SPERMIDINE IS A NOVEL MODULATOR OF SRC KINASE-MEDIATED SIGNALING PATHWAY IN DENDRITIC CELLS

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Background and aim: Polyamines (i.e. putrescine, spermidine and spermine) are highly bioactive polycations capable of modulating several signaling pathways. Although polyamines functions have been mainly studied in tumors, it has been recently shown that spermidine can be produced by, and exert effect on, dendritic cells (DCs; professional antigen-presenting cells of the immune system). Specifically, spermidine promotes the non-enzymatic functions of indoleamine 2,3-dioxygenase 1(IDO1), an immunoregulatory molecule endowed of both catalytic activity (i.e., degradation of the essential amino acid tryptophan) and signal transducing properties (i.e., induction of IDO1and TGF-β expression). However, the specific mode of action of spermidine is not yet known.

Methods: To broadly assess the activation of tyrosine kinases involved in IDO1-non-enzymatic functions, we performed a kinomic analysis using a microarray of phosphorylable peptides. Molecular modeling approaches, mutagenesis and co-immunoprecipitation studies were used to evaluate the key residues on Src kinase involved in the interaction with the activator (namely, spermidine) and with the substrate (i.e., IDO1). Results: We found that spermidine activates Src kinase that, in turn, phosphorylates IDO1and thus confers immunoregulatory properties on DCs. In particular, spermidine-treated DCs foster the differentiation of T cells into $LAP^{+}Foxp3^{+}$ regulatory T lymphocytes in IDO1-dependent fashion. At molecular level, we found that Src kinase directly interacts with the substrate IDO1. Moreover, we identified a stretch of negative surface on the SH2domain of Src kinase involved in the binding with spermidine. Specifically, when the key amino acidic residues are mutated, the interaction between Src and spermidine is lost as well as the substrate binding and catalytic activation of the enzyme is blunted.

Discussion: Our data suggest that spermidine may act as positive allosteric modulator of Src kinase. Moreover, although it has been previously shown that IDO1undergoes tyrosine phosphorylation, we demonstrated, for the first time, that this is a direct effect through physical interaction between Src and IDO1.

Conclusion: Overall, this study may pave the way toward the design of novel allosteric modulators able to switch on/off the Src-mediated pathways, including those involving the immunoregulatory protein IDO1.