Appendix: Abstract of research project

RESEARCH AREA 3: Neurodevelopmental disorders

3.1. Congenital disorder of Glycosylation (CDG type II): A *Drosophila* model to study associated neurological defects (Frappaolo A., Sechi S., Giansanti M.G.-IBPM)

Congenital Disorders of Glycosylation (CDG) comprise a family of human diseases caused by mutations in genes required for the synthesis of glycoconjugates. More than 80% of these diseases display severe neurological impairment. The Conserved Oligomeric Golgi (COG) complex mediates tethering of vesicles carrying glycosylation enzymes across the Golgi cisternae. Mutations affecting human COG1, COG2, COG4-COG8 cause monogenic forms of inherited, autosomal recessive CDGs. Typical clinical manifestations of COG-CDGs include psychomotor delay, epileptic seizures and hypothonia. Yet, it is unknown how the glycosylation defects cause the neurological aspects of CDGs.
We have generated a *Drosophila* COG7-CDG model, which closely parallels the pathological characteristics of COG7-CDG patients, including pronounced neuromotor defects and altered N-glycome profiles. We have demonstrated that the COG complex cooperates with Rab1 and Golgi phosphoprotein 3, to regulate Golgi trafficking and that overexpression of Rab1 can rescue the locomotor defects associated with loss of Cog7. Our results suggest that the *Drosophila* COG7-CDG model can be used to test novel potential therapeutic strategies by modulating trafficking pathways.
Collaborations:
Tiemeyer M. (Complex Carbohydrate Research Center, The University of Georgia, Athens, USA)
Fraschini R. (Università degli studi di Milano Bicocca)

2. Autism Spectrum Disorders (ASD)

3.2.1 Role of regulators on synapse formation and activity (Cestra G.-IBPM)

Synapse is a cell junction specialization responsible of directional communication between neurons. In the chemical synapse, the pre-synaptic neuron releases neurotransmitters into the cleft that, by binding to membrane receptors, elicits post-synaptic currents. Activity and dynamics of neurotransmitter receptors it tightly controlled by the elaborate network of adapter proteins of the postsynaptic density. The actin cytoskeleton extends throughout the post-synapse and controls its morphology and activity.
In our laboratory we are currently studying the role of the adapter protein named neuronal Arg Binding Protein 2 (nArgBP2), which interacts and is phosphorylated by the Arg/Abl kinase protein family. nArgBP2 is implicated in the regulation of actin dynamics at the post-synaptic specialization and it is involved in the formation of glutamatergic synapses. Since preliminary observations have suggests a possible interaction of nArgBP2 with proteins involved in ASD, such as FMRP, Shank3 and SAPAP3, we are currently investigating its possible implication in these pathways.

3.2.2 Role of noncoding RNA in ASD altered plasticity mechanisms (Mannironi C.-IBPM)

The formation and maintenance of neural circuits in the mammalian central nervous system requires a fine control of gene expression at post-transcriptional level, with regulatory RNAs, such as microRNAs (miRNAs), as key modulators of brain function. Circular RNAs (circRNAs) belong to a re-emerged highly abundant class of neuronal RNAs, that may act as regulatory factors of

neuronal mechanisms. Our research is focused on the study of regulatory RNAs, i.e. miRNAs and circRNAs, in physiological and pathological synaptic mechanisms. We have identified miRNAs involved in neuronal transmission, we are currently analysing their role in complex behaviours, such as anxiety and stress-related diseases. We aim to deepen microRNA molecular mechanisms, analysing the involvement of specific RNA binding proteins (RBPs) in miRNA regulatory circuits. The function of circRNAs in the aetiology of the Autism Spectrum Disorder (ASD) is currently investigated in the Btbr ASD mouse model. ASD-related circRNAs are characterized in vitro and in vivo, in term of mode of action and function in synaptic mechanisms, exploring their potential application as new markers for ASD in biological biofluids.

Collaborations

Presutti C., Rinaldi A., Mele A. (Department of Biology and Biotechnology, Sapienza University Rome)

Scattoni A.M., Ricceri L. (Higher Health Institute, Rome)

3.3 Molecular basis of neonatal epileptic encephalopathy (NEE): a severe neurological disease caused by defects in the vitamin B6 salvage pathway enzyme pyridoxine 5'-phosphate oxidase. (Tramonti A. and Nogués I. – IBPM-IBAF)

Vitamin B_6 plays important roles in the metabolism of neurotransmitters such as glutamate, dopamine, serotonin, epinephrine, and gamma-aminobutyric acid. The vitamin derivative pyridoxal 5'-phosphate (PLP) is the cofactor of a plethora of enzymes, whose activity is of pivotal importance for central metabolism and is essential for the correct functioning of the central nervous system. The activity of these enzymes relies on an appropriate availability of PLP in the neuronal cells. Mammals are not able to synthesize PLP but they recycle it through a salvage pathway (in which the enzymes pyridoxal kinase and pyridoxine 5'-phosphate oxidase are key components) from the different B_6 vitamers contained in food and from protein turnover. Once made available, PLP is somehow targeted to apo-PLP-enzymes. Deficiency of vitamin B6 has been implicated in pathologies such as autism, schizophrenia, Alzheimer, Parkinson, epilepsy and Down's syndrome.

The focus of our project is a severe, newly recognized and rare neurological disease known as neonatal epileptic encephalopathy (NEE), determined by autosomal recessive mutations in the gene encoding pyridoxine 5'-phosphate oxidase (PNPO), that result in inadequate levels of PLP. The main feature of the disease is the onset of severe seizures, often within hours from birth, which respond to PLP administration and, in some cases, also to pyridoxine. If not promptly treated, neonates will present a drug resistant epileptic status that might determine death. To date, 18 pathogenic mutations of the PNPO gene and about 40 patients have been reported in the literature. Recently, we have studied the functional effects of the c.347G>A (p.R116Q) mutation of the human PNPO gene, and discussed its pathogenic role in epileptic encephalopathy. Novel missense mutations have been found in children affected by NEE, but have yet not been characterized at a molecular level. We believe that a detailed structural and functional characterization of these mutant forms is very important for a full understanding of the disease and for the rational devise of treatment strategies.

3.4 The role of p21-lacking adult Neural Stem Cells in the neuro-regenerative responses following traumatic brain injury (Farioli Vecchioli S., IBCN).

Adult neural stem cells (aNSCs) play a pivotal role in maintaining a high rate of the adult neurogenesis in the neurogenic niches throughout life. In physiological conditions most of aNSCs are in the reversible G0 phase (also called quiescent state) and only rarely they are recruited in cell cycle to give rise to new neurons. In aging, the number as well as the neurogenic potentiality of aNCS progressively diminish until they disappear within the neurogenic niches. However, after

brain injury, such as ischemic stroke or Traumatic Brain Injury (TBI), there is a strong increase in the activation and expansion of aNSCs, which actively participate in the neurorepair processes. The molecular mechanisms modulating the transition between quiescence and activation of aNSCs are very complex and are subjected of a wide range of studies aimed at enhancing the neurogenic potential of aNSCs to procrastinate brain aging and to increase the neuro-rigenerative responses following brain damage. In this context, our lab is involved in the study of the modulation of the gene p21, which represent one major regulators in the maintenance of the aNSCs quiescence. The purpose of our study is to specifically target the deletion of p21 in the aNSCs, with the goal to expand the NSC pool size and consequently enhance the adult neurogenesis in aging and after brain damage, in particular after TBI. To achieve these aims we developed a multi-approaches strategy including conditional cre-loxP models, adeno associated virus (AAV) specifically infecting aNSCs and containing interference sequences for p21 and the adjuvant proneurogenic action of physical activity.

3.5. Aging of neural stem cells: control by a network of cell cycle inhibitors (Btg1/2, p16Ink4a) and reversal by neurogenic stimuli (running, fluoxetine and diet polyphenols)
(Tirone F., Micheli L.,D'Andrea G., Ceccarelli M. IBBC)

The continued production of new neurons during adulthood in the neurogenic niches (i.e., dentate gyrus of the hippocampus and subventricular zone) enhances the efficiency of episodic/associative memory, as shown by several laboratories including ours. We have found a network of cell cycle inhibitors (Tis21/Btg2, Btg1) that regulate quiescence and proliferation of stem and progenitor cells in adult and aging neurogenic niches. We have shown that neurogenic stimuli, i.e., running, antidepressant, diet, are able to counteract the reduced neurogenesis associated to aging, either physiological or induced by Btg1 knockout. Part of this network is also p16Ink4a, which we have shown to prevent during aging any exit from quiescence elicited by running; therefore, p16Ink4a protects the stem cells pool against depletion after stimulus during aging.

3.6 Postnatal early environment may affect the epigenome, stably improving or exacerbating behavioral and physiological phenotypes in mouse models of developmental disorders
(D'Amato F.R., IBBC)

Early aversive environments (maltreatment, maternal separation, etc.) have deleterious effects on several aspect of offspring development. Few studies explore the effects of "positive" early environments. Environmental enrichment (social and physical) is usually applied to adults and young mouse models of different psychopathologies: positive temporary effects that reduce symptoms are usually detected, but they do not usually last for long time. Rearing newborns in enriched environment, when neural plasticity is maximal, might result in stable rescue of different psychopathologies. Due to infant immaturity at birth, social enrichment/deprivation can be perceived by pups (and mothers) even during vey early postnatal days and can affect later development. Molecular mechanisms responsible/associated to these altered phenotypes are investigated.
Collaboration:
Prof. Marco Battaglia, CAMH, Toronto University
Prof. Rossella Ventura, Sapienza University, Rome
Dr. Bice Chini, IN CNR, Milano

3.7 Roles of non-coding RNAs in myotonic dystrophy type 1
(Falcone G., Cardinali B., Provenzano C., IBBC)

Myotonic dystrophy type 1 (DM1) is a neuromuscular disorder caused by an unstable (CTG)n repeat in the DM protein kinase (*DMPK*) gene. Expanded CUG-repeats have been demonstrated to

be toxic *per se,* sequestering nuclear proteins and disrupting gene transcription and pre-mRNA alternative splicing. Non-coding RNAs such as microRNAs and circRNAs have been found to be important regulators of cellular physiology and pathology by using a variety of mechanisms. Our studies aim at identifying microRNAs and circRNAs that are dysregulated in muscles of DM1 patients and may contribute to the disease mechanisms. In addition, their potential to be used as biomarkers for disease staging and progression is being investigated.
Key words: non-coding RNAs, microRNAs, circular RNAs, disease mechanisms, disease biomarkers
Collaborations:
Fabio Martelli, IRCCS Policlinico San Donato, San Donato Milanese, Milan

3.8 Synaptic dysfunctions and potential therapies in Fragile X syndrome
(Catania M.V., D'Antoni S. IRIB)

Our research is focused on pathophysiological mechanisms of Fragile X Syndrome (FXS) and other neurodevelopmental disorders characterized by Intellectual Disability and autism, with the final goal of identifying possible pharmacological treatments. We found that in the synapses of the Fmr1 KO mouse model of FXS, metabotropic glutamate receptors type 5 (mGlu5) are less coupled to the scaffolding/effector protein Homer, with consequences for the mobility of mGlu5 receptors and their functional interaction with the NMDA receptors. We provide also evidence that activation of serotonin 5-HT7 receptor agonists improves pathological phenotypes of the *Fmr1* KO mouse, namely altered morphology of dendritic spines and audiogenic seizures susceptibility. We are currently extending our findings to other diseases by studying the expression of different receptors and scaffolding proteins in specimens from human brains.

Collaborations:
Dr. Eleonora Aronica, Dept of (Neuro) Pathology, Academic Medical Center, Amsterdam, The Netherlands;
Dr. Sebastiano Musumeci, Dr. Corrado Romano, Dr. Carmela Maria Bonaccorso, Dr. Michela Spatuzza, Oasi Research Institute Troina (EN);
Prof. Ferdinando Nicoletti, Dr. Giuseppe Battaglia, Università La Sapienza, Roma - IRCCS Neuromed, Pozzilli (IS);
Dr. Barbara Bardoni, IPMC, CNRS UMR6097, Valbonne, France
Prof. Marcello Leopoldo, Dr. Enza Lacivita, Università di Bari

3.9 Neurodevelopmental rare diseases: potassium channels mutations and ncRNAs in Zimmermann-Laband and FHEIG syndromes.
(Parisi C., IBBC)

Zimmermann-Laband and FHEIG syndromes are neurodevelopmental genetic rare diseases with overlapping clinical features. A high proportion of mutations reside on K+ channels. We work on the functional characterization of KCNH1 and KCNK4 mutations in human patients fibroblasts, focusing on primary cilium signalling through Hedgehog pathway and its regulation by microRNAs.
Collaborations:
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