Appendix: Abstract of research project

RESEARCH AREA 2: Neurodegenerative diseases and neuro-muscular diseases

2.1) Alzheimer's disease

2.1.1 AD: Impairment of protein homeostasis (Foppoli C.-IBPM)

Disturbance of components of the proteostasis network, that provides a critical protective role against stress conditions, may trigger neuronal death. In Alzheimer's Disease (AD), because of increased oxidative stress, the occurrence of oxidation/dysfunction of specific members of the protein quality control, regulating protein folding, surveillance and degradation has been shown. We aim at identifying the specific pathways involved in the proteostasis network, whose alteration could contribute to the AD development. To this purpose, proteomic techniques will allow to identify specific proteins with altered expression levels or post-translational modifications in AD brain, that could be related to pathways associated with neurodegeneration.
Collaborations:
Perluigi M., Di Domenico F. (Department of Biochemical Sciences, Sapienza University Rome).

2.1.2 AD and MCI: Genetic factors in neurodegenerative 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), which prevent the achievement of advanced age.
Alzheimer's disease (AD) is a neurodegenerative disease associated with a drastic decline in life expectancy. Along with the AD, an additional "clinical phenotype", namely Mild Cognitive Impairment (MCI), has been identified, characterized by memory alterations in subjects with normal global cognitive function. Sporadic AD is a complex disease whose onset depends on the interaction of environmental factors and numerous susceptibility genes. 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. We investigate also the role of APOE gene on AD susceptibility, the subjects were genotyped for apoliprotein E, and plasma ApoE was assayed. The aim of the project was compared the distribution of genetic and biochemical markers observed in subjects with worsening of cognitive status and progression to AD, with subjects with persistence over time of MCI, in order to highlight predicting markers for AD.
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))

2.1.3 AD: miR-101 as a regulator of APP and other genes associated to AD in hippocampal neurons. (Ruberti F. Barbato C-IBCN)

Our group is studying the function of specific microRNAs engaged in the regulation of gene targets and pathways in neuronal cells using lentiviral vectors expressing miRNAs or miRNA sponge inhibitors. Amyloid precursor protein (APP) and its metabolites are associated to Alzheimer's

Disease (AD). MicroRNAs are also involved in AD pathogenesis. We have identified miR-101 as a regulator of APP and other genes associated to AD in hippocampal neurons. By using primary hippocampal neurons and murine models, we are currently studying miRNA-regulated networks implicated in learning and memory impairment, and in neurodegeneration

AD: metabolic disorders, nutraceutical (Di Carlo M.,Nuzzo D.,Picone P.,Galizzi G.(IRIB)

Studies on the effect of diet-induced obesity as risk factor for the onset of neurodegenerative diseases (ND), including Alzheimer's disease (AD). The impact of nutrition and dietary constituent on ND are investigated by using a mouse model (C57BL/6) fed with high fat diet (HFD). The systemic and central metabolic conditions such as insulin resistance, are evaluated. Diet-induced stress and inflammation conditions associated to neurodegeneration are assessed by using specific assays and biomarkers. Expression of proteins involved in AD are also investigated. The effects of the addition of food with high content of bioactive components, including polyphenols, are explored as potential nutraceutical approach for preventing and/or reducing both metabolic and neurogenerative disorders.
Collaborations:
Prof. Flavia Mulè and Prof. Antonella Amato Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Palermo University

2. Amyotrophic lateral sclerosis (ALS)

2.2.1 Investigating ncRNA roles in ALS pathogenesis (Caffarelli E., Laneve P.-IBPM)

The main interest is on the ALS-linked RNA binding proteins TDP-43 and FUS. They widely control RNA metabolism and make ALS an RNA disorder. Transcriptome analysis, carried out on FUS-ALS murine motoneurons, unveiled different classes of RNAs (microRNAs, long noncoding RNAs and circular RNAs) affected by FUS-ALS mutations and allowed the construction of regulatory networks relevant for the pathology.
Collaborations:
Prof. Bozzoni I. (Department of Biology and Biotechnology, Sapienza University Rome)

2.2.2 ALS *Drosophila* models (Cestra G.-IBPM)

Drosophila melanogaster has emerged as a valuable in vivo system in the study of ALS. Flies carrying mutations in genes essential for motor neuron survival show reduced life span and defective locomotive behaviour. We are currently involved in different research projects on ALS in fly:

1) the identification of novel interactors of FUS and analysis of their in vivo role in motor neuron function;
2) to study the in vivo effect of different chemical chaperons, which exhibit reverse aggregation of misfolded proteins, on different Drosophila models of ALS-mediated proteinopathy;
3) to collaborate in the identification and characterization of the mRNAs with altered nuclear-cytoplasm distribution in Drosophila models of C9orf72-mediated ALS.

2.2.3 ALS: Aberrant H2S metabolism (Giuffrè A.-IBPM)

Hydrogen sulfide (H2S), the third gaseous signaling molecule along with nitric oxide (NO) and carbon monoxide (CO), exerts neuroprotective antioxidant, anti-inflammatory and anti-apoptotic

effects at physiological concentrations, while leading to toxic impairment of energy metabolism at higher levels. Aberrant H2S metabolism was interestingly found to be implicated in the pathogenesis of several neurodegenerative diseases. Namely, H2S levels proved to be pathologically low in Alzheimer's and Parkinson disease, and abnormally high in the central nervous system of ALS patients. The project aims to gain molecular insights into H2S metabolism and its regulation by investigating the human enzymes responsible of endogenous H2S production and catabolism, studied both as isolated proteins and in cellular models of ALS. The ultimate goal of this research is to develop innovative therapeutic interventions based on the pharmacological modulation of H2S levels by natural or synthetic modulators of H2S biosynthesis/catabolism.
Collaborations:
Vicente J.B. (ITQB, New University of Lisbon, Portugal)
Forte E. (Department of Biochemical Sciences, Sapienza University Rome)
Saso L. (Department of Physiology and Pharmacology, Sapienza University of Rome)

2.2.4 Role of endothelin 1 in motor neuron degeneration: a possible therapeutic target for amyotrophic lateral sclerosis (D'Antoni S., Catania M.V. IRIB)

Evidence indicates that factors released by activated astrocytes may contribute to motor neurons (MNs) degeneration. We have found that the expression of endothelin-1 (ET-1), a vasopeptide produced in the CNS by astrocytes and microglia, is increased in reactive astrocytes in the spinal cord of SOD1-G93A mice and ALS patients. Importantly, ET-1 is toxic for MNs in an *in vitro* model of mixed spinal cord cultures enriched with reactive astrocytes. The toxic effect of ET-1 on cultured MNs is associated with a reduced activation of the PI3K pathway and requires NO synthesis. Our study suggests that the modulation of ET-1 signaling could be a therapeutic strategy to slow down MN degeneration in ALS.
Our mixed spinal cord cultures can be used to validate other potential targets for ALS.
Collaborations:
Dr. Eleonora Aronica, Dept of (Neuro) Pathology, Academic Medical Center, Amsterdam, The Netherlands; Dr. Patrizia Longone and Dr. Alida Spalloni, Molecular Neurobiology Unit, Experimental Neurology, Fondazione Santa Lucia, Rome

2.2.5 *Mechanisms of neuroinflammation in ALS pathogenesis and the role of microRNAs* (Parisi C., IBBC)

Neuroinflammation is a feature of ALS and RNA dysregulation a mechanisms related to the pathology. We study how microRNA-mediated post-transcriptional regulation affect neuroinflammation in ALS focusing on microglia in the SOD1-G93A mouse. We revealed that termination of A20 function by miR-125b prolongs the activation of NF-κB in ALS. Given the known NF-κB involvement in ALS we are extending our analysis to other pathway components and to human ALS models.
Collaborations:
Dr. Roberto Rizzi ITB, CNR/INGM Milan, IT
Dr. Claudia Bearzi IBBC, CNR/INGM Milan, IT
Dr. Sara Marinelli IBBC, CNR Rome, IT
Dr. Tommaso Mazza, CSS Mendel Rome, IT

2.2.6 Effects of voluntary exercise in the SOD1G93A low copy mouse model of ALS: sex differences, metabolism, inflammation (Mandillo S.,Golini E., IBBC)

ALS is a fatal disease in which the role of environmental factors and their interactions with affecting genes are not well understood. Effective treatments are lacking and the need of new therapies and early diagnostic tools is urgent. In our projects we use the classical SOD1G3A high-copy transgenic mouse model and the less studied SOD1G3A low-copy line. The latter gives the opportunity to examine the very early stages of pathology and allows a longer therapeutic window to test new interventions (Mandillo et al., 2014; Garbugino et al., 2018).

We focus on the behavioral aspects of ALS to elucidate its neurobiological mechanisms in reference to:

Projects
1) Influence of environmental factors (physical activity) on onset, progression and survival
2) Potential pharmacological treatments to alleviate symptoms : Preclinical validation of candidate drug target for ALS
3) Detection of early behavioral pathological signs via automated monitoring systems : Home cage monitoring using the DVC Tecniplast system: validation in ALS mouse models

Collaborations:
Sara Marinelli, IBBC
Prof. Antonio Musarò, Università Sapienza Roma
Sebstiano Cavallaro, IRIB-Catania
Fabio Mammano, IBBC Associate
Marcello Raspa, Ferdinando Scavizzi, IBBC
Fabio Ianniello, Tecniplast, Varese

2.3) Juvenile Hungtinton Disease: Rhes, a key protein in motor and cognitive impairments (Ilari A., Morea V., Colotti G.-IBPM)

Human Rhes (Ras-homolog enriched in striatum) has SUMO-E3 ligase function and among its targets include mutated huntingtin (mHtt) which upon sumoylation becomes more soluble and hence more cytotoxic. mHtt is the well-known cause of Huntington disease (HD), an inherited neurodegenerative disease. The mutation consists in a >35 CAG repeats in exon 1 of the Htt gene. The expansion of the CAG repeats stretch makes mHtt very unstable and able to aggregate with itself and/or other proteins interfering with different metabolic pathways. In addition to its catalytic activity, Rhes exerts a role in autophagy by activating autophagy inhibitor mTOR and by directly interacting with autophagy activator Beclin-1. Rhes bidirectional regulation of autophagy induction, together with the SUMO-E3 ligase activity on mHTT and specific expression in corpus striatum, make Rhes an attractive target for HD treatment. We aim at determining Rhes three-dimensional structure and at identifying its interactors in human cells.

2.4) Hereditary Spastic Paraplegia HSP: Mechanisms underlying spastin regulation (Rinaldo C.-IBPM)

HSP neurodegenerative disease: the most common type is due to heterozygous mutations in spastin gene. Precise levels of spastin appear crucial for its biological functions. A gene-dosage rescue of neurite defects in HSP patients' neurons has been recently reported, stressing the relevance of studying the mechanisms that regulate spastin to develop therapeutic approaches. The aims of this research project is investigate the molecular mechanism underlying spastin regulation mediated by the kinase HIPK2 and evaluate whether manipulating this pathway is possible to restore spastin physiological levels and rescue pathological phenotype in neurons from HSP patients

2.5) Motor Neuron Disorders MNDs: The role of RNA-binding proteins in synaptic plasticity (Onori A., Passananti C., Pisani C., Corbi N.-IBPM)

Transcription, mRNA localization and translation are key events at the synaptic region, determining proper neuron/neuron and muscle/neuron communication. In this context, one of our research interest is to understand how RNA-related proteins, such as the survival motor neuron protein SMN, the apoptosis antagonizing transcription factor AATF/Che-1 and the master gene NF-κB operate within cellular networks implicated in the RNA processing, trafficking and in local translation. We are also studying the potential involvement of AATF in memory-related events in which NF-κB is known to play a fundamental role. Defects in RNA metabolism have been linked to a variety of neuropathological conditions including intellectual disabilities and motor neuron diseases (MNDs), while memory deficits correlate with severe neurodegenerative disease, such as Alzheimer's disease.
These studies will provide new insight into molecular and cellular mechanisms underlying neuronal development, synaptic plasticity and cognitive processes, as well as to develop therapeutic strategies for severe neurological and neuromuscular diseases.
Collaborations:
Di Certo M.G., Gabanella F. Institute of Cellular Biology and Neurobiology (IBCN) CNR, Rome.
Prof. Romano A., Dr. Freudenthal R. (University of Buenos Aires UBA/CONICET)

2.6. Duchenne Muscular Distrophy (DMD)

2.6.1 Glucocorticoids as regulatory signals in neuronal functions in DMD (Fragapane P.IBPM)

Stressful events induce activation of the autonomic nervous system and the hypothalamus-pituitary-adrenal axis, with increase in glucocorticoid (GC) synthesis and release. The ubiquitous expression of GC receptors (Gr) confer to this system an essential role in the response to stress and restoration of homeostasis. These stress-induced changes are mediated by modifications in gene expression and protein synthesis. Precise regulation of the HPA (Hypothalamic-Pituitary-Adrenal) axis activity is very important for the organism; indeed, chronic exposure to GCs results in various adverse side effects, such as osteoporosis, diabetes, hypertension and neurodegeneration. The goal of this research project is to uncover the effect that GCs exert in brain regions susceptible to stressful stimuli as hippocampus, through gene expression analysis of the Gr and his target genes by administration of different glucocorticoid hormones. The study will be performed in vitro neuron cultures from wild-type and in mdx mice (a Duchenne Muscular Dystrophy animal model) and neuroblastoma cell lines administered with Dexamethasone and Cortisone. RNAs and proteins factors involved in this regulation will be the subject of this study.
Collaborations:
De Stefano E., Camilloni G., Bozzoni I. (Department of Biology and Biotechnology, Sapienza University Rome)

2.6.2 The role of histone methyltransferases in the epigenetic regulation of neuronal and muscle stem cells in DMD (Mozzetta C.-IBPM)

Epigenetic regulation of gene transcription is crucial to shape the gene expression programs and cell fate choices in stem cells. Therefore, understanding how these processes are regulated both at the physiological and pathological level is paramount to devise strategies aimed to improve the regenerative capacity of organs affected by degenerative diseases, such as dystrophic muscles. Histone lysine methyltransferases (KMTs) are crucial chromatin-modifying enzymes that have been implicated in the maintenance of cell-specific transcriptional homeostasis in a variety of cell types,

including neuronal and muscle progenitors. Among this class of enzymes, we are interested in studying the role of H3K9 KMTs in shaping higher ordered chromatin structures (i.e. nuclear-lamina associated domains) both during muscle and neuronal differentiation. More specifically, we aim to understand the specific transcriptional programs regulated by H3K9 KMTs in the different population of muscle progenitor cells involved in skeletal muscle regeneration and degeneration, with the final goal to elucidate the epigenetic control of their phenotypical plasticity in relation to the environmental changes imposed by DMD progression. Moreover, since H3K9 KMTs have been firmly implicated in the maintenance of neuronal transcriptional homeostasis, we aim also to understand how these enzymes influences the different fates of neuronal progenitor cells. Given the availability of small molecules inhibiting specifically the action of the different KMTs, our study will help to identify possible new pharmacological targets to be exploited to revert pathological epigenetic states associated with DMD.
Collaborations:
Prof. Sigmar Stricker (Freie Universitat, Berlin)
Dr. Giovanna Peruzzi (IIT, Rome)
Prof. Stefano Biagioni (Department of Biology and Biotechnology, Sapienza University Rome)

2.6.3 Role of cell cycle regulators in the control of skeletal muscle metabolism and regeneration in DMD (Caruso M., Agnese Bonato A., IBBC)

In addition to their well-known function in cell proliferation, there is increasing evidence that cell cycle regulators play important roles in metabolic control. 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). Recently, we observed that *cyclin D3-/-* mice display an increased number of oxidative myofibers and increased basal metabolism and muscle performance. Interestingly, oxidative fibers in patients with DMD are more resistant to the dystrophic pathology in comparison with glycolytic fibers.
Our objective is to evaluate whether inactivation of cyclin D3 in the *mdx* mouse model of DMD can attenuate the dystrophic pathology by promoting a slower, more oxidative muscle phenotype.

2.7 Possible role of p65 iso5, a new isoform of the NF-kB complex, in neurodegenerative diseases (Francesco Di Blasi IRIB)

Nuclear Factor kB (NF-kB) consist of a family of transcription factors that regulates the expression of genes involved in many processes as inflammation. Among the various NF-kB complex, the most characterized is the heterodimer p65/p50. NF-kB is ubiquitously expresses in neurons and when constitutively activated is associated with neuronal information. A dysregulation of NF-kB activity is related to neurodegenerative diseases. Synthetic glucocorticoids (Gcs) are the most frequently prescribed anti-inflammatory drugs, associated with a reduction in the AD risk. In our lab we have founded a new spliced form of p65, named p65 iso5. This protein is able to bind Dexamethasone and doubling glucocorticoid response GR-mediated. We hypothesize that this natural protein, could act synergistically with synthetic glucocorticoids, thus reducing the dosage and time therapy and consequently minimize synthetic glucocorticoids side effects.