Appendix: Abstract of research project

RESEARCH AREA 4: Innovative diagnostic and therapeutic strategies

4.1 Adeno associated viral delivery system targeting nervous system (Onori A., Passananti C., Pisani C., Corbi N.-IBPM)

Recombinant adeno-associated virus (rAAV) vectors are versatile tools for gene transfer to the nervous system and in particular for central nervous system (CNS). Recombinant AAVs exhibit important advantages, including: safety profile due to the non-pathogenic nature of their wild-type form, stable transgene expression in post-mitotic cells, low risk of insertion mutagenesis, minimal immune responses and capsid-dependent tissue-specific tropism. Over the last years, twelve natural serotypes and more than one hundred variants of AAVs have been detected and isolated from humans and other primates. In particular, specific capsid serotypes, including serotype "9" bypass the blood-brain barrier, raising the possibility of intravascular administration as a non-invasive delivery route to achieve widespread CNS gene expression. To further lower any possible host immune response the use of peculiar AAV serotypes can be combined with tissue specific promoter/enhancer regions. We have successfully designed, produced and tested several rAAV vectors containing different human tissue specific promoters/enhancers. We intend to develop additional rAAV vectors carrying human promoter/enhancer regions exhibiting high nervous system tissue specificity. The AAV vector administration has been tested in several clinical trials for motor neuron disorders, lysosomal storage disorders, neurotransmitter disorders, glycogen storage disorders, neurodegenerative disorders and eye disorders.
Collaborations:
Di Certo M.G., Gabanella F. (Institute of Cellular Biology and Neurobiology (IBCN) CNR Rome)

4.2 DMD: Innovative therapeutic strategy for Duchenne Muscular Dystrophy by AAV-mediated delivery of Zinc Finger Artificial Transcription Factors (ZF-ATFs). (Onori A., Passananti C., Pisani C., Corbi N.-IBPM)

Up-regulation of the dystrophin-related gene "utrophin" is a promising therapeutic strategy for the treatment of Duchenne Muscular Dystrophy (DMD). In order to re-program the utrophin expression level in muscle, we engineered the artificial ZF-ATF named "JZif1" that targets and up-regulates utrophin promoter. JZif1, delivered by AAV viral vectors, induces remarkable amelioration of the pathological phenotype in dystrophic mice (mdx). The molecular mechanisms underlying ZF-ATF induced muscle functional rescue is partially explained by the ZF-ATFs positive impact on the neuromuscular Junction (NMJ). Our results candidate our ZF-ATFs as novel therapeutic molecules for DMD treatment.
Mattei E., Strimpakos G. Institute of Cellular Biology and Neurobiology (IBCN) CNR, Rome.
Fanciulli M., SAFU, Translational Research Area, Regina Elena National Cancer Institute, Rome.

4.3 Exosomes in the neurodegenerative diseases (Alzheimer’s and Parkinson’s diseases) (Fiorucci G.IBPM)

Extracellular vesicles (EVs) comprise a wide variety of membrane-limited vesicles released from cells. Exosomes are the best characterized EVs. Neuronal cells release exosomes and for this reason these EVs have been proposed as novel means for intercellular communication which takes part to the transmission of pathogenic proteins in the neurological diseases, such as Alzheimer’s and Parkinson’s diseases.

In both diseases exosomes can propagate the misfolded proteins, but there is also evidence that EVs can have a protective role in neurodegeneration. The possible effects of exosome-associated pathogenic proteins on the recipient cells are important: how these proteins are internalized and how they induce protein aggregation and neurodegeneration in the recipient cells.
Moreover, exosomes can deliver microRNAs. Exosome-mediated microRNA signature could be utilized in the diagnosis of these diseases.
On the basis of these evidence, our aim is to deeply investigate the role of exosomes in the pathogenesis and in the progression of neurodegenerative diseases for a concrete use of exosomes for diagnosis and as novel therapeutic approach in these diseases.
Collaborations:
Chiantore M.V. (ISS)
Romeo G., Dr. Mangino G., Dr. Iuliano M. (Università Sapienza Roma)

4.4 Ferritin nanoparticles for innovative therapies against brain tumors (Falvo E., Colotti G., Ilari A., Morea V., Ceci P. IBPM)

We will develop nanoparticles based on the human protein ferritin to selectively deliver therapeutic and/or diagnostic agents to brain tumors. The human protein ferritin is endowed with a number of favourable properties that make it an ideal nanocarrier for biomedical applications: it is present both in plasma and within cells in physiological conditions, scarcely immunogenic, non-toxic and long circulating *in vivo*; it can incorporate small compounds, shielding them from the external environment; it is imported in all cell types by a specific receptor, which is overexpressed by many types of cancer cells; it can be produced in high yields with low costs in bacterial cells. As far as delivery to brain regions is concerned, ferritin-based nanoparticles have been recently shown to be able to cross the endothelium, epithelium, and blood-brain barrier layers. Additionally, in brain tumors, such as glioblastoma, the integrity of the blood-brain barrier is compromised, allowing an increased passage of proteins between peripheral vasculature and central nervous system.

4.6 GBM: Characterization of Axitinib treatment on glioblastoma angiogenesis (Falchetti ML, -IBCN)

Axitinib, a tyrosine kinase inhibitor, works as a specific inhibitor of Vascular Endothelial Growth Factor Receptors 1, 2 and 3. It is FDA-approved for the treatment of metastatic renal cell carcinoma. In vitro, chronic treatment with Axitinib induces senescence in different glioblastoma cell lines. It is known that, following radiotherapy, endothelial cells of GBM patients may undergo cellular senescence releasing a variety of pro-inflammatory chemokines and citokines eventually resulting in a huge modification of their SASP (Senescence Associated secretory Phenotype), able to strongly affect tumor microenvironment. Starting from these premises, we want to perform a characterization of the consequences of Axitinib treatment on endothelial cells. We are currently performing a gene expression profiling by RNAseq of HUVEC cells treated with Axitinib.

4.7 Isolated peptides from mt-leucyl-tRNA synthetase as novel therapeutic instruments against neurodegenerative mitochondriopathies (Morea V., Colotti G., Ceci P.-IBPM)

Mitochondrial encephalopathy, lactic acidosis, and stroke (MELAS) syndrome and myoclonic epilepsy with ragged red fibers (MERRF) syndrome are multisystem and progressive neurodegenerative disorders due to mutations in mitochondrial DNA genes (mt-tRNALeu^{UUR} and mt-tRNALys, respectively). MELAS neurodegeneration involves the cerebellar purkinje layer and cortical neurons, while MERRF neurodegeneration involves the cerebellar purkinje layer and dentate nucleus.We have identified several short peptides derived from human mitochondrial

LeuRS (leucyl tRNA synthetase) that represent attractive new candidates for future therapeutic applications against MELAS and MERRF syndromes: overexpression of LeuRS-derived peptides is able to rescue the defective phenotype of cells bearing mt-tRNA mutations that cause MELAS and MERRF. Additionally, LeuRS-derived peptides directly interact with the mutated tRNAs and stabilize their functional conformations. We will implement strategies to deliver LeuRS-derived peptides to the mitochondria and will identify small non-peptide molecules able to both mimic the biological activity of the peptides and spontaneously diffuse to the mitochondrial matrix.
Collaborations:
D'Amati G., Prof. Giordano C. (Sapienza University)
Cantatore P. (Bari University)
Bresciani A. (IRBM)

4.8 LTL as a biomarker in MCI and AD diseases (Scarabino D.-IBPM)

The main interest is to investigate susceptibility genes for age-related complex neurodegenerative diseases, in particular Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI). Alzheimer's disease (AD) is a neurodegenerative disease associated with a drastic decline in life expectancy. Mild Cognitive Impairment (MCI), a clinical entity considered prodromal of AD, is characterized by memory alterations in subjects with normal global cognitive function. Our recent data have shown that some polymorphisms of the telomerase genes TERC and TERT are involved in susceptibility to AD confirming the relationship between the onset of AD / telomerase / telomere length. Numerous studies have addressed the analysis of LTL in neurodegenerative diseases, the overall data suggest that LTL could be a marker for cellular pathology in neurodegenerative diseases. Moreover, in a recent investigation, we have shown that LTL measurement may be a useful marker to follow the dementia progression . The possibility of detecting and following the disease before the onset of the most serious symptoms, could provide a window of opportunity for actions aimed at preventing or delaying the disease.
Collaborations:
Corbo R.M. (Department of Biology and Biotechnology, Sapienza University)
Gambina G., Brogio E. (Alzheimer's Disease Center, Department of Neuroscience, University and Hospital of Verona)
Businaro R. (Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University)
Maida C., Gaudio M.R. (S. Giovanni –Addolorata Hospital, Rome)
Mantuano E.,Veneziano L. (CNR Institute of Translational Pharmacology (IFT))

4.9 Pathological memory: from disease' mechanisms to therapeutic strategies (De Leonibus E-IBCN).

We study the neurobiology of learning and memory in normal and pathological conditions, which include aging, ageing associated neurodegenerative disorders (Alzheimer' disease, Parkinson' disease and synucleinopathies), paediatric neurodegenerative disorders (lysosomal storage disorders), and neurodevelopmental disorders (autism, ADHD, and schizophrenia). The goal of our projects is to identify early cognitive deficits preceding the onset of neuronal loss in neurodegenerative disorders; working at this early stage we can identify the synaptic and molecular disease' mechanisms leading to neuronal loss. This information is used to identify novel restorative or symptomatic therapeutic approaches (pharmacological, gene-therapy or the combination of the two) to correct the cognitive deficits and/or preventing the conversion to dementia-like symptoms.
Techniques routinely used in the lab: 1. *In vivo*: Behavioural testing probing different types of memory in rodents (mice and rats), cannula brain permanent implantation, optogenetics and we are setting up electroencefalografic recording in mice. 2. *Ex-vivo*: Western blot, brain tissue fractionations, immunohistochemistry, immunofluorescence, RNAseq and Mass Spectrometry in

collaboration with the Telethon Institute of Genetics and Medicine (TIGEM) bioinformatic core; 3. *In vitro*: primary neuronal cultures and directed reprogrammed neurons from mouse or human fibroblasts, adeno-associated viral vector production in collaboration with TIGEM-aav core.
Collaborations:
Mele A. (University of Roma,Sapienza), Calabresi P., (University of Perugia) Picconi B. (University San Raffaele la Pisana), Ballabio A., Alessandro Fraldi A. (TIGEM), Jezek K.(University of Pilsen, Chzec Republic),Di Angelantoio S. Ruocco G. (IIT, Sapienza), Gardoni F. (University of Milano), Sulzer D. (Columbia University, USA).

4.10 QMT: Modulation of neurotrophins' and cytokines' levels in human subjects (Caserta M., Verdone L.-IBPM)

This field of research is related to the analysis of the effects of Quadrato Motor Training, a specific motor-cognitive training, on the levels of relevant molecular markers in humans: neurotrophins, such as Brain-derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF), both known to be involved in the development and function of a wide variety of neuron populations, and cytokines as markers of neuroinflammation. Experiments enrolling different groups of healthy volunteers have shown that the training induces changes of proBDNF and proNGF salivary levels. In a subset of participants, proBDNF increase was shown to correlate with changes in morphological parameters at the level of defined brain areas, as observed by fMRI performed in collaboration with the Department of Physiology and Pharmacology at Sapienza University. The recent implications of neurotrophins and their receptors in the pathogenesis and therapy of neurodegenerative diseases suggest this training could have an impact on their prevention and treatment.
Collaborations:
Ben-Soussan T.D. (Research Institute for Neuroscience, Education and Didactics, Patrizio Paoletti Foundation)
Venditti S. (Department of Biology and Biotechnology, Sapienza University Rome)
Raffone A. (Department of Psychology, Sapienza University of Rome)

4.11 Epigenetic drugs as therapeutic approach in DMD (Mozzetta C. -IBPM)
Duchenne Muscular Dystrophy (DMD) is the most severe form of dystrophy that leads to progressive muscle weakness because of a gradual replacement of functional muscle with fat and fibrotic scars. Pharmacological therapies for DMD should therefore aim to counteract this fibro-adipogenic degeneration and to promote the compensatory regeneration to slow down progression of pathology. Previous works proved pre-clinical efficacy of pan-histone deacetylase inhibitors (HDACi) in the treatment of murine models of DMD, showing the ability of HDACi to counter disease progression and induce functional and morphological recovery. These studies paved the way for an epigenetic therapy as a potential therapeutic approach in DMD prompting our interest in exploring pre-clinical efficacy of more selective compounds, such as specific inhibitors for class I HDACs (I-HDACi). Moreover, other epigenetic modifiers, such as Histone Lysine Methyltransferases (KMTs) are emerging as particularly relevant in myogenesis, given their high specificity for precise histone residues, the development of KMTs specific inhibitors might be a strategy to increase selectivity of epigenetic pharmacology in the context of DMD.
Collaborations:
IRBM (Pomezia, Italy)
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Prof. Maria Egle De Stefano (Department of Biology and Biotechnology "C. Darwin", Sapienza University Rome)

4.12 AD: mitochondrial dysfunction and transplantation therapy (Di Carlo M., Picone P., Galizzi G., Nuzzo D. IRIB)

Studies on mitochondrial dysfunction as early event in neurodegenerative diseases including Alzheimer's disease. In particular, mitochondrial dynamics, biogenesis, homeostasis and degradation (mitophagy) are analyzed by using specific biomarkers and imaging techniques. Use of antioxidant molecules and insulin as therapeutic approach to prevent or reduce mitochondrial dysfunction is contemplated. It is also studied the role of mitochondria-associated ER membranes (MAMs) in the control of calcium perturbation, signal transduction, mitochondrial and ER stress, mitochondrial dysfunction, and Amyloid Precursor Protein (APP) cleavage. A new approach, called mitochondrial transplantation, based on the possibility of replacing damaged mitochondria with healthy exogenous mitochondria, is currently being studied. Development of techniques to isolate and characterize neuro-microvesicles to be used as mitochondria delivery carrier are considered.

Collaborations:
Dr. Donatella Bulone and Pierluigi San Biagio Biophysic Institute (IBF), CNR, Palermo; Prof. Valeria Vetri Chemistry Physics Department (DCF) Palermo University

4.13 CRISPR/Cas9-mediated gene editing in in vitro and in vivo models of Myotonic Dystrophy type 1: assessment of efficiency, safety and therapeutic effect of CTG-repeat deletion.
(Falcone F., Cardinali C., Provenzano C., Mandillo S., Golini E., Strimpakos G., IBBC)

Myotonic dystrophy type 1 (DM1) is a dominantly inherited, multisystemic disorder caused by expanded CTG repeats in the 3'UTR of the *DMPK* gene. *DMPK* mutated transcript accumulates into nuclear foci that affect the localization and activities of RNA-binding proteins involved in splicing regulation. No effective therapy is yet available for DM1. Our project focuses on the design, application and molecular characterization of a CRISPR/Cas9-mediated gene editing approach for the permanent elimination of the toxic mutant repeats in both *in vitro* and *in vivo* disease models, in order to reverse the pathologic phenotype. A deep understanding of the efficiency and specificity of the CRISPR/Cas9 gene editing strategy is essential to evaluate its potential application as gene therapy in DM1patients.
Key words: CRISPR/Cas9, DM1 models, repeat expansion disease, gene therapy, adeno-associated virus (AAV)
Collaborations:
Fabio Martelli, IRCCS Policlinico San Donato, San Donato Milanese, Milan
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4.14 Neurodegenerative and neuromuscular diseases, Innovative diagnostic and therapeutic strategies (Manni L., Soligo M. ITP)

Nerve growth factor has been indicated as possible therapeutic for neurodegenerative diseases. We recently explored its therapeutic efficacy after intranasal delivery in pediatric patients affected by severe brain traumas. However, the predominant form of NGF expressed in the brain is proNGF. The physiology of the different proNGF variants is still unexplored. We aimed our recent work at exploring proNGF variants production, processing, release and action, in animal models of neurodegeneration. We recently demonstrated, both in vitro and in vivo, that different native proNGF variants may challenge different receptor(s), exerting selective biological roles. We also recently received financial support to explore the role of different proNGFs as surrogate biomarkers in pediatric traumatic brain injury.

Collaboration:
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Institute of Pediatrics, Università Cattolica del Sacro Cuore, Rome – Italy
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Division of Rheumatology and Immuno-Rheumatology Research Laboratories, Bambino Gesù Children's Hospital, Rome, Italy
Dept of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden.

4.15 A multi-omics perspective of neurodegeneration: new clues for personal diagnostics and therapies (Cavallaro S., Guarnaccia M., Gentile G., Spampinato A.G. IRIB)

Our research group investigates neurodegeneration through a systems biology approach and multi-omics integration. Different layers of genomic information arising from the analysis of human pathology, together with *in vitro* and *in vivo* models of neurodegenerative disorders, are allowing a systems biology portrait of neurodegeneration. This approach is not only helping to identify key drivers and pathways underlying neurodegeneration, but is laying the foundation for a molecular taxonomy and patient-tailored therapies. This new perspective allows an innovative pharmacology focused on downstream targets, networks and transcriptional modules controlling neuronal fate. The final goal of our research is to develop breakthrough therapies for neurodegenerative diseases through integration of diverse omics data, stringent and reliable drug target selection and validation.

Collaboration:
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Prof. Eleonora Aronica, University of Amsterdam, The Netherlands.
Prof. Velia D'Agata, University of Catania, Italy.
Prof. Francesca Luisa Conforti, University of Calabria, Cosenza, Italy.

4.16 Olfactory Ensheathing Cells, a glial stem cell type, as promising tool for cell therapy (Pellitteri R.- IRIB)

Cell therapy has attracted considerable interest as a promising approach for repair in neurodegenerative diseases. Olfactory Ensheathing Cells (OECs), are glial cells able to secrete trophic factors, to exert neuroprotective effects and to promote plasticity in the lesioned area. OECs have capability to express stem cell marker, such as nestin, showing stem cell characteristics, indicating them as useful potential clinical agents to support nerve injured. In the recent years, several combined treatments have been studied: they include the use of OECs plus phitochemical molecules (curcumin), or neuropeptides, (ghrelin), or use of OECs plated on silicon-carbide substrate, a highly biocompatible material. Therefore, OECs might be considered as clinical tool for lesioned neural areas, representing a future perspective of novel cell therapies that would to be useful for the neuro-degenerated patients.
Collaboration:
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Prof. Vincenzo Silani, Dr. Patrizia Bossolasco, Dr. Lidia Cova - Dep. Neurology and Laboratory of Neuroscience, IRCCS, Istituto Auxologico Italiano, Milan (Italy).

Prof. Maria Teresa Moreno-Flores - Dip. Anatomia, Histologia y Neurociencia Facultad de Medicina, Universidad Autonoma de Madrid, Madrid (Spain)

4.17 Therapeutic effects of novel drugs for spinal muscular atrophy (Crescimanno G., Marrone O., IRIB)

Spinal muscular atrophy (SMA) is characterized by reduction of the survival motor neuron protein (SMN), which is normally found mainly in the spinal cord and plays a critical role in the survival of spinal motor neurons. In this disease, skeletal muscles become progressively hypotrophic and weaker. Eventually, respiratory muscles impairment leads to death. Two drugs for SMA have been recently developed. Nusinersen, which was developed with «anti-sense RNA» biotechnology, seems to be less effective on respiratory than on other skeletal muscles; its administration was often associated with respiratory tract infections and atelectasis. Risdiplam, an investigational SMN2 splicing modifier, is expected to be more effective on respiratory function. We are interested to evaluate long-term effects of these drugs in patients with SMA, paying particular attention to the performance of respiratory muscles.
Collaborations:
NEMO Center Milano and Messina.