INSTITUTE OF BIOCHEMISTRY
AND CELL BIOLOGY

IBBC

Highlights

Shaping Knowledge and Innovation

National Research
Council of Italy

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Contents

About Us

Who We Are

The Institute of Biochemistry and Cell Biology (IBBC) belongs to the National Research Council (CNR) of Italy, the first Italian public research center for number of researchers, and it's one of the 91 Institutions located throughout the country. At IBBC, through a multidisciplinary approach, scientists tackle a broad range of biological questions to pursue knowledge of basic research in the field of cancer biology, immunology, muscle biology and neurobiology, facing also environment-related issues according to a holistic approach. On the basis of scientific interests, IBBC actively joins the activities promoted by the CNR Biomedical Sciences Department.

Founded in 2019 from the merger of the Institute of Biochemistry of Proteins (IBP) and the Institute of Cell Biology and Neurobiology (IBCN), so that IBBC research groups are dedicated to the understanding of the molecular mechanisms regulating cell processes under physiological and pathological conditions, to developing new therapeutic approaches, as well as to implementing innovative technologies to care for public health and environment safety. Therefore, the study of biodiversity and the mechanisms responsible for life adaptation in extreme habitats are met as well.

Research at IBBC is supported by a number of in-house facilities, including the European Mouse Models Archive (EMMA) infrastructure, and by national and international funding agencies deriving from collaborative-research projects with national or international Institutions.

Where We Are

The Institute of Biochemistry and Cell Biology is located in two fascinating Italian regions: Campania and Lazio. IBBC is based in Naples at Castellino Campus and in Monterotondo at Adriano Buzzati-Traverso International Campus. Two ideal sites to live, work and enjoy art and culture.

Castellino Campus - Naples - Campania

The Castellino Campus, namely the Research Area CNR-NA1, is based on the hill of Naples (Vomero) in the central neighborhood known as the "Hospital Area", at very close distance (less than 1 km) from the "University of Naples Federico II" Medical School, the Cancer Research Hospital "Pascale", the "Cardarelli" General Hospital, the Children Research Hospital "Santobono" and the "Center for Genetic Engineering and Advanced Biotechnologies" (Ceinge).

Different types of expertise as diverse as humanities, life sciences and technologies are present across the Campus community. Collaborations in the Campus are routinely carried out for success in competitive projects. Expert researchers in genetics, cell and molecular biology, biochemistry, biophysics and immunology operate at IGB and IBBC; in life sciences at IBBR; in mathematics, bioinformatics, engineering science, physics at IAC, ICAR and ISASI; in environmental sciences at IRET to cite a few. Scientific dialogue and discussion are fostered by the presence of other internationally known Institutions in Naples old city center. These are the "Anton Dohrn" Marine Biological Station, the Physics, Chemistry, Life Sciences and Humanities Departments of the Federico II University and of the Luigi Vanvitelli Campania University among others all contributing to shape a dynamic scientific discussion through collaborative approach.

"A. Buzzati - Traverso" International Campus - Monterotondo - Lazio

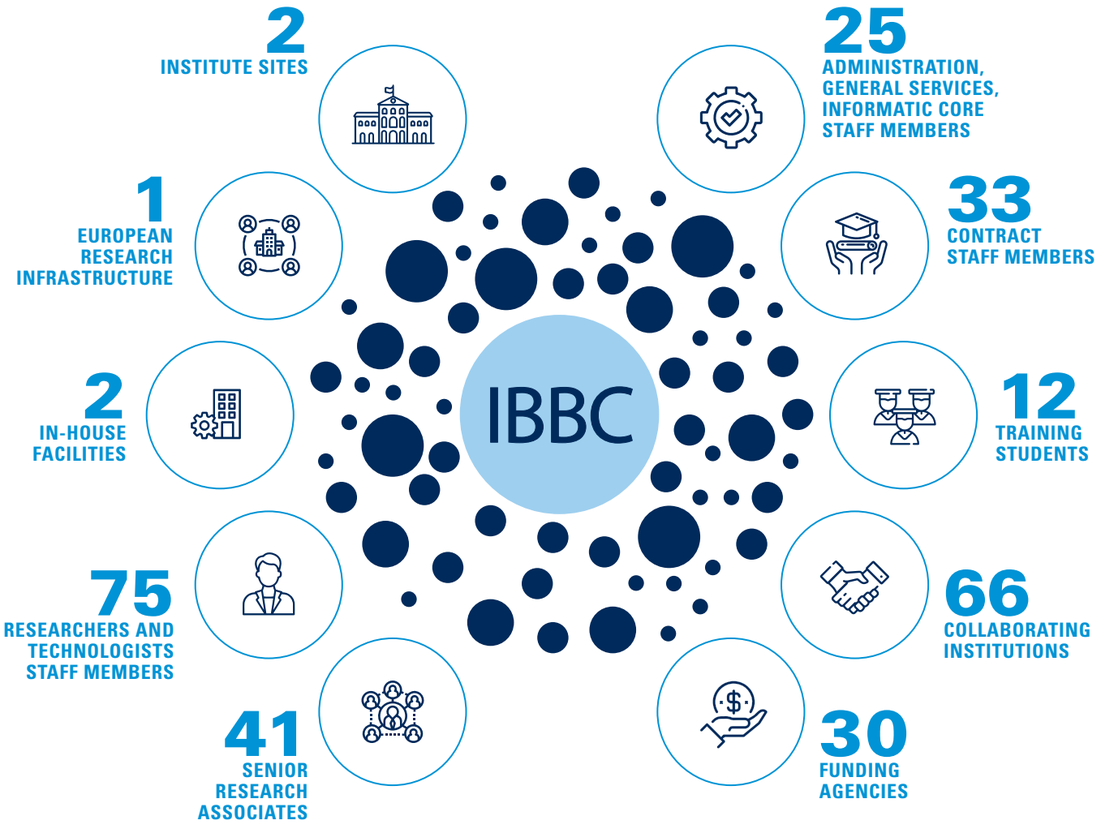
The "A. Buzzati-Traverso" Campus is located in the proximity of four National University Campuses and several private Universities in Rome with strong molecular biology, biochemistry and genetics programmes, and several CNR Research Institutes. The international "A. Buzzati-Traverso" Campus, inaugurated by the President of the Italian Republic in 1999 is located within the technology park of Monterotondo in the North-East of Rome. It also hosts the Core Structure European Mouse Mutant Archive (EMMA), the Mouse Clinic, and the EMBL Rome Epigenetics & Neurobiology laboratory managed by the European Molecular Biology Laboratory (EMBL) dedicated to basic research in molecular life sciences. All the activities foreseen in the Medical Research Council (MRC) Science and Innovation - CNR Cooperation Agreement take place here as well. Scientific discussion and collaboration are fostered by the presence of other national research centers. The Higher Institute of Health, the biological laboratories of ENEA (Italian Energy and Environment Agency) in Casaccia (30 km far from Monterotondo), and the National Institute of Nuclear Physics (INFN) in Frascati are easy to reach. Other CNR institutions, such as Rome 1-Montelibretti Research Area and CNR Headquarters in Rome, can be quickly reached by car from Monterotondo in about 7 minutes and 30 minutes, respectively. The international campus is linked to the GARR Italian Research and Academic Network via the main CNR Headquarter node in Rome.

What We Do

The Institute of Biochemistry and Cell Biology is a multi - and interdisciplinary scientific center committed in basic research in life sciences and biomedicine. Cellular processes investigated at molecular, cellular and *in vivo* level aim at defining the mechanisms regulating cell physiology that when altered cause severe or rare diseases including cancer, immunological, muscular or neurological disorders. Fundamental discoveries often can pave the way for translational research opening to the development of new therapeutic treatments or novel technologies for advanced diagnostic approaches. Particular attention is also deserved to environmental issues with the aim to preserve environment safety and human health into a holistic approach through novel technologies implementation. Exploitation of knowledge acquired form the study of life in extreme environments serves this purpose.

The scientific activities run at IBBC meet the great challenges outlined by the United Nations Organization and European Commission agenda by 2030 and look relevant to address some of the global concerns needed to solve including zero hunger, health and wellbeing, as well as to promote innovation to pursue sustainable development. By combining scientific knowledge and expertise, IBBC's main goal is to reach important achievements on: *i)* basic research; *ii)* translational medicine and biology; *iii)* training the next generation of scientists in biomedicine; *iv)* communication of research and innovation to engage citizens and stakeholders.

IBBC in Numbers



EMMA European Infrastructure

The Mouse Clinic and Archive Core Structure, located in Monterotondo, participates in the Infrafrontier-European Mutant Mouse Archive (EMMA) European Network Infrastructure (Landmark Project of the European Strategy Forum on Research Infrastructures - ESFRI Roadmap). The Infrafrontier European Network aims at building a world-class research infrastructure that provides the international biomedical research community with effective tools to unravel the role of genes function in human diseases.

Infrafrontier-EMMA Mission intends to: *i)* shape the European Research Area in the field of mouse functional genomics in order to give an important contribution to the study of human disease; *ii)* establish reference standards for systemic phenotyping of mouse models and for archiving and distribution of mouse mutants in Europe; *iii)* offer highest-quality services and cutting-edge technologies provided by the leading labs in Europe; *iv)* disseminate knowledge by state-of-the-art training courses. Infrafrontier-EMMA's operations are integrated with the leading International Mouse Phenotyping Consortium (IMPC), the G7 Science Ministers' Mature Global Initiative for the Life Sciences.

The Health and Welfare of animals are of paramount importance to Infrafrontier-EMMA that operates following all the standard procedures and relevant European and national rules and regulations. Animals generated and distributed are bred in Specific Pathogen Free barriered facilities in which all materials are sterilized before entry.

The Infrafrontier-EMMA International Networks and Consortia are coordinated and managed by Infrafrontier Gmbh-Helmholtz Zentrum Muenchen and comprise the leading biomedical research Institutions of most European countries, Israel and Canada, including the National Research Council (CNR) as the Italian promotor and founding member.

Cultivating Innovation

Startups and Technology Transfer

The IBBC is proactive in fostering the technology transfer activity. Within the areas of applied biotechnologies and *in vivo* imaging few startups and spin-offs have been established combining different expertise and approaches deriving from IBBC research center and small-medium sized enterprises (SMEs).

Patent applications have been filed and granted for development of innovative tools based on enzymes, natural compounds, nano-carriers and repurposed drug molecules to cite a few. IBBC's patents cover a wide range of inventions in the field of human health such as therapeutic treatment of tumours, muscular and neurological pathologies, neglected diseases, and also targeted immunotherapy, diagnostic tools for *in vivo* imaging, food safety and biosecurity.

Startups & Spin-offs

Detoxizymes

Startup

Manager Giuseppe Manco

Detoxizymes is a biotech company that develops and produces enzymatic systems for environmental decontamination and safety of human health.

Imagensys

Startup

Manager Alessandro Soluri

Imagensys mission is the development of new detection systems used in nuclear and diagnostic surgery to respond to important challenges in radio-guided surgery: greater speed and greater accuracy in localising lesions and sentinel lymph nodes.

Li-Tech

Spin-off

Manager Alessandro Soluri

Li-tech SpA was created from a National Research Council (CNR) research spin-off. The company's principal aim is the production of high-technology scintigraphic cameras.

Technology Development

Super Spatial Resolution-SPECT

Design and production of some specimens of PET-SPECT detectors, based on SSR (super spatial resolution) to be inserted in an advanced device to four modes (PET-SPECT-CT-Fluorescence), pre-clinical imaging for small animals.

Patents

Anti-Parasitic Compounds

Title: USE OF PERHEXILINE
Code: RM2014A000390 16.07.2014; WO/2016/008977; PCT/EP2015/066264 Publication number: US20170157102A1
Date: 21.01.2016
Application; Filed: 16.07.2015; Publication date: 8.06.2017
Inventors: Ruberti G., Lalli C., Guidi A., Bresciani A., Gennari N., Paonessa G., Nizi E.
Description: The present invention relates to Perhexiline, or a pharmaceutically acceptable salt thereof, for use in the treatment of a pathology caused by trematodes.

Cancer Therapeutic Approach

Title: PYRIMIDO[5,4-d]PYRIMIDINE OR PYRIMIDINE DERIVATIVES COMPOUNDS AND USES THEREOF IN THE TREATMENT OF CANCER
Code: Patent Cooperation Treaty, PCT28627,
Date: September 2012
Inventors: Zollo M., Galeone A., Virgilio A., Spano D., De Antonellis P.
Description: The patent concerns the functional characterization of pirimidopirimidine derivatives able to inhibit specifically the pro-tumoral activity of prune protein and the identification of the more efficient molecules

Title: PEPTIDES ABLE TO IMPAIR THE INHIBITING ACTIVITY OF MDM2/MDM4 HETERODIMER TOWARDS P53 AND USE THEREOF FOR CANCER TREATMENT
Code: EU Patent N.EP 2639240 B1. Patent Classification: INV.C07K14/47
Date: 21.02.2013
Inventors: Moretti F., Mancini F., Pellegrino M., Macchiarulo A., Pellicciari R.
Description: My group has demonstrated that the MDM2/MDM4 heterodimer is an efficient and alternative approach for reactivation of the oncosuppressor p53 in cancer characterized by wildtype p53. This approach is characterized by increased specificity and reduced toxicity compared to other p53-activating approaches.

Title: PROTEASES TARGETING AGENTS
Code: Rif. CNR 10003 PCT/IT2009/000478
Date: 29.04.2010
Inventors: Rossi M., Catara G., Palmieri G., Ruvo M.
Description: The strategy underlining the present invention relies on the knowledge that modulation of the activity of some proteases, namely AARE and elastase, produces negative cellular effects inducing a number of diseases including cognitive, enhancement, cardiovascular diseases, cancer, inflammation, hematological diseases, neurological and urological diseases.

Diagnostic Devices

Title: SURGICAL PROBE FOR LAPAROSCOPY OR INTRACAVITARY TUMOUR LOCALIZATION
Code: ITRM95A000481 13.07.1995 - PCT/IT96/00142 30.01.1997 - US6021341A 1.02.2000
Inventors: Scibilia F., Soluri A.
Description: Proposal of a gamma counter for Radio Guided Surgery that can be used in either intracavitary mode, or in laparoscopic mode, by the use with a specific trocar. The device may automatically subtract the background and provide visualization on a monitor. The probe can also include a flexible tip, in order to enlarge the field of view.

Title: MINIATURISED GAMMA CAMERA WITH VERY HIGH SPATIAL RESOLUTION
Code: ITRM97A000233 23.04.1997 - PCT/IT98/00096 29.10.1998 - WO98/48300 29.10.1998 - US6242744B1 05.06.2001
Inventors: Soluri A., Pani R.
Description: The invention relates to a miniaturized gamma camera with high spatial resolution allowing the localization of tumours of small area in diagnostic or during surgical operations.

Title: FLAT SCINTILLATION GAMMA CAMERA, WITH VERY HIGH SPATIAL RESOLUTION, WITH MODULAR STRUCTURE
Code: ITRM97A000256 02.05.1997 - PCT/IT98/00097 22.04.1998 - WO1998050801A2 12.11.1998 - US6232605B1 5.05.2001
Inventors: Soluri A., Pani R.
Description: Proposal of a gamma camera made up of several modules, so as to obtain a device of the desired size. Each module is based on a position sensitive photomutiplier (PSPMT); the patent shows how to solve the issues related to the dead zones between PSPMTs. Applications may range from the medical field (PET, SPECT, etc.) to the use in astrophysics.

Title: MODULAR HIGH SPATIAL RESOLUTION SCINTIGRAPHIC DEVICE WITH MULTIPLE INDEPENDENT PHOTOMULTIPLIERS AND WITH EXTENSIBLE VISUALISATION AREA
Code: ITRM2001A000280 23.05.2001 – EP1265079A3 11.12.2002 – US6608310B2 19.08.2003
Inventors: Soluri A., Scafè R., Burgio N., Schiaratura A.
Description: The invention relates to a modular high Spatial resolution scintigraphic device with multiple independent photomultipliers and with extensible visualisation area. The device can be applied in Nuclear Medicine as localisation and diagnostic devices to identify neoplasias with high Spatial resolution. Moreover it can be used for Small Animal imaging.

Title: HIGH SPATIAL RESOLUTION SCINTIGRAPHIC DEVICE HAVING COLLIMATOR WITH INTEGRATED CRYSTALS
Code: ITRM2001A000279 23.05.2001 – EP1262796 12.04.2002 – US6734430B2 11.05.2004
Inventors: Soluri A., Scafè R., Burgio N., Schiaratura A.
Description: The invention relates to a modular high Spatial resolution scintigraphic device with multiple independent photomultipliers and with extensible visualisation area.

Title: SCINTIGRAPHIC DEVICE WITH HIGH VARIABLE COLLIMATOR
Code: ITRM2004A000271 31.05.2004 - US7274022B2 25.09.2007
Inventors: Soluri A., Scafè R., Piano M., Scopinaro F.
Description: The patent proposes a scintigraphic device that vary automatically, or manually, the overall length of the collimator in relation to the characteristics of the lesion to be examined (diameter, depth and lesion/bottom radioactivity concentration ratio). Image contrast and spatial resolution can be optimized without replacing the collimator.

Title: METHOD FOR OBTAINING A SCINTILLATION STRUCTURE
Code: ITRM20080169 28.03.2008 - US7928396B2 01.10.2009
Inventors: Soluri A., Massari R., Trotta C., Scopinaro F.
Description: Description of construction method regarding a structure composed by scintillation crystals mutually separated by a metallic material having high atomic number and high density. The assembly is intended to be used into scintigraphic devices.

Title: SCINTIGRAPHIC DEVICE WITH SPATIAL SUPER-RESOLUTION
Code: ITMI20081798 10/10/2008 - US7939807B2 10.05.2011
Inventors: Soluri A., Massari R.
Description: The patent relates to a scintigraphic detector exploiting a super resolution technique which enables to enhance the achievable spatial resolution of the imaging system. The device can be used in small animals imaging, as well as in Nuclear Medicine both in SPECT and PET techniques or in Astrophysics.

Title: A SCINTIGRAPHIC DEVICE WITH HIGH SPATIAL RESOLUTION
Code: ITRM20090666 18.12.2009 - WO2011074022A1 23.06.2011
Inventors: Soluri A., Massari R., Scandellari M., Trinci G.
Description: The patent relates to a scintigraphic device with high spatial resolution through the use of a collimator having moving septa. The described gamma camera could be used for several applications such as diagnostic systems (PET, SPECT and traditional scintigraphy), in Astrophysics or for industrial non-destructive tests.

Title: DIAGNOSTIC DEVICE FOR MORPHO-FUNCTIONAL INVESTIGATIONS
Code: ITRM2011A000543 13.10.2011 - PCT/IT2012/000314 11.10.2012 - WO2013054369A1 18.04.2013 - US9414789B2 16.08.2016
Inventors: Soluri A., Massari R.
Description: Proposal of a diagnostic device for morpho-functional investigations that includes a plurality of detectors such as SPECT, PET and CT. The device is capable of performing investigations on different areas of the patient's body while maintaining high spatial resolution and providing short scan times. Its compact size allows easy installation.

Title: PORTABLE GAMMA CAMERA
Code: ITRM2012A0491 16.10.2012 - PCT/IT2013/000285 15.10.2013 - WO2014061047A1 24.04.2014 - US9408583B2 09.08.2016.
Inventors: Soluri A., Massari R.
Description: This invention relates to a portable fully integrated gamma camera, i.e. designed to operate without any cable for external connections, having shape and dimensions so as to be easily handled by an operator, and integrating the display.

Title: SCINTIGRAPHIC DIRECTIONAL DETECTOR
Code: PCT/IT2013/000131 08.05.2013 - WO2013168188A3 27.11.2014
Inventors: Soluri A., Massari R.
Description: Proposal of a gamma detector for radio-guided surgery. The device comprises a plurality of sensing elements to simultaneously detect gamma rays along different directions. This allows the directional detection of gamma rays to guide the surgeon more effectively.

Title: MULTIFUNCTION GAMMA RADIATION DETECTOR
Code: PCT/IB2021/056322 14.07.2021
Inventors: Soluri A., Massari R.
Description: The invention relates to a multifunctional gamma detector for radio-guided surgery (intra-operative and laparoscopic) for localisation of lymph nodes and tumours and/or other pathologies. Besides a directional gamma radiation detection system, an optical spectrometric analysis system is integrated to analyse the patient's tissue.

Environment/Biosecurity

Title: INTEGRATED SYSTEM FOR THE DETECTION AND DEGRADATION OF NERVE AGENTS BY THERMOSTABLE BIOCATALYSTS"
Code: 102017000059318
Inventors: Manco G., Porzio E., Febbraio F., Suzumoto Y., Palchetti I., Carusone T. M.
Description: The present patent describes the realization of an integrated system consisting of a node biosensor/actuator, possibly expandable in a network, for the defense of civilians from a terrorist attack with nerve agents. The innovative aspect lies in the use of thermostable enzyme formulations, both for the detection (as biosensors) and for the degradation of toxic agents.

Title: HUMAN PARAOXONASE 2 MUTANTS AND ITS FORMULATIONS FOR THE DEGRADATION OF DIFFERENT ORGANO(THIO)PHOSPHATE COMPOUNDS
Code: 102015000027699
Inventors: Mandrich L., Porzio E., Manco G.
Description: The present invention relates to a polypeptide (recPON2), by means of protein engineering techniques, unexpectedly endowed with thio-phosphoesterase activity, and its mutated version (K126R) that is resistant to a post-translational modification and has higher stability. The object of the patent is also the use in appropriate formulations of these polypeptides, also with other enzymes, for degradation of different organothio-phosphate compounds and also for the treatment of biofilm produced by pathogenic bacteria, as P. aeruginosa.

Title: THERMOPHILIC AND THERMOSTABLE PHOSPHOTRIESTERASES OBTAINABLE IN RECOMBINANT FORMS
Code: 102007901484273
Inventors: Manco G., Mandrich L., Merone L., Porzio E., Rossi M.

Description: In this invention mutants of the thermostable phosphotriesterase from Saccharolobus solfataricus (strain MT4), as well as new homologous genes have been described equipped with: increased activity, altered termofilia, pH optimum and substrate specificities that widen the repertoire of enzymes usable especially in the field of organophosphates decontamination, detoxification and detection.

Food Safety/Celiac Disease

Title: TREATMENT OF CEREAL FLOURS FOR FOOD CONSUMPTION BY CELIAC PATIENTS
Inventors: Rossi M., Gianfrani C. , Siciliano R. A.
Code: MI2006 A 002080 C12N A21D, 30.10.06. Ref CNR 1838. PCT2008/053310 A2 of 05.08.2008.
Description: This invention relates to the use of an enzymatic method for treatment of flour and protein extracts derived from cereals that stimulate a pathological immune response in Celiac Disease patients. The method uses the catalytic activity of microbial tissue transglutaminase and alkylated lysine. The treated cereal flour/proteins lose their immunetoxicity for celiacs.

Immunostimolatory Compounds

Title: PHAGE CONJUGATES AND USES THEREOF
Code: EP 3573667; 140869BE
Date: 27.01.2021
Inventors: De Berardinis P., Sartorius R.
Description: The invention consists of a pharmaceutical formulation based on a natural nanoparticle, the bacteriophage fd, specifically targeted to dendritic cells for the simultaneous delivery of immunomodulating lipids and of antigenic peptides. This delivery system enhances anti-tumor immune responses and is protective in preclinical models of vaccination.

Muscle Pathology

Title: COMPOSITIONS AND METHODS FOR TREATMENT OF MUSCULAR DYSTROPHY
Code: Granted European Patent n EP3194600B1, validated (protected) in French, United Kingdom, Germany, Ireland.
Assignee: CNR
Date: 26.07.2017
Inventors: Passananti C., Corbi N., Di Certo M. G., Mattei E. Pisani C., Strimpakos G., Luvisetto S.
Description: The present invention features modified human transcription factors capable of increasing utrophin expression, recombinant adeno-associated vectors for delivery of the modified human transcription factors, and methods of treating muscle diseases, including Duchenne's muscular dystrophy.

Title: COMPOSITIONS AND METHODS FOR TREATMENT OF MUSCULAR DYSTROPHY
Code: International Granted USA Patent US10301367B2
Assignee: CNR
Date: 03.08.2017
Inventors: Passananti C., Corbi N., Di Certo M. G., Mattei E. Pisani C., Strimpakos G., Luvisetto S.
Description: The present invention features modified human transcription factors capable of increasing utrophin expression, recombinant adeno-associated vectors for delivery of the modified human transcription factors, and methods of treating muscle diseases, including Duchenne's muscular dystrophy.

Title: COMPOSITIONS AND METHODS FOR TREATMENT OF MUSCULAR DYSTROPHY
Code: International Granted USA Patent n. US10286085B2
Date: 20.10.2016
Inventors: Passananti C., Corbi N., Di Certo M. G., Mattei E. Pisani C., Strimpakos G.
Description: The present invention features recombinant adeno-associated vectors for delivery of genes to both skeletal and cardiac muscle and methods for treatment of muscle defects, including Duchenne's muscular dystrophy.

Neurobiology/Medulloblastoma Treatment

Title: CXCL3 CHEMOKINE FOR THE THERAPEUTIC TREATMENT OF MEDULLOBLASTOMA
Code: IT000858A patent request filed by CNR on 02.10.2012; CNR European patent WO 2014053999 A1;
Date: 10.04.2014; recording 02.10.2012
Inventors: : Tirone F., Cavallaro S., Farioli-Vecchioli S., Micheli L., Leonardi L., Cinà I., Ceccarelli M.
Description: The invention relates to a novel therapeutic agent, the Cxcl3 chemokine, to treat medulloblastoma (MB), one of the most widespread pediatric brain tumors. Cxcl3 was identified as MB suppressor by genomic analysis of a high frequency MB mouse model (Farioli-Vecchioli e al. J.Neurosci, 2012, 32:15547).

Title: NEW THERAPEUTIC USE OF BOTULINUM NEUROTOXIN SEROTYPE A
Code: International, Nr. WO2016170501_A1
Date: 28.02.2018
Inventors: Marinelli S., Pavone F., Luvisetto S., Vacca V.
Description: A new therapeutic use of botulinum neurotoxin serotype A (Bont/A) is described, therapeutic treatment of paralysis caused by spinal cord injury.

Title: METHOD FOR THE POTENTIATION OF OPIOID ANALGESICS EFFECTS ON PAIN
Code: WO2006137106
Date: 28.12.2006
Inventors: Pavone F., Marinelli S., Cattaneo A., Ugolini G.
Description: According to the invention there is provided use of an anti-TrkA antibody capable of inhibiting the binding between NGF and TrkA combined with at least one opioid analgesic for the preparation of a medicament for treating and/or preventing pain.

Title: NOVEL ANALGESIC TREATMENT WITH PROLONGED EFFECT
Code: WO2006131952
Date: 14.12.2006
Inventors: Pavone F., Marinelli S., Cattaneo A., Ugolini G.
Description: Use of an anti-TrkA antibody capable of inhibiting the binding between NGF and TrkA, in particular capable of blocking the biological activity of TrkA, for the preparation of a medicament for treating and/or preventing chronic pain.

Science Communication

Science in the City

Science communication at IBBC is actively run by senior and young scientists through implementation of activities designed to promote passion and awareness of research to the lay public, aiming to raise trust in science among the youngest generation. Routinely, IBBC welcomes students, teachers and sciencephiles to visit laboratories, discover more about the IBBC research, and understand why science and innovation matter to society.

Through a complex approach relying on collaboration and co-design between IBBC researchers and stakeholders, science communication practice points to establish a dialogue with citizens to foster their participation into debates around public issues. Therefore, dedicated meetings are organized to tackle diverse topics as the role of vaccinations, the prevention from old or emerging infectious diseases, or the importance of food safety to cite a few. Recent Covid-19 outbreak has strongly pushed IBBC science communication activity to move to digital formats reaching a wider audience.

IBBC is involved in a number of science communication projects directly or in partnership and participates in national and international science festivals. Since June 2021 it is member of CREO - CNR the Campania Research Outreach network, which includes 24 CNR Institutes based in Campania region and CNR Single Guarantee Committee.

AUTHORS:

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Covid-19 and science communication: an opportunity to assess new formats in public engagement

Covid-19 breakout has highlighted the pivotal role played by science in the society. Among the effects caused by pandemic, the acceleration rate toward digitalization process of information, including-science communication, represents one of the most evident cases. Suddenly, one had to move from interactive educational approaches to distance learning education by the adoption of dedicated platforms within virtual rooms, searching for new forms of dialogue between the players: digital immigrates (baby boomers and x generation) and digital borns (z generation), more prone in the use of technological devices (i.e. smartphone, videogames).

This digital transformation process has let to reconsider the organization of our science communication events, including educational activities for the secondary grade school students. Therefore, the cycle "School meets the research" (1) has been turned in "Research goes to school", which foresees synchronous meetings tackling important topics of interest for the society within a virtual space. From vaccination for healthcare of citizens to adoption of eco-friendly measures for environmental protection, from innovative technologies addressed to diagnosis and treatment of diseases to marine and antartic biology. Digital transformation has led us also to participate to international events such as "European Biotechnoloy Week 2020" (2) through the implementation of the asynchronous meeting "Doing BIOTECH: Healthcare that will come", to allow teachers to use the educational tools according to tailored modalities and needs, exploiting alternative channels such as YouTube.

In this context, the "hands on" approach, key element characterizing the visits at the research laboratories, has been substituted by video-tutorials performed according to the logic of screen, with the aim to catch young digital borns' attention. Besides, being in streaming has allowed to start new collaboration programmes with schools based in other regions in Italy and again to participate into broader european communities such as "European Schoolnet" (3) tackling the innovative trends in education of STEM (Science, Technology, Engineering, Mathematics).

Concerning the public engagement events, the management is more complicated because the "facetoface" factor, distinctive of the engagement events (two-way process), has to be reinvented using new formats and social media. Futuro Remoto 2020 and European researchers' night - Meet Me tonight 2020 are currently ongoing experiments, although constrained to propose a format closer to a dissemination event (one-way process).

In this scenario, the risk is that part of the general public goes missed, because not confident with information and communication technologies. Therefore, the "spillover" of a high number of citizens to digitalization process represents the challenge to cope with getting science communication more inclusive and opened to everyone.

KEYWORDS:

Covid19, science communication, digital transformation, public engagement

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2. https://www.cnr.it/it/evento/16943/fare-biotech-la-salute-che-verra-la-ricerca-va-a-scuola
3. https://www.cnr.it/it/news/9388/il-cnr-ibbc-aderisce-alla-2020-stem-discovery-campaign

Research Topics

IBBC activity falls into 9 topics: Biochemistry and applied biotechnologies, Cell and molecular biology, Drug discovery, Immunity and infection, *In vivo* imaging, Murine models, Muscle biology and pathology, Neurobiology and Translational research.

The research topics face relevant challenges such as the cure of cancer, neurological or muscular disorders, as well as immune-mediated pathologies. At the same time novel approaches for drug delivery, technologies to apply in preclinical studies or to use for monitoring environment safety are also implemented. Discovering the mechanisms of life adaptation in extreme habitats allows to enrich the collection of novel molecules to harness in diverse biotechnological fields. Furthermore, the possibility to work with novel murine mutant models of human diseases strongly supports the transition from basic to translational research.

- BIOCHEMISTRY AND APPLIED BIOTECHNOLOGIES
- CELL AND MOLECULAR BIOLOGY
- DRUG DISCOVERY
- IMMUNITY AND INFECTION
- IN VIVO IMAGING
- MURINE MODELS
- MUSCLE BIOLOGY AND PATHOLOGY
- NEUROBIOLOGY
- TRANSLATIONAL RESEARCH

Biochemistry and Applied Biotechnologies

Biochemistry and applied biotechnologies research activities focus on protein structure-function relationship and on protein-protein interaction dynamics and localization of cellular proteins. To these aims, different approaches are undertaken as diverse as *in vitro* biochemical and biophysical studies including the 3D-structure resolution, identification of post-translational modifications, and protein-protein interactions including protein interactions with other cellular components (RNA, DNA, lipids, carbohydrates, metabolites). Biochemical screenings relying on functional assays, enzymatic analyses, virtual screenings on the basis of bioinformatic approaches and mass spectrometry analysis are routinely carried out to identify new proteins as molecular targets to develop targeted-based therapies related to human health and environment safety as well. The development of diagnostic tools for human health and environment is also tackled.

AUTHORS:

- F. Febbraio

Development of new bioreceptors to be used in biosensors and/or diagnostic kits for the monitoring of toxic substances in humans and in the environment

Biosensors are analytical devices which use a biological mediator (bioreceptor) to selectively detect, with high sensitivity, chemical or biological analytes without the need for sample pretreatment. This is usually achieved by coupling the bioreceptor to a suitable transduction system, which converts the biochemical response into a quantifiable and processable physical signal. Diagnostic kits for the detection of biomarkers are based on the same principles, serological tests for SARS-COV2 are an example (Hussein et al., 2020). The advantages of biosensors can be summarized in three simple concepts: easy, fast and cheap. In fact, thanks to their specificity and easy of use, the biosensors are also suitable to be used by less experienced users. In addition, they allow to perform an analysis in a relatively short time, few minutes or seconds, while maintaining high reliability at remarkably low costs.

Starting from the need to develop new bioreceptors for toxic molecules, that are until now identified only by liquid chromatography and mass spectrometry approaches, new protein bioreceptors have been studied. Also, their use in multi-biosensor systems for the detection of toxic substances, has been evaluated. A database of 3D structures of the most widespread toxic molecules, belonging to the main families of pesticides (the most widespread toxic molecules), was built. Similarly, a database of 3D protein structures was constructed on the basis of sequence and structure similarity with proteins that bind some analyzed compounds or similar molecules. In order to identify new bioreceptors to be used in biosensors to perform large-scale monitoring analyzes, the databases were analyzed through "inverse virtual-screening" bioinformatic studies. These studies were performed on an ad hoc workstation, using some software for structural analysis and protein-ligand docking, and using scripts to automate the analysis processes. The results were manually analyzed and verified in a second round of analysis. Some proteins or protein domains were selected with a higher binding efficiency towards the toxic compounds analyzed. The selected proteins were proposed as possible candidate bioreceptors, so we started with the cloning and expression in E. coli of an HRDC-RecQ domain, which showed high affinity towards famoxadone, a fungicide widely used in agriculture and which seems to have harmful effects on bees. The protein was then purified and the bond with pesticide molecules was studied by means of composition gradient - multi angle light scattering measurements. This technique allows to analyze the oligomeric state of the protein in solution, the formation of protein-ligand complexes and their binding affinity. This technique allowed to demonstrate that the protein domain maintains a monomeric form in solution. These results were also confirmed by measurements on native and glutaraldehyde cross-linked proteins analyzed by SDS-PAGE. Finally, the protein was labeled with fluorescent probes to be used for pesticide detection by means of fluorescence quenching measurements. Preliminary data showed a linear decrease in the fluorescence of protein-bound probe at increasing concentrations of pesticide. These results, although still preliminary, support the detection of the pesticide molecule by the bioreceptor, opening new perspectives for the development of new biosensors.

KEYWORDS:

Bioreceptors, Biosensors, Toxic molecules, CG-MALS, Fluorescence.

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Biochemical and molecular studies on enzymes involved in cell detoxification

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KEYWORDS:

Regulation of Catalysis; Structure-Function Relationships; Detoxification.

Despite the important role pesticides play in modern agriculture, an increasing number of reports concerning the negative impact of organophosphate (OP) pesticides have appeared. In fact, OPs poisoning has become a global health problem, affecting millions of people if one considers acute and chronic unintentional intoxications. Toxicity of OPs is determined by targeting acetylcholinesterase (AChE), one of the key enzymes in cholinergic transmission. OPs act as inhibitors of AChE through the covalent phosphorylation of a serine residue within the active site. This results in the abnormal accumulation of acetylcholine at the neuron–neuron junctions or the neuromuscular junctions, leading to lacrimation, hypersalivation, ataxia, convulsions, and respiratory failure, which is the primary cause of death in most cases. Besides, several other studies have suggested a correlation between OP pesticides and neurodevelopment disorders as well as neurodegenerative diseases, including Parkinson's or Alzheimer's diseases. In particular, long-term health implications of even low levels of OP pesticide exposure have been highlighted. Other studies on the toxic effects of OPs have implied the involvement in DNA damage, cancerogenesis, and endocrine disorders. In many of these effects, ROS production seems to be also involved (1). Our studies in this field proceed along different lanes of research starting from the structure-function relationships of microbial homologs of AChE useful for the construction of specific biosensors and traps, not only for pesticides but also for the deadly nerve gases, and going to the study of phosphotriesterases/lactonases for the degradation of OPs (2-5) or microbial lactones involved in the formation of the antibiotic-resistant microbial biofilm (6). In the vein of the last research topic, we have undertaken the study of human paraoxonase 2 (PON2). PON2 is a member of the paraoxonase/lactonase family of genes, which also includes PON1 and PON3. Only PON2 is ubiquitous and located on plasma membranes, where it seems to represent the first line of defense against infections. In the mitochondria, PON2 has anti-ROS activity. The activity of PON2 is rapidly reduced in cells incubated with the bacterial quorormone 3-Oxo-dodecanoyl Homoserine Lactone (3OC12HSL), an observation that led to hypothesize a fast PON2 post-translational modification (PTM). We detected a 3OC12HSL-induced PTM in a cell-free system in which a crude extract from 3OC12HSL-treated HeLa cells was able to inactivate and ubiquitinate at position 144 a recombinant PON2 (6). Lately, employing nanoLC/MsMs we have shown the occurrence of this and new PTMs of PON2 in HeLa cells. In particular, we detected ADP-ribosylation (D124) and many ubiquitinations (K29; K156; K159). Furthermore, we proposed a link between gene expression and regulation of activity mediated by PTMs and SNPs related to diabetes type 2 (7). PON2 is unique among PONs to be expressed in the brain tissue. Due to its cellular localization and anti-oxidant and anti-inflammatory actions it may represent a relevant enzyme involved in neuroprotection. This would represent an important area for future developments.

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Connexin hemichannel-mediated ATP release drives spontaneous Ca2+ signaling in the greater epithelial ridge of the developing cochlea

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KEYWORDS:

Whole Cell-Based Biosensors, ATP Release, Connexin Hemichannels, Calcium Signaling, Developing Cochlea, Microfluidic Chip.

In the inner ear, extracellular ATP plays a fundamental role in the regulation of both sensory and non-sensory cells. During cochlear development, ATP triggers oscillations of intracellular calcium (Ca2+) concentration and propagation of intercellular Ca2+ waves that carry crucial biochemical information[1]. The greater epithelial ridge (GER) is one of the two embryologically distinct regions into which the cochlear sensory epithelium is divided. In non-sensory cells of the GER, Ca2+ waves arise spontaneously and seem to be key to functional maturation of hair cells, hearing acquisition or age-related hearing loss[2].

A variety of experimental data supports a crucial role for the two major cochlear connexins, connexin 26 (Cx26) and connexin 30 (Cx30), in the propagation of spontaneous Ca2+ waves in the developing cochlea[3]. However, there has been much speculation on whether extracellular ATP driving spontaneous Ca2+ signaling is released by connexin hemichannels or by other membrane channel, such as pannexin 1 (Panx1) hemichannels[4, 5].

To address this issue, we used cochlear organotypic cultures (COCs) from two global knock out (KO) mouse strains, namely Cx30 KO (Gjb6–/–), in which Cx30 is absent and Cx26 is strongly downregulated, and Panx1 KO (Panx1–/–). We developed whole cell-based biosensors (ATP-WCBs) with nM ATP sensitivity, suitable to detect ATP release events exceeding the baseline ATP concentration ([ATP]) in the endolymph (low nM range). Then, we used COCs and ATP-WCBs coupled in an innovative transparent microfluidic chamber, designed ad hoc to host two samples, separated from each other by an average distance of 50 µm. In each experiment, the COC was located in the bottom site of the microfluidic chip and a coverslip plated with the ATP-WCBs was placed upside down at the top. Both samples were previously loaded with Fluo8H, a cytosolic Ca2+ indicator, to perform dual-plane multiphoton Ca2+ imaging. By oscillating the objective of the two-photon microscope between the two focal planes, we monitored in real time spontaneous intercellular Ca2+ waves in the GER of COCs, paralleled by ATP-dependent Ca2+ responses in ATP-WCBs. Addition of ATP diphosphohydrolase to the shared extracellular medium inside the microfluidic chamber abolished Ca2+ signals in both COCs and ATP-WCBs. Importantly, spontaneous Ca2+ signals were strongly depressed in experiments with Gjb6–/– COCs, but they were not affected in the case of Panx1-/- COCs. We further confirm these results using a classical luciferin–luciferase bioluminescence assay to evaluate the amount of ATP released by Gjb6–/– or Panx1–/– COCs compared to wild type COCs from respective siblings.

Our findings provide strong evidence that connexin hemichannels -and not Panx1 channels- mediate the release of ATP that drives spontaneous Ca2+ wave propagation in the developing cochlea[6].

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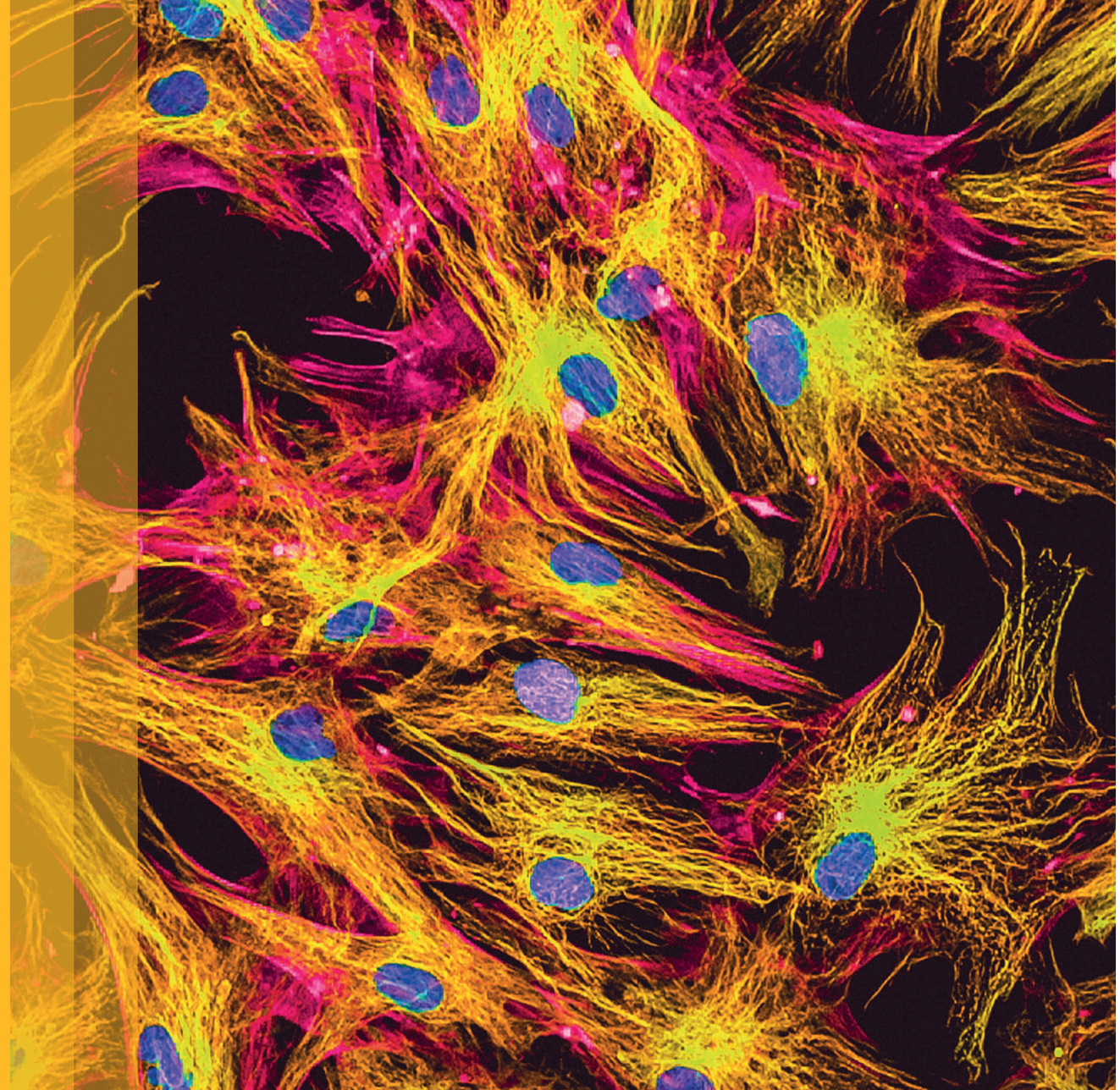
Cell and Molecular Biology



Cell and molecular biology research activities aim at understanding the basic biological processes and the cellular regulatory mechanisms, namely signal transduction, that, when altered, lead to rare diseases and cancer. This knowledge is further harnessed to design novel drug-based therapies upon identification of new molecular targets, or to shape innovative diagnostic tools to implement translational medicine strategies. By a multidisciplinary approach, relevant cellular processes are investigated, which include among others genome stability and cell proliferation; the regulatory mechanisms responsible for osteoclastogenic process; the thyroid-specific gene expression to unravel the regulatory patterns involved in the thyroid tumors onset.

Epidermal cells observed under fluorescence microscopy. Nucleus are in blue, actin filaments are in pink, tubulin was labeled with green.

Thyroid hormone receptors as co-repressors of TTF-2/FoxE1 activity

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KEYWORDS:

Thyroid, Estrogen, FoxE1/TTF-2, Thyroid-Specific Gene Expression.

Gender medicine indicates that diseases can manifest differently in men and women and thus diagnosis, therapy and medication have to be gender-specific. Namely, thyroid diseases are common afflictions, with a much higher prevalence in females than in males. Both benign and malignant thyroid tumors are more prevalent in women than men, so female sex hormones may have an etiological role in these conditions. Immunohistochemistry and binding assays have identified estrogen receptor (ERs) isoforms (ER and ER) in thyroid tissue and overexpression of ERs has been associated with neoplastic thyroid tissues. Furthermore, it has been shown that 17beta-estradiol (E2) enhances cell growth in FRTL-5 cells (rat thyrocytes) where it inhibits the sodium/iodide symporter (SLC5A5, NIS) gene expression. However, our knowledge on how E2 affects thyroid function and namely the thyroid-specific gene expression is very scarce.

The tissue-specific expression of the thyroid differentiation markers has been extensively studied and their transcriptional regulation results mediated by a set of transcription factors including TTF-1/Nkx2-1, Pax8 and TTF2/FoxE1. During development and in the adult life, these transcription factors are present in other cell types, but their concomitant expression is restricted to thyrocytes only. Their expression is required for the early stages of thyroid morphogenesis and is crucial for the normal thyroid function and homeostasis.

In this study we have analysed the role of E2 in regulation of thyroid-specific gene expression. In order to verify the interaction between ER and TTF-2, we have performed the two - hybrid assay in mammalian cells. We show that Gal4TTF-2 has modest trans-activation activity on Gal4-luc vector. Similarly, the estrogen receptors, expressed alone, have weak activity on the GAL4 -dependent promoter. However, when Gal4-TTF-2 and ER or Gal4-TTF-2 and ER are coexpressed, Gal4-TTF-2 is able to recruit the ERs on the promoter, resulting in a robust stimulation of the reporter gene transcription. These experiments strongly suggest that TTF-2 is able to form a complex with either ER or ER. It is worth to point out that these experiments have been performed in HeLa cells that do not express endogenous ERs . To determine whether estrogen could affect TTF-2 activity in thyrocytes, we used p4xZ-Luc vector, a TTF-2-specific artificial promoter driving the luciferase gene expression. We have performed, in FRTL-5 cells, transfection experiments of p4xZ-Luc in presence or in absence of E2. The experiments show that E2 down-regulates the promoter activity and so strongly suggest that E2 inhibits TTF-2 activity. To confirm this result in the contest of a thyroidspecific promoter we have used the pNIS-luc vector. We have co-transfected the pLKO-i77 vector, expressing the shRNA able to silence TTF-2 expression. In absence of estrogen, the silencing of TTF-2 down-regulates the luciferase gene expression. This result confirms that TTF-2 regulates NIS gene transcription. Interestingly, in presence of E2, NIS promoter activity is very similar in presence and in absence of TTF-2. Hence, in presence of E2, TTF-2 is not able to trans-activate the promoter. This result suggest that E2 inhibits TTF-2 activity on NIS promoter as well.

Thus, we have provided some experimental evidence that the estrogen receptors interact with TTF-2 and E2 inhibits its transcriptional activity. Taken together, these data suggest that estrogen receptors may acts as a TTF-2 corepressor.

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sPLA2-IIA regulates osteoclast differentiation and function

Introduction.
Bone metastasis development can be blocked by inhibition of bone resorption, which defines osteoclasts as targets for anti-metastatic treatments. Secreted phospholipase A2 group IIA (sPLA2-IIA) is involved in osteoclastogenesis, which leads to formation of professional bone-resorbing osteoclasts, but its mechanism(s) of action is still unknown.

Material and method.
To deepen the role of this glycerophospholipid-hydrolytic enzyme, the Raw264.7 macrophagic cell line was chosen as osteoclast precursors, which can be differentiated by 5-day treatment with Receptor Activator of Nuclear Factor kappa-B Ligand (RANKL). As sPLA2-IIA is a dual-function protein, inhibitors with distinct selectivities against sPLA2-IIA actions were used to dissect out its mechanism(s), together with down-regulation of sPLA2-IIA expression using small-interfering-RNAs (siRNAs), and precursor cells from sPLA2-IIA knock-out mice.

Results and discussion.
During RANKL-induced osteoclastogenesis of Raw264.7 cells, two sPLA2-IIA inhibitors, the pentapeptide [c(2NapA)LS(2NapA)R; 20 M] and the small molecule [KH064; 40 M], decreased transcription of osteoclast markers and multinucleated cell formation. Down-regulation of sPLA2-IIA using siRNAs in precursor cells confirmed these data. Instead, treatment with an alkylating reagent of the catalytic histidine of sPLA2-IIA [p-bromophenacyl bromide; 10 nM] reduced osteoclast maturation without blocking syncytium formation. These data indicate sPLA2-IIA participation in osteoclast maturation and control of syncytium formation by mechanisms that may be both catalytically dependent and independent via interactions with an unidentified partner. Data obtained with addition of both wild-type and catalytically inactive recombinant sPLA2-IIA throughout the differentiation of Raw264.7 cells reinforces this interpretation. Further support comes from primary osteoclast precursors isolated from sPLA2-IIA knock-out BALB/cJ mice. Indeed, RANKL-induced differentiation of sPLA2-IIA knock-out precursors generated less TRAP-positive osteoclasts, with lower transcription of osteoclast markers and bone resorbing activity, compared to wild-type controls. Of note, several data indicate the involvement of p-38 signalling downstream of sPLA2-IIA in osteoclast fusion.

Conclusion.
These studies provide more complete understanding of the still enigmatic osteoclastogenic process, and pave the way to more targeted drug interventions for treatment of bone metastases.

KEYWORDS:

Phospholipase A_2, Osteoclastogenesis, p-38 SAPK.

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"Shedding laser light" on the molecular and cellular functions of DDX11 and FANCJ DNA helicases

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KEYWORDS:

DNA Replication, Genome Stability, Cell Cycle, DNA Helicases, Fork-Protection Complex.

In the recent years, the interest of our laboratory has been focused on investigating the molecular and cellular functions of DDX11 and FANCJ, two iron-sulphur (Fe-S) cluster-containing super-family 2 DNA helicases, whose mutations are linked to the genome instability disorders Warsaw breakage syndrome (WABS) and Fanconi anaemia (FA), respectively [1-3] . DDX11 and FANCJ are both associated to the ongoing replisomes and cooperate with the fork-protection complex (FPC, formed by the replication factors Timeless, Tipin, Claspin and AND-1) in assisting smooth progression of the replication forks at difficult to replicate templates [4, *our unpublished data*]. These latter are genomic sites prone to give rise to stable secondary structures (such as G-quadruplexes, triplexes, i-motifs and hairpins) that represent roadblocks to the replication machinery progression. While the replicative DNA helicase (the MCM2-7 complex) is unable to dismantle these alternative structures, DDX11 and FANCJ can untangle them *in vitro*. Thus, they are believed to play a key role in allowing the replisomes to overcome these obstacles in cell nuclei [5].

Our present research aims to discover how DDX11 and FANCJ cooperate with the fork-protection complex in promoting duplication of difficult to replicate genomic regions. To this aim we are using complementary experimental approaches that include biochemistry, molecular and cell biology and biophysics techniques. We are analysing genome stability maintenance pathways by means of various functional readouts in DDX11- or FANCJ-depleted cell lines. Using a combination of FISH and karyotypic analyses we are examining if any phenotypic anomaly (such as cohesion and mis-segregation defects, gaps, breakages, anaphase bridges) can be associated with specific chromosomes and/or genomic loci in the above cell lines.

Besides, we plan to examine recognition/binding/resolution of alternative nucleic acid structures (such as G-quadruplex DNA) by DDX11 and FANCJ DNA helicases and the role played by components of the fork-protection complex in this process by biophysical single-molecule experiments with *in vitro* reconstituted protein systems. We will use the correlative optical tweezers-confocal fluorescence microscope that will be installed at the IBBC in 2021 in the framework of the PON IMPARA project. With this cutting-edge instrumentation we will visualise real-time binding of fluorescent-labelled proteins of interest to DNA/RNA molecules. Changes of the mechanical properties of these nucleic acid ligands, finely micro-manipulated by the optical tweezers, can be monitored with high sensitivity and resolution and directly correlated to the interacting protein activity.

The results of these studies are expected to reveal unprecedented details of DDX11 and FANCJ action mechanism and elucidate the role played by the fork-protection complex in counteracting replication stress at difficult to replicate genomic loci.

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Unravelling the signaling and molecular pathways downstream Golgi fragmentation

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The Golgi apparatus is composed of stacks of cisternae laterally connected by tubules to form a ribbon-like structure. At the onset of mitosis, the Golgi ribbon is broken down into discrete stacks, which then undergo further fragmentation. This ribbon cleavage is required for G2/M transition. Up to now, the molecular mechanisms and signaling pathways underlying this process are not clearly understood. We previously showed that the Golgi ribbon fragmentation promotes the activation of a Golgi-based pool of Src, which then interacts with and phosphorylates Aurora-A at tyrosine 148 and this specific phosphorylation is required for Aurora-A catalytic activity and recruitment at the centrosomes (1). This process, pivotal for centrosome maturation, is a fundamental prerequisite for proper spindle formation and chromosome segregation. Once activated, Aurora-A can also contribute to ribbon unlinking by activating the tubulin deacetylase HDAC6 (2) and PLK1 (3), which further contribute to Golgi fragmentation. To acquire further insights on the molecular bases and signaling networks that couple the Golgi unlinking to cell cycle progression, the changes in phosphorylation status and expression levels of several proteins (including kinases, phosphateses, transcription factors, adaptor/scaffold proteins, stress proteins and other target proteins) will be analysed in G2 phase Hela cells untreated and treated with inhibitors of the upstream signaling pathways (PKD, JNK2, MAPK) (3) responsible for Golgi ribbon unlinking by Antibody Microarray approach. Then, the more significant changes in protein expression and/or phosphorylation status will be checked by western blot analyses and maps of protein signaling networks will be generated. The data collected not only will improve our knowledge on the signaling and molecular networks of G2/M transition, but also will provide new potential therapeutic targets to impair the cell proliferation, which is one of the main features of cancer cells.

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KEYWORDS:

Golgi Complex, Cell Cycle Checkpoint, Signaling, Antibody Microarray.

Drug Discovery

Drug discovery activity is part of the molecular oncology and pathology topic, whose primary goal is the understanding of molecular and cellular mechanisms leading to cell transformation, cancer development and progression. To gain insight into this topic several tumor models are mainly investigated including leukemias, epithelial tumors (breast, lung, thyroid, head and neck, prostate, ovarian), brain and nervous system tumors.

An integrated approach consisting of profile-based screening of gene expression, bioinformatic analysis, phenotypical and biochemical characterization of genetically modified cellular and murine models is carried out to tackle this complex issue. The identification of new targets serve the development of innovative anti-cancer drugs and treatments for unmet medical needs.

Re-Using old drugs against cancer: Computational drug repurposing identified decitabine for targeted therapy in KRAS-dependent Pancreatic Ductal Adenocarcinoma by exploiting pyrimidine biosynthesis addiction

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KEYWORDS:

Pancreatic Cancer, Functional Genomics, Cell Death and Senescence, Drug Targets, Oncoprotein, Small Molecule Agents, Oncogenes, Cancer Metabolism, Pyrimidine Biosynthesis, Drug Discovery Technologies, in Silico Evaluation of Drug Repurposing.

Pancreatic cancer is an aggressive malignancy and is the fourth cause of death by cancer with a 5 years survival rate of only 8%. Effective targeted therapies to treat Pancreatic Ductal AdenoCarcinoma (PDAC) patients are still awaiting clinical validation. Activating mutation of the KRAS oncogene occur in 90-95% of PDAC and is an initiating genetic event in PDAC. Although KRAS could represent an important therapeutic target, there is a lack of effective KRAS inhibitors. The analysis of KRAS-associated gene signatures in pancreatic cancer cell lines has revealed the presence of subtypes of PDAC tumors cells whose survival exhibit a strong dependency on KRAS.

Drug repositioning (i.e., the use of old drugs for a novel therapeutic indication) is a cost-effective approach to rapidly offer new therapeutic opportunities in the clinic. The repositioning of U.S. Food and Drug Administration (FDA)-approved drugs to target oncogenic pathways that still lack effective inhibitors, such as K-RAS, would be a relevant pharmacologic approach to inhibit oncogene-dependent tumor growth. Using a specific K-RAS-dependent gene signature, we implemented a computer-assisted inspection of a drug-gene network to in silico repurpose drugs that work like inhibitors of oncogenic K-RAS. We identified and validated decitabine - a FDA-approved drug - as a potent inhibitor of growth in pancreatic cancer cells and patient-derived xenograft models that showed K-RAS dependency. Mechanistically, decitabine efficacy was linked to K-RAS-driven dependency on nucleotide metabolism and its ability to specifically impair pyrimidine biosynthesis in K-RAS-dependent tumors cells and induce DNA damage. Preliminary data suggested that DEC could sensitize PDAC cells to drugs inhibiting DNA repair, such as the PARP inhibitor OLAPARIB, in selected PDACs.

Based on these findings, the ongoing project, developed through the strong clinical collaboration with the National Cancer Institute Regina Elena (Rome, Italy), together with other national and international cancer centres, will layout the basis for the repurposing of DEC in selected PDAC through the following research lines: 1) To understand the molecular mechanism of the cytotoxicity of DEC in KRAS-dependent PDAC tumor; 2) To investigate the pre-clinical efficacy of DEC or DEC plus OLAPARIB combined treatment by using Patient-Derived Xenograft (PDX)-PDAC models, orthotopic, and immunocompetent mice models of PDAC; 3) To analyze the frequency of KRAS-dependent tumors in PDAC cohorts and the prognostic value of the KRAS dependency scores.

Overall, our research aims to extensively investigate the preclinical efficacy of a tailored drug repositioning in PDAC. If the preclinical efficacy is confirmed, results from this project will promote a phase II clinical trial of decitabine in selected PDAC patients.

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Pyrvinium Pamoate drug Induces Death of Triple-Negative Breast Cancer Stem–Like Cells and Reduces Metastases through its effects on Lipid Anabolism

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Cancer stem-like cells (CSC) induce aggressive tumor phenotypes such as metastasis formation, which is associated with poor prognosis in triple-negative breast cancer (TNBC).

Repurposing of FDA-approved drugs that can eradicate the CSC subcompartment in primary tumors may prevent metastatic disease, thus representing an effective strategy to improve the prognosis of TNBC.

We have investigated spheroid-forming cells in a metastatic TNBC model. This strategy enabled us to specifically study a population of long-lived tumor cells enriched in CSCs, which show stem-like characteristics and induce metastases. To repurpose FDA-approved drugs potentially toxic for CSCs, we focused on pyrvinium pamoate (PP), an anthelmintic drug with documented anticancer activity in preclinical models. PP induced cytotoxic effects in CSCs and prevented metastasis formation. Mechanistically, the cell killing effects of PP were a result of inhibition of lipid anabolism and, more specifically, the impairment of anabolic flux from glucose to cholesterol and fatty acids. CSCs were strongly dependent upon activation of lipid biosynthetic pathways; activation of these pathways exhibited an unfavorable prognostic value in a cohort of breast cancer patients, where it predicted high probability of metastatic dissemination and tumor relapse.

Overall, our studies describe a new approach to target aggressive CSCs that may substantially improve clinical outcomes for patients with TNBC, who currently lack effective targeted therapeutic options.

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KEYWORDS:

Breast Cancer, Cancer Progression, Metastasis, Stem Cell Biology, Molecular Markers of Metastasis and Progression, Cancer Metabolism, Lipid Anabolism, Mitochondria, OxPHOS, Computational Methods, Gene Expression Profiling, Molecular Modeling.

An integrated platform for specific and selective histone deacetylase inibitors (HDACiPLAT)

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KEYWORDS:

Istone Deacetylase Inhibitors, Schistosoma Mansoni, Cancer, Integrated Drug-Discovery Platform.

In the course of evolution, parasites improve their fitness as a result of the selection of traits relevant for their relationships with hosts. Adaptation to a multitude of signals of the environment (i.e. temperature, pH, osmolarity, and also host cell signals) is essential. Parasites as cancer cells have the ability to survive in the host by hiding and escaping the immune system and by adjusting their metabolic pathways to environment constrains. Post-translational modifications may provide parasites and cancer cells with the ability to readily adapt to changes in gene expression required for their development and adaptation to the host environment.

Acetylation and deacetylation of histones play pivotal roles in chromatin structure and in the regulation of transcription in eukaryotic cells. Moreover histone deacetylase inhibitors (HDACi) modulate acetylation of several other proteins localized both in the nucleus and in the cytoplasm and therefore impact on many signaling networks and biological processes. Changes in the expression levels of HDAC have been associated with various infectious and chronic diseases including schistosomiasis and cancer; the challenge today is to understand the mechanisms that regulate HDACs activity and to obtain selective and effective inhibitors.

We identified and characterized several HDACi active on multiple stages of S. *mansoni* (1, 2) some of those are also able to inhibit the activity of recombinant SmHDAC8 protein in vitro (1). Thanks to our expertise, model systems of S. *mansoni* and cancer cells and our ability in the development of enzymatic and functional assays for the identification and characterization of novel active compounds, we aim, to develop and validate an integrated technological platform. Through a multidisciplinary approach including in silico virtual screening on selected target, innovative drug design and synthesis, phenotypical screening on multiple developmental stages of S. *mansoni* and on epithelial and nervous system cancer cell models, *in vitro* improved inhibitory deacetylase assays with recombinant HDAC and structural data of HDAC-HDACi complexes we expect to gain valuable insight into the mechanisms of action of HDAC6 and HDAC8 and the regulation of their activity. We also aim to develop an integrated platform for the discovery of specific and isoform selective HDACi already in the early pre-clinical stages of drug development open to collaboration with academic and industrial partners.

HDACi have many potential therapeutic applications in parasitic, oncological, neurodegenerative, muscle and rare diseases; with the possibility of being used also in combination with other drugs for synergistic effects and for drug-resistance prevention. Therefore we envisage further development of the HDACiPLAT to other research and innovation areas.

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Characterization of novel lncRNA associated to EMT intermediate phenotypes and resistance to target therapy in NSCLC (LEARN)

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KEYWORDS:

Long Non Coding RNA (lncRNA), Non Small Cell Lung Cancer (NSCLC), Target-Therapy Resistance, Epithelial to Mesenchimal Transition (EMT).

Lung cancer is the leading cause of cancer death among both men and women, making up almost 25% of all cancer deaths worldwide. Non-small cell lung cancer (NSCLC) is the most frequent type of lung cancer (~85% of cases). The treatment of NSCLC harbouring epidermal growth factor receptor (EGFR) activating mutations has benefited from the use of EGFR tyrosine kinase inhibitors (TKis). Unfortunately, the onset of resistance is unavoidable and most patients relapse. Therefore, the identification of mechanisms of resistance to target therapy, of prognostic markers and the search for novel treatment represent today great challenges to be addressed.

The association between epithelial-mesenchymal transition (EMT) states and resistance to EGFR inhibitors is an emerging issue, and a comprehensive picture of the molecular mechanisms regulating this complex process still needs to be further investigated. Importantly, EMT is not a binary process and cancer cells with intermediate or hybrid epithelial/mesenchymal (E/M) phenotypes characterized by a mixture of epithelial and mesenchymal traits have been described also in our laboratory (1).

LncRNAs are non protein-coding transcripts longer than 200 nt, highly conserved in evolution with a regulated expression. lncRNAs are involved in many cell processes and function by forming complexes with proteins or nucleic acids, both inside and outside the nucleus, acting as regulators of gene expression at epigenetic, transcriptional, and post-transcriptional levels. For this their relevant function in cells, lncRNAs disfunctions play important roles in various cancers.

Our group generated and characterized a NSCLC cellular model of resistance to EGFR-TKi (1,2). Interestingly, cell lines resistant to EGFR inhibitors show E/M features, according to morphology, motility and invasiveness capability, and expression of EMT markers. A whole-genome expression profiling of our NSCLC cell lines resulted in the identification of a list of lncRNAs, some of which not yet been associated with EMT or EGFR-TKis resistance. The gene expression profiling of our cell lines, followed by a focused bioinformatics analysis of data of NSCLC patient tumours of the The Cancer Genome Atlas, spotlighted a set of lncRNAs differentially expressed between drug-resistant and -sensitive NSCLC cell lines, and significantly associated to poor survival and/or metastasis.

A combination of in silico and wet approaches is currently used to validate and functionally characterize the novel lncRNAs associated to EMT intermediate phenotypes and/or to target drug-resistance in NSCLC.

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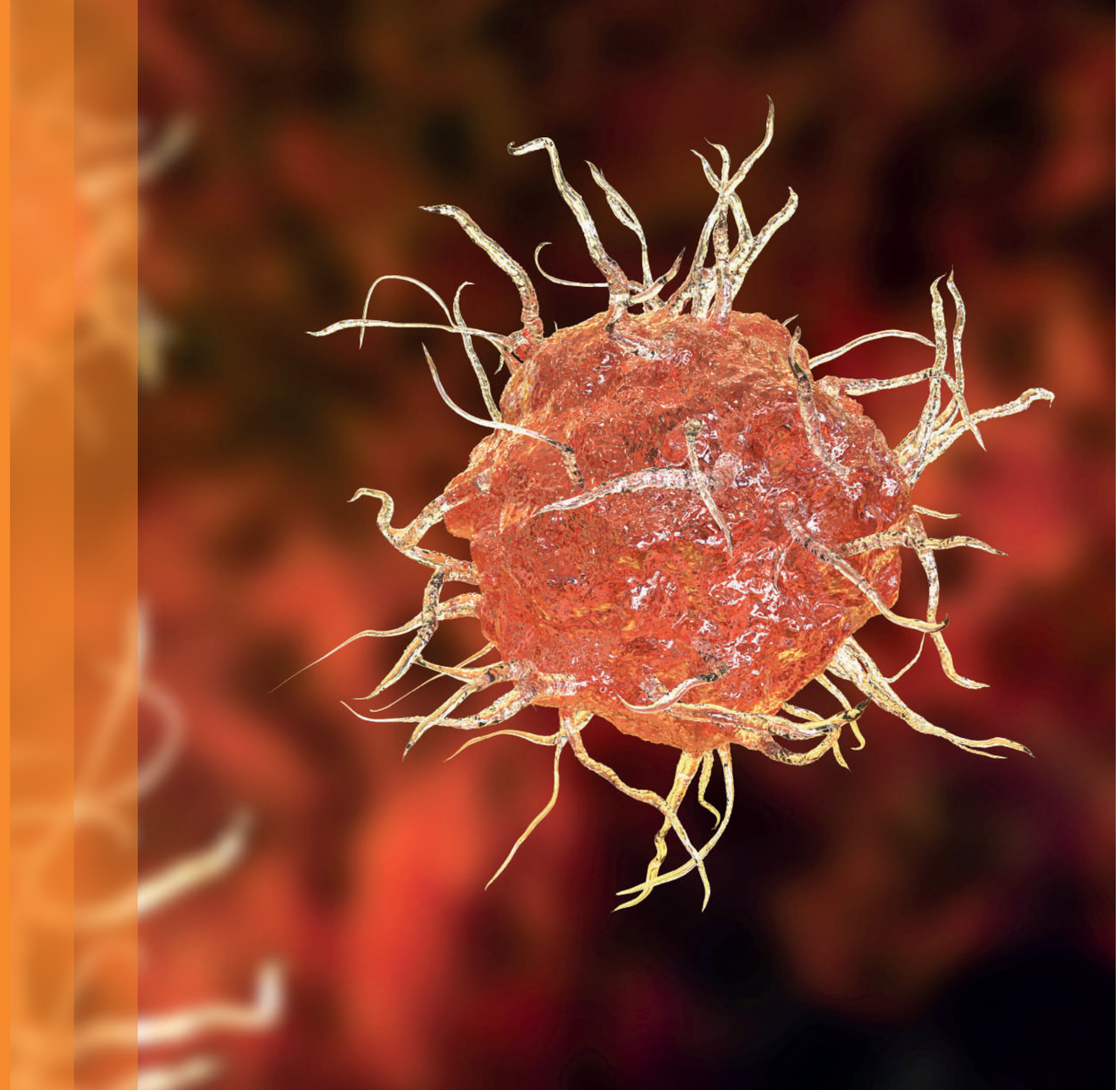
Immunity and Infection



Immunity and infection research activities explore different aspects of immunology as diverse as evolution, physiology and pathology from basic life science to molecular mechanisms studies. Evolutionary studies aim at better understanding the mechanisms underpinning the adaptive immune response, and cover the design of novel diagnostic/therapeutic tools (engineered high performance antibodies; innate, B- and T-cell receptors). The development of innovative tools to design preventive and therapeutic interventions for disease treatment is tackled as well. This include targeted vaccination and immunotherapeutic strategies developed by using novel nano-carriers, immunomodulatory approaches for chronic intestinal disease treatment, therapeutic approaches targeting chronic inflammatory, degenerative and autoimmune diseases, identification of novel anti-schistosomal molecules to counteract schistosomiasis, one of the 17 NTDs Neglected Tropical Diseases (NTDs) prioritized by the World Health Organization.

3D illustration of an antigen-presenting cell.

Intestinal T-cell based assays: from basic science to translational application for treatment of celiac disease

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KEYWORDS:

Mucosal Immunology, Celiac Disease, T Cells, Bioassays.

Celiac disease (CD) is an immune-mediated enteropathy, caused by gluten proteins characterized by an increased prevalence worldwide in the last decades. CD4+ T cells are central players in the inflammatory reaction to dietary gluten. In the recent time, T cell lines and clones isolated from the intestinal mucosa of celiac patients, and highly reactive to gluten peptides, have been largely used to explore the inflammatory pathways responsible of CD (1). Not less important, these T cell cultures represent a sensitive bioassay for the in vitro validation of immunomodulatory and gluten detoxifying strategies, a necessary step before the *in vivo* clinical studies (2).

A diet completely deprived of gluten is, currently, the only efficacious treatment for CD. Some limitations of gluten-free therapy, particularly due to the poor compliance for some patients during to social events and travelling, have encouraged the searching of alternative strategies aimed to improve the life quality of young patients. To date, the most promising strategies for the treatment of CD are based on enzymatic approaches aiming to degrade gluten fragments escaping the digestion of gastrointestinal proteases, thus destroying their immune-stimulatory sequences. The "oral enzyme therapy", based on proteases able to cleave the Q-P bonds in gluten proteins (named glutenases), has been proposed to promote complete digestion of disease-inducing gluten peptides in gastric conditions. We have demonstrated, by using celiac T-cell based bioassays, the ability of a novel glutenase, the endoprotease-40 (E40) of microbial origin, to efficiently degrade immunogenic gluten peptides, resulting in a strong reduction of bioactivity on celiac T cells of whole glutens (2).

An additional approach to examine the T-cell mediated response to gluten is the short-term oral challenge, basically an *in vivo* procedure that allows to monitor the gluten-specific T cells in peripheral blood of CD patients in disease remission, after a medical controlled consumption of a low amount of wheat food (3). This bioassay has been successfully applied to the search of wheat species with a null, or very low, content of toxic gluten sequences, suitable for disease prevention in subjects at high genetic risk of CD (4). In particular, we have dissected the immunological properties of gluten from monococcum, an ancient wheat cultivar, by an extensive proteomic, immunoenzymatic and T cell-based analyses. Unequivocally, we demonstrated the capability of gluten from two monococcum cultivars to stimulate T cells from celiac intestinal mucosa, thus not suitable for those people with a CD diagnoses. Interestingly, by a further investigation, we found that, after an in vitro digestion mimicking the gastrointestinal hydrolyses, monococcum gluten proteins retained a reduced immunogenicity compared to that of common wheat species (such as soft wheat). Our results demonstrated that gluten peptides from ancient crops are extensively degraded by gastrointestinal proteases, thus suggesting a marked digestibility of this ancient wheat variety compared to common bread wheat (4).

Currently, our research is focused in different projects aiming to take advantages of our sensitive bioassays, based on gluten-reactive T cells, as listed:

1. to search naturally non-toxic, or less toxic, cereals for CD treatment or prevention;

2. to identify new strategies to detoxify wheat gluten;

3. to validate novel immunomodulatory strategies that aim to inhibit the intestinal inflammatory reaction in gluten-exposed CD patients.

These research projects are done in collaboration with national and international academic institutes or private biotech companies.

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Immune challenge within the European Green Deal: profiling the impact of food-borne micro- and nanoplastics on human immune system

Environmental degradation due to pollution has become a huge health and social threat to worldwide countries. The "European Green Deal" commits to a zero-pollution economy for a toxic-free environment, and it specifically mentions the need to rapidly address the risks posed by very persistent chemicals, such as plastic.

Micro- and nanoplastics (MP and NP) already heavily contaminate the environment, in particular the oceans, and, by contaminating environmental species, they enter the food chain. Human exposure to MP and NP mostly occurs through contaminated food. We ingest on average five grams of plastic per week (the equivalent of a credit card) and the implications for our health are not yet known, either as direct effects or as carriers of microorganisms and toxic chemicals. Moreover, whether they are ingested in micro or nano form, they can also lead to the intake of viruses, bacteria and toxic contaminants inside organisms. So, improving our knowledge on the interactions between MP and NP and the immune system has become extremely important.

The immunology group of IBBC is addressing the interaction of MP/NP with the immune system, from marine invertebrates to human beings, as the knowledge basis required for understanding the MP/NP impact on environmental and human health. The group is examining the interaction of MP/NP with microorganisms, the effects on marine invertebrates *in vivo* and the cellular and molecular mechanisms underpinning the onset of immune reactions to MP and NP in marine species (vertebrates and invertebrates) and in humans, through in vitro models aimed at realistic scenarios of exposure, using human primary cell/tissue systems and gut biopsies. In particular we are studying:

1. the immunological mechanisms of adaptation of polar fishes to MP/NP 1

2. the immunological response to MP/NP in marine invertebrates, such as Ciona robusta 2 ;

3. the predictive value of the human health hazard posed by MP/NP, using human primary cell-based in vitro models of innate immune/inflammatory response and innate memory (i.e., monocytes, macrophages, DC) 3,4 ;

4. the interaction between MP/NP with viruses (e.g., SARS-CoV-2 pseudovirus), to assess changes in pathogenicity and carrier effects;

5. the interaction of ingested MP/NP with the human gastro-intestinal tract (in vitro advanced models and biopsies from healthy subjects and coeliac disease patients) for effects on healthy vs. inflamed/pathological gut mucosa 5 .

The overall aim is to exploit the commonalities and specificities of immune defensive responses throughout living species for a realistic prediction of the health and environmental hazard of MP/NP, including possible indirect effects caused by fine modulation of innate memory, carrier-dependent changes of the kinetics and dynamics of chemical compounds, allergens and microorganisms. As practical goal, we aim at developing simplified assays for a realistic and personalized prediction of MP/NP hazard for human health that consider the variability of immune competence within the human population (chronic diseases, inflammaging, immunobiography).

KEYWORDS:

Immunotoxicology, Microplastics, Predictive Models.

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Induction of Innate Immune Memory by Engineered Nanoparticles

In the last decades two important research fields have crossed paths: nanotechnology and immunology. Nanotechnology has experienced a huge development in many different application areas, including nanomedicine. In this field, the safety needs for nanoparticle (NP) use has fostered the improvement of our knowledge on the interactions between NP and the immune system, in particular the impact on innate immunity.

The capacity of engineered NP to activate cells of the innate immune system, in particular monocytes and macrophages, is at the basis of their toxic/inflammatory effects. It is, however, evident that even NP that do not directly induce inflammatory activation, and are therefore considered as safe, can nevertheless induce epigenetic modifications and affect metabolic pathways in monocytes and macrophages. Since epigenetic and metabolic changes are the main mechanisms of innate memory (the ability of innate immune cells to react differently to challenges based on previous stimulations), we should revise our approach in examining the effects of NP on innate immune responses by including the effects on the establishment and modulation of innate memory.

For the first time, we proposed that NP can induce/modulate innate memory 1 . In light of our data, and of very few data from the literature, it is now possible to support our original hypothesis and show that different types of NP can both directly induce innate memory, priming macrophages for a more potent response to subsequent stimuli, and modulate bacteria-induced memory by attenuating the priming-induced enhancement 2,3 . We have used *in vivo* models in the marine invertebrate Ciona robusta for defining the parameters of innate memory (shared with humans) 4,5 and developed in vitro cell-based assays with human primary cells for evaluating the direct and indirect effects of NP on innate memory. The evidence that NP can modulate innate memory raises two important issues. First, in addition to overt toxic/inflammatory effects, we should consider evaluating the capacity to induce innate memory and the related epigenetic and metabolic changes in the immunosafety assessment of nanomaterials, since modulation of innate memory may be at the basis of long-term unwanted immunological effects. The other important consideration is that this capacity of nanomaterials could open a new avenue in immunomodulation and the possibility of using engineered nanomaterials for improving immune responses to vaccines and resistance to infections, and modulate anomalous immune/inflammatory reactions in chronic inflammatory diseases, autoimmunity, and a range of other immune-related pathologies.

KEYWORDS:

Innate Memory, Nanoparticles, Monocytes/ Macrophages.

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Targeted vaccination using novel nano-carriers

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KEYWORDS:

Filamentous Bacteriophage, Vaccine, Dendritic Cells, Invariant Natural Killer T Cells, Alpha-Galactosylceramide, Anti Tumor Immunity.

The filamentous bacteriophage fd is a powerful antigen nanocarrier due to its intrinsic adjuvant properties. Bacteriophages can be engineered for the expression of exogenous peptides at high density on its surface, and exposed peptides can be processed and presented after bacteriophage internalization by dendritic cells (DCs), triggering strong immune responses.

Previous studies have described the use of bacteriophages for delivering tumor-associated antigens (TAAs) to murine DCs via specific receptor and the ability of these phage particles to induce antigen-specific cytotoxic T-cells.

Analysis of the molecular mechanism underlying the immune response induced by vaccination with filamentous bacteriophages demonstrated the ability of these nanocarriers to evoke both innate and adaptive immune responses.
The delivery of stimulatory lipids and antigenic peptides derived from TAAs represents a novel strategy to potentiate anti-tumor immune responses. Bacteriophages can be conjugated to the immunostimulatory lipid alpha-GalactosylCeramide (α-GalCer) to activate invariant natural killer T (iNKT) cells and induce the release of a wide variety of pro-inflammatory cytokines which facilitate DC maturation and cytotoxic T cell priming.

To improve anti-tumor response by bacteriophage delivering TAAs and α-GalCer, we developed phages targeting specific cell subsets such as human DC subpopulations or tumor cells, in order to explore the immune responses to targeted bacteriophage in the human system.

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"Antarctized" antibody: an innovative monoclonal antibody engineered by the CRISPR/Cas9 system

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KEYWORDS:

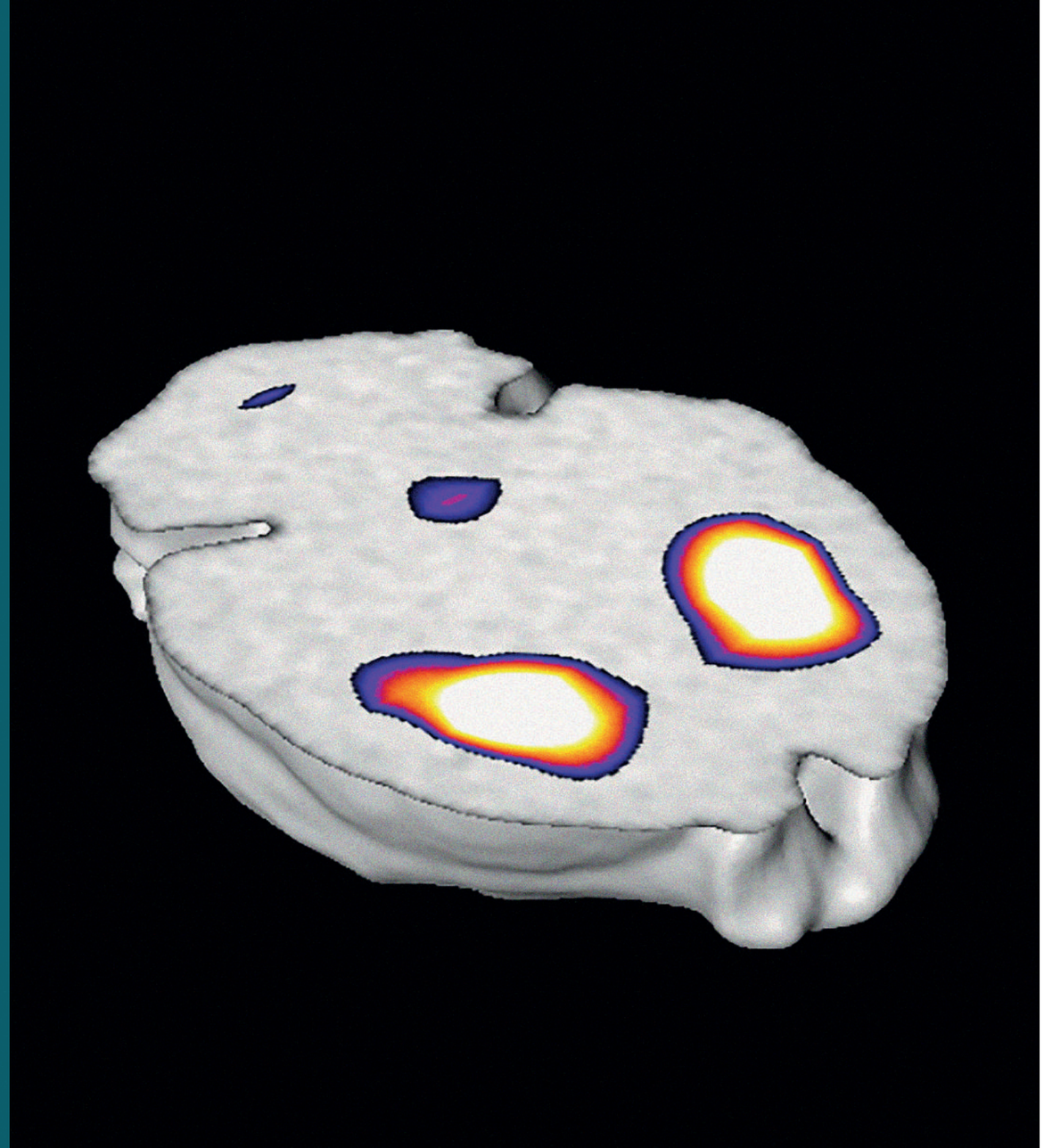
Engineered Monoclonal Antibody, Genome Editing, Immunoglobulin Gene Locus.

Since many years, Dr. Coscia's research activity has been focused mostly on Immunoglobulins (Ig) from Antarctic fish. They are distinguished by some unique features located in crucial parts of the molecule, such as a long hinge region in the central part of the heavy chains, and up to four short repeats at the extracellular membrane proximal domain. These peculiar structural characteristics, not seen in any other vertebrate Ig, can be viewed as a result of adaptive evolution to improve the flexibility and, in turn, the functionality of the antibody molecule under very extreme environmental conditions. In particular, the extended hinge region expands the Fab arms further away from the Fc, providing the molecule with greater flexibility, which, in turn, can assume different conformations to cope a vast array of antigens.

A recent topic includes the design and production of engineered monoclonal antibodies (mAb) with enhanced functions. Previous findings prompted the idea to engineer the Antarctic Ig structural features at the murine IgG heavy-chain gene locus by using the CRISPR-Cas9 system, and then to evaluate their impact on the structure and function of the Ig molecule. The CRISPR/Cas9 system has been chosen since, very recently, it has been successfully applied to edit the mouse and human immunoglobulin gene locus in order to modify the antigenic specificity of mAb or to obtain Fab fragments without using enzymatic digestion (1, 2). Overall, these studies have underlined the potential of the CRISPR/Cas9 technology to advance the field of molecular engineering of antibodies, meeting criteria of developability, reproducibility and efficacy, without off-target effects and at low cost.

Hybridoma genome editing has been performed in our lab, as described above, and the whole "Antarctized" mAb has been purified. Physico-chemical, and functional characterization is currently carried out to assess whether the modifications introduced may enhance antibody performance, offering a wider range of biotechnological applications.

In Vivo Imaging



Small animal imaging is an important tool in preclinical research enabling to investigate new drug molecules for diagnosis and therapeutic treatment of diseases. This approach harnesses high-performance scintigraphic detectors for small animal imaging accomplished with additional imaging modalities such as optical and ultrasound. As a result, the new multi-modality molecular imaging systems with improved performance will provide advanced technologies for morpho-functional investigations.

SPECT/CT imaging of DaTSCAN radioactive drug distribution within the mouse striatum.

Innovative neuroimaging techniques applied to animal models to assess Neuroinflammation in Alzheimer's Disease

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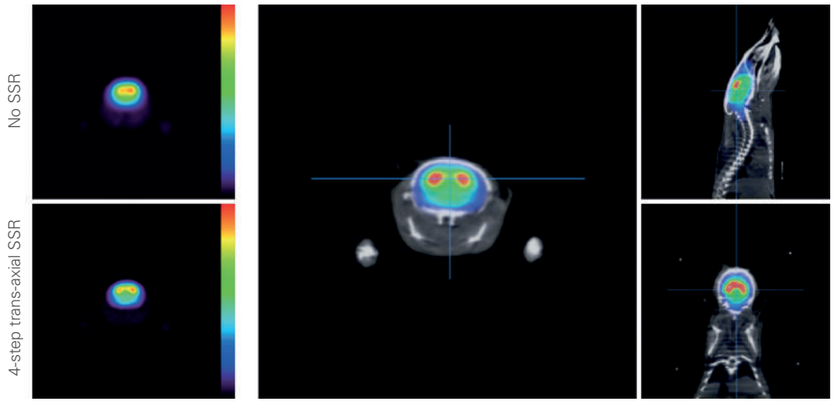
- R. Massari

KEYWORDS:

Small Animal SPECT, Super Spatial Resolution, Neuroimaging Application.

The purpose of this work is the study of the correlation between neuroinflammatory mechanisms and their potential evolution into neurodegenerative diseases through the use of advanced neuroimaging techniques on laboratory animals.

An early, progressive and selective loss of the Ventral Tegmental Area (VTA) dopamine neurons (DA) in a mouse model of familial Alzheimer's Disease (AD), was recently reported before the deposition of amyloid- (A) plaques. Moreover, several longitudinal studies show that neuroinflammation and microglial activation occurs years before the clinical AD manifestation. In this context, the early activation of microglia in AD patients has been demonstrated by performing functional investigations such as Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET). Indeed, neuroimaging applied on an animal model by exploiting both morphological (MRI) and functional (SPECT/PET) techniques, paves the way to a deep circuit-level understanding of the neurological degeneration which may cause pathological alterations.



Digimouse phantom neurological imaging simulation. Left: SPECT imaging with and without the super spatial resolution method (SSR). Right: SPECT/CT obtained with 4-step trans-axial SSR.

For these reasons, a dedicated small-animal SPECT system will be developed to provide a high-resolution brain imaging pointing out the VTA region of interest. Our target is to perform a longitudinal study during the AD progression in animal models by focusing on the correlation between the microglia physiological activity and its inflammatory condition. This implies the use of long decay isotopes as 125I (60 days half-life). The Super Spatial Resolution (SSR) technique will be also applied. Finally, new pharmacological targets will be evaluated accordingly with the experimental results to put together two key concepts: the precocious death of VTA DA neurons and the early inflammatory response. The objective of this work is finalized to find new pharmacological targets for early inflammatory response.

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SSR-SPECT/CT device: a new method for the small animal imaging study

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KEYWORDS:

Small Animal SPECT, Monte Carlo Simulation, Super Spatial Resolution, Neuroimaging.

Over the past decade, there has been a rapid rise in the use of small animal models in in-vivo biomedical research. As a consequence, small animal imaging has become an important tool in preclinical research, because the use of these techniques enables to investigate new drug molecules for disease diagnosis and therapy. This increasing interest was driven by the development of new multi-modality molecular imaging systems with improved performance. Indeed, a multi-modality system can effectively integrate functional morphological techniques.

Recently, new generation preclinical imaging systems have been introduced that combine traditional techniques like positron emission tomography (PET) or the single-photon emission computed tomography (SPECT) and computed tomography (CT) or magnetic resonance imaging (MRI), with other imaging modalities such as optical and ultrasound.

Our group has developed in the past several high-performance scintigraphic detectors for small animal imaging. Consequently, our interest is focused on developing small animal SPECT suitable for integration into multi-modality imaging systems. We modelled a four-headed preclinical SPECT scanner capable of the proper movements to obtain the SSR acquisition sequences. The whole system sensitivity was 164.1 cps/MBq. To assess the impact of the SSR this value must be divided by the number of the images acquired to perform this method. The average value of the trans-axial resolution improves from 2.4 mm to 1.54 mm, 1.21 mm and 1.03 mm by respectively applying the SSR based on two, three or four images. While the average axial resolution changes from 1.69 mm to 1.49 mm, 1.15 mm and 0.98 mm by respectively using the SSR based on two, three or four images. The mouse images obtained by using the voxel phantom have demonstrated the good capability of the system as a suitable tool for small animal imaging. Finally, a comparison with commercial preclinical scanners has proved that the presented SSR scanner provides an alternative to pinhole SPECT systems for many preclinical research studies.

Moreover, we expect to apply SPECT/CT in neurosciences aiming to demonstrate a correlation between neuroinflammatory mechanisms and their potential evolution into neurodegenerative diseases.

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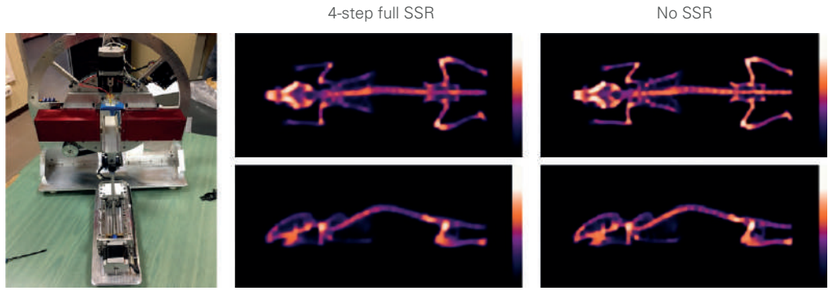

Digimouse phantom bone scan simulation with and without SSR.

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Study of new pharmacological targets for autism spectrum disorder by using innovative neuroimaging techniques applied to the conscious laboratory animal

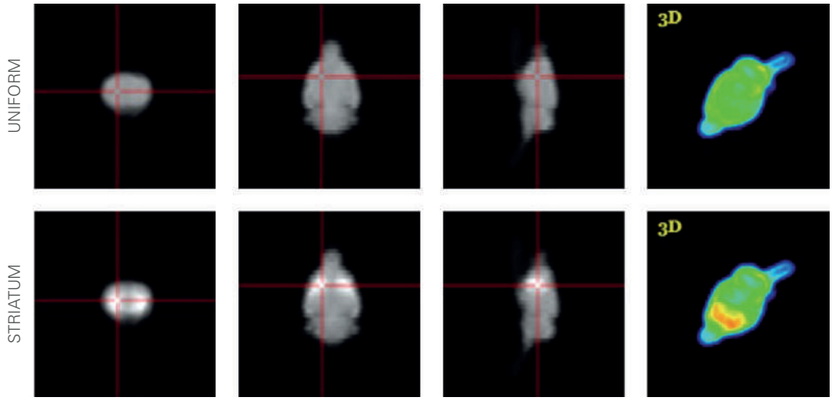
KEYWORDS:

Autism Spectrum, Social Behaviour, Small Animal SPECT, Lightweight Scintigraphyc Devices.

Autism spectrum disorder (ASD) is one of the psychiatric diseases of the developing age, with greater incidence in the population. It is characterized by the concomitant presence of deficits in social interaction and social communication associated with behavioural stereotypies. It has recently been hypothesized that the endocannabinoid system may play an important role in the pathogenesis of ASD, especially for disorders of the social sphere. In this context, in the last few years, the use of devices dedicated to imaging on laboratory animals has grown exponentially, integrating different techniques to investigate the correlations between pharmacological effects and neurological reactions. Behavioural neuroimaging is an emerging discipline that merges neuroscience behavioural and functional neuroimaging. To provide a deep, circuit-level understanding of social brain functioning it is necessary to perform *in vivo* animal analysis.

The Single Photon Emission Computed Tomography (SPECT), provides a quantitative, real-time measure of drugs biodistribution. It is able to image biochemical processes and as a highly sensitive technique, it requires very small amounts of radiolabels, which minimizes the disruption of cell function and surrounding tissue.

This study has been devoted to the design of a new class of SPECT detectors with high performance suitable for preclinical imaging. In particular, the imaging on awake animals has many advantages, in fact actually: (a) animals need to be anaesthetized during SPECT imaging due to their inability to lie motionless in the scanner; (b) anaesthesia can greatly depress brain functions and affect the neurochemistry which is the object of the study; (c) simultaneous images acquisition is precluded; (d) the immobilization may cause stress to the experimental subject and alter the measurements; (e) there is an ethical problem associated with the animal immobilization.



Brain regions in which neurotransmitters are involved in social behavior. Uniform and stiatum activation.

To overcome all these issues, we carried out several computer simulations to understand the best geometry and performance to apply.
A preliminary experimental study with rodents has been performed with good results, opening a new and wide field of research in the knowledge in deep brain structure and drugs bio-distribution. Study and design of a miniaturized, lightweight system that allows reasonable freedom of movement.

We expect, in the nearest future, to miniaturize a SPECT system to apply it to behavioural studies. For this reason, we will develop a new detector based on silicon photomultipliers (SiPMs) that could reach 1.2 mm of spatial resolution. Our aim will be the identification of the brain regions and neurotransmitters involved in social behaviour to better understand the neural underpinnings of autism.

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Murine Models

The European Mouse Mutant Archive (EMMA) Core Structure and Monterotondo Mouse Clinic (MMC) are advanced international infrastructures for the life sciences. Both infrastructures are devoted to the design, production, functional characterization and world-wide dissemination of mutant strains, as *ad hoc* innovative *in vivo* models of diseases and related phenotypic traits with more than 8000 mutant strain models currently available. EMMA Core Structure and MMC belong to the INFRAFRONTIER network infrastructures, a Landmark Project of the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap, collaborating with the leading international biomedical research institutions. They also take part in a selected project of the Italian Ministry of Research's Roadmap and National Programme on Research Infrastructures (PNIR).

The Infrafrontier Landmark Research Infrastructure and the European Mouse Mutant Archive (EMMA) - Mouse Disease Models for the Scientific Community

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KEYWORDS:

Bio-Repository, Phenogenomics, Preclinical Research, Translational and Personalised Medicine.

Infrafrontier is the ESFRI Landmark Network Research Infrastructure (RI) for the development, phenotyping, archiving and distribution of mammalian models of human diseases, with 29 partners at the leading biomedical research Institutions in Europe, Israel and Canada. The European Mouse Mutant Archive (EMMA) is a core activity of Infrafrontier, with a world-level public cryo-repository of murine mutant models. EMMA and Infrafrontier have gained continued support by European Framework Programmes 4-7 and Horizon2020, as well as MIUR/MUR RI's Roadmap and National Plan and annual Research Institutions' Fund. CNR is the Italian promotor and founder of the EMMA and Infrafrontier Networks. Since 1996 it has established and developed the original EMMA Core Structure and the new Mouse Clinic at the international Monterotondo Campus. Infrafrontier-EMMA has so far produced and/or archived more than 7000 mouse mutant models and served over 6000 model requests from scientists worldwide. EMMA maintains and distributes its mutant resources at the highest-level standards, with quality-controlled genetic/health status, according to relevant ethics/animal welfare laws/regulations. EMMA collections include thousands of constitutive/conditional embryonic stem cells-targeted or CRISPR-mediated mutants, transgenic strains, cre/flp/dre recombinase driver or tet-inducible lines, etc. with ample sets of specific models for rare, genetic and multifactorial diseases. Large assortments of lines for cancer modeling and COVID-19 studies are also available, according to current research priorities of the European Union Programmes and the global biomedical community. Infrafrontier also provides access to systemic phenotyping in mouse clinics, with large-scale/standardized analysis of models' phenotypes, while the resulting genotypic/phenotypic data are extensively annotated and linked to complementary preclinical and clinical datasets. Infrafrontier is a major component of the International Mouse Phenotyping Consortium (IMPC), the selected mature global initiative of the G7 Science Ministers, which aims at generating a comprehensive, open-access model catalogue of all mammalian genes' functions. Infrafrontier participates in the leading Horizon2020 Open Data Cloud for Life Sciences (EOSC-Life) and the curated EMMA models' datasets have been recognized to constitute a FAIR (findable, accessible, interoperable, reusable) reference resource. Infrafrontier-EMMA also develops and applies new methods and techniques for refining procedures, reducing animal use and increasing reproducibility. Innovative training activities for the global research community are routinely carried out, including the new Erasmus+ PATHBIO Knowledge Alliance. The Infrafrontier-EMMA group at CNR-IBBC Monterotondo actively contributes to IMPC, EOSC-Life and PATHBIO. With over 1000 produced and/or archived model strains and capacity up to ca. 50,000 live animals and 100,000 germplasm samples, the Monterotondo facilities are the largest in Italy and uniquely combine in-house development, analysis, processing and quality control of the banked strains. Advanced *in vivo* and ex vivo imaging modalities are also major research areas, benefiting from strong integration with IBBC's multi-sited cellular/biological node of the EuroBioImaging RI.

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CNR-IBBC Monterotondo mutant strain production: open doors for new mouse models

CNR-IBBC Monterotondo mutant strain production unit (MMP), from 2008 participated in EUCOMM (European Conditional Mouse Mutagenesis) project, that is a cornerstone of the International Knockout Mouse Consortium (IKMC). We produced more than 200 mutant mouse lines, by ESC blastocyst injection, and CrispR/Cas9 editing technologies, feeding of mutant mice, the large scale mouse phenotyping projects EUMODIC (European Mouse Disease Clinic), and IMPC (International Mouse Phenotype Consortium). We was also member of EUCOMM Tools project in which we produced as requested from our work package more than 50 mouse mutants CRE drivers line, as well as dissection from these lines to detect the effective tissue specific CRE expression of the mutants produced.

These lines are already cryopreserved in EMMA/INFRAFRONTIER and ready for distribution, to the scientific community. We also have an active "Research and Development" project, with the aim to improve transgenic technologies.

The CNR Monterotondo Mouse Production facility, has greatly increased the possibilities of researchers from all over the world to obtain the mouse model more suitable for their research, already checked both genotypic and often also phenotypic traits, with highly standardized methods, contributing to the reduction and replacement (3R's), avoiding the wasteful attempt to generate new models, sometimes attempted by personnel inexperienced or individual research groups with little knowledge of the mouse model in general.

At the same time the great experience acquired for more than 14 years and the ability to cryopreserve the mouse models generated, in a public repository such as INFRAFRONTIER, allows to increase the generation efficiency with internal control procedures, keeping track and records of each procedure, and external, thanks to the comparison of data and results with different production centers within the consortium. Furthermore, access to standardized cryopreservation avoids unnecessary sacrifices and repetitions. As a reference for the CNR in murine trans-genesis, we make available to the researchers of the Department of Biomedicine and their related project ideas, our skills, gained in these years of active participation in European projects for the production of large-scale mouse models.

KEYWORDS:

Genetically Modified Mouse Models.

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The International Centre for Genetic Engineering and Biotechnology (ICGEB) - European Mouse Mutant Archive (EMMA). Workshops and courses on mouse models of disease and genome editing

KEYWORDS:

Mouse Models, Genome Editing, Embryo Manipulation, Human Diseases.

The International Centre for Genetic Engineering and Biotechnology (ICGEB) is an intergovernmental organization that offers a unique technological and educational platform to promote scientific learning and innovation, helping its Member States progress towards the achievement of the 2030 Agenda on Sustainable Development. It runs 46 state-of-the-art laboratories, in Trieste, Italy, New Delhi, India and Cape Town, South Africa, with more than 600 scientists, and forms an interactive network with over 65 Member States. The ICGEB Meetings and Courses Programme funds, organizes and promotes participation in scientific meetings, workshops and theoretical and practical courses in the Life Sciences in ICGEB Member States. Every year the Centre funds and organises over 30 scientific events on cutting-edge topics in the field of Life Sciences to facilitate interaction between internationally renowned scientists and young researchers from Member States. Since 2008 the ICGEB has organized theoretical courses focused on mouse genetics with outstanding impact on the students. Fellowships for selected students are available to promote science in less developed countries. The ICGEB, in collaboration with the European Mouse Mutant Archive (EMMA, as part of INFRAFRONTIER consortium) proposes workshops focused on the use of cellular and animal models, and in the use of innovative approaches such as the CRISPR/Cas9 platform for the generation of these models. These models represent precious tools for their ability to reproduce human diseases, the development of novel therapeutic approaches, and the implementation of precision medicine approaches. "Genome editing" is booming in molecular biology laboratories. In order to offer the students with the necessary hands-on training, in the next courses we will include very complete and ambitious practical sessions covering techniques that are becoming a standard requisite in laboratories and animal facilities all over the world, such as generation of cellular and animal models by genome editing platforms, and addressing key aspects of embryo and sperm handling and in vitro fertilization, with the collaboration of the EMMA personnel, and with the expert support of Jackson's lab (USA) staff.

The next two courses of the series will be organized at Monterotondo, Rome ("Genetics and model organism in human disease research: workshop on Assisted Reproductive Technologies (ART) in laboratory mouse") and at Cape Town, South Africa ("Genome editing to generate cellular and animal models of human diseases") in September and October 2021, respectively. These courses intend to provide a learning platform for scientists willing to implement the technology in their research, and a forum for discussion on both scientific and regulatory challenges. Participants from the ICGEB Member States will have facilitated access to the Workshops, with the aim of fostering their access to the technology, understanding their need and possibly establishing future services or capacity building actions.

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D2M – Digital Mouse Monitoring

(Improving animal welfare, model translation and data reproducibility in preclinical trials using a 24/7 A.I. true home-cage monitoring system)

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KEYWORDS:

Artificial Intelligence, Mouse Monitoring, Sensors, Home Cage Monitoring.

Despite growing incentives to find new ways of reducing the usage of animals for scientific purposes, preclinical *in vivo* studies remain an essential means of characterizing and predicting the human response to novel therapeutic interventions and are required by health authorities to assess safety prior to clinical development. However, pre-clinical studies still follow conservative procedures that mostly rely on direct human observation in controlled research environments. Over the past years, animal testing has been the subject of significant critique, due to insufficient reporting of animal strains used, husbandry practices, design errors and lack of statistical power. Animals display a vast repertoire of behavioural responses to experimental testing, but it is surprisingly rare that these responses are recorded because animals are only monitored for limited periods of time outside their home-cage environment. Cage monitoring devices have been used on limited scale for decades, but the deployment of active monitoring on a large and industrious scale has been inefficient and challenging because of cost and reliability issues. In addition to animal behaviour, there is an unmet need for systems that automatically control the environment and conditions of the cage (e.g. bedding conditions, water and food levels, temperature, relative humidity, light, etc.), which can have a significant impact on animal welfare and studies outcome. Moreover, large research centres and international infrastructures of universities, contract research organisations (CROs), hospitals and/or pharmaceutical companies have facilities with thousands of animal cages. Facility managers need to keep track of how many cages are in use, how many cages are associated to a given owner/researcher, where each cage is located and for how long the cage has been in each specific position. Automated home cage monitoring is a key technology that enables researchers to monitor animals over long periods of time without human intervention. Home cage monitoring systems have potential impact not only in extracting relevant information regarding spontaneous animal activity, but they also provide support for improving animal welfare by decreasing animal anxiety levels. D2M will be the first smart and modular digital ventilated home-cage for enhancing the threshold in animal welfare, users experience and management, and data interpretation. The D2M automated 24/7 monitoring and advanced data analytics projects the pre-clinical vivarium into the digital era and artificial intelligence (A.I.) providing standardization and advanced animal data analytics, in addition to promoting a significant improvement in animal welfare and scalability of the experiments. D2M consists of a scalable home-cage monitoring system based on an electrical capacitance sensing technology and an advanced data analysis platform. The system is designed to gather 24/7 animal data directly from the home- cage while keeping cages into conventional individual ventilated cage (IVC) racks. D2M technology is composed of a next generation backboard to which the end-user can attach a variety of optional modules that collect both animal activity and behavioural data, including locomotion, weight, food and water availability/ intake, physiological parameters (e.g. temperature, blood pressure, glucose levels), and environmental conditions (e.g. cage temperature, relative humidity, noise).

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Sensory Decays and Aging (Sensaging)

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The aging process is associated with a decline in the function of our senses (Age Related Sensory Decline - ARSD). Sensory deficits (i.e. single or multiple) are main drivers for quality of life deterioration in the elderly and impairing interactions with the environment and other people. Twin and heritability studies suggest a relevant genetic component for ARSD that is still largely unknown. Thus, there is a strong need in carrying out research activities aimed at identifying possible genetic determinants as well as their interplay with environmental/lifestyle factors. In such scenario we will address the biology of ARSD employing wild type and genetically engineered mouse models with a convergent methodology that includes a combination of sensory test and behavioural evaluation, clinical and histopathology analysis, and genetics/molecular biology studies in a very innovative unbiased approach never applied before for the study of ARSD. Thanks to this project we plan to increase our knowledge on: i) the impact of ARSD in the aging laboratory (lab) mouse ii) on the genetic determinants, both causative and protective, and how they can predict individual ARSD, iv) on the interactions between ARSD genetic variants, individual molecular phenotype and sensory deficits, and those between aging mechanism and genetic determinants, and also to collect possible data on age-associated diseases that may arise with the aging process. To reach this goal a series of research activities that involves clinical evaluation, sensory function and deficit test, behavioral, molecular and histopathology analysis have been planned to involve both inbreed and outbreed mouse models within a time lapse of 6-18/24 months period. The laboratory mouse (Mus musculus) is the most common and predictive animal model for the study of aging and neurodegenerative diseases. As reported by the Jackson Lab (https://www.jax.org/news-and-insights/jax-blog/2017/november/when-are-mice-considered-old) on the life expectancy of mice normally used in the laboratory; animals of 3-6, 10-14 and 18-24 months old are respectively considered adults (equivalent to 20-30, 38-47 and 56-69 years of humans). The mouse genome is also very similar to our own, making mouse genetic research particularly useful for the study of human diseases. Research in mice provides insights into the genetic risk factors for these diseases in the human population. Until now, in the literature, all published studies have partially examined this topic, addressing mainly in some senses (for example hearing), or focusing only on one sex or analyzing transgenic mouse lines of genes of specific interest. The originality of our study consists not only in a complete and contemporary analysis of the five senses in both sexes, in the course of aging, but also in the choice of using both inbred murine strains, C57BL6/N as it is a strain used in global level as a background for the generation of transgenic lines (Simon et al. 2013, doi.org/10.1186/gb-2013-14-7-r82), and outbred murine strains, CD1 as it better represents the genetic variability normally present in human population (Aldinger et al. 2009, doi: 10.1371 / journal.pone.0004729). Recent epidemiological, population and genetic screenings (GWAS and SNPs) studies carried out by the other PRIN - SENSAGING units on cohorts of normal elderly and patients of both sexes have defined a series of gene variants, restricted to a list of 15 genes to influence the aging process in humans. These defined genes together with 16 biomarker genes will be subjected to the role in mice aging to specific tests for the analysis of the five senses (hearing, sight, smell, taste, tactile sensitivity), and social interaction and behavioural tests clinical evaluation and and the relevant organs harvesting for gene expression and histopathology analysis of mice at ages 6, 12 and 18 months. Concluding, SENSAGING will not only lay the foundation for a thorough understanding of ARSD but also provide tools for its prevention, early diagnosis, and clinical management.

KEYWORDS:

Sensory System, Aging, Genomics, Metabolomics, Functional Analysis, Sensory Decay.

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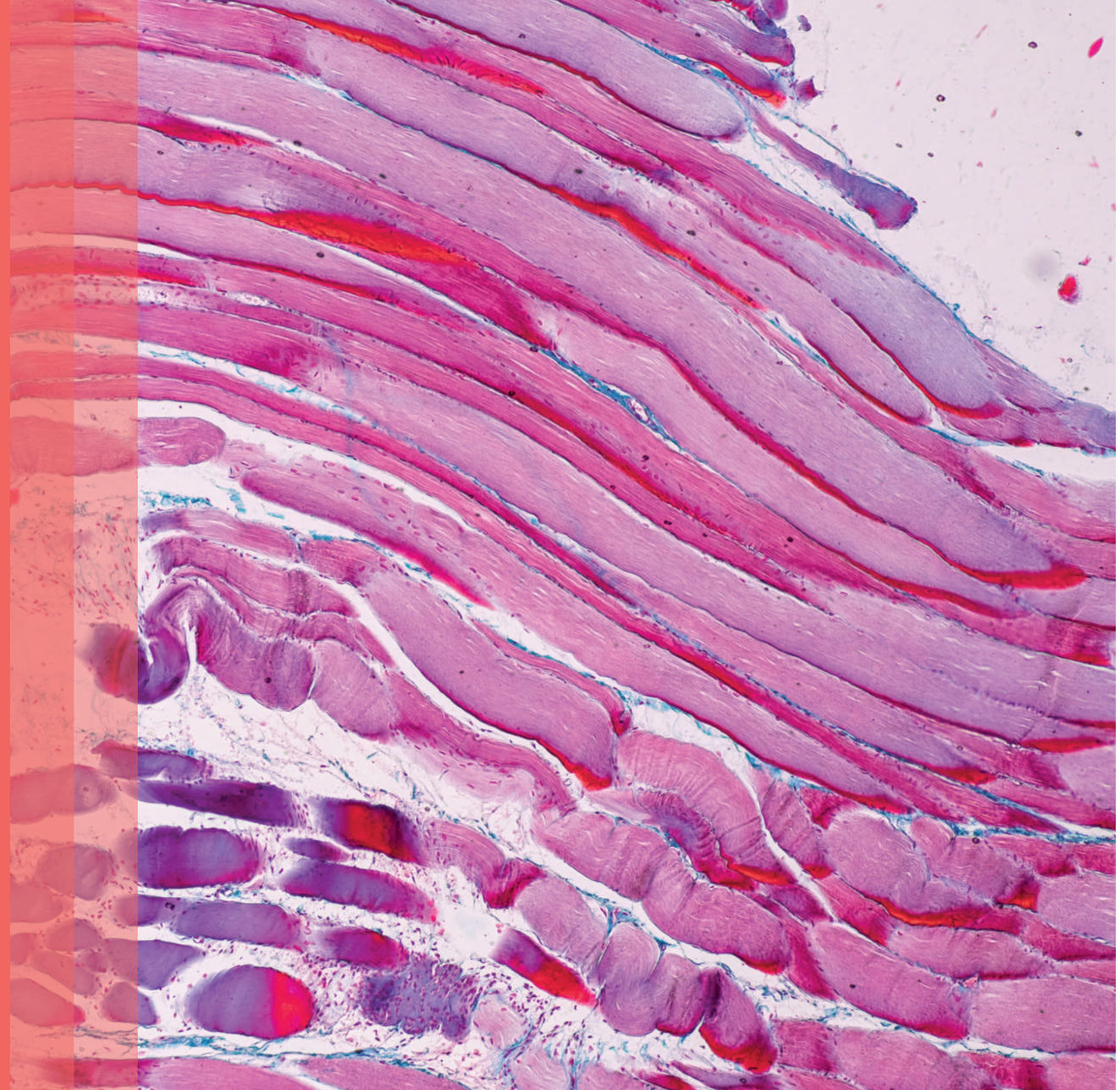
Muscle Biology and Pathology



Muscle biology research projects focus on the definition of pathogenetic regulatory mechanisms and on the identification of therapeutic strategies to cure severe pathological conditions. These refer to genetic and traumatic origin diseases, systemic metabolic disorders, neuromuscular and age-associated chronic pathologies. The ultimate goal of these different research interests rely on the development of novel strategies valuable for tissue regeneration and for neuromuscular diseases treatment. The design of innovative prognostic/diagnostic tools is implemented as well. To these aims, home-generated or existing cellular and animal models of neuromuscular diseases, such as Duchenne, Myotonic and Facioscapulohumeral (FSHD) muscular dystrophies, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are currently adopted in research activities.

Histological sample of mammal striated muscle tissue observed under the microscope.
Staining of muscular cells is performed with eosin.

Role of cell cycle regulators in the control of skeletal muscle function in physiological and pathological conditions

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KEYWORDS:

Muscle Metabolism, Cell Cycle Regulators, Muscular Dystrophy.

For many years, the lab has been studying the interplay between cell cycle regulation and skeletal muscle cell differentiation, with a specific focus on the role played by the retinoblastoma growth suppressor and mitogen-induced D-type cyclins, the regulatory subunits of the cyclin-dependent kinases CDK4 and CDK6 that drive cell cycle progression (1, 2).

More recently, by using a cyclinD3 knockout mouse model, we demonstrated that cyclin D3 plays a unique function in controlling the developmental program of satellite cells, the skeletal muscle stem cells responsible of post-natal muscle growth and adult muscle regeneration (3). Furthermore, we discovered a novel function for cyclin D3 in regulating muscle fiber type phenotype and wholebody energy metabolism. Indeed, mice lacking cyclin D3 display an increased number of myofibers with high oxidative capacity, increased basal metabolism and running endurance and enhanced fatty acid oxidation (4).
Skeletal muscles are composed of heterogeneous myofibers that differ in their contractile response to motor nerve action (slow or fast) and metabolism (oxidative or glycolytic). It has been known for many years that slower, oxidative muscle fibers in patients with Duchenne Muscular Dystrophy (DMD) are more resistant to the dystrophic pathology in comparison with faster, more glycolytic fibers. Therefore, a proposed therapeutic strategy for treating muscular dystrophy is designed to remodel skeletal muscle toward a slower, more oxidative phenotype (5).

Based on the above observations, our current research activities aim to:

- Elucidate the role of cyclin D3 in modulating the metabolic response of skeletal muscle to functional demands and physiopathological conditions, such as nutritional challenges, exercise, aging.

- Evaluate whether genetic or pharmacological inactivation of cyclin D3 in the mdx mouse model of DMD can attenuate the dystrophic pathology by promoting a slower, more oxidative muscle phenotype.

The overall objective is to verify whether cyclin D3 is a potential molecular target for therapeutics in muscular dystrophies and age-related muscle pathologies.

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The intriguing life of SMN at the plasma membrane: not only a matter of Spinal Muscular Atrophy

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KEYWORDS:

SMN, SMA, RNA Metabolism, Plasma Membrane, Ribosome.

The RNA life cycle is accomplished through distinct phases promoting synthesis, modifications, traffic, and translation. Each of these phases takes place in a specific subcellular location, arguing that "where", and not only "how" these processes occur, can be crucial for RNA homeostasis. Cytoplasmic touring of individual mRNAs coupled with localized translation may ensure a common mechanism regulating gene expression at the subcellular level. Several findings suggest that the plasma membrane may contribute to compartmentalize protein synthesis in subcellular sites. It is therefore reasonable to suppose that membrane dynamics may influence RNA homeostasis as well as mRNA translation underlying specialized subcellular activities. In this framework, RNA-related proteins may be key determinants dictating the proteome profile in time and space.

Low levels of the Survival Motor Neuron (SMN) protein cause Spinal Muscular Atrophy (SMA), a neurological disease leading to infant mortality. Clinical manifestation of SMA reflects degeneration of motor neurons in the brain stem and spinal cord. Although SMA is widely known as a motor neuron disease, additional organs may be perturbed by SMN deficiency, especially in the most severe forms of the disease. This is consistent with the notion that SMN is an essential protein whose loss becomes deleterious in all cell types. SMN plays a key role in the RNA life cycle. We have previously reported a relationship between SMN and membrane-bound ribosomal proteins (RPs) (Gabanella et al., 2016). We found that SMN coexists with RPs in caveolin-rich membrane domains and promotes spatially restricted protein production underlying membrane remodelling. Recently, we confirmed the SMN-mediated crosstalk between plasma membrane dynamics and translation machinery (Gabanella et al., 2020). We have shown that SMN associates to and affects subcellular distribution of RPS6 mRNA. We also found that SMN knockdown perturbs the translation rate of RPS6 during active membrane and cortical actin remodelling. We observed that membrane compartments sequester a subset of RPS6 transcripts in an SMN-dependent manner. Moreover, we provide evidence that SMN could bridge RP-coding transcripts to caveolin-rich membrane domains. Importantly, for the first time we have obtained a spatial mapping of RPS6 mRNA in single cells by using the target RNA-initiated rolling circle amplification method ("Padlock Assay"). Overall, our studies point to a potential role of SMN in the ribosome assembly pathway by selective RPs synthesis/ localization in both space and time.

Ongoing investigations aim to bridge critical gap in SMN networks. Since SMN mediates membrane compartmentalization of RP-coding transcripts, we hypothesize that SMN could promote specialized translation underlying membrane plasticity. In this regard, it is important to mention that the dysregulation of neuromuscular junction (NMJ) may be an early event in SMA pathogenesis. Coherently, abnormalities of NMJ precede the motor neuron degeneration in SMA patients. NMJ is a highly specialized plasma membrane domain by which motor neuron communicates with muscle. Furthermore, proper NMJ morphology/plasticity requires activity-dependent local translation. Again, the molecular context in which SMN appears strongly implicated includes plasma membrane-related networks. Our ongoing studies aim to (1) obtain a complete list of RP-coding transcripts associated to membrane compartments; (2) identify a potential transcriptional profile of plasma membrane- enriched fractions, and reveal changes occurring in SMN deficiency. In addition, our major challenge is to understand how plasma membrane domains sequester mRNAs.

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Pathogenetic mechanisms and therapeutic approaches for myotonic dystrophy

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KEYWORDS:

Myotonic Dystrophy, Circular RNAs, Pathogenetic Mechanisms, Gene Therapy.

Myotonic dystrophy type 1 (DM1) is a dominantly inherited, multisystemic disorder caused by expanded CTG repeats in the 3' untranslated region (3'UTR) of the *DMPK* gene. DM1 is the most common adult-onset muscular dystrophy characterized by progressive skeletal muscle weakness, myotonia, cardiac arrhythmia, smooth muscle dysfunction, and neurological abnormalities. The repeat regions do not result in protein mutation because they are located in the 3 UTR, but lead to transcript accumulation into nuclear foci that affect the localization and activities of RNA-binding proteins involved in splicing regulation. Therapeutic strategies aimed at neutralizing the toxic RNA provide only short-term effects unless repeated administration of the inhibitory molecules are applied. No effective long-lasting therapy is yet available for DM1.

Our research focuses on the study of the pathogenetic mechanisms of DM1 disease and the development of new therapeutic strategies, through two different but related projects:

1. Circular RNAs (circRNAs) are emerging as key new members of the gene regulatory milieu, which are produced by back-splicing events within gene transcripts. Many circRNAs have been found to be important regulators of cellular physiology and pathology by a variety of mechanisms, and perturbations of circRNA expression have been recently reported in association with disease, including DM1(1). Analysis of publicly available gene-expression datasets indicate a pervasive dysregulation of circRNA levels and we identified a subset of circRNAs that are significantly increased in muscle biopsies of DM1 patients (2). With the aim of understanding the role of circRNAs in DM1 pathogenetic mechanisms, we are further expanding the search for circRNAs dysregulated in muscle biopsies of DM1 patients and plan to functionally validate them in DM1 *in vitro* and *in vivo* models.

2. Being DM1 a monogenic disease, gene therapy approaches aimed at eliminating the pathogenetic mutation are feasible. We have previously obtained permanent elimination of the toxic mutant repeats by using the CRISPR/Cas9 methodology in DM1 patient-derived fibroblasts (3), and recently developed inducible and tissue specific CRISPR/Cas9 complex components that ensure a time-limited and cell specific gene editing. Now, we are applying the same strategy in a well characterized DM1 mouse model carrying a mutated human DMPK transgene. These mice exhibit a pathologic neuromuscular phenotype similar to that observed in human DM1 disease and we expect that genome editing in diseased animals will lead to reversal of the pathologic phenotype. Given that this treatment should potentially result in a durable therapeutic response in postmitotic adult tissue, this technology could open the way for future gene therapy application in humans, alone or in combination with other therapies.

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Cardiac stromal cells biology and contribution to tissue repair and regeneration

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The cardiac muscle is composed of muscular as well as non-muscular cells, such as fibroblasts and more general stromal cells of different nature. The latter have been studied, in the past decade, as a promising source of therapeutic cells for heart failure treatment. My research interest currently focuses on how patients' clinical conditions affect cardiac stroma, possibly impairing its physiological role in repair.

Recently I have been investigating whether patients' clinical record might be associated with specific features of resident stromal cells. In particular, we have demonstrated that specific pharmacological treatments aiming at preserving cardiac function, can affect the phenotype of resident cardiac stromal cells, possibly keeping their potency in myocardial regeneration after cardiac injury (1–3). We demonstrated a positive correlation between beta blockers treatment and cardiac stromal cells phenotype which showed upregulation of cardiogenic genes and miRNAs paralleled by a decrease in markers related to lower regeneration potency.

Currently I am investigating the effects of dysmetabolism on the cardiac stroma, by assessing the features, potency and stress response in stromal cells isolated from patients with diabetes or metabolic syndrome. The data suggest an involvement of dysmetabolic condition, regardless of pharmacological intervention in our patients' cohort, in modulating the phenotype and paracrine profile of the stromal cells. The data are encouraging, and the project is currently running in collaboration with Sapienza University.

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KEYWORDS:

Cardiac Repair, Stromal Cells, Regeneration.

Neurobiology

Neurobiology research activities focus on mechanisms regulating brain functional states under physiological and pathological conditions in cells and mouse models. Main themes include the study of development and plasticity, emotionality, movement disorders, learning and memory, response to acute and chronic stress and pain, central control of energy homeostasis and reward, depression, aging and neurodegeneration, brain tumors. The role played by mouse genetic background, neurogenesis, microbioma, hormonal, neuroimmune systems, LncRNA/miRNA-mediated regulation and neurotrophins are investigated. Genetically manipulated animals and cellular models are used to clarify the role of genes in neural stem cell control and in different pathologies related to Central Nervous System (CNS) and Peripheral Nervous System (PNS) functions. Transcriptomic and proteomic analyses are carried out in order to identify new target molecules involved in physiological and/or pathological processes to evaluate rescuing strategies, in a translational perspective.

3D illustration of a nerve cell.

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Altered metabolism of heparan sulfate leads to developmental dopaminergic abnormalities responsible for autistic-like symptoms in lysosomal storage disorders

Lysosomal storage disorders (LSD), characterized by altered metabolism of heparan sulfate (HS), including Mucopolysaccharidosis (MPS) III and MPS-II, exhibit lysosomal dysfunctions leading to neurodegeneration and dementia in children. In LSD, dementia is preceded by severe and therapy-resistant autistic-like symptoms (ALBSs) of unknown cause.

Using the *Sgsh-/-* mouse model (hereafter called MPS-IIIA), bearing a spontaneous mutation in the sulfamidase gene, we identified endophenotypes of ALBSs, preceding DLSs, in young MPS-IIIA mice and discovered that they are due to hyperdopaminergia and upregulation of D1 receptor (D1R) pathway in the striatum. Pharmacological treatments aimed at inhibiting hyperdopaminergia or D1R activation correct ALBSs in MPS-IIIA, thus providing a novel therapeutic strategy to manage ALBSs in HS-defective metabolic disorders. Following the developmental trajectory of the hyperdopaminergia we found that it is due to an increased DA neurogenesis originating during the embryonic development; using different *in vitro* models of MPS-IIIA (primary mesencephalic neurons, induced dopaminergic neurons from embryonic fibroblasts and neuroblastoma cell line knocked-out for the gene *SGSH* by CRISPR/Cas9), we provide here mechanistic evidence that increased proliferation of DA cells is due to a loss of HS function consequent to the loss of function of the *SGSH* gene.

These findings identify for the first time embryonic dopaminergic neurodevelopmental defects due to defective function of HS leading to ALBSs in LSD and support evidence showing that altered HS related genes function are causative of autism.

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KEYWORDS:

Lysosomal Storage Disorders, MPS-IIIA, Dopamine, Autism.

Identification of miRNA and/or circRNA involved with Parkinson Disease

Cell death in Substantia Nigra appears to be the central pathophysiologic mechanism of Parkinson's Disease (PD). While the complete picture of the molecular basis remains to be elucidated, several recent studies have observed dysregulation of the autophagy pathway in the brains of PD patients, leading to emerging interest in the role of this cellular process in the disease. Genetic studies on PD patients have identified mutations in genes encoding components of the autophagy-lysosomal pathway. However, the selectivity for most agents targeting autophagy is limited. Because upstream autophagic-lysosomal components are involved in many other pathways, a broad stimulation of autophagy could result in a wide spectrum of side effects.

We recently identified PD familial cases that carry new mutations in the PGRN gene [1], and our preliminary data suggest that its mutant isoforms can affect normal autophagy in the brain. In addition, we have evidence of PGRN involvement in macroautophagy through TOR dependent regulation.

To understand this mechanism, we will Induce Pluripotent Stem Cells (iPSCs) using PBMC's isolated from patients diagnosed with PD with known and unknown mutations. Specific midbrain dopaminergic (mDA) neurons made by this technique are able to recapitulate key phenotypes of PD creating a patient-derived disease model which allow us to identify coding and non-coding transcriptional signatures associated with neurodegenerative phenotype. Then we will be devoted to the characterization of differentially-expressed miRNA and/or circRNA extracted from these cells and finally we will create neuronal cellular models of PD by CRISPR-Cas9 genome editing technique to identify any dysregulated molecular network involved in a specific autophagy step.

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KEYWORDS:

Progranulin, Parkinsonism, Genetics, Dementia, Gaze Palsy, CRISPR-Cas9, Induce Pluripotent Stem Cells (iPSCs).

Identification and treatment of early cognitive deficits during ageing to prevent dementia

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KEYWORDS:

Ageing, Dementia, Alzheimer Disease, Autophagy, Sex Differences.

Individuals do not all show a reduction in mnemonic capacity with advancing age, but the ones who do, show it very early and, in general, the symptoms of mental decline are associated with the accumulation, in neurons, of alpha-synuclein and beta amyloid protein aggregates that can form fibrils - or filaments - potentially toxic to cells. In a young cell these aggregates, considered cellular waste, are enclosed within a vesicle (autophagosome) that carries them into lysosome, an organelle that breaks them down and recycles their constituents. With ageing, the aggregates increase and the lysosome's degradative capacity is reduced.

One of the lines of research in our laboratory deals with identifying the early mechanisms that precede the development of dementia in animal models. To identify middle-aged subjects with vulnerable memory, we set up and used a memory test in which we were able to manipulate the amount of information to remember (the number of objects), in order to make the task more difficult. This allowed us to separate subjects of the same age able to remember up to 6 different objects from those who are able to remember a maximum of 2 objects. In middle-aged subjects that fail the six different objects recognition task, the neuronal lysosomes are enlarged and engulfed with alpha-synuclein and beta amyloid aggregates in the hippocampus, a particular region of the brain that is crucial for memory.

The ultimate goal of our studies is to identify new therapies with symptomatic efficacy on cognitive symptoms and for this reason we have been working for some time on the use of dopaminergic drugs; at the same time we try to find molecules able to slow down the progression from mild cognitive symptoms to dementia.

In a recent study, we have shown that Spermidine, a polyamine naturally present in many foods, stimulates autophagy, and thus improves the degradative capacities of the cells. The study showed that one month treatment with Spermidine stimulates the expression of the transcription factor EB (TFEB), which controls the expression of genes responsible for autophagy/lysomal degradation and therefore promotes the cell cleaning from alpha-synuclein and beta amyloid aggregates. Once cleared the cell from these aggregates, we also observed that the synaptic communication, through the AMPA receptor, is restored and it allows the memory to function even under conditions of high information load in subjects with the deficit. In fact, in subjects with deficit in memory capacity we saw that the engulfment of lysosomes is associated with a defect in activating those communication processes between neurons that are necessary in young subjects to create new memories and that are transmitted by synapses through the glutamate receptor AMPA. Instead, these processes are preserved in young subjects, or in those aged but with intact memory. We will continue to study the effects of Spermidine in neurodegenerative diseases, alone and in combination with other treatments, and we will try to verify whether an enrichment of the diet may be sufficient to prevent the onset of dementia.

In the same project line we are studying how sex differences can affect not only the course of cognitive symptoms, but also the efficacy of pharmacological and behavioral treatments. Among the behavioral ones we are working on the efficacy of exercising and of neuronal stimulation; preliminary data in our laboratory suggest that both mechanisms are regulated by the sex of the subject.

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Identification and treatment of neural mechanisms underlying early behavioral symptoms in Parkinson's disease

Parkinson's disease is the most common neurodegenerative disease after Alzheimer's disease. The disease is characterized from the histopathological perspective by the degeneration of neurons that produce dopamine and the presence of Lewy bodies, protein aggregates that contain mainly alpha-synuclein. Recent studies have shown that an increase in the amount of alpha-synuclein in the brain is sufficient to induce the death of dopaminergic neurons. The death of dopaminergic neurons in the midbrain leads to a reduction in the quantity of dopamine released in the striatum and consequent motor impairment. However, when motor symptoms occur, the degeneration is already excessively extended, which makes compelling the need to identify early symptoms and the biological mechanisms underlying them for commencing restorative therapies.
One of the research lines of our laboratory aims at identifying early alterations of Parkinson's disease and associated symptoms to develop therapeutic strategies that can slow down the disease course. Among these early symptoms, there are motor learning deficits in complex tasks, alterations in working memory and more recently it has been noted that specific vision deficits can also be a warning bell.

In our lab we study the physiology of motor memory; motor memory is characterized by a slow and progressive learning that requires massive training until movements become automatic. The striatum is crucial for motor learning; however, it is not clear, how practice modify the striatal cells activity to progressively reach plateau performance.

Using a protocol of behavioral metaplasticity, which consists of identifying the synaptic signature of behavioral experiences, we found that if we apply an electrical stimulus to the striatum neurons in untrained animals, they give an inhibitory response; if the same stimulus is applied to animals subjected to the first learning sessions, the neurons respond by getting excited, identifying a new form of cellular memory stored in striatal cells. However, once the motor exercise is perfectly learned and the movement is performed automatically, the neurons return to give an inhibitory response to the electrical stimulus. From biochemical analysis we found that this mechanism of metaplasticity is regulated by the dopamine transporter, DAT. Using alpha-synuclein over-expression models, we then discovered that long before the death of dopaminergic neurons, the excess of alpha-synuclein led to a transcriptional reduction of DAT and stopped the animals from performing the learned movements automatically. These results identify for the first time a very early clinical manifestation in motor learning prior to the death of neurons in Parkinson's disease. Low levels of DAT, measured by imaging methods in patients, are used in the clinic to predict the level of neurodegeneration, based on the assumption that striatal DAT is reduced due to the degeneration of dopaminergic cells in the midbrain. The results of this research suggest that low levels of DAT do not necessarily represent the death of dopaminergic neurons, but may instead indicate a sinucleinopathy, a diagnostic hypothesis that deserves targeted investigation with genetic investigations and cerebrospinal fluid sampling, in order to predict its evolution.

We then observed similar defects in Parkinson's disease models generated by directly injecting into the striatum aggregates of alpha-synuclein protofibrils, that in an early stage lead to specific visual-spatial cognitive defects and synaptic plasticity defects that depend on the amount of aggregates. We are using these models to verify the efficacy of monoclonal antibodies to inhibit alpha-synuclein and other new generation treatments.

More recently we've started to study visual defects in Parkinson's disease and we have discovered that intra-vitreus alpha-synuclein over-expression in healthy mice, induces at an early stage the death of dopaminergic neurons of the retina, the amacrine cells, visual acuity defects and altered adaptation to darkness measured by electroretinogram. We are using this model to understand if the retina can be used as one of the models of choice to test new cell therapies for Parkinson's disease

KEYWORDS:

Motor Memory, Synaptic Plasticity, Parkinson's Disease, Retina, Alpha-Synuclein.

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Neurobiology of memory capacity

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KEYWORDS:

Memory Capacity, Neuronal Circuits, AMPA Receptors, Dopamine, Sex Differences, Cortical-Subcortical Plasticity, Optogenetics.

One of the focus of our lab is to understand the neurobiology of learning and memory under normal and pathological conditions. Memory is a fundamental brain function for animal survival. In our lab we strive to answer questions related to the neurobiology of memory and focus on the roles of dopamine, glutamate and their interactions in different brain regions.

Recently, we have attempted to understand how the brain processes increasing information loads. This is a question of fundamental importance, especially in the information era in which we are living. It has been estimated, that because of the information revolution, we are receiving (via television, internet, etc) five times as much information as we did in 1986. This fact is related to *memory capacity* (MC), which in psychology refers to the limited capacity of working memory (WM), and more in general to the limited capacity of our mental resources. Although, memory capacity is a hot topic in neuropsychology, the neurobiology of normal and pathological MC is almost completely unexplored. George Miller was the first to suggest that humans have a limited MC of about 7 plus/minus 2 items, which is defined as memory span.

We have recently developed a novel behavioral procedure to study MC in rodents (Sannino et al 2012), the DOT-IOT. Using the DOT-IOT we have demonstrated that mice, as well as humans, also have a limited MC. Using this task, we have discovered that under low memory load, male normal mice do not recruit the hippocampus (a region of the medial temporal lobe that is crucial for long-term memory formation) into the task. However, when the information load increases up to the highest limit the hippocampus is necessary for memory maintenance event at short-time intervals (minutes). By combining behavioral, genetic, optogenetics, chemogenetic, pharmacological and molecular approaches, we have discovered that this process requires post-translational modifications of AMPA receptors in the hippocampus and it can be modulated by the differential activation of dopamine receptors subtypes.

More recently we are studying sex differences in memory capacity and using mice, we discovered that although both sexes have the same memory span at short delay, female mice tend to activate negative regulatory feedback for the consolidation of this memory.

Based on the identification of these pathways we are testing drugs capable to expand memory capacity in physiological conditions, in models of pathologies such as schizophrenia and aging presenting reduce memory capacity as a core cognitive deficit.

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Protective effect of a multi-strain probiotic (BP-002) in a mouse model of neuroinflammation induced by LPS: role of gut-brain axis

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KEYWORDS:

Gut Brain Axis, Neuroinfammation, Adult Neurogenesis, LPS, Probiotics.

Neuroinflammation promotes neurochemical and hormonal changes, resulting in profound effects on motivational states (anhedonia), mood (depression and anxiety disorders) and cognitive functions (decrements in learning and memory). One of the processes mainly affected by the neuroinflammation is the hippocampal adult neurogenesis, the mechanism of production of new neurons in the adult mammalian hippocampus.

Indeed, injection of LPS triggers an inflammatory response leading to several dysfunctions in the hippocampal neurogenic niches: an increase of astrogliosis with a consequent depletion of the neural stem cells pool, a decrease of progenitor proliferation and an altered migration and arborization of neuroblasts. These events result in an impaired adult hippocampal neurogenesis with a consequent deficit of hippocampal memory tasks.

In this context, several studies have highlighted that gut microbiome not only contributes to the regulation of metabolism and immunity, but also plays a key role in regulating the neuro-inflammatory pathways through its interaction with gut-brain axis. During inflammation, several intestinal cell signaling pathways are persistently active and lead to the overproduction of pro-inflammatory mediators such as cytokines, which may cause intestinal injury, and interfere with the production of critical factors involved in brain functions, such as short chain fatty acids (SCFA), neurotransmitters and gut hormones, compromising neural functions and contributing to the development of brain disorders.

For this reason, the modulation of microbiota-gut-brain axis represents an appealing approach to develop novel therapeutic strategies for disorders characterized by cognitive decline.

Our research aims at evaluating the putative neuroprotective and proneurogenic role of a new probiotic mixture, called BP-002, in a mouse model of LPS-induced neuroinflammation.

BP-002 was administered to 3 month-old mice for 15 days and then the mice were treated with a single dose of LPS by ip injection. The sacrifice and the tissue collection was carried out at 2 and 24 hours after LPS injection. Before the sacrifice, we performed behavioral tests in order to analyze the sickness behavior.

Collected tissues were used to analyze the early stages of adult neurogenesis and microglia activation, the modulation of neuro-inflammatory patterns and intestinal homeostasis:

- The proliferation analysis clearly indicates that the treatment with BP-002 induced a significant increase in newly-generated neurons in the dentate gyrus mainly in DCX + neural progenitors.

- LPS induced a clear modification of the microglial population towards a state of activation, a symptom of an ongoing pro-inflammatory state in hippocampus and cortex. BP-002 prevents the LPS-dependent inflammatory activation of microglia both in Dentate Gyrus and Cerebral Cortex by maintaining the cells in a resting/surveillance state.

- The expression analysis of neuro-inflammatory patterns demonstrated that the treatment with BP-002 decreases the expression of proinflammatory genes in response to LPS.

- The analysis of the colonic inflammatory state showed that the high level of pro-inflammatory cytokines induced by LPS treatment, was reduced by BP-002 consumption. BP-002 alone was able to increase gut barrier integrity.

Collectively, our data clearly indicate that BP-002 exerts a powerful neurogenic stimulus in terms of increase of proliferation and differentiation of neural progenitor pool, independent of LPS treatment. The treatment with BP-002 could attenuate the inflammatory response under acute inflammatory condition and prevent the pro-inflammatory over-activation leading to neuronal damage.

This project is part of a wider IBBC-Actial Pharmaceutical collaboration, with a more general perspective of implementing public-private interaction aiming at expanding the scientific potentiality of the IBBC.

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Antioxidant action of plant polyphenols in the counteraction of alcohol-abuse induced damage: Impact on the Mediterranean diet

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KEYWORDS:

AUD, NGF, BDNF, Oxidative Stress.

Polyphenols are a structural class of more than five thousand chemicals, including organic but also synthetical and semi-synthetical components. Their molecules are characterized by multiples phenolic structures that contribute to their functional activity. Polyphenols can be found in a large variety of plants, including foods like fruits, vegetables, cereals, tea, coffee, olive oil and red wine which are part of the Mediterranean diet.

The phenolic fraction in the olive includes among others oleuropein, tyrosol, hydroxytyrosol, polyphenols, secoiridoids and ligands while in the wine is the resveratrol. However, the quite toxic effects of alcohol abuse on health shouldn't be downrated: alcohol consumption may cause various kinds of tissue damage in several regions of the body as the brain, liver, kidney, endocrine glands and its intake can disrupt the synthesis and functionality of neurotrophins, proteins that play an important role in nerve cells development and growth, immune and endocrine functions and also in the fine tuning of memory and learning processes.

Data have shown that the antioxidant properties of polyphenols can play a pivotal role in counteracting alcohol-induced damage in animal models and during alcohol withdrawal in humans by reducing oxidative stress and by the modulation of neurotrophins as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF).

In conclusion, the Mediterranean diet is globally known as the dietary pattern that provides the greatest number of positive effects on health because using food and drinking rich in polyphenols as vegetables, fruits, extra-virgin olive oil and a moderate intake of wine. Thus, the detrimental effects of ethanol contained in alcoholic beverages seem to be partly counterbalanced by the presence of polyphenols in the foods and extra-virgin olive oil which yield an important antioxidant action. However, further studies on humans will be necessary to fully disclose if and how it will be possible to include polyphenols supplementation in the treatment of patients affected by alcohol use disorders and in the management of abstinence.

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Epigenetics of alcohol consumption and abuse during gestation

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Ethanol exposure during gestation is an early life stressor that profoundly dysregulates structure and functions of the embryonal nervous system, altering the cognitive and behavioral development. Such dysregulation is also achieved by epigenetic mechanisms, which, altering the chromatin structure, redraw the entire pattern of gene expression. In parallel, an oxidative stress response at the cellular level and a global upregulation of neuroendocrine stress response, regulated by the HPA axis, exist and persist in adulthood. This neurobehavioral framework matches those observed in other psychiatric diseases such as mood diseases, depression, autism; those early life stressing events, although probably triggered by specific and different epigenetic mechanisms, give rise to largely overlapping neurobehavioral phenotypes. An early diagnosis of prenatal alcohol exposure, using reliable markers of ethanol intake, together with a deeper understanding of the pathogenic mechanisms, some of them reversible by their nature, can offer a temporal "window" of intervention. Supplementing a mother's diet with protective and antioxidant substances in addition to supportive psychological therapies can protect new- borns from being affected.

KEYWORDS:

Epigenetic, Alcohol Use Disorders, Fetal Alcohol Spectrum Disorders.

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Neuroinflammatory Markers in the Serum of Children with Down Syndrome, DiGeorge Syndrome, and Klinefelter Syndrome

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Down Syndrome (DS), DiGeorge Syndrome (DGS), and Klinefelter Syndrome (KS) are common chromosomal disorders. Although DS, DGC and KS individuals are mostly perceived as characterized by some distinct physical features, cognitive disabilities, and cardiac defects, they also show important dysregulations of neuro-immune functions associated with changes in oxidative stress. While critical information is available for DS, DGC and KS adults, little literature is available on the neuroinflammation in prepubertal children. We aimed to evaluate in prepubertal and post-pubertal DS, DGC and KS children the serum levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), oxidative stress as free oxygen radicals defense (FORD), free oxygen radicals test (FORT), and cytokines playing key roles in neuroinflammation and oxidative processes as TNF-α, TGF-β, MCP-1, IL-1α, IL-2, IL-6, IL-10, and IL-12. No differences were found in NGF between DS, DGC and KS children and controls. However, we found changes in BDNF DS, DGC and KS subjects compared to controls. We also did not reveal changes in FORD and FORT. Quite interestingly, the serum of DS, DGC and KS children disclosed marked alterations in all analyzed cytokines with evident differences in serum cytokine presence between male and female DS children. In conclusion, the present study evidences in DS, DGC and KS prepubertal and post-pubertal children a disruption in the neurotrophins and immune system pathways.

KEYWORDS:

NGF, BDNF, TNF-α, TGF-β, MCP-1, IL-1α, IL-2, IL-6, IL-10, IL-12, Oxidative Stress, Genetic Rare Diseases.

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Promising therapeutic applications of *botulinum* neurotoxins: nerve regeneration and functional recovery in a spinal cord injury mouse model

Treatment of spinal cord injury (SCI) is a dramatic health and social challenge that needs urgent attention by the medical and scientific community. Different experimental approaches are currently being tested, from axon growth promoting or neuroprotection to rehabilitative measures, but none has been able to reverse the consequences of SCI. Considering that the molecular and cellular environment of the spinal cord is constantly changing from the moment of injury until several weeks, or even months later, spinal cord repairing is particularly complex.

Our challenge is represented by the identification of pharmacological therapies able to exert neuroprotection reducing the extension of damage and inducing regeneration even through the stimulation of spinal stem cells.

Over the last 20 years, therapeutic utilization of *botulinum* neurotoxins (BoNTs) has successfully expanded. Established and emerging applications of BoNTs (mainly of serotype A, BoNT/A), from muscular to neurological and pain disorders, are already present in clinical practice. Our previous studies demonstrated that BoNT/A effectively contrasts neuropathic pain inducing analgesic and anti-inflammatory effects and exerting its action on both neurons and glial cells.

Currently, we are studying novel and unexpected aspects of BoNT/A that may represent a new and concrete possibility for fighting spinal trauma induced paralysis. We demonstrated the extraordinary capacity of BoNT/A to neutralize the complete paralysis and pain insensitivity induced in a murine model of SCI. We showed that the toxin, spinally administered within one hour from spinal trauma, exerts a long-lasting protective action, up to 60 days after its administration, and induces a complete recovery of muscle and motor function. BoNT/A modulates SCI-induced neuroglia hyperreactivity, facilitating axonal restoration, and preventing secondary cells death and damage. The great power of stimulating axonal regeneration and nerve sprouting of BoNT/A is the new therapeutic potential discovered, which can be of great general interest for the important biological and biomedical implications. Because of the well-documented BoNT/A pharmacology, safety, and toxicity, these findings strongly encourage clinical translation.

KEYWORDS:

Botulinum, Spinal Cord, Regeneration, Neuroprotection, Anti-Inflammatory Action.

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Detection of sleep-related disturbances in mouse models of neurological diseases using a novel automated home cage monitoring system

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KEYWORDS:

ALS, Sleep, Circadian Rhythm, Home Cage Monitoring $_4$, Neurodegeneration, Neuromuscular Diseases, Mouse Behavioral Phenotyping.

Sleep disturbances are common in patients with neurodegenerative and neuromuscular diseases (AD, PD, ALS, DM-1) leading to even further deteriorated quality of life. Investigating methods to potentially assess sleep and rest disturbances in animal models is thus of crucial interest.

Here we show our initial study testing an animal model of ALS (Amyotrophic Lateral Sclerosis), a devastating neurodegenerative disease that affects both central and peripheral nervous system, leading to the degeneration of motor neurons, which eventually results in muscle atrophy, paralysis and death. In ALS sleep disruption it is often related to hypoventilation, hypoxia, hypercapnia, restless legs, immobilization, nocturnal cramps and pain.

We used an automated home cage monitoring system (DVC®) to capture irregular activity patterns that can potentially be associated with sleep and rest disturbances and thus to the progression of ALS in the SOD1G93A mouse model. DVC® enables non-intrusive 24/7 long term animal activity monitoring, which we assessed together with body weight decline and neuromuscular function deterioration measured by grid hanging and grip strength tests in male and female mice from 7 until 24 weeks of age.

We show that as the ALS progresses over time in SOD1G93A mice, activity patterns start becoming irregular, especially during day time, with frequent activity bouts that are neither observed in control mice nor in SOD1G93A at a younger age. The increasing irregularities of activity pattern are quantitatively captured by designing a novel digital biomarker, referred to as Regularity Disruption Index (RDI). We show that RDI is a robust measure capable of detecting home cage activity patterns that could be related to rest/sleep-related disturbances during the disease progression. Moreover, the RDI rise during the early symptomatic stage parallels grid hanging and body weight decline.

The non-intrusive long-term continuous monitoring of animal activity enabled by DVC® has been instrumental in discovering novel activity patterns potentially correlated, once validated, with sleep and rest disturbances in the SOD1G93A mouse model of the ALS disease. We are confident that this novel automated system can be of great help in the study of this important symptom often neglected in animal models of ALS or even other neurodegenerative diseases. Additionally, circadian and sleep dysfunctions are often premorbid and can serve as early diagnostic markers of neurodegeneration. In the future we aim to further investigate such disturbances in other models as for example DMSXL mice, model of myotonic distrophy type 1, and Alzheimer's or Parkinson's diseases models.

Moreover the use of home cage monitoring systems in the wider scientific community is expanding, as in the International Mouse Phenotyping Consortium (IMPC), a large-scale phenotyping endeavor devoted to the creation of a comprehensive catalogue of mammalian gene function. In this case such monitoring systems could be important to uncover sleep-related or circadian activity relevant phenotypes associated to specific genes in potential models of many human diseases.

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G protein-coupled receptors' signaling, cerebellum development and medulloblastoma occurrence

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https://medicineinnovates.com/genetic-ablation-gpr37l1-delays-tumor-occurrence-ptch1-medulloblastoma/

KEYWORDS:

Cerebellum, Proliferation, GPCRs.

Tight spatiotemporal coordination of developmental signaling is required for building the characteristic shape, morphology and connectivity of the mammalian cerebellum. The sonic hedgehog (Shh) mitogenic pathway is a major regulator of both prenatal and postnatal stages of cerebellar development. Shh signaling is transduced by non-motile primary cilia (PC) and controls cerebellar morphogenesis by promoting the expansion of the granule cell progenitor pool (GCP). We have characterized the G-protein coupled receptor 37-like 1 (Gpr37l1; a putative receptor for prosaposin and derived cytoprotective peptides) as specifically expressed in murine cerebellum's Bergmann astrocytes, where it interacts at peri-ciliary membrane compartments with patched 1 (Ptch1), a co-receptor of the Shh signaling complex (1, 2).

The aim of our studies is to understand the role played by Gpr37l1 and related receptors in modulating Shh signaling during development and adulthood, upon production and characterization of novel mouse mutant lines, carrying specific targeted mutations of these receptors' and interacting proteins' genes. Our work has highlighted the specific involvement of Gpr37l1 in regulating Shh's early post-natal proliferative signals in Bergmann glia astrocytes, which in turn critically modulate the differentiation and maturation of cerebellar neurons in the external granule layer (EGL):

1. Gpr37l1 controls GCP proliferation during postnatal cerebellum development, as Gpr37l1 KO (*Gpr37l1-/-*) mice exhibit an altered Shh mitogenic cascade, with premature down-regulation of GCP proliferation and precocious post-natal cerebellar development.

2. Genetic ablation of the Gpr37l1 protein significantly delays medulloblastoma occurrence in *Gpr37l1-/-,Ptch1+/–* double-mutant murine models, with concomitant marked reduction in GCP proliferation and EGL thickness, extensive expression of the Shh-induced mitogenesis' inhibitor wingless-type member 3 (Wnt3) and decrease in the level of glioma-associated oncogene family 2 (Gli2), a major transcriptional activator of Shh mitogenic signaling, at the early stages of postnatal cerebellar development (3).

3. Cultured *Gpr37l1-/-* cerebellar primary astrocytes display striking increases in proliferative activity and Ptch1 protein expression and internalization, as well as marked synthesis and secretion of active Shh (4).

The availability of appropriate *in vivo* and *ex vivo* mutant models will be instrumental for the detailed biochemical and physiological analysis of the Gpr37l1-regulated molecular mechanisms that modulate the Shh signaling pathway during cerebellum development and in adulthood.

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Molecular study of GLUT1 deficiency syndrome: functional characterization of causative mutations and new genotype-phenotype correlations

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KEYWORDS:

GLUT1, Non-Coding RNA.

GLUT1 DS (OMIM 606777, GLUT1 deficiency syndrome, GLUT1 DS) is a metabolic brain disorder with great clinical heterogeneity caused by different types of SLC2A1 mutations, thereby making clinical and genetic diagnosis challenging in some cases. The pathogenic effects of coding altering sequence mutations is usually evaluated using deleteriousness scoring systems, but an effective approach to study dynamic effects of mutations on molecular mechanism of GLUT1 is highly required.

Moreover, 10% of patients with signs suggestive of GLUT1 DS, are actually negative to SLC2A1 mutations. On this regard, the occurrence of pathogenic mutations in promoter and intronic regions of SLC2A1 has been recently reported (1;2).

For patients with GLUT1 defects, the ketogenic diet, which produces ketone bodies as an alternative energy source for brain metabolism, is administered.

The purpose of our study is to bring new insights into the GLUT1 DS molecular pathology to clarify genotype-phenotype correlations and improve the diagnostic yield of genetic test, as establishing the diagnosis is crucial for the ketogenic diet effectiveness.

GLUT1 is found primarily in the cell membrane and on the cell surface and is located at the blood-brain barrier where transports glucose into the brain. Thus, first aim of the study is to explore the functional impact of SCL2A1 pathogenic missense mutations on GLUT1 sub-cellular localization using cellular and molecular biology approaches. To this aim we generated a plasmid system to express and visualize wt and mutated GLUT1 in fusion with GFP protein. Through this system we will be also able to assess the effects of mutations on cell glucose uptake.
Additionally, we hypothesize that at least a part of patients, negative for SLC2A1 mutations, could harbor mutations in regions not routinely screened in standard analyses. To this aim we are implementing sequencing data analysis workflow specifically designed to evaluate also non-coding and regulatory sequences and large deletions.

Finally, phenotype heterogeneity and occurrence of mutations in regulatory regions suggest that factors that modulate gene expression could potentially contribute to pathophysiology mechanisms.
On this regard, analysis of the genome region of SLC2A1 disclosed an annotated transcript that could potentially act as a natural antisense transcript. In order to characterize the functional role of this transcript we verified its expression in different tissues and cell lines. Moreover, we assessed its subcellular distribution. We will characterize non-coding antisense RNA structure and expression in cell lines and patients. Additionally, experiments of non-coding antisense over-expression and silencing will be performed to assess the effects on SLC2A1 expression levels.

The knowledge on this non-coding RNA functional roles could shed light on molecular pathogenesis and clarify genotype-phenotype correlations. Moreover, it could be used for therapies development as modulation of gene expression levels has been suggested as a potential therapeutic approach.

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Zimmermann-Laband Syndrome: functional studies to characterize causative mutations in KCNH1 gene

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KEYWORDS:

Neurodevelopmental Disorders, Primary Cilium, KCNH1.

KCNH1 is a member of the EAG (ether-à-gogo) family of voltage-gated potassium channels. Recent studies have demonstrated that gain-of-function mutations in KCHN1 are implicated in Zimmermann-Laband syndromes (ZLS; MIM 135500; 1) and other forms of developmental deficits that include mental retardation and epilepsy (2;3;4;5). These findings suggest that KCNH1 might be important for cognitive development in human. KCNH1 is highly expressed in adult central nervous system and in most human tumours, at low levels in placenta, testis, adrenal gland and transiently in myoblasts. It is aberrant expressed in cancer cells in contrast to its restricted distribution in healthy tissues. As its transforming activity and ectopic expression occurs in 70% of human cancers, KCNH1 is considered a clinically relevant ion channel and a marker and therapeutic target of several tumours. Cancer cells overexpressing KCNH1 acquire selective advantages that promote cancer progression, such as chronic cell proliferation and migration, interference with oxygen homeostasis, regulation of cell cycle and modulation of ciliogenesis. Control of cell cycle is associated with ciliogenesis. In tissues, quiescent cells assemble a primary cilium, an antenna like sensory organelle that regulates multiple cell signalling pathways during development and in the adulthood. Mutations in genes that impair ciliary biogenesis, protein trafficking and function lead to a dysregulated signalling and cause diseases known as ciliopathies, which may affect nearly all organs during development or in adulthood (6). It has been suggested that some clinical features of ZLS patients, as orofacial and digital abnormalities, together with severe mental retardation and morphological features, are at least in part reminiscent of ciliopathies (7). Most functional studies on K+ channels focused on their electrophysiological properties. Notably, while electrophysiological studies were performed to shed light on the mechanisms underlying KCNH1 in ZLS, no functional data have been collected on the consequences of mutations on cell functions, i.e. localization, structure, and altered pathways. Based on these considerations, we explored the functional impact of two KCNH1 mutations associated with ZLS (1) using cellular and molecular biology approaches in primary skin fibroblasts derived from ZLS-patients carrying mutations in KCNH1. We demonstrated impaired proliferation of KCNH1 mutant fibroblasts, confirming the role of KCNH1 as a regulator of cell cycle and also found a significant increase of cilia number and cilium-related pathways, i.e. SHH pathway, activation in all the mutant fibroblasts, thus suggesting functional role of KCNH1 in cilia regulation. Moreover, confocal microscopy analysis in fibroblasts from ZLS patients further confirmed defects in cilia morphology and mislocalization of IFT components of the cilium directional transport. Confocal analysis refined also the reported KCNH1 localization in the cilium (7) to be concentrated at the centrosome and ciliary pocket regions for both wild-type and mutant fibroblasts.

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MicroRNAs and LncRNAs in brain ageing and Alzheimer Disease

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KEYWORDS:

miR-101, LncRNAs, Neurons, Hippocampus, Ageing, Alzheimer's Disease.

Non-coding RNAs and in particular microRNAs tune brain cell differentiation and synaptic development, and modulate brain functions such as synaptic plasticity, memory formation and behavioral performances. Our research, aims to identify long noncoding RNAs (lncRNAs) and/or microRNAs as well as their interactions, and to investigate their roles in neuronal and glia cells during physiological and pathological conditions. Main focus is the study of these molecules in Ageing and Alzheimer's Disease (AD).

Primary rodent neural cell cultures, cell lines, human neuronal and glia cells differentiated from iPSC (induced pluripotent stem cells)-derived NSC (neural stem cells) as well as mouse models are instrumental to our studies.

Previous findings in our laboratory have shown that miR-101 regulates directly AD-related genes, Amyloid precursor protein (APP) and Ranbp9, in rodent hippocampal neurons in vitro and *in vivo* (Vilardo et al., 2010; Barbato et al., 2014). More recently we found that inhibition of miR-101 post-transcriptional regulation in CA1 hippocampal neurons of adult C57BL/SJ mice, by stereotaxic injection of a lentiviral miRNA sponge, leads to cognitive decline (Barbato et al., 2020). The cognitive impairment features were associated with increased hippocampal expression of relevant miR-101 target genes, APP, RanBP9 and Rab5 and overproduction of amyloid beta (Aβ) 42 levels, the more toxic species of Aβ peptide. Notably, phosphorylation-dependent AMP-activated protein kinase (AMPK) hyperactivation is associated with AD pathology and age-dependent memory decline, and we found AMPK hyperphosphorylation in the hippocampus of pLSyn-miR-101 sponge mice.

Further characterization of the molecular pathways involved in hippocampal dependent cognitive decline after miR-101 downregulation are ongoing. We are also planning to search for functional effects of miR-101 inhibition in neurons on glia cells using co-culture models.

Finally, we are also searching for lncRNAs that might regulate miR-101 and/or other microRNAs and pathways associated to Ageing/AD by bioinformatics and wet studies.

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Neuroprotective and inflammatory factors in neurodegenerative diseases: potential biomarkers

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KEYWORDS:

Alzheimer's and Parkinson's Diseases, Chemokines, Biomarkers, Blood, Cerebrospinal Fluid.

A member of the chemokines family, the Bv8/Prokineticin (PROK2), interacting with two G-protein coupled receptors (PKR1 and PKR2) has recently emerged as a critical player in immune system and inflammatory diseases (Negri and Ferrara, 2018).

In the contest of neurodegenerative diseases, an involvement of PROK2 and its receptors has been demonstrated in Aβ toxicity, indicating a deleterious role of this chemokine, up-regulated by Aβ mainly in astrocytes (Severini et al., 2015; Maftei et al., 2019). More recently, we demonstrated a significant up-regulation of PROK2 levels in brain tissues of both Aβ1-42 i.c.v. injected rats and of transgenic Alzheimer's disease (AD) mice (Tg2576) and in the hippocampus of AD patients. Additionally, by a pilot study, a significant increase of PROK2 levels has been proved in the serum of AD patients, as compared to control subjects, identifying a potential plasma marker of the disease (Lattanzi et al., 2019).

Conversely, PROK2 seems to exert a neuroprotective activity in Parkinson's disease (PD), as demonstrated by Gordon et al. (2014). PROK2 expression was highly induced in nigral dopaminergic neurons during early stages of degeneration in multiple models of PD, including PK2 reporter mice and MitoPark mice and brain of PD patients.

In the proposed research, we are planning to test the serum, CSF and olfactory neuroepithelium levels of PROK2, comparatively in a cohort of PD patients and sex/age-matched controls, correlating biochemical findings to clinical parameters, in order to dissect the role of PROK2 in PD.

Additionally, to confirm and extend data from patients, we aim to study a PD rat model overexpressing α-synuclein, analyzing the amount of the mRNA of this chemokine and of its cognate receptors by qPCR, respect to the corresponding controls, and examining their localization by immunofluorescence studies.

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This project research is carried out in collaboration with the Department of Physiology and Pharmacology, Sapienza University of Roma (Prof. R. Lattanzi, Dr. D. Maftei), and with the Department of Systems Medicine, University of Rome Tor Vergata (Prof. N. Mercuri, Prof. R. Possenti, Dr. T. Schirinzi).

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Experimental approaches to investigate the Nerve Growth Factor receptor-mediated actions

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KEYWORDS:

Biological Activity, Cleavage, NGF Processing, Small Peptides.

The Nerve Growth Factor (NGF) is largely known as a differentiative and trophic factor whose actions on targets cells are mediated by the interaction with two class of receptors: the Tropomyosin Kinase Receptor A (TrkA) and the p75 Neurotrophin Receptor (p75NTR). Apoptosis, impairment of cell proliferation and migration, as well as altered differentiation of cell precursors have been indeed reported as a consequence of defective NGF signalling in different pathological conditions including neurodegenerative diseases and cancers, but also in cognitive and mood impairment in humans (1,2,3). To further support the biological value of NGF homeostasis, increasing evidence demonstrates that the balance between NGF receptors and their ligands regulates the NGF activity in different tissues and organs, including eye and brain.

Similar to brain, ocular tissues synthetize neurotrophins, and their receptors are expressed in neuronal and non-neuronal cells, including neuronal cell precursors. NGF and Brain Derived Neurotrophic Factor (BDNF) are anterograde and retrograde transported through the optic nerve, and maintain homeostasis in brain and eye (retina; 1). For these reasons we have recently proposed the neurotrophin & retina-brain system as a paradigm to further explore the mechanism regulating NGF processing, and the TRKA e p75NTR-mediated intracellular pathway activation, and evaluate their impact on brain/body functions, including the maintenance of circadian rhythmicity, and the response to environmental changes in physio and pathological conditions.

Into this framework, different approaches, including primary neuronal cells, DRG and retina culture system, animal models of optical nerve injury, diabetes, and ocular or systemic inflammation, are used in our laboratory to investigate:

- The biological functions of peptides derived from NGF processing

- The impact of p75NTR cleavage on the TrkA activity and mediated functions

- The possible functional interaction of TrkA with receptors other than p75NTR, as for example the SorCS receptors, or ligands, including LINX (also known as islr2). LINX is a group of proteins belonging to the leucine-rich repeat (LRR) superfamily which display highly specific and dynamic expression patterns, particularly in the nervous system.

In cooperation with clinicians, including psychiatrics and neurologists, our laboratory is also exploring the role of Neurotrophins as potential risk or therapeutic markers for mood disorders by analyzing the individual profile of NGF and BDNF release in tears, saliva and serum, and their correlation with psycho-cognitive parameters and personality traits.

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Medulloblastoma, tumor of the cerebellum: novel *in vivo* models and preclinical therapies

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KEYWORDS:

Cerebellar Precursor Cells, Cerebellum Development, Medulloblastoma, Gene Therapy.

We are studying since many years the process of development of the cerebellum and of its tumor, medulloblastoma (MB), which arises from a developmental misregulation and pathology of the cerebellar granule cell precursors (GCPs). GCPs proliferate in the cerebellar external granular layer (EGL) and then migrate inwardly, forming the internal cerebellar layers. We study a MB subtype carrying activation of the Sonic Hedgehog pathway (Shh-type), which makes GCPs prone to transformation and to tumorigenesis. In fact, Patched1 heterozygous mice (with activated Shh pathway) are spontaneously developing low frequency MB. This tumor hits mostly children and is very difficult to cure, as standard treatment with radiotherapy and chemotherapy after surgical resection frequently leaves permanent cognitive damage in young patients. Thus, new, less aggressive therapies are needed.

We have previously identified the antiproliferative gene Tis21 as a MB-suppressor gene, by demonstrating that a Patched1+/- mouse model with conditionally activated expression of Tis21 in GCPs, generated by us, shows a significant reduction of MB frequency (1). The underlying mechanism relies on the ability of Tis21 to selectively inhibit cyclin D1 expression. Given that a down-regulation of Tis21 is observed in human MBs, we also generated a new Patched1 heterozygous mouse model with deletion of Tis21 (Patched1+/-Tis21KO), and observed that it develops MB with high frequency (about 85%) (2). By genomic analysis we identified as responsible for the increased MB penetrance a defect of migration of the GCPs out of the proliferating region of the EGL, consequent to reduced expression of the chemokine Cxcl3. We observed that Cxcl3 is transcriptionally activated by Tis21 and it drives the GCPs to migrate out of the EGL, thus making the GCPs less prone to the transforming local influence of Shh (2).

In view of these findings, we recently performed two preclinical experiments involving Tis21 and Cxcl3. Concerning Tis21, by means of an adeno-associated virus (AAV) vector, we vehiculated Tis21 to MB tumor cells allotransplanted into the flank of immunodepressed mice, and observed a strong decrease of MB growth and proliferation (3). As for Cxcl3, we found that the chronic intracerebellar administration of Cxcl3, for 1 month, in the high frequency MB model Patched1+/- Tis21KO, prevents totally the growth of medulloblastoma lesions by forcing neoplastic cells to migrate and differentiate (4).

We are currently analyzing the effect of chronic intracerebellar administration of Cxcl3 in older high frequency MB mice (3-month-old), in order to check the ability of Cxcl3 to counteract MB development at more advanced stages.

Since Cxcl3 is not toxic and is well tolerated, we hope that these studies may lead to a novel therapy, which could be used alone or together with other anti-MB drugs.

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KEYWORDS:

Neural Stem Cells Activation, *In Vivo*, Neurogenic Niches, Hippocampus, Memory, Aging, Neurodegenerative Diseases.

Interaction Between Neurogenic Stimuli and the Gene Network Controlling the Activation of Stem Cells of the Adult Neurogenic Niches, in Physiological and Pathological Conditions

Adult neurogenesis is the process by which new neurons are generated throughout life. They develop from stem cells, located in two neurogenic niches, i.e., the dentate gyrus of the hippocampus and the subventricular zone (SVZ).

This process appears to take place also in adult humans and is severely reduced during aging, depression, and during neurodegenerative pathologies; the first consequence of this reduction is a decrease of associative memory. In fact, the newly generated neurons within the dentate gyrus are integrated in memory circuits and play an important role in counteracting processes such as aging or neurodegeneration, whereas the new neurons generated in the subventricular zone are redirected, in case of brain trauma, to the damaged neural area to counteract the ongoing degeneration.

In this scenario, in the last years we have investigated the process of adult neurogenesis, seeking to identify neurogenic stimuli as well as the gene network responsible for the maintenance of the quiescence of stem cells and for the migration and differentiation of the new neurons. Stem cells in fact mature into progenitor cells that in turn develop into new neurons.

Concerning the neurogenesis gene network, we identified and studied four genes involved, all cell cycle inhibitors, namely Tis21/Btg2, Btg1, p16Ink4a, p21Cip1, and also the chemokine Cxcl3. We found that the gene Tis21/Btg2 is responsible for the exit from cell cycle of progenitor cells (neuroblasts) and for their differentiation in new neurons. A misregulation of the timing of activation of Tis21/Btg2 generates new neurons unable to encode new memories (1).

We found also that another gene of the Btg family, Btg1, maintains the quiescence of the stem cells in the dentate gyrus as well as in the subventricular zone, as its deletion triggers their proliferation (2).
Recently we showed that p16Ink4a is specifically responsible for preventing the exit from quiescence of aged hippocampal stem cells, in aged mice undergoing the neurogenic stimulus of running. Thus, p16Ink4a appears to prevent the depletion of the pool of stem cells of the hippocampus in aging subjects, since p16Ink4a expression becomes detectable only during aging (3).

On the other hand, we observed that p21Cip1 is required to regulate neurogenesis in the subventricular zone (4).

Moreover, Tis21/Btg2 directly binds and regulates the promoter of cyclin D1 and of the chemokine Cxcl3, which controls the migration of progenitor cells (5). Thus, the coordinated action of all these cell cycle inhibitory genes contributes to orchestrate the process of adult neurogenesis.

In parallel, we analyzed the possibility to activate stem cells by neurogenic stimuli as a function of the intensity of the stimulus (i.e., whether this is physiological or pathological), and of the deregulation of the system (i.e., whether the model is aged or carrying genetic mutations in the gene network controlling quiescence). While neurogenic stimuli such as running or antidepressants (e.g., fluoxetine) or diet nutrients (e.g. hydroxytyrosol) are normally unable to activate stem cells in the dentate gyrus or SVZ, when the system is aged and/or carrying mutations of quiescence-maintaining genes, such as Btg1 or p16Ink4a or p21Cip1, preservation of the quiescent state of stem cells is more critical and stem cells can be activated by neurogenic stimuli ineffective in normal conditions (3,4,6,7). This indicates that stem cells retain a high proliferative capability and plasticity, and suggests that stem cells are protected against the response to stimulus and are resilient to exhaustion (see for review: 8).

We are currenly studying different functional degrees of deregulation of the stem cell quiescence-maintaining system. These studies are relevant to find strategies counteracting neural aging and neurodegenerative diseases.

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Targeting metabolic disturbances of central cholinergic circuits to recover cognition in AD and T2D

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KEYWORDS:

NGF, BFCN, Brain Insulin Resistance, Oxysterols, Alzheimer's Disease, T2D.

The signalling pathway activated by NGF, a key neurotrophin for the metabolism of basal forebrain cholinergic neurons (BFCN), is one of the first homeostatic systems affected in prodromal Alzheimer's Disease (AD). BFCN projections to cortical and hippocampal target circuits are key to LTP, learning and memory and higher cognition in the mammalian brain. In line with this, we recently demonstrated the molecular mechanisms underlying NGF control of Amyloid Precursor Protein (APP) phosphorylation at key C-terminal sites, shuttling APP to the Golgi system and leading to preferential anti-amylodogenic processing in physiological conditions (Triaca et al., 2016; Triaca et al., 2018).

Of note, BFCN demise and NGF signalling dysfunctions have been thought for decades to occur in AD late stages, as a mere consequence of amyloid-driven disruption of the retrograde axonal transport of neurotrophins to BFCN. Nowadays, a wealth of knowledge from neuroimaging studies in humans affected by Mild cognitive impairment (MCI) and AD is potentially opening a new scenario: neurotrophic pathways impairment in BFCN occurs, possibly because of their higher metabolic requirements, at the onset of AD and correlates better than amyloid load with cognitive decline. Central metabolic dysfunction is considered a well-established feature of AD, and brain glucose hypometabolism is associated with AD dementia (Whitmer et al. 2008; Craft 2009) even before early AD manifestation, and proposed as good predictor of MCI progression toward AD, although the underlying mechanisms are still under debate.

To investigate these mechanisms, we developed an in vitro model of insulin resistance in rat cholinergic neurons. In particular, employing hyperinsulinemic culture conditions, BFCNs were shown to develop insulin resistance that was revealed by reduced activation of both Insulin receptor (IR) and insulin substrate receptor 1 (IRS1). Further, insulin-resistant neurons expressed a higher level of serine phosphorylated IRS1, a well-known hallmark of insulin resistance, and showed reduced glucose transporter 2 (Glut2) translocation to plasma membrane resulting in lower glucose uptake. Also, neuronal activity was repressed as seen by a decrease in nuclear c-Fos expression. Significantly NGF was shown to improve insulin resistance by TrkA-driven tyrosine phosphorylation of IRS1 and its consequent activation, both in the in vitro model and 3xTg-AD mice. Indeed, nasal administration of NGF in 3xTg AD mice rescued insulin resistance in the medial septum in the pre-symptomatic phase several months before it is detectable in the neocortex and hippocampus (Sposato et al. 2018).

Further, the involvement of cholesterol derivatives is under scrutiny as key driver of neuronal insulin resistance in AD and T2D, focusing on the 27-hydroxycholesterol (27-OH), a peripherally generated cholesterol metabolite able to enter the brain modulating local cholesterol biosynthesis and detoxification. Increased 27-OH level in brain, blood and CSF is a characteristic AD trait and our preliminary data indicate a role for 27-OH in perturbating NGF and insulin signalling in BFCN in vitro and *in vivo* (Fico et al., 2019).

Taken together, these findings are of potential clinical relevance for both AD and Type 2 diabetes (T2D) cognitive deficits. Thus, neurotrophins and NGF have been long time ago suggested for neuroprotection in cholinergic diseases, although with poor clinical outcomes.

To overcome the well-known limitation of Blood-Brain Barrier transport to the brain of a macromolecules like NGF, brain targeting via the nasal route is currently under investigation. The use of small peptides like human NGF 1-14 (hNGF) to reach relevant brain target areas in animal models of age and/or diet- related neurodegeneration has recently been addressed in vitro in NGF responsive neurons (BFCN and DRG; Triaca et al., 2020). We investigated the NGF-like properties of the human NGF 1-14 sequence (hNGF-1-14), by resorting to primary dorsal root ganglia and cholinergic neurons. We found that hNGF-1-14 peptides retain biological activity of the whole NGF molecule fully sustaining survival and neurites elongation of primary dorsal root ganglia (DRG) neurons. Further, hNGF-1-14 peptides activate the early and late NGF-TrkA pathway signalling intermediates, resulting in CREB nuclear translocation, Immediate Early Gene c-Fos trascription, ChAT level increase and finally miniEPSP potentiation.

Thus, the findings here reported pinpoint the hNGF (1-14) peptide, and in particular its acetylated monomeric form, as novel promising therapeutic tools to achieve TrkA-specific efficient activation of the NGF pathway in NGF-target neurons in vitro. The feasibility and efficacy of nasal administration of the NGF mimetic hNGF *in vivo* will open the way to novel and non-invasive therapeutic approaches with the final goal to improve brain resilience to cognitive ageing and AD pathology.

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Translational Research

Knowledge and discoveries gained from fundamental research are translated into applicable results that can improve human health at large following the "bench-to-bedside" framework. Translational research activity deals with implementation of novel diagnostic tools, therapeutics, as well as to the development of trials and clinical procedures in preclinical studies.

Perspectives For Future Innovation in Tendon repair (P4 FIT)-H2020-MSCA-ITN-EJD-955685

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KEYWORDS:

Tendon Regenerative Medicine; Nanomedicines; Stem Cells; Bioactive Molecules, Immunomodulation; Artificial Intelligence; Biosensors; Bioimaging, Preclinical Studies.

P4 FIT was selected within the highly competitive H2020-MSCA-ITN-EJD actions (mean rating score 3-5%) which aim at financing research networks involved in creating international Doctoral Programmes. Innovation in tendon medicine is a promising frontier to respond to the urgent societal/economic healthcare demand determined by the worldwide growing incidence of tendinopathy. Perspectives For Future Innovation in Tendon repair (P4 FIT) fosters to build a new generation of 15 early stage researchers with adequate skills to explore non-conventional therapeutic and diagnostic solutions by exploiting the technological advances in nanomedicine. The University of Helsinki brings together world-renowned academic and non-academic EU institutions, covering most of the basic and technological disciplines of the fields to launch a unique EJD. The inter-disciplinary, inter-sectoral, and international high quality educational environment will booster innovation-driving training and research leadership grounded in excellence for widening success in P4 medicine (predictive, preventive, personalized and participatory), promoting tendinopathy resolution. P4 FIT will encourage cross-disciplinary working under the coordination of human and veterinary orthopedics addressing innovation and R&D facilities to combine multidrug nanovectors/nanotheranostic devices with tissue engineering. The translation of innovative nanodevices carried out on integrated pre-clinical and vet/human clinical settings will produce solid evidence-based datasets able to reduce fragmentation still limiting the impact of biomedical discoveries and to offer a unique opportunity for identifying new predictive biomarkers through the use of AI and deep learning data analysis. Working across disciplines and sectors, P4 FIT will foster ESRs to be creative, critical, autonomous intellectual risk takers at the frontiers of research with the R&I mind-set necessary for thriving careers. P4 FIT will allow to fill the EU gap in tendon healthcare, building up a generation of researchers able to develop nano-based biomedical devices by integrating biology advances to technology innovation, and to computational resolution. These aims will also be possible thanks to the collaboration with the CNR-EMMA-Infrafrontier - International Network and Mouse Clinic (EU - ESFRI), which with the preclinical studies on mice, will increase solidity and accelerate the technological transfer of the P4 FIT proposed novel devices/diagnostic path.

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Nanogel-delivered neuroprotective drugs to hamper degeneration of dopaminergic neurons in a mouse model of Alzheimer's disease

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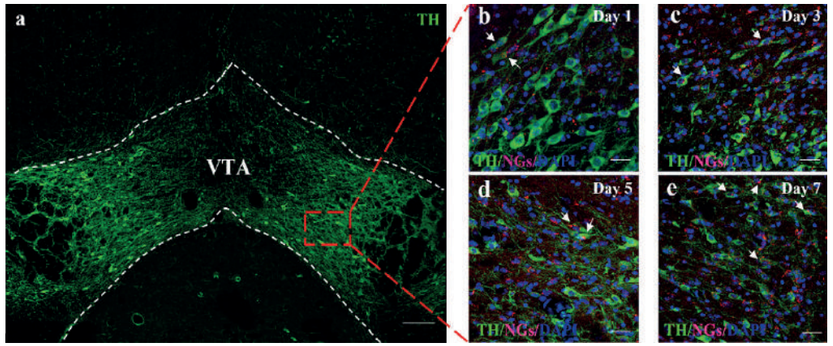
KEYWORDS:

Alzheimer's Disease, Dopamine, Ventral Tegmental Area, Neuroinflammation, Nanogels, Polyphenols.

An early, progressive and selective dopaminergic (DA) neurodegeneration in the Ventral Tegmental Area (VTA) of the Tg2576 (Tg) mouse model of familiar Alzheimer's disease (AD), from 3 months of age, has been recently reported. Such neurodegenerative process, which occurs far before the deposition of amyloid-β (Aβ) plaques, may be correlated to neuroinflammation and could be one of the first events occurring in AD [1]. However, the causes of this increased vulnerability and resolutive treatments are still missing; thereby, targeting DA neurons together with activated astrocytes and microglia using nanogel (NG)-delivered Hydroxytyrosol (HT) in a more controlled fashion [2], could rescue the early neuroinflammatory condition as well as functional, structural and metabolic DA neuron degeneration, improving the observed AD neural and cognitive impairments.



Confocal immunofluorescence analysis of NG localization (at 1.2mg/mL concentration) within the midbrain: thyrosine hydroxylase (TH)-positive DA neurons in the VTA stained in green (a), 10x magnification; images in (b-e) show the progressive NG uptake from day 1 to day 7 in different neural cell populations of the VTA as follows: TH+ DA neurons in green with NGs-Cy5 in purple (white arrows, 20x magnification), nuclei are stained in blue with DAPI; Scale bar = 100μm in (a), 25μm in (b-e).

Objective of this work is to help natural polyphenolic anti-inflammatory and antioxidant compounds at crossing the blood-brain barrier of Tg mice and targeting VTA DA neurons, harnessing biocompatible NGs as effective drug carriers, with the final goal of slowing down or even hampering AD. To this aim, we will perform NG synthesis, characterization and assessment of *in vitro* and *in vivo* biocompatibility before HT-loaded NG injection and *ex vivo* analysis of pharmacological effect in Tg brain sections by confocal fluorescence microscopy, IHC, molecular biology and biochemical assays as well as electrophysiological and behavioral studies.

Through this alternative nanotechnological approach, we set out to discover novel potential therapeutic treatments against AD neurodegeneration.

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Nutrition, metabolic disorders, aging, gender-differences, maternal and post-natal environment in incidence of neuropathy and pain: revealing new and selective pharmacological targets for a personalized medicine

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The personalized medicine is the last frontier in the patient care. Depending on the patient age and sex, it offers a "tailor-made" approach that can revolutionize the therapies in the next ten years.

In this context, the physiological differences, related to different categories of subjects, intertwine with those of age and life's style in determining the choice of effective and appropriate therapy. The same approach can be applied to the comorbidities associated to different pathologies and disorders that may occur in different way depending on the subject considered.

KEYWORDS:

Autophagy, Myelin, Glia, Disease-Related Biomarkers, Immune Cells.

Several clinical and experimental studies remark a higher incidence of neuropathies and chronic pain development in females and aged people. Adverse environment during pre- and post-natal period is also a factor of vulnerability for these diseases, both in terms of comorbidity of neurological, metabolic and traumatic disorders and of spontaneous onset.

Researchers only recently began to study the factors that predispose female gender, elderly and patients with metabolic disorders (such as obesity and diabetes) to develop neuropathy. Furthermore, innovative studies are trying to figure out the influence of early social interactions (risk and protective factors for pre- and postnatal phases) on inflammatory and chronic pain.

Our studies take advantage of murine models and multidisciplinary approach: behavioural, pharmacological, histological, molecular and biochemical results are integrated to obtain a preclinical research with higher translational power. Moreover, thanks to consolidated collaborations, -omics experiments support our project facilitating the search for biomarkers and new molecular targets for a personalized medicine.

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A monoclonal antibody targeting connexin hemichannels as novel therapeutic approach for the treatment of Clouston syndrome

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Mutations in connexin (Cx) genes are responsible for several human diseases. Some of these pathological mutations generate abnormally active hemichannels (HCs), referred to as "leaky" HCs [1]. When the Cx mutation is expressed in epidermal keratinocytes, it can cause a variety of rare and incurable genodermatoses that range in severity from increased skin thickness, to life-threatening and fatal barrier break down [2]. Therefore, there is an urgent need to develop therapeutic approaches targeting Cxs. The availability of specific mouse models for Cx-related dermatosis combined with the epidermal Cx accessibility offers a significant opportunity to validate new potential therapies.

Our previous results demonstrated that abEC1.1, a human-derived scFv-Fc antibody selected from a combinatorial phage display library [3], can inhibit activity of HCs formed by Cx26, Cx30 and Cx32 expressed in HeLa DH or HaCaT cell lines, as well as in cochlear organotypic cultures [3,4]. Hence, we decided to test abEC1.1 therapeutic potential as treatment for Clouston syndrome, a rare epidermal disease caused by hyperactive HCs formed by Cx30 carrying the point mutation A88V ($Cx30^{A88V}$) [1].

We first investigated antibody operating mechanism in vitro, studing its binding to mutant $Cx30^{A88V}$ HCs expressed in HaCaT cells or mouse primary keratinocyte by patch clamp recordings, Ca^{2+} imaging and ATP release assay. Incubation with sub-micromolar concentration of abEC1.1 significantly reduced both Ca^{2+} influx and ATP release through leaky HCs. Then, we tested the *in vivo* efficacy of abEC1.1 on the only available mouse model for Clouston syndrome [5]. We treated two experimental groups of $Cx30^{A88V/A88V}$ mice with abEC1.1 either topically or systemically for two weeks. In both cases, our results showed that treatment remarkably reduces hyperproliferation of mutant epidermal keratinocytes and restores normal size of hypertrophic sebaceous glands (SGs) without significant side effects.

In summary, we showed the therapeutic potential of a monoclonal anti-Cx antibody in the treatment of the symptoms of Clouston syndrome in a mouse model. Our findings pave the way for a new therapeutic strategy for rare Cx-related dermatoses based on restoration of abnormal keratinocyte physiology using antibodies that bind the extracellular domain of mutant Cx HCs [6]. These findings support the further development of antibodies as drugs to address unmet medical needs for Cx-related diseases.

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Translational medicine in HEAD/NECK CANCER: Laryngeal carcinoma

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Laryngeal carcinoma (largely (95%) squamous cell carcinoma) is the second most common head and neck cancer. Comprehensive treatment measures such as surgery, radiotherapy, chemotherapy, and gene therapy have gained a higher 5-year survival rate for patients with laryngeal cancer. However, 30-40% of these patients still dies for tumor recurrence or local (mainly) and distant metastasis. The identification of the transduction pathways involved in this tumor progression and of novel independent prognostic markers is of paramount importance for the advancement of research, accuracy of preoperative diagnosis and improvement of survival rate in patients with laryngeal cancer.

To this aim, by the use of oropharyngeal squamous cell lines, human primary tumor bioptic tissues and blood samples from the same patients, we are investigating the following aspects:

1. Molecular and cellular mechanism involved in carcinogenesis (p75NTR pathway)

The low-affinity p75 neurotrophin receptor (p75NTR) has been recently found to be overexpressed in primary tumour cells from several squamous cancers, including Laryngeal Squamous Cell Carcinoma. Accordingly, p75NTR has been proposed as a reliable index of tumorigenicity, invasiveness and chemotherapy resistance [see Triaca et al.(2019) for review]. Considering the pivot role suggested for p75NTR in the regulation of cell survival and functions, in both healthy and pathological conditions, the study of p75 pathway to the acquisition and maintenance of stem cell properties in LSCC might contribute to better profiling of LSCC patients, and to improve their outcome.

2. Identification of potential laryngeal carcinoma biomarkers (VGF-derived peptides and other neuropeptides)

Chromogranin A is commonly used as a marker of neuroendocrine tumors, including laryngeal tumors. A member of the Chromogranins family, the Secretogranin VGF (Bartolomucci et al., 2011), has been shown to trigger epithelial-to-mesenchymal transition in non-neuroendocrine tumors (lung adenocarcinoma), suggesting its potential utility as a marker for acquirement of tumor invasiveness (Rindi et al., 2007). The aim of this study is to potentially identify the VGF precursor and its derived peptides as new markers of the active/proliferating status of the tumor, thus detecting neoplastic lesions with higher risk of tumor invasiveness also in non-neuroendocrine tumors.

3. RNA metabolism and related proteins in HN cancer.

Alterations of RNA homeostasis can lead to severe pathological conditions. Our ongoing findings highlight a potential role of the RNA-binding protein SMN in tumorigenesis of HN cancer. By in vitro studies, using cell lines derived from human larynx carcinoma, we observed that SMN could be implicated in cell adhesion and motility. Taking into account the key role of SMN in local translation control (Gabanella et al., 2016, 2020), we suppose that SMN could promote the local expression of invasiveness-related mRNAs affecting cell adherence and polarity. Although preliminary, these results point to SMN as a novel therapeutic target for larynx carcinomas.

OTHER:

Studies founded by Projects Sapienza:

1. Characterization of laryngeal squamous cell carcinoma: molecular profiling of p75 neurotrophic receptor in cancer stem cells and circulating tumour cells to predict tumour aggressiveness and treatment response to Prof. Antonio Greco (PI) prot. RG11816436834B1;
2. Ion mobility mass spectrometry: a new high-potential device for new life science ways. to Prof.R. Currini prot. GA11816492EF0E14;
3. Characterization of the secretogranin VGF (SgVII) nerve growth factor inducible gene in laryngeal carcinoma to identify a new potential tumor marker and independent prognostic factor to Dr. M. Ralli, prot. RM11916B7E5A0D24.

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The Transgenic Animals in-house facility, active since 1999, focuses on the generation of genetically modified mice (transgenic, knockout, knockin), applying all the relevant standard transgenic technologies including the latest methods to introduce genetic modifications *in vivo*. Zinc Finger nucleases (ZFNs), Transcription activator-like effector nucleases (TALENs), Clustered regulatory interspaced short palindromic repeat (CRISPR/Cas-based RNA-guided DNA endonucleases) are routinely used. The service, offered as collaborative and/ or contributive activity, gives support in the strategic design and in providing existing animal models or generating new ones. Theoretical/ practical courses on genetically modified animals are tackled during the dedicated annual course 'Science of Laboratory Animals' (Recognition FELASA, Federation of European Laboratory Animal Science Associations) Cat B (No:023/09).

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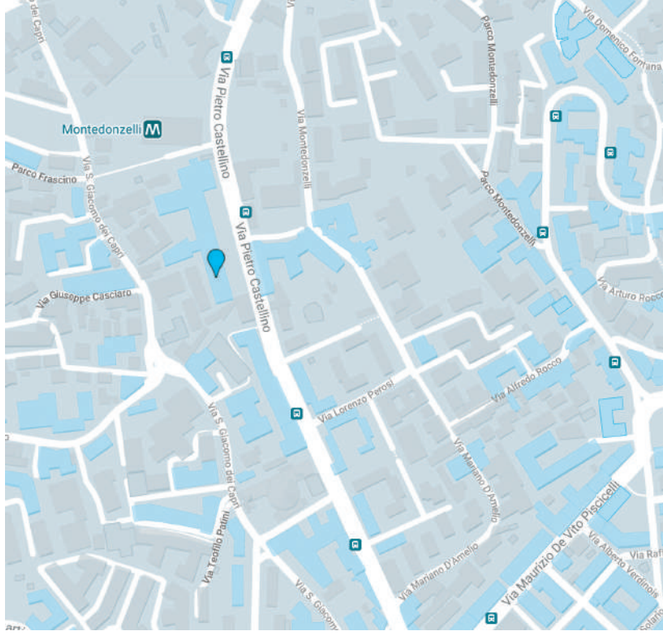

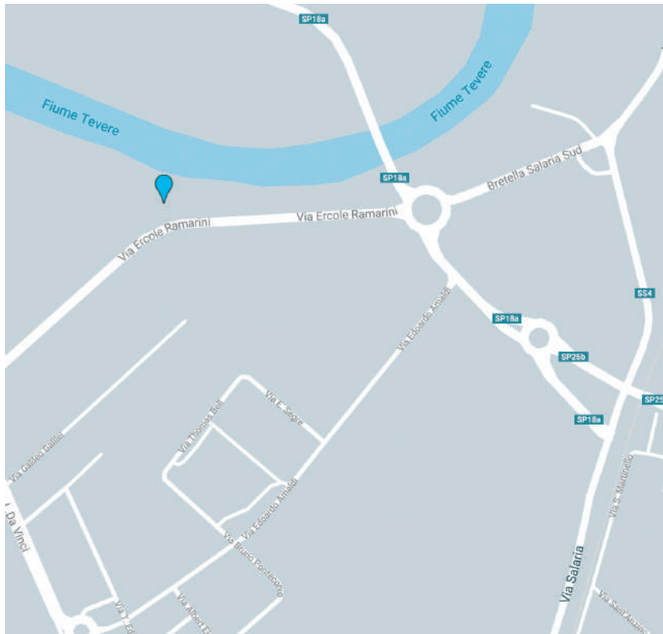

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