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Acute myeloid leukemia - Biology II

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IDENTIFICATION OF NOVEL MUTATIONS IN ACUTE MYELOID LEUKEMIA USING WHOLE TRANSCRIPTOME SEQUENCING

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Background. Acute myeloid leukemia (AML) is a genetically heterogeneous disease. While approximately half of AML patients have at least one chromosomal aberration, the other half classifies as cytogenetically normal (CN-AML). In CN-AML patients an increasing number of recurring somatic mutations have been identified during the last decade. Despite their pathological and prognostic relevance, none of these mutations are sufficient to cause AML on their own. Furthermore, in approximately one quarter of the CN-AML patients none of the known mutations can be detected. *Aims*. To identify novel mutations in AML by transcriptome sequencing using a next generation sequencing machine. *Design and Methods*. The term transcriptome summarizes all mRNA transcripts present in the cells from a certain patient sample. To identify tumor-specific somatic coding mutations, we sequenced the transcriptome of a CN-AML and a remission sample from the same patient using an Illumina GAII machine. Poly-A selected, fragmented RNA was used to synthesize double stranded cDNA, which was then sequenced. SNPs were called with the MAQ software. *Results*. We generated 20.4 and 15.6 million 32 bp paired-end reads of the CN-AML and remission sample, respectively, which mapped to exons of UCSC genes. 8.9% of reads for the AML and 5.0% reads of the remission sample mapped to intergenic regions. Of the 11,178 transcripts with a higher expression than 60 reads per gene (corresponding to approximately 1 transcript per cell), we sequenced 5,911 with an average coverage of greater than seven. By comparing the 63,159 SNPs discovered in the CN-AML sample with the remission sample, we identified 5 non-synonymous mutations not present in either the remission sample or in dbSNP. One of these point mutations affected the RUNX1 gene which forms a well known fusion gene in AML (RUNX1/RUNX1T1) and is a known mutational target in AML. The second mutation affected a gene which encodes a RUNX1 interacting protein, and a third mutation was found in the cellular homolog of a viral oncogene. The two other mutations occurrred in a phospholipase gene and a nuclear chaperon gene. The five genes identified as mutational targets are currently studied in a larger patient cohort of 200 patients with normal karyotype in order to determine the frequency of mutations in these genes. *Conclusions*. Transcriptome sequencing of AML patients is a pioneering application of the latest sequencing technology that may allow the unbiased detecting and understanding of the majority of genetic lesions that contribute to the onset and progression of AML. For mutation screening in the coding regions of expressed genes, whole transcriptome sequencing is currently 5-10 times faster and more cost effective than whole genome sequencing.

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SETBP1 OVEREXPRESSION IS A NOVEL LEUKEMOGENIC MECHANISM THAT PREDICTS ADVERSE OUTCOME IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemias (AML) are clonal malignant disorders that result from multiple genetic alterations in normal hematopoietic stem cells. In recent years, several genetic markers with prognostic impact in AML have been identified, permitting a better understanding of the biology of this disease and, in some cases, providing targets for molecular therapies. However, the outcome of older patients with AML has not improved in the last three decades, due to both patient-specific and disease-specific factors. Here, we describe a novel t(12;18)(p13;q12) involving ETV6 in a patient with AML. The translocation resulted in no functional fusion gene, indicating that a different mechanism might be acting. The SETBP1 gene (18q12), located close to the breakpoint, was overexpressed in the patient, suggesting that expression of this gene was upregulated by the translocation. Overexpression (OE) of SETBP1 through retroviral insertion has been reported to confer a growth advantage in hematopoietic progenitor cells, and SETBP1 interacts specifically with the SET protein, a potent inhibitor of PP2A; however, the role of this gene at the molecular level remained unknown. We demonstrate that SETBP1 OE leads to higher levels of SET due to formation of a SETBP1-SET heterodimer that protects SET of the protease activity, increasing the amount of full-length SET protein of 39 KDa, and decreasing the shorter SET processed forms. We also observed the formation of a SETBP1-SET-PP2A complex that, eventually, results in PP2A inhibition. Furthermore, the deregulation of PP2A activity promotes proliferation of the myeloid leukemic cells. We also analyzed the prevalence of SETBP1 OE in a series of 192 patients with AML at diagnosis. SETBP1 was overexpressed in 28% of patients with AML, and it was associated with unfavorable cytogenetic prognostic group, monosomy 7, and EVI1 OE ($p<0.01$). We found a significant shorter overall survival (OS) in patients with SETBP1 OE. The impact prognosis was especially remarkable in the group of patients older than 60 years in both OS ($p=0.015$) and event free survival ($p=0.015$). In conclusion, our results show a novel leukemogenic mechanism: SETBP1 overexpression would lead to the formation of a SETBP1-SET-PP2A complex that increases the amount of full-length SET protein, resulting in PP2A inhibition and, therefore, promoting the proliferation of the cells. Moreover, we have shown that SETBP1 overexpression is a recurrent molecular event with prognostic impact in AML, especially in the subgroup of elderly patients. Advanced age is the most important prognostic factor for determining outcome in AML, therefore, it is important to identify genetic markers that could categorize cases within this subgroup. Systematic molecular genetic studies in AML patients not only are useful for the evaluation of biomarkers for prognostication, but also for the identification of predictive factors for response to novel therapies. Our data suggest that SETBP1 overexpression could be a predictive factor for response to PP2A activators such as FTY720, which has been proposed as a new alternative for treating blast crisis CML and Philadelphia chromosome-positive ALL.

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STUDYING THE ROLE OF PIM KINASES IN FLT3-ITD-INDUCED LEUKEMIA REVEALED PIM1 AS REGULATOR OF CXCL12/CXCR4-MEDIATED HOMING AND MIGRATION

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Background. The PIM1 and PIM2 serine/threonine kinases are overexpressed in most human hematological malignancies. By expression of siRNAs or dominant-negative mutants, we have previously shown that PIM kinases are important for proliferation and survival of hematopoietic cells transformed by oncogenic protein tyrosine kinases such as the FLT3-ITD mutant associated with acute myeloid leukemia (AML). *Aims*. To study the role of PIM kinases in FLT3-ITD mediated leukemogenesis. *Design and Methods*: Reconstitution assays with FLT3-ITD expressing PIM-/- bone marrow cells were performed. We also modulated the function of PIM kinases in cell lines by small molecule PIM inhibitors and isoform-specific siRNAs. *in vitro* kinase assays as well as masspectrometry was used to characterize putative PIM phosphorylation sites. *Results*. Unexpectedly, bone marrow cells deficient for PIM1 failed to reconstitute lethally irradiated wild-type recipients, whereas, the absence of PIM2 did not interfere with the induction of a FLT3-ITD-mediated leukemia-like disease. PIM1-/- bone marrow cells were impaired in early homing to bone marrow and spleen. PIM1-/- but not PIM2-/- bone marrow cells displayed decreased surface expression of the CXCR4 receptor and were defective in migration towards the CXCL12 ligand. By blocking PIM1 function or re-expression in PIM1-/- bone marrow cells, we found that PIM1 activity was essential for proper CXCR4 surface expression and migration towards CXCL12. By expression of wild-type and mutant GST-CXCR4-C-terminal mutants we identified Serine 339 in the CXCR4 intracellular domain as being phosphorylated by PIM1 and essential for proper recycling of the receptor to the surface. In addition, expression of S339 CXCR4 mutants in cell lines functionally