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Annual Retreat 2019

Abstract book

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PEDIATRICA

Annual Retreat

October 4th - 5th 2019
Villa Condulmer,
Mogliano Veneto - TV

The 2nd Annual Retreat edition of the Istituto di Ricerca Pediatrica Città della Speranza was held on October, 4th – 5th, 2019 at the Villa Condulmer in Mogliano Veneto (Treviso) and hosted about 120 people between Professors, Principal Investigators, Post-docs, PhDs and students.

This event consisted of excellent young researchers that presented their work, through oral and poster presentations, on a variety of scientific topics ranging from pediatric oncology, regenerative medicine, nanomedicine, genetic and rare diseases, immunology and neuroimmunology, and predictive medicine. The meeting was not only an occasion for scientists to directly interact at professional level, build a network for future collaborations and share research experiences, but also a unique opportunity for colleagues and friends to relate personally.

During the two-day event, a keynote lecture on Hematopoietic Stem Cells (HSC) gene therapy in neurodegenerative diseases was given by Prof. Alessandra Biffi, Director of the Pediatric Onco-hematology Clinic in Padua and Coordinator of the Onco-hematology, stem cell transplant and gene therapy research area in IRP.

A special thanks goes to Promega, Miltenyi Biotec and Tecan, who presented their companies' innovation and last-generation technologies.

Moreover, all participants enjoyed coffee breaks and a social dinner that created an even more friendly and relaxing atmosphere.

The 2nd IRP Retreat has been an important platform for leading researchers in IRP to present their latest findings, but also a way for the PIs to keep track of the big picture of the Institute.

I congratulate all the scientists for conducting their research at the highest levels and for the great standard of their oral and poster presentations. The exceptional quality of the event highlights how productive is the cooperation among the different groups in IRP. I believe indeed that creating a good network benefits scientists in accessing tools, expertise and the support they need, thus improving our research programs and developing new strategies.

This is the conclusion of a very intense year in terms of commitments and results. We all have the clear feeling of being in fast and constant growth, and representing, with increasing force, a fundamental reference point, both in Italy and internationally, in the pediatric research field.

Prof. Antonella Viola
Scientific Director Istituto di Ricerca Pediatrica Città della Speranza

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ONCO-HEMATOLOGY, STEM CELL TRANSPLANT AND GENE THERAPY

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GENETICS AND RARE DISEASES

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MEDICAL BIOTECHNOLOGY

Coordinator: Marco Agostini
Elisa Cimetta
Lucia Delogu
Filippo Romanato

IMMUNOLOGY AND NEUROIMMUNOLOGY

Coordinator: Antonella Viola
Marcella Canton
Stefano Sartori

Press review – Highlights

il mattino di Padova

«Città della Speranza, un sogno Tutti dobbiamo sostenerlo»

L'Istituto di Ricerca pediatrica si fa "radiografare" da un comitato di saggi Ilaria Capua: «Straordinaria operazione che deve affermarsi a livello europeo»

Viola: «Tre nuovi microscopi per un laboratorio condiviso»

GIORNATA CONTRO IL CANCRO INFANTILE

Centinaia di palloncini lanciati dai ricercatori alla Città della Speranza

CITTÀ DELLA SPERANZA

Istituto ricerca pediatrica Parbonetti presidente Voto unanime del Cda

Martina Pigazzi di Oncoematologia pediatrica e i pazienti guariti in corsa con la Città della Speranza: «È il nostro messaggio di vita»

Vinta la battaglia con la leucemia corrono per chi ancora combatte

SINERGIE

Malattie rare nei bimbi la Pediatria fa rete con tante associazioni

Città della Speranza

La diagnosi molecolare tra gli strumenti avanzati della ricerca pediatrica

Cinque gradi dalla fine. Su clima e salute dei bimbi l'allarme di Mercalli e IRP

Press review – Highlights

CORRIERE DELLA SERA

«Sconfiggeremo il male invincibile»

IL GAZZETTINO

Città della Speranza

Torre della ricerca, cresce il bilancio: 2,5 milioni investisti per la scienza

LA MANIFESTAZIONE. Venerdì e sabato la prima edizione cittadina che si inserisce in un calendario di eventi dedicati alla scienza in tutta Europa

Idee e provette, la Notte della ricerca

IL GIORNALE DI VICENZA

SCIENZA. La ricercatrice bassanese Silvia Bresolin lavora all'Istituto per la ricerca pediatrica

La "lingua" delle cellule Arma contro le leucemie

CITTÀ DELLA SPERANZA. L'iniziativa voluta da tre famiglie vicentine

Cinque premi di ricerca per scienziati under 35

Press review – Highlights

“Viaggio al centro della Scienza”: a Padova il 24 maggio Federico Taddia e Pleiadi incontrano le scuole primarie

MeteoWeb

“MY FIRST IRP GRANT”, L’ESPERIENZA DI BIANCA CALÌ ALL’INSTITUT CURIE DI PARIGI

SETTIMANA MONDIALE DI SENSIBILIZZAZIONE SULLE MALATTIE MITOCONDRIALI

Fondazione ISTITUTO DI RICERCA PEDIATRICA

PADOVAOGGI

2 milioni di euro per avviare 13 innovativi progetti di ricerca: il "dono" della Città della Speranza

Fondazione Cassa di Risparmio di Padova e Rovigo

Faccia a faccia con la ricerca pediatrica

PADOVANDOMAGAZINE

IRP dona 25 alberi alla Città di Padova nell’anniversario di Città della Speranza

La scienza nascosta nei luoghi di Padova: la Torre della ricerca

POSTSCRIPTUM

ILBO LIVE UNIVERSITÀ DI PADOVA

Un **modello 3D** *per* **combattere** *i* **tumori**

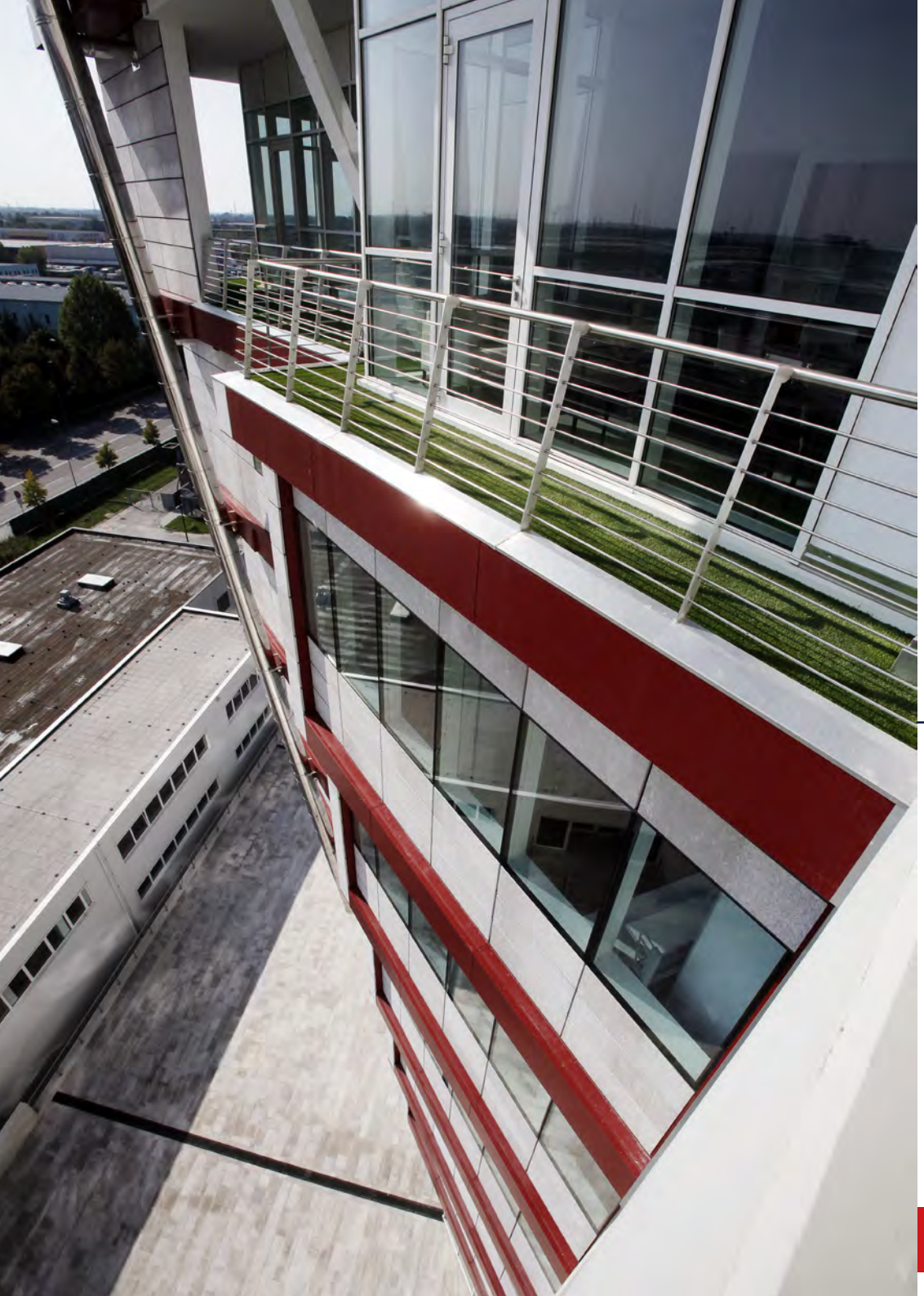
Press review – Highlights

G. Viola a 7 Gold
www.youtube.com/watch?v=PljEcAB5uQk

L. Salviati per IRP
www.youtube.com/watch?v=cNzzFS86RLs

M. Piccoli per Cariparo
www.youtube.com/watch?v=yqJNWl1OKU8

Assemblea IRP su Rete Veneta
www.youtube.com/watch?v=lwclT9JMHOM



Abstracts

Onco-Hematology, Stem Cell transplant and Gene Therapy

LIN28B INCREASES NEURAL CREST CELL MIGRATION AND LEADS THE TRANSFORMATION OF TRUNK SYMPATHOADRENAL PRECURSORS

Corallo D, Donadon M, Pantile M, Sidarovich V, Cocchi S, Ori M, De Sarlo M, Candiani S, Frasson C, Distel M, Quattrone A, Zanon C, Basso G, Tonini GP and Aveic S

The RNA-binding protein LIN28B regulates developmental timing and stem cell identity by suppressing the Let-7 family of microRNAs. Postembryonic reactivation of LIN28B is a hallmark of poor prognosis and metastasis in different types of cancer, including neuroblastoma, a pediatric solid tumor deriving from neural crest cells (NCC). However, the extent in which LIN28B activation participates in modulation of NCC behavior in neuroblastoma remains unclear. In this study, we provide evidence that the overexpression of LIN28B inhibits sympathoadrenergic cell differentiation and increases the velocity of NCC migration in the two vertebrate models, Xenopus leavis and Danio rerio. Moreover, we show that LIN28B sustains the invasive motility of neuroblastoma cells in vitro and in zebrafish xenotransplanted embryos, where tumor cells show increased spread throughout the body. Our data support a growing body of evidence that LIN28B may re-establish neuroblastoma heterogeneity through epithelial-to-mesenchymal transition (EMT), leading to tumor progression and metastatic tumor cell invasion. The EMT/PI3K/AKT regulatory axis is triggered by the up-regulation of the two integrins, ITGA5 and ITGA6, upon LIN28B overexpression, sustaining the metastatic phenotypes of this disease. Taken together, our results establish a relevance of LIN28B in the regulation of NCC migration, and suggest a mechanism by which long-term expression of LIN28B supports aggressive neuroblastoma phenotype.

TUMOR-ASSOCIATED AUTOANTIBODIES IN RHABDOMYOSARCOMA PATIENTS: DETECTION, CHARACTERIZATION AND SIGNIFICANCE

Poli E, Barbon V, Cattelan M, Zin A, Bisogno G, Bonvini P

Oncogenesis is a complex process characterized by the accumulation of mutations and changes in gene expression which provide a selective advantage to cancer cells by boosting their genetic divergence and evolutionary fitness. This diversity comes at a cost and the cost is to be recognized as foreign by the immune system. Among the consequences of the immunological recognition of cancer is the production of autoantibodies raised against tumor-associated antigens, also known as tumor-associated autoantibodies (TAABs). TAABs, which can be easily obtained from a simple and non-invasive blood withdrawal, have a long half-life in the bloodstream, are generated even in presence of small amount of antigens and can be detected prior to clinical disease manifestation. In this study, proteomic profiling of antibody signatures using high-content protein arrays was assessed in children with PAX3-FOXO1A-positive alveolar rhabdomyosarcoma (P3F+ ARMS) tumors and age-matched healthy donors. By performing such an immune response biomarker profiling we were able to demonstrate the presence of antigens exhibiting stronger immunoreactivity in children with P3F+ ARMS compared to healthy donors, as well as circulating autoantibodies capable of distinguishing metastatic patients from those with localized disease at diagnosis. Thus, we characterized the humoral immune response against such an aggressive malignancy and provided evidence that TAABs are useful for diagnostic and prognostic purposes, likewise they can be effective at revealing expression of tumor antigens involved in cancer growth, survival and expansion.

LINKING CIRCRNA TO LEUKEMOGENESIS: CIRCRNA ROLES IN HEMATOPOIESIS AND ONCOGENIC FUNCTIONS IN MLL TRANSLOCATED ALL

Tretti C, Dal Molin A, Gaffo E, Boldrin E, Meyer LH, Biffi A, te Kronnie G, Bresolin S, Bortoluzzi S

CircRNAs are covalently closed RNA molecules, regulating cellular processes. MLL-AF4 translocation is associated with high-risk infant pro-B-cell acute lymphoblastic leukemia. The oncogenic mechanisms downstream MLL-AF4 translocation have been only partially unveiled, and the effect of the fusion protein on circular RNA (circRNA) expression and their possible oncogenic roles are unknown. Our study investigates circRNAome in MLL-AF4 patients in order to clarify their involvement in leukemogenesis. We generated RNA sequencing data of MLL-AF4 positive infant patients. CircRNAs were detected using the CirComPara pipeline. We compared circRNA expression in MLL-AF4 samples and in a previously collected series of samples representing normal hematopoiesis. Overall, in leukemia patients and in different blood cell subpopulations, 74,004 circRNAs were identified. Of 38 circRNAs expressed only in MLL-AF4 patients, circPROM1 and circFLT3 resulted specific of MLL-AF4 leukemia screened in leukemia cell lines. Furthermore, 1,116 and 205 circRNAs were deregulated comparing MLL-AF4 versus B-cells and versus CD34+ cells, respectively, with 85 circRNAs resulting specific of malignant cells. Several circular isoforms derived from FLT3 and generated by alternative backsplicing were amongst the circRNAs most deregulated in MLL-AF4. In parallel, our investigation of fusion-circRNAs (isoforms containing exons of MLL and of AF4 genes) by in silico analysis of RNA-seq data and PCR-based custom screening succeeded in identifying a fusion circRNA. In summary, we validated circRNAome specificities in MLL-AF4 leukemia and prioritized circRNAs for further investigations, by computational predictions of circRNA interactions and by functional in vitro and in vivo studies, aiming to unveil the contribution of deregulated circRNAs in MLL-AF4 pathogenesis

COMPREHENSIVE CHARACTERIZATION OF PLASMATIC EXOSOMES IN ANAPLASTIC LARGE CELL LYMPHOMA OF CHILDHOOD

Lovisa F, Gaffo E, Di Battista P, Garbin A, Damanti C, Gallingani I, Carraro E, Pillon M, Biffi A, Bortoluzzi S, Mussolin L

Anaplastic Large-Cell Lymphoma (ALCL) is a T-cell non-Hodgkin lymphoma mainly affecting children. Despite current treatment protocols lead to cure >70% of patients, disease relapse is associated to severe prognosis. Based on recent findings in solid tumors, plasmatic exosomes represent a promising source of biomarkers and are directly involved in disease mechanisms. The aim of this research is to identify ALCL exosome-associated biomarkers and to decipher the role of exosomes in ALCL pathogenesis. The small RNA (sRNA) profiles of plasmatic exosomes were characterized by RNA-seq and selected differentially expressed sRNAs validated by qRT-PCR. Exosomal proteomic profiles were investigated by Mass Spectrometry. Specific sRNA profiles were identified in exosomes from ALCL compared to healthy donors (HD) and in exosomes from ALCL with different prognosis. miR-122-5p, previously shown to promote metastasis by suppressing glucose uptake by niche cells, was found up-regulated in exosomes of ALCL compared to HD and in patients with metastatic disease. Overexpression of miR-122-5p or treatment with miR-122-5p-loaded exosomes resulted in PKM downregulation in mouse fibroblasts. In addition, proteomic analyses discovered 50 exosomal proteins up-regulated in ALCL compared to HD. Pathway enrichment analysis identified 8 significantly enriched pathways, mostly involved in extracellular matrix remodeling and cell adhesion. In the next future, the role of exosomal miR-122-5p in promoting disease dissemination by reducing glucose utilization in niche cells will be investigated in vivo. Moreover, the association of candidate sRNA with microenvironment modulation (miR-146a-3p) and immunosuppression (RNY4) will be evaluated in vitro, while candidate exosomal proteins will be validated by Western blotting

HDAC INHIBITORS COUNTERACTS GLIOBLASTOMA STEM-LIKE CELL BY ENAHNCING WNT SIGNALING ACTIVATION

Rampazzo E, Frasson C, Della Puppa A, Biffi A, Persano L

Glioblastoma (GBM) is the most malignant brain tumor and considered one of the deadliest tumors occurring in humans. In a recent study, we reconstructed how a signaling crosstalk between HIF-1αand Wnt pathway components controls phenotype plasticity in GBM cells by enhancing their pro-neuronal differentiation capability. Interestingly, we demonstrated that high molecular weight (hMW) isoforms of the αcatenin co-factor TCF4 are responsible for counteracting this differentiation potential. All these data, prompted us to investigate if the pharmacological modulation of hMW TCF4 with a compound-based interference of its functions could be a successful strategy in order to induce GBM cell differentiation. In this context, the Histone Deacetylase Inhibitors (HDI) of the hydroxamate class Trichostatin-A (TSA) and suberoylanilide hydroxamic acid (SAHA) have been demonstrated to promote a proteasome-dependent decrease of TCF4 levels in colon cancer cells, thus significantly affecting their proliferation and viability. Here, we show that the administration of TSA and SAHA in GBM cells strongly decreases TCF4 protein levels and significantly reduce their stem-like cell properties. Moreover, in these conditions, we measured a dramatic impairement of GBM cell proliferation accompained by a significant sensitization effect toward temozolomide chemotherapy. Notably, in agreement with our previous data, reporter experiments clearly indicate that an HDI-dependent increase in Wnt singaling activation may be a fundamental molecular mechanism underlying these effects. These results, strongly suggest that a fine modeling of the molecular players controlling GBM cell differentiation

could provide novel effective phamacological targets for GBM treatment, with the final aim of improving GBM patient survival

AML BLASTS SUPPORT A LEUKEMIA-PERMISSIVE MICROENVIRONMENT REVEALING THE STROMAL CONTRIBUTION ELIGIBLE FOR INNOVATIVE 3D TARGETING

Borella G, Da Ros A, Porcù E, Tregnago C, Benetton M, Bisio V, Campodoni E, Panseri S, Sandri M, Montesi M, Cairo S, Locatelli F, Biffi A, Pigazzi M

Pediatric acute myeloid leukemia (AML) is a disease in which refinements in diagnostic approaches for patient stratification have resulted into remarkable progresses during the past decade, but chemotherapy still remains the pillar of treatment. Novel anti-leukemia agents failed during clinical validation phases, due to the inappropriateness of current preclinical models used to study drug efficacy. For this purpose, we developed a new protocol for long-term 3D-AML cultures to perform more predictable drug screenings in vitro. We used mesenchymal stromal cells derived from AML patients (AML-MSCs) documenting their support to AML cell growth. We observed that AML-MSCs did not exert anti-inflammatory activity and expressed a peculiar secretome profile. Thus, a drug targeting of AML-MSCs would be desirable, and we performed a screening of 480 compounds. This screening identified 17/480 active compounds capable of reducing AML-MSCs proliferation without toxicity over h-MSCs and AML blasts. We identified one main compound able to selectively reduce AML-MSCs proliferation, that, when combined to novel therapeutic agents for AML blasts showed a synergistic effect in 3D (CI=0.5, $p<0.05$). We implanted 3D scaffolds in the back of NSG mice and monitored leukemia engraftment in the scaffolds and documented this as a novel useful in vivo system to screen selected drugs in loco. In conclusion, our data support the possibility to work with long-term 3D cultures of AML in vitro to identify new drugs, and we attribute to AML-MSCs a crucial supportive role to be further considered in in vivo settings for novel combo strategies

IN VITRO MODELING OF MEDULLOBLASTOMA DRUG RESISTANCE: IDENTIFICATION OF NEW THERAPEUTIC TARGETS BY A MULTI OMICS-BASED APPROACH

Mariotto E, Bortolozzi R, Rruga F, Rampazzo E, Biffi A, Persano L, Viola G

Medulloblastoma (MB) is the deadliest brain tumor of childhood, intrinsically characterized by fast growth, high invasiveness and resistance to treatments. In the recent years, MB tumors have been extensively characterized for their genetic landscape and transcriptional phenotype, thus allowing a reproducible classification of these tumors into four molecular subgroups. However, this new information provided only limited improvements to the therapeutic success and the clinical management of MB patients. Many studies ascribe the appearance of resistant cell clones arising after treatment cycles as the leading cause of relapse. In this context, we aimed to model in vitro the selection of resistant MB cells surviving after chemotherapy and to characterize the molecular events associated to the acquisition of such a resistant phenotype by an integrated -omics approach. Here we show that a defined treatment schedule comprising four commonly used chemotherapeutics for MB therapy, such as vincristine, etoposide, cisplatin and cyclophosphamide, induces resistance to further treatments in MB cell lines and primary culture through a mechanism of clone selection/resistant phenotype acquisition which is still under investigation. Moreover, a preliminary screen of the protein kinases potentially involved in this phenomenon significantly points at VEGFR and SRC-dependent cascades as likely early events underlying resistance. Finally, MB drug resistant cell models together with the full integration of functional and -omics data will provide the molecular basis for the identification of targeted inhibitors of the cellular mechanisms underlying MB cell resistance, allowing the reversal of their aggressiveness.

PHOSPHOPROTEOMIC ANALYSIS OF PEDIATRIC T-LBL

Veltri G, Serafin V, Lovisa F, Gallingani I, Cortese G, Bresolin S, Biffi A, Mussolin L, Accordi B

Lymphoblastic lymphoma (LBL) is the second most common type of childhood Non Hodgkin Lymphoma (NHL), with the vast majority being of T cell origin (T-LBL). With current treatment regimens, more than 80% of patients achieves a stable remission. However, the dismal prognosis of relapsed patients remains a big challenge to be addressed. The identification of molecular markers to early recognize patients at high risk of relapse is mandatory to improve patients' stratification and propose new targeted therapies.

Our research tried to address this question by phosphoproteomic analysis of 23 T-LBL primary tumor samples from relapsed (n=7) and non-relapsed (n=16) patients. We compared the expression/activation of 59 proteins by Reverse Phase Protein Arrays and detected the upregulation of c-MYC (q-value<0.1) and JAK2 Y1008 (q-value<0.1) in relapsed patients. ROC curve analysis revealed that a c-MYC value of 109378 AU is able to predict relapse with 71.43% sensitivity and 93.75% specificity (AUC=0.89, p=0.003). Next, since other proteins belonging to the JAK/STAT pathway were more activated in relapsed patients, we performed a Global Test analysis and confirmed JAK/STAT signaling upregulation in relapsed T-LBLs (p=0.031). In vitro treatment of 3 cell lines with the JAK inhibitor Ruxolitinib was able to induce cell death, synergize with Dexamethasone and revert drug resistance. In this study, we identified c-MYC as a promising biomarker of disease aggressiveness, which will be validated in an independent cohort of T-LBL patients by immunohistochemistry. Moreover, we will test Ruxolitinib efficacy in primary T-LBL cultures, to assess its possible application as a new therapeutic agent

LCK/NFAT AXIS AS KEY REGULATOR OF GLUCOCORTICOID RESISTANCE IN PEDIATRIC T-ACUTE LYMPHOBLASTIC LEUKEMIA

Veltri G, Bresolin S, Ntziachristos P, Biffi A, Accordi B, Serafin V

Glucocorticoids (GC) are widely used in T-Acute Lymphoblastic Leukemia (T-ALL) therapy, but, unfortunately, around 40% of patients display GC-resistance, resulting in a worse outcome compared to other T-ALL patients. Mechanisms that trigger GC-resistance are not yet fully elucidated, thus we first focused on the identification of deregulated signaling pathways in GC-resistant patients. We previously demonstrated that in these patients LCK is hyperactivated and its inhibition reverses GC-resistance. Furthermore, we revealed that LCK controls the activation of the Calcineurin/NFAT axis, and we confirmed the pivotal role of this pathway in regulating GC-resistance treating resistant cells with dexamethasone+CiclosporinA, a Calcineurin/NFAT inhibitor. The aim of this study is thus to characterize LCK/NFAT-regulated molecular mechanisms responsible of GC-resistance in T-ALL, in order to unravel key molecular players that could also represent biomarkers of GC-resistance already at diagnosis useful to improve patients stratification and to develop new therapeutic approaches. We thus plan to perform ChIP-Seq analysis to identify NFAT family members-deregulated targets in GC-resistant cells. As a first step, to select which NFAT isoforms are principally involved in inducing GC-resistance, we are now combining single NFAT silencing with dexamethasone treatment. Simultaneously, we are setting up NFAT ChIP-Seq and we will compare NFAT-regulated binding sites between GC-resistant and sensitive T-ALL cell lines, treated or not with GC. Results will be next validated by Gene Expression Profiling. Most promising differently NFAT-regulated genes will be investigated by RQ-PCR in a cohort of T-ALL patients, and this will be followed by validation experiments and functional studies.

INHIBITION OF AUTOPHAGY ENHANCES THE CYTOTOXIC EFFECT OF THE TYROSINE KINASE INHIBITOR PONATINIB

Corallo D, Pantile M, Caicci F, Sidarovich V, Mariotto E, Longo L, Quattrone A, Tonini GP, Viscard E, Aveic S

Ewing sarcoma (EWS) is a highly pediatric malignant tumor arising from both bone and soft tissues. The survival of EWS patients has improved over the years, but the prognosis for those who relapse or develop metastases during therapy or at the follow-up is still poor. Therefore, the identification of new diagnostic and/or prognostic biomarkers is needed to improve risk stratification and predict event occurrence in these children. For this purpose, we employed a high-throughput protein microarray technology comprising of >9,000 human recombinant proteins spotted under native conditions onto glass slides, in order to detect autoantibodies raised against tumor antigens, starting from a simple blood drawn obtained at diagnosis. Nineteen plasma samples from children with EWS, who experienced (n=10) or not (n=9) an event during therapy or at follow-up, were selected and compared to plasma samples from 12 age-matched healthy children. From this analysis, 149 tumor-associated antigens (TAA) were found, 120 of which to be more immunoreactive in EWS patients than in matched controls. Circulating autoantibodies capable of distinguishing patients at risk of disease progression or relapse (TAA=82) from those who did not report any event during therapy or at follow-up (TAA=477) were also characterized, providing evidence that immune response in EWS patients with progressed disease is strikingly different from the others. Antigens exhibiting stronger immunoreactivity were selected for further validation by indirect ELISA assay and used to generate panels suitable to improve disease detection and patients' risk stratification, as to provide more insights into tumor growth and evolution

IDENTIFICATION AND CHARCATERIZATION OF CIRCULATING TUMOR CELLS AND CELL-FREE DNA IN PEDIATRIC RHABDOMYOSARCOMA

Tombolan L, Zin A, Rossi E, Binatti A, Bortoluzzi S, Zamarchi R, Bonvini P and Bisogno G

Rhabdomyosarcoma (RMS) is an aggressive solid tumor that in children disseminates frequently hematogenously, giving rise to metastases, the most adverse known prognostic factor. Chances of an effective cure rely on the capacity to make an early and accurate diagnosis, detect metastatic disease or relapses, and predict response to treatment. Liquid biopsy analysis, in particular detection and characterization of circulating tumor cells (CTCs) and cell-free DNA (cfDNA), is a very promising blood-based approach for cancer detection and noninvasive disease monitoring. Herein, we evaluated EpCAM positive (CellSearch CS) and EpCAM low/negative (Autoprep-Sample-Collection-Device, ASCD) circulating cells in peripheral blood and bone marrow of 19 pediatric RMS patients, implementing the standard assay with a specific RMS marker (desmin). Notably, we detected CTCs in the blood of RMS patients and identified a significant proportion of cells positive to desmin. Total cfDNA was also isolated and quantified in plasma samples of RMS patients, providing evidence that the latter have higher levels of cfDNA compared to healthy subjects. Finally, we performed whole exome sequencing (WES) in selected primary tumors and matched control blood cells. Analysis of sequencing data identified variants already associated to disease growth and survival, as well as novel genomic alterations. A network of altered genes was built, revealing that MAPK signaling pathway is strongly compromised in RMS patients. Importantly, mutations identified in primary tumor cells were confirmed by ddPCR in matched cfDNA and CTC DNA, providing new insights into disease dissemination and highlighting the potential of using liquid biopsies for RMS detection and monitoring.

AUTOANTIBODY PROFILING IN EWING SARCOMA PATIENTS

Lucchetta S, Cattelan M, Zin A, Bisogno G, Poli E and Bonvini P

Ewing sarcoma (EWS) is a highly pediatric malignant tumor arising from both bone and soft tissues. The survival of EWS patients has improved over the years, but the prognosis for those who relapse or develop metastases during therapy or at the follow-up is still poor. Therefore, the identification of new diagnostic and/or prognostic biomarkers is needed to improve risk stratification and predict event occurrence in these children. For this purpose, we employed a high-throughput protein microarray technology comprising of >9,000 human recombinant proteins spotted under native conditions onto glass slides, in order to detect autoantibodies raised against tumor antigens, starting from a simple blood drawn obtained at diagnosis. Nineteen plasma samples from children with EWS, who experienced (n=10) or not (n=9) an event during therapy or at follow-up, were selected and compared to plasma samples from 12 age-matched healthy children. From this analysis, 149 tumor-associated antigens (TAA) were found, 120 of which to be more immunoreactive in EWS patients than in matched controls. Circulating autoantibodies capable of distinguishing patients at risk of disease progression or relapse (TAA=82) from those who did not report any event during therapy or at follow-up (TAA=477) were also characterized, providing evidence that immune response in EWS patients with progressed disease is strikingly different from the others. Antigens exhibiting stronger immunoreactivity were selected for further validation by indirect ELISA assay and used to generate panels suitable to improve disease detection and patients' risk stratification, as to provide more insights into tumor growth and evolution

HYPOXIA ENHANCES JUVENILE MYELOMONOCYTIC LEUKEMIA PROGENITOR CELLS PROLIFERATION AND SURVIVAL IN VITRO

Cani A, Tretti C, Rampazzo E, Frasson C, Barbieri V, Rosato A, Persano L, Basso G, Biffi A, Te Kronnie G and Bresolin S

Juvenile myelomonocytic leukemia (JMML) is a rare clonal myelodysplastic and myeloproliferative disorder of infancy and early childhood characterized by a rapid accumulation of mature cells and hyperactivation of RAS signaling pathway. The only therapeutic treatment available is represented by hematopoietic stem cells transplantation (HSCT) but JMML patients undergo an aggressive clinical course in 50% of cases. Relapse is attributed to a minor subpopulation of cells, referred to leukemic stem cells (LSCs) that resides in the bone marrow (BM) niche, where low oxygen tension levels have been recognized as being a key element that critically selects and maintains hematopoietic progenitors with stem cell potential.

Here, it is shown an in vitro 3D system, able to mimic the hypoxic extracellular microenvironment. Our model promotes the growth of primary JMML cells under hypoxia condition (<2% O2) and supports the propagation of JMML myeloid progenitor, which represents about 2% of total bone marrow cells at diagnosis. JMML cells grown in our model are able to recapitulate patient features at onset, such as patient mutation, GM-CSF hypersensitivity and, if present, monosomy of chromosome 7. Furthermore, cells sprouted out from the 3D system maintain a long-term proliferative potential for almost 3 months and are characterized by an immature immunophenotype. This model will help the study of resistant cells that reside in the bone marrow hypoxic niche and the progression and clonal evolution of JMML disease

UNCOVERING NEW MOLECULAR MECHANISMS OF THERAPY RESISTANCE IN PEDIATRIC B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA RELAPSES

Coppe A, Serafin V, Cani A, Veltri G, Binatti A, Anselmi F, Oliviero S, te Kronnie G, Bortoluzzi S, Biffi A, Basso G, Accordi B and Bresolin S

In the last decades the improvements in multi-agent chemotherapy strategy and the development of new drugs for the treatment of childhood acute lymphoblastic leukemia (ALL) resulted in a survival of more than 80%. However, a considerable number of patients still do not benefit from the current therapies presenting drug resistance and relapse; innovative treatment approaches are therefore mandatory for them. We performed whole exome sequencing analysis of paired samples at diagnosis and at relapse of high resistant B-ALL pediatric patients, identifying several relapse-associated mutated genes, mostly (84%) patient-specific. Recurrence in more than two patients was observed of 70 relapse-associated genes, including CREBBP. Further, clones dynamic analysis showed two main different models of clonal evolution from diagnosis to relapse. In 20% of the patients, we also observed relapse-specific aberrations in the NR3C1 gene encoding the glucocorticoids receptor (GR). The aberrations lead to a complete absence of GR protein. Proteomic analysis of an enlarged cohort of patients revealed a down expression or a complete absence of GR protein in most of the samples at relapse, although the mRNA expression remains at high levels. RNA-seq did not indicate the presence of alternative splicing or mutations in the UTR regions of the NR3C1 gene. In conclusion, genetic profiling has shown to be a powerful approach to unravel aberrations responsible of drug resistance at relapse and thus to develop a personalized target therapy in ALL relapsed therapy resistant patients.

EVALUATION OF TOTAL AND TUMOUR-DERIVED CFDNA IN PEDIATRIC NON-HODGKIN LYMPHOMAS

Damanti C, Lovisa F, Garbin A, Gallingani I, Carraro E, Pillon M, Biffi A, Mussolin L

Levels of plasma cell-free DNA (cfDNA) can be found in small amount in healthy individuals, whereas increased levels have been reported in patients with different malignancies, originated from both tumour and non-tumour cells. In addition, cfDNA integrity index can be correlated to the apoptotic or necrotic origin of circulating DNA. In this work, cfDNA was analysed in plasma samples of pediatric patients affected by Burkitt Lymphoma (BL, n=60), Lymphoblastic Lymphoma (n=12) and Anaplastic Large Cell Lymphoma (ALCL, n=33) and compared with healthy donors (HD). Quantitative real-time PCR was used to amplify actin-αshort and long fragments and to calculate total cfDNA concentration and integrity index. The amount of tumour-derived cfDNA in BL samples was evaluated by droplet digital PCR using patient-specific clonotypic markers already employed for Minimal Disseminated Disease (MDD) analysis. Total cfDNA amount was significantly higher in lymphoma patients compared to HD, while the integrity index was significantly lower in BL compared to HD and the other lymphoma subtypes. This finding suggests a prevalent apoptotic origin of cfDNA in this specific tumour characterized by rapid proliferation of mature B-cells. Interestingly, total amount of cfDNA did not correlate with disease stage. Indeed, cfDNA concentration was generally low in high-stage MDD-positive patients, who were also characterized by higher levels of tumour-derived cfDNA. In contrast, patients with high amounts of total cfDNA showed mainly non-tumour cfDNA. In the next future, we will evaluate the association between cfDNA and tumor hypoxia, to clarify if cfDNA can be used to estimate hypoxia-promoted disease aggressiveness.

MIR-939 ACTS AS A TUMOR SUPPRESSOR BY MODULATING JUNB TRANSCRIPTIONAL ACTIVITY IN PEDIATRIC ANAPLASTIC LARGE CELL LYMPHOMA

Garbin A, Lovisa F, Holmes AB, Damanti C, Gallingani I, Carraro E, Accordi B, Pizzi M, d'Amore ESG, Pillon M, Biffi A, Basso K, Mussolin L

Anaplastic large cell lymphoma (ALCL) is characterized by CD30 overexpression and in most of the cases by t(2;5)(p23;q35) translocation and NPM-ALK chimeric protein expression. We previously identified two distinct subgroups of ALCL patients based on different gene expression signatures related to NPM-ALK transcript levels (ALK-low and -high). Interestingly, most of the relapsed patients were ALK–high cases and their median time of relapse was 5 months, compared with 30 months of the ALK–low cases. To further characterize these two subgroups, we performed an array-based miRNA profiling and we identified 19 miRNAs upregulated in ALK-low patients. Since high ALK expression was associated to a more aggressive ALCL phenotype, miRNAs up-regulated in the ALK–low cases could probably act as tumor suppressors. Microarray data were validated in 53 ALCL cases, including 42 ALK–high and 11 ALK–low. Focusing on miR-939, the most differentially expressed miRNA, we demonstrated that its overexpression was able to impair migration and colony formation ability in cell lines, suggesting its role as an oncosuppressor. Moreover, we found that the transcription factor JunB is a target of miR-939. PDGFRB, a known JunB transcriptional target, was found downregulated in cell lines transfected with miR-939 mimic and in patients with higher levels of miR-939. Since the combination of ALK and PDGFRB inhibitors has been recently suggested as a valuable strategy to reduce relapse rates in ALCL, our data could contribute to define the best treatment option in ALCL patients based on NPM-ALK and miR-939 expression levels.

NEXT-GENERATION SEQUENCING OF PTEN MUTATIONS FOR MONITORING MINIMAL RESIDUAL DISEASE IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Germano G, Valsecchi MG, Buldini B, Cazzaniga G, Zanon C, Silvestri D, te Kronnie G, Biffi A, Basso G, Paganin M

Around 15% of newly diagnosed pediatric cases of acute lymphoblastic leukemia (ALL) have a T-cell immunophenotype. Currently, minimal residual disease (MRD) quantification has become crucial in clinical management of patients with T-ALL for treatment decisions as powerful independent prognostic factor for relapse at the end of induction (day 33; TP1) and after induction-consolidation (day 78; TP2). Next-generation sequencing (NGS) technologies have been tested for MRD detection in hematologic malignancies but as yet no workflow standardization and data interpretation are available. Thus, new studies are necessary to compare NGS and standard methods and also to search and test other MRD markers suitable for NGS-based MRD detection. In T-ALL patient samples, PTEN has been found mutated with a frequency of around 10-15%. In the AIEOP cohort of T-ALL patients, the presence of PTEN aberration is associated with an increased risk of relapse. In this context, we evaluated the applicability of NGS of PTEN mutations to detect MRD. We analyzed 30 patients previously identified to carry an insertion/deletion in PTEN and with DNA samples available at TP1 and/or at TP2. Four of the PTEN mutations resulted not suitable for NGS analysis. In total 40 PTEN mutations from 27 patients, were available for NGS-PTEN MRD analysis. Both TP1 and TP2 samples where available from 20 patients, whereas of 4 patients only TP1 and of 3 patients only TP2 samples were available. By comparing the NGS-PTEN results with current quantitative PCR of antigen receptor gene rearrangements (qPCR-Ig/TR), an overall concordance of 80% was found between the two methods. However, the NGS-PTEN qualified a lower number of high-risk patients than qPCR-Ig/TR according to the AIEOP-BFM ALL protocol in which patients with MRD levels ≥5×10-4 at TP2 were considered high-risk (MRD-HR) cases. Ongoing studies in the field of NGS MRD are mandatory to improve the work-flow strategy, establish conditions for optimal detection sensitivity, definition of quantifiability and, most importantly, explore the significance of disease quantification by mutation analysis in terms of therapy response in larger patient cohorts. However this study, suggest that NGS-PTEN is a promising tool that could potentially be used to support current MRD analysis for T-ALL patients.

THE LONG NONCODING RNA BALR2 CONTROLS NOVEL TRANSCRIPTIONAL CIRCUITS CONFERRING CHEMOTHERAPY RESISTANCE IN PEDIATRIC ACUTE MYELOID LEUKEMIA

Benetton M, Bisio V, Porcù E, Bordi M, Zanon C, Borella G, Da Ros A, Germano G, Manni S, Tregnago C, Campello S, Rao DS, Locatelli F, Biffi A, Pigazzi M

In pediatric acute myeloid leukemia (AML), the achievement of complete remission is the most important endpoint during treatment, and many efforts have been spent to develop sensitive and specific assays to monitor immunophenotypic and genetic markers in minimal residual disease (MRD). However, previous studies evidenced that MRD negativity cannot be considered completely reliable in predicting clinical outcome since it does not exclude a relapse. Thus, we hypothesized that disease recurrence may be due to other markers. We identified the long noncoding RNA BALR2 being highly expressed in AML patients at diagnosis (n=132) and associated with higher incidence of therapy resistance. We explored BALR2 functional role demonstrating that it bidirectionally controlled its own and its neighbor gene CDK6 promoter activity. BALR2 and CDK6 overexpression was shown to modify RUNX1 transcriptional activity, causing the block of myeloid hematopoietic differentiation process that was restored by BALR2 silencing. Moreover, by gene expression analysis of 58 AML patients classified on the basis of BALR2 levels, we identified that among the differentially expressed genes (Fold Change|2|, $p<0.01$), most were involved in mitochondrial synthesis pathways according to Gene Ontology analysis. Thus, we documented BALR2 levels directly influencing AML cells mitochondrial mass in vitro. Then, using a mitochondrial inhibitor, we confirmed this strategy able to stimulate differentiation and induce AML cell death, both being significantly enhanced by BALR2 silencing. This study supports evidences of BALR2 direct control of transcriptional networks impairing AML cells chemo-sensitivity, and opens for further mitochondria-oriented therapeutic approaches to improve patients' response to treatment.

ESTABLISHMENT OF PATIENT-DERIVED XENOGRAFTS (PDXS) FOR THE IDENTIFICATION OF NEW THERAPEUTIC STRATEGIES IN PEDIATRIC CANCER

Da Ros A, Porcù E, Borella G, Benetton M, Tregnago C, Bisio V, Michielotto B, Campodoni E, Panseri S, Sandri M, Montesi M, Buldini B, Locatelli F, Biffi A, Cairo S, Pigazzi M

Pediatric cancer therapy achieved numerous improvements in the last decades, but a high number of patients still experience relapse, this latter limiting patients' chance of survival mainly due to the absence of reliable second line treatments. The urgency to identify new drugs represents the main unmet clinical need, and the scientific world identifies the causes in the rare incidence of aggressive tumors in childhood, and in the lack of predictive in vivo models. We aim to develop Patient-Derived Xenografts (PDXs) from two detrimental pediatric tumors: acute myeloid leukemia (AML) and hepatoblastoma (HB), To date, we have implanted 47 AML samples at diagnosis and 7 primary HBs collected at surgery in the framework of the European project ChilTERN. As for AML there are no previous robust indications for generating PDXs, we used several inoculation strategies: intrafemoral-intravenous-intrahepatic injection of 1x106 blasts, or subcutaneous implantation of humanized 3D scaffold pre-seeded with AML blasts. Instead, HB fragments were implanted in the interscapular fat pad of immunodeficient mice. Our HB models are currently under evaluation, whereas 6 AML-PDXs have been obtained so far. We documented primary AML immunophenotype and genetic markers being maintained in AML-PDXs. Moreover, whole exome and transcriptome sequencing revealed similarity between patient-AML and tumor at third passage in mouse, suggesting that at this step PDXs can be considered as "patient's avatars" and expanded for phase II-like clinical trials. This study will improve cancer driver mutations catalogue and facilitate the identification of new argeted therapies with the aim to ameliorate patient outcome.

NRF2 SIGNALING SUSTAIN CHEMOTHERAPY RESISTANCE IN MEDULLOBLASTOMA

Bortolozzi R, Rruga F, Mariotto E, Persano L, Rampazzo E, Biffi A, Viola G

Nrf2, a key orchestrator of cell defense mechanisms against oxidative stress and xenobiotic insults, has been linked to chemotherapy resistance and recurrence in many human cancers. Through the establishment of an in vitro model of chemoresistance, we investigated the role of Nrf2 in sustaining resistance to chemotherapy in medulloblastoma (MB), a highly aggressive pediatric brain tumor characterized by fast growth, high invasiveness and resistance to treatments. MB DAOY cells were weekly exposed to a cocktail of drugs commonly used in MB treatment, vincristine, etoposide, cisplatin and cyclophosphamide (VECC) inducing the emergence of a resistant phenotype after 5 cycles of VECC treatment. Starting from the second round of VECC exposure we found increased both Nrf2 expression and transcriptional activity. Interestingly, the activity of Nrf2 spiked up when resistance to chemotherapy emerged. Furthermore, resistant cells were more able to scavenge oxidative insults in comparison to their naïve counterpart and at the same time showed an overactivation of enzymes belonging to glycolytic and PPP pathways that are direct target of Nrf2. The increased sensitivity of resistant cells to glycolysis and PPP inhibition suggest that a metabolic switch occur in resistant cells. Nrf2 silencing in DAOY resistant cells was able to restore chemosensitivity, reduced the ability to manage oxidative stress and finally restored the expression of glycolytic and PPP enzymes. All this data suggest an important role of Nrf2 in the onset and in the maintenance of a resistant phenotype in medulloblastoma cells.

PROTOCOL BIOPSIES AND INTRARENAL VIRUS INFECTION: IS THERE A RELATIONSHIP BETWEEN PARVOVIRUS B19 AND HUMORAL REJECTION?

Bertazza Partigiani N, Negrisolo S, Carraro A, Benetti E, Marzenta D, Barzon L, Murer L

The protocol biopsies could identify histological signs of subclinical allograft rejection. It has been known that antibody mediated rejection (ABMR) represents one of major risk factors for reduced transplanted kidney survival. Viral infections can lead to transplant dysfunction and through the cytopathic effect and immune response they can lead to acute/chronic allograft rejection. In order to evaluate if local virus mediated inflammation could be linked to development of anti-donor antibody and ABMR, we retrospectively reviewed histological, donor-specific antibodies (DSA), viremia and intrarenal virus data of transplanted patients at our pediatric center from 2011 to 2018. Histological data were obtained from protocol biopsies performed at 6, 12 and 24 months after transplantation in association with DSA+ (MFI>3000). The detection of virus DNA was done by Real-time PCR for CMV, EBV, BKV, PVB19 in blood and bioptic tissue samples at the time of transplant (T0) and in protocol biopsies. Analysis included 106 patients: 266 protocol biopsies, 52 diagnosed as acute/chronic rejection (69.2% cellular vs 30.8% humoral). The study did not highlight correlation between systemic viral infection and humoral or cellular rejection. Conversely, our data emphasized that the presence of PVB19 is associated with humoral rejection compared to the cellular one (50%vs13%, p=0.04) and we could observe that only PVB19 has a prevalence in T0 biopsies (25.5%). In situ Hybridization confirmed the PVB19 endothelium localization. This retrospective study highlights a possible association between the presence of PVB19 intragraft and ABMR. The creation of a new multicentre study will allow increasing statistical power.

CHARACTERIZATION OF THE GENETIC BASES OF HEARING LOSS IN AN ITALIAN COHORT

Cesca F, Bettella E, Polli R, Leonardi E, Aspromonte MC, Cameran M, Bigoni S, Sensi A, Scimemi P, Santarelli R, Murgia A

Congenital sensorineural hearing lossaffect 1-2/1000 children.Non-syndromic hearing loss (NSHL) is genetically heterogeneouswith more than 100 genes so far identified. With the aim of obtaining an advanced efficient diagnostic tool we developed an NGS targetedpanel of 59 genes,strongly associated, in Caucasians, with NSHL or with syndromic HL,which onset is usually characterized by isolated deafness (i.e. Pendred and Usher syndrome). The Ion Torrent PGMTM platform and a customized bioinformatics pipeline have been used for the analysis of DNA samples of200subjectsnegative for GJB2 mutations/ GJB6deletions. 82 individuals were found positive and a diagnostic yield of 41%was obtained. In 21 of the positive subjects (25.6%)we identified mutations in genes involved both in NSHLand Usher syndrome. We found 38novel likely pathogenic mutations,that were in silico characterized. The most frequently mutated gene in our cohort was CDH23with 14pathogenic mutations identified, of which 6novel ones. We encountereda fewcases withdual molecular diagnoses, which exemplifies the complexity of hearing loss transmission. Our NGS analysis,combined with a careful data processing,allowed the identification of homozygous and heterozygous CNV in OTOAand STRCgenes, proving the effectiveness of the high coverage in targeted NGS. Our targeted approach, coupled with a solid bioinformatics pipeline, has provena sensitive molecular tool with high diagnostic yield.We obtain precise early diagnosisandimportant prognostic and follow-up information for affected individual and better counseling for their families.

ARE THE PRODUCTS OF COQ10A AND COQ10B INVOLVED IN COENZYME Q METABOLISM IN HUMAN CELLS?

Baschiera E, Morbiato L, Desbats MA, Salviati L

In Saccharomyces cerevisiae the COQ10 gene encodes a START domain protein, thought to be required for the proper localization of CoQ within the inner mitochondrial membrane. Yeast lacking COQ10 display impaired mitochondrial respiration, with normal CoQ levels. Respiration could be restored by addition of quinone analogues, or by overexpression of other COQ genes. Mammals have two COQ10 paralogues: COQ10A, constitutively expressed in two isoforms, and COQ10B, regulated by circadian rhythms. Neither COQ10A, nor COQ10B can efficiently complement yeast COQ10 mutants. To study their precise function, we generated cell lines lacking either or both genes using CRISPR-Cas9 technology. We detected a partial complex III deficiency, similar in COQ10AKO and COQ10BKO cells and more pronounced in the double mutant. CoQ levels were normal in all cell lines. ATP levels were reduced and could not be rescued by CoQ supplementation. Only COQ10A longer isoform could restore respiration in double KO cells. Our colleagues identified a homozygous COQ10A missense mutation in a patient with myopathy. Cultured skin fibroblasts recapitulated the biochemical phenotype observed in COQ10AKO. Transduction of patient's cells with wild type COQ10A rescued complex III deficiency. Overall these results indicate that COQ10A is relevant for human pathology and data obtained in yeast on COQ10 function cannot be translated to mammalian cells. In human cells, COQ10A and B seem to be important for the assembly of complex III, but the partial complex III deficiency observed in KO cells cannot account for the important respiratory defect. These data suggest that COQ10 proteins could have also other important roles in mitochondrial homeostasis, but their relationship with CoQ metabolism is still unclear.

A NOVEL DROSOPHILA MELANOGASTER MODEL FOR MUCOPOLYSACCHARIDOSIS TYPE I DISPLAYS NEUROLOGICAL AND AUTOPHAGY ALTERATIONS

De Filippis C, Napoli B, Orso G, Tomanin R

Mucopolysaccharidosis type I is a rare, inherited disease caused by mutations in the gene coding for the lysosomal enzyme alfa L-iduronidase (IDUA). Deficit in IDUA activity leads to the accumulation of the undegraded glycosaminoglycans (GAG) heparan- and dermatan-sulfate, in most organs. Clinically, there is a spectrum of phenotypes from attenuated to severe, with different ages at onset, severity and rate of progression, with neurological involvement characterizing the severe forms. Here, we investigated the IDUA function using Drosophila as model system. Ubiquitous downregulation of the Drosophila IDUA (D-IDUA) by RNAi caused lethality at pupal stage, and biochemical analysis of third instar larvae showed increased GAG levels. Downregulation of IDUA in muscle caused lethality at pupal stage; loss of IDUA in glial cells was semi-lethal, whereas in neuronal tissue did not cause lethality, indicating that D-IDUA in Drosophila is required in glial cells and in muscles for a correct development of the organism. Moreover, when D-IDUA was downregulated in neuronal and glial cells, we observed comparable progressive defects in locomotor activities, a hallmark of neurological dysfunction.

A preliminary analysis of D-IDUA-deficient tissues showed an increased number and size of lysosomes in muscles and nervous system, a typical feature of lysosomal storage diseases (LSDs). Lysotracker staining displayed a significant reduction of the correctly acidified lysosomes. In addition, we observed a block of autophagosome-lysosome fusion and the accumulation of autophagic structures, a sign of autophagy activation. These data suggest that absence of IDUA may impair the autophagy-lysosomal pathway, a highly regulated mechanism necessary for cell survival and functions. Results so far obtained propose Drosophila as an interesting model to investigate the molecular mechanisms underlying LSDs, useful to identify appropriate therapeutic targets.

C. ELEGANS AS A MODEL TO INVESTIGATE NOVEL GENES WITH UNKNOWN FUNCTION AND POTENTIALLY INVOLVED IN HUMAN GENETIC DISORDERS

Morbidoni V, Pannone L, Franco A, Martinelli S, Sandri M, Trevisson E

Our research group employs Caenorhabditis elegans, which combines the advantage of a significant genomic evolutionary conservation with the possibility to obtain relevant data from a whole animal setting, to study genes with unknown function and potentially involved in rare inherited conditions. We started to investigate the function of MYTHO, a recently identified FOXO-dependent gene, which seems to be involved in autophagy, through T01G9.2 (the worm orthologue) knock-down and knock out (KO) models. Since RNAi experiments did not detect a clearcut phenotype, we generated a KO model by CRISPR/Cas9 technology, highlighting a precocious aging phenotype which resulted in movement impairment and eventually reduced lifespan in KO animals compared to controls. We are now performing experiments aimed at the analysis of the autophagic flux and at the characterization of the pathway linking IGF/Akt/FOXO to MYTHO.

We are also investigating the functional consequences of a novel genetic variant identified in TOGARAM1 in patients with a severe malformation of the central nervous system (compatible with the Meckel-Gruber ciliopathy), using a knock-in model generated by CRISPR/Cas9 technology and harboring the same variant in the worm orthologue che-12. Although we did not observe in che-12 knock-in mutants an impairment in behaviors controlled by sensory neurons where the gene is specifically expressed (chemotaxis on a NaCl gradient, dye-uptake frequency), preliminary results indicate that the cilium of these neurons is shortened. The demonstration of the variant pathogenicity could establish an important link between mutations in TOGARAM1 (that has not been so far associated with human diseases) and ciliopathies.

URINARY EXTRACELLULAR VESICLES: A PROTOCOL OPTIMIZATION FOR URINARY PEDIATRIC SAMPLE

Negrisolo S, Carraro A, Marzenta D, Collino F, Simonato M, Giambelluca S, Cogo P, Bussolati B, Murer L

Urinary extracellular vesicles (UEVs) are lipid nanoparticles release from the different cell's type of nephrons. They carry different type of cargo (e.g miRNA, proteins etc) that are involved in cell to cell communication and could reflect the physio pathological status of organ they originated from. In this optic it has a great importance to precisely define UEVs size/concentration, lipid and miRNA content in urine samples of transplanted children. Therefore, we started to optimize a specific and accurate protocol for pediatric sample collected retrospectively. UEVs isolation was performed on 10 ml urine samples by using ultrafiltration technique (Amicon Ultrafiltration column). Vesicles size and concentration was determined by NTA technology (Nanosight 3000). Lipid fraction were extracted from UEVs (60µg of total proteins) using a Bligh & Dyer extraction procedure. Samples were analyzed using UHPLC/ESI-MS based untargeted lipidomics. Vesicles miRNA fraction was extracted from UEVs using a commercial kit (miRNease mini kit QIAGEN), reverse track and amplified by RealTime PCR using MIRCURY LNA miRNA PCR kit. NTA analysis showed a range of concentration between 1.81^109—7.65^109 and a size ranging 122 – 222 nm. After data filtering, 3056 and 865 compounds with unique molecular weight and retention times were annotated from the positive and the negative modes, respectively. The RealTime PCR confirmed the presence of UEVs' miRNAs. Furthermore, to confirm UEVs presence we performed electron microscopy assay. Together, these results sustained that UEVs and their content could be considered a reliable source of biomarkers in nephrological area.

TARGETED GENE PANEL FOR PEDIATRIC MOVEMENT DISORDERS

Aspromonte MC, Leonardi E, Bellini M, Bettella E, Cesca F, Cameran M, Polli R, Murgia A

Childhood Movement Disorders (MDs) are clinically and genetically heterogeneous conditions characterized by abnormal movements often associated with comorbid conditions including intellectual disability (ID) and autism spectrum disorders (ASD). We developed a diagnostic strategy for a paediatric population affected by MDs and the associated comorbidity, to improve knowledge about the genes involved in these phenotypic spectra. We performed targeted NGS of 74 ID/ASD genes, in a cohort of 34 paediatric patients, referred for ID and/or ASD but also affected with MDs. Subsequently, we developed a 59 gene-panel, specific for childhood onset MDs and Cerebral Palsy (CP) and tested 22 patients: 12 pz negative to the ID/ASD panel and 10 referred for early onset MD. In 3 out of 34 pz, we detected de novo disease causing mutations in DYRK1A, GRIN2B and CASK. In 3 patients, we identified 4 rare variants in GAD1 and SCN2A, predicted in silico to be deleterious and classified as likely pathogenic (LP); genes known to cause neurodevelopmental disorders (NDD) with MD recurrence. Among the 22 patients tested with MD/CP panel, one homozygous known truncating mutation in AP4S1 was found in a child with spasticity and in his newborn, mildly symptomatic sister. Two brothers with similar phenotypes, inherited from unaffected mother a pathogenic variant in COL4A2. A novel LP variant in SCN8A, was detected in a boy with ataxia and tremor, without epilepsy. The analysis of a cohort of subjects suffering from NDDs with and without MD could help clarify the biological bases of complex and interconnected conditions.

THE USE OF NEXT GENERATION SEQUENCING FOR THE DIAGNOSIS OF EARLY ONSET EPILEPSY AND EPILEPTIC ENCEPHALOPATHIES

Bettella E, Polli R, Leonardi E, Cesca F, Aspromonte MC, Cameran M, Danieli A, Osanni E, Boniver C, Toldo I, Sartori S, Murgia A

Epilepsy comprises a wide range of etiologically heterogeneous clinical conditions ranging from benign forms to treatment-refractory progressive epileptic encephalopathies, which clinical features, seizure type, age of onset, electroencephalographic features and response to anti-epileptic drugs are very diverse and may vary over time. At present, targeted NGS is the elective choice for early and efficient etiological diagnoses. 242 consecutive subjects have been sequenced on the Ion PGM™ platform, using a customized panel of 31 genes designed to particularly focus on early onset epilepsy. Sequence variants were interpreted according to ACMG guidelines. The diagnostic yield resulted 20% in patients with seizure onset within 36 months of life and 22% in patients with seizure onset within 12 months of life. 40 pathogenic/likely pathogenic variants were detected: 25 in genes encoding for ion channels proteins (HCN1, KCNQ2, SCN1A, SCN2A and SCN8A), 5 in enzyme/enzyme modulators (ALDH7A1, CDKL5, CHD2, PNPO), 4 in nucleic acid binding (FOXG1, MECP2, MEF2C), 2 in transporters (SLC13A5, SLC2A1) and one in a transcription factor (TCF4). 15 inherited Variants of Unknown Significance (VUS), 6 novel VUS and 9 ultra-rare VUS, have been detected. The peculiar phenotypes of individuals we found to carry disease-causing variants contribute to further expanding the genotypic and clinical spectrum of these disorders. Our findings highlight the pleiotropy of early onset epilepsy genes and indicates in a subset of few genes the major players for these disorders. Our results are a proof of concept, demonstrating the relevance of combining an accurate clinical characterization with a significant gene content.

USE OF NEXT GENERATION SEQUENCING IN CLINICAL GENETIC SETTING

Bertolin C, Cassina M, Trevisson E, Rigon C, Rossi S, Forzan M, Boaretto F, Salviati L

NGS have provided new possibilities for diagnostic genetic testing, making it possible to examine a large number of genes in a single reaction and to perform large-scale analyses of the patient's exome.

In the Clinical Genetics and Epidemiology laboratory, we routinely performed diagnostic analysis to detect causative mutations of human diseases and since 2015 we started to use NGS. Here we reported our experience with the use of NGS.

We adopted tree different kind of panels: small custom-genes-panels (~30/50 genes) suitable for oligogenic conditions, large custom-genes-panels (~120/150 genes) for diseases with high genetic heterogeneity and clinical-exome-panel for all other cases.

In the last 4 year, we changed several times the design of our panel to add new disease genes and we switched from amplicon-based to hybridization-based enrichment method in order to improve the quality of NGS data.

As results we can achieve two important goals:1) the time-to-results dramatically decrease; 2) in the last 4 years, we have increased exponentially the number of patients analyzed and consequently the number of causative mutations identified.

In clinical settings, it is important to balance the security of validate protocols and the continuous upgrade of methods routinely used to reach higher quality of clinical reports and to accelerate the time-to-result. NGS methods and kits undergone to a rapidly evolving innovation and it is important to choose every time the best approach in order to improve accuracy and sensitivity of our pipeline of mutation identification.

DEVELOPMENT OF A YEAST MODEL TO VALIDATE COQ4 PATHOGENIC MUTATIONS ASSOCIATED WITH HUMAN PRIMARY COENZYME Q10 DEFICIENCY

Calderan C, Sartori G, Salviati L

Defects in genes involved in coenzyme Q10 (CoQ10) biosynthesis cause primary CoQ10 deficiency, a severe multisystem mitochondrial disorder. COQ genes have been initially characterized in the yeast Saccharomyces cerevisiae and subsequently their human counterparts have been identified. In yeast and in mammalian cells Coq proteins assemble in a multi-subunit complex; however, its composition and the role of some Coq polypeptides are not completely clear yet. For example, the precise function of COQ4 is unknown, even if it seems to be crucial for the formation and stability of the complex. Here we tested the pathogenic role of 9 COQ4 autosomal recessive mutations, found in patients with primary CoQ10 deficiency, using a recombinant yeast model. The working model was achieved co-expressing the human WT or mutant COQ4 with high quantities of yeast COQ8 in a Coq4-knockout strain. The overexpression of COQ8, a putative regulatory kinase, supported the assembly of the complex and allowed the human WT COQ4 to restore coq4-mutant growth on respiratory media. Conversely, all the COQ4 mutants except one lost this ability, indicating that 8 out of the 9 mutations reported cause a virtually complete loss of function of the corresponding protein and should have a pathogenic role in the disease. Interestingly, the yeast Arg102His relatively milder phenotype is consistent with the later onset slowly progressive disease of the patient carrying this variant: this good correlation gives strength to our COQ4 yeast model. Moreover, all these findings support the putative central role of the COQ4 polypeptide in COQ10 production.

OPTIMIZING NANOPARTICLE-MEDIATED ENZYME DELIVERY FOR BRAIN TREATMENT IN MUCOPOLYSACCHARIDOSIS TYPE II

D'Avanzo F,Pederzoli F, Rigon L, Ruozi B, Tosi G, Scarpa M and Tomanin R

Mucopolysaccharidosis type II (MPSII) is an inherited disease due to the deficit of the lysosomal enzyme iduronate 2-sulfatase (IDS), this causing a pathological accumulation of undegraded glycosaminoglycans (GAG) in most organ-systems, including CNS in the severe forms. Recombinant IDS, used as Enzyme Replacement Therapy, has shown partial efficacy in different organs, except the CNS given the enzyme inability to cross the blood-brain barrier (BBB). We recently showed that PLGA nanoparticles (NPs), functionalized with a CNS-targeting eptapeptide (g7), can deliver the enzyme across the BBB in the MPSII mouse model, reducing GAG levels and some neuro-pathological and inflammatory biomarkers in brain parenchyma. However, a complete reversion to the healthy condition was not observed. To further improve the therapeutic efficacy of this system, we tested the modification of different parameters of the NPs formulation process (emulsions' sonication power and time, emulsions'phase volumes, addition of BSA and Tween20 as stabilizers). Following this optimization, a new formulation of NPs has been produced and characterized in cell-free systems and in human fibroblasts from MPSII patients. Preliminary results showed an encapsulation efficiency of 28%, almost double compared to previous formulations, and corresponding to 2 mg IDS/100 mg NPs. Also, a good improvement in enzyme stability has been obtained, maintaining the 76% of the starting solution activity. Following confirmation of these results by further in vitro experiments, now ongoing, this new formulation is ready to be assayed in the MPSII mouse model.

A DROSOPHILA MELANOGASTER MODEL FOR MUCOPOLYSACCHARIDOSIS TYPE II: PROOF OF PRINCIPLE

Rigon L, Kucharowski N, Tomanin R, Bauer R

Mucopolysaccharidosis type II (MPSII) is an X-linked lysosomal storage disorder due to the deficit of the lysosomal hydrolase iduronate 2-sulfatase (IDS), leading to undegraded glycosaminoglycan storage. MPSII is characterized by important clinical features, including organomegaly, cardiopathies, joint stiffness and bone deformities. In about 2/3 of the cases, also a heavy neurological impairment occurs. The applied therapy is the enzyme replacement, which however does not cure the brain disease, due to its inability to cross the blood-brain barrier. Moreover, the pathogenic mechanisms are not fully understood. Therefore, brain disease remains one of the critical issues still unresolved. Drosophila melanogaster is a very suitable model to study neurological impairment, thanks to its simplicity, the well-known neurological development and the numerous tools available. Therefore, we are developing knock-down and knock-out fruit fly models for MPSII, using 4 different approaches. 1) The RNAi interference, by using the UAS-Gal4 binary system to knock-down the IDS gene in specific tissues/cells. 2) The CRISPR/Cas9 system to knock-out ubiquitously the IDS gene, targeting the first conserved active site of the protein. 3) A combination of the CRISPR/Cas9 approach and the UAS-Gal4 system, using commercially available Gal4-Cas9 fruit fly lines and a UAS-gRNA line for IDS, to obtain conditional knock-out model. 4) The CRISPR/Cas9 system combined with a homologous direct repair approach to totally delete the IDS gene and substitute it with an exchangeable cassette for future varied replacement. Preliminary data with the RNAi approach is negative, as IDS knock-down flies do not show pathological phenotype. Other analyses are in progress on this model, but surely the total knock-out models (currently under first evaluation) will allow better results.

VITAMIN K2 CANNOT SUBSTITUTE COENZYME Q10 AS ELECTRON CARRIER IN THE MITOCHONDRIAL RESPIRATORY CHAIN OF MAMMALIAN CELLS

Cerqua C, Casarin A, Pierrel F, Vazquez Fonseca L, Viola G, Salviati L and Trevisson E

Coenzyme Q10 (CoQ10) deficiencies are a group of heterogeneous conditions that respond to ubiquinone administration if treated soon after the onset of symptoms. However, this treatment is only partially effective due to its poor bioavailability. We tested whether vitamin K2, which was reported to act as a mitochondrial electron carrier in D. melanogaster, could mimic ubiquinone function in human CoQ10 deficient cell lines, and in yeast carrying mutations in genes required for coenzyme Q6 (CoQ6) biosynthesis. We found that vitamin K2, despite entering into mitochondria, restored neither electron flow in the respiratory chain, nor ATP synthesis. Conversely, coenzyme Q4 (CoQ4), an analog of CoQ10 with a shorter isoprenoid side chain, could efficiently substitute its function. Given its better solubility, CoQ4 could represent an alternative to CoQ10 in patients with both primary and secondary CoQ10 deficiencies.

Immunology and Neuroimmunology

AUTOIMMUNE ENCEPHALITIS DIAGNOSIS: FROM THE BENCH TO THE CLINIC

De Gaspari P, Zoccarato M, Nosadini M, Zuliani L, Sartori S

Currently, about 90,000 people around the world develop Autoimmune Encephalitis (AE) each year. AE are severe neurological disorders characterized by seizures, cognitive deficits and psychosis, that affect patients of all ages, frequently children. AEs are associated with neuronal antibodies (NAbs) that are either markers or the direct cause of the disease. The identification of NAbs allows a prompt start of the therapy, possibly leading to a complete recovery. Commercial assays are available for some NAbs detection but, development and implementation of in-house highly sensitive diagnostic methods, to detect most Nabs, is yet an unmet need. During the last year, our laboratory analysed samples from 31 patients with suspected AE having doubtful and negative diagnostic results on commercial assays. 45 samples (serum and cerebrospinal fluid) were screened by indirect immunohistochemistry (IHC) on frozen rat brain and cerebellum sections. Analysis of the patterns of IHC reactivity on rat hippocampus and cerebellum led to define the presence of NAbs and suggested the possible neuronal or glial antigenic target. Further, we are now working on different assays including cryoconserved mouse neurons cells and home-made cell-based assays (eg. IgLON5 and Glycine receptor) to detect, confirm or discover other nAbs. In conclusion, the correct and rapid identification of neuronal antibodies and their pathogenic role requires the development of a novel set of diagnostics assays based on antigenic expression analysis on rodent brain tissue and mammalian cells cultures.

CD73+ EXTRACELLULAR VESICLES INHIBIT ANGIOGENESIS THROUGH ADENOSINE A2B RECEPTOR SIGNALLING

Angioni R, Herkenne S, Liboni C, Sánchez-Rodríguez R, Borile G, Muraca M, Calì B, Viola A

Pathological angiogenesis is a hallmark of several conditions including eye diseases, inflammatory diseases, and cancer. Stromal cells play a crucial role in regulating angiogenesis through the release of soluble factors or direct contact with endothelial cells. Here, we analysed the properties of the extracellular vesicles released by bone marrow mesenchymal stromal cells (MSCs) and explored the possibility of using them to therapeutically target angiogenesis. We demonstrated that in response to pro-inflammatory cytokines, MSCs produce extracellular vesicles that are enriched in TIMP-1, CD39 and CD73 and inhibit angiogenesis targeting both extracellular matrix remodelling and endothelial cell migration. We identified a novel anti-angiogenic mechanism based on adenosine production, triggering of A2B adenosine receptors, and induction of NOX2-dependent oxidative stress within endothelial cells. Finally, in pilot experiments, we exploited the anti-angiogenic extracellular vesicles to inhibit tumor progression in vivo. Our results identify novel pathways involved in the crosstalk between endothelial and stromal cell and suggest new therapeutic strategies to target pathological angiogenesis.

TARGETING MONOAMINE OXIDASE TO DAMPEN NLRP3 INFLAMMASOME ACTIVATION IN ACUTE AND CHRONIC INFLAMMATION

Sánchez-Rodríguez R, Munari F, Agnellini A, Brun P, Menga A, Castegna A, Nogara L, Luisetto R, Blaauw B, Viola A, Canton M

Inflammasome activation is one of the main cellular strategies to control pathogens and cellular damage, though their uncontrolled activation drives progression of inflammatory, metabolic and neurodegenerative diseases. Several stimulus trigger NLRP3 inflammasome activation, however, the underlying mechanisms involved are still unclear. Even though mitochondrial reactive oxygen species (ROS) trigger the NLRP3 inflammasome, the specific source of ROS is undefined. Our work demonstrates that ROS produced by the mitochondrial enzyme monoamine oxidase-B (MAO-B) induce NLRP3 inflammasome activation in human macrophages. The ROS produced by MAO-B causes mitochondrial dysfunction and NFkB activation, resulting in the overexpression of NLRP3, IL-1α and the increase of Caspase-1 activity. In vivo, MAO-B inhibition by Rasagiline reduces IL-1 α plasma levels in LPS-mediated endotoxemia mouse model. The decrease in Il-1α plasma levels was confirmed in MAO-B knock-out mice under the same sepsis model. Importantly, in the most common murine model of Duchenne dystrophy, Rasagiline reduces inflammasome activation in muscle-infiltrating macrophages, along with muscle performance recovery. Overall, our findings identify MAO-B as a specific producer of mitochondrial ROS fuelling the NLRP3 inflammasome, thereby providing the basis for repurposing MAO-B inhibitors to treat inflammasome-mediated pathologies.

FATTY ACID DYSREGULATION IS AN EARLY EVENT ON HEPATOCARCINOGENESIS: TRANSCRIPTOMIC ANALYSIS OF EARLY AND LATE LESIONS

Castro-Gil MP, Torres-Mena JE, López CD, Muñoz-Montero S, Salgado RM, Krötzsch E, Sánchez-Rodriguez R, Pérez-Carreón JI

Hepatocellular carcinoma (HCC) transcriptomics focuses primarly in late stage lesions while early lesions transcriptomics remains obscure. In a healthy liver, Gamma Glutamyltransferase (GGT) and Keratin 19 (K19) are only expressed in cholangiocytes and hepatic progenitor cells, meanwhile, hepatocytes that go through tumorigenic transformation express both proteins. Our aim was to determine the transcriptome profile of early and late GGT/K19 positive lesions in a hepatocarcinogenesis model, to generate a molecular signature associated to carcinogenic progression and glimpse the transcriptome of early lesions. Hepatocarcinogenesis was induced in Fisher 344 rats by weekly intraperitoneal injections of diethylnitrosamine (DEN) producing early lesions in 12 weeks and late HCC lesions at 18 weeks. Livers from non-treated rats were used as controls. GGT/K19 lesions were laser microdissected. The transcriptome Sequencing of microdissected lesions was performed in Illumina Nextseq 500 sequencer. Global Gene Expression (GGE) and Differential Gene Expression (DGE) analysis were performed using in-house RNA analysis (https://github.com/said3427/rnaseq). Enrichment Analysis was performed by Ingenuity Pathway Analysis (IPA) software, considering genes with the thresholds: FDR < 0.05 and |log2FoldChange| > 1.5. Two samples clusters were evident after GGE analysis: Normal Liver and Liver Lesions. After DGE analysis; 585 and 632 Differentially Expressed Genes (DEG) were found in early and late lesions respectively. The molecular signature associated to carcinogenic progression comprised 438 DEG and it showed a Fatty Acid Metabolism dysregulation. Fatty Acid dysregulation is an early and persistent event in hepatocarcinogenesis, suggesting its importance in metabolic reprogramming, a hallmark of Hepatocellular Carcinoma

CXCL12 ALTERNATIVE SIGNALING PATHWAYS MODULATE THREE DIMENSIONAL NEUTROPHIL MIGRATION

Calì B, Marcuzzi E, Cassarà A, Toffali L, Vetralla M, Gagliano O, Laudanna C, Thelen M, Elvassore N, Molon B, Piel M, Vargas P, Viola A

Neutrophil migration represents a critical step for the establishment of immune responses. To reach the inflamed tissues leukocytes have to face several physical obstacles represented by the endothelial barrier and the dense fibrillar interstitial spaces. Although the cytoplasm can quickly change consistence and form to allow cells to interact with and penetrate the endothelium, the deformation of the nucleus, the largest and stiffest cellular organelle, represents the most challenging step during trans-migration. Several studies have already described mechanical adaptation of the cell nucleus under 3D physical confinement, however, the contribution of biochemical cues, such as chemokines, to the modulation of nuclear mechanical properties during cell migration in tissues remains unexplored. Exploiting live cell imaging and original micro-fabricated devices, we investigated the role of CXCL12 in the modulation of the biomechanical properties of the neutrophil nucleus, and the impact of nuclear adaptation on their migration through physically confining microenvironments. Here we show that CXCL12 induces neutrophil nuclear deformation and sustains transendothelial migration towards inflammatory stimuli. Intriguingly, going deep into the signaling pathway, we found that atypical receptors and unexpected protein kinases are involved in the modulation of nuclear deformability. On this basis, we propose that, in addition to mechanical signalling, chemical cues regulate the deformation of the nuclei of migrating leukocytes during their migration inside the tissues.

Nano medicine

MESENCHYMAL STROMAL CELLS CONTRIBUTES TO THE ARRHYTHMOGENIC CARDIOMYOPATHY

Liboni C, Scalco A, Angioni R, Di Bona A, Bertoldi N, Pilato CA, Thiene G, Judge D, Sommariva E, Pompilio G, Basso C, Mongillo M, Viola A, Zaglia T

Arrhythmogenic cardiomyopathy (ACM) is a rare cardiac disease that causes life-threating arrhythmias and sudden cardiac death. The aetiology is still unknown and no treatments are available. The fibro-fatty replacement of the myocardium is the main feature of this disease. A recent publication identifies the mesenchymal stromal compartment (MSC) as the source of adipocytes in the ACM-heart. Thus, we dissected the role of MSCs in this the ACM. We found that ACM progression is characterized by alterations in the MSC compartment in a DSG2mut/mut murine model. At the early stage of the disease, DSG2mut/mut mice show higher number of MSCs in the bone marrow compared to wild type animals, whereas at a later stage they are characterized by accumulation of MSCs in the heart. In this line, in vitro experiments demonstrated that, MSCs from DSG2mut/mut mice have a higher proliferation index, in comparison to the healthy counterpart, when isolated from the bone marrow, but not when derived from the heart. Thus, we demonstrated that the mutation alters the behaviour of the BM-MSCs. This data was confirmed by a competition homing assay in vivo, showing an increased homing of injected DSG2mut/mut BM-MSCs to the DSG2mut/mut heart, in comparison to the wild type MSCs. The molecular signal driving the specific homing of BM-MSCs to the DSG2mut/mut heart is still under consideration. Taken together our results suggest that mutations in desmoglein-2 affect the functional properties of BM-MSCs. Importantly, this study shed light on the pathogenesis of ACM and on the role of MSCs in the disease.

PATIENT-DERIVED SCAFFOLDS OF COLORECTAL CANCER METASTASES AS AN ORGANOTYPIC 3D MODEL OF LIVER METASTATIC COLONIZATION

D'Angelo E, Natarajan D, Sensi F, Fassan M, Bresolin S, Spolverato G, Piccoli M, Urbani L, Agostini M

The liver is the most common site for colorectal cancer (CRC) metastasis and new tissue culture models are needed to study colorectal cancer liver metastasis (CRLM) as none of the current models mimic the biological, biochemical and structural characteristics of the metastatic microenvironment. Decellularization provides a novel approach for the study of cancer extracellular matrix (ECM) as decellularized scaffolds retained their tissue-specific features and biological properties. In the present study we created a 3D model of CRC and matched CRLM using patient-derived decellularized ECM scaffolds seeded with HT-29 and HCT-116 CRC cell line. Here we show increased cell proliferation and migration capability when cultured in cancer-derived scaffolds compared to same-patient healthy colon and liver tissues. CRLM scaffolds also induced activation of epithelial-mesenchymal transition (EMT) with cancer cells showing loss of E-cadherin and increased Vimentin expression, coupled with an induction of Snail-1-mediated up-regulation of Zeb-1. EMT was confirmed by gene expression profiling, with the most represented biological processes in CRLM-seeded scaffolds involving cellular response to stress metabolic processes, response to oxygen level and to starvation. The complex 3D culture environment reduced the CRC cell response to 5-fluorouracil and FOLFIRI (5-fluorouracil combined with irinotecan), when used at standard IC50 determined in 2D cultures. Our 3D culture system with patient-derived tissue-specific decellularized ECM better recapitulates the metastatic microenvironment compared to conventional 2D culture conditions and represents a relevant approach for the study of CRLM formation and progression.

HYPOXIA-DERIVED EXOSOMES IN NEUROBLASTOMA DISSEMINATION AND AGGRESSIVENESS

Fusco P, Zanella L, Esposito MR, Manfredi M, Cimetta E

Neuroblastoma (NB) is a heterogeneous pediatric malignancy accounting for up to 10% of childhood cancers with a strong tendency to metastasize. Hypoxia is a key feature of solid tumors, and is specifically known to i. favor NB metastasis and dedifferentiation and to ii. stimulate release of exosomes (EXOs), facilitating intercellular communication at distant sites. In this study, we investigated the functional role of EXOs secreted by NB cells cultured at different oxygen concentrations on the surrounding stromal cells and characterized the proteomic and miRNAs cargo to identify an exosomal signature associated with metastatic dissemination. EXOs were analyzed by electron microscopy and qNANO to confirm morphology and to determine the diameters. We also evaluated a panel of surface proteins revealing that hypoxic-EXOs expressed proteins associated with angiogenesis, adhesion, stemness and immune function. We hypothesize that the expression of these markers contributes in directing the EXOs towards specific target tissues, where EXOs drive the formation of the pre-metastatic niche. We confirmed that treatment with hypoxic-EXOs increased the expression of stemness markers in MCSs, and stimulated tube forming ability and motility in HUVECs. Moreover, we demonstrated that hypoxic-EXOs enhanced the migration ability of NB cells and increased the number of colonies compared to normoxic EXOs-treated cells. Proteome and miRNA cargo profiles were analyzed by quantitative mass spectrometry and FirePlex Discovery Panel, respectively. These promising results are the starting point for the identification of 1. the mechanisms and of 2. potential predictive biomarkers for metastatic spread in NB.

RECELLULARIZED COLORECTAL PATIENT-DERIVED SCAFFOLD AS IN VITRO PRE-CLINICAL 3D MODEL FOR DRUG SCREENING

Sensi F, D'Angelo E, Piccoli M, Corallo D, Biccari A, Pucciarelli A, Agostini M

Colorectal cancer (CRC) shows a highly ineffective therapeutic management. An urgent needing not yet addressed is the random assignment to adjuvant chemotherapy of high-risk stage II and stage III patients. In the field of drug discovery the critical step is the preclinical evaluation of drug cytotoxicity and efficiency. We purpose to develop a patient-derived three-dimensional (3D) preclinical model useful for drug evaluation. Surgically resected healthy and matched CRC were recellularized with HT29 cells. Qualitative and quantitative characterization were evaluated through histology, immunofluorescences and DNA amount quantification. Chemosensitivity test was performed using 5-Fluorouracil (5-FU). In vivo studies were carried out using the zebrafish (Danio rerio) animal model. Drug absorption and perfusion along scaffolds were evaluated qualitative using autofluorescence of doxorubicin and quantitative calculated by Darcy's Law. Five days after recellularization the 3D CRC model exhibited reduced sensitivity to 5-FU treatments compared with conventional 2D culture. Calculated IC50 resulted in 11.5 µM and 1.3 µM of 5-FU, respectively. In the zebrafish transplantation model we obtained an IC50 concentration fully comparable with that observed in 3D CRC. Using confocal microscopy, we demonstrated that doxorubicin diffuses through the 3D CRC model and co-localize with the cell nuclei which repopulate the scaffold. 3D CRC model could be preclinical reliable tool to bridge the gap between in vitro (2D) and in vivo (zebrafish) preclinical drug testing assays.

MICROBIOREACTOR PLATFORMS AND 3D BIOPRINTING TO PROBE THE ROLE OF NEUROBLASTOMA-DERIVED EXOSOMES IN CANCER DISSEMINATION

Bova L, Micheli S, Sorgato M, Fusco P, Esposito MR, Cimetta E

This work exploits two different engineering approaches, microfluidics and 3D bioprinting, to study the influence of exosomes (EXO) in Neuroblastoma (NB) dissemination. Both methods help providing cultured cells more specific, tunable and in vivo-like physiological stimuli than standard 2D cultures. Bioprinting allows for the creation of 3D structures using a cell-laden hydrogel as printing material. Among the tested hydrogels, gelatin methacrylate (GelMA) had the ideal characteristics in terms of printability and maintenance of cell viability for long-term cultures. The printed structure was characterized by 2 parallelepipeds connected by 8 narrow channels; only 1 side was printed with NB cells (SK-N-AS), while the rest was EXO-laden or hydrogel only. Although cells didn't invade empty channels, the presence of EXOs induced motility leading to the formation of cell clusters and 3D aggregates. The microfluidic platform (μBR) operates generating a diffusion-driven concentration gradient. The μBR consists of two main lateral channels connected to a culture chamber through an array of micro-channels. An aluminum master with the desired geometry is produced via micro-milling, and a large number of identical devices can be replica-molded in Polydimethylsiloxane (PDMS). Once culture media of different composition (with and without EXOs) is fed through the inlets, the effects, magnitude and directionality of the induced concentration-dependent signal on cultured cells can be simultaneously screened. A biological validation on SK-N-AS NB cells shows successful graded EXOs internalization and paves the way to more in-depth studies on the role of EXOs in NB metastatic dissemination.

A DATA-DRIVEN APPROACH TO ASSESS THE ROLE OF NEUROBLASTOMA-DERIVED EXOSOMES IN CANCER DISSEMINATION

Zanella L, Fusco P, Esposito MR, Facco P, Bezzo F, Cimetta E

Neuroblastoma (NB) is a pediatric malignancy with a strong tendency to metastasize. The regulation of tumor progression is considerably affected by microenvironmental cues. Tumor secreted exosomes, extracellular vesicle-like structures whose cargo includes miRNAs and proteins, deliver signals that act by regulating cell-cell communication, promoting tumor progression, invasion and metastasis. In addition, hypoxia has been identified as a potent inducer of NB metastasis and exosomes release. This work deals with the characterization of the miRNAs and proteomic cargo of exosomes secreted by two NB cell lines cultured at different oxygenation conditions: i. 20% O2, ii. 1.5% O2 and iii. reoxygenation (24 h at 1.5% O2, followed by 24 h at 20% O2). The application of multivariate statistical techniques, mainly PCA (unsupervised) and PLS-DA (supervised), has allowed identifying a list of miRNAs and proteins differentially expressed in 1. each cell line and 2. each oxygenation level. These preliminary results are subject to validation and will represent the starting point for the identification of predictive biomarkers for the metastatic spread in Neuroblastoma.

PUMPLESS MICROFLUIDIC SYSTEM FOR BONE MARROW NICHE-ON-A-CHIP IN VITRO MODELLING AND IMAGING IN LEUKEMIA

Borile G, Borella G, Charoy C, Filippi A, Romanato F, Pigazzi M, Anderson K

Bone marrow niche is one of the major contributing factors in Pediatric Acute Myeloid Leukemia development and drug resistance. Among the major challenges in Pediatric AML drug discovery and testing there is the lack of in vitro models of the bone marrow niche. Here, we aim to design and fabricate a microdevice capable of improving a scaffold-based 3D in vitro model bone marrow niche, in terms of engraftment and proliferation. Combining biophysics, microfabrication and cancer biology, we developed a prototype of pumpless microfluidic device that is: simple to produce; amenable for multiphoton microscopy live imaging; low cost; independent on external power. The device fits onto a microscopy glass slide where the scaffold-based 3D culture of leukemic cells is maintained and imaged through a glass window. A multiphoton microscopy pipeline has been developed to perform live imaging of mesenchymal stromal cells and leukemia cells injected inside the scaffold. The engraftment and proliferative capacity of cells into cultured in this system is currently under evaluation. This system would improve leukemic cell niche knowledge and may serve to explore innovative drug screenings.

EFFECTS OF MATERNAL CHORIOAMNIONITIS ON EPITHELIAL LINING FLUID LIPIDOMICS IN NEWBORNS WITH RESPIRATORY DISTRESS SYNDROME

Giambelluca S, Verlato G, Simonato M, Vedovelli L, Santoro F, Najdekr L, Dunn WB, Carnielli VP, Baraldi E, Cogo P

Chorioamnionitis is an inflammation of fetal membranes often associated with preterm delivery and morbidities. The role in lung disease is controversial. It is generally accepted that chorioamnionitis correlates with decreased risk of respiratory distress syndrome (RDS) and increased risk of chronic lung disease. To assess the effect of chorioamnionitis on lipid profile of epithelial lining fluid (ELF) of preterm newborns with RDS. The study involved 30 newborns with RDS, born from mothers with (HCA+, N=10) or without (HCA-, N=20) histological chorioamnionitis. Patients had a gestational age ≤30 weeks and the groups were matched for age and birth weights. Tracheal aspirates (TAs) were collected within the first 24h and analyzed using UHPLC/ESI-MS based untargeted lipidomics. Blank samples were used to exclude contaminants, QC samples to determine within-experiment precision. Filtered data were analyzed with PCA and differences between groups were assessed by multivariate analysis. Compounds were annotated using formulas to search against the Human Metabolome Database and Lipid Maps. After data filtering, we detected over 4000 unique features. The most relevant changes among complex lipids involved sphingolipids metabolism, with lower levels of all annotated sphingomyelins in HCA+. Differences in ceramide levels were detected between the two groups according to ceramide classes. Lipidomics of TAs suggested changes in specific areas of metabolism between HCA+ and HCA-. However, this is an exploratory study on a small size population. The hypothesis of an effect of chorioamnionitis on ELF lipidomics deserves further investigations on a larger group of patients.

AN INTEGRATED SOFTWARE PLATFORM TO IMPROVE THE IDENTIFICATION OF METABOLITES, ON THE UNTARGETED LC-MS METABOLOMICS

Poloniato G, Pirillo P, Stocchero M, Gucciardi A, Naturale M, Baraldi E, Giordano G

Metabolomics is the science that measures the metabolic response of a living system to chemical, biological and environmental stimuli, providing information about the genetic, enzymatic, and physiological activities of an organism following exposure to dietary or pharmacological interventions, for example. This is possible through the analysis of low molecular weight constituents in biofluids and tissues, by using high-throughput techniques like liquid chromatography coupled to mass spectrometry (LC/MS) and nuclear magnetic resonance (NMR). Untargeted metabolomics allows monitoring of the whole metabolic composition of a specimen, without a preliminary selection of specific metabolites, producing a large amount of data. A well-defined workflow is essential to guarantee accurate and high quality data, however comprehensive software solutions for untargeted metabolomics data management are limited. Currently, different software platforms are used to implement individual workflow steps. The main challenge is to integrate them in a unique, user-friendly architecture, where automatic procedures for data processing are applied to ensure data stability. This work aims to create a customized solution where different freely available and commercial software platforms are integrated to implement our workflow for untargeted metabolomics. Specifically, mass spectrometry data and sample information are collected and stored in a single database based on ACD/Spectrus technology. The designed data model allows a simple integration with Progenesis, aiming to permorm a faster component identification process reducing time and interpretation errors. Importantly, data capture from MassLynx without data conversion guaranteed data integrity, while in-house data were merged with the available databases to improve the quality of data annotation/identification.

URINARY METABOLOMICS REVEALS KYNURENINE PATHWAY PERTURBATION IN NEWBORNS WITH TRANSPOSITION OF GREAT ARTERIES AFTER SURGICAL REPAIR

Simonato M, Fochi I, Vedovelli L, Giambelluca S, Carollo C, Padalino M, Carnielli VP, Cogo P

Transposition of the great arteries (TGA) is a cyanotic congenital heart defect that requires surgical correction, with the use of cardiopulmonary-bypass (CPB), usually within three weeks of life. The use of CPB in open heart surgery results in brain hypoperfusion and in a powerful systemic inflammatory response and oxidative stress. We aimed to develop a novel untargeted metabolomics approach to detect early postoperative changes in metabolic profile following neonatal cardiac surgery. We studied 14 TGA newborns with intact ventricular septum undergoing arterial switch operation with the use of CPB. Urine samples were collected preoperatively and at the end of the surgery and were analyzed using an untargeted metabolomics approach based on UHPLC-high resolution mass spectrometry. Since post-surgery metabolic spectra were heavily contaminated by metabolites derived from administered drugs, we constructed a list of drugs used during surgery and their related metabolites retrieved from urine samples. This library was applied to our samples and 1255 drugs and drug metabolites were excluded from the analysis. Afterward, we detected over 39000 unique compounds and 371 putatively annotated metabolites were different between pre and post-surgery samples. Among these metabolites, 13 were correctly annotated or identified. Metabolites linked to kynurenine pathway of tryptophan degradation displayed the highest fold change. This is the first report on metabolic response to cardiac surgery in TGA newborns. We developed an experimental design that allowed the identification of perturbed metabolic pathways and potential biomarkers of brain damage, limiting drugs interference in the analysis.

TARGETED LC-MS BASED METABOLOMICS IN PEDIATRICS

Gucciardi A, Pirillo P, Poloniato G, Stocchero M, Naturale M, Baraldi E, Giordano G

Metabolomics is the science that measures the metabolic response of a living system to chemical, biological, and environmental stimuli, providing information about the genetic, enzymatic, and physiological activities of the system. This is possible through the analysis of low molecular weight constituents (i.e. metabolites) in biofluids using techniques like liquid chromatography coupled to mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR). Metabolomics enables us to detect diseases and characterize their phenotype, stratify patients on the basis of their biochemical profiles, and to monitor the progression of a particular disease. Being a very informative technique that can be applied to samples collected non-invasively, metabolomics has considerable appeal for the study of pediatrics. There are two different approaches to metabolomics: untargeted and targeted. The former is largely applied as a hypothesis-free approach and tries to provide a whole description of the metabolic composition of a specimen using semi-quantitative methods, whereas the latter quantifies a small set of known metabolites focusing on specific metabolic pathways. Targeted metabolomics is often applied to confirm the results of untargeted metabolomics improving the quality of the analytical data and that of the characterization of the metabolomic perturbations at a pathway level, or it is the basis for hypothesis-driven investigations. Usually, the targeted methods suitable for adult patients cannot be directly applied to children because of the small sample size available. For this reason, new methods must be developed or existing methods adjusted. Here, we present the targeted methods available in our laboratory for the quantification of compounds involved in many key-metabolic pathways.

UNTARGETED METABOLOMICS AND NEONATAL SEPSIS

Pirillo P, Mardegan V, Stocchero M, Naturale M, Poloniato G, Gucciardi A, Baraldi E, Giordano G

Neonatal sepsis is one of the main concerns in neonatology and its prompt detection is mandatory. To date, there are no reliable and effective biomarkers of neonatal sepsis. Metabolomics can be defined as the analysis and interpretation of the global metabolic data expressing the multiparametric metabolic response of living systems to genetic modification, pathophysiological stimuli and environmental influences. Metabolomics has been successfully applied to paediatrics because it required samples non-invasively collected and small sample sizes. In this study, untargeted metabolomics based on ultra performance liquid chromatography-mass spectrometry (UPLC-MS) has been applied to compare the urinary metabolic profile of samples collected within 24 hours of birth from preterm neonates with and without early-onset sepsis. In untargeted metabolomics a large number of metabolites are measured with the aim to obtain a full description of the metabolic composition of the samples. The raw data must be heavily processed and the obtained data structures are complex. Noise and redundancy are present in the data, and artefacts could be generated during the analytical session. For these reasons, a well-defined workflow for data pre-processing is essential to guarantee accurate and high quality data, and suitable statistical approaches must be applied to avoid false discovery. In this study, a new data correction procedure to normalize the data during the analytical session based on quality controls with different concentrations, and a new method to discover relevant and irrelevant features from the extracted data have been applied.

SURFACE MODIFICATIONS OF MESENCHYMAL STROMAL CELL-DERIVED EXTRACELLULAR VESICLES ENHANCE THEIR ANTI-INFLAMMATORY ACTIVITY

Grassi M, Tolomeo AM, Giarraputo A, Scarpa M, Incendi D, Castagliuolo I, Porzionato A, Jurga M, Pozzobon M, Muraca M

Mesenchymal stromal cell (MSC)-derived extracellular vesicles (EVs) are being increasingly tested as immune modulatory agents. However, insufficient anti-inflammatory activity was reported in some animal models. Annexin A5 (An5) is a physiological molecule with tolerogenic properties. Since An5 can bind to negatively charged phosphatidylserine on the surface of EVs, we reasoned that this molecule could enhance the anti-inflammatory effects of MSC-EVs and we tested this hypothesis both in vitro in a macrophage polarization assay and in vivo in a murine model of colitis. Clinical-grade human Wharton jelly-derived MSC-EVs were obtained from The Cell Factory (Esperite NV, Niel, Belgium) and were quantified by Resistive Pulse Sensing analysis before and after An5 labelling. An5 was bound to human MSC-EVs using a commercial kit. For the in vitro assay, macrophages were isolated from C57BL6/j mouse bone marrow and activated by IFNand LPS. Then, 5,0E +05 macrophages were treated with PBS (vehicle only), 5,0E+08 hMSC-EVs, 5,0E+08 hMSC-EVs added with 2 ug An5 or with free An5. EV uptake by macrophages was evaluated by labelling the nanoparticles with CMTMR. For the in vivo assay, mice with dextran sulphate sodium-induced colitis (3% in drinking water for 6 days) received via enema 2.0E+10 hMSC-EVs, 2.0E+10 hMSC-An5-EVs, 2.0E+10 fibroblast (F)-derived EVs or free An5. In vitro, activated macrophage incubation with both hMSC-EVs and hMSC-An5-EVs significantly increased both the iNOS2/Arg1 mRNA expression ratio and iCAM/CD206 markers ratio, suggesting a shift to a more tolerogenic phenotype. In vivo, only administration of hMSC-An5-EVs improved both clinical and morphometric/histological scores. hMSC-EVs, F-EVs and free An5 administration had no effect on colitis severity. Moreover, hMSC-An5-EVs induced the mRNA expression of the anti-inflammatory cytokines IL-10 and Tgfb1 in colon mucosa and promoted polarization of mucosal macrophages to a more tolerogenic phenotype. An5 binding enhanced the anti-inflammatory activity of hMSC-EVs in vivo. Additional studies are required to investigate the mechanisms of action of these surface-modified nanoparticles.

3D BIOPRINTING AS THE NEW ERA OF TISSUE ENGINEERING IN SKELETAL MUSCLE REGENERATION

Boso D, Maghin E, Carraro E, Todros S, Pavan P, Piccoli M

Tissue engineering (TE) was defined by Langer and Vacanti in early 90s as "an interdisciplinary field which applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue formation". During last decades, TE opened wide horizons for tissue and organ replacement overcoming drawbacks related to organ transplantation as donor shortage or need of immunosuppressive therapies. TE approaches were developed to effectively obtain a 3D living construct that is structurally, mechanically and functionally equal to the tissue to be replaced in vivo. The 3D in vitro model should be properly designed to recapitulate the particular conditions that are intended to be mimicked in vivo, such as the tissue-specific environment in which cells live and their interaction with the surrounding extracellular matrix (ECM). The recent evolution of TE is represented by 3D bioprinting. Our team is exploiting this new groundbreaking technique to develop a functional skeletal muscle tissue for treating large defects as the congenital diaphragmatic hernia. The bioprinting involves layer by layer deposition of biomaterials, living cells and controlled motor systems for creating complex and precise structures. In our lab, we are working to produce a new cell-laden bioink starting from porcine diaphragm decellularized ECM and human muscle precursor cells to fabricate a 3D printed diaphragm. This approach allow us to produce batch-to-batch identical scaffolds that will be repopulated with the cells obtained directly from each single patient, pursuing the goal of an advanced personalized regenerative medicine in a fast, automated and on demand way.

ALVEOLAR RHABDOMYOSARCOMA 3D MODEL DEVELOPMENT TO MIMIC PHYSIOLOGICAL CELL-ECM INTERACTION WITH FOCUS ON INTEGRINS

Saggioro M, D'Agostino S, Rampazzo E, Muraro MG, Crotti S, Zanon C, D'Aronco S, Corallo D, Germano G, Agostini M, Aveic S, Bisogno G, Gamba P, Martin I, Pozzobon M

Alveolar Rhabdomyosarcoma (ARMS) is one of the most aggressive pediatric soft tissue sarcoma with a high tendency to metastasize. New models to study cell-extracellular matrix (ECM) interactions are needed. In this work, we considered 2 different approaches to establish a 3D culture of ARMS that mimics the interaction between ECM and cell adhesion molecules with specific attention on integrins. In the first approach, we used a xenogenic tumor mass derived from the injection of ARMS cell line (RH30) in immunodeficient mice, we optimized a decellularization protocol of the tissue and tested two different strategies of recellularization: static and dynamic. These cell-seeding strategies into ECM, that maintained architectural and molecular features of the original tissue, are considered a "conservative approach". In parallel, to gain more knowledge on ARMS protein ECM composition; we develop a "deconstructive approach" where the ECM ultra-structure was not preserved since the fresh tissue was digested for proteins extraction. Decellularization and proteomic analysis were performed and a list of the most present proteins in ARMS ECM was identified. Two different scaffolds were developed to study the influence of these proteins in cell migration: one based on protein enriched hydrogel and the other based on commercially available Ultrafoam collagen I sponge. Preliminary results on hydrogel showed 3D cell distribution and long term cell survival, while up-regulation of ITGA5 and CXCR4 was detected using Ultrafoam in dynamic perfusion bioreactor U-Cup. We then studied the role of ITGA5 in motility with migration and invasion assays, wound healing assay of ITGA5 wild type and ITGA5 siRNA in RH30 cells. In parallel, cells expressing high levels of ITGA5 (RH30 ITGA5high) were isolated from cells expressing low levels of ITGA5 (RH30 ITGA5low). Injections of these two cell populations in Zebrafish allowed to study the distribution in the vascular system and the frequency of extravasation, while injection in immunodeficient mice were used to evaluate the in vivo tumour growth. This study is the first that developed a rhabdomyosarcoma model with particular attention toward ECM protein composition and cell cross talk. The final goal is to deep the knowledge on how ARMS cells relate and interact with their microenvironment, in particular with ECM, and if this interaction has a role on metastatic migration. This could identify new therapeutic targets to hit in order to improve the outcome of metastatic ARMS patients.

IN VITRO DYNAMIC CONDITIONS AMELIORATE 3D DIAPHRAGM-LIKE TISSUE GENERATION AND MATURATION

Carraro E, Maghin E, Boso D, Caccin P, Giagante M, de Cesare N, Pavan P, Piccoli M

Congenital diaphragmatic hernia (CDH) is a severe birth defect that causes the abnormal closure of the diaphragmatic muscle during foetal life. Current treatments include the application of synthetic patches, which often leads to hernia recurrence due to the materials' lack of growth and integration with the native tissue. In this perspective, an innovative approach is offered by tissue engineering. Recently we have demonstrated the advantages of using decellularized extracellular matrix (dECM) in vivo to overcome the above-mentioned drawbacks. To raise dECM resistance and performance, we focused on recellularization of dECM with paediatric human muscle precursor cells (phMPCs) to generate an in vitro tissue-like construct able to augment patch integration and muscle regeneration in vivo. Today, one of the main challenges in skeletal muscle 3D models is to achieve the correct cell maturation and alignment to obtain functional constructs. Combining engineering and biological knowledges we made a specific bioreactor platform able to mimic the physiological movement of the diaphragmatic muscle. We tested different protocols of mechanical radial strain individually to dECM and phMPCs, evaluating the integrity and mechanical properties of the matrix, and the myotubes alignment, respectively. Then, we recellularized the diaphragm and cultivated in dynamic conditions. We mechanically stimulated the 3D constructs for 21 days, demonstrating both the maintenance of proliferating cell pool and the enhanced myotubes alignment and maturation compared to the standard static condition. In conclusion, our bioreactor is a tunable platform to study dECM and cell behaviour and to improve cell homing, differentiation and alignment in a 3D diaphragmatic muscle model.

DEVELOPMENT OF PORCINE-DERIVED DIAPHRAGMATIC DECELLULARIZED EXTRACELLULAR MATRIX SCAFFOLDS FOR CONGENITAL DIAPHRAGMATIC HERNIA REPAIR: TOWARD THE CLINICAL TRANSLATION

Maghin E, Carraro E, Boso D, Giagante M, Caccin P, de Cesare N, De Coppi P, Pavan P, Piccoli M

Congenital diaphragmatic hernia (CDH) is a severe malformation of the developing diaphragm. An innovative approach for the repair of this congenital defect is the use of a portion of decellularized diaphragm extracellular matrix (dECM) to close the large muscle defects, as alternative solution to the gold standard treatments (e.g. synthetic patches). Recently we have demonstrated the advantages of using tissue specific decellularized extracellular matrix (dECM) in vivo in a mouse model. Here we translate our approach to obtain porcine-derived dECM as relevant acellular scaffold for future preclinical studies and clinical translation. We are working on different strategy (perfusion/agitation) for the decellularization of porcine diaphragm that will allow us to use this scaffold for different purposes. From one hand, the perfusion of reagents through the muscle vascular tree will allow us to use this scaffold as intact acellular patch; from the other hand, decellularization obtained after reduction in small parts of the original diaphragm would be useful as starting material for specific ECM derived bioink. We have characterized both the methods of decellularization: histological and biochemical validation confirmed successful removal of cells and DNA, as well as the preservation of native ECM components and structural architecture. Moreover, we performed pilot experiments of scaffold repopulation using human muscle precursor cells to explore the possibility of recreating a xenograft-derived muscle. Starting from Piglet diaphragm dECM we also obtained a specific bioink in order to explore a different approach to fabricate a 3D bioprinted diaphragm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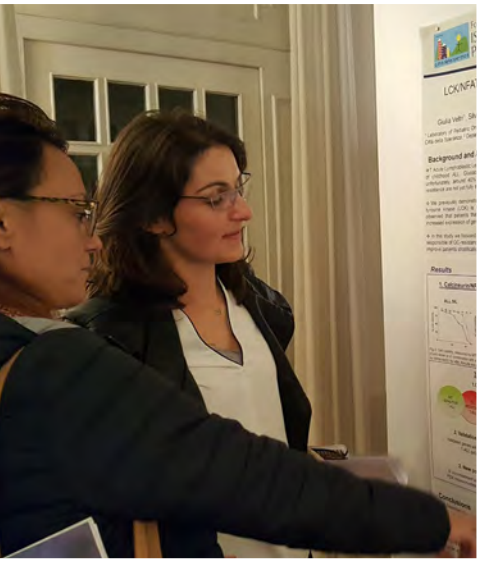


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7
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Background and Aim
Methods
Results
8
Evaluation of total and tumour-derived cfDNA in pediatric non-Hodgkin lymphomas


Mechanism of HSC contribution to microgliosis


1. Expression of CaV1.2
3. CaV1.2 agonist (antagonist + Lerco-HCl
4. Wash/out experiment

Scientific Prizes

“Famiglia Masello in memoria di Rita Masello e Massimo Zilio” for the two best oral communications

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“Elisa Camporese” and “Matteo Fochesato” for the three best posters

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