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P190$^{BCR-ABL1}$ Signaling Modulates the Function of Tumor Suppressor Protein IKZF1

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Background: The chromosomal translocation *BCR-ABL1* is frequently present in adult B cell precursor acute lymphoblastic leukemia (BCP-ALL) in about 30% of the patients, while in pediatric BCP-ALL it occurs only in 3% of the patients. However, in both cases the disease is characterized by the almost obligatory presence of *IKZF1* gene deletions and mutations, arguing that loss of IKZF1 function is required for oncogenic transformation by p190$^{BCR-ABL1}$. The *IKZF1* gene encodes the transcription factor IKZF1 (Ikaros), which mainly acts as a transcriptional repressor protein through the recruitment of both HDAC-dependent and HDAC-independent co-repressor molecules. However, in some cases IKZF1 has also been shown to transcriptional activate specific target genes through association with the SWI/SNF chromatin remodeling complexes. We hypothesized that IKZF1-mediated transcription in a direct or indirect manner is modulated by BCR-ABL1 signaling. Therefore, cell biological assays and proteomic studies were performed to investigate the effect of p190$^{BCR-ABL1}$ expression on IKZF1 protein function.

Results: Using a luciferase reporter assay employing the human Bax- promoter, we establsihed that IKZF1-induced transcriptional repression was alleviated by p190$^{BCR-ABL1}$ expression. This effect could be reversed by Imatinib treatment, suggesting that BCR-ABL1 signaling interferes with the normal function of IKZF1. Next, we assessed the effect of p190$^{BCR-ABL1}$ on doxycycline-induced expression of IKZF1 using the murine lymphoid Tet-on Ba/F3 (TonB) cell line. Gene expression analysis showed that several target genes that are repressed by IKZF1 in TonB cells, such as *p16Ink4a, Cnot6, Dscc1* and *Tspan5*, are transcriptionally induced by co-expression of p190$^{BCR-ABL1}$. In order to understand how p190$^{BCR-ABL1}$ signaling affects IKZF1 protein function, mass spectrometry was performed on FLAG-affinity purified IKZF1 from transiently transfected HEK293 cells in the absence or presence of p190$^{BCR-ABL1}$. These analyses revealed that p190$^{BCR-ABL1}$ expression induces phosphorylation of IKZF1 on specific serine, threonine and tyrosine residues as well lysine acetylation. Transient transfection of lysine acetyltransferase PCAF (KAT2B) confirmed that IKZF1 is modified by lysine acetylation. Western blot analysis using phospho-specific antibodies showed that IKZF1 is subject to

tyrosine phosphorylation by $p190^{BCR\text{-}ABL1}$, both in HEK293 cells and TonB cells. Using an *in vitro* kinase assay, we demonstrated that IKZF1 can be directly phosphorylated by active recombinant ABL kinase.

Conclusion: Our studies show that $p190^{BCR\text{-}ABL1}$ signaling induces a multitude of different post-translational modifications on IKZF1, which could modify its properties as transcriptional regulator. We hypothesize that modulation of IKZF1 tumor suppressor function by $p190^{BCR\text{-}ABL1}$ signaling is the driving force for *IKZF1* gene deletions in BCP-ALL patients harboring a *BCR-ABL1* translocation.