From model systems to therapies

IBPM Annual Meeting

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Aula Marconi CNR

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SUMMARY

PROGRAMME

9.00 - 09.20 ***Registration***

9.20 - 9.30 **Patrizia Lavia, Direttrice ff IBPM**
Welcome message from the IBPM Acting Director

9.30 - 9.40 **Tullio Pozzan, Direttore Dipartimento Scienze Biomediche (DSB)**
Greetings from the Director, CNR Department of Biomedical Sciences

Session 1: Drug Discovery (part 1) *Chair: Dr P Laneve*

9.40 - 10.30 **Ferdinando Squitieri, IRCSS Casa Sollievo della Sofferenza and CSS-Mendel. Invited speaker**
Huntington's disease: from biology to clinics towards new therapies

10.30 - 10.50 **Elisa Caffarelli**
The European Paediatric Translational Research Infrastructure (EPTRI): the bridge towards the future of paediatric medicine

10.50 - 11.10 **Pierpaolo Ceci**
Preclinical evaluation of a stimuli-sensitive nanocarrier based on the human ferritin for cancer therapy

11.10 - 11.40 ***Coffee break***

Session 1: Drug Discovery (part 2) *Chair: Dr A Ilari*

11.40 - 12.00 **Theo Battista**
A double approach for anti-trypanosomatid drug discovery targeting Trypanothione reductase, a key enzyme for the redox equilibrium in Trypanosomatids

12.00 - 12.20 **Adele Di Matteo**
Exploiting nucleophosmin interactions for the treatment of acute myeloid leukemia

12.20 - 12.40 **Veronica Morea**
Rational design of peptides for biomedical applications

12.40 - 13.00 **Linda Celeste Montemiglio**
Cryo-EM structure of the human Ferritin-Transferrin Receptor 1 complex

13.00 - 14.30 ***Lunch break and poster session***

Session 2. Model Systems in Basic and Applied Reserch (part 1) *Chair: Dr R Piergentili*

14.30 - 15.20 **Giulio Cossu, Regenerative Medicine Chair, University of Manchester, UK). Invited speaker**
Ex vivo gene therapy for Duchenne Muscular Dystrophy

15.20 - 15.40 **Claudio Passananti**
Artificial Transcription Factors

15.40 - 16.00 **Chiara Mozzetta**
H3K9 methylation controls Fibro-Adipogenic Progenitors identity and skeletal muscle repair

16.00 - 16.20 ***Coffee break***

Session 2. Model Systems in Basic and Applied Reserch (part 2) *Chair: Dr MP Somma*

16.20 - 16.40 **Maria Grazia Giansanti**
Investigating the molecular mechanisms underlying the neurological defects associated with Congenital Disorder of Glycosylation type IIe using Drosophila as a model system

16.40 - 17.00 **Davide Marzi**
COnstitutive Photomorphogenic 1 (COP1) mediates light-controlled stamen growth in Arabidopsis thaliana

17.00 - 17.20 **Patrizia Filetici**
From yeast to cancer cells, epigenetic cross-talk and metabolism

17.20 - 17.40 **Ambra Natalini**
Cell cycle analysis of clonally expanding antigen-specific CD8 T cells at different times after vaccination

17.40 - 18.00 **Cinzia Rinaldo**
Pancreatic tumor formation and progression studies in the era of organoid models

18.00 ***Concluding remarks and meeting closure***

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Posters

ORAL PRESENTATIONS

O-1: EPTRI - European Paediatric Translational Research Infrastructure: the bridge towards the future of paediatric medicine

Elisa Caffarelli, *IBPM group for paediatric disease*

Istituto di Biologia e Patologia molecolari del CNR

The EPTRI project is coordinated by the Consortium for Biological and Pharmacological Evaluations (CVBF) and funded within the European Framework Program for Research and Innovation Horizon 2020. It arises from the need to fill the big gap in paediatric research and to find answers to the serious lack of medicines for children in EU and worldwide.

In this context, the nascent European Paediatric Translational Research Infrastructure has been designed. It aims to put together and to network all the available competences and the most innovative methodologies in the field of life sciences, for triggering the development cycle of new drugs and therapies for the paediatric population. This will ultimately lead to a reduction of costs of long-term health by improving care during the neonatal and pediatric phases.

In consideration of the expertise of numerous IBPM researchers in several paediatric areas, our Institute has been identified as a structure potentially interested in participating, both as users and as providers, in the creation of the future paediatric infrastructure.

O-2: Preclinical evaluation of a stimuli-sensitive nanocarrier based on the human ferritin for cancer therapy

Pierpaolo Ceci[a,b], Gianni Colotti[a], Martina Pitea[c], Giuseppe Cipolla[c], Giulio Fracasso[e], Veronica Morea[a], Alberto Boffi[c], Patrizio Giacomini[e], Gianluca Sala[f], Elisabetta Falvo[a]

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Protein-based nanocarriers have increasingly been used as drug delivery carriers for cancer therapy. We developed a genetically engineered nanocarrier based on human ferritin heavy chain (HFt) able to incorporate and deliver drugs. These variants contain a masking polypeptide (named PAS) that improves both the drug encapsulation ability and the bloodstream permanence in comparison to the native HFts, enabling their accumulation in tumors where the drug payload is released. In particular, this HFt-based system (HFt-MP-PAS) is designed to be relatively inactive versus non-cancer cells during blood circulation, and subsequently can be activated by multiple proteases (MMP-2/9) in the tumor microenvironment where the therapeutic cargo must be released. This limits unwanted damage to healthy cells.

In our previous studies we demonstrated the antitumor effects of the novel stimuli-sensitive nano-ferritin containing doxorubicin (HFt-MP-PAS40-Dox) in pancreatic and head and neck cancer malignancies, showing an excellent therapeutic efficacy.

Now, in collaboration with a private biotech (Thena Biotech) we are evaluating the preclinical performances of the stimuli-sensitive nano-ferritin carrying a topoisomerase I inhibitor (HFt-MP-PASE-Topo). Different cancer models have been evaluated, including a relevant pancreatic cancer xenograft (PDX) model. Our findings demonstrate that HFt-MP-PASE-Topo is able to reduce more efficiently tumor growth compared both to the naked drug and to the current standard of care Abraxane. Altogether, our results highlight the enormous potential of this approach for *in vivo* cancer treatment.

O-3: A double approach for antitrypanosomatidal drug discovery targeting Trypanothione reductase, a key enzyme for the redox equilibrium in Trypanosomatids

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Trypanosomatids are the causative agents of vector-born neglected diseases as Leishmaniasis, Chagas disease and Human African Trypanosomiasis. Currently no effective vaccines are available for prevention, while treatment with both first line (Antimonials, Amphotericin B) and second line drugs (Paromomycin), toxic and expensive, are becoming ineffective in endemic areas because of drug-resistant protozoan strains. For all these reasons, it is important to identify new active compounds against these parasites.

Trypanothione metabolism represents an ideal drug target as it is essential for parasites survival, absent in mammals and druggable. This pathway is based indeed on Trypanothione (N_1,N_8-bis-glutathionyl-spermidine, (TS_2)), a small dithiol synthesized in a double step reaction catalysed by the ATP-dependent Trypanothione synthetase (TryS), which conjugates two molecules of glutathione to a molecule of spermidine. Trypanothione in its reduced form, $T(SH)_2$, acts as a scavenger for reactive oxidative species, produced by the immune system of the host, preventing the parasites from oxidative damage. Trypanothione reductase (TR), a NADPH-dependent flavoenzyme, keeps trypanothione in the reduced form $T(SH)_2$ ensuring an intracellular reducing environment, playing therefore the same crucial role of the homologous human system Glutathione/Glutathione reductase.

The inhibition of TR is known to disrupt the parasite redox balance, inducing its death. For this reason, TR can be regarded as a validated drug target.

In order to discover new lead compounds active against TR, two different approaches were used:

1. An High Throughput Screening (HTS) of a library of 120.000 compounds, carried out in collaboration with the pharmaceutical company IRBM (Pomezia, Rome);

2. A ligand-based drug design which led to a family of diarylsulfide derivatives, realized in collaboration with Prof. Roberto Di Santo (Sapienza, University of Rome).

Both the approaches led to the identification of new TR inhibitors: IRBM3 and RDS562, specifically active against both the Trypanothione reductase from *Leishmania infantum* and from *Trypanosoma brucei*, endowed with antiproliferative activity against *L. donovani* promastigotes. Kinetic parameters (IC_{50}, K_i) were determined and the crystal structure of TR from *T. brucei* in complex with IRBM3 was solved, showing the structural basis of its interaction with Trypanothione reductase and paving the way for structure based drug optimization in order to obtain more potent inhibitors.

O-4: Exploring nucleophosmin interactions for the treatment of acute myeloid leukemia

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Nucleophosmin (NPM1) is a nucleus-cytoplasm shuttling protein implicated in processes such as ribogenesis, centrosome duplication, cell cycle control and response to stress. It is one of the main “hub” proteins in nucleoli and, as such, it interacts with a plethora of other proteins including the tumor suppressors p14arf and Fbw7γ, the main E3-ubiquitin ligase of c-MYC [1].

NPM1 is the most frequently altered protein in Acute Myeloid Leukemia (AML). Mutations are located in the NPM1 C-terminal domain and lead to the stable and aberrant delocalization of the protein in the blasts’ cytoplasm [2]. In doing so, both p14arf and Fbw7γ are also delocalized and consequently degraded thus impairing a critical p14arf-HDM2-p53 tumor suppressor axis and leading to c-MYC stabilization [3].

By combining NMR, molecular dynamics, fluorescence spectroscopy and site-directed mutagenesis, we analysed the interaction of NPM1 with p14arf and Fbw7γ at the molecular level and identified critical residues in both NPM1 and protein partners [3,4]. Moreover, the ability of N6L, a positively charged pseudopeptide, to disrupt NPM1 protein-protein associations was tested [5]. The effect of N6L on AML cell lines bearing NPM1 mutations or not was also analysed. Targeting NPM1 interactions, in combination with conventional chemotherapy, may be a novel route for the treatment of AML.

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O-5: Rational design of peptides for biomedical applications

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Peptides are highly effective research tools and attractive therapeutic agents *per se*, as well as convenient lead compounds for the rational development of non-peptidic small molecule drugs. Indeed, since interaction surfaces are selected by evolution to be highly specific and, in many cases, low energy, peptides mapping on protein regions involved in interactions with macromolecular partners are effective interaction mimics and/or inhibitors. Whenever the experimentally determined three-dimensional (3D) structure of a protein complex is available, or a reliable molecular model can be built for it, the design of inhibitory peptides is straightforward, provided a few simple rules – mostly aimed at preventing solubility problems – are followed. Additionally, when a 3D structure or model of a protein is only available in the free state, peptides aimed at binding the protein interaction partners and/or inhibiting protein interactions will be comprised within a set of peptides designed to cover essentially the whole protein surface. Even when targeting protein interactions with small molecules, peptides can be effective binders/inhibitors, as long as the ligand binding site is not too small or difficult to access. Once peptides endowed with the desired biological activity are identified, they can be administered to cells to assess their ability to penetrate the cell membrane and, if required, the membrane of the intracellular organelle where they have to exert their activity, either by themselves or upon conjugation with a short cell penetrating peptide (e.g., the four-residue Phe-Arg-Phe-Lys sequence). The pharmacokinetic properties of peptides upon *in vivo* administration can be estimated based on their susceptibility to degradation by plasmatic proteases. This can be investigated both experimentally, by incubating the peptides with human plasma and identifying the resulting peptide fragments by mass spectrometry, and computationally, by searching for the presence of peptide bonds cleavable by plasma proteases available from public databases. In case potentially scissile peptide bonds are identified in the bioactive peptides, the bioavailability of the peptides can be increased by replacing these bonds with non-peptide moieties. Further, alanine-scanning mutagenesis can be performed to assess the individual contribution of each peptide residue to target binding and design shorter peptides comprising the structural elements required to exert the biological activity. By following this rational, structure-based protocol, it is possible to obtain short peptides able to exert the desired activity at low doses, penetrate biological membranes and resist enzymatic degradation, ready to use for *in vivo* studies.

We present three studies were we have exploited the aforementioned procedure to

rationally design peptides aimed at therapeutic applications: 1) severe mitochondrial (mt) syndromes, such as Mitochondrial Encephalopathy, Lactic Acidosis and Stroke (MELAS) and Mioclonal Epilepsy and Ragged Red Fibers (MERRF); 2) diseases related to either impaired or pathological (*e.g.*, tumour) angiogenesis; 3) Huntington disease (HD).

1) In the first case, 15- and 16-residue long peptides aimed at binding mt-tRNAs bearing single-point mutations responsible for MELAS or MERRF were designed based on the analysis of the 3D structure of a bacterial $tRNA^{Leu}$-leucyl-tRNA synthetase complex. The designed peptides bind to both mt-tRNALeu(UUR) and mt-tRNALys *in vitro*, stabilize a wild-type-like structure of the tRNA mutants and rescue the pathological phenotype of mutant cells at low μM concentration.

2) In the second case, twelve peptides were designed that covered the whole domain surface of the second immunoglobulin-like domain of the vascular endothelial growth factor receptor (VEGFR)-1, whose 3D structure with VEGF-A, but not with other ligands, was available. One of the peptides resulted to have a strong pro-angiogenic activity, mediated by the interaction with α5β1 integrin, both in vitro and in rabbit cornea. Two other peptides have anti-angiogenic activity in cellular models, which is exerted by hampering sVEGFR-1 homo-dimerization in one case, and sVEGFR-1 interaction with NRP-1, in the other. All of these peptides bind the interaction partners, inhibit target interactions and exert pro- or anti-angiogenic activity on endothelial cells at low μM concentration.

All the aforementioned peptides are ready to be tested for the presence of scissile peptides bonds before undergoing *in vivo* studies.

3) Recently, we have designed peptides covering the whole surface of the 3D homology model that we had previously built for the Ras-Homologue Expressed in the Striatum (RHES) protein. RHES is an E3-ligase that catalyses the sumoylation of both wild-type (Htt) and mutated (mHtt) huntingtin, thereby preventing mHtt aggregation and allowing it to exert its cytotoxic effect in HD. These peptides will be tested for their ability to revert the HD phenotype in cellular models of HD by inhibiting RHES interaction with mHtt.

O-6: Cryo-EM structure of the human Ferritin-Transferrin Receptor 1 complex

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The transferrin receptor 1 (CD71) is one of the key regulator of iron homeostasis for most higher organisms. It mediates cellular iron import through a constitutive clathrin-dependent endocytosis mechanism and by recruiting iron-loaded transferrin, HFE and serum ferritin in response to cellular demand (1). The receptor is also opportunistically exploited by several viruses and malaria parasites as a preferential entry for cell invasion (2, 3). The structural features of CD71 interaction with transferrin and viruses have been recently clarified and specific transferrin and viruses recognition epitopes have been identified (4, 5).

Here, we provide the molecular basis of the CD71 ectodomain-human ferritin interaction by determining the 3.9 Å resolution single-particle cryo-electron microscopy structure of their complex and by validating our structural findings in a cellular context. We observed that two short motifs upon the H-ferritin homopolymer recognize a precise epitope on CD71 that overlaps the arenaviruses and Plasmodium vivax binding region in the apical part of the receptor ectodomain. Our data account for transferrin-independent binding of ferritin to CD71 and suggest that select pathogens have adapted to enter cells by mimicking the ferritin access gate (6).

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O-7: Artificial Transcription Factors

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The possibility to re-program the expression of a specific endogenous gene represents a revolutionizing tool in the field of molecular biology and medicine. Transcriptional targeting of disease-related genes could provide beneficial effect for diseases that still lack a cure. In particular, the combination of Zinc Finger Artificial Transcription Factor (ZF-ATF) technology and adeno-associated virus (AAV) vectors gene delivery represents an innovative tool that may have relevant applications in the study and cure of selected genetic disorders. We are approaching several muscular dystrophies focusing mainly on Duchenne Muscular Dystrophy and Congenital Muscular Dystrophy Type1A.

Duchenne Muscular Dystrophy (DMD) - A promising approach for DMD treatment aims to modulate utrophin gene expression. Utrophin has been evaluated as a potential replacement for defective dystrophin in DMD patients. Thus, ongoing studies searching for natural or synthetic small molecules targeting utrophin could accelerate the clinical translation process, holding new hope for individuals with DMD. We designed and engineered several ZF-ATFs able to drive the transcription of endogenous utrophin gene. Our preclinical studies candidate ZF-ATF technology as a promising and feasible therapeutic approach for DMD pathology. Adeno-associated viral muscle vector (mAAV) delivery of our ZF-ATF genes, "Jazz" and its up-graded version "JZif1", significantly corrects dystrophic pathology in dystrophin-deficient mice (mdx), inducing muscle functional rescue. To investigate the molecular mechanisms underlying Jazz and JZif1 induced muscle functional rescue, we focused on utrophin related pathways. Coherently with utrophin subcellular localization and role in neuromuscular junction (NMJ) plasticity, we found that our ZF-ATFs positively impact the NMJ. We report on ZF-ATF effects on post-synaptic membranes in myogenic cell line, as well as in wild type and mdx mice. These results candidate our ZF-ATFs as novel therapeutic molecules for DMD treatment.

Congenital Muscular Dystrophy Type1A (MDC1A) is a rare severe muscle-wasting disorder characterized by the involvement of not only skeletal muscle, but also of central and peripheral nervous systems. MDC1A is the most prevalent form of Congenital Muscular Dystrophy (CMD) and is due to a defect in a gene named: "laminin alpha2" (LAMA2). As novel therapeutic strategy for MDC1A treatment, we propose to activate the "embryonic copy" of LAMA2 gene named "LAMA1" that can act as functional substitute. In order to reactivate LAMA1 gene we use artificial genes, named “ZF-ATFs”, designed ad hoc and engineered in our laboratory. We obtained promising results in MDC1A-patient derived cells treated with our ZF-ATF artificial genes. We are going to test our ZF-ATF genes in MDC1A animal models with the aim to approach gene therapy protocols.

O-8: H3K9 methylation controls Fibro-Adipogenic Progenitors identity and skeletal muscle repair

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Keywords: Duchenne Muscular Dystrophy, FAPs, H3K9 methylation, skeletal muscle regeneration, lysine methyltransferases, KMTs.

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. Fibro-Adipogenic Progenitors (FAPs) are crucial regulators of muscle homeostasis as they possess the intrinsic ability to either support muscle regeneration or to contribute to fibro-adipogenic degeneration of dystrophic muscles. Therefore, the elucidation of the molecular mechanisms controlling their phenotypical plasticity holds therapeutic potential.

Here, we provide evidence that histone H3 lysine K9 methyltransferases (H3K9 KMTs), G9a, GLP and PRDM16, are key stabilizing epigenetic factors of FAPs-specific gene expression programs. Our data support a role for H3K9 KMTs in preserving FAPs identity by repressing alternative transcriptional programs through deposition of H3K9 di-methylation (H3K9me2). Specifically, we show that PRDM16 controls G9a/GLP's genomic recruitment and H3K9me2 deposition at muscle-specific loci. Of note, we found PRDM16, G9a and GLP enriched at the nuclear lamina of FAPs suggesting that they organize heterochromatin at the nuclear periphery to maintain the stable repression of genes encoding alternative developmental regulators. Accordingly, pharmacological inhibition or RNAi-mediated knock-down (KD) of H3K9 KMTs de-repress master myogenic genes in FAPs and induce the muscle differentiation program. These data are corroborated by transplants experiments showing that FAPs isolated from mice treated with G9a/GLP specific inhibitors, participate in myofibers formation in regenerating recipient mice. Together, our findings reveal a FAPs-specific epigenetic axis of therapeutic relevance since we demonstrate that *in vivo* inhibition of H3K9 methylation in dystrophic mice enhances skeletal muscle regeneration, inducing an increase in myofibers size and reduction of adipogenic and fibrotic scars.

O-9: Investigating the molecular mechanisms underlying the neurological defects associated with Congenital Disorder of Glycosylation type IIe.

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Keywords: Congenital Disorders of Glycosylation, Golgi trafficking, glycosylation, *Drosophila*, neurological impairment

Congenital Disorders of Glycosylation (CDG) comprise a family of human diseases caused by mutations in genes required for synthesis of glycoconjugates. More than 100 distinct forms of CDGs have been identified and more than 80% of these diseases display severe neurological and neuromuscular impairment. CDGs comprise two large groups. Type I CDGs affect the synthesis of the dolichol-linked oligosaccharide, the major precursor of N-linked glycoproteins, and its transfer to acceptor proteins. Type II CDG (CDG-II) diseases disrupt either the processing of N-linked glycans, the biosynthesis of O-linked oligosaccharides or the addition of glycans to lipids. 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-II. Typical clinical manifestations of COG-CDGs include psychomotor delay, epileptic seizures, general hypotonia and myopathy and failure to thrive. Most of the published studies, focused on clinical and biochemical characteristics of COG-CDG, documented the N-linked and O-linked glycosylation defects associated with this disease. Yet the correlation between defective glycosylation and the neuropathology is unknown and there is no effective therapy for this disorder.

Animal models can help to study the correlation between COG-dependent glycosylation defects and malfunction at the neuromuscular junction. 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. The *Drosophila Cog7*-CDG disease model, together with COG-CDG patients' cells, offer unique opportunities to clarify the molecular mechanisms underlying COG-CDGs and help identify novel therapeutic strategies.

O-10: CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) mediates light-controlled stamen growth in *Arabidopsis thaliana*

Davide Marzi[a,b], Erica Spaziani[a], Lorenzo Calò[a], Nadia Napoli[a,b], Giovanni Mele[c], Minami Matsui[d], Simone De Panfilis[e], Paolo Costantino[a], Giovanna Serino[a], Maura Cardarelli[b]

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In *Arabidopsis thaliana* stamen and hypocotyl have a similar simple anatomy, follow a similar growth pattern, and their elongation is triggered by auxin.

In hypocotyls, light repress elongation through a conserved regulatory module centered around COP1, a well characterized repressor of light-mediated responses. Here we provide evidence that a similar regulatory module, which includes COP1, its targets LONG HYPOCOTYL 5 (HY5) and HY5 HOMOLOG (HYH), and the downstream effector Auxin-RESPONSIVE PROTEIN IAA19 (IAA19), controls stamen elongation. Furthermore, blue light receptors CRYPTOCHROME 1 and 2 (CRY1 and CRY2) repress stamen elongation mainly before flower bud disclosure, while red and far-red light receptors PHYTOCHROME A and B (PHYA and PHYB), repress stamen elongation mainly during flower disclosure. These results suggest that when stamen elongate within the closed flower bud they are in a shade-like environment, perceived mostly by CRY1 and 2, while they are increasingly exposed to higher light intensities – perceived mainly by PHYA and B – during flower disclosure. Thus, our results highlight a novel role for photoreceptors and the COP1-HY5/HYH module in promoting stamen elongation and suggest that light may control both stamen and hypocotyl growth through a shared set of signaling components.

O-11: From yeast to cancer cells, epigentic cross-talk and metabolism

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Our experimental work is based on a long lasting research in yeast, an inspiring model where it is easy to dissect complex regulatory networks driving to translational future directions. Post-translational modifications write the epigenetic code necessary to remodel and alleviate the barrier of chromatin structure for gene expression. Modifying factors are able to deposit or erase epigenetic marks on histone tails involved in chromatin dynamic.
We have been focusing our research on the other side of the code, the PTM modifications of non histone proteins. We will discuss novel findings on the role of some epigenetic regulators involved not only in chromatin signaling but also in cell metabolism. Our efforts are focused in translating our discoveries from yeast to cancer cells, test novel compounds and understand regulatory circuitries as strategic approaches to study tumorigenesis.

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O-12: Cell cycle analysis of clonally expanding CD8 T cells at different times after vaccination

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Keywords: antigen-specific CD8 T cell response, clonal expansion, vaccination, cell cycle, blood

After infection or vaccination, antigen-responding T cells clonally expand and differentiate, generating effector and memory T cells. Although we have learned a great deal about clonally expanding T cells, we still lack essential spatial information, particularly as to the location of T cells during each phase of cell cycle.

By using a combination of DNA and Ki67 staining together with a novel strategy for analysis of flow cytometry data, we investigated the clonal expansion of antigen-specific CD8 T cells in BALB/c mice vaccinated via the intramuscular route with antigen-expressing viral vectors.

At early times after vaccination we found that most antigen-responding CD8 T cells in S-G_2/M phases of cell cycle had high Forward and Side Scatter, thus resembling monocytes and granulocytes. Cells with these characteristics are usually excluded from the analysis of normal lymphocytes ex vivo. By including them, we highly increased sensitivity of antigen-specific cell detection and discovered a previously missed population of cycling antigen-specific CD8 T cell in spleen, lymph nodes and also in the blood which is not expected to be a site for antigen-responding CD8 T cells proliferation. At late times after vaccination, the vast majority of the antigen-specific CD8 T cells reverted to a quiescent state, and only a few of them were dividing, mostly in the bone marrow.

These results have implications for prior and future immunological studies and also for haematological analysis of blood cells in humans.

O-13: Pancreatic tumor formation and progression studies in the era of organoid models

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Pancreatic ductal adenocarcinoma (PDA) is one of the most difficult human malignancies to treat. It is the fourth cause in cancer-related death in Western countries. PDA is often diagnosed late, therefore the majority of the studies on human samples provide information only on the last stage of tumorigenesis. Thus, to investigate PDA pathogenesis the gold standard is considered the KC mouse (Kras LoxStopLoxG12D/-; Pdx1-Cre+/-) in which the Cre recombinase activates the expression of oncogenic KRas-G12D specifically in pancreatic cells. It is possible to derive pancreatic organoid models that mimic the pathophysiological features of human PDAC from KC mouse, offering an opportunity to study PDAC tumorigenesis ex-vivo, by reducing animal experimentation.

PDA is a complex disease in which cells progressively accumulate mutations/dysfunctions disrupting their cellular processes. A fraction of these mutations/dysfunctions drive tumorigenesis, but some of them are passengers with no clear contribution to tumor development. The oncosuppressor HIPK2 (Homeodomain Interacting Protein Kinase2) is a kinase involved in the cell fate decisions in development and response to stress. We have developed mouse pancreatic organoids from opportune mice to induce HIPK2 deficiency and investigate the role of this kinase during tumor formation/progression. Parallel studies are ongoing in animals in vivo.

POSTERS

P1: Intranasal rapamycin treatment causes restoration of aberrant mTOR signalling and reduces oxidative protein damage in a mouse model of Down Syndrome

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The accumulation of oxidized/misfolded proteins due to the impairment of intracellular degradative machinery that ultimately results in the deposition of protein aggregates is one of the a key pathological aspects of Alzheimer disease (AD). Interestingly, Down syndrome (DS) neuropathology shares many features with AD, such as the deposition of both amyloid plaques and neurofibrillary tangles. Studies from our group and others demonstrated in DS brain the dysfunction of both proteasome and autophagy degradative systems, coupled with increased oxidative damage. Further, we observed the aberrant increase of mTOR signaling and of its down-stream pathways in both DS individuals and Ts65Dn mice (transgenic mouse model of DS). Administration of the mTOR inhibitor rapamycin by intranasal route (InRapa) in Ts65Dn mice was able to rescue aberrant mTOR signaling, restore autophagosome formation and reduce amyloid production and tau hyperphosphorylation.

Considering that oxidized proteins are significantly increased in DS brain and the crosstalk between mTOR and oxidative stress, we have tested if pharmacological rescue of mTOR hyperactivation might result in the decreased accumulation of oxidized proteins. By proteomics approach, we were able to identify specific proteins that showed decreased levels of HNE-modification (protein oxidation marker) after InRapa treatment compared with vehicle group. Among these, we found arginase-1 (ARG-1) and protein phosphatase 2A (PP2A), enzymes affecting different pathological aspects of the disease. The reduction of ARG-1 protein-bound HNE levels following rapamycin treatment rescued the enzyme activity, conceivably contributing to the recovery of arginase-regulated functions, such as synaptic transmission and metabolic control of neurotransmitters synthesis and tau phosphorylation . This latter process is also crucially regulated by PP2A, whose inhibition has been demonstrated to induce tau hyperphosphorylation and spatial memory deficits. InRapa treatment caused a reduced oxidation and an increased expression of PP2A, accompanied by a reduction of phosphorylated -tau levels. Therefore, the InRapa effects might play a role in reducing brain damage associated with synaptic transmission failure and tau hyperphosphorylation.

In summary, considering that mTOR pathway is a central hub of multiple intracellular signaling, we propose that InRapa treatment is able to lower the oxidation-mediated damage to proteins, thus representing a valuable therapeutic strategy to reduce the early development of AD pathology in DS population.

P2: Identification and characterization of miR-135-CPEB1 interactions

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MicroRNAs (miRNAs) are small non coding RNAs that act as post-transcriptional regulators of gene expression. RNA-binding proteins (RBPs) are important modulators of RNA metabolism. Growing evidences indicate a regulatory interplay between miRNAs and RBPs. miR-135a is a neuronal microRNA that we have recently characterized as regulator of neuronal transmission and anxiety behavior (Mannironi et al, 2018). CPEB1 is an mRNA-specific translational control factor, that is localised in dendrites and that regulates local translation. It binds the CPE consensus sequence present in specific mRNAs. The observation that miR-135a harbours a U rich sequence that perfectly matches the CPE sequence suggested us a possible interaction between miRNA135a gene transcripts and CPEB1. To address the hypothesis of an intermolecular interaction we performed gel binding assays of reconstituted pre-miR-135 - CPEB1 complexes. We measured the dissociation constants (K_d) between pre-mRNAs of the miR135 family and the tandem RRM domain (RRM1-RRM2) of CPEB1 and we obtained values in the 25-60 nM range. The K_ds that we obtained are the lowest determined so far between CPEB1 and a target RNA.

We suggest that the molecular interaction between CPEB1 and pre-miR135 might be relevant for miR-135 function and metabolism. Ongoing experiments are aimed to address this hypothesis.

P3: Quadrato Motor Training: change in neurotrophin levels in healthy subjects and in altered neurological conditions

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Neurotrophins are a family of growth and survival factors regulating the development and the maintenance of functional phenotype of neural cells, in both the central and peripheral nervous system. Neurotrophins and their receptors are implicated in several human pathologies such as neurodegenerative and inflammatory diseases, making these proteins target for treatment. These molecules are also closely related to stress and well-being.

Brain plasticity, or neuroplasticity, is the ability of the brain to recover and restructure itself. This adaptive potential of the nervous system allows the brain to regenerate from disorders or injuries and to reduce the effects of altered structures due to pathologies.

The neurotrophins analyzed in this study are Brain-Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF).

The *Quadrato Motor Training* (QMT) is a specifically-structured sensorimotor training, based on verbal commands, which was found to improve neuronal synchronization (increase alpha, 8-12 Hz) and to increase creativity, reflectivity, attention, as well as neuroplasticity in healthy subjects.

The molecular studies presented here show that QMT-induced morphological and behavioral changes are correlated with the modulation of salivary proNGF and proBDNF levels in healthy subjects. More interestingly, in two different case studies we observe increased levels of both neurotrophins. The cases analysed refer to dyslexia and to chronic inflammatory demyelinating polyneuropathy (CIDP).

We are currently approaching the investigation of the possible epigenetic mechanisms underlying the effects of QMT, in order to provide novel insights into the relationship between neural and molecular mechanisms.

P4: *Drosophila melanogaster* as model system of neurodegeneration

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P5: Leukocyte telomere length in Huntington's disease: a study in fully penetrant and reduced penetrant alleles

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Keywords: Huntington's disease (HD), neurodegeneration, CAG repeat expansion, leucocyte telomere length.

Introduction Huntington's disease (HD), an autosomal dominant neurodegenerative disease, is caused by an expanded CAG repeat in the first exon of the Huntingtin gene. The disease is fully penetrant in individuals with 40 or more CAG repeats, whereas it has reduced penetrance in the range of 36–39 repeats. Overall the onset of symptoms occurs at midlife and inversely correlates with the CAG repeat expansion, but it can vary widely between individuals and it accounts for about 56% of the variation in age at clinical onset, while the remaining variance is probably due to genetic, stochastic and environmental factors (1).

Human telomeres consist of repeated TTAGGG nucleotide sequences, located at the extremities of chromosomes, and play a role in preserving genome stability. Telomere shortening occurs progressively with repeated cell division because of the inability of DNA polymerase to replicate the 3′ end of the DNA strand. A cellular multiprotein complex, called telomerase, counteracts telomere shortening, but its activity, usually present in the early stages of embryonic development, is silenced in several human somatic tissues immediately after birth. As a consequence, the telomeres shorten progressively with increasing age in the replicating cells of adult tissues (2). This phenomenon may indicate cellular senescence and reflect an organism's biological age. Peripheral blood mononuclear cells (PBMC) provide an easily accessible source of cells in which telomere length can be analyzed.

Numerous studies have provided evidence for the hypothesis that leukocyte telomere shortening is associated with aging and with age-related chronic diseases. Leukocyte telomere length (LTL) was also investigated in connection with neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson disease (PD) (3,4).

In the present study we analyzed LTL in a sample of fully penetrant and reduced penetrant pre-manifest HD (FP pre-HD and RP pre-HD) and fully penetrant and reduced penetrant manifest HD (HD) patients, relating it with relevant disease parameters (number of repeats, age at disease onset, disease duration) with the aim to gain a clear picture of the relationships between telomere length and HD development and progression.

Methods. LTL (T/S ratio) was measured in manifest HD patients (HD, n=62), pre-manifest HD patients (FP pre-HD, n=38) with fully penetrant alleles, in subjects with reduced penetrant alleles (RP pre-HD, n=23), and age-matched controls (n= 76). The LTL was measured by real-time PCR quantitative analysis (qPCR) on a 7300 real-time PCR instrument (Applied Biosystems). This method allows the determination of the number of copies of telomeric repeats (T) compared to a single copy gene (S), used as a quantitative control (T/S ratio) (5).

Results. Mean LTL values of controls, pre-HD and HD patients were significantly different ($p < 0.0001$), in the order: HD (0.58±0.07) <FP pre-HD (0.78 ± 0.16) <controls (0.92 ± 0.09). Mean LTL values of RP pre-HD subjects (0.82±0.16) were significantly lower than controls ($p=0.003$), but similar to pre-HD patients (Fig. 1).

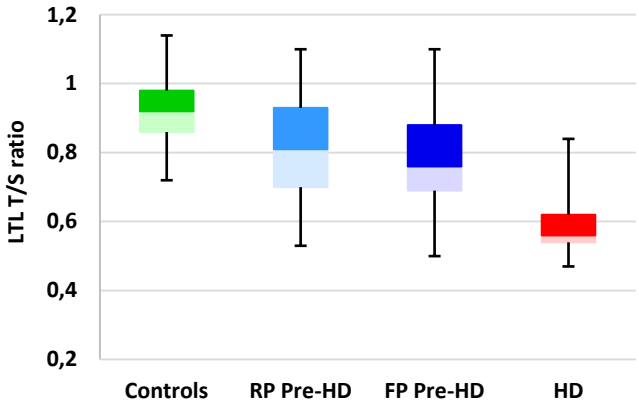

Fig. 1 Box Plot showing the distribution of LTL (T/S ratio) in controls, RP Pre-HD, FP Pre-HD, and HD patients.

The relationship between LTL and age is different in the four groups (Fig. 2). As expected, there was a downward trend of LTL with increasing age in the controls, which began to increase with advancing ages.

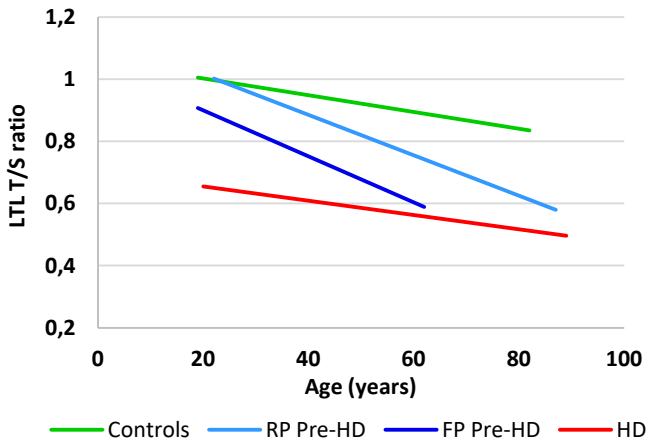

Fig. 2 LTL expressed as T/S ratio as a function of age in controls, in RP pre-HD, FP pre-HD and HD patients.

LTL of FP pre-HD and controls are not different below 30 years, but, after 30 years, LTL values are lower than those that, in controls, can be found after 80 years. In contrast, LTL of RP pre-HD and controls are not different up to 50 years, but, after 50 years of age, LTL values decrease quickly and become lower than those of 80 year controls (Fig. 3). The delay in the beginning of telomere shortening in RP pre-HD, seems to reflect literature data (6) indicating that there is a probability of about 64-74% that the disease may arise between 65-75 years respectively.

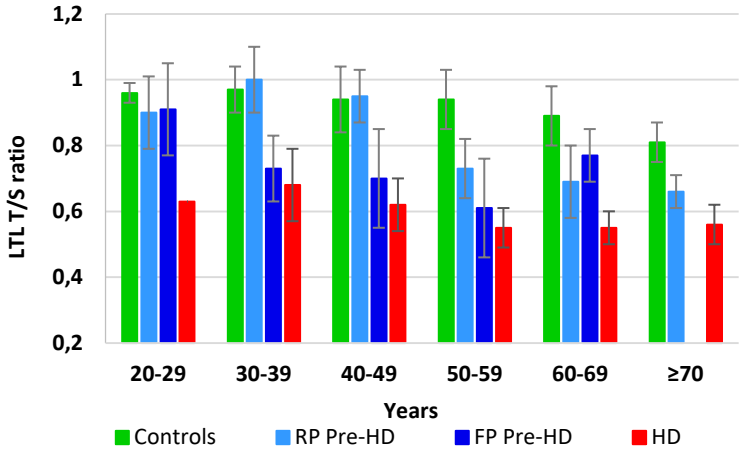

Fig. 3 LTL distribution (T/S ratio) in controls, RP pre-HD, FP pre-HD, and HD patients by age class.

Looking at HD patients, it was observed that the youngest manifest HD subjects examined (with age mostly over 30 years) showed already very short telomeres. In addition no relationship was observed between disease duration for the HD patients and LTL adjusted for age.

The CAG repeat number has also been shown to contribute to telomere shortening, mostly in FP pre-HD, with an action which seemed slightly less relevant than age (Fig. 4).

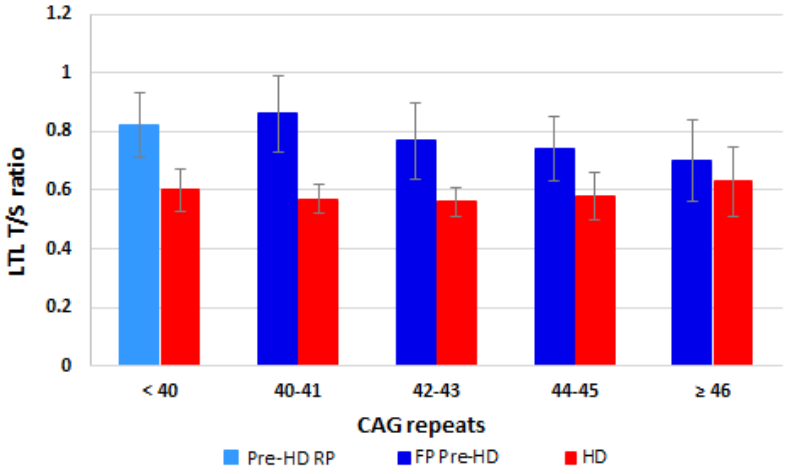

Fig.4 Relationship between LTL (T/S) and CAG repeat number.

In premanifest HD individuals, LTL was then examined in relation to the estimated time to clinical diagnosis. The estimated age at onset was calculated according to the formula of Langbehn et al. (7), at different probability values, given CAG repeat number and age at blood sampling. The years to clinical diagnosis were calculated as the difference between estimated onset and age at blood sampling. A significant linear positive relationship was observed between LTL and estimated years to diagnosis, indicating that the fewer the estimated years to HD onset, the shorter the LTL and vice-versa (Fig. 5).

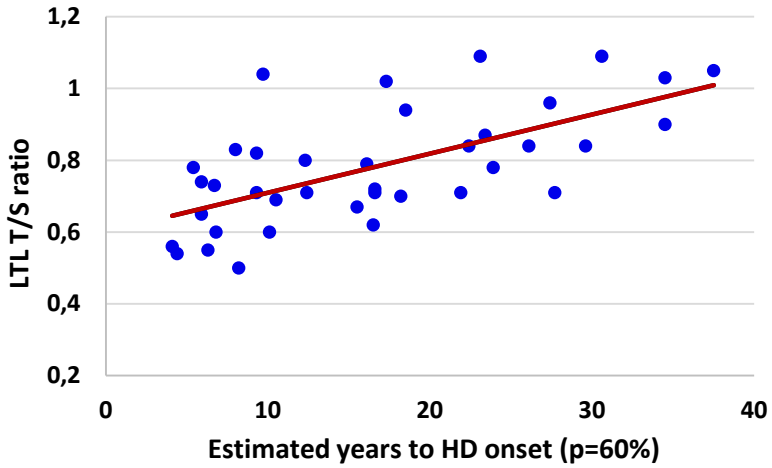

Fig. 5 Relationship between LTL (T/S) and estimated years to HD diagnosis (p=60%) in pre-manifest HD patients.

Conclusions. In pre-HD patients, LTL shorten gradually according to advancing age and CAG number, up to the low values observed in HD patients. A similar LTL shortening seems to be present in RP-HD, but at more advanced age. The different LTL shortening trends observed in FP and RP pre-HD subjects provide strong support to the hypothesis that LTL could be a reliable biomarker of HD progression. LTL measurement seems to possess distinctive features required for a suitable biomarker to detect HD progression: it is easy to obtain, readily quantifiable and reproducible, and closely linked to the pathophysiology of HD. In premanifest HD individuals, LTL shows a very significant linear relationship with the estimated years to the clinical onset of HD and could predict the time at clinical diagnosis with good probability levels (8).

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P6: TRF1 Poly ADP-ribosylation by PARP1 is required for the accomplishment of telomere replication

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Keywords: Telomeres, DNA replication, TRF1, PARP1

PARP1 is the most abundant chromatin-associated protein (1–2 million copies/cell) after histones. It is activated upon DNA damage and in turn regulate different pathways of DNA repair. PARP1 is also a regulator of chromatin structure being a component of insulator complexes, and consequently modulating transcription. At telomeres, the increased topological stress generated by G-quadruplex stabilization was reported to increase PARP1 recruitment at teleomeric sites. The Telomere Repeat Binding Factor 1 (TRF1) is a telomere specific binding protein essential for telomere replication since its lack induces telomere fragility. However, the mechanisms by which TRF1 ensures a complete error free telomere replication are not clearly understood. We found and characterized an S-phase dependent interaction between TRF1 and PARP1 leading to TRF1 PARylation. This modification impinge on TRF1 dynamics at replicating telomeres and its lacks induces an increase of telomere fragility comparable to TRF1 downregulation by RNAi, as shown by telo-FISH analysis on metaphase spreads. Moreover, PARP1 interaction with TRF1 is necessary for the recruitment of helicases known to be required for unwinding G4 structures at the G-rich lagging strand of replicating telomeres.

P7: A small molecule targeted to the microtubule–kinetochore interaction promotes autophagy by impairing autophagosome-lysosome fusion

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Autophagy is a cellular process responsible for the turnover of misfolded proteins or damaged organelles, and it also recycles nutrients to maintain energy levels for cell survival. In cancer, the role of autophagy is complex, as autophagy can either promote or inhibit tumorigenesis. Accordingly, autophagy activation can be a mediator of therapeutic resistance or play a positive role in cancer therapy by promoting autophagic cell death in tumors. Hence, the identification of novel autophagic enhancers for use in oncology is highly desirable. In our previous study, we identified SM15 as a small molecule able to bind microtubules (MT) at the interaction domain of the kinetochore protein Hec1 with high cytotoxic, pro-apoptotic and anti-mitotic activities. Herein, we investigated the molecular and functional effects of SM15 on autophagic pathways of cancer cells.

By using human tumor cell lines with different origin, we demonstrated that SM15 induces, in a dose-dependent manner, a significant increase in autophagic features, as revealed by the appearance of GFP- LC3B-II-associated autophagosomal puncta and by the immunoblot detection of membrane-bound form of LC3 (LC3B-II). Conversely, autophagic markers were unmodified after treatment with SM16, an inactive SM15 analog compound.

Combined treatment of SM15 with chloroquine, a late-stage autophagy inhibitor, demonstrated that SM15 affects autophagosome turnover through an impairment of their degradation pathway under basal conditions. Accordingly, SM15 increased the levels of the autophagy substrates p62 and NBR1 by preventing their degradation once captured at the autophagosomes. Finally, autophagosome maturation assay using RFP-EGFP-tandem fluorescent-tagged LC3B-II and autophagosome intracellular localization experiments demonstrated that SM15 blocked the fusion of autophagosomes with lysosomes. Overall, SM15 could represent a promising agent for the treatment of cancer, not only by inducing mitotic catastrophe but also by affecting autophagy.

P8: TPX2 interaction enhances the oncogenic potential of the Aurora-A kinase and can be exploited for innovative inhibition strategies

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The Aurora-A kinase is a key regulator of mitosis. The microtubule binding protein TPX2 stabilizes both Aurora-A protein levels and its active conformation, thus representing the main regulator of Aurora-A. Aurora-A and TPX2 co-overexpression has been reported in many tumor types, and their known mitotic roles suggest that chromosomal instability contributes to their transforming potential. Based on these observations we proposed the Aurora-A/TPX2 complex as an oncogenic unit and an attractive novel therapeutic target in cancer.

To address the oncogenic functions of the Aurora-A/TPX2 complex we are investigating the effects of Aurora-A and TPX2 overexpression, or co-overexpression, on chromosome segregation in non-transformed chromosomally stable cell lines. We observe cell division defects and chromosome mis-segregation events, particularly when overexpressing the whole Aurora-A/TPX2 complex. Furthermore preliminary results indicate that overexpression of the complex yields reduced p53 levels in response to prolonged prometaphase. This observation suggests that impairment of the p53-mediated response to mitotic defects may be an additional route through which Aurora-A and TPX2 overexpression contribute to tumorigenesis.

In parallel, as an innovative approach for Aurora-A inhibition, we searched for protein-protein interaction inhibitors that can disrupt the Aurora-A/TPX2 interaction. A workflow based on virtual screening followed by in vitro and cell culture assays enabled us to identify promising small molecules that are able to compete with TPX2 for Aurora-A binding. The hit-to-lead optimization of these molecules, as well as the search for the most suitable cellular backgrounds for their potential therapeutic use, is ongoing.

Overall, these results are contributing to the understanding of the effects of the Aurora-A/TPX2 complex overexpression on chromosome stability and cell transformation and to the development of innovative approaches to target the complex for anticancer therapies.

P9: The thiazole derivative CPTH6 suppresses cancer cell migration by impairing tubulin acetylation

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Recent advances in the identification and quantification of lysine acetylation by mass spectrometry have increased our understanding of acetylation, implicating it in many biological processes beyond gene transcription, through the regulation of protein interactions, activity and localization. Because these processes are often subverted in cancer, many laboratories are studying the effect of modulators of acetylation in cancer cells and are identifying their precise molecular mechanisms, producing impressive achievements in the field. Although histones are prime targets of histone acetyltransferase enzymes (HATs), and HATi were initially proposed to act as modifiers of gene expression, it is now clear that HATs target many classes of cellular proteins. In addition to chromatin, a major cellular component that is affected by modulators of acetylation is tubulin. Although it has long been known that acetylated tubulin is of crucial importance to cell division, migration, and vasculogenesis, the effect of HAT modulators on these processes is comparatively under-investigated. Herein we investigated the effect of 3-methyl-cyclopentylidene-[4-(4'-chlorophenyl)thiazol-2-yl)]hydrazone, i.e. CPTH6, which has proved very promising in our previous studies with lung cancer, on tubulin acetylation and cell migration. Using A549 lung cancer cell line as a model system, we found that CPTH6 treatment inhibits tubulin acetylation at lysine 40 residue in a concentration- and time-dependent manner. CPTH6 treatment also impairs cell migration of A549 and reduces the density of F-actin fibers and reduced the number of focal adhesions. Notably A549 expressing the K40R α-tubulin-GFP mutant show similar cell migration behaviour as compared with α-tubulin-GFP control cells. Despite this, CPTH6 effect on cell migration is reduced in A549 cells expressing the non-acetylatable K40R α-tubulin mutant. Overall, this study adds information to the role of tubulin acetylation in tumor progression, and proposes HAT inhibition as an attractive target for metastatic behavior of lung cancer.

P10: N-acetylcysteine stimulates H2S metabolism in colon cancer cells

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Hydrogen sulfide (H_2S) was recently discovered to be, along with nitric oxide (NO) and carbon monoxide (CO), a gaseous signalling molecule. Endogenously synthesized by the cytosolic enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) along with 3-mercaptopyruvate sulfurtransferase (MST) partly localized in mitochondria, H_2S is catabolized by a mitochondrial sulfide-oxidizing pathway comprising sulfide quinone reductase (SQR). Both MST and SQR contribute to formation of species with zero-valent sulfur (S^0), such as persulfides and polysulfides, collectively known as “sulfane sulfur”. H_2S plays a key signaling role in human (patho)physiology, and alterations of H_2S metabolism were reported in several cancer types, including colon cancer, where H_2S supports cellular proliferation and energy metabolism. N-acetyl cysteine (NAC) is used as a pharmacological antioxidant, though recent clinical trials showed that antioxidants may not be beneficial or even adversely affect anticancer therapy. Recently, the antioxidant properties of NAC were suggested to stem from the ability of this drug to increase the intracellular levels of sulfane sulfur. Here, working on SW480 colon cancer cells, we evaluated the effect of NAC on H_2S metabolism. After exposing cells to 10 mM NAC for 24 hours, increased expression of MST and SQR (but not of CBS or CSE) was observed, as evaluated by immunoblotting. Accordingly, after exposure to NAC, higher MST and SQR activities were detected in cell lysates, based on colorimetric and fluorimetric assays. In addition, NAC was shown to persist for at least 24 hours inside colon cancer cells, following administration at 10 mM concentration, and be able to act as a substrate for human MST, as shown with the isolated enzyme recombinantly produced in *Escherichia coli*. In conclusion, this work shows that chronic exposure of colon cancer cells to NAC stimulates H_2S metabolism, thus shedding new light on the mechanism of action of this drug and providing a possible explanation for its failure in anticancer therapy.

P11: Ochratoxin A inhibition via three synthesized flavonoids

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Ochratoxin A (OTA) is a mycotoxin whose dangers have been sufficient for many countries to regulate its presence in various foods. In Mediterranean countries, the black Aspergilli group, in particular *Aspergillus carbonarius*, causes the highest OTA contamination in fruit. Oxidative stress plays a key role in OTA biosynthesis(1), so several studies have tested food-grade antioxidant molecules, such as polyphenols, to control the biosynthesis of the toxin. Here we describe the synthesis of three molecules with polyphenolic flavonoid structure: 5,6 dihydroxy-flavone (DF), 5,6-dihydroxy-7-methoxy-flavone (negletein, NEG), and 5-hydroxy-6,7-dimethoxy-flavone (mosloflavone, MOS). The first two compounds are present in some plants used in folk medicine(2), the third one, as far as we know, has not been detected in nature. These molecules were added to OTA-producing *Aspergillus carbonarius* cultures in order to verify whether they might have a control effect on the biosynthesis of the toxin.

The most effective control of OTA biosynthesis was achieved with DF and with NEG. Fungal cultures treated with these compounds at 5, 25, and 50 ppm, showed an inhibition of OTA biosynthesis by 63% to 97% respectively, until the experiment's conclusion (8 days). On the other hand, the effect obtained by treating fungalculture with MOS was not as effective.

This behaviour is probably due to the effect of radical stabilization exerted by the hydroxy groups in cathecolic position present only in DF and NEG.

Natural compounds present in edible plants having a polyphenolic flavonoid structure may be effective inhibitors of OTA biosynthesis.

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P12: Magnetite NPs functionalized with iminosugars as drug delivery system

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Magnetic nanoparticles have been widely studied for biomedical applications, such as magnetic resonance imaging or drug delivery thanks to their superparamagnetic properties, which allow them to exhibit a magnetic behaviour under the influence of a magnetic field. This characteristic, in association with the high dispersibility in various solvents in absence of magnetic fields, provide many advantages in drug delivery, as the possibility to direct the drug-functionalized nanoparticles toward the targeted organ, reducing sides effects.[1] Among the various materials of which nanoparticles are composed, magnetite (Fe_3O_4) is biocompatible, has great chemical stability and the surface of Fe_3O_4 nanoparticles can be modified with a wide range of functional group through covalent bonds.[2]

Given the great experience of our research group developed in functionalization of magnetite nanoparticles and in the synthesis of biological active compounds called iminosugars,[3] recently we have undertaken the study of the immobilization of iminosugars on nanoparticles in order to develop a superparamagnetic drug delivery system (Figure 1). The iminosugars are carbohydrate analogues in which the endocyclic oxygen is replaced by a nitrogen atom; they are the most attractive class of sugar mimics because of their high glycosidase and glycosyltransferase inhibitor activity and hence their therapeutic potential in a vast array of diseases, such as cancer, diabetes, glycosphingolipid storage disorders and viral infections (HIV, hepatitis B and C...).[4]

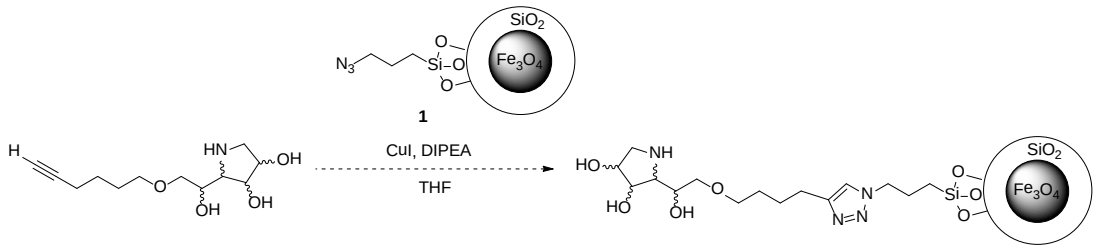

Figure 1

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P13: Artificial Intelligence in functional genomics and proteomics

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Keywords: Artificial Intelligence, Deep Learning, Machine Learning, Functional Genomics, Proteomics

Exponential increase in the volume of genomic data outputed by massive sequencing technologies, as well as their diversification (e.g. structural and functional genomic data, massive DNA sequencing as well as biomedical literature itself) are fueling proliferation of "omics" applications in biomedical science. In this fertile soil, artificial intelligence (AI) techniques have been widely applied, especially machine learning (ML), for example for pattern identification, data clustering and classification, and deep learning (DL), for example for the development of fully-automated pipelines for feature prediction (end-to-end models). This expanding research field also face several challenges that need to be addressed. These challenges arise from inner characteristics of "omics" data, such as noise, incompleteness, complexity of types and formats, and high dimensionality (consider for instance new single-cell sequencing technologies). Another fundamental yet different issue in the field of artificial intelligence methods applied to biomedical research is posed by high degree of specialization in quite heterogeneous research areas that is needed to develop adequate analytical tools. Thus the challenge to first develop a common language to productively communicate among these diverse domains of knowledge, and the accompanying risk of achieving suboptimal application methodologies due to inability of an effective interaction between these skills.

Recently (March 2019), the Italian Ministry for University and Research (MIUR) has recognized research and training in AI as a core investment for the next future in several broad areas, such as data science, cyber security, industry 4.0, environment and agriculture, and health and life science. The National Research Council (CNR), the largest public research institution in Italy, that comprises various research groups engaged to different extent and in different ways in each and every strategic area outlined by the MIUR, has immediately launched an observatory on its AI activities, as well as various thematic work tables therein. In the context of this newly established observatory, the Institute of Molecular Biology and Pathology (IBPM) has been the proponent and it is now the referent for the table on "AI for Functional Genomics and Proteomics".

The above thematic table has gathered together candidate partecipants with different backgrounds (including biochemistry, bioinformatics, biology, engineering, mathematics and physics) from many CNR's Institutes (such as IAC, IBIOM, IBPM, ICAR, IFC, IIT and IMATI), and promotes the following aims:

1. to establish a multidisciplinary work group able to meet the high synergy required in AI research among different domains (e.g mathematics, engineering, molecular biology, biochemistry)
2. to survey current applications of AI methodologies in the field of functional genomics and proteomics, ranging from base research to translational applications
3. to identify and document current 'best practices' for AI techniques applied to functional genomics and proteomics
4. to apply and, whenever needed, develop integrative, effective and innovative AI-based methods to analyze and visualize heterogeneous and multidimensional genomic data
5. to develop high-level teaching and hands-on material as well as to project and possibly realize advanced courses for training researchers in AI methodologies for functional genomics and proteomics

Proceedings of the CNR's Observatory table on "AI for Functional Genomics and Proteomics" can be accessed at the following WWW address: https://bit.ly/2ZpcBAW.

P14: Non-enzymatic oligomerization of 3'-5' cyclic ribonucleotides in prebiotic conditions

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Extant biological DNA and RNA syntheses are based on template copying by highly evolved polymerases and high levels of adaptation and refinement have been achieved in studies on the *in vitro* evolution of polymerizing enzymes (1). Complex chemistries involved in the non-enzymatic polymerization of high-energy monomers to nucleic acids are also well described in the literature (2). However, these are all compounds whose likelihood of prebiotic availability and accumulation is inversely proportional to their intrinsic stability and the elaborate chemistry necessary for their synthesis. In brief, the prebiotic generation of RNA remains undeciphered.

We explored in prebiotic conditions the non-enzymatic polymerization of cyclic nucleotides. Such a template-free polymerization reaction is preceded by the self-assembling of the cyclic precursors utilizing stacking interactions, which mediate the trans-phosphorylations among the pillared monomer units, resulting in covalently bound oligonucleotides. The conditions allowing this chemistry necessarily differ among the different nucleotides and depend on the propensity of the monomers to participate in various intermolecular interactions. Thus, in order to reconstruct the series of chemical events that eventually led to the prebiotic non-enzymatic synthesis of mixed-sequence RNA, the polymerization of each cyclic nucleotide requires a dedicated specific analysis. Starting by our observation of abiotic phosphorylation of nucleosides using phosphate minerals as source of phosphate and the spontaneous formation of cyclic nucleotides, the non-enzymatic polymerization of 3',5'cGMP, 3',5'cAMP, and 3',5'cCMP has been obtained and characterized (3,4,5).

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P15: The long noncoding RNA HOTAIRM1 contributes to neuronal differentiation by regulating Neurogenin 2 expression

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Neuronal differentiation is a timely and spatially regulated process, which relies on precisely orchestrated gene expression control. The sequential activation/repression of genes driving cell fate specification is achieved by complex regulatory networks, where transcriptional factors and noncoding RNAs often work in a coordinated manner. Here we assign a new function to the long noncoding RNA HOTAIRM1 as a regulator of the proneural transcription factor *Neurogenin 2*, that is key to the commitment to neuronal fate and critical for normal brain development.

We characterize the neuronal isoform of HOTAIRM1 and demonstrate that the nuclear transcript controls the highly transitory expression of *Neurogenin 2*. Exploring the underlying mechanism, we show that HOTAIRM1 acts as an epigenetic regulator that, recruiting the repressive complex PRC2, contributes to limit the time-window of *Neurogenin 2* expression in differentiating neurons. The regulatory effect on the downstream neurogenic gene cascade activated by NEUROGENIN 2 reveals that HOTAIRM1 contributes to the achievement of proper neuronal differentiation timing.

P16: Comprehensive analysis of β and γ Human Papillomaviruses in actinic keratosis and apparently healthy skin of elderly patients

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Background. Actinic keratosis (AK) is an in situ carcinoma occurring in photo-damaged skin, which can potentially progress to invasive squamous cell carcinoma (SCC). Many findings support the role of cutaneous β human papillomaviruses (HPVs) in AK and cutaneous SCC.

Objective. In this cross-sectional study, the presence of β and γ-HPVs was investigated in apparently healthy skin (HS) and AK samples of immunocompetent elderly patients.

Methods. DNA was extracted from AK and HS samples collected from 244 patients. Prevalence of cutaneous HPVs was determined through a highly sensitive assay based on type-specific multiplex PCR and Luminex technology, which detects 46 β- and 52 γ-HPV genotypes.

Results. The majority of the patients (85.8%-98.3%) were positive for β and γ-HPVs and harbored multiple infections (77.6%-98.6% of the HPV-positive subjects) both in HS and AK. The median number of genotypes per sample was significantly higher in HS than AK (11 vs 6 β-HPVs, $p<0.0001$; 4 vs 2 γ-HPVs, $p<0.0001$). HPV38-β2 and HPV5-β1 were the most frequent β-types, while HPV-SD2 was the most prevalent γ-type both in AK and HS. Apart from HPV4-γ1, prevalence of the individual β and γ-types was higher in HS than AK samples. Semi-quantitative viral loads were also lower in AK compared to HS.

Conclusions. A large spectrum of β and γ-HPVs was detected in HS and AK. For both genera, prevalence, number of genotypes, and semi-quantitative viral loads were higher in HS than AK samples, further supporting the hypothesis for an early-stage role of viral infection in skin carcinogenesis.

P17: Nuclear transport receptors Importin beta-1 and CRM1 synergise with microtubule-targeting drugs used in cancer therapy

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The design of synthetic lethal therapies for cancer aims to exploit molecular vulnerabilities present in cancer cells that may sensitise them to specific therapeutic molecules, so as to increase the selectivity and specificity of the treatment(s) in a personalized medicine perspective. This idea currently represents a major avenue in the cancer therapy field.

Importin beta-1 and CRM1 (exportin-1) are nuclear transport receptors that respectively transport proteins into, and out of, interphase nuclei. After nuclear envelope breakdown, they both localize to the mitotic apparatus and are required at many steps of mitotic progression. Both importin beta-1 and CRM1 are overexpressed in many cancer types that display high levels of genetic instability as shown by published data and bionformatic screening (TCGA atlas).

We have engineered stable cell lines overexpressing either importin beta-1 or CRM1 in an inducible manner to characterize their mitotic functions. We find that both, when overexpressed, induce abnormalities in spindle organization, chromosome misalignment, and chromosome segregation. We identify both common targets (e.g. components of the SUMO pathway), and specific interactors that may mediate these effects: importin beta-1 interacts with mitotic microtubule regulators, while CRM1 interacts with survivin, a chromosomal passenger protein. These experiments suggest that both importin-β and CRM1 have roles in regulating the spindle microtubule dynamics via the interactors with which they interplay.

We surmised that both nuclear transport receptors may also modulate the cancer cell response to microtubule-targeting agents used in cancer therapy. Indeed, time-lapse recording assays show that overexpression of both Importin beta-1 and CRM1 sensitise cancer cells to anti-microtubule drugs (e.g., taxol, nocodazole), and synergise with these drugs to increase mitotic cell death. The data reveal a dual role of nuclear transport receptors: they act as pro-oncogenic factors in inducing chromosome segregation errors and genetic instability, a major cancer hallmark, yet at the same time confer a therapeutic vulnerability to anti-mitotic drugs in cancer cells in which they are overexpressed.

P18: Protein SUMOylation in control of cell division

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Mitosis is a finely tuned cell cycle stage, during which replicated chromosomes are equally partitioned into daughter cells. Spatial and temporal regulation of mitotic factors is essential to proper division. Several post-translational modifications (e.g. phosphorylation, ubiquitination) regulate the functional state of mitotic regulators. Growing evidence highlight the importance of protein conjugation with SUMO peptides (SUMOylation) which regulates their subcellular localization and interactions with partners, and is frequently associated with dynamic processes.

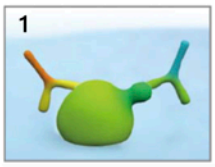
RANBP2 is a large nucleoporin with SUMO E3 ligase and SUMO-stabilizing activity. In interphase it contributes to regulate the directionality of nucleo-cytoplasmic transport. After mitotic onset and disassembly of nuclear pore complexes, it localizes to the mitotic spindle and in part to kinetochores.

We have combined in situ proximity ligation assays (PLA), live imaging and phenotype analysis, and have identified at least three RANBP2-guided mitotic processes that depend on timely SUMOylation:

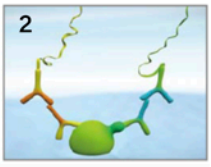
- Topoisomerase II-alpha recruitment at centromeres, required for decatenation of sister kinetochores prior to anaphase onset;
- Concentration of the Aurora-B kinase at kinetochores, which monitors errors in kinetochore-microtubule attachments;
- Localisation of NuSAP1 (nucleolar and spindle-associated protein) at microtubule plus-ends, required to stabilise microtubule interactions with kinetochores.

These results show that RANBP2 is required for SUMOylation of key kinetochore factors, and hence ensures their localisation and function during mitosis. Failure of these mechanisms affects chromosome segregation and can facilitate the onset of genetic instability typical of cancer cells.

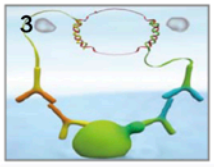
The intramolecular PLA (Proximity Ligation Assay) depicts SUMOylated proteins *in situ*



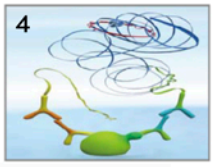
Primary antibodies against the target protein (here, Aurora-B) and SUMO peptide



Secondary antibodies conjugated to oligo tails

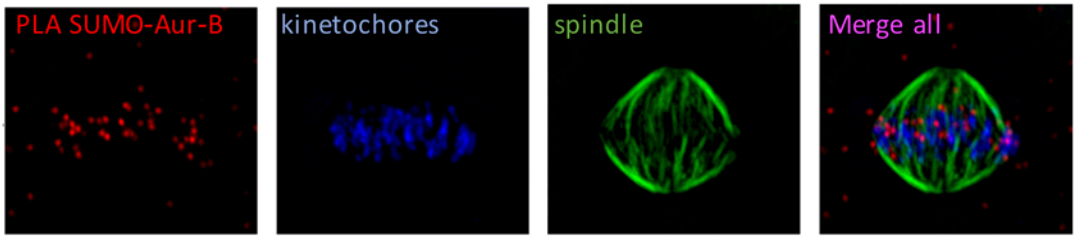


Add connecting oligos + DNA ligase → DNA circle forms → amplification



A complementary fluorescent probe reveals rolling circle amplification → depicts sites of protein SUMOylation

PLA visualization of SUMO-conjugated Aurora-B in mitotic cells



P19: Spindle orientation is regulated by the Aurora-A/TPX2 axis and contributes to faithful chromosome segregation

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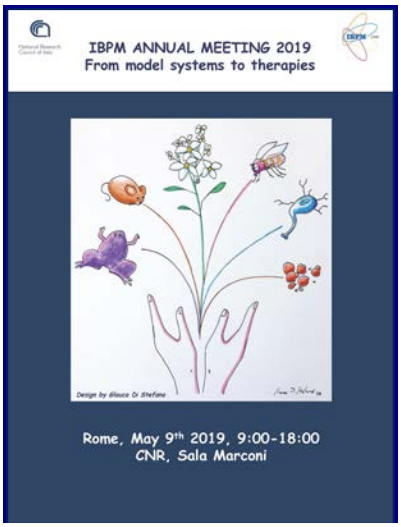
Mitotic spindle orientation is a key process that determines the orientation of cell division, and is essential for cell fate decisions and tissue morphogenesis; whether it also contributes to the fidelity of chromosome segregation is not yet clarified. The Aurora-A kinase (Aurk-A) is a key regulator of spindle assembly; it is overexpressed in tumours together with its major regulator TPX2 and their involvement in tumorigenesis is under active investigation.

We previously characterised a role of Aurk-A in spindle orientation in human cells, through phosphorylation of the NuMA protein, a major regulator of this process. We now show that formation of the Aurk-A/TPX2 complex is required for correct NuMA localization and spindle orientation; we also find that TPX2 and NuMA are part of a protein complex at the mitotic spindle that we are currently characterising. Excess Aurk-A and TPX2 in non-transformed cells also affect spindle orientation, although in opposite ways, depending on their distinct influence on microtubule stability. In parallel, we observe that mis-oriented cell division induced by Aurk-A inhibition increases the occurrence of chromosome mis-segregation events. Independently interfering with spindle orientation, through inactivation of the orientation factor LGN, yields telophase chromosome bridges and micronuclei, and a subsequent G2 arrest in p53-proficient cells. These results suggest that Aurk-A and TPX2 are required for spindle orientation through regulation of both NuMA localisation and microtubule stability, and support the hypothesis of a link between spindle mis-orientation and chromosomal instability, of potential relevance in cancer.

CONCLUDING REMARKS

Patrizia Lavia, IBPM Acting Director



The aim of our annual meeting is to present the Institute's most promising studies and discuss our research perspectives.

From the outline of the forthcoming EU research programmes to be launched in 2021 to the topics covered in scientific conferences of broad vision, such as the Nature Conferences for example, which are important venues to set the trends of future research in life sciences, we see an increasing demand to take the depth of scientific knowledge to translational applications in biotechnology and health. In particular, there is a need to expand our understanding of the molecular mechanisms of vital processes towards developing innovative precision therapies for various diseases.

Reasoning along these lines, in the 2019 edition of the IBPM annual meeting we have chosen to focus on two interconnected themes:

- **The field of drug discovery**, including the analysis and identification of novel targets, the design of bioactive molecular tools, and the development of approaches guided by structural biology, molecular biology, genetics, epigenetics and the knowledge of the non-coding genome;

- **Model systems**, a research area that IBPM has pioneered and in which it is internationally recognized; several systems are being developed to understand molecular pathways relevant to design disease and therapy models.

http://www.cnrweb.tv/ibpm-cnr-focus-su-malattie-neurologiche-e-e-oncologia/

The first session on drug discovery was opened by Prof. Ferdinando Squitieri, head of the Huntington Unit and rare diseases of the IRCCS Casa Sollievo della Sofferenza, and scientific director of the LIRH-Italian League for Huntington research. Prof. Squitieri

has recently become an IBPM associate.
Prof. Squitieri illustrated the historical development of our understanding, since its early discovery, of Huntington's chorea, a severe neurodegenerative genetic disease that affects muscle coordination, causing typical uncontrolled movements and a progressive cognitive decline. The disease is caused by a mutation in the huntingtin protein gene; the mutation alters the conformation of the protein, which forms aggregates in the central nervous system with severe neurotoxic effects. Squitieri emphasized the power of networking and multidisciplinarity, from classical genetics to next generation sequencing, from clinical observations to experimental neurosciences, from developmental biology to the engineering of models capable to recapitulate the phenotypical heterogeneity of the disease. Current research efforts aim to counteract the effects of mutant huntingtin, trying to prevent its accumulation in toxic concentrations. Squitieri summarized the most promising directions: "we will try it first in symptomatic adults, next in people who do not yet display the symptoms and eventually in early onset patients, as recent research shows that the disease can affect even young children, with developmental alterations in the nervous system that produce invalidating characteristics even more severe than in adults". A collaborative research project on the juvenile form of Huntington's disease includes IBPM researchers who use structural biology methods to understand the nature of the aggregates formed by huntingtin mutant versions.

Still in the first session, IBPM researchers also discussed novel results obtained from diversified approaches to drug discovery, including:

- molecular modeling studies of potentially targetable proteins and their mutations in cancers,
- the screening of libraries of molecules based on computer-guided target recognition, including at the high throughput level, a promising approach to identify molecules of potential therapeutic value against specific antigens associated with infectious agents,
- the design of targeted peptides as potential drugs in several pathological contexts,
- developments in cryo-electron microscopy to resolve macromolecular complexes of diagnostic or therapeutic significance (target/drug or drug/receptor complexes);
- progress in research on optimized nanovectors based on the human ferritin protein. The nanovectors are being assayed as novel drug delivery systems in cancer models in vivo for their ability to bind the ferritin receptor, which is overexpressed in cancer cells: after encapsulating chemotherapeutic drugs, they can selectively release them in cancer tissues that preferentially express the ferritin receptor. Preclinical trials show far higher therapeutic efficacy compared to conventional drug administration protocols.

The second session on model systems was opened by Prof. Giulio Cossu, Chair of Regenerative Medicine at the University of Manchester and member of the CNR Scientific Council.
Prof. Cossu reviewed the challenges in the journey towards an effective therapy for Duchenne dystrophy. He recalled key steps in understanding defects in the genes responsible for muscle function and, subsequently, in devising a therapy based on providing the patient with cells carrying the healthy gene that would colonize and repair

dystrophic muscles. A highly valuable conceptual and therapeutic achievement came with his realization that it was possible to "instruct" mesangioblasts, multipotent cells derived from blood vessel endothelia, to behave as muscle cells. This cell-based therapy approach has proved effective in animal models of dystrophy (Mdx mice). Many challenges, however, remain unresolved in human therapy, entailing multiple issues, not least the therapy starting time with respect to the age of onset. Cossu has retraced the complexity, from the molecular to the organismic level, that can affect the therapeutic outcome. The lecture was a highly inspiring science lesson in illustrating an instructive and difficult path.

IBPM groups followed up onto the complexity described by Cossu. They discussed alternative therapeutic approaches to Duchenne dystrophy, including strategies based on transcriptional up-regulation of compensatory genes, and epigenetic modulation of genes that modify the phenotype severity.

Other important aspects in this session reviewed the use of model systems to address complex pathological and physiological processes, including Drosophila models to understand the genetic pathways altered in rare genetic pathologies, yeast to study epigenetic alterations implicated in human tumorigenesis, Arabidopsis to reconstruct the molecular mechanisms underlying morphogenetic and developmental processes driven by the response to light.
New cellular methods with a potential to reach new information levels, developed at IBPM, were also illustrated:

- in immunology, the development of new cytofluorimetric protocols has enabled a novel understanding of the dynamics of proliferation and expansion of T-cell populations responsible for immune memory,
- in oncology, protocols for cultivating three-dimensional organoids have been successfully developed; in the future, deriving organoids from biopsies of cancer patients will be highly beneficial to the design of personalised therapies.

It was not possible in our one-day meeting to present all research studies relevant to the objectives addressed by the conference. Many new results and ongoing projects were presented as posters.

Synthesis and synergies.

To keep up with current challenges and advances in molecular biology and pathology worldwide, it is necessary to systemize all the scientific, experimental and instrumental competences available to IBPM researchers. The complementarity of scientific and experimental expertise at IBPM can expand the amplitude and variety of experimental approaches and help to develop a global view, at the "systems" level, of complex processes and pathways. In this direction, IBPM researchers have already presented the "Neuronet" consortium in the 2018 conference, aiming to enhance synergy and complementarity across disciplines and competences in the field of neurooncology and neurological and neuromuscular diseases. A similar effort has now been made in the field of pediatric diseases (comprising oncological and developmental syndromes) with the establishment of the "IBPM group for pediatric diseases". The group, illustrated at the

2019 conference, includes 26 IBPM researchers who participate both as providers and users of the EPTRI network (European Pediatric Translational Research Infrastructure), an infrastructure dedicated to pediatric medicine of the future.

The future.

An aspect that could not be discussed in the conference, but worth following up in the near future, concerns the development of the IBPM portfolio of facilities: from cellular imaging, which we intend to develop at the high-throughput level to analyze large numbers of cells in dynamic processes (e.g., response to external stimuli, differentiation, response to drugs, cell death induction) to cryogenic electron microscopy (Cryo-TEM); from structural biology to bioinformatics; part of these facilities will expand thanks to two PON grants for research infrastructures, EuroBioImaging and Elixir, to which IBPM participates. Recently, IBPM has also joined the CNR "observatory for artificial intelligence", and therein coordinates the working group on functional genomics and proteomics.

Highlights of the 2019 conference were also given with the excellent presentations delivered by three young researchers (PhD students or recent PhD awardees). They demonstrated scientific proficiency and insight in structural biology, immunology, and plant biology, as well as passion and momentum.

The IBPM, as other CNR institutes, is now at a time of transition. IBPM has attracted many young researchers who have recently been recruited to permanent positions, now representing more than 10% of permanent staff. The recruitment program ought to be completed by the end of 2019 and we expect that still 4 or 5 more researchers will join. These researchers will now start their own independent scientific careers at CNR. There are concerns related primarily to the lack of sufficient resources and also to more general issues on research policy. Despite these concerns, the 2019 conference has shown that, with everyone's contribution, solid foundations are present to strengthen synergies and develop the Institute's scientific potential, with a younger generation ready to kick off.

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