P9

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Different iron expression signature in B-ALL patients cohorts

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Background and Aim

Iron is an essential element for several physiological activities. Recent reports have suggested that an association exists between altered intracellular iron homeostasis and iron-related protein functioning, and cancer. Furthermore, in acute myeloid leukemia the dysregulation of iron proteins may influence the response to treatment as shown by the increased sensitivity to chemotherapy in the presence of low levels of ferroportin-1 (FPN1) expression [1].
The aim of our study was to study iron metabolism in childhood B acute lymphoblastic leukemia (B-ALL) by analyzing the expression profile of iron-related genes in patients, and iron-related proteins in B-ALL *in vitro* models.

Materials and Methods

Patients. Cohort was composed of n=93 childhood B-ALL and n=8 healthy donors. Patients were stratified according to chromosome translocations [t(1;19), t(12;21), t(9;22) and t(4;11)]; patients without chromosome translocation were stratified according to minimal residual disease risk (MRD) prognosis [high risk (HR), medium risk (MR) and standard risk (SR)]

In vitro models. SEM and RS4;11 cell lines, both carrying t(4;11), were grown in RPMI (Roswell Park Memorial Institute Medium) with 10% heat-inactivated fetal bovine serum (FBS), with glutamine and combined antibiotics. All cells were maintained in an incubator at 37 ° C and 5% CO_2.

qRT-PCR. cDNA was retro-transcribed starting from the total bone marrow (BM) mRNA. Iron-related genes of BM samples (FTL (L-Ferritin), TFR1 (Transferrin receptor 1), SLC40A1 (Ferroportin-1), and CP (Ceruloplasmin)) were analyzed by qRT-PCR (2^-ΔCt method) using QuantStudio Real-Time Software (Thermofisher, Waltham, MA, USA).

MTT Assay. 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to evaluate the effect of iron as ferric ammonium citrate (FAC) on cellular viability. Cell lines were treated with FAC [100 µM] for 24h and 48h.

Labile Iron Pool Measurement (LIP). LIP was measured by flow cytometry using Calcein-AM technique after 24h exposure to FAC [100 µM] [2].

Flow Cytometry Analysis (FACS). FPN1 and TFR1 protein expression was measured by flow cytometry using Ab-FPN1 PE Rabbit Anti-Human and Ab-TFR1 APC-H7 Mouse Anti-Human before and after exposure to iron.

Statistical analysis. All values are expressed as means ± SD of three independent experiments performed in triplicate. Results were compared by One-Way Anova and Student t test using Prism 5 software (GraphPad Software, San Diego, CA, USA). Differences were considered significant at $p<0.05$.

Results (1)

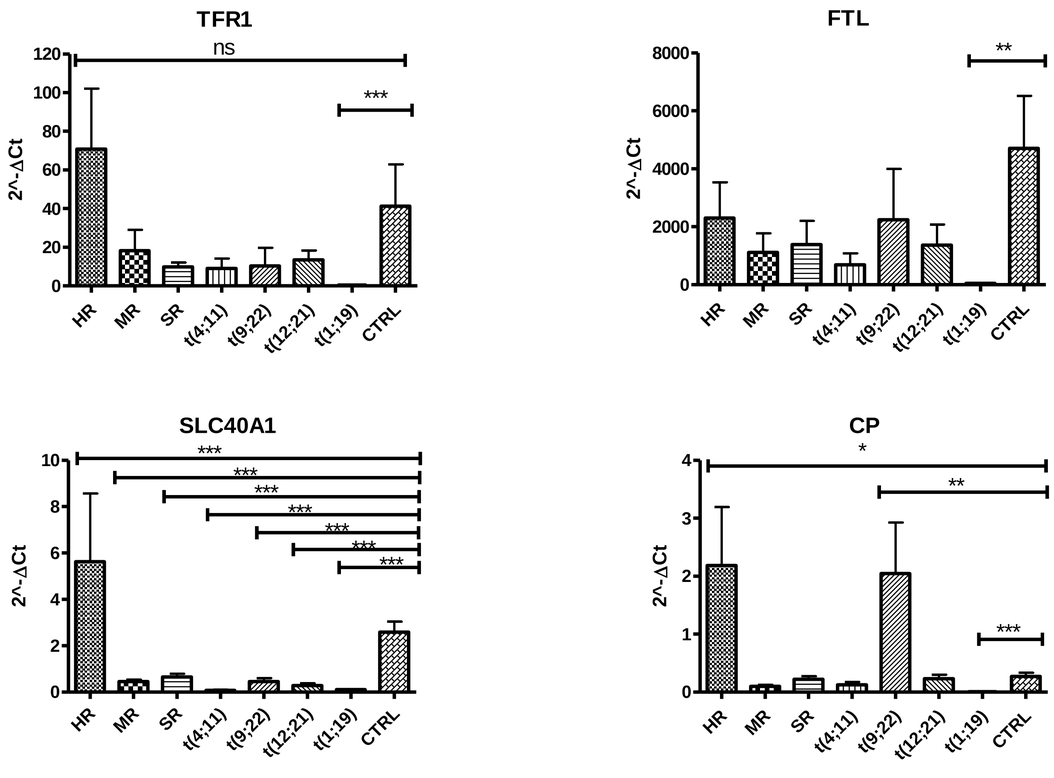

Figure 1A. Expression of iron-related genes in B-ALL childhood at the diagnosis, according to prognostic risk and chromosome translocations. Data are expressed as mean ± SD of three independent experiments performed in triplicate.
HR = High Risk, MR = Medium Risk, SR = Standard Risk, CTRL = Control

Iron genes are differently expressed in B-ALL subgroups.
TFR1 is downregulated in all classes, except for HR group with a high expression of TFR1. FTL is differentially downregulated in all subgroups compared to controls. SLC40A1 is significantly upregulated in HR, while it is downregulated in the other classes. CP is significantly upregulated in HR and in t(9;22) classes **(Figure 1A)**.
*p<0,01 **p<0,001 ***p<0,0001

Results (2)

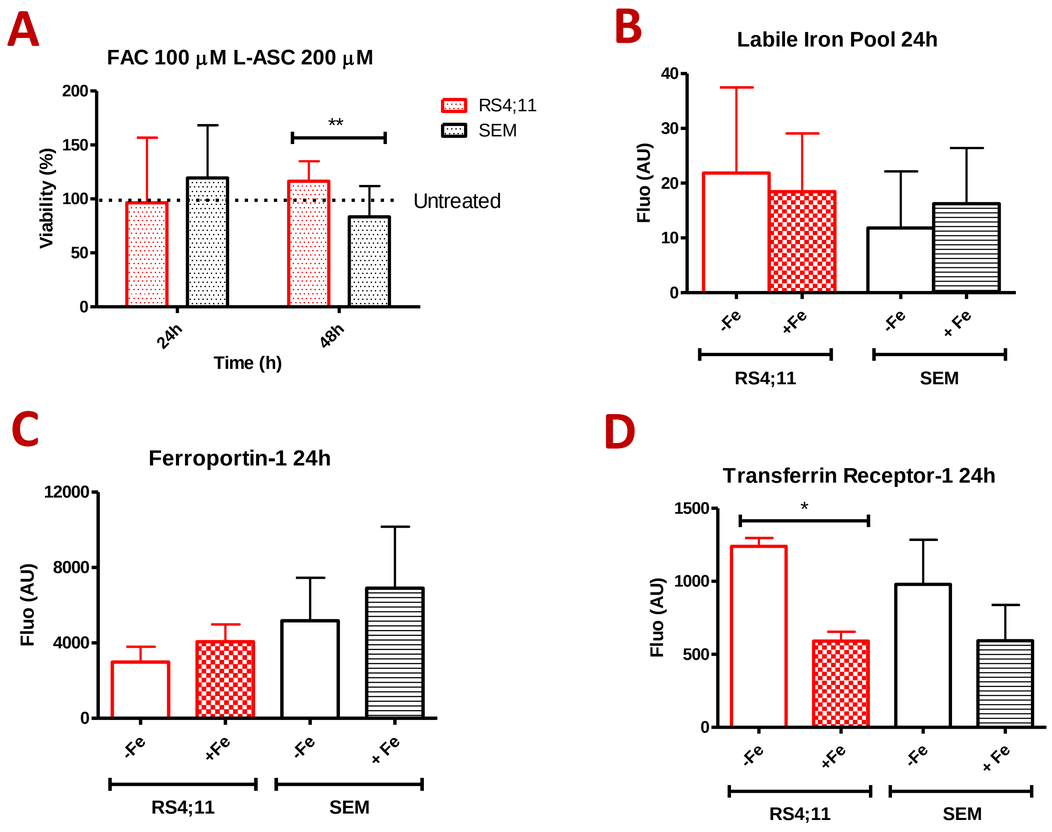

Figure 2A, 2B , 2C, 2D. SEM and RS4;11 viability at 24h and 48h after treatment with iron **(2B)**. LIP level, FPN1 and TFR1 expression measured by FACS after 24h iron supplementation **(2C and 2D)**. Data are expressed as mean ± SD of three independent experiments performed in triplicate.

Response to iron supplementation in B-ALL cellular models.
After 24h treatment, SEM viability slightly increases, while RS4;11 viability does not change **(Figure 2A)**. At 48h RS4;11 viability is higher compared to SEM **(Figure 2B)**. At 24h treatment, FPN-1 is slightly upregulated vs. untreated, while TFR1 is downregulated vs. untreated in both cell lines **(Figure 2C)**. SEM presents a small increase in the level of LIP vs. untreated condition after iron administration **(Figure 2D)**.

Conclusion

Patients' gene expression analyses show dissimilar expression signature of iron-related genes among B-ALL subclasses, where HR and t(1;19) classes have two opposite tendencies: HR shows high expression of *CP*, *SLC40A1* and *TFR1* genes. Vice versa, patients carrying t(1;19) translocation present very low expression of iron-related genes. These findings suggest different and significant dysregulation of iron homeostasis that can be related to the severity of the diseases and that might have implications in patients' prognosis.
In vitro data also suggest an involvement of iron dysregulation in SEM and RS4;11 cell lines, which represent B-ALL bad prognosis models. Despite they carry the same translocation t(4;11), RS4;11 and SEM are differently susceptible to the action of steroids, used in chemotherapy. While RS4;11 are sensible, SEM are resistant to the treatment. However, the two cellular subpopulation might have different response to iron exposure. From the cellular viability and labile iron pool graphs, it is possible to deduce that both RS4;11 and SEM are able to handle iron: in particular, RS4;11 takes advantage of the metal using it as proliferation factor, avoiding iron overload. In conclusion, further studies are needed to understand the heterogeneity and the molecular mechanisms, which are behind these differences.

References

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