

0983**ETV6/RUNX1 DIRECTLY DYSREGULATES GENES WITH RUNX1 BINDING SITE VIA MECHANISM REVERSIBLE BY HISTONE DEACETYLASE INHIBITORS**J. Starkova,¹ J. Madzo,¹ M. Zaliova,¹ G. Cario,² A. Ford,³ O. Hrusak¹
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RUNX1 is implicated in over 30 different translocations in human acute leukemia. *RUNX1*, can either activate or repress transcription of key regulators of cell growth and differentiation through binding to promoters or enhancer elements. The *ETV6/RUNX1* chromosomal translocation is the most common chromosomal aberration in paediatric cancers (25% of ALL). The *ETV6* part of the fusion protein contains domains interacting with the mSin3, N-CoR and HDAC-3 corepressors. A part of the *RUNX1* gene involved in the fusion carries DNA-binding domain. *RUNX1* regulates haematopoietic myeloid cell differentiation and transcriptional activation but the role in lymphoid development is not yet fully understood. We hypothesize that *ETV6/RUNX1* causes pathological differentiation block in lymphoid cells. In the current project, we utilized treatment with histone deacetylase inhibitors (HDACi). We have previously confirmed specific effect of HDACi (valproate-VPA, Trichostatin A-TSA) on *ETV6/RUNX1* leukaemic cells in comparison with lymphoblastic leukaemias with different mechanism of leukaemogenesis (BCR/ABL and PDGFR α /ETV6). To prove the direct effect of HDACi on *ETV6/RUNX1* *in vitro*, we utilized a target gene of *RUNX1*, granzyme B (GZMB). To determine whether *ETV6/RUNX1* represses GZMB via direct interaction with RUNX1-binding site at GZMB promoter, luciferase activity was measured in HeLa cells transfected with *pcDNA3.1-ETV6/RUNX1Myc* and compared with HeLa with *pcDNA3.1* empty vector. Cells were transfected with pGZMB-luc or pGL3-basic to normalize the luciferase activity (pGZMB-luc/pGL3-basic). Fold change of ~3 FRU indicated that GZMB was downregulated by *ETV6/RUNX1*. To test the direct effect of HDACi on *ETV6/RUNX1*, after incubation of HeLa cells with VPA and TSA, luciferase activity was monitored again. Repression activity was reduced in treated transfected HeLa cells to 53% after VPA administration and 49% after TSA administration when compared to untreated cells. We used effect of HDACi on *ETV6/RUNX1* leukaemic cells and identified *ETV6/RUNX1* target genes in lymphoid cells. Analysis of expression profiling of treated (VPA, TSA) vs untreated (control) *ETV6/RUNX1*[+] REH cells showed genes with significantly changed expression after HDACi treatment. This group of genes was compared with a group of genes associated with *ETV6/RUNX1* phenotype selected by meta-analysis of expression data of ALL patients. Microarray data of selected genes showed downregulation of JunD, ACK1, PDGFRB in *ETV6/RUNX1*[+] patients as well as in our cell line model with increased expression after HDACi treatment. TCF4 gene was upregulated in the studied group and the administration of HDACi lead to its downregulation. Expression levels of chosen genes were validated by qRT-PCR: JunD - TSA $p=0.013$, VPA $p=0.0008$; PDGFRB - TSA $p<0.0001$, VPA $p=0.016$; TCF4 - TSA $p<0.0001$, VPA $p=0.0002$; ACK1 - VPA $p=0.07$. Selected genes have a fundamental role in cell proliferation and cell cycle progression therefore their role in leukaemogenesis is presumptive. We show for the first time direct transcription repression by *ETV6/RUNX1* on GZMB gene model. These data also support our hypothesis that HDACi affect *ETV6/RUNX1*[+] cells via direct interaction with *ETV6/RUNX1* protein, and that treatment with HDACi may release pathological differentiation block caused by *ETV6/RUNX1* aberrant transcription factor.

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0984**MINIMAL RESIDUAL DISEASE STATUS IS THE MOST IMPORTANT PREDICTIVE FACTOR IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA. PALG 4-2002 PROSPECTIVE ALL-MRD STUDY**J. Holowiecki,¹ M. Krawczyk-Kulis,¹ S. Giebel,¹ K. Jagoda,¹
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Current therapeutic protocols for adult acute lymphoblastic leukemia (ALL) take into account the risk of relapse, in order adjust the treatment intensity to individual patient needs. It is postulated that in addition to classical risk criteria including age, cytogenetics, immunophenotype, and tumor burden, also minimal residual disease (MRD) should be considered for treatment decisions. The aim of this prospective study was to evaluate the feasibility and prognostic significance of MRD detected with the use of immunophenotyping for disease-free survival (DFS) of ALL patients treated according to 4-2002 protocol of the Polish Adult Leukemia Group (PALG). Induction therapy included prednisolone, asparaginase and 4x epirubicin+vincristin. Consolidation consisted of 2x high-dose AraC+cyclophosphamide, 2x methotrexate+etoposide, mercaptopurine, and CNS prophylaxis including irradiation. Patients stratified to high risk group according 'classical' criteria based on those formerly developed by GMALL (bcr/abl(+), WBC>30 G/L, prepreB or preT phenotype, age>35 years, or 2 courses of induction required to achieve CR) were further referred for bone marrow transplantation, whereas those assigned to standard risk group (none of the above factors present) were treated with maintenance for two years. MRD was tested at the level of 0.1% after completion of induction and consolidation therapy in patients achieving CR, employing multicolor flow-cytometry. For patients with specific antigen combinations a standard *quadrans* method was used, for the remaining ones we applied a new *empty spaces* method taking into account an individual antigen expression on blast cells. The *forbidden* gates were established with the use of triple staining by comparison with the pattern obtained for healthy volunteer bone marrow donors. At least two antigen combinations were tested for each patient. One-hundred-ten ALL patients (B-lineage 82%, T-lineage 17%), aged 30 years (17-61) treated in 16 hematological centers were included in the analysis. CR rate equaled 80%. Among patients who achieved CR, 24% were assigned to standard risk, 76% - to high risk group, according to classical criteria. MRD evaluation was possible in all CR patients. In 50% of patients MRD was negative after both induction and consolidation - MRD(-) group, whereas in the remaining 50% of cases MRD was detected at least once - MRD(+) group. At 3 years the probability of DFS in MRD(-) and MRD(+) group equaled 58% and 28%, respectively ($p=0.04$). The prognostic value of MRD status for DFS was more pronounced in patients with standard risk ALL: 80% for MRD(-) vs. 0% for MRD(+)($p=0.048$), than in those with high risk ALL: 51% vs. 33%, respectively ($p=0.23$). In a multivariate analysis including classical prognostic criteria the MRD status remained the only significant predictive factor (HR: 1.33 (1.24-22.46), $p=0.04$). We conclude that immunophenotyping employing *empty spaces* method is feasible for MRD evaluation in adults with ALL. MRD status after induction and consolidation is the most important predictive factor for DFS. In particular, patients assigned to standard risk according to classical criteria can be further stratified and those with MRD detected after induction and/or consolidation should be offered intensified treatment with the use of hematopoietic cell transplantation.