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Organizers: V Scarlato, D Roncarati

Tuesday, July 23, 2019

Giacomo Vezzani (34, Scarlato / Frigimelica / Merola):
Selection and characterization of human monoclonal antibodies to HCMV isolated from convalescent and/or chronically-infected subjects to guide vaccine antigen design

Human cytomegalovirus (HCMV) is a herpesvirus that causes serious damages in immunocompromised individuals or in fetuses of mothers experiencing primary infection. To date, there are no efficacious vaccines licensed, although several different vaccine candidates are being developed.
Many HCMV antigens are under investigation as vaccine candidates, as the glycoprotein-B (gB).
This protein is essential to virus entry into eukaryotic cells and undergo a change between its prefusion conformation (before the virus entry in the cells) and postfusion conformation (after the virus entry). To date, only postfusion conformation has been utilized as vaccine candidate, because no one determined the structure of the prefusion gB. We believe that, as previously shown with respiratory syncitial virus (RSV), the protein in its prefusion conformation will lead to a higher immune response than the one postfusion.
Therefore, we propose to isolate, from human plasmablasts of cronic HCMV infected donors, anti-gB humAbs to be used as stabilizers of prefusion gB conformation, taking advanttage of the B cell platform developed in our laboratories. Antibodies will be expressed in eukaryotic cells and screened for their capability to neutralize HCMV infection in an in vitro system. Neutralizing anti-gB pre-fusion Fabs will be cloned into eukaryotic expression vector to be transiently co-expressed with the secreted soluble gB ectodomain, based on the concept that Fabs with relative high affinity to prefusion gB should be able to prevent its irreversible conformational change to postfusion.
Purified complexes will be subjected to X-ray crystallography and cryoEM to determine the structure of prefusion gB to drive the preclinical design of a novel vaccine antigen, a potential game-changer in the field of HCMV vaccinology.

Viola Viviani (33, Scarlato / Bartolini):
Dissection of the meningococcal protective immunosignature elicited by 4CMenB vaccination

Neisseria meningitidis detergent-extracted outer membrane vesicles (DOMVs) are one of the components of the 4CMenB vaccine. DOMVs have been shown to be safe, highly immunogenic and able to raise bactericidal antibodies directed against multiple antigens. Nevertheless, except for the immunodominant PorA protein, the relative contribution of other DOMV minor antigens in eliciting protective antibodies has not been fully elucidated.
Here, we investigate DOMV immunogenicity by dissecting the functional role of associated antigens. Thirty out of the 31 proteomic-prioritized DOMV-specific proteins have been cloned and expressed in *E. coli* OMVs and 11 were also purified as recombinant proteins. Obtained antigens were then included in a protein-microarray and used to fingerprint sera response to DOMVs. Simultaneously, the purified OMVs and the recombinant proteins were also tested in vivo to assess the immunogenicity and the functionality of DOMV-specific antigens. Western-blot analysis highlighted that generated antisera recognize the antigen expressed by different meningococcal strains and ELISA results revealed comparable immunogenicity levels when the antigens were expressed in OMVs or administered as recombinant. However, none of the proteins was positive when tested in serum bactericidal assay. These results suggested either that the anti-DOMV functionality might be mediated by multiple factors that act in synergy rather than single minor antigens or that the panel of potential functional antigens has to be enlarged. To this aim we performed 2D-blot and *E. coli* surface display library studies.

Collectively, these synergistic strategies can lead to the deconvolution of novel immunodominant antigens contributing to the protection induced by the DOMV-component of the 4CMenB vaccine.

Benedetta Manca (34, Scarlato / Giuliani):
Genetic and proteomic investigation of the role of the Surface Lipoprotein Assembly Modulator (SLAM) proteins in translocation of immunogenic surface lipoproteins in *N. gonorrhoeae*

Surface lipoproteins (SLPs) are often very successful antigens in protecting against bacterial diseases as they are immunogenic and play key roles in pathogenesis. SLAM (surface lipoprotein assembly modulator) has been recently identified as an outer membrane protein (OMP) that translocates or flips lipoproteins across the outer membrane, and multiple SLAM homologues are present in many Gram-negative bacteria presumably with different specificities for lipoproteins that they flip.
In gonococcus, there are 3 SLAM homologues and the goal of this project is to characterize the role of each of these proteins in translocating lipoproteins onto the surface of gonococcus. Knockout mutants have been generated to each of the SLAM homologues in FA1090 reference strain. In order to identify the specific substrates for each of the SLAM translocators several techniques will be applied and in particular Proteinase K will be used to 'shave' the bacterial surface while a non-permeable fluorescent dye will be applied to specifically stain exposed protein residues for pull-down experiments. Both techniques will be coupled to tandem mass spectrometry experiments to identify the lipoproteins that are surface exposed and mislocalised in the absence of each SLAM variant.
The specificity of each SLAM for its substrate SLP will be examined through analysis of conserved domains and site directed mutagenesis to reveal the specific trafficking signals of the SLPs to the surface via the SLAM translocators.
Finally, deletion mutants of the new SLPs will be generated in order to perform phenotypic analyses and identify possible functions for each of the lipoproteins.

Stefano Miglietta (33, Porcelli):
MCJ/DNAJC15: role in mitochondrial metabolism of chemoresistant ovarian cancer cells

Ovarian cancer (OC) is the seventh most common cancer in women and the eighth most common cause of cancer death. OC cells display flexibility in mitochondrial bioenergetics that has been related to metastatization and chemoresistance. Recently, it has been reported that chemoresistant ovarian cancers are more dependent on glycolytic metabolism rather than on oxidative phosphorylation. Further, several studies have showed that the hypermethylation of *DNAJC15* gene is associated with cisplatin-chemioresistance in OC (cisR-OC). This gene encodes for MCJ protein, a mitochondrial co-chaperone protein involved in the assembly of supercomplexes of the respiratory chain and able to modify the bioenergetic profile of cells. However, whether and how MCJ impacts on the cisplatin-chemoresistance mechanisms is still not understood. Hence, the aim of this study is to clarify the role of MCJ in the molecular mechanisms underlying the cisplatin-chemoresistance and investigate its impact on mitochondrial metabolism and dynamics. The overexpression of DNAJC15/MCJ in chemoresistant and chemosensitive ovarian cancer cells, leads to increases levels of supercomplexes and isolated complexes of the respiratory chain, and mitochondrial protein content in cisR-OC cells. Further, the MCJ overexpression has been associated with the stimulation of oxygen consumption rate, the increases of oxidative stress levels and, in turn, the expression of antioxidant enzymes. Overall, these preliminary data suggest that MCJ triggers an oxidative metabolic switch in cisplatin-chemoresistant OC cell line similar to what occurs in chemosensitive counterpart. Hence, this study may provide useful insights on OC metabolism rewiring, revealing MCJ as a new potential target for cancer therapy.

Serena Jasmine Aleo (34, Rugolo / Ghelli):
Mitochondrial function in the α-galactosidase A knock out mice model of Fabry Disease

Fabry disease (FD) is a rare genetic disorder associated with mutations in the GLA gene, located on the X chromosome, causing partial or complete loss of function of the lysosomal enzyme α-galactosidase A (α-GAL A), and resulting in multi-systemic clinical manifestations. The cellular pathophysiological mechanisms underlying α−GAL A deficiency are still poorly known.

Recent evidence uncovered the role of mitochondrial dysfunction on the pathophysiology of several lysosomal storage diseases. Interestingly, a strong reduction in the activity of complexes I, IV and V of oxidative phosphorylation was previously reported in fibroblasts derived from FD patients. To prove the occurrence of a mitochondrial dysfunction, we decided to take advantage of the α-GAL A-knock out mice, a recognized model of this disease. Heart, kidney and brain tissues, known to be affected in FD patients, were collected from α-GAL A-KO mice of 8, 16-20 and 52 weeks and age-matched controls (WT). The CI, CIII and CIV activities were determined in isolated mitochondria by spectrophotometric assays, after normalization for citrate synthase activity. No significant difference in the respiratory complexes activities was observed, except in the brain of the 52 weeks-old α-GAL A-KO mice, where a marked increase in CIII and CIV activities was apparent compared with WT, age-matched mice. Western blot analysis failed to reveal any significant change in the level of representative subunits of oxidative phosphorylation complexes, suggesting that enhanced CIV activity was not due to increased mitochondrial biogenesis. Perturbation in the levels of mitochondrial and/or cytosolic antioxidant defenses is under investigation.

Simona Paglia (33, Grifoni / Pession):
Genetic and molecular analysis of the contribution of cell polarity disruption to brain cancer

The poor prognosis associated with adult brain tumours accounts on resistance to therapy and consistent relapse. Primary glioblastoma, a severe form of brain cancer, displays early PTEN inactivation, and cancer stem cells show a dysfunctional PTEN/aPKC/Lgl axis. After showing this axis is conserved in Drosophila, I impaired its function in the fly's neural stem cells, causing neoplastic growth and formation of tumour masses that kept growing in the adult brain, leading to premature death.
The glioblastoma cell of origin is still under debate; then, in order to understand what neural population is more susceptible to alterations in the PTEN/aPKC/Lgl axis, I induced its dysfunction in distinct progenitor subtypes of the Drosophila brain, so as to identify those responsible for cancer initiation. My results demonstrated that only the type II neuroblasts (NBs) are prone to undergoun restrained growth following dysfunction of this axis. Of note, those type II NBs generate mature neurons and glia through transit-amplifying progenitor cells, as it happens in the human brain.
It is our interest to identify the contribution of the PTEN/aPKC/Lgl axis to the complex molecular pathogenesis of brain cancer: to this aim, we are going to carry out an RNAseq analysis of brain cancers from our fly model, in the hope to isolate some relevant molecole which,in collaboration with the Ottawa Hospital Research Institute, will be validated and studied in patient-derived glioblastoma cell lines. The manipulated cells will also be injected intracranially in SCID mice to observe major changes in cancer growth and aggressivity.

Ylenia Beniamino (34, Zambelli / Ciurli):
Nickel and human health: structural and biophysical characterization of NDRG1, a protein involved in nickel-dependent carcinogenesis

Nickel, the twenty-fourth most abundant element in the Earth's crust, is an essential element for bacteria and lower eukaryotes that use this metal as co-factor in important metallo-enzymes. In humans, nickel exposure can induce the hypoxic response, promoting cancer and metastasis de-velopment [1] and activating the expression of different genes, including N-myc downstream regulated gene 1 (NDRG1) [2].
This gene codes for the human protein NDRG1, belonging to NDRG family protein. The members of this family share a N-terminal domain with an α/β hydrolase fold, lacking the catalytic triad residues. Only NDRG1 features a three-times repeated sequence of ten residues in the C-terminal domain. This sequence presents Ni(II) binding activity, suggesting a link between the induction of NDRG1 by nickel compounds and its activity as metal binder [3][4]. Despite the numerous studies highlighting the role of NDRG1 in carcinogenesis, its structure, interactions and mechanism of ac-tion are undiscovered [5].
My work focuses on these aspects, at the molecular level, involving a structural, biophysical and functional characterization of the protein. I developed protocols for heterologously expressing and purifying the wild type NDRG1, the α/β hydrolase-like N-terminal domain, and the C-terminal Ni-binding domain from Escherichia coli. Disorder prediction analysis in silico, circular dichroism, light scattering and isothermal titration calorimetry were performed to investigate the structural properties of the purified proteins and to study their metal binding activity. A model structure of the N-terminal domain was generated through homology modelling.

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Anna Galea (32, Norais / Scarlato):
Development of new analytical tools for characterization of bioconjugate vaccines in *E. coli*

Glycoconjugate vaccines have been proven to be efficacious and cost effective in the prevention of several infectious diseases. In the last decade, among the approaches for glycoconjugate vaccine, a new technology which exploits the bacterial N-glycosylation is emerging. Bioconjugation technology is based on the production of glycoproteins in vivo, in which the glycosylation machinery PglB-based from *Campylobacter jejuni* is co-expressed in *Escherichia coli* cells, with a pathogen polysaccharide chain and a target carrier protein, acceptor of the polysaccharide. PglB is an oligosaccharyltransferase able to covalently link an oligosaccharide chain to Asparagine residue in the specific consensus sequence on the carrier protein-acceptor by covalent linkage (1). The consensus sequence required for bioconjugation by *C. jejuni* PglB has been well characterized and consists in D/E(-2)-X(-1)-N-Y(+1)-S/T(+2), where X and Y can be any amino acid except for Proline (2).
Despite the increasing interest to produce bioconjugate vaccine candidates, the analytical tools required for their characterization are still missing. The quantification of the extent of glycosylation remains a challenging task to achieve, although this information is fundamental to respond to potential regulatory expectations and to monitor antigen production and characterization.
In this work, we report an analytics driven design approach to develop bioconjugate vaccine candidates suitable for their characterization by Mass Spectrometry. The *Staphylococcus aureus* Hla antigen, substrate for bioconjugation with *S. aureus* serotype 5 capsular polysaccharide (3), was used as a proof of concept to design new consensus sequences suitable for the quantification of the extent of conjugation by Selected Reaction Monitoring-MS approach. Three new consensus sequences were designed and the degree of glycosylation of Hla was determined. Moreover, to achieve a multiple glycodisplay on Hla protein, the design of new engineered Hla protein, carring two or more different consensus sequences is under investigation.

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Jacopo Rossi (34, Trost):
Structural comparison and biochemical analysis of alcohol dehydrogenases from *Arabidopsis thal*iana

Alcohol dehydrogenases (ADHs) are Zn-binding enzymes part of a well-characterized protein superfamily called medium-length dehydrogenase/reductase (MDR). In its active dimeric form, ADH is mostly known for its involvement in the final step of the alcoholic fermentation where it reduces toxic acetaldehyde into ethanol regenerating NAD+. This mostly occurs in plants under stress conditions such as oxygen deprivation. To date, plants carry various ADH genes, among which it is possible to find glutathione-dependent formaldehyde dehydrogenase part of a so-called group of class III ADHs. This enzyme has been described for its ability to reduce the nitrosylated form of glutathione (GSNO) using the reducing power of NADH and thus being one of the major regulator of protein S-nitrosylation in plants. In the most common model organism for plant biology, Arabidopsis thaliana, this enzyme, namely ADH2, shares high sequence and structural homology with the classic alcohol dehydrogenase ADH1, although they are involved in completely different metabolic pathway while also sharing differential expression patterns. As part of my first PhD year I analyzed and compared these two enzymes from *A. thaliana*. We started with an in silico structural comparison

followed by a heterologous expression and a detailed biochemical characterization. In the end, we also evaluated the activity of the two ADHs using protein extracts from mutant plants lacking one of the two gene also considering the effect of hypoxic stress on the expression and functionality of the proteins.

Wednesday, July 24, 2019

Marco Russo (32, Capranico / Marinello):
A multi-omics analysis of G-quadruplex as a novel target to modulate innate immune response in cancer cells

G-quadruplexes (G4s) are non-canonical DNA structures, notably located at telomeres and oncogene promoters. G4 motifs can be structurally compatible with R-loops, which consists of a DNA-RNA hybrid and a displaced ssDNA. G4s stabilization and R-loops accumulation have been associated with genomic instability and DNA damage. Recently, we proposed that stabilization of G4 structures with specific ligands leads to accumulation of DNA damage and genome instability in cancer cells and that this process is mediated by R-loop stabilization. Moreover, we found that G4 ligand pyridostatin stimulates formation of micronuclei, a hallmark of genome instability. Micronuclei accumulation has been associated with innate immune response triggering through cGAS/STING pathway activation. Activation of this pathway leads to the expression of cytokines, interferons and interferon-stimulated genes. Innate immune system modulation has been proposed as a promising therapeutic strategy for cancer treatment. To clarify the role of cGAS/STING pathway and the effects of its perturbation in human tumor tissues, we analyzed mutations and expression levels of genes involved in this pathway across 31 cancer types and ~7800 tumor samples from The Cancer Genome Atlas (TCGA). Alterations in mutation status or expression in these genes have been related with innate immune response activation and patient survival and other immune tumor microenvironment features. Our findings indicate that these genes are rarely mutated in human cancers, while their expression may affect the interaction of the tumor with host immune cells affecting disease progression and patient survival.

Ottavia Tartagni (34, Zuccheri):
Reproducible growth of 3d cell spheroids for drug testing

The generation of three-dimension (3D) in vitro cultures has emerged as a new approach in cancer cell, stem cell and developmental biology. This method has the potential to mimic crucial features of in vivo environment, providing a more physiologically relevant model to better understand the biology of healthy and diseased tissue. 3D cell cultures, show essential cellular functions of living tissues, potentially predicting cellular responses of real organisms with higher fidelity. However, growing, imaging and analyzing consistent and reproducible spheroids could be challenging: this method requires the development of standard protocols, different cell lines and quantitative analysis methods, which include well suited three-dimensional imaging techniques.
Our research project is focused on the development and implementation of advanced 3D cellular systems for in vitro assay of the effects and toxicity of pharmaceuticals, nanostructures and chemical agents. In order to obtain a homogeneous population of spheroids, we are employing microstructured devices made of a silicon elastomer. Devices can be conveniently fit into a multiwell plates and their surface is characterized by several hundred conical microwells of 500 µm diameter. These can collect the desired number of cells and lead to the formation of a single spheroid in each microwell. We have tested our devices with different cell lines and compared our results with what can be obtained with microstructured commercial plates.
We have tested a number of different cell types for their ability to form spheroids and to do so in our devices. We will present our preliminary results on standardizing and characterizing the growth conditions.

Andrea Miti (32, Zuccheri):
Development of DNA based biosensors employing DNA self-assembly for microRNA detection

MicroRNAs are found to be greatly informative about the status of pathological phenomena, such as infections and cancer. Conventional techniques for sensitive miRNAs quantification are often time consuming,

complex and specialized operators are needed. DNA based biosensors could overcome these drawbacks and replace common techniques in diagnostic applications thanks to the possibility to develop simple sensitive point of care devices for the real time and specific detection of biomarkers. Since self-assembling reactions could lead to consistent enhancement of the signal in this class of biosensors, we designed and tested Hybridization Chain Reaction (HCR), a self-assembling isothermal reaction, that is able to specifically detect miRNA sequences with good sensitivity leading to the formation of DNA nanostructures with higher molecular weight. We also thought to test a new alternative approach using a non-B DNA structure to extend the potential of this strategy by further enhancing target detection and signal amplification. We designed a triple helix probe able to mediate the amplification after the interaction with the specific miRNA. This probe is able to trigger a double response, in this case a double HCR amplification after the target recognition. Focusing on electrochemical and optical transduction, we worked on different tools for the implementation on a biosensor. We tested the HCR-based detection on surface using electrochemistry and Localized Surface Plasmon Resonance (LSPR), an optical technique based on the peculiar interaction of light with immobilized gold nanoparticles.

Renée Concetta Duardo (34, Capranico):
Genetic and molecular mechanisms of genomic instability induced by DNA Topoisomerase 1 poisons leading to innate immune response

Immunotherapy of human cancers has progressed considerably in recent years and its combination with other clinical interventions, such as chemotherapy, is expected to reduce potential clinical failure.
R-loops are non-B DNA structures which can cause collision between replication and transcription machineries leading to genome instability. Topoisomerase 1 (Top1) minimises such conflicts modulating DNA topology. The enzyme is targeted by anticancer agents that trap DNA-enzymes intermediates triggering persistent DNA breaks, cell death and genome instability. DNA damage and DNA repair factors have been recently linked to cellular activation of the innate immune response, therefore raising the possibility to exploit the mechanism for human cancers treatment. However, whether and how Top1 poisons induce genomic instability stimulating the innate immune response it has not been fully established yet.
In this study, we aim to define the genetic and molecular mechanisms of genomic instability induced by specific DNA Top1 poisons and leading to innate immune response. By immunofluorescence assays, we found that Top1 poisons treatments result in a short-term increase of nuclear R-loops. This produces an increase of DNA damage and, later after cell cycle, a micronuclei rate enhancement. Interestingly, DNA damage increase is also transcription dependent. In the future, I will investigate if these phenotypes are cell cycle phase specific and will map unscheduled R-loops formed after Top1 poison treatments.
Our preliminary data suggest that Top1 poisons induce DNA damage and micronuclei in a R-loop dependent manner, highlighting previously unknown aspects of the mechanisms of Top1 poison activity. This may help in developing new strategies for effective personalized interventions using Top1-targeted compounds as immuno-modulators in cancer patients.

Sonia Nicchi (34, Scarlato / Maione):
Unravelling the mechanisms exploited by *Moraxella catarrhalis* to tackle the host antimicrobial defense

Moraxella catarrhalis is second most prevalent bacterium found in sputum of individuals with chronic obstructive pulmonary disease which is characterized by a potent inflammatory immune response with infiltration of macrophages and neutrophils. These phagocytic cells are the main producers of reactive oxygen species (ROS) which boost the antimicrobial response also by promoting extracellular traps formation. In this context, copper appears to play a unique role by acting as a component of the antimicrobial arsenal. In fact, the concentration of this metal is elevated at sites of lung infection and infants with copper deficiency disorder, Menkes disease, exhibit higher incidences of lung infection. Despite the growing evidence that copper and ROS tolerance pathways are general determinants of virulence of different pathogens, these have not been studied extensively in *M.catarrhalis*. Hence, the aim of my PhD project is to investigate these virulence mechanisms both at molecular and cellular level. First, we selected by bioinformatic analysis homologue genes involved in these pathways. Then a lab strain of *M.catarrhalis* was exposed to different stimuli in in vitro culture that mimic the respiratory burst. The next step will be the analysis of gene expression of the selected genes by dd-PCR. In parallel to molecular characterization, we have investigated the interactions between M. catarrhalis and phagocytic cells. The extracellular ROS production was evaluated by a luminol assay while viable adherent and intracellular bacteria were quantified at different time points both in differenti-

ated HL-60 and THP-1 stimulated and non-stimulated for ROS production both by cell counting and microscopy

Paolo Emidio Costantini (33, Zannoni / Cappelletti / Fedi):
Phenotypic characterization and antipathogenic activities of Lactobacillus strains

Lactobacillus is the largest genus of the lactic acid bacteria group and includes over 50 species that can colonize different niches of the human body and play a protective key role through different mechanisms such as competitive exclusion, production of various antimicrobial compounds, immunomodulation and release of biosurfactants. *Lactobacillus crispatus* BC5 and *Lactobacillus gasseri* BC12, recently isolated from vaginal swabs of healthy women, possess intrinsic antipathogenic activities that make them good candidates as next generation probiotics. Indeed, it was demonstrated that this couple of Lactobacilli can carry out a fungistatic and fungicidal activities against *Candida albicans*, inhibit the *Chlamydia trachomatis* infectivity, reduce the *Neisseria gonorrhoeae* viability and inhibit HIV-1 Replication.
The aim of this study is to characterize BC5 and BC12 strains from genotypic and phenotypic point of views. Whole genome sequencing was performed followed by gene annotation, genome comparison and single gene analysis, with a specific focus on genes involved in antibiotic and toxic metal resistance and production of antimicrobial compounds. Phenotypic characterization was performed using Phenotype Microarray analysis, revealing carbon sources used by these two strains as well as their specific sensitivity to several drugs and chemical compounds. Lastly, additionally specific functional assays demonstrated that biosurfactants produced by *Lactobacillus* spp. were able to eradicate and inhibit the biofilm formation of multi-drug resistant *S. aureus* strains.
Taken together, these results demonstrate that *L. crispatus* and *L. gasseri* have possible biotechnological applications for the treatment and/or prevention of pathogen infections.

Daniele Ghezzi (32, Zannoni / Cappelletti):
Unexplored caves for the discovery of novel microbial taxa and bioactive molecules

Subsurface microbial ecosystems are unexplored habitats where microbial survival depends on the interaction with minerals and limited organic sources. In this context, caves are considered pristine ancient and natural environments populated by peculiar microbial species mainly uncharacterized. Low carbon sources and absence of light represent unfavourable conditions for life development in caves, where microbes have to exploit alternative metabolic strategies including bioactive molecules production. In this work, metagenomics analyses were performed to investigate the microbial communities colonizing three different cave systems, i.e. the quartzitic Imawari Yeuta cave (Auyan-Tepui, Venezuela), the sulfidic Fetida cave (Apulia, Italy) and the high-altitude ice cave Cenote Abyss (Dolomites, Italy). Peculiar microbial groups characterize each cave system, mostly associated to specific chemolithotrophic activities and oligotrophic growth conditions featuring each site under analysis. Among these, novel *Chloroflexi Ktedonobacterales* members were identified in biological deposits collected from Imawarì Yeuta quartzitic rocks, and still undescribed *Gammaproteobacteria Beggiatoaceae* strains dominated sulphuric microbial mats in Fetida. Additionally, isolation procedures were conducted and the isolates were tested for the production of inhibitors of common human pathogens growth. As a result, ten strains, belonging to different taxonomic groups, were found to inhibit the growth of the pathogens in top-agar assays. Chemical extracts obtained from liquid and solid cultures of these ten strains were explored through HPLC and LC-MS analyses, revealing the presence of strain-specific anti-microbial compounds. Taken together, these results open new insights into pristine caves as reservoir of novel microbial taxa and novel metabolic activities that could be exploit for biotechnology applications.

Marta Palombo (32, Ciurli / Scarlato):
Molecular characterization of the heat-shock regulatory circuit in *Campylobacter jejuni*

The heat-shock response is a mechanism of cellular protection that triggers a sudden increase in the cellular concentration of different proteins, including molecular chaperones and proteases, to preserve the protein folding that is damaged by the stress conditions. In the human pathogen *Campylobacter jejuni*, the response to thermic stress is controlled by a regulatory circuit, which acts at the transcriptional level and involves the repressors HspR and HrcA. In order to characterize the molecular mechanism underpinning HspR and HrcA

regulatory function, we investigated in detail the HspR and HrcA interactions with their operator sites by DNase I footprinting assays. These analyses allowed the identification of their binding sites, highlighting a complex architecture of protein-DNA interaction that is composed of multiple recognition sites on several heat-shock promoters. Our results indicate that HspR interacts cooperatively with high and low affinity DNA binding sites mapping on each promoter. To elucidate the role of this complex architecture of DNA-binding, we tested the HspR binding capacity to several DNA probes harbouring mutations within the target sequences. We also explored the DNA-binding properties of HspR and HrcA in a competitive manner to their common targets and observed for the first time that, on co-regulated promoters, each regulator has a positive effect on DNA-binding capacity of the regulatory partner. This mutual cooperative effect on DNA binding to HspR and HrcA specific operators could explain the synergic repressive effect of the two repressors observed *in vivo* on co-regulated promoters.

Annamaria Zannoni (33, Scarlato / Roncarati):
HP1043, the *Helicobacter pylori* essential transcriptional regulator

HP1043 is an OmpR-like dimeric orphan response regulator essential for the viability of the human pathogen *Helicobacter pylori* that has been shown to control the expression of several genes involved in key cellular processes. As such, it could represent an ideal target for the design of novel antimicrobial strategies.
Based on structural information, amino acids involved in HP1043 dimerization or target DNA recognition were mutagenized and the mutant proteins assayed for functionality. Specifically, a bacterial two-hybrid system was used to investigate the ability of HP1043 mutants to dimerize, while forms of HP1043 mutated in DNA-binding determinants were assayed both in in vitro by DNase I footprinting and in vivo using a lacZ-based reporter system. This information, along with the HP1043 consensus binding motif, was used as restraints to perform an in-silico protein-DNA docking and generate a structural model of the interacting HP1043 dimer and its target DNA. The model will be exploited to carry out a virtual screening of small molecule libraries to identify compounds potentially able to interfere with HP1043 function and likely block *H. pylori* infection.
To further characterize the mechanism of regulation in vivo, a conditional mutant strain overexpressing a synthetic copy of the gene altered in nucleotide sequence yet encoding the wild type amino acid sequence was generated. Preliminary data, combined with in vitro transcription analyses, suggest that HP1043 is a transcriptional activator and its abundancy is regulated at the post-transcriptional level.

Roberto Ciaccio (33, Perini):
The novel long non-coding RNA lncNB1 interacts with Ribosomal protein L35 to promote E2F-1 protein synthesis and N-Myc protein stabilization in high-risk neuroblastoma

Neuroblastoma is the third most common neurogenic-extracranial solid cancer occurring in childhood. The genetic aberration most consistently associated with poor prognosis of this pathology is genomic amplification of MYCN, encoding for the N-Myc protein, a bHLH-LZ transcriptional regulator of several pro-tumorigenic coding and non-coding genes. Long non-coding RNAs (lncRNAs) are expressed in specific tissue patterns and exert key functions in both physiological and pathological contexts. These molecules could control several processes such as protein synthesis, RNA maturation, as well as transcriptional gene silencing and chromatin modification, which critically influence the fate of the cell. In this study, we have investigated whether and how N-Myc can regulate transcription of lncRNAs by comparing transcriptional profiles between non-amplified and MYCN-amplified neuroblastoma cell lines. Among the several lncRNAs stimulated by N-Myc, we singled out lncNB1, a lncRNA that in addition to being selectively highly expressed in high MYCN cells only, is also strongly and almost uniquely transcribed in neuroblastoma among all types of cancer. Our data showed that N-Myc directly activates transcription of lncNB1, which accumulates in the cytoplasm to physically interact with RPL35. This interaction enhances translation of the E2F-1 transcription factor, whose accumulation in the nucleus up-regulates in particular the expression of DEPDC1B gene, a GAP protein that stimulates ERKs to phosphorylate N-Myc at Ser62 to increase N-Myc half-life. Overall, our findings show that N-Myc can instruct a complex network of molecular interactions through transcriptional stimulation of lncNB1, ultimately resulting in increased stability of the N-Myc oncoprotein to reinforce N-Myc oncogenetic program

Houda Abla (33, Porcelli):
Investigating αKG derivatives in preventing metabolic adaptation in tumorigenesis

Solid tumours sustain their high growth rate through a metabolic and hypoxic adaptation mainly orchestrated by Hypoxia Inducible Factor-1α (HIF-1α), a transcription factor that controls the transcription of target genes implicated in glycolysis, angiogenesis and survival. The stability of this protein is regulated by Prolyl Hydroxylases (PHDs), enzymes responsible of hydroxylating two proline residues in HIF-1α and thereby leading to its proteasomal degradation. PHDs activity and thus HIF-1α destabilization are strictly dependent on O2 levels and ketoglutarate (αKG)/succinate (SA) ratio, as these metabolites respectively constitute the substrate and product of the PHDs-catalysed reaction. We previously reported that the lack of respiratory Complex I causes the unbalance of NAD+/NADH ratio and the accumulation of αKG leading to HIF-1α degradation. This metabolic signalling renders cancer cells unable to adapt to hypoxia with a strong inhibition of tumour growth in vivo (Calabrese et al., 2013; Kurelac et al., 2019). Hence, the aim of this study is to provoke PHDs-mediated degradation of HIF-1α by using novel cell permeable αKG ester derivatives to prevent the hypoxic and metabolic adaptation of cancer cells, and in turn, tumor progression. Moreover, to mimic nutrients and oxygen restriction conditions of the in vivo microenvironment, we investigate the best compounds effects on spheroids as well as on *Drosophila melanogaster* cancer models with a final aim to develop new metabolic-based therapeutic approaches to associate to conventional treatments.

Calabrese C., et al. (2013). Respiratory complex I is essential to induce a Warburg profile in mitochondria-defective tumor cells. Cancer Metab., 1:11.
Kurelac I., Iommarini L., et al., (2019). Inducing indolence in aggressive cancers by targeting mitochondrial complex I is counteracted by macrophage-mediated adaptive responses, Nat. Commun. 1–18

Sara Monticelli (32, Maestrini):
The power of the model system Drosophila melanogaster: from Neuroblastoma tumor to Autism Spectrum Disorder

A robust prognostic marker of the childhood tumor Neuroblastoma is the amplification of the transcription factor MYCN that dimerizes with MAX and activates gene transcription promoting cell proliferation. Besides, MYCN has a still elusive function as repressor. We found that MYC overexpression in Drosophila eye cell precursors inhibits cell differentiation, affecting ommatidia organization and inducing ectopic expression of Antennapedia (the wing HOX gene) in the eye primordium. Further increase of MYC/MAX ratio results in an eye to wing homeotic transformation. Moreover, MYC overexpression phenotype is suppressed by low levels of the transcriptional co-repressors HDAC1 or Smr/N-Cor and MYCN can bind the promoter of Deformed (eye HOX gene) in canonical repression sites. Since mutual repression between HOX genes is required for their proper expression, we envisage that upon MYC/MAX ratio increase, MYC might inhibit Deformed leading to Antennapedia expression and to the homeotic transformation.
Recently, we chose Drosophila to elucidate the association found between the duplication of 16p13.11 genomic region and a sample of patients affected by Autism. This region includes ABCC1 gene encoding MRP1: an ATP-binding cassette transporter known to confer Multidrug Resistance. ABCC1 physiological role is still poorly explored, then we started assessing its putative role in Nervous System. Interestingly, the overexpression of ABCC1 in neurons, but not in glial cells, leads to an altered fasciculation of embryonic nerves. Furthermore, it seems to affect neuromuscular junctions, a well-established model of peripheral synapses. Altogether, these preliminary data provide the basis for further investigating ABCC1 role in Autism susceptibility.

Giulia Gobbi (33, Ciarrocchi /Ambrosetti):
Dissecting susceptibility to BET inhibitors in lung cancer

Lung cancer is the main cause of cancer related mortality worldwide. Despite the introduction of innovative drugs in the last years, including target therapy and immunotherapy, the success of pharmacological treatment is often impaired by resistance, leading to poor prognosis. Inhibitors of the Bromodomain and Extraterminal domain containing Proteins (BETi) are novel drugs currently in clinical trials for lung cancer. Despite the positive effect of BETi on patients, a comprehensive characterization of the response mechanisms to these drugs is still lacking.

Through a CRISPR/Cas9 screening in non small cell lung cancer cell line, we identified three Hippo Pathway members, LATS2, TAOK1 and NF2 as genes implicated in susceptibility to BETi. We demonstrated that these genes confer sensitivity to these drugs by inhibiting TAZ effector nuclear localization and activity. Conversely, TAZ overexpression increases resistance to these drugs. Moreover, we displayed that BETi downregulate the expression of YAP and TAZ.
We also found that two poorly characterized proteins, FRYL and PDCD10, are strongly associated with sensitivity to BETi. This finding may suggest a completely new mechanism of response to these drugs.
Overall, our data demonstrate that Hippo Pathway is involved in BETi response through modulation of TAZ activity. In addition, we identified two novel proteins that mediate sensitivity to BETi through still undiscovered mechanisms.

Eugenia Lorenzini (34, Ciarrocchi / Ambrosetti):
Identification of key genes in the development and progression of malignant pleural mesothelioma

Malignant Pleural Mesothelioma (MPM) is a rare but aggressive cancer arising from the mesothelial cells lining the pleura. It is characterized by high mortality and dismal prognosis due to limited treatment options available. MPM is associated with chronic asbestos exposure. Its widespread use in the 20th century and the long latency make MPM a current clinical challenge.
Genome-wide profiling has provided new clues into the genetics of MPM but the molecular bases of this disease remain unknown. This poor characterization is currently the most relevant limitation to development of MPM targeting strategies. The aim of this project is to identify genes essential for MPM survival with the intent to: get new clues into the MPM pathobiology and identify targets for new MPM pharmacological strategies.
To this aim, we performed a CRISPR-Cas9-genome-wide screening in the MPM cells MSTO-211H, obtaining a list of essential and suppressor genes. We filtered these data using the MPM-dataset of the DEPMAP-database confirming 233 essential and 45 suppressor genes. GO analysis identified membrane trafficking and chromatin modification pathways as significantly enriched in the MPM-specific essential genes. Several tumor suppressors were identified among the enriched genes, confirming the validity of the analysis. Using publically available drug-genes interaction databases we found pharmacological compounds potentially targeting a subset of identified essential genes.
Starting from these lists we are currently validating the top scoring gene candidates in additional already engineered Cas9-MPM cell lines, as well as the effect of predicted drugs on MPM growth and survival.

Simona Salimbeni (32, Capranico):
TDP1 deficiency and genomic instability in non replicating cells

Tyrosyl-DNA phosphodiesterase-1 (TDP1), the deficiency of which causes the severe neurodegenerative disorder spinocerebellar ataxia with axonal neuropathy-1 (SCAN1), is primarily involved in the repair of transcription-blocking topoisomerase I cleavage complexes (TOP1cc). Yet, our understanding of how TDP1 deficiency promotes neuronal cell death is not known. DNA double-strand breaks (DSBs) are lethal genomic lesions and transcription-blocking TOP1cc can lead to DSB formation in post-mitotic neurons by a mechanism that depends on R-loops, which are RNA/DNA hybrids plus a displaced DNA strand. Hence, we investigated whether TDP1-deficient cells accumulate transcription- and R-loop-dependent DSBs. We generated TDP1 knockout (KO) primary lung WI-38 hTERT cells and U2OS cells by CRISPR-Cas9 and showed that they accumulate DSBs primarily in G1 phase, measured by H2AX and p53BP1 foci formation. Similar results were obtained in the colon cancer HCT116 cells TDP1 KO and RNAi-mediated depletion of TDP1 in quiescent WI-38 hTERT cells further suggest the replication-independent nature of DSBs. We also showed by DNA/RNA immunoprecipitation (DRIP) experiments that TDP1 KO WI-38 hTERT cells increase R-loop levels in two out the five genes analysed. We are planning to explore whether the accumulation of DSBs in TDP1-deficient cells depends on TOP1 and R-loops and we will further search for the extend of R-loop level changes in TDP1 KO cells at the genome level by DRIP-seq. These findings might provide insights on the molecular pathogenesis of the SCAN1 syndrome.