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ABSTRACT BOOK

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ABSTRACT BOOK
cence demonstrated the de-localization of the protein within the nucle-
ous in AML. In an analysis of CD19 positive blasts, with normal karyotype and in MDS. Transfection experiments with PR3 plas-
mid demonstrate that overexpression of PR3 results in a significant
increasing of the proliferation rate and reduced apoptosis. By contrast
transfection with siRNA triggers apoptosis and cell growth inhibition.
Conclusion: PR3 gene expression and protein are significantly increa-
sed in AML and MDS, particularly in CBF leukemias in which the protein
is completed delocalized within the nucleous. PR3 overexpression is asso-
ciated with CEBPa/tA downmodulation. Ectopic expression of PR3
induces increased proliferation and apoptosis arrest. The mechanism
underlying the nuclear localization of PR3 in CBF leukemias and its patho-
genic role remains unexplored.

0026
CXCR-4 POSITIVE ACUTE MYELOID LEUKEMIAS (AGE < 60 YEARS): IMMUNOPHENOTYPIC, CYTOMORPHOLOGIC, CYTOGENETIC, AND MOLECULAR CHARACTERISTICS

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Background. The interaction of the CXCR-4 receptor with its ligand
SDF-1 plays a key role in the process of homing and mobilization of
stem cells and might be involved in the trafficking of leukemic blasts, too. Aims. The aim of our study was to correlate the CXCR-4 expression with the main diagnostic features of AML. Methods. 682 AML patients (other than AML M5) under the age of 80 were included in our investiga-
tion. CXCR-4 expression was measured in whole bone marrow or
peripheral blood performing multi-color flow cytometry (CD45
FITC/CXCR-4 PE/CD34 PerCP5.5). The cut-off was set at 20% CXCR-
4 positive cells. Cytomorphologic, cytogenetic, and molecular analyses
have been done in parallel. Results. Ninety percent of all patients express
CXCR-4 (616/682 patients). These leukemias were significantly more
assigned to a monocytic phenotype (38% vs 19%, p=0.0228). The per-
centage of CD34+, HLA DR+, or aberrant CD19 +, or CD7 + patients was
higher in the CXCR-4 positive group (38% vs 22%, p=0.0330; 6% vs
17%, p=0.0002). Furthermore, in CXCR-4 positive patients low risk cytoge-
netics was present less frequently (10% vs 22%, p<0.0002), whereas
patients with high risk cytogenetics were equally distributed in both
groups (22% vs 27%). There were significantly more NPM1 mutated or
FLT3-ITD mutated cases in the CXCR-4 positive group (38% vs 8%,
p<0.0001; 24% vs 6%, p=0.0005). In addition, the median expression lev-
el of CXCR-4 was higher on blasts of FLT3-ITD positive vs negative patients (81% vs 71%, p=0.0115). Interestingly, patients with NPM1
mutations expressed significantly more CXCR-4 compared to non mutat-
ed cases (87.9% vs 62.7%; p<0.0001), independent from the FLT3 sta-
tus. Conclusions. CXCR-4 positive vs negative acute myeloid leukemias
displayed distinct immunophenotypic and molecular characteristics.
Especially the NPM1 mutation status seems to be correlated with
CXCR-4 expression.

0027
FLT3 INTERNAL TANDEM DUPLICATION INVOLVING ITS UBQUITIN
DEPENDENT ENDOCYTOSIS MOTIF SUSPEND MODULATION BY HDM2
AND ARE ASSOCIATED WITH INFERIOR SURVIVAL IN AML

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Background. Internal tandem duplications in the juxtamembrane region
of the receptor tyrosine kinase Flt3 (Flt3-ITD) and elevated expression
of the oncogenic E3 ubiquitin ligase Hdm2 are frequent features of acute
myeloid leukemia (AML). Hdm2 is a well known suppressor of p53, but
is also associated with endocytosis of cell surface receptors. Recently we
have shown that Hdm2 and Flt3 are reciprocally modulated upon DNA
 damage therapy of AML cells in vitro and in vivo. Aims. To elucidate the
mechanism behind the Flt3-Hdm2 modulation and the impact of vari-
ous Flt3-ITDs. Furthermore, demonstrate if the Flt3-Hdm2 modulation
may have a clinical impact in AML. Methods. Primary AML cells and cell
lines (NB4 and MV4-11) with wild type Flt3 (Flt3-wt) or mutated Flt3
(Flt3-ITD) were used with the ligand (FL), small molecular inhibitors and
small interfering RNA (siRNA) to elucidate the relation between Flt3 and
Hdm2 on protein level, mRNA expression and modulation of apop-
tosis. Results. Kinase inhibition of Flt3 increase Hdm2 in Flt3-wt cells, but
not in Flt3-ITD cells. Modulation of Flt3 and Hdm2 by siRNA indicates
that their protein levels are mutually dependent. Cell lines with Flt3-ITD
have a more rapid cycling of receptors on the cell surface than cells with
Flt3-wt. A sequence alignment of the juxtamembrane region of Flt3 and
other related tyrosine kinase receptors lead to the identification of a
putative Ubiquitin dependent endocytosis motif (UbE) in the juxtamem-
brane region of Flt3. Based on transfection studies of mutant Flt3 and
Hdm2, internalisation of Flt3 is dependent on both Hdm2 E3 ligase activ-
ity and Flt3 UbE domain. A subset of AML patients with Flt3-ITD has
a duplication of this domain (Flt3-2xUbE). These patients have a reduced
level of surface Flt3 which significantly correlates to an elevated level of
Hdm2, and survival data suggest a negative prognostic impact in the
presence of Flt3xUbE. Conclusions. Flt3 receptor turnover involves
Hdm2, and Flt3-ITD resulted in dysregulated receptor turnover atten-
ced for Hdm2-induced Flt3 down-regulation. A novel type of Flt3 muta-
tions (Flt3-2xUbE) is associated with the Hdm2-Flt3-signalling pathway
and may have prognostic impact within the group of Flt3-ITD positive
AML patients.

0028
MICRNA PROFILING OF EVI1 DEREGULATED MYELOID LEUKEMIA

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Background. Chromosomal rearrangements involving the EVI1 gene
are a recurrent finding in malignant myeloid disorders. These transloca-
tions or inversions contribute to ectopic expression of the EVI1 gene.
EVI1 transcriptional activation has also been reported in approximately
5% of acute myeloid leukemia (AML) patients without chromosomal
defects affecting the EVI1 locus. Survival of patients with EVI1 overex-
pressing leukemias is poor and new insights into the molecular pathol-
ogy are needed as a basis for development of targeted therapies. Recent-
ly, microRNA deregulation was identified as a major contributor to can-
cer initiation and progression. Moreover, microRNA genes were shown
to be directly regulated by activated proto-oncogenes. Aims. The aim of
this study was to investigate which microRNA genes are implicated in
the transcriptional pathways governed by the EVI1 oncogene. Methods.
Silencing mediated by siRNA knockdown was performed in EVI1 overex-
pressing AML cell lines Kasumi-3 and U937-AML1 and validated by qRT-
PCR and Western blotting. A total of 384 microRNAs were profiled
through automated qRT-PCR using high-throughput quantitative stem-
loop RT-PCR (Applied Biosystems). Integrated statistical analysis (SAM
analysis, delta Ct and confidence interval analysis) was performed to
distinguish up- and downregulated microRNAs. Results. After siRNA treat-
ment, a reduction of 90% on mRNA level and a reduction of 70% on protein level were observed for both EVI1 overexpressing cell lines Kasu-
mi-3 and U937-AML1. Through integrated analysis several statistically
significant up- (mir-184 and mir-182#) and downregulated (mir-
155, mir-222 and mir-210) microRNAs were identified. Conclusions. We
identified several EVI1 regulated microRNAs after siRNA treatment of
EVI1 overexpressing cell lines which are currently under further inves-
tigation in a large panel of AML patients. Furthermore, microRNA pro-
file of 5q26 rearranged EVI1 overexpressing patient samples and non-
rearranged EVI1 overexpressing bone marrow samples is underway. Fur-
ther studies will also include electroperation of antagonisms or microR-
NA mimics for EVI1 regulated microRNAs to assess their contribution to
the leukemic phenotype. The discovery of functionally relevant microR-
NAs may provide new targets for therapeutic intervention in AML.