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ELEVENTH ANNUAL MEETING

THE DOWNREGULATION OF BOTH LARGE E3 UBIQUITIN LIGASES, *HERC1* AND *HERC2*, IS A COMMON AND UNAMBIGUOUS FEATURE OF CHRONIC MYELOIDLEUKEMIA (CML)

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INTRODUCTION

Protein degradation by the ubiquitin-proteasome system (UPS) has been shown to regulate hematopoietic stem cell (HSC) self-renewal, differentiation, and survival[1]. The balance between HSC self-renewal and differentiation is critical to maintain hematopoiesis and its dysregulation is often associated with malignancies, including chronic myeloid leukemia (CML).

AIM

Large HERC E3 ubiquitin ligase family members, HERC1 and HERC2, are staggeringly complex proteins that can intervene in a wide range of physiological processes, such as cell proliferation, DNA repair, neurodevelopment, and inflammation. Therefore, mutations or dysregulation of large HERCs are associated with neurological disorders, DNA repair defects, and cancer[2]. Their roles in non-solid neoplasms remain unknown. Hence, in the present study, we explored the roles of the large HERCs, in CML.

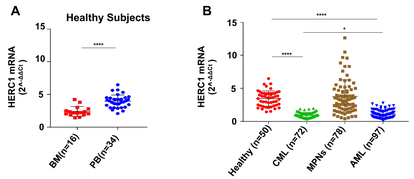
METHOD

After written informed consent, specimens from CML patients were collected at diagnosis, remission and relapse.

- All the patients were screened by RT-qPCR for the presence of the most frequent fusion transcripts.
- Differentiation was performed by incubation of myeloid leukemia cell line i.e K562 with PMA and EPO.
- HERCs mRNA quantification was done by RT-qPCR.
- Immunofluorescence and western blots were performed to assess the protein expression.
- Flow cytometry was performed to assess cell surface differentiation markers.

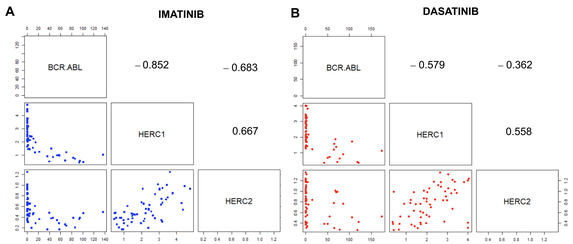
RESULTS

THE *HERC1* GENE EXPRESSION IS DYSREGULATED IN HEAMATOLOGICAL MALIGNANCIES



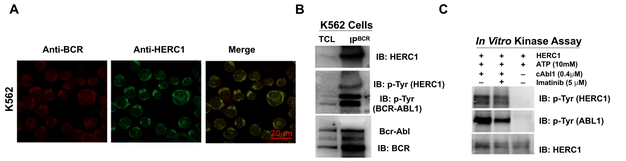
The expression of the *HERC1* gene is differently regulated in healthy blood specimens. **A)** PB samples exhibited higher *HERC1* amount (median=4.01) when compared to the BM (median=2.2). **B)** *HERC1* transcript was assayed in healthy donors and in a panel of myeloid related disorders at diagnosis, namely Chronic Myeloid Leukaemia (CML), Acute Myeloid Leukaemia (AML), Myeloproliferative Neoplasms (MPNs), by RT-qPCR. The *HERC1* mRNA quantity was expressed as $2^{-\Delta\Delta Ct}$ 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. $p \leq 0.05$ = *; $p \leq 0.01$ = *** ; $p \leq 0.01$ = **** .

INVERSE AND POSITIVE CORRELATIONS AMONG LARGE HERCs AND BCR-ABL IN CML PATIENTS UNDER TKIs TREATMENT



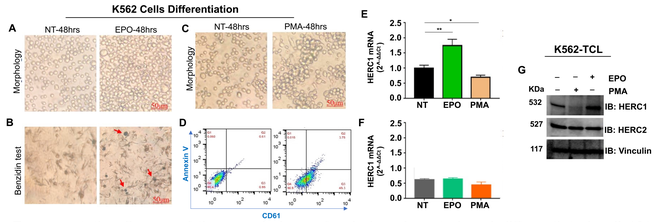
Gene expression correlation analysed among *HERC1*, *HERC2,* and *BCR-ABL* from CML patients treated with two different TKIs. The scatter plots and Spearman coefficient (rho) showed the correlations occurring among the *BCR-ABL*, *HERC1,* and *HERC2* transcripts in CML patients' specimens collected at different time points starting from the beginning of the Imatinib (**A**) and Dasatinib (**B**) treatments up to 12 months (0, 3, 6, and 12). Values are expressed either as $2^{-\Delta\Delta Ct}$ for both *HERC1* and *HERC2* and as I.S. for *BCR-ABL*, respectively. Each blue and red solid circle refers to a patient who has been treated either with Imatinib or with Dasatinib, respectively.

HERC1 IS A NOVEL INTERACTOR AND A POTENTIAL SUBSTATE OF BCR-ABL1



HERC1 is a novel interactor and a potential substrate of BCR-ABL1. (**A**) The subcellular localization of either the HERC1 or BCR-ABL1 (p210) proteins were assessed by staining cytospun 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 both are co-localized. (**B**) In K562 cells BCR-ABL1 and HERC1 proteins form a complex. The immunoprecipitated BCR-ABL1 was employed to pull-down HERC1 from K562 total cell lysate. The presence of HERC1 in the immunoprecipitated sample was determined by immunoblotting the membrane with HERC1 antibody (upper panel). Subsequently probing with a tyrosine phospho-specific antibody proved the phosphorylated HERC1 status. (**C**) In vitro kinase assay with purified c-ABL and HERC1 substrate, obtained after HERC1 immunoprecipitation on HEK-293T cell lysate, performed in the absence or presence of 5 μM Imatinib treatment for 30 min.

LEUKEMIC CELLS' DIFFERENTIATION TOWARDS ERYTHROID AND MEGAKARYOCYTE-LIKE LINEAGES ANTAGONISTICALLY AFFECT THE HERC1 LEVELS



Representative figures of the morphology of uninduced control and differentiated cells(**A**); K562 cells were stained with benzidine after EPO induction. Untreated K562 cells served as a control(**B**). Representative cell morphology images and flow cytometry analysis of PMA treated K562 cells (**C**,**D**). An increase in HERC1 protein and mRNA level was seen in EPO-treated K562 cells, while a decrease in HERC1 gene expression was observed in PMA treated cells. Interestingly, there is not any change in HERC2 protein level in K562 differentiated cells (**E-G**). $p \leq 0.05$ = *; $p \leq 0.01$ = **.

CONCLUSIONS

Both HERCs were down-modulated at CML onset, with a tight and unambiguous inverse correlation between the presence of the fusion oncogene *BCR-ABL1*. Upon the achievement of remission, both *HERC1* and *HERC2* mRNAs return to levels comparable to those of the healthy counterparts. Additionally, our survey unveils that their gene expression is sensitive to different Tyrosine Kinases Inhibitors (TKIs) in a time-dependent fashion. Interestingly HERC1 protein physically interacted with BCR-ABL1. A differential *HERC1* expression was also observed when the leukemic cell line induced to differentiate towards different lineages. Remarkably, our findings suggest that, at least in blood cells, HERC1 might be a novel differentiation marker.

REFERENCES

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