Young Researchers Retreat

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Abstract book

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SUMMARY

SESSION I: Pharmacology

Electronic cigarettes: smoking by any other name

Fabio Vivarelli

Tobacco smoking remains a leading cause of premature death worldwide. In combination with conventional smoking cessation protocols, Electronic Nicotine Delivery Systems (ENDS) (including the e-cigarette and the newest heat-not-burn devices) have been proposed as a useful smoking cessation tool. Although the levels of toxicants in the mainstream are significantly lower than those observed in tobacco smoke, our research team raised some concerns about the alleged health risk associated with their use. Our studies show the co-mutagenic and cancer-initiating effects of ENDS exposure in rat lung. We found that ENDS produce extensive DNA damage in leukocytes measured by the comet assay as well as increased rate of micronuclei formation in reticulocytes. Furthermore, electron microscopy images indicate severe remodeling of the smaller and larger airways. ENDS exposure increases oxidative stress in the prefrontal cortex with an increased level of 8-hydroxyguanosine, a reliable marker of DNA oxidation positively associated with smoking habit. Consistently, xeroderma pigmentosum group C protein complex and 8-oxoguanine DNA glycosylase-1 (OGG-1), two crucial proteins involved in major DNA repair pathways were found upregulated in exposed animals. ENDS can induce neuroinflammation through activation of the NF-κB signaling pathway and the down-regulation of PPARα and PPARγ as negative regulators of inflammation. Interestingly, ENDS affect male gonadal function by increasing markers of oxidative stress including those associated with DNA damage, as well as NF-kB dependent pro-inflammatory mediators; inactivation of key androgenic enzymes such as 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD), together with reduced testosterone synthesis suggest, potential impairment of male gonadal function. Collectively, our findings reveal that ENDS consumption mimics some pathological mechanisms typically triggered by conventional cigarettes suggesting caution in proposing them as a healthy alternative to smoking or as an aid to smoking cessation.

miRNAs as potential diagnostic and predictive biomarkers in gynecological malignancies

Gloria Ravegnini

miRNAs can represent potential biomarkers for cancer diagnosis and treatment response. Recently, a growing interest for miRNAs in gynecological malignancies has been observed. Ovarian Cancer (OC) is often diagnosed too late, and there are no effective screening methods. Women with ovarian endometriosis (EMS) have an increased risk of developing EMS-related OC (EAOC), but there is currently no way to predict which patients are most likely to develop it. We analyzed 81 samples from 44 patients, of which 9 (20.5%) with ovarian EMS while the rest had either borderline lesions (3, 6.8%) or EAOC (32, 72.7%). Global miRNA profiling was evaluated and showed that 30 miRNAs were significantly upregulated already in the borderline lesions (p-val FDR adjusted <0.05). Comparison of EROC vs borderline samples yielded a total of 118 significantly upregulated miRNAs and a total of 72 downregulated. Finally, differential expression analysis of EROC vs EMS lesions showed 199 upregulated and 120 downregulated. Both borderline and EROC lesions presented 14 miRNAs commonly upregulated with respect to EMS. Moreover, the discriminatory capacity of these miRNAs showed excellent results with accuracies ranging from 0.92-1 and sensitivities from 0.9-1. In a different project, we also analyzed circulating miRNAs for monitoring chemotherapy response in OC patients. Plasma miRNA profiles of 15 patients were analyzed before (t0) and after 6 cycles of chemotherapy (t1). Comparison between t0 and t1 showed 15 miRNAs significantly deregulated. The most significant deregulated ones, miR-200b-3p (p=6.20E-06) and miR-429 (p=8.07E-05), were further validated in 30 OC patients and both maintained the statistical significance with a down-regulation after chemotherapy. Moreover, patients were divided in 2 groups by using medium level as a cut-off values. For miR-429, patients with lower levels showed a mean overall survival (OS) of 499 days while patients with higher expression had a mean time of OS of 288 days (p = 0.0284); for miR-200b-3p the OS was not significantly different between the two groups (446 vs 310 days, p =0.166).

New Biomarkers in Alzheimer's disease: innovation for diagnosis and treatment

Giulia Sita

With a rapidly ageing population, Alzheimer's disease (AD) represents the largest unmet medical need with no disease-modifying treatments approved to date. Moreover, considering the increasing health and environmental costs due to increased prevalence of the disease, new directions in the identification of novel targets and new biomarkers to improve early detection and innovative drug development are an urgent need. Recently, a considerable effort has been focused on the dysregulation of small non-protein-coding microRNA (miRNA) and the associated post-transcriptional gene alteration in AD. miRNAs are involved in the post-transcriptional control of gene expression and are the most important fine-tuning regulators for many cellular processes. Their aberrant expression is often associated with the development of different pathologies for which circulating miRNA could be used as early biomarkers of disease. The present project aims to identify circulating miRNAs within serum and cerebrospinal fluid (CSF), which can be used as diagnostic biomarkers that facilitate the early detection of disease, the identification of drug targets, and potentially the continuous monitoring of disease progression. In this study, 20 AD patients and 20 healthy controls, were recruited at the Neurology Unit, Hospital of Brescia, Italy. Plasma and CSF samples were obtained and total RNA was isolated for miRNA sequencing. RNA sequencing analysis in serum and CSF samples allowed us to identify some differentially expressed miRNAs. The significant miRNAs identified in the sequencing were validated using quantitative PCR, and the miRNA significantly modulated in the first cohort of patients was investigated in a separate cohort of 80 other patients. Validated miRNAs regulate essential pathology pathways, making them therapeutic targets. Such information may not only support disease diagnosis but also provide the opportunity to evaluate therapeutic interventions earlier in the disease process. Overall, this study highlights the potential of miRNAs as non-invasive biomarkers and helps identify new therapeutic targets.

Protein engineering for higher crop yield: testing the approach on an algal enzyme and ongoing results

Libero Gurrieri

The increasing frequency of extreme climate events poses a challenge to plant survival. At the same time, population growth increases demand for food and tests crop productivity. Numerous approaches have been attempted to enhance plant productivity by improving carbon fixation. Our research regards the Calvin-Benson cycle, which is responsible for fixing CO2, with the aim of increasing carbon flux through the cycle and consequently the amount of carbon fixed into sugars. We focused on improving the enzyme phosphoribulokinase (PRK). PRK catalyses the final step of the Calvin-Benson cycle, regenerating the initial substrate. Chlamydomonas reinhardtii, a green algae, is a well-studied model organism with a wide range of available biotechnological tools. Its metabolic pathways have been extensively researched, reaching a deep understanding, for example the concentrations of numerous enzymes and metabolites are known. This feature makes Chlamydomonas an invaluable platform for testing our approach. The experimentally determined KM of Chlamydomonas PRK (CrPRK) for its substrate ribulose-5-phosphate (Ru5P) is approximately twice the concentration of Ru5P in Chlamydomonas chloroplast. In vivo, the presence of inhibitors and reaction products reduces the affinity, resulting in calculated in vivo KM that are even higher than experimental ones. In our work we modified CrPRK to increase its affinity for the substrate. The computational approach MM-GBSA was used to simulate the Ru5P trajectory from the enzyme's outer shell to the catalytic site, identifying some interesting residues. PRK was mutagenized and the catalytic parameters measured. The first round of mutants highlighted the reliability of the in silico analysis and provided a first promising mutant to improve with further cycles of in silico/in vitro analysis.

SESSION II: Drug discovery and development

Molecular characterization of SARS-CoV-2 Mpro activity and inhibition, a new toolbox for drug discovery

Luca Mazzei

SARS-CoV-2 is the causal pathogen of COVID-19, which is responsible for more than seven million deaths worldwide and created a huge threat to the global economy and healthcare system. In the host infection by SARS-CoV-2, the two viral proteases Mpro and PLpro play the key role of catalyzing the hydrolysis of polyproteins pp1a and pp1ab, which are translated by host ribosomes upon recognition of the viral positive single-stranded RNA, resulting in the release of an entire set of viral proteins crucial for SARS-CoV-2 replication. For this reason, Mpro and PLpro are recognized as potential primary targets for antiviral drug development. In this work an integrated procedure for a biochemical and structural characterization of Mpro inhibition is presented. The protein was expressed as a fusion with an N-terminal His-SUMO tag, which is exploited for purification purposes and then cleaved to obtain the native protease. The obtained native Mpro was successfully crystallized, and the resulting X-ray crystal structure was determined at 1.54 Å. Moreover, a novel approach involving Isothermal Titration Calorimetry (ITC) was developed to characterize the kinetics of Mpro as well as its inhibition by Ensitrelvir, a known non-covalent inhibitor. The developed ITC-based assay provided a rapid response to Mpro activity, which was used to derive the kinetic enzymatic constants KM and kcat reliably, as well as their temperature dependence, from which the activation energy of the reaction was obtained for the first time. The assay further revealed the existence of a noncompetitive inhibition of Mpro by Ensitrelvir, further yielding the inhibition constant with high precision. The calorimetric method described, together with the established crystallization and structural determination setup, is thus proposed to be generally and widely used in the development of novel drugs targeting Mpro, thus providing a useful toolbox for the biochemical and structural investigation of protein – inhibitor interactions.

Genetically modified M13 bacteriophages for precise photodynamic cancer eradication

Paolo Emidio Costantini

Phage-based targeting platforms have emerged as versatile tools in the field of biomedical research and therapy. Here, we present the development of a novel theranostic platform using the M13 phage. M13 was genetically engineered to display the 7D12 nanobody in fusion with the pIII protein on the phage tip, enabling for precise molecular recognition of the Epidermal Growth Factor Receptor (EGFR), which is overexpressed in several cancers with severe outcomes. The acquired tropism of M137D12 was demonstrated on both single and co-culture experiments performed with EGFR positive and negative cell line. In addition, we have successfully chemically conjugated photosensitizers (Rose Bengal) or fluorescent dyes (CF594) onto pVIII proteins, resulting in a nanovector capable of delivering hundreds of molecules exclusively to cancer cells overexpressing EGFR. After demonstrating that chemical conjugation did not alter the tropism of the engineered phage, the therapeutic performance in photodynamic therapy (PDT) of M137D12-RB was evaluated on both 2D and 3D cell culture models. As a result, M137D12-RB exhibited potent and selective photodynamic killing activity at picomolar concentration of phage upon irradiation with white LED lamp or laser on 2D cell culture. Notably, in 3D models, the phage successfully penetrated the spheroid core and led to complete disaggregation of the spheroid cytoarchitecture upon light irradiation. This innovative strategy has enabled the development of a modular and multifunctional platform for theranostic applications.

Tricyclic-based bifunctional compounds for neurodegenerative diseases

Elisa Uliassi

Neurodegenerative diseases (ND) represent a leading biomedical and societal challenge. The current lack of effective treatments for ND is likely due to their multifactorial etiology, which calls for alternative drug discovery approaches. Bifunctional compounds, featuring two different chemical scaffolds for the simultaneous engagement of synergistic proteins involved in ND pathogenesis, may address their multifactorial and complex nature and generate effective therapies [1]. In our previous efforts, we validated the tricyclic arylalkylamine structures as a privileged chemotype against neurodegeneration [2]. We report herein on the development of tricyclic-based bifunctional compounds for ND, in which this chemotype has been combined with valproic acid (VPA). Our strategy is based on the reported beneficial properties of VPA in various ND models, as its epigenetic action and glycogen synthase kinase-3 beta (GSK-3β) modulation provide a wide range of neuroprotective and neuroregenerative effects. Thus, the primary amines of selected tricyclic analogues were combined to the carboxylic function of VPA through an amide bond, leading to a small set of 7 bifunctional compounds. Docking studies and preliminary evaluation of GSK-3β inhibition showed a promising inhibitory profile. Following the positive results of neuro- and hepato-toxicity, neuroprotection was evaluated in a serum and potassium deprivation neuronal cell model, displaying favorable neuroprotective effects. Collectively, a small series of 7 tricyclic-based bifunctional compounds is being developed with ND-modifying potential.

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Pursuing the PROTAC approach to fight emerging flavivirus infections

Eleonora Diamanti

Flaviviruses are vector-borne RNA viruses causing severe diseases, including epidemics of Dengue, West Nile Virus (WNV), and Zika virus. Measure to control and cure flavivirus-induced infections are largely underestimated. To mitigate the current health burden and limit future outbreaks, drugmakers need to be ready and avoid the spread of virus will materialize, in the worst-case scenario, in a pandemic, as recently experienced with the Covid-19. Currently, there is neither vaccine nor drug to treat flavivirus infection, making drug-discovery efforts extremely urgent. Despite different families of small molecules or peptidomimetic inhibitors have been reported, none of them reached clinical phases. Here, as game-changer, we are proposing the proteolysis targeting chimeras (PROTAC) approach to target the NS2B-NS3 protease. PROTAC are heterobifunctional molecules with the ability to harness ubiquitination and subsequent proteasomal degradation, by putting in close proximity the protein of interest (POI) and an E3 ligase. In fact, PROTAC are structurally constituted of a ligand, directed to the POI, bridged to an E3 ligase by a suitable linker. The NS2B-NS3 protease is essential for the replication of flaviviruses and, importantly, is conserved among WNV, Dengue and Zika viruses, making our final PROTACs pan-flaviviral degraders. We successfully manage to set out the multi-step synthetic pathway to access final PROTAC molecules, to establish the conditions for co-crystallization and, to assess the cellular degradation. Furthermore, we envisaged to leverage the innovative PROTAC strategy to develop a new class of small molecule degraders targeting the nsp1 Chikungunya protein.

Obeticholic acid consumption in AUSL Romagna

Lorenzo Evangelisti

Introduction: Ocaliva® is indicated for the treatment of primary biliary cholangitis (PBC) in combination with ursodeoxycholic acid. OCA annual expenditure in AUSL Romagna has doubled from 2021 (451.155€) to 2023 (897.297€) and the consumption in 2023 (0,29 vs 0,17 DDD*10.000 ab pes die) is higher than regional average.

Objective: The aim of this study is to analyze Ocaliva® consumption, considering that scientific literature showed that less than half of the patients (47%) were responder to OCA. The data were discussed with the clinicians to define and share common usage criteria and to program the expenditure for the 2024.

Method: We analyzed all AUSL Romagna patients treated with Ocaliva® in 2023 first eight months, processing age, previous therapy with UDCA and its duration, the motivation of transition to OCA, ALP and BT values at the baselines, after 6-12 months of OCA, number of responders (measured as ALP value 15% reduction compared to the baseline after 12 months) and the reasons of the suspension, off-label usage of fibrates.

Results: We found 48 patients, 13 starting OCA in 2023: 27 were over 65 years old. All patients were treated with UDCA for at least 1 year before OCA and everyone started with 5mg dosage, 45 because of inadequate response to UDCA and 3 for intolerance. 6 were not evaluable, 2 of 8 with 6 months-assessment did not reach the target; 22 of 34 with 12 months-assessment (65%) were responders; only 3 non responders suspended the therapy. 8 used off-label fibrates.

Conclusions: Our analysis shows higher percentage of responders in real life than in clinical trials thanks to a strict monitoring of the treatment and continuous counseling to patients. Considering therapy revaluation in non responder and elderly patients, clinicians planned a 2024 drug budget similar to 2023, even considering possible incident patients.

SESSION III: Neuroscience

Study of molecular mechanisms underlying neuronal alterations in chronic pain

Francesco Formaggio

Chronic pain is a clinical condition associated with various pathologies (oncological, neurological, musculoskeletal), which lasts for more than 3 months. It creates enormous individual suffering and entails high social and health impact due to the consequences linked to disability and absences from work. The neuropathic component (prevalence 8-10% in Italy) derives from a lesion of the somatosensory system which frequently affects the primary sensory neurons. These neurons, located in the dorsal root ganglia (DRG), play a fundamental role in the genesis and maintenance of neuropathic pain (DN), as they are primarily responsible for ectopic/spontaneous or evoked activity. DN is associated with various neuropathies involving the peripheral nerves such as diabetic neuropathy, lumbar spine pain, cancer pain, HIV and Herpes Zoster infections, neuropathies resulting from the use of chemotherapy drugs and various hereditary neuropathies. Currently, available treatments are largely ineffective and can induce severe side effects. In this project we aim to elucidate the molecular mechanisms behind the genesis and maintenance of neuropathic pain. Particularly we focus on Fabry disease (FD), an X-linked metabolic disease due to a deficiency in α-galactosidase A (α-gal A) activity. This causes the accumulation of glycosphingolipids, especially globotriaosylceramide (Gb3), in different organs. Neuropathic pain and gastrointestinal symptoms, such as pain crises arising from the extremities, abdominal pain, are the most frequent symptoms reported by patients. Numerous pieces of evidence, including a reduction in small Aδ nerve fibers and C nerve fibers, describe Fabry pain as primarily neuropathic. Even though deposits of Gb3 in DRG neurons have been previously observed, the origin of these symptoms is complex and multifactorial, and the exact mechanisms of pathogenesis are still poorly understood. In this project we aim to elucidate the molecular mechanisms behind the genesis of neuropathic pain and explore new therapeutic interventions. The research lines related to this project are: 1) Calcium dysregulation in DRG neurons; 2) New pharmacological approaches; 3) Brain-gut axis; 4) Central nervous system involvement; 5) New in vitro models to study pain.

Microsampling and mass spectrometry for targeted metabolic profiling in murine models of amyotrophic lateral sclerosis

Michele Protti

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive deterioration of motor neurons in the brain and spinal cord, leading to muscle weakness, paralysis and ultimately respiratory failure. Currently, there is no cure for ALS, and available treatments only provide limited symptomatic relief without halting or reversing the disease progression. Emerging evidence suggests neuroinflammation within central nervous system (CNS), driven by dysregulated immunoregulatory responses, may play a critical role in the disease pathogenesis. Tryptophan (Trp) catabolism has been identified as a key physiological immunoregulatory mechanism, with several Trp metabolites implicated in neurological conditions. This study, within a multidisciplinary research project, aimed to develop analytical approaches based on miniaturized sampling and pretreatment strategies. The objective was to assess Trp metabolites in biological samples obtained from ALS-bearing mice and wild-type mice, including a step after pharmacological treatment. By examining Trp catabolism and identifying potential biomarkers of disease progression, the goal was to elucidate ALS pathogenic mechanisms. Our focus was on designing innovative microsampling and miniaturized pretreatment procedures to reduce solvent and reagent usage and accommodate limited sample volumes. A workflow for miniaturized sample collection was developed using volumetric absorptive microsampling (VAMS). This microsampling strategy was coupled to liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) and high-resolution mass spectrometry (HRMS) systems for identity confirmation and quantitative assessments. Method validation demonstrated good results in terms of linearity, extraction efficiency, precision and accuracy. Within this collaborative project, we applied the method to analyze miniaturized samples from ALS-bearing mice and controls, including those treated with edaravone, the first drug to show effective inhibition of ALS motor function deterioration. This research aims to uncover therapeutic targets for ALS and advance the knowledge about the disease, leading to innovative treatments to slow its progression and improve patients' life quality.

Characterization of a novel humanized knockin mouse model carrying the E364X patient mutation for CDKL5 deficiency disorder

Corinne Quadalti

CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental syndrome caused by mutations in the X-linked CDKL5 gene. To date, several knockout and a few knockin mouse models have been generated, the latest of which modelling a mutation in the N-terminal catalytic domain of the protein. Here we present a new CDD mouse model carrying a humanized nonsense variant (c.1090G>T; p.E364X) described in the C-terminal domain of the CDKL5 protein in a female patient with a milder phenotype. Cdkl5E364X mice of box sexes were analysed. The Cdkl5E364X mouse showed an altered behavioural profile in line with that previously described for other CDKL5 mouse models, with Cdkl5E364X mice of both sexes showing altered neurological and motor neuron maturation, hyperactivity, defective coordination and impaired memory and cognition. Gene expression analysis highlighted an unexpected reduction of Cdkl5 expression in Cdkl5E364X mice brain tissues, with a significant increase in overall neuron-specific gene expression and an area-dependent alteration of astrocyte- and oligodendrocyte-specific transcripts. The ex vivo isolation and differentiation of neural stem cells from the subventricular zone of Cdkl5E364X mice showed a significant alteration of neuronal maturation in both sexes and, interestingly, a marked reduction in number and maturation of oligodendrocyte lineage in Cdkl5E364X females, compared to sex-matched controls. In line with our previous findings, we confirmed that both the cerebellum and the hippocampus are significantly affected by CDKL5 protein loss. An in-depth analysis of synaptic plasticity in the cerebellum and hippocampus showed reduced Gabra1 and Gabra5 expression in females, whereas Gabra1 expression was increased in males, suggesting an opposite, sex-dependent regulation of the GABA receptor expression already described in humans. The novel Cdkl5E364X mouse model is characterized by robust neurological and neurobehavioral alterations, with a neurochemical profile indicative of a cerebellar GABAergic hypofunction, pointing to Gabra1 and Gabra5 as novel druggable target candidates for CDD.

Microglia across neurodegenerative diseases: role of EVs-miRNAs in neuroinflammation

Francesca Massenzio

Neuroinflammation is a crucial pathogenic mechanism that commonly underlies most neurodegenerative diseases. Microglia, the brain's immune cells, play a critical role in the neuroinflammatory process according to the stage of pathology. The primary role of extracellular vesicles (EVs) is a distinctive hypothesis. EVs, a heterogeneous population of membrane vesicles, which contain, and transfer bioactive molecules play a role in many of the major pathological pathways altered in neurodegeneration, including Aβ aggregation, neuroinflammation, synaptic transmission, cell death, and senescence. Of note, most EV effects are mediated by encapsulated miRNAs, small non-coding RNAs that negatively regulate the expression of target mRNAs. On the one hand, with the progression of neuropathology, the inflammatory response of microglia can influence the expression of EV-miRNAs, whose release could promote neuroinflammatory processes. On the other hand, EVs from neural stem cells, (NSC-EVs) have been explored for their ability to modulate neuroinflammation and neuronal-glial functions in neurodegenerative disorders. In this regard, we proved that microglia have the capacity to self-sustain its active state, by releasing vesicular and non-vesicular pro-inflammatory factors contributing to the spreading of neuroinflammation. We therefore demonstrated that the intracellular misregulation of selective inflamma-miRNAs in response to inflammatory stimuli is mirrored in the composition of EVs-miRNAs, proving their role in exacerbating the neuroinflammatory response in vitro.

Evaluation of the role of the NEGR1 pathway and IgLON family members in a mice model of depression based on stress exposure

Marco Salluzzo

Major Depression Disorder represents a disabling mental disorder whose pathophysiological mechanisms are not yet fully understood. Previous GWAS studies revealed that NEGR1, a cell adhesion molecule belonging to the IgLON subfamily, is one of the most significant risk genes for depression. In the present study, we investigated the role of NEGR1 and other IgLONs in the Unpredictable Chronic Mild Stress (UCMS) model in adult C57BL6J male and female mice. Animals were exposed to several mild stresses presented in a random fashion for 10 weeks. The impact of this protocol was evaluated through different behavioural tests starting from week 7. Both sexes showed impaired grooming behaviour and reduced sucrose preference, the latter more evident in females. Open Field tests detected increased locomotor activity in females, while Morris Water Maze test revealed changes in spatial memory in male animals. At the end of the stress exposure period, alterations in gene and protein expression were assessed by real-time qPCR and Western blotting, respectively. Brain areas relevant for depression were analysed: hippocampus, pre-frontal cortex, striatum, and hypothalamus. qPCR experiments detected In stressed male animals a reduction in the gene expression levels of LSAMP in the hippocampus, an increase in the hypothalamic levels of NTM and an increase of OPCML levels both in the hippocampus and in the prefrontal cortex were detected. In stressed female animals, significant increases in gene expression levels of FGFR2, LSAMP, OPCML, and NTM were detected in the hypothalamus. Western Blot experiments revealed significant variations for NEGR1 protein levels in the striatum of males, while an increase in the striatum and a reduction in the prefrontal cortex for LSAMP protein levels were observed in females. These results provide evidence of the involvement of the NEGR1 pathway and IgLON proteins in the depressive-like phenotype in the UCMS model.

Understanding the molecular mechanisms at the basis of AGC1 deficiency pathogenesis

Eleonora Poeta

AGC1 deficiency is an ultra-rare demyelinating disease caused by mutations in the SLC25A12 gene, which encodes for isoform 1 of the mitochondrial aspartate-glutamate carrier (AGC1). The main hallmark is secondary hypomyelination, along with impaired proliferation of brain cells. Probably, abnormal myelin production is due to a reduced synthesis of N-acetyl-aspartate (NAA), from which the acetyl groups mainly derive. This, in turn, leads to epigenetic alterations in the brain precursor cells and resulting in transcriptional dysregulation, causing proliferation and differentiation defects, as demonstrated on in vitro AGC1 deficiency models (precursor cells of mouse oligodendrocytes -OPCs- where SLC25A12 is silenced by a shRNA, neurospheres from AGC1 heterozygous mice, and neural precursor cells -NPCs- derived from human iPSCs of AGC1 deficiency patiens) of our laboratory. Along with epigenetic alterations, lower production of NAA leads specifically to reduced levels of acetyl-CoA, involved in the synthesis of fatty acids, major components of the myelin sheath. Thus, an alteration of their production leads to hypomyelination. This is confirmed by RNA-seq analysis and in vitro validation on OPCs and neural stem cells model, which show altered expression of transcriptional factors and enzymes involved in the fatty acid synthesis pathway. Meanwhile, metabolomics analysis shows altered concentrations of many metabolites, as TCA cycle intermediates, amino acids and cofactors (NAD, CoA); whereas ATAC-seq confirmed the presence of abnormal epigenetic landscape, which could affect genes involved in many biological pathways. To try to rebalance the epigenetic and metabolic alterations, supplementations with ketogenic compounds, as branched-chain amino acids and ketone bodies, are being performed on OPCs, neurospheres and hiPSCs-derived NPCs to induce a potential recovery of differentiation/proliferation defects, through epigenetic modifications restoration.

Silent Killers in Your Everyday Products: Unveiling the Neurotoxic Effects of Endocrine Disrupting Molecules

Agnese Graziosi

Endocrine disruptors (EDs) are ubiquitous compounds that can interfere with endogenous hormones. Molecules identified as EDs are highly heterogeneous, but most are man-made chemicals, such as ethinyl estradiol (EE), medication, and diethyl-phthalate (DP) plasticizers. The constant exposure of the global population to these molecules can lead to an induction of toxic effects, especially on the central nervous system. Among the different mechanisms by which EDs could influence human health, the modulation of microRNA (miRNA) is of interest. The present project aimed to study the impact of exposure to subtoxic concentrations of EE and DP on the mechanisms of neurotoxicity, focusing on the modulation of miRNAs implicated in neuronal functions. The human neuroblastoma cell line SH-SY5Y was exposed to the molecules in the study. Afterward, profiling unveiled a modulation in the expression of some miRNAs implicated in neurotoxicity. Computational analysis to determine the target genes revealed that most were involved in the RAS pathway, which regulates cell proliferation, survival, and differentiation. RT-PCR to validate the pathway showed an upregulation of the genes EGFR, IGF1R, AREG, SH3BP4, and BTG2, while Western Blotting identified an upregulation in the phosphorylation of Akt and mTOR, Bcl2, and RAS, while PTEN and BAX underwent downregulation. These preliminary analyses allow to investigate the role of EE and DP as EDs able to induce modulation in pathways involved in neurodegeneration and development of cancer.

Biochemical and computational approaches to dissect the effect of MT-CYB pathogenic mutations on respiratory chain activity and assembly

Gaia Tioli

Complex III (ubiquinol:cytochrome c oxidoreductase) is a multisubunit membrane bound enzyme and in its native form is a symmetrical homodimer (CIII2). CIII2 is central for mitochondrial respiratory chain and is associated with different stoichiometry with Complex I and Complex IV to form supramolecular assemblies, called supercomplexes (SCs). Defects in CIII2 are rare and mostly associated with mutations in MT-CYB gene that encodes for one of the catalytic core subunits, cytochrome b (cyt b). It has been suggested that pathogenic mutations in MT-CYB are mitigated when CIII2 is assembled in SCs [1]. Therefore, we applied biochemical approaches in human cellular models carrying pathogenic point mutations in cyt b to analyse the structural stability and enzymatic activity of CIII2 in its isolated form or assembled in SCs. Our results show that these mutations affect the kinetics of the assembly of CIII2 and its supercomplexes after the treatment with a reversible mitochondrial translation inhibitor, suggesting a role of these pathogenic mutations not only in CIII2 activity but also in its biogenesis. In addition, we applied the Protein Stability Prediction with a Gaussian Network Model (PSP-GNM) approach [2] to evaluate global changes in the unfolding Gibbs free energy change and study the effects of single amino acid mutations on cyt b stability on the available CIII2 structures both in its isolated and bound form. Preliminary results indicate that some pathogenic mutations may affect the unfolding free energy of CIII2, stiffening the structure of the enzyme, in agreement with the reduction of CIII2 activity. This dual experimental and biocomputational approach may be very useful to better understand the effect of these rare pathogenic mutations and to design new strategies for possible therapeutic options.

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SESSION IV: Computational chemistry and biology

Genetic and functional characterization of rare sequence variants in Autism Spectrum Disorder

Marta Viggiano

Autism Spectrum Disorder (ASD) is a highly heritable neurodevelopmental disorder, affecting 1% of the population. It is a multifactorial disease, with a complex genetic architecture, consisting of a combination of common low-risk and more penetrant rare variants. Particularly, both de novo and inherited rare variants highly contribute to individual risk for ASD, and their analysis has led to the identification of more than 100 candidate genes. However, exploring the role in ASD development for the majority of these genes is highly complex, and so far it remains largely unknown. This project is focused on the functional characterization of highly penetrant rare coding variants emerging from genomic sequencing in a sample of ASD families, in order to clarify the role of known ASD genes and highlight new candidates. From whole genome and exome sequencing in a cohort of 435 individuals from 116 ASD families, we identified a set of 37 rare potentially damaging de novo single nucleotide variants. Among these, we detected a novel de novo missense variant in the RAB11B gene (p. R33H). RAB11B plays an essential role in numerous neurodevelopmental processes, and it has been recently associated with a neurodevelopmental disorder including intellectual disability and white matter anomalies. Thorough a review of public databases and literature, we found two additional RAB11B uncharacterized missense variants in individuals with ASD/NDD. Thus, we carried out immunofluorescence analysis to functionally characterize these variants and better understand RAB11B role in the ASD phenotype. Particularly, co-localization experiments using ARPE-19 showed that after induction of the primary cilium assembly there was enriched localisation of the R33H mutant to the Golgi network, similar to inactive RAB11B. These results provide evidence that de novo missense variants close to RAB11B functional domains affect the protein sub-cellular localization during primary cilium assembly, suggesting a role for RAB11B in ASD aetiology.

Associating multifunctional proteins with diseases and pathways

Giulia Babbi

We recently propose a new portal called Bioinformatic Sweeties (https://bioinformaticsweeties.biocomp.unibo.it), collecting resources ranging from databases for human protein annotation to computational methods for predicting the impact of variants. The tools, included in the portal, allow computing different protein properties, ranging from solvent accessible surface to stability and interactions, to protein functional annotation. Here we focus on a newly released resource in the portal, called MultifacetedProtDB (https://multifacetedprotdb.biocomp.unibo.it) a curated database providing a collection of 1103 multifunctional human proteins. The characterization of multifunctional proteins is an expanding research area aiming to elucidate the complexities of biological processes. In our resource, we merge information from UniProt, Humsavar, Monarch, and ClinVar, reporting disease nomenclatures as MONDO, ICD10, OMIM and Orphanet catalogues. Some 30% of multifunctional proteins in our database (321 enzymes and 110 non-enzymes) are associated with 895 MONDO diseases classified into 213 ICD10 categories and in 17 out of the 19 ICD10 main chapters. Out of the 895 diseases, 323 are included in the Orphanet catalogue of rare diseases. Over the 431 multifaceted proteins with MONDO disease annotation, 212 are associated with multiple diseases, and 56% are associated also with multiple Reactome pathways. Performing different functions in different pathways could explicate why a protein is associated with different diseases, giving insight to the molecular mechanism leading to disease insurgence. MultifacetedProtDB is useful for characterizing the involvement of a multitasking protein in the cell molecular complexity and associated disease, and possibly future research will enlarge the actual collection and improve its annotation.

Large-scale comparison of AI-based protein structure prediction tools on the human reference proteome

Castrense Savojardo

Recently published results from the Critical Assessment of methods for Structure Prediction (CASP15) confirm the relevance of Artificial Intelligence (AI)-based modeling on the accuracy of protein structure prediction. On selected CASP targets, impressive performance was achieved by methods derived from the original AlphaFold2 release, which in CASP14 paved the way to highly accurate large-scale structure predictions. AlphaFold2 is based on deep neural networks (transformers) trained to produce protein structures from amino acid sequences, multiple sequence alignments, and, remarkably, homologous proteins with experimental structure used as templates. As an alternative, recent methods take advantage of protein Language Models (pLMs), and sequence embeddings to develop end-to-end models of protein structures based only on sequence information, such as ESMFold. This talk presents results of a large-scale comparison of AlphaFold2 and ESMFold carried out on the extended set of proteins in the human reference proteome. Structural models predicted with AlphaFold2 and ESMFold are analyzed and compared from different perspectives, evaluating the reliability of intrinsic model quality associated by the two tools (pLDDT), and computing superimposition scores between predicted models as well as a their direct comparison with experimental information available at the Protein Data Bank (PDB). Our results provide a comprehensive snapshot of the state-of-the-art of protein structure prediction for the human reference proteome. Remarkably, we identify a “dark” proteome region characterized by a scarce availability of experimental structural information, in which predictions provided by AI tools significantly diverge, suggesting some level of uncertainty in the in-silico structure determination. This highlights the need to further improve AI-based prediction methods in this region. All models are included in 3DhRPx2, a dedicated database available at https://3dhrpx2.biocomp.unibo.it. The database is particularly useful for those proteins lacking an experimental reference structure, allowing a direct evaluation and comparison of predicted models with two alternative state-of-the-art tools. Moreover, it helps in highlighting high-quality model regions and can support research in different areas, such as identification of active sites, protein-protein interface analysis, functional annotation, analysis of disease-related variations and improvement of protein structure prediction.

Geometric Deep Learning Methods for Biological Data

Ferdinando Zanchetta

Geometric Deep Learning models are particular Deep Learning algorithms leveraging some geometric structure present or discoverable in the data they appy to (such as a graph structure). To start with, I will introduce some basic ideas and results to explain why this very important family of algorithms is becoming so popular and successful. I will then focus on the algorithms of this type that I am using to study two types of biological time series that I am studying: ECG and calcium imaging time series of neurons. I will report our findings and point out to future developments.

DNA damage and genome instability induced by Topoisomerase I cleavage complexes

Marco Russo

DNA Topoisomerase 1 (Top1) has an important function in preventing transcription/replication conflicts (TRCs) caused by RNA polymerases elongating in the opposite (head-on) or same direction (co-directional) of advancing replication fork. As poisoning of Top1 can lead to DNA damage and genome instability, we have focused on molecular mechanisms underlying DNA double strand breaks (DSBs) formation induced by Top1 cleavage complexes (Top1ccs) in HCT116 cell line. We have mapped genome-wide DSBs by END-Seq at very early time of treatment with camptothecin (a Top1 poison) to evaluate the immediate effects of Top1ccs. We demonstrated that thousands of DSBs appear on the genome soon after 10 minutes of treatment, and most of them persist for longer treatment times (20 min). These DSBs can be either double- or single-ended. Persistent DSBs induced by Top1ccs are present at euchromatin regions, highly transcribed genes, overlap RNA Pol II accumulation sites and are strongly associated with topological associating domains (TADs) and early replication origins. Furthermore, as seDSBs originate at replication forks and taking advantage of strand specificity of END-seq technology, we inferred TRC directionality. We found that seDSBs show a very close relationship with Top1ccs levels, that accumulate immediately downstream of the break, suggesting a direct involvement of Top1cc in seDSB formation. Moreover, seDSBs that originates from co-directional TRC are more enriched at 5' end of highly transcribed genes and are associated with strong accumulation of RNAPII levels upstream of the DSB. These data demonstrate that Top1ccs mediate the onset of DSBs at sites with a specific genomic context, that is different depending on the directionality of TRCs.

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SESSION V: Microbiology

Culture supernatants from beneficial vaginal lactobacilli: old friends with new perspectives

Carola Parolin

The correct functioning of the human organism is strictly dependent on its microbiota, women's health is also closely connected to the composition of the vaginal and genital microbiota. Microbiota alterations have been correlated with the onset of genital infections, inflammatory state and infertility, which have a strong impact on the women's quality of life. In healthy condition, the vaginal microbiota is characterized by low microbial diversity and dominance of lactobacilli, which contribute to the maintenance of vaginal health preventing pathogenic species overgrowth and maintaining the cervicovaginal epithelium functionality. In recent years, the paradigm of probiotic strains -including Lactobacillus- which are beneficial for human health has shifted towards the identification of probiotics-derived compounds with specific health-promoting activities. In this context, postbiotics, which include isolated cellular components or metabolites, have gained attention in terms of chemical characterization and functional activity assessment. In the postbiotics field, we have been working on the isolation of cellular components from vaginal Lactobacillus crispatus and Lactobacillus gasseri strains, and studied chemical and functional properties: we have thus demonstrated biosurfactants, exopolysaccharides, extracellular vesicles and that heat-killed lactobacilli are able to prevent pathogen adhesion/growth and promote beneficial bacteria. In the context of metabolites produced by probiotic bacteria, we have extensively studied lactobacilli spent culture supernatants, correlating their composition with antimicrobial activity and mode of growth, and leading to the identification of culture supernatants particularly active towards genital pathogens, such as Candida spp, Chlamydia trachomatis and HSV-1. Our research aims to deepen the chemical characterization of lactobacilli supernatants, in relation to bacterial metabolism and functionality. Through the comprehension of the beneficial activities of the vaginal microbiota, we can identify bioactive molecules or metabolites to be proposed as to improve women's health.

Deciphering the interactions between bacteria and macrophages in ex vivo human organ perfusion models

Daniele Ghezzi

Tissue resident macrophages are a heterogeneous population that provides innate cellular immunity by performing pathogens removal from the bloodstream and protects from sepsis during systemic infections. Previous studies in mouse models showed that invasive bacterial disease occurs after rare events of macrophage functionality failure that led to within-macrophage replication of bacteria and consequent systemic infection. However, the mechanisms of interaction between macrophages and bacteria in the human spleen and liver are still poorly known, particularly in the early phases of the infection process. To this end, we used ex vivo human spleen and liver perfusion models to investigate the interactions between macrophages and a cocktail of Streptococcus pneumoniae serotypes with high or low invasive disease potential. Human spleens and livers were infected and perfused for 6 hours. Biopsies were collected at different timepoints, processed for viable bacterial cell count, and stained for immunohistochemistry image analysis. Microscopy of spleen biopsies revealed that most bacteria were detected inside the red pulp macrophages, but the periarteriolar sheath macrophages were relatively more efficient at capturing and clearing bacteria. During spleen perfusion, macrophage removed and killed over 90% of the bacteria introduced into the perfusion liquid. Apoptosis was observed about eight times more frequently in perifollicular macrophages, which are among the first cell types to encounter invading pathogens. Spleen macrophages also showed high levels of lysosome activation and GM-CSF production. Differently to the spleen, the liver macrophages were less efficient in pneumococcal clearance. No changes were observed in apoptosis signal in liver macrophages over the infection time, as less than 1% of the apoptosis marker colocalized with macrophages in both infected and uninfected livers at all time points. This set of data provide the first functional analysis on how human splenic and liver macrophages control pneumococcal infection.

Zonation of the Vitis vinifera microbiome in Vino Nobile di Montepulciano production area

Simone Rampelli

The uniqueness of the relationship between wine and its territory of origin is defined by the concept of terroir, which includes local pedoclimatic, biotic and abiotic factors combined with traditional agricultural practices to explain the distinctive regional characteristics of the product. Among these determinants, the components of the vineyard microbiome have been proposed as possible and previously neglected new key determinants of terroir characteristics, associated with geographic location and reported to be directly relevant to viticulture, grape quality and winemaking. In the context of the EU project CIRCLES H2020, we had the opportunity to define the microbial terroir of vineyards scattered in the 12 sub-areas (or Additional Geographic Unit - AGU) of the "Consorzio del Vino Nobile di Montepulciano DOCG" (Italy), a world-renowned wine-producing area. To this end, rhizospheres of Vitis vinifera cultivar Sangiovese and soil samples were collected throughout the 2022 viticultural season and analyzed by an integrated metabarcoding/shotgun metagenomics approach targeting both bacteria and fungi. In addition to highlighting a peculiar taxonomic configuration of the microbial terroir in the Montepulciano territory compared to other vineyards worldwide, our data show that microbiomes vary according to geographic location also at a more local scale (i.e., the AGUs). Furthermore, the reconstruction of metagenome-assembled genomes revealed the potential for plant growth-promoting functionalities in some of the microorganisms of the Montepulciano territory, with some differences related to the AGU of origin. In addition to enriching our understanding of the importance of soil and root-associated microbiomes in defining wine terroir within the Vino Nobile di Montepulciano DOCG area, this project may provide further economic incentives for agricultural and oenological practices that preserve regional microbial terroir and biodiversity.

Routes of dispersion of antibiotic resistance genes from the poultry farm system

Daniel Scicchitano

Poultry farms are hotspots for the development and spread of antibiotic resistance genes (ARGs), due to high stocking densities and extensive use of antibiotics, posing a threat of spread and contagion to workers and the external environment. Here, we applied shotgun metagenome sequencing to characterize the gut microbiome and resistome of poultry, workers and their households - also including microbiomes from the internal and external farm environment – in three different farms in Italy during a complete rearing cycle. Our results highlighted a relevant overlap among the microbiomes of poultry, workers, and their families (gut and skin), with clinically relevant ARGs and associated mobile elements shared in both poultry and human samples. On a finer scale, the reconstruction of species-level genome bins (SGBs) allowed us to delineate the dynamics of microorganism and ARGs dispersion from farm systems. We found the associations with worker microbiomes representing the main route of ARGs dispersion from poultry to human populations. Collectively, our findings clearly demonstrate the urgent need to implement more effective procedures to counteract ARGs dispersion from poultry food systems and the relevance of metagenomics-based metacommunity approaches to monitor the ARGs dispersion process for the safety of the working environment on farms.

Blueprinting Gene Regulation in Helicobacter pylori

Andrea Vannini

Bacterial pathogens interact with the host, its immune defenses, other co-commensal microorganisms, and adapt to environmental fluctuations by orchestrating the expression of arrays of genes, including those coding for virulence factors, toxins, detoxification systems, and for metabolic and homeostatic adaptations. This modulation of gene expression occurs through complex networks of specialized regulatory systems, influencing transcription, transcript stability, translation, as well as the turnover of the proteins, their localization and activity. In the widespread human pathogen Helicobacter pylori, known as a class 1 carcinogen due to its role in promoting stomach cancer, the modulation of gene expression is primarily achieved through transcriptional and post transcriptional circuits controlled by a limited number of transcription factors (TRs) and multiple non-coding RNAs (ncRNAs), respectively. This project aims to unravel the direct regulons and molecular functioning of selected TRs essential for H. pylori survival and host infection, specifically Fur and the orphan regulator HsrA. This is accomplished by integrating the identification of TR binding sites on the genome (ChIP-seq) with the analysis of transcriptional responses (RNA-seq) mediated by the TR. Results of the study (re)define the regulons of these transcription factors, propose additional biological functions for the TRs, and identify groups of ncRNAs controlled by the TRs, whose levels are modulated in response to specific environmental signals. Additionally, the study extends to elucidating interactome and functioning of these ncRNAs through transcriptome (RNA-seq) and proteome analysis (iTRAQ-based) and the identification of direct targets using the MS2 affinity purification coupled with RNA sequencing (MAPS) genomics approach.

Continuous antibiotic prophylaxis has long-lasting effects on the gut microbiome structure and functionality in infants with vesicoureteral reflux

Federica D'Amico

The development of the gut microbiome (GM) during childhood can be influenced by several factors, including antibiotic treatment, which may have long-term consequences for host health (e.g., development of allergy, asthma, and obesity). Vesicoureteral reflux (VUR) is a typical disorder of early childhood, which is treated with continuous antibiotic prophylaxis (CAP) to limit the development of urinary tract infections and subsequent damage to the renal parenchyma. However, the use of CAP is controversial. In this context, the aim of this study was to investigate the long-term effects of CAP on the GM of children with high-grade (III-V) VUR. Specifically, the GM of 122 children with VUR treated or not with CAP was longitudinally characterized from enrolment to 72 months by 16S rRNA amplicon sequencing and targeted metabolomics (e.g., short-chain fatty acids, amino acids, sugars). Moreover, shotgun metagenomics was performed in a subset of samples for resistome profiling over time.

Comparative subgroup analysis revealed temporal changes in GM diversity in CAP-exposed infants compared to the non-CAP group. From the taxonomic standpoint, CAP-related GM was characterized by an initial dysbiosis, i.e., an increased relative abundance of opportunistic pathogens and decreased proportions of health-associated taxa. The dysbiosis tended to resolve over time, also from a functional point of view, but some potentially harmful signatures persisted.

This study demonstrates that even a short exposure to CAP in children with VUR can lead to long-term GM alterations, with potential risks to host health.

Characterization of Limosilactobacillus vaginalis derivatives as food supplements

Barbara Giordani

Early gut colonization by Bifidobacterium spp., as it generally occurs in vaginally born infants but not in those born by C-section, is strictly related to a general state of health. Considering that naturally delivered babies come into contact with material's lactobacilli, which dominate healthy vaginal microbiota, we unraveled the bifidogenic properties of such beneficial bacteria, with the ultimate aim of proposing novel postbiotic-based strategies to shape the gut microbiota.

In this perspective, postbiotics (cell-free supernatants, CFS, and heat-killed cells, HKC) were recovered from vaginal Lactobacillus gasseri, Lactobacillus crispatus and Limosilactobacillus vaginalis isolates and screened for the stimulating capacity of Bifidobacterium spp. free-floating cells and biofilms. Although CFS/HKC from all vaginal lactobacilli demonstrated bifidogenic activities, postbiotics from L. vaginalis BC17 consistently proved to be the most effective and were consequently selected to be further characterized in cell models.

Regarding this, CFS and HKC from L. vaginalis BC17 CFS/HKC from L. vaginalis BC17 improved bifidobacteria adhesion to Caco-2 cells (up to +200% for CFS and +290% for HK), whilst gastrointestinal pathogens' adhesion resulted impaired by 25-80%, thus confirming their prebiotic potential. The impact of postbiotics on intestinal cells (Caco-2 and HT-29) was considered and, importantly, they resulted not cytotoxic. On the contrary, after 48 h of incubation the viability of Caco-2 cells was higher in the presence of CFS (+40%) and HKC (+20%) while the proliferation of HT-29 resulted increase after 24 h of incubation with CFS (+60%) and HK (+40%).

Finally, postbiotics from BC17 reduced the nitric oxide production in Raw264.7 macrophages by 50% (CFS) and 40% (HKC), thus showing good anti-inflammatory activity.

In conclusion, L. vaginalis BC17 derivatives exhibited excellent prebiotics features, opening up the possibility of their incorporation in food supplements to trigger a favourable bifidogenic shift in the infant gut. In this perspective, formulative studies are currently ongoing.

SESSION VI: Drug discovery and development

Nanosystems for the treatment of bacterial local infections

Angela Abruzzo

In the last decade, the incidence of bacterial local infections has been increasing at an alarming pace, representing one of the major healthcare issues affecting people worldwide and often determining frequent emergency hospitalisation. Moreover, the global spread of multidrug-resistant strains has determined the insurgence of infections that are difficult to eradicate, posing further a global threat to public health. The use of conventional dosage forms for the treatment of local (mucosal or cutaneous) infections presents some drawbacks mainly related to the difficulty to reach adequate drug concentration at the infected site. This in turn requires multiple administrations, thus impairing patient compliance and worsening the antibiotic-resistance phenomenon. In this context, the encapsulation of anti-infective agents into innovative drug delivery systems, like polymeric or lipid-based nanocarriers, can be a promising approach to overcome the mentioned drawbacks associated with the use of conventional dosage forms. Scalable and suitable manufacturing methods as well as the use of adequate excipients can be exploited in order to design nanosystems with peculiar characteristics. Particularly, the selection of nanosystems with specific size and surface charge, characterized by the ability to interact with the main components of the tissue and capable to control drug release, can allow to improve the accumulation of the delivered drugs at the infected site and to improve drug efficacy. Therefore, several polymeric and lipid-based release nanosystems, intended for mucosal and cutaneous delivery of antimicrobials, will be presented highlighting the impact of the employed preparative method and excipients on their main physico-chemical, technological and functional properties.

Spray congealed microparticles for oral drug administration: recent developments, current challenges and future directions

Serena Bertoni

Spray congealing (SC), a process that transforms a melt into solid microparticles (MPs), is receiving great attention as environmentally-friendly manufacturing technology characterized by avoidance of organic or aqueous solvents, simple scale-up and high product yield. SC has been used in a wide range of applications including food and pharmaceutical area. Recently, our research group has explored for the first time the utilization of SC for the preparation of hybrid MPs consisting of two phases, a lipid and a polymer. This was possible by using, instead of one molten material, non-aqueous melt emulsions, which were transformed by SC into MPs showing different internal architecture. Due to the combination of lipids and polymers, hybrid MPs showed a wide array of drug release profiles that depends on their composition, the type of loaded drug, the drug loading amount and location, providing a more versatile delivery platform compared to traditional one-phase MPs. However, one of the main challenges related to lipid-based MPs regards their complex solid state behaviour, characterized by multiple crystalline structures (polymorphism), which can cause instability of the formulation during storage. For example, solid triacylglycerides (TAG) present three polymorphs, namely α, β' and β, from the less stable to the most stable one. Small amounts (5-10 % w/w) of fatty acids and esters with different chain length were found to significantly speed up the polymorphic transition of MPs based on a solid TAG, tristearin, from α to β-form. The ability to promote the crystallization of the lipid into the most stable crystalline form can be exploited to avoid uncontrolled transitions over storage. The kinetic of the polymorphic transition was investigated by simultaneous synchrotron SAXS/WAXS analysis as well as by Raman microscopy. Finally, the effect of additives on the nano- and micro-structural properties and on the release behaviour of MPs was evaluated.

Searching for inhibitors of the DNA repair protein RAD52

Federico Falchi

Synthetic lethality presents an innovative paradigm for the discovery of novel cancer therapeutics. Notably, PARP inhibitors have shown efficacy in cancers harbouring BRCA mutations. Our recent findings extend the concept of synthetic lethality beyond mutational landscapes, demonstrating its induction through the combination of Olaparib with small molecules able to disrupt the RAD51/BRCA interaction. Leveraging the recent structure obtained using Cryo-EM of the RAD52 DNA repair protein, we conducted a structure-based virtual screening, leading to the identification of small molecules capable of inhibiting RAD52's DNA repair function. These compounds can potentially induce synthetic lethality when used synergistically with PARP inhibitors.

Playing covalent: looking for valid chemical probes to study neurodegenerative diseases

Filippo Basagni

The complex etiopathology of neurodegenerative diseases remains one of the biggest unsolved questions of modern neuroscience which results in lack of effective treatments able to halt the cognitive/motor decline. Intertwined neurotoxic mechanisms (i.e. misfolding and protein aggregation, oxidative stress, neuroinflammation) in concerted manner lead to synaptic loss and neuronal death in important functional brain regions related to the characteristic clinical symptoms. In this context, the development of chemical probes able to selectively modulate specific cellular pathways could result in pivotal contribution toward a deepen comprehension of cellular machinery under neurodegenerative conditions. Particularly, tailored covalent modulators trapping critical functional nucleophilic residues of relevant molecular targets are gaining increasing interest in chemical biology thanks to the peculiar prolonged (in)activation mechanism, and the opportunity to lock specific nucleophilic residues in proteins. On this basis, we developed a series of nature-inspired polyphenol hybrids that, properly decorated, were able to selectively modulate different targets, moving from non-covalent to covalent mechanisms of action. Particularly, by systematically modifying the reciprocal position of the aromatic hydroxy functions we achieved potent antiaggregating compounds, ranging from covalent to non-covalent inhibition modes. From this point, small structural modifications were performed on the scaffold responsible of the antiaggregating properties, which switched the selective inhibition of Aβ aggregation into the activation of Nrf2-mediated antioxidant activities. Furthermore, the chemical manipulation of the electrophilic fragments within this class of compounds offered the opportunity to selectively and alternatively modulate inducible transcriptional responses, moving from Nrf2 activation to NF-κB inhibition. Based on these premises, we are seeking to perform wider structure-activity relationship studies with the aid of biophysical techniques in order to define the molecular mechanism for activities of electrophilic compounds towards the three different targets and further optimize the corresponding target engagement underpinning antiaggregating and neuroprotective properties.

Metabolomic patterns in response to abiotic stimuli of Cladonia lichens from dry grasslands

Ilaria Chiocchio

Lichens are complex organisms, defined as a self-sustaining ecosystem generated from the symbiosis between photobionts and mycobionts (Hawksworth & Martin, 2020).

This study focuses on 4 species of genus Cladonia (C. foliacea, C. polycarpoides, C. rangiformis, C. rei), endowed with numerous bioactivities (Adenubi et al, 2022). Each species was sampled monthly for one year, in 5 different sites alongside dry grasslands of the Ticino River valley (Italy). The objectives were: 1) to acquire and elucidate (for the first time) the 1H NMR profile of these species, 2) to picture intraspecies metabolomic differences, and 3) interspecies metabolomic variation due to environmental factors.

By chemometrics were obtained validated models, and the species were clustered not only based on their biomarkers (i.e. usnic, protocetaric, fumarprotocetaric, rangiformic, norrangiformic, sekikaic and homosekikaic acids), but also for the enrichment in primary metabolites.

For instance, fumarate and sucrose were more concentrated in C. polycarpoides. C. rangiformis had the highest concentration of arabinitol, acetate and alanine, while glucose and trehalose were more abundant in C. rei. Several intraspecies differences, both related to time-point and harvesting site, were also detected. Moreover, the metabolome of all species resulted significantly affected by the temperature gradient. Arabinitol emerged as one of the most important metabolites produced in response to chilling.

The overall results offer a metabolomics-based discrimination method for Cladonia species, bringing insights both on when to harvest these lichens in view of a specific medicinal use, and on how they might adapt to environmental changes.

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The use of pre-exposure prophylaxis (PrEP) in the control of HIV infection and other sexually transmitted diseases (STDs). Descriptive-observational analysis in an italian polyclinic

Davide Cicetti

Between Aug 2023 and Feb 2024, 517 patients had access to the medication dispensary of the Infectious Diseases Department to collect PrEP. The average age of the population was 38 years and 98% were men. The analysis of 424 patients showed that the average duration of PrEP use was 17 months and that the preferred mode was “daily” in 56.8% of cases. Only 1 patient contracted HIV-1 during PrEP on demand, showing a 99.8% success rate of prophylaxis. However, in 357 patients, there are significant data on the acquisition of other sexually transmitted diseases. In particular, gonorrhea is the most frequently recorded infection with at least one episode in 30.5% of patients; followed by Chlamydia trachomatis infections 26.8%, and Luetica 12.6%. 11 patients had at least one episode of each of the three STDs. No new HBV infections were diagnosed, while 1 case of HCV acquisition was diagnosed. Of the population analyzed, only 50.7% of cases were not infected with sexually transmitted pathogens. 77.6% of the total 424 patients analyzed were vaccinated against HBV, with 78.4% of cases having a protective antibody titer; in the remaining 8.2% serology was consistent with previous infection. Regarding protection against HPV, 42.9% of patients had been vaccinated with 3 doses, 27.1% had not been vaccinated and 7.3% was completing the vaccination cycle.

It appears that pre-exposure antiretroviral chemoprophylaxis is indeed an effective means of preventing the acquisition of HIV-1 infection, as of all 357, patients only 1 patient acquired the infection during the study period, with a treatment success rate of 99.8%. However, 49.3% of patients were diagnosed with at least one STD. Therefore, although PrEP is an effective means of reducing the spread of HIV-1 infections, this treatment does not eliminate the possibility of contraction all other sexually transmitted diseases.

The role of the counselor pharmacist in the management, education, and care of the patient in chronic obstructive pulmonary disease (COPD)

Silvia Bartoli, Mariangela Parca

Objective: According to the World Health Organization, COPD is currently the third most common cause of death globally. Non-adherence to treatment leads to an exacerbation of disease symptoms and increased frequency of medical consultations and hospital admissions. The counselor pharmacist must educate the patient in the correct use of the device to promote better therapeutic adherence (which averages 50% in developed countries). From this perspective, the purpose of the project is to reduce the risks of misuse and increase the beneficial effects of new therapies based on the GOLD guidelines (global initiative for chronic obstructive lung disease) improving patient management and differentiating the therapeutic approach.

Materials and methods: After specialist examination, the patient is sent to the direct distribution of the hospital pharmacy for a structured interview. At this point, the counselor pharmacist performs pharmacological reconnaissance, promotes adherence to therapy, carries out educational activities on the correct techniques of device use, and gives the patient information sheets on the prescribed device (nebulizers, pre-dosed sprays, MDIs with spacers or powder inhalers). The first course of therapy is documented by patients in a therapeutic diary that must be presented at the next appointment to record any problems encountered with the device or adverse reactions to the drug (adhesion%).

Expected results: As pharmacists are easily accessible to both patients and health care providers, they are ideally placed to play an important role in the enhancement of education, and continuous assessment of, optimal inhaler technique, thereby improving adherence (get to at least 70%), disease control, and quality of life.

Keywords: adherence; chronic obstructive pulmonary disease; counselor; device; education; inhalers; patient; pharmacist; therapy.

Leveraging N-Acylhydrazone-based dynamic combinatorial chemistry to develop RAD51-BRCA2 protein-protein inhibitors as potential synthetic lethal anticancer agents

Greta Bagnolini

Synthetic lethality (SL) has been consolidated as paradigm for anticancer therapy, as proved by the use of PARP inhibitors (PARPi) in BRCAness tumors. In this context, we envisioned SL as paradigm to design synergistic combinations of small molecule drugs, targeting the synthetic lethal pair PARP/BRCA2. BRCA2 acts upon a DNA double-strand break, by recruiting RAD51, a cytosolic ATP-dependent recombinase, and transporting it into the nucleus where it performs DNA repair by homologous recombination. Cancer cells are subjected to a high rate of DNA damages and can become dependent on RAD51 for their survival. Indeed, RAD51 is overexpressed in many tumors. Therefore, we reasoned to inhibit the protein-protein interaction (PPI) between RAD51 and BRCA2 using small molecules, in order to mimic BRCAness and trigger SL in combination with PARPi in cancer. Among the hit identification strategies to design PPI inhibitors, we used the protein-templated dynamic combinatorial chemistry (ptDCC), as first application on oligomeric RAD51. This technique uses dynamic combinatorial libraries (DCLs), generated by reversible reactions of appropriate building blocks. Upon reaching an equilibrium state, the addition of the target protein shifts the equilibrium in favor of protein ligands, an event named templating effect. We designed three N-acylhydrazone-based (NAH) DCLs and, upon addition, RAD51 showed a clear templating effect, amplifying 19 NAHs. We screened amplified NAHs in an ELISA assay to evaluate their inhibitory activity against RAD51-BRCA2 PPI and identified ten NAHs as micromolar inhibitors. The subsequent 19F-NMR experiment suggested the interaction of one NAH in the same binding pocket of BRCA2, supporting the results of the ELISA assay. Here, ptDCC could support the early stage of the development of small molecule PPI inhibitors. Lastly, upon an appropriate chemical exploration, the identified hit NAHs may be potentially players in the synthetic lethal combination with PARPi to treat cancer.

SESSION VII: Cancer biology

New insights in the G-Quadruplex-mediated genome instability to promote an innate immune response in cancer cells

Giulia Miglietta

Micronuclei (MNi) are extranuclear bodies containing chromatin enveloped by a defective nuclear membrane and they are caused by an aberrant chromosomes segregation during mitosis. In our lab, we discovered that the unproper stabilization of a DNA non canonical structure called G-quadruplex (G4) result to micronuclei accumulation in cancer cells [Miglietta et al. Nucleic Acids Res (2021), Marzano et al, J. Med. Chem. (2022), Amato et al. J. Med. Chem. (2020)]. G4 folding regulates fundamental cellular processes indeed the non-physiological stabilization of these structure results to DNA damage and consequently to micronuclei formation [Miglietta et al. Nucleic Acids Res (2020)]. By the screening of G4 binders we disclosed that G4-dependent micronuclei do not stimulate the same cellular outcome. We set up a protocol to purify with high efficiency the micronuclei promoted by G4-dependent genome instability in cancer cells, and we performed a genome-wide proteomic mass spectrometry. Our data indicate that pyridostatin (PDS)-stimulated micronuclei are enriched in autophagosomes, lysosomal proteins, protein involved in innate immune response and cytosolic sensors of pathogen-associated DNA. Differently MNi stimulated by a chemical unrelated G4 binder (RHPS4) are enriched of mitochondrial proteins and they are not responsible for an interferon-related transcriptional cascade as showed by ELISA assay and RNA sequencing experiments. Furthermore, through transcriptional profiles stimulated by the G4 binders we show that G4-mediated MNi result to different cellular responses, and we disclose the role of autophagy in the cGAS-STING pathway activation and the suppressive role of damaged mitochondria to trigger an innate immune response. Our data suggest that G4-stimulated micronuclei are crucial in regulating an innate immune response revealing therapeutic challenge for G4 binders [Miglietta et al. Mol. Cancer. (2022)] especially in non-responsive tumors. The research is funded by AIRC (ID 23032 project) and by the European Union (PNRR - M4C2-I1.3 Project PE_00000019 "HEAL ITALIA" CUP (J33C22002920006)).

SPARC release upon respiratory Complex I impairment supports survival and migration of ovarian cancer cells

Manuela Sollazzo

In the last years the involvement of mitochondrial respiration has been increasingly recognized as pivotal player during tumor progression and chemoresistance. In particular, targeting respiratory Complex I (CI) has been proposed as a new therapeutic approach to hinder cancer growth. In this context, we have demonstrated that a severe CI impairment promotes a delay of tumor expansion but not its complete eradication. Indeed, over time CI-defective cancer cells survive and reactivate their proliferation, allowing us to speculate that adaptive mechanisms occur to overcome the metabolic and molecular consequences of mitochondrial impairment. This scenario might be relevant in ovarian cancer (OC), where about 85% of patients develop relapses after standard surgical and pharmacological treatments. Here, we show that under metabolic stress condition the activation of AMPK allows CI-defective cancer cells to experience the downregulation of mTORC1 activity and the block of protein synthesis which could explain their proliferation slowdown. The survival of CI-defective quiescent cells may be supported by an upregulation of a matricellular protein SPARC associated with the cytoskeleton remodeling likely mediated by PKC activation. The dissection of such different pathways may offer potential molecular players in synthetic lethality with CI inhibition, thus providing new synergistic strategies for cancer treatment and, in particular, for OC.

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Molecular and functional characterization of the novel master regulator RCOR2 in MYCN-driven tumors

Giorgio Milazzo

MYCN is certainly one of the most well- known master regulators in cancer. Given the challenge associated with targeting MYCN, the identification and characterization of MYCN-associated functional partners with greater druggability represent a strategic approach to surmounting this obstacle. Preliminary studies on RCOR protein family highlighted the unique nature of RCOR2 as compared to its paralogs RCOR1 and RCOR3, revealing RCOR2 as a new member of the NB core regulatory circuitry (CRC), the essential-for-cell-state transcriptional network that positively drives tumorigenic programs. Strikingly, we also noticed a distinctive functional and physical relationship with MYCN, but not with MYC. Transcriptomic analyses upon CoRESTs KD revealed a non-functionally redundant role of these factors, as they seem to drive the expression of unique sets of target genes. Taken together, these findings highlight a prognostic role of CoREST proteins in NB and revealed them as essential factors for NB cells survival, suggesting new possible and promising targets for cancer therapies to tackle NB.

3D multicellular osteosarcoma models for drug screening

Martina Rossi

Osteosarcoma (OS) is the most common malignant bone tumor in children and adolescents. To date, treatment for this neoplasm focuses on surgical therapy, combined with pre- and post-operative adjuvant chemotherapy. However, the prognosis for patients remains very poor, especially in cases where surgery is not possible, or drug resistance sets in. Therefore, research for alternative therapeutic approaches is particularly important. The studies of our research group are focused on the development of a 3D model, capable of mimicking the entire tumor microenvironment, to test new therapeutic approaches. A 3D model consisting of a co-culture of OS cells and stromal cells has been optimized in order to recreate the tumor microenvironment in vitro. Firstly, OS spheroids were generated from two different cell lines, MG63 and 143B, using the hanging drop technique and then combined with stromal cells. Finally, cells were included in a hydrogel, Vitrogel-RGD, in order to recreate the extracellular matrix. After one week of culture, it is possible to observe the invasion of the matrix by OS cells. At this stage, the models were treated with licochalcone A (Lic-A), a molecule already known for its anti-tumor activity against OS. To evaluate the anticancer effect of Lic-A firstly metabolic and viability assays were conducted. Second gene and protein expression of different marker, as metalloproteinase and BMP2, were evaluated. The results showed that Lic-A is able to reduce both the viability of tumor cells and the expression of markers typical of the metastatization process. In conclusion, we successfully developed an advanced 3D multicellular model of OS and demonstrated the efficacy of Lic-A in reducing viability and metastasis-associated markers.

Impact of osteosarcoma cells differentiation on biomineralization and mitochondrial morphology

Francesca Rossi

Osteosarcoma (OS) is the most common primary malignant bone tumor, and its etiology has been recently associated with osteogenic differentiation dysfunctions. A deeper understanding of the relationship between defects in osteogenic differentiation and malignant bone tissue formation could help the comprehension of osteosarcoma tumorigenesis and the development of new treatment strategies. To characterize the defective biomineralization process that occurs in OS, we studied osteoblast-like SaOS-2 cells during the early stages of differentiation. Cryo-soft X-ray tomography (cryo-SXT) and cryo-XANES imaging at the CaL2,3 edges were combined to investigate the evolution of the Ca-depositions during differentiation, allowing to simultaneously track morphological variations in intracellular organelles at nanometric resolution. SaOS-2 cells were grown on electron microscopy grids, treated for 4 and 10 days with a differentiating cocktail, plunge frozen in liquid ethane and finally imaged in a quasi-native state using the soft X-ray transmission microscope installed at the Mistral beamline of the Alba synchrotron. Untreated SaOS-2 produced calcite depositions as previously observed in early differentiated bMSCs (bone mesenchymal stem cells). This confirms that the early phases of osteoblastic differentiation are similar in both bMSCs and SaOS-2 cells, supporting the hypothesis that OS cells arise from MSCs unable to undergo complete differentiation. The evolution of mineral deposits from calcium phosphate to hydroxyapatite induced by the differentiating treatment suggests a partial restoration of the physiological biomineralization process in SaOS-2 cells, as previously assessed in differentiating bMSCs. At 4 days after induction, mitochondria contain small Ca structures and are linked to vesicles containing calcium phosphate depositions highlighting calcium transfer between the two intracellular organelles. At 10 days after induction, no Ca minerals are detected in the mitochondria, supporting the interplay between mitochondria and vesicles in calcium trafficking. Interestingly, during differentiation, mitochondria show a change in morphology from elongated to rounded indicating a metabolic reprogramming of OS cells possibly linked to an increase in glycolysis contribution to energy metabolism.

These findings contribute to the understanding of OS genesis giving new insights on the development of therapeutic strategies able to restore the physiological mineralization in OS cells.

The metabolic setting of ovarian cancer cells modifies response to chemotherapy

Luigi D'Angelo

Epithelial ovarian cancer (EOC) is the most lethal and silent gynaecological malignancy. Despite initial response to conventional platinum- and taxane-based chemotherapy, 85% of treated patients develops chemoresistance and often relapses. Therefore, the understanding of the still elusive mechanisms leading to therapeutic resistance is a major challenge for the development of more effective treatments. In the last few years, metabolic reprogramming has emerged as crucial for tumour progression with growing evidence showing that EOC cells switch their metabolism between aerobic glycolysis and oxidative phosphorylation (OXPHOS) to sustain tumour proliferation, invasiveness, and chemoresistance. However, which metabolism is preferred by chemoresistant cells is still debated. Our analyses revealed that metabolically energetic chemo-naïve EOC cell lines, relying both on glycolysis and OXPHOS, were more responsive to platinum-based treatment, whereas purely glycolytic cells were chemoresistant. Indeed, we found higher abundance and activity of mitochondrial respiratory complexes in chemosensitive EOC cell lines. Such high-OXPHOS state correlated with the expression of PGC-1 family of transcriptional co-activators, the master regulators of mitochondrial biogenesis. In hypoxia, we observed an increase in chemoresistance that was prevented by HIF1α ablation. Importantly, the acquisition of cisplatin-resistance in two energetic cell lines induced a shift to a quiescent metabolism with the reduction of both glycolytic and OXPHOS function when compared to their syngeneic sensitive counterparts. Overall, our findings suggest that a high-OXPHOS condition is associated with chemosensitivity and upregulation of OXPHOS may be a possible therapeutic strategy to overcome chemoresistance.

Generation of complex tumor spheroids for in vitro drug toxicity, towards personalized medicine

Emanuela Mensà

The tumor microenvironment (TME) is a complex environment of different cell types (tumor and non-tumor), extracellular matrix and chemicals, crucial for the development of cancer and for its response to drug treatments. Bi-dimensional in vitro cell models (2D) are generally limited as they currently fail to represent the complexity of TME.

For this reason the three-dimensional (3D) multicellular tumor spheroids (MCTs) model is becoming an essential tool because they closely simulate in vivo solid tumors (e.g. mimicking cell to cell and cell-extracellular matrix interactions) and they can increase the predictive value of pre-clinical drug research. However, their integration into more sophisticated drug discovery initiatives is still at a nascent stage owing to the absence of reproducibility and the limited efficiency in terms of time and cost.

In this study, we established a reproducible short-term 3D culture system addressing the various complexities of the colorectal carcinoma (CRC) microenvironment. HCT116 cells were co-cultured with immune cells (THP-1) and normal epithelial colon cells (FHC) in 3D spheroids. To generate homogeneous MCTs we used homemade thin microstructured poly-dimethyilsiloxane (PDMS) supports, fitted on the bottom of commercially sourced multi-well plates.

We observed the grow and the architecture of all spheroids generated by Incucyte and lightsheet technology respectively. Moreover we analysed the response of all spheroids to 5-fuorouracil (5−FU), one of the most clinically relevant drug in colon cancer treatment, by flow cytometry.

The step forward is to develop a new technology platform that synergizes the custom bioprinting technology with 3D cell culture in microwells designed to obtain a large number of structurally homogeneous 3D cancer spheroids.

The short-term 3D co-culture system developed in this study will be used for screening therapies. Understanding of signalling in 3D co-cultures and the response upon drug treatment will open the path to better disentangle the TME and to develop a promising tool for cost-effective studies on novel anticancer drugs for the colon cancer to be used in the precision medicine."

Personale tecnico

Poster session

A40

Labirinto tecnico

Dedalo della filiera tecnica Fabit

Filiera tecnica FABIT

Il labirinto è un percorso di corridoi non lineari in cui ci si potrebbe perdere, anche se è possibile scoprire nuovi spazi e opportunità inaspettate mentre si cerca l'uscita.
La filiera tecnica di un Dipartimento può sembrare un dedalo intricato nei cui meandri si possono celare aspetti inattesi. In questo poster vi descriviamo il labirinto tecnico del FaBiT, le caratteristiche della nostra filiera, la sua composizione e come si articolano le diverse attività del personale.
Scoprite le possibili vie per non perdervi nella ricerca delle soluzioni alle vostre esigenze.

A41

Indovina Chi

Filiera tecnica FABIT

A chi mi devo rivolgere per organizzare un'esercitazione di un laboratorio microbiologico?
Chi è il tecnico che può analizzare i miei campioni allo spettrometro di massa?
Ho usato il ghiaccio istantaneo della cassetta di primo soccorso a chi devo comunicarlo?
Come si chiama il tecnico che mi può attivare il badge?
Dove trovo informazioni sulle visite mediche?
A chi mi posso rivolgere per pubblicare una notizia sul sito del Dipartimento?
Ho finito la carta per la fotocopiatrice, a chi mi devo rivolgere?

Con questo poster indovina chi fa cosa!

A42

Può essere utile? Strumenti condivisi

Filiera tecnica FABIT

Il FaBiT dispone di attrezzature all'avanguardia per la ricerca in campo biochimico, biologico e chimico-farmaceutico. Queste strumentazioni sono state acquistate tramite finanziamenti dedicati e con il coinvolgimento di altre strutture e gruppi di ricerca dell'Ateneo, per soddisfare le sempre più specifiche e complesse richieste della ricerca sperimentale. Tali attrezzature sono messe a disposizione dei ricercatori e ricercatrici del nostro Dipartimento. Scopri quale strumento può essere utile alla tua ricerca.

A43

RM: Research Manager o Razza Maledetta? Un compendio dell'Ufficio Ricerca

Ufficio ricerca FABIT

Una descrizione della figura dell'RM e dello specifico lavoro all'interno dell'Ufficio Ricerca del FABIT

A44

CILDIC - ovvero il Mistero della Torre UE5

R.Bacchilega, V.Sanginario, A.Venturini, L.Verardi

Vogliamo provare a raccontarvi cosa accade nella grigia e misteriosa UE5: con immagini, keywords e soprattutto numeri, vi faremo affacciare ai 6 piani di laboratori didattici (anche se i piani sono 8...) e vi faremo intravedere ciò che accade quotidianamente durante la "Stagione" della didattica.

Nei piani 2, 3 e 4 vengono ospitate le esercitazioni che non hanno bisogno di aspirazione forzata mentre i piani 5, 6 e 7 sono arredati con 56 postazioni singole dotate di cappa aspirante che, se maltrattate, emettono sinistri e fastidiosi lamenti rendendo la vita impossibile.

In ciascun piano sono inoltre sempre presenti le megacappe di servizio, un'accogliente sala bilance e, in ordine sparso, si nascondono diversi strumenti spesso seviziati dagli studenti, altre volte dimenticati sotto strati di polvere...

Al piano -1 si apre il magazzino, composto da antri e corridoi bui e malsani, ma questo proibito ai più....

Ma cosa accade nella UE5 nelle lunghe e buie giornate di didattica...ma anche quando c'è il sole?

Arrivano classi (tra i 35 e 50) di ignari studenti provenienti da tutti i mondi chimici conosciuti: il FABIT, il CIAMICIAN e il TOSO e si cimentano in preparazioni alchemiche ignote...visto che, ahinoi, praticamente TUTTI non leggono le dispense prima delle esercitazioni.

Possono arrivare assonnati alle prime luci dell'alba oppure, (o anche), appesantiti dal pasto; qualcuno sembra già saperci fare, qualcun altro sembra guardarsi attorno smarrito...

Siamo comunque abbastanza certe che a tutti, in qualche modo, il laboratorio piaccia: si resta sempre stupiti per la vastità degli spazi, per l'intrico di tubi e fili che passano sopra le proprie teste, per gli ostici armadietti con i simboli chimici, per i suoni, o forse rumori.

Speriamo sempre che le giornate passate nella UE5 siano istruttive e divertenti, spesso però per noi sono vorticose ed estenuanti. Leggete e guardate tutto ciò che abbiamo provato a raccontarvi, ma cercate di focalizzare la vostra attenzione sui numeri e magari comprenderete il mistero!

A45

Dal macro al micro: i retroscena dei laboratori didattici della Beverara

Laboratori didattici – Beverara

Il Plesso del Navile ospita cinque laboratori didattici, a postazione singola, per gli studenti dei corsi di laurea biomedici e biotecnologici. La gestione di questi laboratori è affidata a personale che, pur non avendo responsabilità didattiche, offre agli studenti le proprie competenze tecniche per supportare la valenza formativa delle esercitazioni.
Più nel dettaglio, la quotidiana attività di questi professionisti consiste in una attiva collaborazione con i docenti per far fronte alle richieste di materiali, reagenti, preparazioni e strumentazioni al fine di offrire un buon allestimento laboratoriale. A tale scopo, recentemente le richieste di plasticheria e terreni è stata resa più snella con una procedura di condivisione digitalizzata tra tecnici e docenti.
Inoltre, mantengono aggiornato il reagentario online per poter garantire interscambio di reagenti tra diversi laboratori dell'Ateneo. Si preoccupano di garantire il giusto livello di scorte, monitorando le giacenze in magazzino ed eseguono una comparazione tra prezzi limitando gli sprechi per l'acquisto dei materiali.
Si occupano della manutenzione ordinaria della strumentazione cercando anche di destinare i fondi per la didattica per l'acquisto di nuove strumentazioni al fine di rendere i laboratori sempre più efficienti.
Nella conduzione generale del laboratorio, assicurano che siano sempre rispettate le norme e i regolamenti relativi alla sicurezza ed effettuano una corretta gestione dei rifiuti prodotti, assumendo ruoli di responsabilità all' interno del Nucleo Tecnico Rifiuti. Inoltre, prendono parte della Commissione didattica collaborando con docenti e ricercatori per definire obiettivi e razionalizzare risorse al fine di migliorare l'offerta didattica.
In aggiunta, partecipano a corsi di formazione e di aggiornamento, i quali costituiscono momenti essenziali per aumentare le loro competenze tecnico professionali.
Pertanto queste figure, con il loro contributo pratico e tecnico, sono un anello fondamentale durante l'attività formativa di laboratorio degli studenti, i quali riescono a finalizzare ciò che hanno imparato durante le lezioni teoriche in aula.