3066 – DUAL REPLACEMENT OF LNC-MEG3 AND MIR-155 TRIGGER TUMOR SUPPRESSIVE ACTIVITY IN MULTIPLE MYELOMA

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Background: Multiple Myeloma is an aggressive hemopoietic malignancy with high morbidity and mortality. Although new therapeutic strategies have improved the clinical outcome, the disease is still incurable, therefore, the development of promising molecular targets becomes an urgent need. Recent evidence demonstrated a definitive tumor suppressor role of Long non-coding RNA maternally expressed gene 3 (MEG3) and miR-155 in Multiple Myeloma (MM), however, their biological role remains unclear. It has been demonstrated that both markers are downregulated in MM patients compared to healthy. Objectives: We aimed to investigate the biological effect of double hit replacement for both lnc-MEG3 and miR-155 in MM cells, and compare it with the effect of each biomarker separately. Methods: MM cells were transfected by MEG3 overexpression plasmid and miR-155 mimic, the miR-155 and lnc-MEG3 expression levels were measured by qRT-PCR. MTT assay was performed to assess the cell proliferation, cell cycle, and apoptosis were monitored by Flow cytometry analysis. Results: lnc-MEG3 and miR-155 were downregulated in MM cells. In- vitro overexpression of lnc-MEG3 and miR-155 suppresses cell proliferation, induce cell cycle arrest, and promote apoptosis, the effect was more prominent with upregulation of both markers than with each individual biomarker. Conclusion: the present data demonstrate that dual overexpression of lnc-MEG3 and miR-155 elicits a powerful dual tumor suppressor effect in MM cells, in spite they have different signaling pathways, thus providing a promising therapeutic strategy in MM patients.

3068 – LINK BETWEEN BRANCHED-CHAIN AMINO ACIDS, IRON METABOLISM AND ANEMIA – A NEW PATHOPHYSIOLOGICAL CONCEPT

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Background Branched-chain amino acids (BCAAs) are reported to influence erythropoiesis and the human iron status. This study is aimed at investigating potential interactions between blood concentrations of all three BCAAs valine, leucine and isoleucine and indicators of human iron status.

Methods Overall, 430 outpatients referred for a medical health check-up were included in this study. They underwent investigation of valine, leucine and isoleucine and biomarkers of iron metabolism (i.e., hemoglobin [Hb], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], iron, transferrin, ferritin, transferrin saturation, soluble transferrin receptor [sTfR]). Linear regression models were performed to assess possible associations between variables.

Results All three BCAAs were positively correlated with Hb, ferritin and the sTfR (r-values: 0.145 – 0.382; P-values: <0.001 – 0.003). The strongest correlation was observed between valine and Hb ($r = 0.382$; P-value < 0.001). Linear regression models showed a statistically significant influence of all three BCAAs on Hb and ferritin (-coefficients: 0.173 – 0.351; all P-values: < 0.001). Seventeen patients with anemia (4%) were found with significantly lower serum BCAA concentrations compared to 413 non-anemic individuals ($P < 0.05$).

Conclusions These data indicate a pathophysiological link between the three BCAAs valine, leucine and isoleucine and the human iron indicators hemoglobin and ferritin. Anemic individuals had significantly lowered serum BCAA concentrations compared to non-anemic subjects and lowered BCAA concentrations were observed with lowered ferritin levels.

3069 – GRANULIN, REVEALED BY SINGLE-CELL RNA-SEQUENCING OF DEVELOPING HEMATOPOIETIC CELLS, DRIVES MACROPHAGE AND NEUTROPHIL DIFFERENTIATION

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Myeloid cell differentiation leads to the formation of neutrophils and macrophages, which are critical cells for host defenses against infections. In addition, a multitude of other functions beyond their classical inflammatory roles have been assigned to these cells, including wound healing, neurodegenerative diseases and tumorigenesis. Thus, the identification of novel genes that regulate myeloid cell development has undoubtedly the potential to impact diverse areas of human health. Here, we have used deep single-cell RNA-sequencing during the active phase of myelopoiesis in the zebrafish embryo to create a platform for the identification of novel genes required for myeloid cell formation. Using this platform, we have uncovered a role for granulin (GRN) during neutrophil and macrophage differentiation. Leveraging the restricted expression of the zebrafish orthologue grna to the myeloid cell lineage, enabled us to perform loss of function experiments without altering its function in non-hematopoietic cells. With the generation of a zebrafish model of grna deficiency, we reveal that myeloid progenitors are unable to differentiate into neutrophils and macrophages. These grna deficient progenitors are also incapable of triggering a myelopoiesis emergency response, resulting in abnormal wound healing with aberrant collagen depositions. Utilizing CUT&RUN, we identified that Pu.1 directly binds grna enhancers, triggering its expression. Similar to the zebrafish grna, the mammalian granulin is also upregulated in myeloid cells, and controlled by the myeloid transcription factors PU.1 and IRF8. Altogether, our findings uncover the role of Granulin during myeloid cell differentiation, opening a new field of study that will help elucidate the pleiotropic role of this enigmatic protein in inflammation, wound healing, tumor progression and neurodegenerative disease.

3070 – IL-3 RESCUES PROLIFERATIVE DEFECTS IN INFLAMMATION-SENSITIVE RUNX1 DEFICIENT HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Although loss-of-function RUNX1 mutations are commonly found in hematopoietic malignancies, how RUNX1 functions during hematopoietic and leukemic development is unclear. Using a CRISPR/AAV6 system to target the RUNX1 locus in human CD34+ hematopoietic stem and progenitor cells (HSPCs), we show that RUNX1 deficiency causes monocytic skew at the expense of erythro-megakaryocytic potential and stem cell activity, including a severe in vivo stem cell competitive defect. RNA-seq and ATAC-seq review that these effects are mediated by broad upregulation of PU.1 and NFKB transcriptional programs; downregulation of GATA1- and TAL1-dependent erythro-megakaryocytic differentiation; and downregulation of cell cycle programs mediated by MYC and E2F. Treatment with IL-3 rescues RUNX1-deficient cell proliferative and stem cell defects. Together, these results show that RUNX1 controls transcription factor activity and cytokine signaling, and loss of RUNX1 causes monocytic skewing and hypersensitivity to IL-3-dependent expansion. We are currently studying how the IL-3 selects for inflammation-sensitive RUNX1 deficient cells and whether targeting IL-3 signaling may be a viable therapeutic in the prevention or treatment of RUNX1 mutant malignancies.