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ABSTRACT BOOK

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Mx-Cre+ mice (CD45.2+) were transplanted with the equal number of competitor cells (CD45.1+CD45.2+) into lethally irradiated CD45.1+ recipient mice. 4 weeks after transplantation, pI-pC was administered to the recipient mice to delete floxed alleles. The result showed that the percentage of *Ogt*-deleted cells was remarkably decreased at 2 weeks after pI-pC injection and it progressively decreased over time. These data suggest that *Ogt* is essential for competitive and long-term repopulating capacity of BM-HSCs.
Summary and Conclusions: The *O*-GlcNAcylation by *Ogt* plays a critical role for maintaining homeostasis of FL and BM hematopoiesis in mice.

LB365

EXPLORING THE ROLE OF KIT LIGAND AT THE ONSET OF HEMATOPOIESIS

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Background: Hematopoietic stem cells (HSCs) are generated during mammalian development from hemogenic endothelium in the major arteries through a process called endothelial-to-hematopoietic transition. The extrinsic and intrinsic factors that orchestrate this process are poorly understood. Kit ligand (KitL; also known as Stem Cell Factor/SCF) is a cytokine that plays a pivotal role in the adult bone marrow HSC niche, and it is thought to act on several cell types during embryogenesis. It is known that the absence of KitL causes a significant reduction in the fetal liver HSC pool and leads to death *in utero* with severe anaemia. However, it is currently unclear where, when and how the hematopoietic defect originates in embryos lacking KitL, and what is the precise role of this cytokine in the first steps of hematopoiesis.
Aims: Here we use a novel KitL transgenic reporter mouse line to map expression of this cytokine in the microenvironment of the Aorta-Gonad-Mesonephros (AGM) and yolk sac hematopoietic sites in the developing embryo, and investigate the effect of loss of KitL in these pre-liver sites of hematopoiesis.
Results: We show that KitL expressing cells are found in the microenvironment of all hematopoietic sites of the E8.5-E10.5 mouse embryo. Loss of KitL as assessed in *Steel* mutant (*Sl/Sl)* embryos showed smaller and less proliferative hematopoietic clusters in the dorsal aorta and in the yolk sac. Already at E10.5, homozygous *Steel* mutants show a reduction in pre-HSC numbers, yolk sac erythropoiesis, and fetal liver cellularity. At E11.5 these mutants display decreased progenitor numbers in all hematopoietic sites.
Summary and Conclusions: Our data suggest that, contrary to earlier reports, Kit signalling plays a role in early pre-liver hematopoiesis. In addition, our data identified a previously unrecognized role for KitL in the maturation and/or expansion of hematopoietic progenitors originating from the yolk sac, that could contribute to the anaemia observed later in development. Ongoing studies aim at elucidating the mechanism by which KitL exerts its role in early hematopoiesis.

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DEREGULATION OF GENES RELATED TO IRON AND MITOCHONDRIAL METABOLISM IN REFRACTORY ANEMIA WITH RING SIDEROBLASTS

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Background: The presence of *SF3B1* gene mutations is a hallmark of Myelodysplastic Syndrome with ring sideroblasts (MDS-RS) and could be related to the iron accumulation in these patients. However, the mechanisms underlying that characterize the MDS-RS are not completely understood.
Aims: In order to gain insight in the molecular basis of MDS-RS, an integrative study of the expression and mutational status of genesrelated to iron andmitochondrial metabolism was carried out.
Methods: A total of 231 low-risk MDS patients (50,3% MDS-RS, 49,7% refractory cytopenia with unilineage dysplasia patients-RCUD) and 81 controls were studied. The gene expression profile was analysed using the Human Genome Expression Array (U133 Plus 2.0) from Affymetrix. Array-based sequence capture (Roche NimbleGen) followed by next-generation sequencing (Roche GS FLX Titanium sequencing platform) was used to analyze 39 genes related to iron and mitochondrial metabolism. The genes were selected according to our previous gene expression data. Spliceosome-related genes were studied using an amplicon sequencing design in GS Junior Instrument (Roche Applied Science). In addition, Sanger sequencing was carried out.
Results: Patients with refractory anemia with ring sideroblasts (RARS) showed a differential expression of 1145 genes compared to controls. Interestingly, 38% (266 genes) of the over-expressed genes were related to iron and mitochondrial metabolism. The comparison between RARS and RCUD patients showed a set of 192 differentially expressed genes: 33% (42 genes) of the over-expressed genes were also related to iron and mitochondrial metabolism. *ALAD* and mitochondrial transporters *SLC25* (*SLC25A37* and *SLC25A38*) genes were overexpressed in RARS. Most of the MDS-RS patients (94,3%) carried mutations in any spliceosome-related gene. In addition, our analysis of the *ALAD* gene identified two polymorphisms (rs8177807 and rs2228083) in exon 6 and located 49 bases from each other. The occurrence of both polymorphisms ("variant haplotype") was more frequent in MDS-RS (12%) than in members of the other groups analyzed (5%). ($p=0.07$). Furthermore, an un-described variant in the exon 7 was found in the *ALAD* gene in one RARS patient with a mutation in *SF3B1*. The positively charged arginine residue (R174) was replaced by an uncharged cysteine residue. The three-dimensional structure showed that R174 residue is completely buried into the monomeric structure and the protein was predicted to be potentially damaging. The variant was not found in any of the control samples or in those analyzed from RCUD patients. An over-expressed gene-signature of 71 genes was identified between patients with *SF3B1* mutations and patients without the mutations. Interestingly, *GDF15* was overexpressed in patients showing *SF3B1* mutations. In addition, other genes such as *PPP2R5B*, *PPP1R16A* and *DDIT4L*, related to *SF3B1* and *GDF15*, were up-regulated in the mutated group. A functional analysis with this gene set showed two deregulated pathways: porphyrin biosynthesis and heme biosynthesis ($p<0.001$).
Summary and Conclusions: The deregulation of genes involved in iron and mitochondrial metabolism provides new insights in our knowledge of MDS-RS. Our study revealed mutations in spliceosome-related genes in almost 100% of the MDS-RS. The presence of variations identified in *ALAD* gene could have a possible role in the predisposition to disease (as a first event) as well as contributing to the pathogenesis of MDS-RS where spliceosome-related genes mutations could be the trigger cause.

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THE PRESENCE OF HEMOGLOBIN S IS ASSOCIATED WITH MIRNA DEREGULATION IN CD34+-DERIVED ERYTHROID CELLS

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Background: Sickle cell disease (SCD) is a recessive genetic abnormalitycaused by a point mutation of the *HBB* gene (Glu6Val) that results in theproduction of hemoglobin S (HbS), leading to chronic hemolysis andvaso-occlusion. In addition to its ability to polymerize under hypoxicconditions, HbS may give rise to increased oxidative stress caused by freeheme and iron secondary to its intrinsic instability. MicroRNAs (miRNAs) canmodulate post-transcriptional erythroid-specific regulators.

Aims: The aim of thisstudy was to investigate miRNA expression profiles and the possiblepost-transcriptional role of these molecules associated with the presence ofHbS in sickle cell trait and in compound heterozygosity for HbS and δβ-thalassaemiaSicilian type.
Methods: We obtained samples from individuals from the same family with sickle cell trait (β^S/β^A),two sickle-δβ-thalassaemia Sicilian type patients ($\beta^S/\beta^{\delta\beta}$), two δβ-thalassaemia Sicilian type heterozygotes($\beta^{\delta\beta}/\beta^A$) and normal control ($\beta^A/\beta^A$).CD34$^+$-derived erythroid cells werecultured for 13 days and used to determine the miRNA expression profile.miRNAs were hybridized using an Agilent miRNA microarray platform and theprofiles were subjected to bioinformatics data analysis using GeneSpringsoftware (v11.0). Different databases, such as miRBase (Release 21),TargetScan (Release 6.2) and microRNA.org (Release 2010) were used todetermine the predicted targets of miRNAs. The RNAhybrid tool (BiBiServ) wasused to find the minimum free energy hybridization of each miRNA and apossible target gene.
Results: Twenty five miRNAs were up-regulated in$\beta^S/\beta^{\delta\beta}$ and in β^S/β^A compared to β^A/β^A and $\beta^{\delta\beta}/\beta^A$. *In silico* analysis showedthat eight miRNAs target genes involved in cellular adhesion (*CD36, ICAM4*,and *ITGB1*), antioxidant defense mechanisms (*CAT*, catalase), and hemoglobinaffinity for oxygen (*BPGM* and *MINPP1*, 2,3-bisphosphoglycerate mutase andphosphatase, respectively). *CD36, ICAM4*, and *ITGB1* are possible targets ofthe miR-17, 20a and 20b and the latter is additionally targeted by miR-29b,29c and 30e. The *CAT* gene is also targeted by miR-30e as well as by miR-148aand 148b. *BPGM* is targeted by miRs 20a and 20b, while *MINPP1* is onlytargeted by miR-30e. We also found hemopexin (*HPX*), a major heme-bindingprotein, is a possible target of miR-26b.
Summary and Conclusions: Our data suggest that,in an *in vitro* model, the presence of HbS is associated with miRNAderegulation that may be associated to pathologic processes found *ex vivo* in reticulocytes and mature erythrocytes from SCD patients. Financial supportby FAPESP and CNPq/INCTS.

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ORAL IRON SUPPLEMENTS INCREASE HEPCIDING AND DECREASE ABSORPTION FROM DAILY OR TWICE DAILY DOSES: STUDIES WITH STABLE IRON ISTOPIC LABELS IN YOUNG WOMEN

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Background: Oral iron supplementation is used to treat iron deficiency, but absorption is often low and correction is variable.
Aims: Determine the duration and magnitude of the plasma hepcidin (pHep) response induced by oral iron (Fe) supplements and concomitantly measure bioavailability in healthy iron depleted young women.
Methods: After randomization of 42 subjects with plasma ferritin<20 µg/L, pHep (measured by C-ELISA), iron status and inflammation markers were monitored at regular intervals. On day 1, no supplements were given (control day). On days 2 and 3, subjects received iron supplements containing 40, 60, 80, 160 or 240 mg Fe as $FeSO_4$ as either single or two consecutive daily doses extrinsically labeled with stable iron isotopes $^{54}FeSO_4$, $^{57}FeSO_4$ or $^{58}FeSO_4$. Iron bioavailability was measured by assessing the isotopic enrichment of erythrocytic iron 14 days after administration.
Results: Both Fe dose (P<0.05) and time of day (P<0.05) were associated with increase in pHep. Compared to control days, pHep was significantly higher at 8h and 24h after administration for 60, 80, 160 and 240 mg (P<0.05) but not for 40 mg Fe. Total Fe absorption from the Fe dose on the second day of the consecutive administration compared to a single Fe dose on the first day was not significantly decreased for 40 mg, but was decreased by 37% for 80 mg, 31% for 160 mg and 45% for 240 mg (for all, P<0.01). Fractional absorption was highest from the 40 mg dose. With twice per day dosing (60 mg Fe) the afternoon dose was less bioavailable (P<0.05).
Summary and Conclusions: In Fe-depleted women, consecutive day doses of supplemental Fe at 60 mg or above increase pHep and decrease fractional Fe bioavailability, while a dose of 40 mg does not. These important new data will help guide optimal dosing regimens for Fe supplements in women.

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RED BLOOD CELL GLYCOLYTIC INHIBITION ALTERS INTRACELLULAR ION CONCENTRATIONS, LEADS TO DECREASED DEFORMABILITY, AND UPREGULATES EXPRESSION OF CLEARANCE SIGNALS

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Background: enzymopathies is group of metabolic disorders of the erythrocyte that leads to decreased intracellular energy production. Energy deprivation ultimately results in premature clearance from the circulation in the spleen (extravascular hemolysis). The precise mechanism by which energy-deprived cells are recognized and cleared is largely unknown. One candidate mechanism could involve disturbed ion balance due to defective function of ATP-dependent ion channels in the red cell membrane, with consequent expression of clearance signals, and loss of red blood cell deformability.
Aims: To investigate the effect of energy-depletion on ion channel function, the exposure of clearance signals on the surface of the red cell, and red blood cell deformability in a model for energy-depleted red blood cells.
Methods: Isolated erythrocytes were resuspended in HEPES buffered Ringers solution (pH=7.4) and incubated overnight at 37ºC with 1mM D-glucose and various concentrations (5-10mM) of 2-deoxy-D-glucose. 2-deoxy-D-glucose (2dDg) is a glycolytic inhibitor acting on hexokinase, thereby serving as a model for energy-depleted red blood cells. Intracellular calcium was analysed by flow cytometry with fluo-4 AM as a fluorescent calcium probe and intracellular sodium and potassium concentrations were measured by flame photometry. ATP was measured using the CellTiter-Glo Luminescent Cell Viability Assay. Deformability of erythrocytes was measured using osmotic gradient ektacytometry by LoRRca. Expression of phosphatidylserine (Annexin-V) and CD47 on the erythrocyte membrane was examined by FACS.
Results: Upon incubation with various concentrations of 2-deoxy-D-glucose (2dDg) sodium levels (39 mM *vs* control 8 mM) were increased in a dose-dependent manner. Potassium (51 mM *versus* control 82 mM) and ATP concentrations (23 nM *versus* 7 nM) were decreased following treatment with 2dDg. FACS analysis revealed increased intracellular concentration of calcium and increased expression of CD47 and phosphatidylserine after incubation with 2dDg. Osmotic gradient ektacytometry showed decreased deformability after incubation with 2dDg, reflected by a decreased EI_{max} (maximal Elongation Index, Figure). When compared with ektacytometry curves from a patient with either hexokinase or pyruvate kinase deficiency, similar curves were obtained. Importantly, osmotic gradient ektacytometry curves of erythrocytes incubated with 2dDg are comparable (Figure).

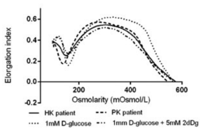

Figure 1.

Summary and Conclusions: Glycolytic inhibition alters intracellular red blood cell ion concentrations, and causes decreased deformability of erythrocytes. The decreased deformability as seen in 2dDg-incubated erythrocytes is comparable with the decreased deformability seen in patients with hexokinase and pyruvate kinase deficiency. Moreover, glycolytic inhibition is associated with upregulation of removal signals CD47 and phosphatidyl serine on the erythrocyte membrane. Loss of deformability and upregulation of removal signals in energy-deprived red blood cells may play a role in the premature removal of erythrocytes in red blood cell enzymopathies.

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MODIFIED ACTRIIB-MFC FUSION PROTEIN (RAP-536) INCREASES FUNCTIONAL RED BLOOD CELLS AND IMPROVES SICKLE CELL DISEASE PATHOLOGY WITH OR WITHOUT HYRDOXYUREA IN A MURINE MODEL

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Background: Sickle cell disease (SCD) is characterized by the presence of the sickle hemoglobin variant (HbS) of the b-globin gene. Under hypoxic conditions, HbS polymerizes, causing irreversibly sickled red blood cells (RBC). Manifestations of SCD include increased reticulocytes, splenomegaly, impaired blood flow due to intravascular sickling, and vaso-occlusive crises. The only approved therapy for SCD is hydroxyurea (HU), which decreases irreversibly sickled cells and painful events.. However, myelosuppression is a dose limiting toxicity and ~1/3 of patients do not respond to HU therapy, thereby highlighting the need for alternative treatment strategies
Aims: Luspatercept (ACE-536) is a modified type IIB activin receptor-Fc fusion protein[1] which acts as a ligand trap for members of the TGF-b superfamily to promote late-stage erythroid differentiation. In a murine model of b-thalassemia, RAP-536 (murine ortholog of ACE-536) corrected anemia and mitigated disease complications of b-thalassemia[2]. In this study, we evaluated RAP-536 as a monotherapy and in combination with HU in a murine model of SCD
Methods: SCD mice[3] (β^S/β^S, 6 and 12 weeks old) were dosed with RAP-536 (10 mg/kg, twice weekly SC) or TBS (VEH) control (N=4-6/group). Combination treatment with HU (100 mg/kg, i.p.) and RAP-536 (10 mg/kg SC) twice weekly for 3 months was compared with vehicle or HU monotherapy. Non-symptomatic compound heterozygote (β/β^S) littermates were used as controls (N=5/group).