SUBCELLULAR DISTRIBUTION OF THE TRANSCRIPTION FACTOR STAT5 IN CHRONIC MYELOID LEUKEMIA ANALYZED BY CONFOCAL MICROSCOPY

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Chronic myeloid leukemia (CML) is caused by the persistent activation of the ABL-tyrosine kinase in the BCR-ABL fusion protein. Among many other substrates, BCR-ABL phosphorylates STAT5 on Y694. STAT5 is an essential BCR-ABL substrate for initial transformation and maintenance of the disease. In cytokine-induced STAT5 signaling, phosphorylation of Y694 leads to nuclear accumulation of the transcription factor, followed by DNA-binding and gene induction. However, in CML cells pSTAT5 is cytoplasmic. We analyzed the subcellular distribution of endogenous pSTAT5 in non-adherent CML cells and of fluorescent STAT5 fusion proteins in adherent cell lines cotransfected with BCR-ABL (p210) and SRC family kinases (SFK). BCR-ABL alone is not sufficient to cause cytoplasmic retention of pSTAT5. Using various combinations of fluorescently labeled fusion proteins and immunofluorescence stainings we found that the SFK SRC and HCK but not LYN mediate cytoplasmic retention of STAT5 in BCR-ABL-positive cells. Confocal analysis of cotransfected cells revealed that pSTAT5 strongly interacts with membrane-bound SRC. Using STAT5 mutants it is shown that the STAT5/SRC interaction is mediated by the SH2 domain of STAT5 and a phosphotyrosine residue of SRC. Moreover, SFK interfere with dimerization of pSTAT5, which is a prerequisite for nuclear accumulation of the transcription factor. Interestingly, SFK cannot prevent the nuclear translocation of STAT5 phosphorylated by FLT3-ITD, a mutant receptor tyrosine kinase often found in acute myeloid leukemia (AML). The functional consequence of the subcellular distribution of pSTAT5 in disease progression will be further studied.