Accumulating evidence shows the HERC1 gene expression is down-regulated both in acute and in chronic myelogenous leukemia patients, while its expression is peculiarly regulated in Ph negative neoplasms. Additionally, we observed that in CML cells the regulation of the HERC1 gene expression is sensitive to the Bcr-Abl kinase activity and that a physical interaction between HERC1 and Bcr-Abl proteins occurred. On the whole our findings indicate that HERC1 is associated with hematological malignancies and a novel player in the regulation of blood cells differentiation in lineage specific manner.

**THE HERC1 GENE EXPRESSION IS DYSREGULATED INHEMATOLOGICAL MALIGNANCIES**

The expression of the HERC1 gene is differently regulated in healthy blood specimens. PB samples exhibited higher HERC1 amount (median=4.01) when compared to the BM (median=2.2) (A). HERC1 transcript was assayed in healthy donors and in a panel of myeloid related disorders at diagnosis, namely Chronic Myeloid Leukemia (CML), Acute Myeloid Leukemia (AML), MyeloProliferative Neoplasms (MPNs), by RT-qPCR. The HERC1 mRNA quantity was expressed as 2^-ΔΔCt after normalization against GUSB. HERC1 gene expression was significantly down-regulated in newly diagnosed CML and AML, with median values of 0.9 and 1 respectively, while in MPNs the HERC1 transcript amount fluctuated from 0.4 to 12.65 with a median value of 3.3 and thus not significantly differing from healthy subjects. P-values were calculated on the differences in HERC1 gene expression observed between patients and the healthy control specimens (B).

**HERC1 mRNA IS DIFFERENTIALLY AND PECULIARLY EXPRESSED IN MPNS PATIENTS**

On the whole, the expression pattern of HERC1 in MPNs appeared to be rather scattered and variable. Among MPNs, when compared to the healthy subjects the CARL mutated Essential Thrombocytemia (ET) specimens (A) displayed a significantly higher amount of HERC1 transcript (p<0.0001). On the contrary, the JAK2 mutated and non-mutated, specimens showed levels comparable to the control counterpart. In Polycythemia Vera (PV) patients the HERC1 mRNA level was slightly, but significantly, lower than that of the healthy subjects (B). Differently, the Polycythemia Vera (PV) patients displayed a rather steep down-regulation of HERC1 gene expression. However, the JAK2V617F mutated samples showed higher HERC1 levels in comparison to the mutation free cohort (C).

**HERC1 GENE EXPRESSION IS DIFFERENTIALLY REGULATED DURING THE EVOLUTION IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS**

HERC1 gene was differentially expressed during the different CML phases being severely down-regulated at diagnosis, both in BM and PB CML specimens, while its transcript level aroze at remission and declined again at the onset of relapse (A). During the evolution of CML patients an inverse correlation exists between HERC1 and BCR-ABL1 gene expression at different time points of disease (B). Similarly to the mRNA, in primary CML cells the HERC1 protein amount was barely detectable at diagnosis and at the relapse onset. On the contrary, at remission the protein level was comparable to that of healthy control. Immunofluorescence was performed on cytokpun cells by using specific anti-HERC1 antibody. DAPI staining (blue) indicated the cells nuclei. The green signal, corresponding to HERC1 protein (C).

**HERC1 IS A NOVEL ANTAGONIST AND A POTENTIAL SUBSTATE OF BCR-ABL1**

Primary bone marrow-derived leukemic cells from newly diagnosed CML patients showed a moderate increase in HERC1 mRNA level after in-vitro Imatinib treatment (A). HXK-293T cells transiently and exogenously expressing the constitutively active p120 c-Abl tyrosine kinase exhibited a severe down-modulation of either the HERC1 transcript (B) and protein (C) levels. The subcellular localization of either the HERC1 or Bcr-Abl (p210) proteins were assessed by staining cytoplasm K562 cells with anti-HERC1 and anti-Bcr specific antibodies. The immunofluorescence assay revealed that the localization of the two proteins was mostly restricted to the cytoplasmic compartment and they co-localized (D). In K562 cells Bcr-Abl and HERC1 proteins form a complex. The immunoprecipitated Bcr-Abl was employed to pull-down HERC1 from K562 total cell lystate. The presence of HERC1 in the co-immunoprecipitated complex was determined by immunoblotting the membrane with HERC1 antibody (E). Subsequently probing with a tyrosine phospho-specific antibody proved the phosphorylated HERC1 status (B) in vitro kinase assay with purified c-Abl and HERC1 substrate (obtained after HERC1 immunoprecipitation on 293T, performed in the absence or presence of 5 μM Imatinib for 30 min which confirmed HERC1 is phosphorylated by cAbl (F). Actin and Vinculin were used as loading control.

**THE HERC1 GENE EXPRESSION AND ITS ASSOCIATION WITH DIFFERENTIATION IN MYELOID LEUKEMIA CELLS**

Upon incubation with differentiation agents the HL-60 cells change the morphology and adhesion properties, being able to adhere to the substrate. Morphological changes and differentiation-specific cell surface marker expression was confirmed by quantifying CD11b neutrophil cell surface marker with flow cytometry (FACS) analysis, after incubation of HL60 cells with phorbol 12 myristate 13-acetate (PMA), All Trans-Retinoic Acid (ATRA) and Dimethyl Sulfoxide (DMSO). Cell treated with PMA and DMSO showed an increase by approximately 75% and 85% of CD11b, associated with morphological changes respectively, while with ATRA differentiating marker CD11b raised approximately by 30%, with elongated cell morphology and weakly attached to the substrate (A). HERC1 gene expression both at mRNA and protein level upregulated in differentiated cells. Remarkably, the phosphorylation of Erk1/2 was severely impaired in differentiated cells and is tightly linked with the HERC1 protein level when compared to undifferentiated control. These findings revealed that the downregulated HERC1 gene expression might be linked to differentiation arrest at the onset of myelogenous leukemia. The vinculin and α-tubulin were used as loading control in Western Blot (B).

**CONCLUSION:** Our findings revealed that HERC1 gene expression was down-regulated both in acute and in chronic myelogenous leukemia patients, while its expression is peculiarly regulated in Ph negative neoplasms. Additionally, we observed that in CML cells the regulation of the HERC1 gene expression is sensitive to the Bcr-Abl kinase activity and that a physical interaction between HERC1 and Bcr-Abl proteins occurred. On the whole our findings indicate that HERC1 is associated with hematological malignancies and a novel player in the regulation of blood cells differentiation in lineage specific manner.

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**Dipartimento di Scienze Mediche e Chirurgiche**

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