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STUDIES ON ITK-SYK SIGNALING PATHWAYS

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ABSTRACT

Chromosomal alterations are frequent causes of cancer. Until now, SYK is reported in two different chromosomal translocation events generating the ITK-SYK-fusion protein in a subset of peripheral T cell lymphomas and the TEL-SYK fusion protein in a case of myelodysplastic syndrome. T lymphocyte-expressed ITK is the only member of the TEC-family of tyrosine kinases reported as a fusion partner in transforming translocations and here we have studied this fusion. The comparison of ITK-SYK with SYK revealed that related tyrosines of ITK-SYK are phosphorylated at the linker-region and at the activation-loop and that the fusion protein localizes to the plasma membrane and potently phosphorylates the adapter proteins SLP-76 and BLNK. Moreover, membrane localization and phosphorylation of adapter substrates are blocked with PI3K inhibitors. SYK, on the other hand, showed phosphorylation at the linker-region, but not at the activation-loop tyrosines and failed to phosphorylate SLP-76 or BLNK under the same conditions.

Since BTK is the predominantly expressed TEC family kinase in B lymphocytes, we engineered the corresponding fusion kinase, BTK-SYK. We then investigated the role of the N-terminal region in the regulation of fusion kinases ITK-SYK, BTK-SYK and TEL-SYK. Unlike ITK-SYK, BTK-SYK showed more nuclear and cytoplasmic localization and PI3K inhibitors, unexpectedly, did not block its capacity to phosphorylate the adapter substrate SLP-76. Interestingly, non-membrane-tethering PH-TH domain-mutants ITK-SYK-R29C and BTK-SYK-R28C potently phosphorylated SLP-76. On the same ground, a TEL-SYK mutant, lacking the dimerization domain, was equally phosphorylated as the full-length fusion protein, but induced highly compromised CD69 upregulation compared with TEL-SYK or ITK-SYK.

Further investigations revealed that ITK-SYK-mediated activation of T cells was dependent on the adapter function of SYK-family kinases (SYK or ZAP-70), but independent of their kinase activity. Moreover, SLP-76 adapter function was not only indispensible for ITK-SYK-mediated CD69 upregulation and IL-2 secretion, but also for the phosphorylation of activation-loop tyrosines of SYK. Mutagenesis revealed a hierarchical phosphorylation pattern in the activation of ITK-SYK. In spite of loss of phosphorylation of the tyrosines, known to act as targets in SYK, the fusion protein potently retained phosphorylation capacity for substrate adapter proteins. Phosphorylation-independent constitutive activation was further confirmed by ITK-SYK expression in SYF cells (cells lacking SRC-family kinases), since there was no detectable phosphorylation on target tyrosines, yet the substrate SLP-76 was potently phosphorylated. Altogether, our studies indicate that lack of auto-inhibition renders fusion kinase constitutive activation suggesting that many of the tyrosine phosphorylations known to be critical in the activation of SYK are dispensable for ITK-SYK activation.

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