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Developing Active Site SHP2 Inhibitors and Testing their Anti-Cancer Effects

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The Src homology phosphotyrosyl phosphatase 2 (SHP2) is an oncogenic enzyme that plays critical roles in cancer particularly in those cancers driven by dysregulated receptor tyrosine kinase (RTK) signaling. As such, SHP2 is considered as an excellent drug target for the triple-negative BC (TNBC) and the HER2-positive (HER2+) BC subtypes, which are both characterized by RTK overexpression. However, there are no FDA-approved anti-SHP2 drugs that have reached the clinic. To fill this gap, we have designed novel classes of competitive and non-competitive (covalent) active site SHP2 inhibitors primarily based on structural insights obtained from biological phosphotyrosyl substrates. The first-generation competitive inhibitors were design around a biphenyl core with carboxylic functional groups to fit the relatively basic environment of the SHP2 catalytic cleft. These inhibitors exhibited excellent specificity to SHP2, but their polar nature have made them less desirable as lead compounds for future development of anti-SHP2 drugs. To improve their drug-like properties, we have designed less polar compounds around an indazole core, bearing functional groups that mediate similar interactions with the active site specificity determinants. In addition, we have added a covalent warhead to enhance their potency as SHP2 inhibitors and as anti-cancer agents. The acrylamide warhead in these compounds is expected to form a covalent thiol linkage with the nucleophilic Cys residue in the active site of the enzyme; these compounds are referred to as covalent active site SHP2 inhibitors (C-ASSIs). We have used molecular modeling to predict binding modalities and to prioritize those with higher predicted free energy of binding for chemical synthesis. So far, we have synthesized more than 30 C-ASSIs and characterized them by PTPase assays. Of these, we have obtained three C-ASSIs, referred to as C-ASSI-2 and C-ASSI-3, and C-ASSI-5 that showed IC_{50} values of less than 100 nM for SHP2, but more than 25 μM for the close homolog SHP1. Treating breast cancer (BC) cells growing in 2D culture with each of these compounds suppressed cell proliferation and/or induced cell death in a concentration dependent manner, but with very little effect on the normal control MCF-10A cells. Similarly, treating cells growing in soft agar and suspension culture led to suppression and/or blockade of colony and mammosphere formation, respectively. To confirm whether C-ASSIs selectively binds to SHP2 under cellular contexts, we conducted cellular thermal shift assay (CETSA). The results showed selective stabilization of SHP2 in solution by each C-ASSI when compared to the non-target proteins PTP1B and HER2. Analysis of vehicle-treated samples showed absence of SHP2 stabilization when compared to the non-target proteins. Band density measurements from three independent experiments confirmed that the amount of SHP2 that remained in solution was unchanged up to 52^{o}C, when compared to the non-target proteins that were undetectable or reduced to less than 10%. These findings suggest that C-ASSIs can bind to full-length SHP2 expressed in BC cells with hyperactive Tyr kinase signaling. Importantly also, these C-ASSIs are specific to SHP2 as they did not stabilize PTP1B.