ANALYSIS OF AN EXPERIMENTAL AND IN SILICO-GENERATED PROTEIN INTERACTION NETWORK UNVEILS A PRO-PROLIFERATIVE ROLE OF ESRP1 IN HUMAN COLORECTAL CANCER CELLS

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Background: The RNA-binding protein, Epithelial Splicing Regulatory Protein 1 (ESRP1), participates in multiple steps of the post-transcriptional regulation of gene expression. Depending on cell type and disease context, ESRP1 can either exert a tumor suppressive role or participate in the metastatic process. We have shown that aberrantly high ESRP1 expression can drive human colorectal tumor progression.

Methods: In order to unveil the mechanisms by which ESRP1 can modulate cancer traits, we searched for genes and proteins affected by modulation of Esrp1 in two human colorectal cancer cell lines, HCA24 and COLO320DM, by cDNA microarray and proteomics analyses, respectively. RNA-immunoprecipitation followed by MALDI-TOF analysis was also employed to reveal proteins hosted by endogenous ESRP1 ribonucleoprotein complex in HCA24 cells. An integrated experimental-in silico approach was employed to identify a common molecular signature possibly explaining the pro-tumorigenic role of ESRP1.

Results: Gene expression profiling revealed that the oncogenic isoform of Rac1, Rac1b, co-expressed with Esrp1 in HCA24 and COLO320DM cells. A significant increase in Splicing factor 3A subunit 1 mRNA and protein expression was also observed upon ESRP1 expression in COLO320DM cells. Moreover, we found that cell cycle and apoptosis regulator protein 2, a well-known oncogene and cell cycle regulator, interacted with ESRP1 at the protein level. Interestingly, systems biology analysis revealed that ESRP1 interacts and is co-modulated with a group of proteins associated with a pro-mitotic and pro-proliferative phenotype.

Conclusions: Our data provide further insights into the factors affected by and entwined with ESRP1 in determining its pro-tumorigenic role in CRC.
ZFP36 GENE FAMILY IN THYROID DEVELOPMENT AND HORMONOGENESIS

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The aim of our work is to investigate the role of Zfp36l2 gene in thyroid development/activity in vitro and in vivo. Using FRTL-5 immortalized and non-tumorigenic thyroid follicular cells, we generated a stable Zfp36l2 KO cell line and cells overexpressing tagged Zfp36l2. We observed a reduction of proliferation in KO cells and an increased proliferation in overexpressing cells. As FRTL-5 cells resemble thyroid functionality, we analysed cells before and after TSH treatment. Interestingly Zfp36l2 expression/protein increase after TSH treatment, suggesting an involvement of this pathway in its control. We also investigated the expression level of thyroid-enriched genes in KO cells before and after TSH stimulation. We observed Tg overexpression in KO starved cells but none change was detected after TSH treatment. More interestingly, Nis transcript was inhibited (both at gene and protein level) after gene targeting and it was not further modulated by TSH. Tpo presented similar transcription profile. When we analysed the transcript of the main thyroid enriched transcription factors, a reduction of Pax8 and Foxe1 transcripts was detected. In thyroid samples of Zfp36l2 KO mice, we did not observed alteration of thyroid morphology until PND21 vs control mice. KO mice showed a reduction of the number of the follicles and the presence of fat deposits in the gland. In order to identify mechanism underlying the reduction of the thyroid follicles, we analysed caspase-3 cleavage revealing, as expected, an increased apoptosis in KO samples, vs controls. As Zfp36l2 targeting results in death at the PND21, we asked and obtained a conditional triple KO for all member of the ZFP36 family (Zfp36, Zfp36l1, Zfp36l2) that was crossed with Pax8-cre mouse. Preliminary results showed that the single KO and the multiple KO are hypothyroid, as circulating free T4 levels are lower in KO mice vs WT at 3, 6 and 9 months.

Overall, the data supports a role of ZFP36 proteins in thyroid hormonogenesis.
CHARACTERIZATION OF THE CALVARIAL SUTURE SKELETGENIC STEM CELL NICHE IN NONSYNDROMIC CRANIOSYNOSTOSIS

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The osteogenic niche residing in the skull includes calvarial sutures that contain mesenchymal stromal cells (MSC) regulating craniofacial bone growth and repair. Yet, few data are available on the mechanisms governing the homeostasis and cell-cell signaling in the niche. Nonsyndromic craniosynostosis (NCS) is a craniofacial malformation due to the premature ossification of skull sutures, with impairment of the calvarial osteogenic niche. The aim of this study was to characterize the calvarial MSC niche and the paracrine signalling that regulates the homeostasis in human skull sutures, hence to identify suitable targets for selective drug delivery to reprogram stem cell fate aimed at non-invasive treatments. Calvarial tissues were collected from surgical waste of patients undergoing cranial remodelling and served for MSC isolation. MSC isolated from bone marrow served as controls. The following lineage-specific markers were analysed in MSC, under growth and osteoinductive conditions, by immunofluorescence and qPCR: THY1, GLI1, AXIN2. In order to evaluate the paracrine effect of calvarial-derived MSC on bone differentiation, their conditioned medium (CM) was collected and used for in vitro osteogenic differentiation assay. Calvarial MSC homogeneously expressed the THY1+/GLI1+/AXIN2+ phenotype, indicating that explant cultures allow selecting comparable cell populations, regardless of the patient phenotype. Upon in vitro osteogenic induction in calvarial-derived cells, the expression of THY1 and GLI1 decreased, whereas AXIN2 levels increased. MSC grown with calvarial-derived CM, for 21 days, showed an increased expression of the early osteospecific gene RUNX2.

MSC isolated from calvarial sutures expressed a specific marker profile, with THY1+/GLI1+ representing the stem cells within the human calvarial niche. Our data, also suggested that calvarial MSC could exert a paracrine effect able to affect the microenvironment causing enhanced ossification in NCS.
CIRCSMARCA5 REGULATES VEGFA MRNA SPlicing AND ANGIoGENESIS IN GLIOBLASTOMA MULTIFORME THROUGH THE BINDING OF SRSF1


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Circular RNAs are a large group of RNAs whose cellular functions are still being investigated. We recently proposed that circSMARCA5 acts as sponge for the splicing factor Serine and Arginine Rich Splicing Factor 1 (SRSF1) in glioblastoma multiforme (GBM) [1]. After demonstrating by RNA immunoprecipitation a physical interaction between SRSF1 and circSMARCA5, we assayed by real-time PCR in a cohort of 31 GBM biopsies and 20 unaffected brain parenchyma controls (UC) the expression of total, pro-angiogenic (Iso8a) and anti-angiogenic (Iso8b) mRNA isoforms of Vascular Endothelial Growth Factor A (VEGFA), a known splicing target of SRSF1. The Iso8a to Iso8b ratio: (i) increased in GBM biopsies with respect to UC (p-value < 0.00001); (ii) negatively correlated with the expression of circSMARCA5 (r-value = −0.46, p-value = 0.006); (iii) decreased in U87-MG overexpressing circSMARCA5 with respect to negative control (p-value = 0.0055). Blood vascular microvessel density, estimated within the same biopsies, negatively correlated with the expression of circSMARCA5 (r-value = −0.59, p-value = 0.00001), while positively correlated with that of SRSF1 (r-value = 0.38, p-value = 0.00663) and the Iso8a to Iso8b ratio (r-value = 0.41, p-value = 0.0259). Kaplan-Meier survival analysis showed that GBM patients with low circSMARCA5 expression had lower overall and progression free survival rates than those with higher circSMARCA5 expression (p-values = 0.033, 0.012, respectively). Our data convincingly suggest that circSMARCA5 is an upstream regulator of pro- to anti-angiogenic VEGFA isoforms ratio within GBM cells and a highly promising GBM prognostic and prospective anti-angiogenic molecule [2].

References:
LNCRNAS IN CELIAC DISEASE

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Celiac Disease (CD) is a multi-factorial autoimmune enteropathy affecting 1% of the population worldwide, developing after the exposure to an exogenous antigen, gluten, in genetically predisposed subjects.

CD pathogenesis involves the crosstalk of several mechanisms, including the activation of the innate and adaptive immunity.

LncRNAs have been found deregulated in several disorders, including autoimmune ones. We thus performed gene expression profiling of 89 different LncRNAs in Marsh 3C adult patients and healthy controls, identifying a strong downregulation of several LncRNAs in celiac patients, including two already reported associated with autoimmune diseases, NEAT1 and TUG1. Gene expression assays in adult and paediatric patients confirmed NEAT1 and TUG1 downregulation. No difference was detected in NEAT1 and TUG1 expression between patients on gluten-free diet (GFD) and healthy controls, confirming that their deregulation is connected with the active disease.

PT-gliadin stimulation on duodenal biopsies of GFD patients revealed an opposite trend between CD patients and controls, with a upregulation of NEAT1 and TUG1 in GFD patients. This could be explained by the higher expression of NEAT1 and TUG1 found in the epithelial part of the mucosa, usually destroyed in Marsh 3C patients. Regression analysis revealed a strong correlation between these two transcripts, and an in silico analysis of their promoter regions identified binding sites for STAT3 and ERK1/2, that are downstream of some of the deregulated pathways of CD (IL-15, IFNγ, EGF). Regression analysis between the 2 LncRNAs and other genes revealed a correlation with innate immunity cytokines (NEAT1/IL-15 and TUG1/IL-8). IL-15 (but not IFNG) stimulation in healthy controls biopsies caused in fact an upregulation of NEAT1.

Thus LncRNA expression is altered in celiac disease, and NEAT1 and TUG1 could be effectors of the activation of the innate immune response observed in this disease.
MICRORNAS EXPRESSION PROFILES IN SERA FROM HEALTHY SUBJECTS, COMPARED TO EX-EXPOSED ASBESTOS WORKERS AND MALIGNANT PLEURAL MESOTHELIOMA PATIENTS


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MicroRNAs (miRNAs) have different regulatory activities in distinct cellular processes, including cell differentiation, proliferation and transformation and they have been proposed as putative biomarkers for early detection, diagnosis, and treatment of human diseases, such as different tumors. Previously in a recent investigation, the expression profiles of circulating miRNAs from serum samples of healthy subjects (HS), workers ex-exposed to asbestos fibers (WEA) and malignant pleural mesothelioma (MPM) affected patients were analyzed.

MPM is an aggressive and fatal malignancy of the pleural surface and the majority of MPM cases (80%) is due to occupational asbestos exposure. Considering the long latency period of the MPM onset, subjects/WEA potentially at risk to develop this cancer may benefit of an early diagnosis based on specific biomarkers. Earlier investigations allowed us to identify in HS, WEA and MPM cohorts, three main dysregulated miRNAs, named miR-197-3p, miR-1281 and miR-32-3p. In the new phase of the study, with the aim to validate statistically our data, we analyzed the three main differentially expressed miRNAs on a larger sample size composed of serum samples from 60 HS, 60 WEA and 60 MPM with Real-Time quantitative PCR technique. At present, miR-197-3p was found dysregulated; other miRNAs are still under investigation. MiR-197-3p was found to be up-regulated in MPM vs WEA and WEA vs HS. These two comparative analyses are statistically significant. MiR-197-3p could be proposed as novel, non-invasive, predictive biomarkers for MPM. We may speculate that dysregulated miRNAs can be employed in the group of WEA subjects as markers to predict over time the risk of MPM onset. Besides, this signature could also help to design targeted therapies for MPM, exploiting the use of antagonir or mimetic miRNAs, to silence the over-expressed oncomiRNAs or substitute the lost miRNAs in cancer, respectively.
DIFFERENT CLASS IIA HDACS REPRESSIVE COMPLEXES REGULATE SPECIFIC EPIGENETIC RESPONSES RELATED TO CELL SURVIVAL IN LEIOMYOSARCOMA CELLS


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Class IIa HDACs are important regulators of different adaptive and differentiative responses. During embryonic development, these deacetylases influence specific differentiation pathways and tissue morphogenesis. In vertebrates HDAC4, HDAC5, HDAC7 and HDAC9 constitute the class IIa subfamily.

Transcriptional networks supervising class IIa HDAC expression are poorly defined. Here we demonstrate that MEF2D is the key factor controlling HDAC9 transcription. This control, which is part of a negative feed-back loop during muscle differentiation, is hijacked in cancer. In leiomyosarcomas the MEF2D/HDAC9 vicious circuit sustains proliferation and cell survival, through the repression of the death receptor FAS. Comprehensive genome-wide studies demonstrate that HDAC4 and HDAC9 supervise different genetic programs and show both specific and common genomic bindings. Although the number of MEF2-target genes commonly regulated is similar, only HDAC4 represses many additional genes that are not MEF2D targets. As expected, HDAC4/- and HDAC9/- cells increase H3K27ac levels around the TSS of the respective repressed genes. However, these genes rarely show bindings of the HDACs at their promoters. Frequently HDAC4 and HDAC9 bind intergenic regions. We demonstrate that these regions, recognized by MEF2D/HDAC4/HDAC9 repressive complexes, show the features of active enhancers. In these regions HDAC4 and HDAC9 can differentially influence H3K27 acetylation. Our studies describe new layers of class IIa HDACs regulation, including a dominant positional effect, and can contribute to explain the pleiotropic actions of MEF2 TFs.
45A NCRNA EXPRESSION IMPAIRS MICROTBULES DYNAMICS GENERATING CHROMOSOMAL INSTABILITY IN NEUROBLASTOMA

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Recently we described 45A non-coding RNA (ncRNA) which overexpression induces a remarkable modification of cell cytoskeleton leading to a cascade of reactions that interferes with proliferation control, cell migration, tumorigenic potential and cell adhesion properties. As a consequence, 45A-overexpressing neuroblastoma cells show a lung-specific metastatic engraftment whereas the silencing of its expression drives to the production of liver-specific metastasis.

In this work we demonstrate that the tuned regulation of 45A ncRNA expression directly regulates the level of GTSE1 (G2 and S-Phase Expressed 1) synthesis, a protein involved in the regulation of microtubules organisation with relevant effects on cancer development. Indeed, alterations of GTSE1 expression causes an attachment of chromosomes to microtubules stronger than normal, the impairment of spindle maintenance and the misalignment of chromosomes leading to a phenomenon observed in most cancers known as chromosome instability (CIN). In detail, the deregulation of 45A ncRNA/GTSE1 expression drives the impairment of microtubule dynamics/functionality inhibiting MCAK (Mitotic Centromere-Associated Kinesin) activity, a microtubules depolymerase.

In conclusion, our data highlight that 45A ncRNA expression affects CIN and the segregation of chromosomes in mitosis. We also show here that the involvement of Aurora B, p53, MCAK and alpha tubulin in the control of genome stability can be modulated by 45A via GTSE1 showing also that the response to anticancer drugs targeting cytoskeleton polymerization/depolymerization activity (i.e. taxols, vinblastine, vincristine) is affected by 45A ncRNA expression level, suggesting the relevance of 45A in neuroblastoma prognosis and/or therapy.
THREE-DIMENSIONAL TELOMERE ARCHITECTURE IN NIFTP FOR IMPROVED MOLECULAR DIAGNOSIS: A PILOT STUDY


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Well-differentiated thyroid cancers (WDTCs) are the most common endocrine malignancy and papillary thyroid cancer (PTC) represents a large group of WDTC with two main histological variants: classic-PTC (cPTC), and follicular variant PTC (FV-PTC). FV-PTC is divided into two different morphological types: infiltrative and encapsulated nodules. Recently, the encapsulated variant has been reclassified as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), because it shows features similar to non-malignant lesions. At the molecular level, PTC are distinguished into two main groups: BRAF-like nodules with BRAF mutations and/or RET7PTC rearrangements and RAS-like nodules, with RAS mutations. Several studies using quantitative three-dimensional (3D) telomere imaging have shown that cancer cells have an altered 3D telomere organization in contrast to normal cells. In thyroid cancer, this aspect has not been yet fully investigated. To evaluate if specific telomere architecture may characterize PTC histological variants, quantitative fluorescence in situ hybridization (Q-FISH), 3D imaging and 3D analysis were performed in 16 thyroid lesions: 5 cPTC, 3 FV-PTC, 4 NIFTP and 4 FTA (follicular thyroid adenoma), using normal thyroid tissue (NT) as control. Moreover, we investigated RET/PTC rearrangements and BRAF expression (indicative of BRAFV600E mutation) by FISH and immunofluorescence, respectively. We found a different telomere profiles in tumors compared to control (p<0.05). The comparison of 3D telomere profiles of the tumors demonstrated that NIFTP has longer telomeres than cPTC and FV-PTC (p<0.001). No correlation between molecular alterations and 3D telomere profiles was observed. These data suggest that 3D telomere organization might have diagnostic utility and might help the clinical management of NIFTP, which has a still unclear characterization and an outcome often difficult to predict. Supported by FIR2018-UniCa; CancerCare Manitoba.
NOVEL INSIGHTS INTO THE ROLE OF SEMAPHORIN SIGNALLING AND GNRH NEURON BIOLOGY

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Gonadotropin releasing hormone neurons are a small group of scattered hypothalamic neuroendocrine cells that control reproductive functions in all mammals and many vertebrates. Despite their position in the adult hypothalamus, during development they originate in the nasal placode and migrate along the vomeronasal nerve to reach the forebrain and attain their final position in the hypothalamus. Failure of GnRH neurons to migrate lead to Hypogonadotropic Hypogonadism (HH) or Kallmann Syndrome (KS), genetic disorders characterised by GnRH deficiency and absent or delayed puberty. The genes underlying HH/KS are largely unknown but the combination of genetically modified mouse models with exome sequencing may help to identify the unknown genes.

We have previously demonstrated that class 3 semaphorin (SEMA) 3A controls the positioning of the vomeronasal nerve and therefore the migration of GnRH neurons via Neuropilin (NRP1-2) receptors. Accordingly, mice lacking SEMA3A or NRP1 and NRP2 display typical KS features including hypogonadism and mutations of the SEMA3A gene have been subsequently identified in patients with KS.

In the search for additional SEMA3-mediated signalling pathways involved in this developmental process, we found that the SEMA3A co-receptors PLXNA1 and PLXNA3 are expressed during mouse development in territories relevant to GnRH neuron development and that the combined loss of the NRP co-receptors Plexin (Plxn) A1 and PlxnA3 phenocopied the GnRH neuron defects of SEMA3A knockout mice. As previously done for Plxna1, the human ortholog of Plxna3 should therefore be investigated as a candidate gene for inherited GnRH deficiency.
CIRCNAPEPLD IS EXPRESSED IN HUMAN AND MURINE SPERMATOZOA AND PHYSICALLY INTERACTS WITH OOCYTE MIRNAS


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Circular RNAs (circRNAs) have a critical role in the control of gene expression. Their function in spermatozoa (SPZ) is unknown to date. Twenty-eight genes, involved in SPZ/testicular and epididymal physiology, were analyzed by using circBase database to find which of them may generate circular transcripts. CircNAPEPLDiso1, one of the two circular RNA isoforms of NAPEPLD transcript, was found to be expressed at high levels in human and murine SPZ. In order to functionally characterize circNAPEPLDiso1 as potential microRNA (miRNA) sponge, we performed circNAPEPLDiso1-miR-CATCH and then profiled the expression of 754 miRNAs, by using TaqMan® Low Density Arrays. MiRNAs 146a-5p, 203a-3p, 302c-3p, 766-3p and 1260a (some of them previously shown to be expressed in the oocyte) resulted enriched in circNAPEPLDiso1-miR-CATCHed cell lysate: the network of interactions generated from their validated targets was centered on a core of genes involved in the control of cell cycle. Interestingly, computational analysis of circNAPEPLDiso1 sequence also showed its potential translation in a short form of NAPEPLD protein.

In order to hypothesize a paternal contribution in circNAPEPLD transmission to the zygote, we analyzed the expression of both circular isoforms in murine unfertilized oocytes; this analysis revealed low and high levels of circNAPEPLDiso1 and circNAPEPLDiso2, respectively. After fertilization, circNAPEPLDiso1 expression significantly increased, instead circNAPEPLDiso2 expression appeared constant.

Based on these data, we suggest that SPZ-derived circNAPEPLDiso1 physically interacts with miRNAs primarily involved in the control of cell cycle; furthermore, it may function as a miRNA decoy inside the fertilized oocytes to regulate the first stages of embryo development.
IN VITRO PROTECTIVE EFFECTS OF DEXAMETHASONE AGAINST CISPLATIN OTOTOXICITY

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Current anticancer therapies involve drugs whose cytotoxic effects are often non-specific, thus affecting healthy cells and tissues. Cisplatin (cis-diaminedichloridoplatinum (II)), a widely employed chemotherapeutic agent, exhibits relevant ototoxic effects leading to hearing loss, an invalidating outcome with high social impact especially when children or young people are involved. A key event in cisplatin ototoxicity is inflammation, thus anti-inflammatory drugs such as glucocorticoids are currently evaluated as protective agents against cisplatin-induced hearing loss. Following previous studies by our research group, the effects of pre-treatment with different doses of dexamethasone, a widely employed anti-inflammatory drug, were tested on an inner ear mouse cell line derived from the organ of Corti (OC-k3), treated with cisplatin for 24 and 48h. The protective effects of dexamethasone were evaluated by cell viability, cell morphology, production of reactive oxygen species and release of inflammation markers. In the experimental conditions tested, dexamethasone had no cytotoxic effects at any concentration and pre-treatment with dexamethasone for 24h had protective effects on OC-k3 cells treated with cisplatin. Overall, these data support the use of dexamethasone to protect auditory cells from damages caused by exposure to cisplatin, possibly preventing hearing loss.
MONITORING MUSCA DOMESTICA POPULATIONS IN VIGASIO (VERONA)

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Musca domestica (Diptera: Muscidae) is a cosmopolitan fly species typically infesting urban and agricultural environments. It has high medical and veterinary importance, being a nuisance and a carrier of relevant pathogens for human and animal health. Complaints about serious periodical infestations by M. domestica were expressed since 2010 by residents of the municipality of Vigasio (Verona, Italy). The infestations were initially attributed to an intensive poultry farm located in the town suburban area. From 2015 to 2017 a monitoring program of fly populations was established and conducted by the Consorzio Futuro in Ricerca (Ferrara, Italy), in association with the Department of Life Sciences and Biotechnology of the University of Ferrara. Monitoring was performed by non-toxic disposable fly traps with attractor bait, placed in 15 locations inside the poultry farm and in 10 locations within the municipality. Every year the traps were placed twice a month from March to October and collected a week after positioning. The captured individuals of M. domestica were identified and counted and the data were statistically analyzed in relation to temperature, humidity and rainfall. The trend of fly infestations along the three years of monitoring was analyzed by ANOVA. The results not only allowed to improve fly control in the poultry farm, but were relevant to establish the contribution to infestations by other livestock farms and by an incorrect management of urban waste in some municipality areas. Monitoring was resumed and continued up to date to provide useful information for fly management procedures to the farm, in the process of modernization.
MOLECULAR PATHWAYS OF MAJOR DEPRESSION: THE BIOLOGICAL SIGNIFICANCE BEHIND NEW PRS SCORE-DETERMINED CASCADES


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The sheer complexity of diseases associated with brain functioning, like Major depression (MDD) is well known in literature. Several analyses were performed in the years on MDD onset and its treatment, however, despite the evidences of a relatively strong genetic background, the controversy surrounding the validity of findings remain strong. Recently, new approaches in the study of MDD do not support previous candidate genes findings and somewhat question the rational of hypotheses about depression. In the recent years the concept of polygenic risk score and its applications started to supplant the candidate gene association study approach. More than single genes, the risk score is related to entire molecular cascades and evaluates how alterations within these pathways may impact MDD treatment. Our group investigated common and rare variants through whole exome sequencing method in a sample of over 1000 subjects to describe the risk score for each gene (and related pathways) and the possible correlations with treatment resistance.

The preliminary estimates of this multicenter project individuated at least 30 pathway with over 200 genes that could possibly be related to depression treatment. Our section aim is to characterize the biological significance behind these statistic signals in order to find new suggestive correlations between disease and the molecular cascades that may explain the individual differences among treated subjects. In order to find rational pathways, we investigated how the cascades works trying to evidence the mechanisms behind each possible alteration.
UNVEILING THE FUNCTIONS OF THE TELOMERIC LONG NONCODING RNA TERRA IN CANCER CELLS USING A LIVE CELL IMAGING APPROACH


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Telomeric repeat-containing RNAs TERRA are long noncoding RNAs transcribed at telomeres which play key roles in telomere biology, including regulation of heterochromatin formation and control of DNA replication. Interestingly, TERRA transcripts interact with numerous extratelomeric sites to regulate gene expression in mouse embryonic stem (ES) cells. However, the molecular details of TERRA functions remain to be defined. Notably, TERRA interacts with the telomerase enzyme in both yeast and human cells. Nevertheless, the function of TERRA in the regulation of telomerase remains to be determined.

We have developed a live-cell imaging assay based on the MS2-GFP system in order to image single-telomere TERRA transcripts in human cancer cells. To this aim, we used the CRISPR/Cas9 system to generate clones containing MS2 sequences integrated at a single telomere. By expressing a MS2-GFP fusion protein which specifically recognizes MS2 RNA sequences, we observed that MS2-tagged TERRA transcripts form discrete foci within the nucleus. Using this approach, we can now dissect the dynamics of TERRA transcripts in cancer cells. Surprisingly, our results indicate that TERRA molecules relocate from telomeres to Cajal bodies. At Cajal bodies, TERRA molecules co-localize with the telomerase RNA component hTR. hTR localization at Cajal bodies is essential to telomerase maturation and function. Thus, our findings suggest that TERRA can participate to the telomerase maturation processes in cancer cells.

In addition, our preliminary results indicate that TERRA expression and localization are tightly regulated by cellular stress conditions. Interestingly, TERRA expression is induced upon various cellular stress, including chemotherapy treatment. Notably, we found that TERRA molecules can relocate from the nucleus to the cytoplasm in cancer cells under stress conditions. These results suggest the intriguing possibility that TERRA may exert extranuclear functions during cellular stress.
THE SYNAPSE TO NUCLEUS SIGNALING IS IMPAIRED IN FRAGILE X SYNDROME

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Synapto-nuclear messenger proteins couple synaptic activity to changes in gene expression, modulating synapses structure, integrity and connectivity. Growing evidence indicates that impairments in the activity of these proteins affect dendritic spine density and morphology, suggesting that they might contribute to different synaptopathies. Notably, the mRNAs encoding for different synapto-nuclear proteins have been found associated with Fmrp, the protein responsible for the Fragile X Syndrome (FXS), the main cause of inherited intellectual disability. Here, we show that Fmrp regulates transcriptional factors at synapses, modulating their expression and distribution at protein and mRNA levels. Furthermore, the absence of Fmrp triggers defects in nuclear trafficking, ultimately affecting gene expression in Fmr1 KO neurons. Importantly, the targeting of the nuclear import of synapto-nuclear proteins ameliorates the transcriptional impairments in the FXS mouse model. Our study highlights a novel molecular pathway altered in FXS and provides new avenues for future pharmacological intervention.
XCT790 AN INVERSE AGONIST OF ESTROGEN RELATED RECEPTOR ALPHA (ERRALPHA) INHIBITS CELLULAR METABOLISM, MOTILITY AND INVASION OF ADRENOCORTICAL CANCER CELL LINE H295R

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Estrogen Related Receptor (ERR) alpha, is a nuclear receptor involved in onco-metabolism in several tumors, including breast, ovary and colon cancer. We have previously showed that a decreased of ERRalpha protein level using the specific inverse agonist XCT790, caused a dose-dependent inhibition of H295R adrenocortical cancer cell growth, both in vitro and in vivo. Aim of this work was to establish if the degrader of ERRα impaired H295R cell metabolism and ACC cell motility and invasion. Our results demonstrate that XCT790 is able to decrease cell metabolism, assayed by measuring the cellular oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR). The metabolic changes caused by XCT790 affect cell migration and invasion, as verified by also the reduction of EMT markers expression such as Vimentin, N-cadherin and Slug. By contrast, ERRalpha overexpression increased cell motility and invasion as well as resistance to anoikis. This last cell phenotype are also able to growth in non-adherent culture condition originating spheroids. Accordingly, ERRalpha overexpressing cells increased spheroids formation and EMT markers. In order to evaluate the effects of a stable reduced expression of ERRalpha in H295R (H295RERR-), we demonstrated that H295R cells with stable silenced ERRalpha gene displayed a reduced cell proliferation and growth, as well as cell motility and migration. In addition, the H295RERR- showed also a reduced 3D-spheroids formation. Moreover, we obtained the same effects using XCT790. In conclusion, our data suggested that also in ACC, ERRalpha is a master regulator of reprogramming cellular metabolism that positively affect cell motility and invasion. Therefore, the use of drugs that interfere with ERRalpha activity could be a valid therapeutic strategy to contrast ACC progression.
ISOFORM-SWITCHING siRNAs Efficiently Correct Tau Isoforms Imbalance in a Cellular Model of Tauopathy

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Tauopathies are neurodegenerative diseases marked by the abnormal processing of microtubule-associated protein tau and its accumulation as insoluble neuronal deposits. Tau, encoded by the MAPT gene, regulates several neuronal functions, such as neurite outgrowth, microtubule dynamics and axonal transport. The adult human brain contains equal amounts of tau isoforms with three (3R) or four (4R) repeats of microtubule-binding domains, originated from the alternative splicing of exon 10 (E10) in the MAPT transcript. Several tauopathies are associated with imbalances of tau isoforms due to splicing shortfalls.

Selective degradation of E10-containing MAPT mRNA isoforms is, in principle, possible by the use of exon-specific siRNAs. However, very few examples of successful exon-specific siRNAs are available in the literature, probably because of two reasons: 1) secondary siRNAs can be produced by RNA-dependent RNA Polymerases in several organisms and cell types, which would generically silence all mRNA splicing isoforms; 2) siRNAs are known to function also in the nucleus and might target the nascent pre-mRNA, resulting in a decrease of all different splicing isoforms of the target mRNA. Here, we evaluate fourteen E10-targeting siRNAs for their efficiency in reverting the inclusion of E10 in MAPT transcripts and identify three effective siRNAs. We validated the three siRNAs in human neuronally differentiated cell models of tauopathy that produce abnormal excess of 3R tau. Our results suggest a promising potential for the use of RNA interference in human neurodegenerative diseases.
TELOMERE LENGTH VARIATION IN DIFFERENT SEVERE MENTAL DISORDERS


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Background: Association between shortened telomeres and severe mental disorders (SMD) has been reported (Lindqvist et al., 2015), and our group showed that duration of Lithium (Li) treatment positively correlates with leukocyte telomere length (LTL) in patients with bipolar disorder (BD) (Squassina et al., 2016).

Aim: In the current study, we explored the impact of LTL in BD patients Li-treated versus schizophrenia (SCZ) and major depressive disorder (MDD) patients whose therapy is Li free.

Methods: LTL was measured in PHA-stimulated T-lymphocytes cultures from SMDs using quantitative fluorescence in situ hybridization (qFISH). Patient cohort included 20 BD, 20 SCZ and 20 MDD patients, stratified according to the presence of comorbid-age related disorders, and 20 controls.

Results: A statistically significant difference in LTL was observed among these groups (F=11.9, p=2x10^-6): LTL was longer in patients with BD compared to both SCZ and MDD patients (p<0.0001 and p=0.008, respectively). A significant correlation between telomere length and duration of Li therapy (Spearman’s rho = 0.47, p = 2x10^-5) was observed in the BD patients. LTL value indicates that SCZ patients had shorter telomeres compared to controls (p=0.002) and a trend towards shortening compared to MDD. In addition, LTL was shorter in subjects with a family history of psychiatric disorders (t=2.95, p=0.005).

Conclusions Our data further confirmed that: a) long-term treatment with Li in BD patients positively correlates with LTL, b) Li treatment has a protective effect against telomere shortening, and c) patients with SCZ and MDD, who are not treated with Li therapy, are more susceptible to accelerated telomere shortening than patients with BD.

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3D CULTURES OF PRIMARY ASTROCYTES ON POLY-L-LACTIC ACID SCAFFOLDS


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Tissue engineering is an emerging multidisciplinary field that aims at reproducing in vitro tissues with morphological and functional features similar to the biological tissue of the human body. Polymeric materials can be used in contact with biological systems in replacing destroyed tissue by transplantation [1]. Several biopolymers, including poly L (lactic acid) (PLLA), have been used in biomedical applications to set scaffolds with ductile properties and biodegradation kinetics [2]. In particular, the PLLA scaffold topography mimics the natural extracellular matrix and makes it a good candidate for neural tissue engineering.

We report about of 3D system the PLLA porous scaffolds prepared via thermally-induced phase separation (TIPS) [3], and utilized as substrate for primary rat astrocytes 3D growth. Interestingly astrocytes adapt well to these porous matrices, not only remaining on the surface, but also penetrating inside the scaffolds. They colonize the matrix acquiring a typical star-like morphology; they form cell contacts and, in addition produce EVs as in vivo [4]. These results suggest that the chosen conditions could be a good starting point for 3D brain culture systems. PLLA scaffolds could be further enriched to host two or three different brain cell types, in order to set an in vitro model of blood brain barrier. The future use of co-culture systems may be involved in drug delivery studies, and in the formulation of new therapeutic strategies for the treatment of neurological diseases.

THE EARLY RESPONSE OF αB-CRYSTALLIN TO A SINGLE BOUT OF AEROBIC EXERCISE IN MOUSE SKELETAL MUSCLES DEPENDS UPON FIBER OXIDATIVE FEATURES


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Besides its substantial role in eye lens, αB-crystallin (HSPB5) retains fundamental function in striated muscle during physiological or pathological modifications. In this study, we aimed to analyse the cellular and molecular factors driving the functional response of HSPB5 protein in different muscles from mice subjected to an acute bout of non-damaging endurance exercise or in C2C12 myocytes upon exposure to pro-oxidant environment.

To this end, red (GR) and white gastrocnemius (GW), as sources of slow-oxidative and fast-glycolytic/oxidative fibers, as well as the soleus (SOL), mainly composed of slow-oxidative type fibers, were obtained from BALB/c mice, before (CTRL) and at different times (0’, 15’, 30’ 120’) following 1-hour of running. Although the total level of HSPB5 protein was not affected by exercise, we found a significantly increase of phosphorylated HSPB5 (p-HSPB5) only in GR and SOL skeletal muscle with a higher amount of type I and IIA/X myofibers. The fiber-specific activation of HSPB5 was correlated to its interaction with the actin filaments, as well as to an increased level of lipid peroxidation and carbonylated proteins. The role of the pro-oxidant environment in HSPB5 response was investigated in terminally differentiated C2C12 myotubes, where most of HSPB5/pHSPB5 pool was present in the cytosolic compartment in standard culture conditions. As a result of exposure to pro-oxidizing, but not cytotoxic, H2O2 concentration, the p-38MAPK-mediated phosphorylation of HSPB5 resulted functional to promote its interaction with the myofibrillar components, such as β-actin, desmin and filamin 1.

This study provides novel information on the molecular pathway underlying the HSPB5 physiological function in skeletal muscle, confirming the contribution of the pro-oxidant environment in HSPB5 activation and interaction with substrate/client myofibrillar proteins, offering new insights for the study of myofibrillar myopathies and cardiomyopathies.
AN INNOVATIVE GENE PRIORITIZATION APPROACH ON WES DATA UNVEILED NEW CANDIDATE GENES RELATED TO RETINITIS PIGMENTOSA

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Retinitis pigmentosa (RP) comprises a heterogeneous group of progressive inherited retinal dystrophies (IRDs), generally characterized by the early degeneration of rod photoreceptors, followed by the loss of cone photoreceptors. Clinical overlaps represent the biggest challenge in IRDs specific classification. The high heterogeneity showed by RP patients is excellently illustrated by the wide number of genetic defects associated with RP. Today, mutations in more than 80 genes have been involved in non-syndromic RP, and each year new genes are added to this list. Each of these genes belongs to a gene-specific subtype of RP with a specific spectrum of phenotypes.

Furthermore, lots of factors can change widely within each of these particular subtypes, even between affected family members, suggesting the presence of unknown genetic and/or environmental factors that could influence the RP phenotype. In order to clarify this scenario and improve the classification of RP orphan forms, an omic approach based on whole exome sequencing was exploited. After wet laboratory and big data analyses, we focused on a complex pipeline consisting of several computational prioritization algorithms, applied on genes carrying coding sequence variants (synonymous ones were excluded from subsequent analyses). The first four top ranked genes, derived from connections with biological processes, were chosen as best candidates. Such genes coding for proteins mainly involved in cilia formation during neuronal patterning and in microtubular associated vesicle trafficking through the photoreceptor connecting cilium.

Moreover, the integrity of such intracellular transport could influence phototransductional mechanisms in outer segments of both cone and rods, whose physiology is also regulated by the same selected genes. In conclusion, the NGS exploited approach allowed us to detect new possible candidate genes associated/causative of retinitis pigmentosa orphan forms, trying to improve RP diagnosis.
SIRT1-DEPENDENT RESPONSE IS ESSENTIAL FOR RESVERATROL TO UPREGULATE ANTIOXIDANT AND ANTIGLYCATIVE DEFENCES IN HIGH GLUCOSE-CHALLENGED HUVECS

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Reactive oxygen species (ROS) and methyglyoxal (MG) are partly responsible for the endothelial dysfunction observed in diabetes-related vascular complications. Endothelial function in high glucose (HG) is improved by resveratrol (RSV), a natural phytoalexin, however the mechanisms underlying RSV-dependent protection of endothelial cells upon HG are still debated. More importantly, the role of sirtuin 1 (SIRT1) in such cytoprotection remains to be elucidated.

Our work aimed to: a) provide details about the redox- and MG-related mechanisms underlying the protective effects of RSV in endothelial cells upon HG; b) establish whether SIRT1 is essential for RSV to protect endothelial cells against HG-dependent cytotoxicity; c) demonstrate whether SIRT1 is required for RSV to regulate ROS- and MG-targeting enzymatic systems in endothelial cells.

Human umbilical vein endothelial cells (HUVECs) were kept in 5.55 mM glucose (CTR) or 30.55 mM glucose (HG), and co-incubated with either RSV (5 µM) or RSV+EX527 (SIRT1 inhibitor) (5 µM+13.4 µM), on the basis of concentration-response curves. Cell viability and apoptosis were assessed by Trypan blue and Annexin V/PI staining, respectively. Morphological assessment was performed by scanning electron microscopy. Expression and function of SIRT1, SOD1, SOD2, CAT, and GLO1 were studied by quantitative relative real time RT-PCR, Western blotting (WB), and spectrophotometric enzyme assays. ROS- and MG-dependent damage was evaluated by TBARS assay and anti-argpyrimidine-based WB, respectively.

We revealed that: a) HG-challenged HUVECs showed increased oxidative/glycative damage, most likely due to impaired ROS and MG scavenging; b) RSV rescued the HG-induced impairment of ROS/MG scavenging, as well as prevented the pro-oxidant and pro-glycation effects of HG; c) the up-regulation of SIRT1 was essential for RSV to protect HUVECs from HG cytotoxicity, and to elicit antioxidant/antiglycative effects on HG-challenged HUVECs.
VCP-MUTANTS INDUCE LYOSOME DAMAGE IN ALS-MODELS


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Amyotrophic Lateral Sclerosis (ALS) is a lethal neurodegenerative disease caused by upper and lower motoneurons death. All ALS-forms are characterized by presence of protein aggregation in brain of affected patients, representing an hallmark of alteration in proteinostasis. In fact, many mutated genes express proteins that misfold and aggregate. Aggregates, if not removed, lead to neurons death. Moreover, ALS forms are associated to the mutation of genes involved in the protein quality control system. One of these genes is Valosin Containing Protein (VCP). VCP has many roles in proteinostasis, in fact it regulates degradation of misfolded proteins and the turnover of damaged organelles like lysosomes. Lysosomes damage is deleterious for cells for their loss of function and for the toxic effects induced by lysosome leakage. In this study, we study lysosomal-damage response in presence of overexpressed wt VCP, and its ALS-mutants (VCP R155H, VCP R191Q) in moterneuron immortalized cell line. To study VCP contribute in this pathway we chemically induced lysosome damage by treating with trehalose, and biologically overexpressing SOD1 G93A. We demonstrated that the overexpressed wt VCP reduces lysosomal damage after it is induced. Conversely, VCP R155H prevents the clearance of damaged lysosomes when the damage is induced. VCP R191Q can partially reduce lysosomal damage, but it significantly loses its functionality compared to wt VCP. Moreover, we demonstrated that both VCP-mutants induce lysosome damage in basal condition. Correlated to damage of lysosomes, we demonstrate that the overexpression of the VCP-mutants leads the translocation of a specific transcription factor, TFE3 that activates the expression of genes involved clearance of damaged lysosomes by autophagy. These data demonstrate a novel pathogenic mechanism of mutants-VCP. The presence of these mutants alter proteinostasis leading to lysosome damage, preventing their removal.
Tumor-associated macrophages (TAMs) are a prominent component of cancer microenvironment having a key role in promoting tumor progression. Several studies have demonstrated that TAMs phenotypically and functionally correspond to M2-polarized macrophages thus they exert immunosuppressive functions also associated to the expression of programmed cell death ligand 1 (PD-L1). Within the local tumor microenvironment, tumor-derived exosomes (TDEs) are well known to play a key role in modulating the properties and the behavior of surrounding cells such as TAMs. Even if several studies demonstrated the ability of TDEs to induce M2-like macrophage polarization, few data are available about their involvement in regulating the expression of PD-L1 in TAMs.

The aim of the current study was to investigate the ability of exosomes derived from SW480 human colon cancer cells to modulate the properties of TAMs by using non-polarized macrophages (M0-M) differentiated from THP-1 as in vitro model. Our results indicate that after 48h treatment, exosomes derived from SW480 cells (SW480exos) significantly upregulate the expression of surface markers of M2-like phenotype (CD163 and CD206) as well as of PD-L1, inducing macrophages to acquire an immunosuppressive phenotype. In parallel, we found that SW480exos were able to induce a significant increase of interleukin 6 (IL6) expression at both mRNA and protein level. Finally, according to the known ability of PD-1/PD-L1 axis to induce T cell dysfunction, we found that CD3+ T cells co-cultured with M0-M pre-treated with SW480exos significantly increased their apoptotic rate in comparison to those grown in presence of no-treated M0-M.

Cumulatively, these preliminary data suggest that within local colon cancer microenvironment TDEs can act as positive modulators of the immunosuppressive status of TAMs, actively promoting the immunotolerance necessary to favor tumor growth and progression.
NEW HYPOTHESES OF NEUROLSD1 MODULATION: FROM LNCRNA TO CRYPTIC EXON INCLUSION

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The transcriptional corepressor Lysine-Specific Demethylase 1 (LSD1) along with its neuronal specific alternative splicing variant, neuroLSD1, represent a remarkable example of environmental stimuli transducer in mammalian brain. LSD1 is a negative modulator of stress-plasticity and memory formation, while neuroLSD1, acting as a dominant negative isoform, promotes activity-dependent transcription of plasticity-related genes in response to environmental stimuli. Interestingly, LSD1 and neuroLSD1 relative level is directly modified by stress, impacting LSD1 activity. Thus, tuning LSD1/neuroLSD1 relative ratio can be considered a process of transcriptional homeostasis pivotal to environmental adaptation at behavioral level.

NeuroLSD1 differs from the ubiquitously expressed LSD1 isoform only for the presence of the 12nt-long micro-exon E8a. The regulation of this alternative splicing event ensures fine control on neuroLSD1 expression level during development, and is mainly exerted by two neurospecific splicing regulators, nSR100 and NOVA1.

Our interest focuses on understanding how this splicing event is modulated in the hippocampus.

In this regard, we identified a possible role of the IncRNA MALAT1 as stress-induced negative regulator of E8a inclusion.

Recently we discovered the presence of a primate specific cryptic exon (E8b) whose inclusion into LSD1 mature transcript is regulated by a different splicing factor involved in neuropsychiatric disorders. E8b inclusion causes transcripts degradation through nonsense-mediated decay (NMD). Since in the human brain E8b is included preferentially in E8a-containing transcript, NMD might be a further mechanism to specifically regulate neuroLSD1 level. This primate-restricted neuroLSD1 splicing tuner could therefore contribute to the complexity of cognitive and social abilities that distinguishes higher primates from other mammals but might also represents a vulnerability factor for stress-related neuropsychiatric disorders.
ESTROGEN, HYPOXIA AND DOXORUBICIN MODULATE NOTCH1 IN CARDIOMYOCYTES


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Notch signaling is an important regulator of many biological processes, including cell proliferation and survival. In adult myocardium, activation of the Notch signaling following injury has been associated with pro-survival pathways. It has been shown that sex hormones, such as estrogens, or antineoplastic drugs, as doxorubicin can protect or damage the myocardium, respectively, but the molecular mechanisms underlying this effect are still poorly understood. The aim of this study was to investigate the interaction estrogens-Notch and doxorubicin-Notch in a cardiac cell model, and the possibility that this interplay could affect the cell survival.

We treated H9c2 cardiac myoblast cell line with doxorubicin and DAPT (Notch inhibitor) and then evaluated the Notch1 protein levels and the cell viability. We have also cultured H9c2 cells in hypoxic conditions and treated them, before and during hypoxia, with 17beta-estradiol (E2) or DAPT. Then Notch1 protein levels and apoptosis analysis were performed.

We found that treatment with doxorubicin enhances active Notch1. H9c2 co-treated with doxorubicin and DAPT showed reduced viability in comparison with cells treated with doxorubicin only. Similarly, in H9c2, hypoxia enhances active Notch1 and in the presence of DAPT, H9c2 are more sensitive to hypoxia-induced apoptosis. E2 treatment increases active Notch1 both in normoxic and hypoxic conditions. Nevertheless, E2 is not able to protect cells against hypoxia-induced apoptosis. This could be due to: the cellular model used (stem cells-like, slow growing phenotype of H9c2); experimental conditions affect the levels of ERalpha and/or ERbeta and cellular pathways regulated by these receptors. In conclusion, these results suggest that, in cardiomyocytes, both damaging and protective agents are able to modulate Notch1, which is necessary for cell survival. Further studies are needed to dissect the Notch1-dependent molecular mechanisms involved in cardiomyocytes survival.
ROLE OF LICOCHALCONE C ON MODULATION OF NF-KB/INOS/NO SIGNALING PATHWAYS IN LPS STIMULATED H9C2 CELL


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Polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. About 300 polyphenols have been isolated from licorice, including phenolic acids, flavonoids, flavans, isoflavans, isoflavonoids and chalcones. Nowadays, several chalcones are used for treatment of viral disorders, cardiovascular diseases, parasitic infections, pain, gastritis, and stomach cancer, as well as like food additives and cosmetic formulation ingredients. The action mechanisms are not yet clear. Licochalcone C (LicoC), a constituent of Glycyrrhiza glabra, has various biological and pharmacological properties. In saying this, the effect of LicoC on the inflammatory response that characterizes septic myocardial dysfunction is poorly understood. The aim of this study was to determine whether LicoC exhibits anti-inflammatory properties on H9c2 cells stimulated with lipopolysaccharide. Our results have shown that LicoC treatment represses nuclear factor-κB (NF-κB) translocation and several downstream molecules, such as inducible nitric oxide synthase (iNOS), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Moreover, LicoC upregulates the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/endothelial nitric oxide synthase (eNOS) signaling pathway. Finally, a specific PI3K inhibitor (LY294002), blocked the protective effects of LicoC. These findings indicate that LicoC plays a pivotal role in molecular cardiac alteration by reducing inflammation in LPS-activated cells.
PLASMA DERIVED EXOSOMAL MICRONRNAS PROFILE: NEW POTENTIAL BIOMARKERS FOR ALZHEIMER’S DISEASE (AD) DIAGNOSIS


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The aim of the study was to isolate and characterize exosomes in plasma of patients with Alzheimer’s disease (AD) and healthy controls in order to detect specific microRNAs signature as potential peripheral AD biomarkers. In particular, we focused on a specific subpopulation of plasma exosomes derived from neurons (NDEs).

Total plasma exosomes from 20 AD patients and 20 controls were isolated by using ExoQuick exosome precipitation solution (SBI). Total exosomes were enriched for neural sources by immunoprecipitation with anti-L1CAM antibody and analyzed by FACS, Transmission Electron Microscopy (TEM) and Nanosight Nanoparticle Tracking Analysis (NTA). Total and NDEs miRNAs levels were determined by RT-qPCR using TaqMan OpenArray technology in a QuantStudio 12K system (Thermo Fisher Scientific).

A panel of 754 miRNAs was analyzed, and 75 of them reached the optimal expression quality score: miR-146a-5p, miR-23a-3p, miR223-3p. They were expressed in both exosomes categories, but only in the total fraction a statistical significant deregulation was observed (P= 0.03; P=0.02; P= 0.0002). Conversely, miR-1260a, miR-1-3p, miR-190a-5p (P=0.02), miR-448, miR-628-3p and miR-653-5p were expressed exclusively in NDEs.

These preliminary results demonstrated that plasma exosomes, especially NDEs, are easily detectable in biological fluids and are an enriched source of microRNAs. We observed deregulated expression levels of microRNAs, already associated with neurological diseases, supporting their role in the pathogenesis of AD. Moreover, our data showed a specific expression microRNAs pattern in NDEs, that could likely represent reliable early peripheral biomarkers for AD diagnosis. Nevertheless, further studies are required to confirm these preliminary data.
INVESTIGATING THE ROLE OF CXCL12 SPLICING ISOFORMS IN A PRE-TUMOUR MODEL OF PANCREATIC CANCER


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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive tumours. The intense intercellular communication between tumour and stromal component maintains a favourable microenvironment for tumour development. Among exchanged signals, the chemokine CXCL12 plays an important role in masking the pain in the early phases of tumour development, guidance of metastatic cells, promotion of cell survival and proliferation. Most studies have attributed a pro-tumour role to CXCL12, however, some evidence shows an anti-tumour role [1]. Controversial results could be due to the existence of 7 splicing isoforms of CXCL12. Indeed, previous studies investigated CXCL12 as a whole, without focusing on a specific isoform. In order to investigate the role of each isoform in a pre-cancer model of PDAC, we performed a microarray analysis after cell treatments with alpha, beta and gamma CXCL12 isoforms. As model of pancreatic intraepithelial neoplasia, we used the hTERT-HPNE E6/E7/KRasG12D cell line. It harbours the KRas mutation G12D and the inactivation of p53 and Rb tumour suppression genes. Microarray analysis showed that each isoform alters common but also specific pathways confirming their different roles. In particular, alpha isoform down-regulates E-cadherin pathway, beta isoform enhances interferon gamma signalling, gamma isoform down-regulates angiogenesis. A detailed analysis of differentially expressed genes suggests that alpha and beta isoforms can sustain tumour, whereas gamma isoform can hinder its growth. We are currently cloning the other not commercially available isoforms, in order to validate their effects. These results could indicate which CXCL12 isoforms should be targeted in clinics.

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References

Activating WNT Pathway in CdLS in Vitro and in Vivo Models: Possible Future Therapeutic Strategies for Cornelia de Lange Syndrome


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Cornelia de Lange Syndrome (CdLS) is a rare genetic disorder, affecting multiple organs including the central nervous system (CNS) with relatively mild to severe manifestations. CdLS is mainly caused by dominant autosomal or X-linked mutations in at least five genes: NIPBL, SMC1A, SMC3, RAD21, and HDAC8. These genes encode for proteins of the cohesin complex, which plays a pivotal role in gene expression regulation. Indeed, we and others have previously reported that the canonical WNT pathway, a master controller of neurodevelopment, is downregulated in CdLS models.

We have taken advantage of different in vitro and in vivo CdLS models to explore possible ameliorative effects of chemical activation of WNT pathway. First, we treated lymphoblastoid cell lines from healthy donors and CdLS patients with chemical compounds known to be activators of the WNT pathway. We found that lithium treatment restored proliferation rate and induced CyclinD1 gene expression, which are usually found reduced in cells of CdLS patients. We then analyzed an in vitro murine model of CdLS, in which neural stem cells have been tested by chemical inhibition of HDAC8 and siRNA strategies. We found that proliferation and differentiation are significantly reduced. Such defects were rescued upon chemical exposure to lithium. Finally, we used a Drosophila melanogaster CdLS model, mutated in Nipped-B (NIPBL in humans), which shows abnormal development of the mushroom body (MB), a CNS structure involved in learning and memory. Upon feeding on lithium, a significant rescue of MB morphology was observed across generations.

Together, our data confirm the impairment of WNT canonical pathway in CdLS, possibly explaining the typical neurodevelopmental alterations, and reveal that WNT activators such as lithium, could contribute to develop future CdLS therapies.
THE STUDY OF “NANO-BIO” INTERACTIONS TO ENGINEER THE BIOLOGICAL FATE OF NANOMATERIALS

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The rational design of safe and effective drug delivery systems requires deep understanding of the processes that govern the interaction between cells and nanomaterials (NMs). Here, we investigate the mechanisms involved in the nano-bio interplay to find correlation between physical-chemical properties of NMs and their short- and long-term cell fate. This is crucial to improve NM therapeutic potential and limit/avoid their possible toxicological effects. In general, the internalization of NMs mainly occurs through endocytosis, resulting in their endo-lysosomal localization, with possible occurrence of biopersistence/biodegradation and/or vesicular escape, as well as of exocytosis processes. We show that the dispersion status of NMs in the different physiological environments plays a key role in governing all these mechanisms, including cytosolic delivery and transcytosis. Moreover, the assessment of the in situ behavior of the NMs is crucial to define a correct methodological approach to circumvent biased results of in vitro studies, making them more predictive of in vivo outcomes. On the other side, we demonstrated that, by exploiting the “canonic” endocytic pathways, it is possible to achieve a potential therapeutic improvement of NMs confined in lysosomal vesicles by prolonging their intracellular retention time, so to increase the effective intracellular dose of the transported drug.
EPIGENETIC OF RARB TUMOR SUPPRESSOR GENE IN KERATINOCYTES FROM NORMAL CERVICAL MUCOSA AND CERVICAL INTRAEPITHELIAL NEOPLASIA

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The tumor suppressor gene retinoic acid receptor B (RARB) is a key regulator of keratinocyte differentiation and proliferation, whereas its down-expression is associated to cervical cancer [1]. We investigated the promoter methylation profile of RARB gene in keratinocytes from normal cervical mucosa and cervical intraepithelial neoplasia (CIN). Normal cervical keratinocytes (NCK) and neoplastic CIN2 and CIN3 keratinocytes were derived from normal cervical tissues (n=15) and CIN2 (n=11) and CIN3 (n=8) lesions, respectively [2,3]. NCK and CIN2/CIN3 keratinocytes were analyzed for (i) HPV DNA, (ii) its physical status by E2/E6 gene ratio, (iii) viral E6 mRNA expression by qRT-PCR, and (iv) HPV genotype by DNA sequencing. RARB gene expression was analyzed by qRT-PCR. RARB promoter methylation analysis was carried out with bisulfite DNA treatment followed by PCR and DNA sequencing.

All NCK specimens tested HPV-negative, whereas all CIN2 and CIN3 keratinocytes were HPV16-positive; the E2/E6 ratio was higher in CIN2 than CIN3 indicating that HPV DNA integration increased from CIN2 to CIN3. Consistently, E6 gene expression was 3.7-fold lower in CIN2 than in CIN3 keratinocytes (p<0.05). RARB gene was 2.6-fold down-expressed in CIN2 and 16.8-fold in CIN3 keratinocytes compared to NCK (p<0.05, CIN3 vs CIN2). RARB gene promoter was found hypermethylated in 54% CIN2 keratinocytes, 75% CIN3 keratinocytes and 6.6% NCK (p<0.05, CIN2 vs normal; p<0.01, CIN3 vs normal). In conclusion, RARB gene was found to be down-expressed in progression from CIN2 to CIN3 through promoter hypermethylation. This result suggests that RARB promoter hypermethylation may play a role during the progression of CIN lesions. RARB gene promoter methylation could represent a potential new prognostic biomarker of CIN progression.

POSSIBLE EFFECT OF D-TOCOTRIENOL ON NEUTROPHILS RECRUITMENT IN MALIGNANT MELANOMA


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Immunotherapies were approved for the treatment of unresectable melanomas; however, most patients relapse because cancer cells can adapt shaping the immune microenvironment toward a protumor setting. A high neutrophil/lymphocyte ratio is a poor prognostic factor for melanomas, and a marker of drug resistance. Moreover, cancer cells can induce a neutrophil switch from an antitumor (N1) toward a protumor (N2) phenotype. The major chemotaxant factor for neutrophils is CXCL8; its activity is mediated by the receptors CXCR1 and CXCR2. We previously showed that the vitamin E derivative d-tocotrienol (d-TT) is endowed with antitumor activity, in vitro and in mouse xenografts, without toxic effects. It is currently unknown if these effects are mediated by the interaction with a defined molecular target. Exploiting the innovative in silico technology SPILLO-PBSS, a screening on 18000+ protein 3D-structures has been performed, and CXCR1 (PDB ID: 2lnl; UniProtKB AC: P25024) was identified as a potential target of interest. Given the key role of CXCR1 in mediating neutrophil recruitment, in vivo experiments have been performed in order to verify the effect of d-TT. First, B16 melanoma cells were injected s.c. into C57BL6 mice, and the neutrophil infiltrating the tumors were analyzed by flow cytometry (CD11b+/Ly6G+ cells), showing that the percentage of neutrophils increases with tumor dimensions. Mice were then orally treated with d-TT, 200 mg/kg for 1 week, and a trend in the reduction of CD11b+/Ly6G+ cells within the tumor mass was observed. Subsequently, we performed preliminary in vitro experiments on primary mouse neutrophils: treatment with d-TT (15 ug/ml) didn’t affect cell viability, whereas induced a significant reduction in ROS production. In conclusion, these data show the ability of d-TT to affect the neutrophil infiltrate within melanoma tumors in mice, as well as its ability in affecting neutrophil functions, possibly contributing to their antitumor properties.
AMNIOTIC FLUID EXTRACELLULAR VESICLES: NOVEL PLAYER IN THE IMMUNE-MODULATION DURING PREGNANCY

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Increasing interests are focused on immune-modulation during pregnancy. Amniotic fluid (AF) is a complex and dynamic environment essential for fetal development and maturation. AF is a rich source of extracellular vesicles (EVs), membrane enclosed spherical particles, deeply involved in cell-cell communication and in a wide range of biological responses, including immune-modulation. Despite the growing interest on EVs, AF-derived EVs (AF-EVs) role remains largely under-investigated. Here, we demonstrated, for the first time, that AF-EVs induce a significant reduction in THP-1 monocyte cell viability. This effect is accompanied by pyroapototic-morphological changes, suggesting an immune-modulatory role for AF-EVs. At the molecular level AF-EVs exposure induced an increase in Absent in Melanoma 2 (AIM2) expression and Caspase-1 activation, without affecting Nucleotide oligomerization domain-like receptors 1 (NLRP1) and 3 (NLRP3) protein expression. These effects are paralleled by pro-IL1beta up-regulation. FACS analysis demonstrated that AF-EVs are not internalized by THP-1 cells, thus excluding EVs cargo-dependent activity inside recipient cells. We hypothesized that the observed effects may be ascribed to extracellular adenosine production by AF-EVs. Indeed: (i) AF-EVs are capable of producing ATP, (ii) AF-EVs possess all the enzymes necessary to convert the nucleotide triphosphate into adenosine, i.e. CD39 (ecto-nucleoside triphosphate diphosphohydrolase 1) and CD73 (ecto-5’-nucleotidase) and (iii) THP-1 cells express adenosine receptors. In agreement, adenosine receptor agonist NECA mirrors whereas adenosine receptor antagonist Caffeine reverts AF-EVs-induced Caspase-1 activation. All together these results suggest that, by inducing monocyte pyroapoptotic cell death, AF-EVs exert a immune-regulatory function. These data unveil a novel role for AF-EVs in the immune response at the maternal-fetal interface that can contribute to semi-allogenic fetus protection.
SYNERGISTIC DRUG COMBINATIONS PREVENT DRUG RESISTANCE IN ANAPLASTIC LARGE CELL LYMPHOMA PRECLINICAL MODELS


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Anaplastic large cell lymphoma (ALCL) is an aggressive type of Non-Hodgkin Lymphoma, characterized by a specific chromosomal translocation leading to the expression of the NPM/ALK fusion protein, an oncogenic tyrosine kinase essential for growth of the neoplastic cells. When resistant or relapsed (R/R) to front-line chemotherapy, ALK+ ALCL prognosis is very poor. The therapeutic activity of an ALK inhibitor, crizotinib, has been shown in patients with R/R ALK+ ALCL: over 80% of R/R ALK+ ALCL patients obtain a long-lasting response with crizotinib monotherapy. However, 30 to 40% of patients develop resistance to tyrosine kinase inhibitors and relapse within short time, leaving no further therapeutic option than bone marrow transplant, when feasible. This highlights the need to identify new first-line therapies for high-risk patients. We explored the possibility to combine ALK inhibitors with other treatments, such as CHOP chemotherapy, epigenetic drugs, or a MEK inhibitor. Here we present the in vitro and in vivo results, showing strong synergistic interactions of such combinations, which resulted in more profound growth arrest and induction of apoptosis, compared to single agents. Moreover, the combined treatments suppressed the rise of drug-resistant clones. In conclusion, our data suggest that a polypharmacology approach combining ALK inhibition with other agents could be a valuable therapeutic option for ALK+ ALCL patients, to prevent relapses and improve outcomes.
Progestosterone-Receptor (PR) positivity is associated with a better response to breast cancer treatment, conversely cyclin D1 (CD1) is a retained marker of poor outcome. Herein, we demonstrate that hydroxyprogesterone (OHPg) through PR-B reduces breast cancer cell aggressiveness, by targeting the cytoplasmic CD1. Specifically, OHPg reduces CD1 expression through a transcriptional mechanism due to the binding of PR-B at a canonical half-PRE site of CD1 promoter, together with HDAC1, influencing a less permissive chromatin conformation for gene transcription. CD1, together with its kinase partner Cdk4, controls cell migration and metastasis, through the interaction with key components of focal adhesion, such as Paxillin (Pxn). Kaplan-Meier analysis shows that low Pxn expression was associated with increased distant metastasis free survival in luminal A PR+ breast carcinomas. Interestingly, OHPg treatment reduced Pxn content in T47-D and MCF-7 cells, besides the interaction between endogenous cytoplasmic CD1/Cdk4 with Pxn was reduced. This was consistent with the reduction of p-Ser83Pxn levels, crucially causing the delay in cell migration and a concomitant inhibition of Rac1 activity and p-PAK. Collectively, these findings support the role of PR-B in breast epithelial cell integrity and reinforce the importance to target PR-B as potential strategy to restrict breast tumor cell invasion and metastasis.
THE N6-METHYLADENOSINE (M6A) MRNA METHYLATION IS REQUIRED TO SUSTAIN NEUROBLASTOMA TUMOR AGGRESSIVENESS


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Neuroblastoma is the most common extracranial solid tumor in early childhood. It manifests as a very heterogeneous disease, from spontaneous regression to rapid progression. High-risk neuroblastoma is mostly characterized by copy number variation alteration, with very low somatic mutation rate, therefore producing most a quantitative than a qualitative driving imbalance in the transcriptome. Moreover, the ability of some metastatic neuroblastomas at diagnosis to spontaneously regress suggests the involvement of reversible epigenetic and/or epitranscriptomic alterations. The N6-methyladenosine (m6A) is the most prevalent covalent modification of mRNAs, which biological impact remained puzzling until the development of an m6A-wide mapping and the discovery that the modification is reversible and dynamic. From a dataset of more than 700 gene-expression profiles of neuroblastoma clinical samples, we found that the expression of the methyltransferase METTL14 is significantly higher in high-risk neuroblastoma patients and directly correlates with worst clinical feature and poor prognosis. Conversely, the demethylase ALKBH5 shows a very low expression in high-risk patients and it is almost null in all the neuroblastoma cell lines commercially available. We demonstrated that the overexpression of METTL14 increase proliferation and invasion in neuroblastoma cells and induce a significant rise in tumor growth rate in vivo. In the opposite fashion, ALKBH5 constitutive rescue is associated with a reduction in cell proliferation and is able to prevent tumor development in vivo. More importantly, we found that the transient restoration of ALKBH5 with an inducible system causes a dramatic slowdown in the progression of mature tumours. With this project, we demonstrated that the m6A modification and its key regulators are required to sustain neuroblastoma aggressiveness and could possibly offer new hints for novel pharmacological therapies.
INDUCTION OF NECROPTOSIS BY DELTA-TOCOTRIENOL OVERCOMES DOCETAXEL-RESISTANCE IN PROSTATE CANCER CELLS


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Prostate cancer is the most commonly diagnosed cancer in men. Androgen deprivation therapy is the initial line of treatment, but unfortunately most patients will progress to castration resistant prostate cancer (CRPC). The first-line therapy for CRPC is docetaxel, but over time patients develop chemoresistance. Docetaxel resistance has been well studied and lots of studies demonstrate that chemoresistant cancer cells escape from apoptosis. Therefore, the activation of alternative cell death could reduce chemoresistance. Recently, the term necroptosis has been used to designate a programmed caspase-independent cell death induced by a number of stimuli when apoptotic signaling is inhibited. Formation of necrosome leads to activation RIPK-1, RIPK-3 and MLKL. Currently, accumulating evidence shows that natural compounds can induce necroptosis. Previous studies conducted in our laboratory demonstrated that delta-tocotrienol (delta-TT), a member of the vitamin E family, exerts a cytotoxic effect in CRPC cells through apoptosis activation. The aim of this study was to analyze whether delta-TT activates necroptosis in CRPC cell lines. The experiments conducted demonstrated that necrostatin (necroptosis inhibitor) reverted delta-TT cytotoxicity and necrotic cells number and confirmed that delta-TT induces necroptosis. Docetaxel remains the effective therapy to suppress CRPC, but most patients acquired drug resistance; so, we have created a docetaxel-resistant cell line treating DU145 cells with increasing doses of docetaxel (from 10 nM to 200 nM) (DU-DXR) and we then evaluated the effect of delta-TT on this cell line. The results obtained showed that delta-TT induces cytotoxicity in DU-DXR by necroptosis activation. In conclusion, our results strengthen the anticancer role of delta-TT in CRPC. Moreover, delta-TT-mediated necroptosis can represent an interesting mechanism that overcome chemotherapy resistance due to an altered apoptotic response.
REAPPRAISAL OF THE PATHOGENIC CASCADES UNDERLYING MUCOPOLYSACCHARIDOSIS TYPE II (MPSII) BEYOND THE LYSOSOMAL SUBSTRATE STORAGE PARADIGM


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In the past few years the traditional concept that clinical manifestations in lysosomal storage disorders are merely due to lysosomal engulfment has been challenged by the identification of key cellular pathways affected by lysosomal enzymes dysfunction. In particular, given the detectable amount in the human placenta, together with its widespread tissue distribution and function, we previously uncovered a neglected role of iduronate-2-sulfatase (Ids) in zebrafish embryonic development. By using a set of biosensor transgenic, fish, we have recently demonstrated the requirement of Ids for the correct tissue-specific transduction of Shh and FGF pathways. The impairment of these developmental signaling cascades in fish and mouse models for Mucopolysaccharidosis type II (MPSII) underlies a defective differentiation program of cardiac and bone precursors, leading to progressive tissue defects. We have, therefore, started to extend our investigation to the central nervous system, by addressing the analysis of the Shh pathway to verify its potential involvement in the onset of defective neurogenesis and synaptogenesis. Our preliminary results further support the concept that functional impairment of lysosomal iduronate-2-sulfatase negatively affects cell signaling pathways transduction and early cell differentiation programs.
THE USE OF THE MONOCYCLULAR EUKARYOTE SACCHAROMYCES CEREVISIAE AS A MODEL FOR MECHANISTIC AND PHYSIOLOGICAL STUDIES OF HUMAN INSIGHTS: THE CASE OF CADMIUM BASED QUANTUM DOTS


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Among the recent key improvements in drug design and testing is the accepted 3Rs principle, which aims to replace, reduce, and refine animal testing. Along this line, the use of the model Saccharomyces cerevisiae has been exploited for the peculiar features: a short replication time, growth on both fermentable and oxidizable carbon sources, and for the contextual availability of genome wide information in the form of genetic maps, DNA microarray, and collections of single-deleted barcoded mutants. Baker’s yeast can be furthermore utilized for more general medical approaches, focused on the effects of substances or conditions upon the expression of specific cloned human genes potentially involved in fundamental diseases, what is called a humanized yeast model. A specific case of study can be the one of the Cadmium-based Quantum Dots (CdS QDs): although uptake, transport and response mechanisms in different organisms have received increased attention in recent years, many questions regarding their risks to human health and environment remain unanswered. The aim of this study is to use the yeast Saccharomyces cerevisiae as a tool for mechanistic studies of CdS QDs effects. The comparison of the whole genome analysis with the microarray experiments (99.9% coverage), the proteomic analysis, and the phenotypic analysis of 4688 barcoded haploid mutants (80.2% coverage), shed light on the genes involved in the response to CdS QDs, both in vivo and in vitro. The results have clarified the mechanisms involved in the exposure to CdS QDs, in particular related to oxidative stress and to the maintenance of mitochondrial integrity and function, and the differential pathways of response exploited by Cd2+. Saccharomyces cerevisiae remains a versatile and robust alternative for organismal toxicological studies, with a high level of heuristic insights into the toxicology of more complex eukaryotes, including mammals.
DYSREGULATION OF AUTOPHAGY HAS A POTENTIAL ROLE IN SYNJ1-ASSOCIATED EARLY-ONSET PARKINSONISM


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Several genes responsible for hereditary forms of Parkinson’s disease are implicated in distinct steps of the intracellular trafficking regulating the endolysosomal pathway. Among the others, we have recently reported how SYNJ1 mutations, which cause an early-onset parkinsonism (PARK20) are associated to an alteration of endosomal trafficking. In particular, we revealed a crucial role for Synaptojanin 1 (Synj1) in regulating the homeostasis and functions of early endosomal compartments. Importantly, the same alterations of early endosomal compartments and trafficking defects occur in fibroblasts derived from PARK20 patients, highlighting the defective cellular pathways in PARK20 mutant cells.

Furthermore, we also found that the structure of lysosomes resulted altered in Synj1-deficient cells, despite any substantial difference in the levels of two lysosomal markers, Lamp-1 and cathepsin D. Because trafficking toward lysosomes is unaffected upon Synj1 silencing, the alteration of lysosomes could be due to changes in the autophagic pathway, whose activity is critical in many neurodegenerative diseases. We found that autophagy is up-regulated in Synj1 silenced cells, as observed by measuring the levels of some autophagic markers and number of autophagosomes. The increase in autophagosome biogenesis can be, at least in part, explained by the alteration of the mTORC1 signaling, a key upstream modulator of the pathway. Nevertheless, the clearance of autophagy substrates results reduced in Sinj1 depleted cells, suggesting therefore a more complex scenario, which will need further investigations.

Overall, our preliminary data corroborate the existence of a functional link between endosomal trafficking, autophagic pathway and Parkinson’s disease.
CRI DU CHAT INDUCED PLURIPOTENT STEM CELLS: NEW FRONTIERS IN DISEASE UNDERSTANDING


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Cri-du-chat syndrome is a genetic disease caused by partial deletion in the short arm of chromosome 5. The syndrome is named by high-pitched cat-like cry. The features of CdCs are facial dysmorphism, developmental motor delay, including difficulties in mobility, dexterity, and verbal communication. A group of genes (1.6%), TERT, SEMA5A, MARCH6, CTNND2, TPPP, SLC6A3, CDH18, and CDH6, was classified as dosage sensitive leading to haploinsufficiency. The deleted genes, semaphorin F (SEMAF) and δ-catenin (CTNND2), believed to play a role in cerebral development, microtubule associated protein (TPPP) witch expression is finely controlled in the human brain, and telomerase (TERT), a role during early stages of embryonic development, are reported to be implicated in CdC intellectual disability, development and premature aging. Pluripotent stem cells (IPSC) represent a more accurately models to study the physiology of human cells. We reprogrammed peripheral mononuclear blood cells from two CdC patients to IPSCs. Clones were expanded and characterized for stemness expression and pluripotent potential was investigated by spontaneous differentiation capacity in the three germ layers genes expression by q-PCR. At first we showed that the expression, in IPSC, of TERT, CTNND2 and SEMA5A are about half, fifth and quarter respectively compared to control donor. The second approach was to differentiated CdC iPSC into fibroblastic-like cells that phenotypically and functionally resemble MSC for an attractive approach to study these cells in the CdC pathologic tissue homeostasis and premature aging and senescence. We focus our attention on their characteristics as compared with iPSC-MSC like cells from healthy donors and on their ability to differentiate into bone and other cells of mesodermal lineages.
PNPLA3RS738409C>G VARIANT PREDICTS LIVER-RELATED OUTCOMES IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Patients with nonalcoholic fatty liver disease (NAFLD) are at higher risk of developing liver-related complications – liver decompensation (LD) and hepatocellular carcinoma (HCC), including death, with severity of liver fibrosis and metabolic comorbidities as main risk factors. PNPLA3 is a lipase active towards triglycerides in hepatocytes and retinyl esters in hepatic stellate cells. The I148M substitution (rs738409) leads to a loss of function promoting triglyceride cellular accumulation. Although PNPLA3 function has been extensively studied, the molecular mechanisms leading to hepatic fibrosis and carcinogenesis remain unclear. This association has highlighted the fact that liver fat metabolism may have a major impact on the pathophysiology of liver diseases. Conversely, alone, this locus may have predictive value with regard to liver disease outcomes in clinical practice. We aimed to assess whether the common rs738409 variant in PNPLA3 gene can predict the occurrence of liver-related events and death in a large cohort of NAFLD patients.

Methods: We considered 471 consecutive Italian individuals with histological diagnosis of NAFLD or clinical diagnosis of compensated NAFLD-related cirrhosis, prospectively followed for at least 6 months. The occurrence of hepatic and extrahepatic outcomes was recorded. Results: During a median follow-up of 64.6 months 26 LD, 13 HCC and 16 deaths (12 liver-related) were recorded. All liver-related events death occurred in patients with F3 fibrosis or cirrhosis. The prevalence of PNPLA3 rs738409 CC, CG and GG genotypes was 31.8%, 45.6% and 22.6%, respectively. After adjusting for clinic-metabolic and histological risk factors PNPLA3 C>G variant was associated with a higher risk of LD, HCC and liver-related death by multivariate Cox regression analysis. Conclusions: Patients carrying PNPLA3 rs738409 G>C variant are at higher risk of developing liver-related events and death.
HDAC8 AND COHESINS: PROMISING THERAPEUTIC TARGETS FOR MYELOID MALIGNANCIES

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The genes of the cohesin complex (SMC1A, SMC3, RAD21, NIPBL and HDAC8), exert different functions ranging from the adhesion of sister chromatids during the cell cycle, DNA repair, gene expression and chromatin architecture remodelling. In recent years, the improvement of DNA sequencing technologies allows the identification of cohesin mutations in myeloid malignancies such as Acute Myeloid Leukaemia (AML). However, the role of cohesin dysfunctions in AML insurgence remains elusive as cohesin mutations alone are insufficient to induce myeloid neoplasms and they have to co-occur with other causative mutations such as NPM1, FLT3-ITD, and DNMT3A. In our work, using zebrafish as a suitable animal model for cohesins haploinsufficiency, we correlate cohesins activity with dysregulated expression of genes involved in myeloid development and differentiation. The dissection of molecular pathways altered by cohesin dysfunctions, allowed the discovery of new therapeutic targets downstream of cohesins. Moreover, our zebrafish models which presents the combined dysregulation of cohesins and other causative genes of AML, could improve the development of multiple therapies such as those targeting specific genes and those directed on shared targets.
ABSTRACT n. 46

CYYR1 GENE AND HH-MEDIATED MYOGENESIS DURING ZEBRAFISH DEVELOPMENT: POTENTIAL ROLE IN RHABDOMYOSARCOMA DISEASE


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CYYR1 (Cysteine/tyrosine-rich 1) cloned on HC21 defines a family of highly conserved vertebrate-specific genes. The human locus is characterized by a multitranscript-system including at least six alternative spliced isoforms. To date, the function of the CYYR1 product is still unknown even if original results suggest its possible involvement in myogenesis differentiation with a putative role in the tumorigenic process related to the Hh pathway.

Zebrafish cyyr1 orthologue is present in single copy and the predicted protein maintains almost 58% of identity with human protein. By WISH approach, we show a cyyr1 broad expression in central nervous system (CNS), somites and muscles during development. The protein seems to localize in plasma and even nuclear membranes.

We perform cyyr1 knock-down with two different approaches: microinjection of morpholino oligos targeting the ATG and the first splice-site of the transcript, and the generation of cyyr1 null fish through CRISPr/Cas9 technique. Defects in heart and muscle development with a significant rescue in embryo co-injected with cyyr1 mRNA, were observed in morphants, while cyyr1 mutants analyses confirmed morphological and molecular weakness in heart.

Dysregulation of Nodal and/or Hedgehog (Hh) pathways in zebrafish decreased cyyr1 expression and the injection of cyyr1 mRNA was able to partially rescue Hh-defective phenotype in embryos. In order to verify a putative role of CYYR1 in the process caused by dysfunction of cell differentiation we performed experiments in rhabdomyosarcoma cell lines showing an inverse correlation between CYYR1 expression and the range of differentiating capabilities of these cells. Interestingly, treatments with inhibitors of Hh allow us to confirm a correlation between CYYR1 and this pathway.
HYPERGLYCEMIA AFFECTS MiRNA EXPRESSION THUS DRIVING THE PROGRESSION OF RENAL DAMAGE IN DIABETIC PATIENTS


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Diabetic Nephropathy (DN) is the primary cause of end stage renal disease. We demonstrated that an accumulation of lysine63-ubiquitinated (K63Ub) proteins at tubular level is involved in the progression of renal damage in DN (PMID: 27881486; 29806072). The aim of this study was to identify miRNAs regulating the expression of K63Ub proteins and epigenetic mechanisms influencing their expression.

By microarray we profiled miRNA expression in kidney biopsies from 6 DN diabetic pts, 6 diabetic pts with membranous nephropathy (T2D-MN) and 4 subjects with Minimal Change Disease (MCD). We selected those miRNAs with a predicted and validated interaction with UBE2v1, an ubiquitin-conjugating E2 enzyme variant that mediates the formation of K63Ub chains. We measured the expression levels of these miRNAs by qPCR in both tissue and urinary samples and by in situ hybridization. DNA methylation of specific CpG island in promoter regions of selected miRNA was evaluated by quantitative methylation PCR assay.

9 miRNAs deregulated in DN kidneys were also deregulated in urine (p<0.05) compared to T2D, T2D-MN and T2D-FSGS (focal segmental glomerulosclerosis). We selected 2 miRNAs showing a predicted interaction with UBE2v1. Both were downregulated in kidney tissues of DN vs T2D-MN pts (qPCR and in situ hybridization-p<0.04 and p<0.01) as well as in urine of DN pts compared to all the other categories (p<0.03). To evaluate if epigenetic mechanisms were involved in those miRNAs downregulation, we investigated miRNAs DNA methylation levels on both renal biopsies from 3 DN and 3 diabetic patients with other renal damage and in a cellular model of tubular cells under hyperglycemic conditions. We confirmed that hyperglycemia induced miRNA promoter hyper-methylation both in vivo and in vitro.

In conclusion we identified two miRNAs whose expression levels, influenced by epigenetic mechanisms, can play an important role in the pathogenesis and progression of renal damage in the diabetic patient.
PRENATAL EXPOSURE TO BISPHENOL A REDUCES SEX DIFFERENCE IN EXPLORATORY BEHAVIOUR AND HYPOTHALAMIC €2-ADRENERGIC RECEPTORS BUT HAS NO EFFECTS ON SEXUAL BEHAVIOR


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Bisphenol A (BPA) is a manmade chemical that binds intracellular and membrane estrogen receptors. BPA can act either as an estrogenic agonist or androgen antagonist and perinatal exposure to BPA is hypothesized to interfere with the normal development of sex differences. In support of this hypothesis, during the last two decades the role of BPA as an environmental estrogen and endocrine disruptor has been well established. Here, using the house mouse (Mus musculus) as a model, we add to this large literature by presenting the long term effects of early exposure to BPA on behavioral and neuroanatomical traits that are known to be sexually dimorphic. Specifically, we tested the effects of the prenatal exposure to low doses of BPA on the adult’s expression of alpha-2-adrenergic receptors in the medial preoptic area (mPOA) and on adult’s exploratory and sexual behaviors. Our aim was to study if and how sex differences in the noradrenergic system were disrupted by the exposure to BPA and if the hypothesized effect would affect behaviors known to be modulated by noradrenaline. Our results show that while within the mPOA of control animals females have a higher number of alpha-2-adrenergic receptors, prenatal exposure to BPA reduces the sex difference. Behaviorally, prenatally exposed males were not different in sexual motivation and performance from control. Similarly, BPA exposed females were not different from controls in terms of attractiveness. However, while unexposed female mice, when allowed to explore a novel environment, were more reactive and exploratory as compared to unexposed males, BPA exposure reduced these sex differences. In line with current models of early life effects to the exposure to environmentally low doses of BPA, our results demonstrate a reduction or a reversal of sexual differences in exposed mice relative to those displayed by controls.
DECREASED ANTI-OXIDATIVE ENZYMATIC ACTIVITY IN ALZHEIMER’S PATIENTS SERA IS LINKED TO ENDOTHELIAL DYSFUNCTION


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Endothelial dysfunction (ED) comprises changes of endothelial cells (ECs) functional phenotype leading to the dysregulation of vascular homeostasis. Both decreased activity of circulating anti-oxidant enzymes and ED have been linked to Alzheimer’s Disease (AD) progression. On these premises, this study investigated whether alterations in circulating anti-oxidative or pro-oxidant enzymes may be linked to AD-associated ED.

We measured anti-oxidative paraoxonase-1 (PON1) lactonase and arylesterase activities, and the pro-oxidant activity of myeloperoxidase (MPO), in serum from AD patients or age-matched controls. Next, we treated HUVEC with AD or control sera and evaluated the levels of ECs apoptosis, as a marker of ED, and of 4-HNE, as a marker of oxidative stress. We found that AD sera have higher MPO activity, and lower lactonase and arylesterase-PON1 activities. ECs treated with AD sera displayed higher apoptosis compared to controls and their protein extracts had decreased SOD activity. Unexpectedly, AD sera treated ECs also presented lower levels of 4-HNE. Interestingly, we found a correlation between SOD activity and 4-HNE levels (r= 0.527; p = 0.004) and a negative correlation between SOD and ECs apoptosis (R = -0.490; p = 0.006). Finally, PON1 lactonase and arylesterase activities negatively correlated with early apoptosis in ECs treated with controls sera (r= -0.740; and r = -0.719, respectively) but not in ECs treated with controls sera.

Overall, our preliminary results indicate that appropriate endothelial SOD activity, possibly thanks to anti-oxidative PON1 activities, maintains 4-HNE in a range of concentrations promoting ECs survival in healthy subjects. Conversely, insufficient SOD activity in ECs is associated to lower 4-HNE levels favoring ECs apoptosis over survival. In conclusion, our findings suggest that a decreased circulating antioxidant capacity in AD patients results in altered redox homeostasis in ECs that may lead to ED.
EXTRA CELLULAR VESICLES DERIVED FROM STEM CELL AND AMNIOTIC FLUID SUPPRESS PATHOGENIC IMMUNE RESPONSES IN AUTOIMMUNE ENCEPHALOMYELITIS


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Multiple sclerosis (MS) is a complex disease of immune dysfunction and neurodegeneration. MS shows a female preponderance, although late pregnancy is the one condition that most profoundly mitigates MS symptoms. Serum exosomes, released by specific cell types during pregnancy, modulate the immune and central nervous systems and contribute to pregnancy-associated suppression of experimental autoimmune encephalomyelitis (EAE), an induced model of MS. Extracellular vesicles (EVs) are released from a variety of cell types and play important roles both in physiologic cell functions and in diseases. The interest around extracellular vesicles (EVs) is rapidly growing as a consequence of the promising implications in diagnosis and therapy that this matter holds.

Here we demonstrated that administration of EVs, derived either from amniotic fluid stem cells or from whole human amniotic fluids, were able to control inflammatory responses and immune dysfunction in EAE. Specifically, EVs administration significantly ameliorated EAE clinical symptoms and suppressed pathogenic cytokines, compared to control groups, leading to stable immunomodulatory functions.

By addressing potential mechanisms involved in these effects, we demonstrated that both amniotic fluid and stem cell derived EVs could be rapidly captured by specific dendritic cell (DC) subsets but not T cells. Moreover, DCs treated with those EVs acquired a potent tolerogenic phenotype, leading to differentiation of regulatory T cells in vitro.

Taken together, our data suggest that EVs isolated both from stem cells and amniotic fluid can function as active immunoregulatory entities, thus our study may serve as an interesting preclinical opportunity to develop novel biological therapeutics, harnessing autoimmune diseases.
IPSC-DERIVED SENSORY NEURONS FROM CMT2B PATIENT SHOW ALTERATIONS IN LYSOSONMAL ACTIVITY


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Charcot-Marie-Tooth type 2B (CMT2B) is a rare autosomal-dominant axonal disorder affecting the peripheral nervous system. CMT2B is caused by 5 mutations (L129F, K157N, N161T/I, V162M) of the RAB7A gene, encoding a small GTPase that controls late endocytic trafficking and plays also important roles in neurons. Mainly, RAB7A controls maturation of early endosomes in late endosomes, transport from late endosomes to lysosomes, biogenesis of lysosomes and clustering and fusion of late endosomes and lysosomes in the perinuclear region. As several neurodegenerative diseases are caused by lysosomal disfunctions, we decided to investigate whether CMT2B-causing RAB7A mutations alter the activity of these organelles. In CMT2B skin fibroblasts carrying the Rab7V162M mutation we found higher expression of late endocytic proteins and of lysosomal enzymes, higher cathepsins activity, higher receptor degradation and higher lysosomal activity compared to control fibroblasts. CMT2B fibroblast exhibited also an increased number of lysosomes. To confirm these data, we differentiated sensory neurons from induced pluripotent stem cells (iPSC) as iPSCs are increasingly used as model system of neurological disorders. Fibroblasts were reprogrammed using four Sendai viruses, each capable of expressing one of the four Yamanaka factors (KLF4, OCT3/4, SOX2 and c-MYC). iPSCs were then subject to strict quality control checks before starting the differentiation. This included tests for Sendai Virus clearance, PCR for pluripotency markers and tri-lineage differentiation experiments. iPSCs were then subjected to several medium changes to induce differentiation and this differentiation resulted in a pure neuronal culture after 2–3 weeks. Analysis of CMT2B neurons showed higher LAMP1 expression and higher lysosomal activity demonstrated by DQ-BSA dequenching assay. Thus, we confirmed the data obtained on fibroblasts, suggesting that higher lysosomal activity could lead to neurodegeneration in CMT2B.
TOXICITY OF FOOD-GRAGE NANOPARTICLES IN HUMAN IMMUNE CELLS

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Synthetic amorphous silica (SAS) is used in a wide variety of industrial applications including food products. According to the EU specifications, the forms of SAS used as food additive (E551) include pyrogenic or hydrated silica depending on the process (thermal or wet) used for their manufacture, but these processes lead to the production of nanoparticles (NPs) of SAS. In the last few years, there has been increased debate regarding the health and safety concerns related to the use of consumer products containing NPs.

In this work we have characterized SAS NPs produced by wet route (precipitated silica, NM-200) or thermal route (pyrogenic silica, NM-203) and we have observed that NM-203 exhibits greater cytotoxicity than the precipitated form on murine and human macrophage cell lines.

To study the molecular interactions of NM-200 and NM-203 in a human monocytic cell line differentiated into macrophages (THP-1), we have isolated and identified the set of proteins from cell lysates which are adsorbed with a high level of affinity to the SAS NP surface. These proteins form a so-called “hard corona”, the structure of which defines the biological identity of NPs.

We have observed that NM-203 NPs adsorbs on their surface more proteins than NM-200. SDS-PAGE analysis shows similar protein profiles, but a different abundance of specific proteins that form the corona of NM-200 and NM-203. The hard corona of these SAS NPs was composed of a number of distinct proteins involved in crucial metabolic pathways. These proteins show large unstructured regions that provide high flexibility that promotes their adsorption to SAS NPs.

This study supports an increased cytotoxicity and protein binding of pyrogenic SAS NPs compared to precipitated form that could be attributed to the higher surface reactivity of NM-203. The identification of structural determinants of NP toxicity appears to be essential for a “safety-by-design” synthesis of NPs used as food additives or therapeutics.
Adult Renal Stem/Progenitor Cells (ARPCs) show an active role in kidney repair processes during acute or chronic injury. However, little is known about their immunomodulatory properties and their capacity to regulate specific T cell subpopulations. The aim of our study was to investigate the immunomodulatory properties of ARPCs on human T cells.

Human Peripheral Blood Mononuclear Cells (PBMCs) were isolated from healthy subjects and activated with 5 ng/ml Concanavalin A (ConA) for 24h. ARPCs were isolated from renal tissues and activated by triggering TLR2 for 24h with 30ug/ml of Lipoteichoic acid (LTA). PBMC were co-cultured with ARPCs and T cell subpopulations were characterized by flow. The relative expression levels of 36 cytokines in supernatants from T cells and ARPCs, alone and in co-culture, were determined by Human Cytokine Proteome Array.

We found that TLR2-activated-ARPCs were able to decrease T cell proliferation after 24h of co-culture (p=0.017).

In order to investigate changes in subset of T cell populations, we co-cultured ConA-activated PBMCs with TLR2-activated ARPCs for short (5 days) and long period (15 days) of time. After both 5 and 15 days, we observed a significant decrease of CD3+CD4+CD25+CD127- T regulatory cells (Tregs) (p=0.003 and p=0.001, respectively) and CD3+CD4-CD8- double negative (DN) T cells (p<0.0001 and p=0.001, respectively).

Finally, we identified cytokines secreted by ARPCs responsible for the immunomodulatory effect. SERPIN-E1, MCP1, GM-CSF and CXCL1 were significantly modulated in T cells co-cultured with TLR2-activated ARPCs, while were not present in T cells alone.

Our data showed that ARPCs regulate Tregs and DN T cells, which are involved in the balance between immune tolerance and autoimmunity, through TLR2 engagement. Moreover, we identified cytokines with a key role in this system. These findings can help to clarify the role of ARPCs in immunomodulation and could be translated to potential clinical treatment.
UNRAVELLING THE EFFECTS OF PRION AND LAMININ RECEPTOR INHIBITORS ON TRAFFICKING AND PROCESSING OF AMYLOID PRECURSOR PROTEIN APP


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The misfolding and aggregation of different proteins is the neuropathological hallmark for numerous diseases including prion diseases, Alzheimer’s disease, Huntington, Parkinson’s and amyotrophic lateral sclerosis. It is believed that misfolded and abnormal forms of wild-type proteins are the vectors of these diseases by acting as seeds for the aggregation of endogenous proteins. Therapeutics for these disorders do not exist. The growing understanding that the most common neurodegenerative diseases progress by an analogue prion mechanism, highlights the importance of identifying anti-prion therapeutics that, recently, pointed to target the prion protein receptor 37/67 kDa laminin receptor (LR).

Several lines of evidence describes a critical role for LR in both cytotoxicity of amyloid beta and the cellular uptake of prions. We are studying the effect of LR inhibitor compounds (naphtol derivatives) in both neuronal cell line (GT1) and fibroblasts from AD patients.

Our results indicate that new compounds are able to control both prion PrPC and APP trafficking and intracellular localization, with also effects on the ERK signalling pathway.

Our findings strongly indicate that these new small compounds could exhibit effects on APP processing which, in turn, reflect on amyloid beta generation/cytotoxicity.

The current applications for the proposed naphtol derived compounds are based either on the unique properties of the molecules or on their specific inhibitor element able to bind the peptide G domain of LR. However, the enormous potential use of these drugs in modifying the traffic and processing of neurodegeneration-related proteins, has not been generally recognized. Our proposal suggests to challenge these compounds with the misfolding mechanism and eventually with the trafficking/clearance of amyloid intra/extra cellular aggregates.

Thus, the napthol derivatives represent interesting candidates to be tested in the context of prion-related Alzheimer’s disease.
AN INNOVATIVE OPTICAL SENSOR FOR THE INORGANIC MERCURY DETECTION IN DRINKING WATER


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Mercury pollution of drinking water is an important issue in the environmental and public health field. Among all derivative forms, the inorganic mercury (Hg2+) is widely diffused in nature and its ingestion can lead to a series of permanent human diseases, affecting kidneys and central nervous system. All the conventional approaches for the Hg2+ assay shared some limitations in terms of bulky instruments and time/cost consuming for the analysis.

Considering this, we introduce a high throughput miniaturizable whole-cell based sensor for Hg2+ optical detection in drinking water, which combines the specificity of a genetically modified Escherichia coli, used as sensing element, with the performances of a small-active-area (250 µm x 250 µm) Silicon Photomultiplier (SiPM), used as optical detector. The modified E. coli strain act as Hg2+ reporter that emits a 485 nm light if in presence of the pollutant in water sample. This light signal is, then, collected and analyzed by the SiPM detector for the final quantification of water pollution.

The sensor is able to reveal up to 0.25 µg/L of Hg2+, which is well below the worldwide guideline value of 1 µg/L for Hg2+ in drinking water. No additional reagents are required to perform the analysis, leading to important cost savings. Moreover, the small dimensions and the miniaturizability of the entire sensing system pave the basis for a portable detection technology that can be expanded easily with other E. coli strains targeting different priority chemicals.
PCR-FREE ELECTROCHEMICAL BIOSENSOR FOR THE PORTABLE DETECTION OF VIRUS GENOME IN HUMAN SAMPLE


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We present an innovative chemical strategy for the rapid and sensitive detection of a viral genome (i.e. the Hepatitis B Virus circular dsDNA) in human plasma, without any PCR amplification step. The strategy consists in a cooperative hybridization based on the recognition of the two antiparallel strands of the target DNA by two probes immobilized at a platinum surface of a microelectrode. The intercalating agent Os(2,2'-bipyridine) (dipyrido[3,2-a:2',3'-c]phenazine)Cl2 is a redox active compound used to sense the target hybridization by interacting with the electrode surface. The redox signal is, then, monitored by the square wave voltammetry analysis. This approach allows a stable and reproducible hybridization process and a PCR-free detection. Moreover, the technology is fully integrated in a silicon miniaturized device containing three planar-microelectrodes and a resistor for heating and temperature control. All experiments, performed on a HBV synthetic DNA clone and an extracted HBV DNA from biological sample, reported a limit of detection of about 20 copies, comparable with the standard real time PCR approaches. These performances are consistent with the development of a Point-of-Care system for the rapid, portable and low-cost molecular diagnostics.
EGFR SIGNALLING TALKS TO MITOCHONDRIA THROUGH CONTACT SITES


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The integration of distinct internalization routes is crucial to determine the fate of plasma membrane (PM) receptors and the output of their signalling pathways. Contact sites between cellular organelles adds a further layer of regulation by creating microdomains that favour different signalling and metabolic pathways. These regulatory mechanisms are relevant to the epidermal growth factor receptor (EGFR). We found that, while clathrin-mediated endocytosis (CME) is mainly involved in EGFR recycling and sustaining signalling, EGFR internalization through non-clathrin endocytosis (NCE) leads primarily to receptor degradation and signal extinction, representing a crucial safety mechanism to protect cells from overstimulation. Internalization via NCE involves the formation of tripartite contact sites between the PM, the endoplasmic reticulum (ER) and the mitochondria, where EGF-dependent localized Ca2+ signalling occurs and propagate. Ca2+ release is in turn needed for the fission of NCE vesicular structures and their release in the cytosol. Thus, NCE, by targeting EGFRs to degradation, restricts EGFR signalling; it also exerts a positive role in promoting localized Ca2+ signalling, possibly activating a wider cellular response and regulating cellular processes that extend beyond those associated with the canonical EGFR signalling pathway. Through a combination of mathematical modelling and wet-lab experiments, we are currently elucidating how the integration of distinct endocytic pathways and inter-organelle crosstalk regulate the EGFR physiological responses and which are the molecular circuitries involved.
BENEFICIAL EFFECTS OF ACYL-L-CARNITINES ON OOCYTE COMPETENCE AND OVARIAN DYSFUNCTION IN A MOUSE MODEL OF POLYCYSTIC OVARIAN SYNDROME (PCOS)


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Background. PCOS is a complex endocrine disorder that affects ~6-20% of reproductive age women and a major cause of infertility. The PCOS classic phenotype consists of hyperandrogenism, and oligo-ovulation in association with the insulin resistance and metabolic disorders. L-carnitine has free-radical scavenging activity and plays a pivotal role in a mitochondrial oxidation of long-chain fatty acids, which increase cell energy supply and are essential for mammalian oocytes and early embryo development. It is well-known that circulating levels of L-carnitine are low in PCOS woman. In the present study we aimed to evaluate whether oral administration of two formulations of acyl-L-carnitines have beneficial effects on a PCOS mouse model induced by DHEA (dehydroepiandrosterone).

Materials and methods. 25 CD1 female mice aged 21 days received 20 consecutive doses of DHEA (60 mg/kg) in association with oral administration of two carnitine formulations. To monitor the efficacy of DHEA in inducing PCOS phenotype, after 7 administrations, we monitored estrous cyclicity every day by vaginal smears. At the end of the treatment, after induction of ovulation by PMSG and hCG administration, metaphase II (MII) oocytes were subjected to evaluation of mitochondrial potential by JC-1 staining and spindle and chromosomes configuration following immunocitochemistry.

Results. Carnitine administration was effective in reducing PCOS features by ameliorating estrous cyclicity and quality of ovulated oocytes. In particular, both carnitine formulations were able to restore mitochondrial potential that was affected by DHEA and to improve the rate of oocytes with normal metaphase (MII) spindle and chromosomes.

Conclusions. These observations suggest that acyl-L-carnitines may contribute to improved attenuate the effects of PCOS on female reproductive competence probably by improving mitochondrial energy balance in germ cells.
MYOPATHY- AND NEUROPATHY- RELATED P209 MUTATIONS IN THE BCL-2-ASSOCIATED ATHANOGENE 3 CAUSE ITS AGGREGATION AND DYSFUNCTION


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The Bcl-2-Associated Athanogene 3 (BAG3) is a nucleotide exchange factor acting as HSP70 co-chaperone that belongs to the BAG family. BAG3 modulates several pathways, including protein quality control (PQC), cell cycle and apoptosis. In PQC, BAG3 acts in the Chaperone Assisted Selective Autophagy (CASA), a pathway that assure the removal of misfolded proteins, particularly in neurons and muscle cells. In CASA, BAG3 acts as a scaffold by interacting with the small Heat Shock Protein B8 (HSPB8) through two IPV domains, with the Heat Shock Protein HSP70 through its BAG domain, and with the dynein motor complex. By CASA complex formation, misfolded substrates recognized by HSPB8 are routed to the microtubule organization complex (MTOC) where aggresomes form. Mutations in one of the two IPV domains are causative of myopathies (P209L/P209Q) or neuropathy (P209S), while a mutation in the BAG domain is related to cardiomyopathy (E455K). Here, we assess the solubility, localization and activity of BAG3 mutants in respect to the WT protein. First, we demonstrate that in mild detergents, all P209 mutants are less soluble than both the WT and the E455K mutant and form high molecular weight insoluble species. Second, we observe that, while WT and E455K mutant are diffusely localized in the cytoplasm, P209 BAG3 mutants form aggregates. Interestingly, these aggregates mainly localize perinuclearly in a region that resembles the centrosomes where MTOC is located and remain tightly bound to the nuclei even after exposure to a mild detergent. Third, by using the SOD1 G93A mutant, a misfolded protein causative of Amyotrophic Lateral Sclerosis and substrate of CASA, we demonstrate that P209 mutants exert a detrimental activity in the aggregate clearance in respect to the WT and E455K mutant of BAG3. In conclusion, we demonstrate that mutations of the second IPV domain induce BAG3 aggregation and cause dysfunction in proteostasis maintenance.
Tissue engineering–based bone grafting is an emerging viable form of treatment to repair and regenerate disease or injury-damaged bone tissue [1,2]. The structure and composition of scaffolds should modulate classic osteogenic pathways in human stem cells [3]. In this study, investigations were carried out to verify the osteoinductivity properties of a hydroxylapatite-collagen hybrid scaffold in an in vitro model of adipose mesenchymal stem cells (hASCs). Differentially expressed genes (DEGs) induced by the scaffold was analysed using the Osteogenesis RT2 PCR Array. The osteoinductivity potential of the scaffold was also investigated by studying ALP activity, matrix mineralization, OCN and CLEFB3B expression protein. DEGs and osteogenesis-related genes which play an important role in osteogenesis, including BMP1/2, ALP, BGLAP, SP7, RUNX2, SPP1 and EGFR, were found to be up-regulated. Genes to cartilage condensation SOX9, BMPR1B, and osteoclast TNFSF11 were detected as up-regulated at all time-points during the investigation. The scaffold studied herein has a high osteoinductivity as revealed by matrix mineralization rates and ALP activity, as well as OCN and CLEB3B expression proteins. Fifty patients who had undergone zygomatic augmentation and bimaxillary osteotomy were evaluated clinically, radiologically, and histologically over a three-year follow-up period. Clinical data demonstrate that the biomaterial promotes an excellent bone regrowth. Histological results on biopsy specimens from patients showed prominent ossification. Our data indicate that the clinical evaluation of bone regrowth in patients after scaffold implantation was supported by those DEGs implicated in skeletal development, as shown in experiments with hASCs.

References
ADDITIVE EFFECT OF SUBCLINIC VARIANTS IN RASOPATHIES: STUDY OF NOONAN SYNDROME AS MODEL DISEASE

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The RASopathies are a group of genetic syndromes caused by germline mutations in genes encoding components of the Ras/mitogen-activated protein kinase (MAPK) pathway. These disorders include different syndromes among which Noonan syndrome (NS), characterized by an autosomal-dominant inheritance, variable expressivity and locus heterogeneity, being currently known about ten NS genes. Nevertheless, 20-30% of NS patients remains without molecular diagnosis, suggesting the involvement of further genes or mechanisms in the pathogenesis. We selected eight unrelated NS patients, negative after sequence analysis, from a cohort of 60 patients with NS or NS-like phenotype, to investigate genetic causes associated to the disease. We analyzed 26 RAS/MAPK pathway genes by NGS and identified the new c.355T>C mutation in LZTR1 and two new candidate NS genes, LRP1 and RASAL3. Protein modeling and in silico prediction of protein stability allowed us to predict deleterious nonsynonymous variants. Moreover, three patients co-inherited more than one LRP1 and a LZTR1, or LRP1 and SOS1, or RASAL3 and A2ML1 variants, respectively, from their parents. These variants are hypomorphic, being singularly present in healthy parents. Their co-occurrence in NS patients suggests an additive effect on RAS pathway activation and a digenic inheritance of disease, besides the more common monogenic transmission. The co-presence of these variants is not detectable in 1000genome database. Their additive effect on Ras pathway activation is supported by evidence of Erk/Jnk activation on immortalized lymphocytes from patients and their parents. Functional studies based on silencing mutated genes and Ras pathway activation assay are ongoing on immortalized cell lines from NS patients and their parents. The application of massive sequencing on mutation screening pinpoints the role of hypomorphic variants in digenic inheritance, besides explaining variable expressivity or incomplete penetrance in NS.
Clustered protocadherins (PCDHs) map in tandem at human chromosome 5q31 and comprise three multi-genes clusters: alfa-, beta- and gamma-PCDH. A great variety of activities has been reported for clustered PCDHs. These transmembrane proteins regulate Wnt/β-catenin, PYK2 and FAK tyrosine kinases (involved in cell adhesion), and mTOR pathways. The expression of this cluster consists of a complex mechanism involving DNA hub formation through DNA-CCTC binding factor (CTCF) interaction. Methylation alterations can affect this interaction, leading to transcriptional dysregulation and probably to cell dysfunction. In cancer, clustered PCDHs undergo a mechanism of long-range epigenetic silencing by hypermethylation. In this study, we detected frequent methylation alterations at CpG islands associated with these clustered PCDHs in all the solid tumours analysed (colorectal, gastric and biliary tract cancers, pilocytic astrocytoma), but not hematologic neoplasms such as chronic lymphocytic leukemia, maybe related to the cell adhesion function of PCDHs that is not essential in blood cancer for cell contact and tumour mass formation. Importantly, several altered CpG islands were associated with CTCF binding sites. Interestingly, our analysis revealed hypomethylation events in pilocytic astrocytoma CpG islands, suggesting that in neuronal tissue, where PCDHs are highly expressed, these genes become hypomethylated in this type of cancer. On the other hand, in tissues where PCDHs are lowly expressed, these CpG islands are targeted by DNA methylation. In fact, PCDH-associated CpG islands resulted hypermethylated in gastrointestinal tumours. Our study highlighted a strong alteration of the clustered PCDHs...
methylation pattern in the analysed solid cancers, suggesting these methylation aberrations also as powerful diagnostic biomarkers and the involvement of this cluster in tumorigenesis.

TECHNOLOGICAL QUALITY AND NUTRITIONAL VALUE OF TWO TRITICUM DURUM VARIETIES DEPENDS ON BOTH GENETIC AND ENVIRONMENTAL FACTORS


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Durum wheat (Triticum turgidum L. subsp. durum (Desf.) Husn) is a major food source in the Mediterranean countries since it is utilized for the production of pasta, leavened and unleavened breads, couscous and other traditional foods. The technological and nutritional properties of durum wheat semolina depend mainly on the type of gluten proteins and on their amount, which is a genotype and environment dependent trait. Gluten proteins are also responsible for celiac disease (CD), an autoimmune enteropathy with a prevalence of about 0.7-2% in the human population. At this purpose, two Italian durum wheat cultivars, Saragolla and Cappelli, currently used for monovarietal pasta, were chosen to compare: i) the reserve and embryo proteome, ii) the free and bound phenolics, antioxidant activity, and amino acid composition and iii) the content of immunogenic peptides produced after a simulated gastrointestinal digestion. The results obtained from two years of field cultivation, in average showed a higher amount of gluten proteins, amino acids and immunogenic peptides in Cappelli. Saragolla showed a higher abundance in bound phenolics, antioxidant enzymes and stress response proteins in line with its higher antioxidant activity. However, the impact of the year of cultivation, largely depending on varying rainfall regimes through the wheat growth cycle, was significant for most of the parameters investigated. Differences in technological and nutritional characteristics observed between the two cultivars are discussed in relation to the influence of genetic and environmental factors.
CPG ISLANDS METHYLATION ALTERATIONS AS MOLECULAR SWITCHES IN CANCER

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In normal mammalian cells, CpG islands (CGIs), mostly concentrated at promoter regions, are protected from DNA methylation, while intergenic and repetitive regions are normally hypermethylated. In cancer cells, a massive change in the global methylation pattern occurs. Intergenic and repetitive regions of the genome become hypomethylated leading to the reactivation of transposable elements and genomic instability. In contrast, a focal hypermethylation of CGIs at promoter regions occurs and it is normally associated to gene expression downregulation. Thus, aberrant DNA methylation is one of the most striking features of cancer cells and several studies have demonstrated that cancer-specific methylation patterns exist. For this reason, DNA methylation alteration also represents an extremely useful biomarker for several applications, including cancer risk definition, prediction of clinical outcomes, treatment response and cancer relapse. From a functional point of view, the association between DNA methylation and gene expression, although notoriously recognized, is not yet fully known. We identified early DNA methylation alterations in colorectal cancer, localization-specific changes in low-grade gliomas, alterations that predict the risk of developing chronic lymphocytic leukemias years before diagnosis and correlating with the disease aggressiveness and finally specific methylation patterns of biliary tract and gastric cancer. Unexpectedly, a feature shared by almost all these alterations is that hypermethylation targets CGIs associated with genes poorly expressed in the tissue where cancer occurs. We have undertaken expression studies for genes associated with these alterations, showing that they are even further downregulated, both at the transcript and protein level, consolidating the hypothesis that these early alterations likely guarantee more stable and permanent silencing of genes, important for cell proliferation and tumorigenesis (epigenetic switching).