ANTILEUKEMIA EFFECT OF PHARMACOLOGICAL INHIBITION OF THE SPlicing REGULATORY SRPKs


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INTRODUCTION AND OBJECTIVES: Pre-mRNA splicing dysregulation has been related to the genesis of several diseases, including cancer. The serine/arginine-rich protein kinases family (SRPKs) plays a critical role in regulating pre-mRNA splicing through the phosphorylation of serine/arginine-rich proteins (SR proteins). SRPKs have been found highly expressed in several cancer types, suggesting that they would serve as targets for developing novel antitumor strategies. Here we aimed to evaluate the antileukemia potential of a selective SRPKs inhibitor, the compound N-(2-(piperidin-1-yl)-5-(trifluoromethyl)phenyl)isonicotinamide (SRPIN340). Also, we sought for in silico and in vitro structural information that would explain the SRPIN340 inhibitory activity. MATERIALS AND METHODS: MTT assays were performed to evaluate the compound cytotoxicity and to estimate its IC₅₀ in a panel of leukemia cell lineages. Annexin V/PI double staining was approached to investigate cell death. Western blotting, RT-qPCR and RT-PCR were carried out to investigate SRPK1 and SRPK2 gene expression and to access the impact of treatments on SRPKs cellular activity. Finally, analysis of intrinsic tryptophan fluorescence emission, docking and molecular dynamics were performed in order to gain structural information on SRPKs-SRPIN340 complex. RESULTS AND CONCLUSIONS: SRPK1 and SRPK2 were found overexpressed in several leukemia cell lineages but not in non transformed PBMCs. Along with significant cytotoxic activity, the impact of SRPIN340 treatments on SR proteins phosphorylation and expression of MAP2K genes were observed. Also, the treatments triggered early and late events of apoptosis. The in vitro structural data obtained with a recombinant SRPK2 form together with the analysis of docking and molecular dynamics provided plausible mechanisms about SRPIN340 inhibitory activity. These data point that SRPKs pharmacological inhibition should be considered as an alternative antileukemia strategy. Moreover, the structural information on SRPK-ligand complex obtained may pave the way toward the development of novel compounds with improved drug-like properties. ACKNOWLEDGEMENTS: CAPES, CNPq, FAPEMIG, FUNARBE/FUNARPEX.
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