PhD Course in Translational and Molecular Medicine – DIMET XXXVI cycle, a.y. 2020/2021

n.1 scholarship funded by Department of Excellence, PREMIA project, linked to the research project:" *Defining the molecular landscape of reparative cardiac progenitor cell by MS-imaging and advanced MS-proteomics approaches*", curriculum international with Surrey

Tutor: *Prof. Fulvio Magni/Prof. Paola Campagnolo*

Abstract

Epicardial cells form an epithelial monolayer located in the outmost layer of the heart and are known to participate to the repair process after myocardial infarction. Harnessing their activation and differentiation might provide a novel route for myocardial infarction resolution. The pharmacological compounds able to stimulate their activation and the intracellular molecular pathways involved have not yet been elucidated. We have established an ex vivo model based on a thin 3D slice of the heart that can manipulated for several days in culture, providing the perfect platform for pharmacological studies. Using state-of-the- art molecular visualization tools such as MassSpec imaging and proteomic analysis, the project will be focused on the study the protein profile of epicardial progenitor cells in the heart following treatment with novel pharmacological compounds aiming to identify the specific compounds eliciting a reparative response in the epicardium and the underling mechanisms.

n.1 sholarship funded by Department of Biotechnology and Biosciences linked to the research project linked to the project:" *Defining the transcriptional signature in the tumor microenvironment at single cell level resolution*".

Tutor*: Prof. Francesca Granucci*

Abstract

It is nowadays well demonstrated that innate and adaptive immune responses play a fundamental role in tumorigenesis; tumor microenvironment can be very heterogenous in terms of the immune infiltrate abundance, composition and response. The project therefore aims at characterize tumor microenvironment at high resolution, through a combined multi omics approach, based on single cell RNA-seq of immune infiltrate , RNA-seq, chromatin RNA-seq and advanced bioinformatics to uncover improved biomarkers for patient diagnosis and cure selection.

N.2 sholarships funded by Istituto di Tecnologie Biomediche del Consiglio Nazionale delle Ricerche

No. 1 position

Title
Dissecting the role of chromatin conformation in muscular diseases

Abstract

Lamin A is a component of the inner nuclear membrane that, together with epigenetic factors, organizes the genome in higher order structures required for transcriptional control. Mutations in the lamin A/C gene cause several diseases belonging to the class of laminopathies, including muscular dystrophies and progeroid syndromes. Nevertheless, molecular mechanisms involved in the pathogenesis of lamin A–dependent diseases are still largely unknown. We found that muscle stem cells lacking lamin A/C redistribute the Polycomb group (PcG) of proteins, transcriptional factors and key regulators of cell identity, directly involved in muscular homeostasis. This leads to lack of muscle stem cell identity and senescence, determining a premature exhaustion of the muscular stem cell niche. We believe that the genome structure alterations observed during physiological and pathological senescence are dependent on the natural Lamin A/C destabilization and a subsequent Lamin/PcG axis dysfunction. We will extend the study to distinct muscular and cardiac cell populations to unravel the contribution of specific cell types to sarcopenia, heart defects and muscular dystrophy. The identification of epigenetic defects that contribute to disease emergence could open up new scenarios and could indicate time frames in which it is possible to intervene to correct defective molecular pathways.

No. 2 position

Title
Study of chromatin accessibility in health and disease.

Abstract

In recent years, the widespread adoption of experimental techniques based on high-throughput sequencing (NGS) has been instrumental in advancing the knowledge of epigenome structure and function. We developed a novel high-throughput sequencing based method to map lamina associated heterochromatin regions: the SAMMY-seq (Sequential Analysis of MacroMolecules accessibilitY). SAMMY-seq technology relies on the sequential isolation and sequencing of multiple chromatin fractions, enriched for differences in accessibility. We applied SAMMY-seq on several physiological and pathological conditions, in human and mouse, including prostate and colon cancer, muscle pathologies and the immune system. In this project, using bioinformatic tools, we will describe the chromatin accessibility dynamics in distinct models and we will study the role of chromatin structural changes in transcriptional dysregulation and pathology progression. In parallel, we will developed new tailored bioinformatic analysis to improve the use of technology and the interpretation of biological significance of observed data.

n.2 sholarships funded by Fondazione Istituto Nazionale di Genetica Molecolare – INGM

No. 1 position

Title
The role of DNA transposable elements (TEs) in shaping human T lymphocytes identity and

plasticity in immunity and cancer.

As part of the project FRRB n. CP2_12/2018 "A regional oncology network addressing the emerging problem of colorectal cancer in young individuals using an integrative omics approach to decipher mechanisms of cancer immunoediting as candidate targets of new therapies (Acronim: IANG-CRC)".

HOSTING INSTITUTION, LABORATORY and TUTOR: *Fondazione Istituto Nazionale di Genetica Molecolare-INGM, Genome Biology lab, Dr. Beatrice Bodega*

Project

In our lab we are interested in understanding the function of DNA repetitive elements in the epigenetic regulation of the transcriptional response of the cell. In particular, we are asking whether DNA repeats could influence the inter-individual variability between humans in terms of response to environmental cues, adaptation and predisposition to human diseases. DNA repeats cover almost 70% of the human genome [1], and their function was largely ignored for decades. Transposable elements (TEs) (mobile genetic DNA elements) account for the 45% of the genome and are nowadays robustly emerging as novel key molecules acting at different level in the cell type specific genome regulation, co-participating and possibly increasing tissue-specific transcriptional complexity [2-4]. Nevertheless, the study of TEs using next generation sequencing (NGS) approaches pose many computational challenges, in particular due to the ambiguity of mapping the short reads on the genome due to multiple copies of the TE sequences; this is further complicated by the high sequence homology among TEs. In the current project, with the use of integrated multi-omics approaches (RNA-seq; whole genome sequencing, chromatin RNA-seq, etc) we intend to decipher at high resolution tumor microenvironment in order to define the tumor specific transcriptional signature that possibly contributes to the cell-cell networks between malignant and immune cells within tumor ecosystem. Moreover, we aim to identify novel and non-coding transcript that represents an original resource to find targetable RNA molecules, never characterized so far in the tumor immunology field, that could be used as adjuvants.

Bibliography

1. Lander, E.S., et al., Initial sequencing and analysis of the human genome. Nature, 2001. 409(6822): p. 860-921.
2. Chuong, E.B., N.C. Elde, and C. Feschotte, Regulatory activities of transposable elements: from conflicts to benefits. Nat Rev Genet, 2017. 18(2): p. 71-86.
3. Hall, L.L., et al., Stable C0T-1 repeat RNA is abundant and is associated with euchromatic interphase chromosomes. Cell, 2014. 156(5): p. 907-19.
4. Kapusta, A., et al., Transposable elements are major contributors to the origin, diversification, and regulation of vertebrate long noncoding RNAs. PLoS Genet, 2013. 9(4): p. e1003470.

No. 2 position

Title
Circulating tumor-derived T lymphocytes (CTDL) from patients' blood: role in the response to cancer immunotherapy and disease course.

As part of the project MERCK "Novel targets of tumor-infiltrating CD4+ regulatory T cells for immunotherapy".

HOSTING INSTITUTION, LABORATORY and TUTOR: *Fondazione Istituto Nazionale ctdi Genetica Molecolare-INGM, Genome Biology lab, Dr. Beatrice Bodega*

Abstract:

It is now established that the interaction of cancer cells with other cells and tissues present in the tumor microenvironment can nfluence tumor growth. Of utmost relevance is the interaction network between cancer cells and the immune system, with a particular focus on immunosuppressive mechanisms, including regulatory T lymphocytes (Treg), immunosuppressive cytokines / chemokines and immunological checkpoints that inhibit anti-tumor immune responses. Recent studies on the role of the adaptive immune system in the tumor microenvironment have highlighted gene signatures associated with T infiltrating tumor lymphocytes. Recently, our institute carried out a transcriptomics study in CD4 + lymphocytes (Th1 and Th17) and Treg lymphocytes present in the lymphocytic infiltrate of colorectal cancer (CRC) and in non-small cell lung cancer (NSCLC). The study selected a highly suppressive tumor-infiltrating Treg population, over-expressing a molecular signature of approximately 320 highly specific genes (De Simone M., et al, Immunity, 45: 1135, 2016). A new concept stemmed from our previous study is that rare subsets of tumor-derived circulating lymphocytes (CTDL) that maintain some molecular characteristics of intra-tumor lymphocytes may influence the diseases outcome and response to cancer therapies. This study proposes to analyze CTDL of cancer patients undergoing cancer therapies, with particular interest for immunotherapy with antibodies against immunological checkpoints, to identify gene expression profiles and molecular markers useful to monitor cancer progression.

n.3 sholarships funded by Fondazione M. Tettamanti M. De Marchi Onlus

No.1 position

Title
Oncogene induced senescence in ETV6/RUNX1 pre-leukemia: role and targeting

Abstract

The t(12;21)(p13;q22) is the most frequent chromosome translocation in pediatric B cell precursor acute lymphoblastic leukemia (BCP-ALL) and it leads to the ETV6-RUNX1 fusion gene, which encodes

for an aberrant transcription factor with constitutive repressive function. ETV6-RUNX1 expression causes the formation of a clinical silent pre-leukemic progenitor, able to persist in the organism for many years and to be more susceptible to additional mutations, that are needed in order to convert to frank disease. It is important to identify and understand the molecular mechanisms sustaining the pre-leukemic phase in order to develop novel therapeutic treatments against them, with the aim to eradicate the pre-leukemic clone to avoid the leukemia development and its relapse. In previous studies, using an ETV6-RUNX1+ pre-leukemic model in vitro, we showed that ETV6-RUNX1 fusion gene was able to induce the inhibition of the cell cycle, the accumulation of p53 protein (one of the principal proteins involved in the induction of senescence) and alterations of its post-translation modifications. The ETV6-RUNX1+ cells also show the strong activation of p53-dependent cell cycle arrest pathway, whereas the p53-dependent apoptosis is blocked. Moreover, the pre-leukemic cells have a growth advantage if subjected to stimuli leading to DNA damage. Based on this results, it is necessary to further investigate whether the fusion gene is able to induce a cellular state defined as oncogene induced senescence (OIS), and to better elucidate the role of p53 pathway in the induction and maintenance of this state. Especially, we will focus on the function of phosphorylation of serine 392-p53 (which is absent in pre-leukemic cells model) in the induction of p53-dependent apoptosis and on the p53 sub-localization in the cells. We will mimic the transition to leukemic phase by blocking CDKN2A gene, which is up regulated in pre-leukemic cells model (while its deletion is a typical second hit that can be found in leukemic cells), using RNA Interference, in order to evaluate the role of this gene in ETV6-RUNX1-induced senescence. Also, we will verify if the growth advantage of the pre-leukemic cells observed in presence of a inducer of DNA double strand breaks is due to a higher resistance to cell death in response to DNA damage. Moreover, we will test different inhibitors of molecules that can be responsible of this resistance to verify if the treatment can cause pre-leukemic cells apoptosis. In addition, we will use an in vivo pre-leukemic mouse model to confirm all the obtained results. All this information will help us understanding how to avoid the accumulation of further genetic alterations in pre-leukemic cells and how to eliminate these cells from the organism before their transformation into leukemic cells.

No.2 position

Title

Engineered CAR T cells for efficient multi targeting of B-cell acute lymphoblastic leukemia: preventing immune evasion and post-CAR T relapses.

Abstract

Background: B-cell precursor acute lymphoblastic leukemia (B-ALL) is a malignancy which affects both children and adults and, currently, is the most common childhood cancer. Although B-ALL can usually be treated with chemotherapy and bone marrow transplants (BMT), <50% of adult patients survive >5 years and about 15% of children eventually relapse. Relapsed and refractory (r/r) adult and childhood B-ALL patients, have significant unmet medical needs. Adoptive transfer of Chimeric

Antigen Receptor (CAR) T cells represents a revolutionary new cancer therapy. CAR T cells have been used for patients with high-risk hematological malignancies and have shown impressive results in r/r B-ALL, reaching MRD-negative complete remission (CR) rates in 63 to 93% at 1 month and overall survival (OS) of 60 to 80% at 6 months were reported in multiple studies among pediatric and adult patients with r/r B-ALL, whose chance of survival was 10% to 30% with conventional therapies [1-7]. We recently demonstrated the pre-clinical efficacy of donor-derived non-viral cytokine induced killer (CIK) cells transfected with the Sleeping Beauty (SB) transposon CD19CAR (CARCIK-CD19). CARCIK-CD19 cells exerted potent antitumor activity in immunodeficient mouse models engrafted with tumor cells from patients with high-risk leukemia. Infused cells were readily detectable in the BM and spleen and persisted in vivo for 3 months while proving to be safe and well tolerated in a biodistribution/toxicity study [8]. Based on these data we initiated a phase I/II study (EudraCT 2017-000900-38, ClinicalTrials. gov ID NCT03389035), demonstrating the feasibility, safety and efficacy of CARCIK-CD19 cells in pediatric and adult B-ALL patients relapsed after hematopoietic stem cell transplantation (HSCT). Although CD19 is expressed by essentially all cases of B-ALL at clinical presentation [9-10] relapses with loss or diminished cell-surface expression of CD19 are increasingly recognized as a cause of treatment failure [3, 11–13]. Preliminary data from our laboratory has identified the role of BAFF/BAFF-R pathway in supporting B-ALL cell survival and contributing to resistance of leukemic clone to therapy in BM microenvironment [14]. Specifically, we found that BAFF-R is highly expressed on BCP-ALL diagnostic samples and is preserved during drug treatment and at relapse, further supporting his role on blast survival. These findings led to the design of a CAR T cell strategy targeting BAFF-R [15] and to its combination with anti-CD19 approach, demonstrating superior activity also towards CD19-negative B-ALL relapsed leukemia.

<u>Aims</u>: To validate the multiple targeting of BAFF-R and CD19 molecules as novel approach to prevent post-CAR T relapses. Furthermore, to identify and target simultaneously additional pathways of immune evasion according to the results emerging from profile transcriptional signature of CAR T cells in interaction with tumor cells.

<u>General Plan</u>:

1) Optimization of the CAR design for optimal activity of engineered T cells, including modulation of CAR binding affinity and modification of spacer length according to antigen density and target epitope accessibility, respectively. 2) The activity of the multitargeting approach will be validated in vitro and in vivo in xenograft models.

<u>Experimental plan</u>:

1) To generate an optimized anti-BAFFR scFv by exploiting different CAR design.

2) To clone different configuration of Sleeping Beauty pT4 vectors containing BAFFR scFv in combination with additional scFv.

3) Cell phenotype, fold increase, population doubling, and expression of CARs will be evaluated in cells engineered by SB100X transposase.

4) To analyze in vitro CAR T cell activity towards ALL cell line and primary leukemic cells by performing: standard cytotoxic assays, measurement of cytokine production by intracytoplasmic staining and ELISA/Luminex systems, proliferation with CSFE flow cytometric methods, analysis of vector copy number and of SB integration near cancer gene by RT-PCR and SLIM-PCR, respectively.

5) In vivo efficacy of CAR+ T cells will be evaluated in NSG mice, previously intravenously (i.v.) injected with ALL cell lines or with patient samples.

1. Davila ML et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 2014;6:224ra225.
2. Grupp SA et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 2013;368:1509-1518.
3. Lee DW et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 2015;385:517-528.
4. Maude SL et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014;371:1507-1517.
5. Gardner R et al. Acquisition of a CD19 negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T cell therapy. Blood 2016; 127, 2406–2410.
6. Turtle CJ et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. J Clin Invest 2016;126:2123-2138.
7. Majzner eta l. Clinical lessons learned from the first leg of the CAR T cell journey. Nat Med. 2019; 25:1341-1355.
8. Magnani CF et al. Preclinical Efficacy and Safety of CD19CAR Cytokine-Induced Killer Cells Transfected with Sleeping Beauty Transposon for the Treatment of Acute Lymphoblastic Leukemia. Human Gene Therapy 2017;29: 602-613.
9. Raponi, S. et al. Flow cytometric study of potential target antigens (CD19, CD20, CD22, CD33) for antibody-based immunotherapy in acute lymphoblastic leukemia: analysis of 552 cases. Leuk. Lymphoma 2011; 52: 1098–1107.
10. Lucio, P. et al. BIOMED-I concerted action report: flow cytometric immunophenotyping of precursor B-ALL with standardized triple-stainings. BIOMED-1 concerted action investigation of minimal residual disease in acute leukemia: international standardization and clinical evaluation. Leukemia 2001; 15: 1185–1192.
11. Sotillo E. et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov. 2015; 5: 1282–1295.
12. Grupp, S.A. et al. Durable remissions in children with relapsed/refractory ALL Treated with T cells engineered with a CD19-targeted chimeric antigen receptor (CTL019). Blood 2015; 126. abstr. 681
13. Gardner, R. et al. Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. Blood 2016;127: 2406–2410.
14. Fazio G et al. TNFRSF13C (BAFFR) positive blasts persist after early treatment and at relapse in childhood B-cell precursor acute lymphoblastic leukaemia. Br J Haematol. 2018;18:434-436.
15. Turazzi N, et al. Engineered T cells towards TNFRSF13C (BAFFR): a novel strategy to efficiently target B-cell acute lymphoblastic leukaemia. Br J Haematol. 2018;182:939-943.

No.3 position

Title
Analysis of the molecular and cellular mechanisms underlying the impaired angiogenic ability of mesenchymal stromal cells derived from Shwachman-Diamond syndrome patients.

Abstract

Shwachman–Diamond syndrome (SDS, MIM 260400) is a rare autosomal recessive bone marrow (BM) disorder mainly characterized by neutropenia (found in 88-100% of patients), exocrine pancreatic insufficiency, and skeletal abnormalities. Pancytopenia occurs in 10-65% of cases and, similarly to other BM syndromes, SDS patients show an increased risk of myelodisplastic syndrome and malignant transformation. In light of the emerging role of mesenchymal stromal cells (MSCs) in the regulation of BM homeostasis, we previously demonstrated that in vivo implanted cartilaginous pellets derived from SDS-MSCs were unable to generate a complete haematopoietic stem cell niche. Moreover, we demonstrated that SDS-MSCs showed a defective in vitro ability to form correct tube networks after angiogenic stimuli, displaying a marked decrease in VEGFA expression .
Thus, in order to elucidate the potential role of MSCs in vascular and haematological defects typical of SDS patients, two will be the aims of the project:
I) to deeply investigate the molecular and cellular mechanisms underlying the impaired angiogenic capability of SDS-MSCs;
II) to evaluate the possible link between aberrant angiogenesis and the haematological abnormalities observed in SDS patients.
The identification of the mechanisms underlying the defective angiogenic ability of SDS-MSCs could pave the way to new highly targeted strategies for the treatment of this rare BM syndrome.

n.2 sholarships funded by Fondazione Telethon

No.1 position

Tutor: *Marta Serafini*
Co-Tutor: *Anna Villa*

Title
Dissection of immune dysregulation in RAG deficiency and development of novel gene correction strategies

Abstract

Different RAG mutations may cause distinct clinical and immunological phenotypes, with some overlap. Consequently, RAG mutations have been associated with severe combined immunodeficiency with absence of T and B cells (T- B- SCID), Omenn syndrome (OS) with presence of oligoclonal and activated T cells infiltrating and damaging target tissues, atypical SCID (AS) with residual presence of oligoclonal T (and occasionally, B) cells, and combined immunodeficiency

associated with granulomas and/or autoimmunity (CID-G/AI)1. Defining cellular and molecular bases of the severity of immune dysregulation and testing gene correction approaches represent the objects of this PhD project. To this end, taking advantage of hypomorphic RAG1 and RAG2 mouse models2 and samples obtained from patients carrying hypomorphic RAG defects, we will characterize the mechanisms leading to defective immune responses. Our research project aims at understanding the molecular mechanisms through which RAG gene mutations determine immune dysregulation. We will perform extensive use of sequencing and trascriptomic analysis to asses to which extent hypomorphic RAG mutations impair regulation and/or function of T and B cells. Novel conditioning strategies using monoclonal antibodies will be tested and compared to the classical body irradiation3. In parallel to these studies, we will compare efficacy and safety of two novel cell therapy approaches: gene addition vs gene editing approach. We will test RAG1 gene therapy approach using a novel lentiviral vector carrying the human RAG1 codon-optimized cDNA in RAG1 knock-out and in two RAG1 mouse models2 mimicking AS and CID-G/AI and when available in CD34+ cells obtained from RAG1 patients. Results from these studies will be compared with gene-editing strategy performed in human RAG1 CD34+ cells. Human artificial thymic organoid (ATO) culture will be used to asses in vitro differentiation of human CD34+ cells corrected with gene addition vs gene editing approaches and their ability to overcome T cell differentiation defect.

Skills to be acquired by the student*:*
Animal handling, transplantation protocol, flow cytometry, digital PCR, in vitro cell functional assays, ELISA/Multiplex and ELISpot assays, BCR/TCR sequencing, immunocytochemistry, molecular biology, gene editing, VDJ recombination biochemistry, viral (lenti and AAV) transduction, clonogenic assays, hematopoietic stem cell isolation and characterization, transcriptomic analysis.

References
1) Villa A, Notarangelo LD.RAG gene defects at the verge of immunodeficiency and immune dysregulation. Immunol Rev. 2019 Jan;287(1):73-90. doi: 10.1111/imr.12713.
2) Ott de Bruin LM, Bosticardo M, Barbieri A, Lin SG, Rowe JH, Poliani PL, Ching K, Eriksson D, Landegren N, Kämpe O, Manis JP, Notarangelo LD. Hypomorphic Rag1 mutations alter the preimmune repertoire at early stages of lymphoid development. Blood. 2018 Jul 19;132(3):281-292. doi: 10.1182/blood-2017-12-820985. Epub 2018 May 9.
3) Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA, Czechowicz A, Kfoury Y, Ruchika F, Rossi DJ, Verdine GL, Mansour MK, Scadden DT.Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. Nat Biotechnol. 2016 Jul;34(7):738-45.

No.2 position

TUTOR: *Prof.ssa Marta Serafini*
Co-TUTOR: *Prof.ssa Giuliana Ferrari*

Title

Targeting of the altered interactions between hematopoietic stem cells and the bone marrow niche in beta-thalassemia

Abstract

Beta-thalassemia (BTHAL) is a severe congenital anemia caused by reduced or absent production of the beta-globin chains of the adult hemoglobin. Correction of the disease can be achieved by allogeneic bone marrow (BM) transplantation or by autologous transplantation of genetically corrected cells upon gene therapy. As a major achievement of many years of basic and translational research on BTHAL (1-4), we carried out a gene therapy clinical trial at San Raffaele Hospital (5). On the other side, our recent studies unraveled unexplored cellular and molecular processes of BTHAL pathophysiology (6, 7).

BTHAL BM niche is a stress environment due to secondary alterations to the primary genetic defect, as marrow overstimulation, iron overload and hormonal factors. We hypothesized that this altered milieu may interfere with the maintenance of hematopoietic stem cells (HSCs), with potential impact on therapeutic BM transplantation and gene therapy approaches. We have recently discovered alterations in BM niche of BTHAL Hbbth3/+ mice, affecting cycling activity and repopulating potential of HSCs (7). This previously ignored defect of BTHAL HSCs is caused by an impaired crosstalk with both stromal and hematopoietic BM niche components, confirmed also in BTHAL patient-derived samples (6, 7).

Defining the cellular and molecular bases of the altered HSC function and HSC-niche interactions in BTHAL is the objective of this project with the final aim of targeting the identified players and restoring BM niche and HSC function.

The biological mechanisms to be explored are: 1) the crosstalk of HSCs and stromal cells; 2) the interactions of HSCs and mature hematopoietic cells, with key role in HSC maintenance.

Specific aims of the project are:

1) to characterize cellular composition of BM stromal niche (mesenchymal stromal cells, osteoblasts, endothelial cells) and mature hematopoietic cells (megakaryocytes, macrophages, erythroid precursors) and their reciprocal interactions by histological, immunophenotype and gene expression analyses;

2) to evaluate the molecular players involved in the altered HSC-niche crosstalk by transcriptomic profiling and in vitro modeling;

3) to target the identified cellular and molecular mechanisms in order to rescue BTHAL BM niche and HSC function in vivo.

These studies will be conducted by using both Hbbth3/+ BTHAL mice and human cells derived from patients. The student will acquire several cellular and molecular techniques from immunophenotype analyses to in vitro and in vivo functional assays, histological analyses and gene expression profiling. The student will be followed in acquiring specific skills in analysis and critical interpretation of research data.

REFERENCES:

(1) Miccio A., Cesari R., Lotti F., Rossi C., Sanvito F., Ponzoni M., Routledge SJ., Chow CM., Antoniou MN., Ferrari G. (2008). In vivo selection of genetically modified erythroblastic progenitors leads to long-term correction of beta-thalassemia. Proc Natl Acad Sci U S A 105:10547-52.

(2) Roselli EA., Mezzadra R., Frittoli MC., Maruggi G., Biral E., Mavilio F., Mastropietro F., Amato A., Tonon G., Refaldi C., Cappellini MD., Andreani M., Lucarelli G., Roncarolo MG., Marktel S., Ferrari G. (2010). Correction of ß-thalassemia major by gene transfer in hematopoietic progenitors of pediatric patients. EMBO Mol Med. 2:315- 328.

(3) Lidonnici, M.R., Aprile, A., Frittoli, M.C., Mandelli, G., Paleari, Y., Spinelli, A., Gentner, B., Zambelli, M., Parisi, C., Bellio, L., Cassinerio E., Zanaboni L., Cappellini M.D., Ciceri F., Marktel S., and Ferrari G. (2016). Plerixafor and G-CSF combination mobilizes hematopoietic stem and progenitors cells with a distinct transcriptional profile and a reduced in vivo homing capacity compared to Plerixafor alone. Haematologica. 2017 Apr;102(4):e120-e124.

(4) Lidonnici, M.R., Paleari, Y., Tiboni, F., Mandelli, G., Rossi, C., Vezzoli, M., Aprile, A., Lederer, C., Ambrosi, A., Chanut, F., Sanvito, F., Calabria, A., Poletti, V., Mavilio, F., Montini, E., Naldini, L., Cristofori, P., and Ferrari, G. (2018). Multiple integrated non-clinical studies predict safety of lentiviral mediated gene therapy for beta thalassemia. Molecular Therapy: Methods & Clinical Development 2018 Sep 13;11:9-28.

(5) Marktel, S., Scaramuzza, S., Cicalese, M.P., Giglio, F., Galimberti, S., Lidonnici, M.R., Calbi, V., Assanelli, A., Bernardo, M.E., Rossi, C., Calabria, A., Milani, R., Gattillo, S., Benedicenti, F., Spinozzi, G., Aprile, A., Bergami, A., Casiraghi, M., Consiglieri, G., Masera, N., D'Angelo, E., Mirra, N., Origa, R., Tartaglione, I., Perrotta, S., Winter, R., Coppola, M., Viarengo, G., Santoleri, L., Graziadei, G., Gabaldo, M., Valsecchi, M.G., Montini, E., Naldini, L., Cappellini, M.D., Ciceri, F., Aiuti, A., and Ferrari, G. (2019). Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion dependent ß-thalassemia. Nature Medicine 2019 Feb;25(2):234-241.

(6) Crippa, S., Rossella, V., Aprile, A., Silvestri, L., Rivis, S., Scaramuzza, S., Avanzini, M.A., Basso-Ricci, L., Hernandez, R.J., Zecca, M., Marktel, S., Ciceri, F., Aiuti, A., Ferrari, G., and Bernardo, M.E. (2019). Bone marrow stromal cells from beta-thalassemia patients have impaired hematopoietic supportive capacity. Journal of Clinical Investigation 2019 Feb 25;129(4):1566-1580.

(7) Aprile, A., Gulino, A., Villa, I., Beretta, S., Merelli, I., Rubinacci, A., Ponzoni, M., Marktel, S., Tripodo, C., Lidonnici, M.R., and Ferrari, G. Hematopoietic stem cell function in ß-Thalassemia is impaired and is rescued by targeting the bone marrow niche (2019). Blood. 2020 Apr 28:blood.2019002721. doi: 10.1182/blood.2019002721. Online ahead of print.

Posizioni con Percorso Executive

n.1 posizione riservata ai dipendenti presso Fondazione IRRCS Istituto Neurologico "Carlo Besta" dal titolo:" *Microbubble distribution in the brain: a real-time map to optimize imaging and treatments in central nervous system diseases*".

Profilo
dipendente

Progetto

The contrast-enhanced ultrasound technique CEUS can provide us a useful tool to us to achieve the real- time study of lesion contrast enhancement, vascularity of focal lesions during the different dynamic phases, analysis of tissue perfusion and online evaluation of treatment efficacy[2]. As the ultrasound (US) contrast agents, Microbubbles(MBs) consist of air or low solubility complex gas encapsulated in a layer of proteins or polymers. They can be carried into the smallest capillaries and across the lungs, allowing imaging of the whole vasculature using a venous injection. In neurological conditions, MBs associated with US are currently used as an imaging technique (contrast-enhanced ultrasound technique - CEUS) and as therapeutic options (with focused ultrasound -FUS) for tissue ablation, blood-brain barrier (BBB) opening and modulation of targeted region. Importantly, the study of MBs behavior with CEUS allows to characterize vessels and brain lesions, to assess tumor removal and to plan the surgical approach, in particular highlighting the vascular structures and tissue perfusion. MBs quantitative analysis has recently being developed and employed in different clinical settings[3]. The use of intravenously injected MBs associated with focused ultrasound (MBs-FUS) is opening a frontier in treatments with unprecedented advantages in the therapeutic approaches for several brain diseases, especially brain tumors[4]. FUS allows to non-invasively create a focal spot of acoustic energy inside the body with almost no effects to the adjacent tissues. MBs-FUS has been developed for US intracranial procedures to overcome the obstacle posed by the presence of the skull which hampers important treatments [5]. Inertial or stable cavitations with MBs-FUS are produced by US interacting with MBs within tissues inducing the collapse of MBs. The interaction of circulating MBs within US is a stochastic process that depends on MBs distribution, acoustic and physiological parameters. Acoustic parameters have been investigated in vitro and in vivo, while investigations on the effect of MBs and physiological parameters remain scarce. In particular, the spatial and temporal distribution of MBs in the different districts of the human brain is still unknown. This study is aimed at fully characterizing the spatial and temporal distribution of MBs in the healthy and pathological brain in both a pre-clinical and a clinical setting and finding substitutes in the next future the use of CEUS to monitor the MBs behavior. The results of this study would allow: 1) to avoid unwanted side effects in the surrounding and along the US beam brain structures with possible neurological sequelae; 2) to expand the treated area with US (often restricted due to the possible side effects of the unknown MBs distribution); 3) to tailor sonication parameters according to the target area (different MBs distributions in the brain need different sonication parameters); 4) to provide pivotal data correlating MBs distribution dynamics along with existing cavitation data from clinical experiences, ultimately predicting MBs behavior.

References:

1. John W. Henson. Treatment of glioblastoma multiforme: a new standard. J. Arch Neurol, 2006,63(3):337-341.

2. Prada F., Perin A., Martegani A., et al. Intraoperative contrast-enhanced ultrasound for brain tumor surgery. J. Neurosurgery , 2014,74(5):542-52.
3. Lassau N., Coiffier B., Faivre L., et al. Study of intrapatient variability and reproducibility of quantitative tumor perfusion parameters evaluated with dynamic contrast-enhanced ultrasonography. J. Invest radiology,52(3): 148-154.
4. Pi Z., Huang Y., Shen Y., et al. Sonodynamic Therapy on Intracranial Glioblastoma Xenografts Using Sinoporphyrin Sodium Delivered by Ultrasound with Microbubbles. J. Ann Biomed Eng, 47(2):549-562.
5. Aryal M., Fischer K., Gentile C., et al. Effects on P-Glycoprotein Expression after Blood-Brain Barrier Disruption Using Focused Ultrasound and Microbubbles. J. PloS One, 12(1):1-15.

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n.1 posizione riservata ai dipendenti presso ASST Papa Giovanni XXIII, vinoclato al progetto:
" Sviluppo e caratterizzazione di un nuovo anticorpo bispecifico diretto contro il BCMA(CD269) per il trattamento del mieloma multiplo"

Tutor
Dr. Martino Introna

Abstract

Il BCMA (B cell membrane antigen o CD269) è una molecola specificatamente espressa sulle plasma cellule normali e sulle cellule del mieloma multiplo (MM) e della leucemia plasma cellulare (PCL). BCMA è un recettore per il fattore di crescita APRIL, implicato nel controllo di queste neoplasie. BCMA è pertanto un bersaglio ideale per nuove terapie a base di anticorpi monoclonali o bispecifici. Nel laboratorio ricevente, è stato sviluppato negli ultimi 10 anni, in collaborazione con un gruppo di ricerca francese diretto dalla Dr.essa Martine Cèrutti, un nuovo formato di anticorpo bispecifico (BsAb). Questo BsAb è tetravalente, porta un dominio Fc IgG1 pienamente competente ed è stato brevettato (EU n. 12748555.5). Sulla base di questo formato, è stato disegnato, prodotto e purificato un nuovo BsAb, BCMAxPDL1, che riconosce il BCMA, e dovrebbe attivare il sistema immune con la sua porzione Fc (IgG1), e allo stesso tempo inibire i meccanismi di inibizione dell'immunità, tramite inattivazione dell'asse PDL1-PD1 (inibitori di checkpoint immuni). Pensiamo che questo nuovo BsAb possa avere attività specifiche e sinergiche sull'immunità innata e adattativa, per un maggior efficacia sul mieloma multiplo rispetto ad altri anticorpi già in clinica contro questo tumore e che vedono ancora molti pazienti refrattari o resistenti.
Pertanto, il progetto prevede di testare in vitro a e in vivo l'attività anti-tumorale di questo BsAb BCMAxPDL1: misurazione in vitro della specificità e dell'affinità dell'anticorpo contro i suoi 2 ligandi, capacità di attivare la citotossicità cellulo-mediata da parte delle cellule NK, di attivare la lisi complemento mediata, di attivare la fagocitosi da parte dei macrofagi, di bloccare il legame di APRIL al BCMA, di bloccare l'inibizione dei linfociti T attraverso l'interazione di PDL1 con PD1. Inoltre, saranno sviluppati dei modelli animali per valutare l'efficacia del BCMAxPDL1 in vivo, in particolare topi immunocompetenti. La parte in vivo nei topi si svolgerà in collaborazione con laboratori esterni. In parallelo saranno valutati i livelli di espressione di BCMA e PDL1 su campioni clinici di MM (prelievi di midollo osseo da pazienti), usando la citometria a flusso. BCMA sarà valutato sulle cellule

neoplastiche, PD1 e PDL1 anche sulle cellule immuni del microambiente. La modulazione di BCMA e PD1/PDL1 sarà anche valutata in vitro, in particolare in risposta ad inibitori della gamma-secretasi, il BCMA essendo un bersaglio noto di questa proteasi. Possibili correlazioni tra livelli di espressione di BCMA e l'attività anti-tumorale in vitro del BsAb BCMAxPDL1 potranno essere valutate. Strategie di combinazione saranno anche testate in vitro.