ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most common cancers in Asia and Africa, which is lethal in about 75% of cases. In the United States, there are 6,000-9,000 new cases of HCC per year. HCC is one of the highly chemo-resistant cancers, for which systemic treatments have been unsuccessful. The available therapies are surgical (10-20% of cases), locoregional or recently, chemical using Sorafenib, a multi-kinase inhibitor which has been shown in clinical trial to add two months to the lifespan of late stage HCC patients. To combat HCC with its assortment of genomic and cellular aberrations that occur during the progression of the disease, we have developed OSU-A9 and OSU-2S, novel small-molecule targeted agents for HCC therapy, using indole-3-carbinol (I3C) and fingolimod (FTY720) respectively, as scaffolds. In this study, we used pharmacological and molecular genetic approaches to investigate the mechanisms of action of the lead compounds of these classes (OSU-A9 and OSU-2S) and assess both the efficacy and safety in a series of preclinical studies carried out both *in vitro* and *in vivo*.

OSU-A9 exhibits up to 100-fold greater *in vitro* efficacy relative to I3C in HCC cells and provides a considerable therapeutic advantage over I3C with respect to chemical stability. Mechanistic evidence indicates that OSU-A9 antitumor effect is mediated by blocking the Akt-NF-κB signaling network, leading to the inhibition of signaling pathways governing cell cycle progression, survival, and metastasis. Equally important

, oral administration of OSU-A9 suppressed HCC xenograft tumor growth in mice without causing overt signs of toxicity. In addition, sub-toxic doses of OSU-A9 combined with the tumor necrosis factor-related apoptosis inducing ligand (TRAIL) effectively inhibited the resistance of HCC to TRAIL-induced apoptosis. The synergistic apoptotic effect is mediated by the ability of OSU-A9 to antagonize TRAIL-activated NF-κB cell survival pathway and to upregulate death receptor (DR) 5 expression in HCC cells. The ability of OSU-A9-TRAIL combination to selectively target HCC cells regardless of p53 status could shed the light on a promising combination therapy for HCC.

Based on our finding that FTY720 mediates apoptosis in HCC cells by activating the reactive oxygen species (ROS)-protein kinase (PK)Cδ signaling independent of S1P1 receptor, we developed OSU-2S, a novel PKCδ-targeted non-immunosuppressive antitumor agent, by abrogating the S1P1 receptor activity of FTY720. Several lines of pharmacological evidence indicate that OSU-2S exhibits higher potency than FTY720 in suppressing HCC due to the metabolic inactivation of FTY720 through phosphorylation in the context of antitumor activity. As a single agent, OSU-2S exhibits high *in vivo* potency in suppressing xenograft tumor growth without overt toxicity, which supports its clinical promise as a component of therapeutic strategies for advanced HCC. Moreover, PKCδ is a major downstream effector of DNA damage-induced apoptosis, so the activation of PKCδ by OSU-2S is noteworthy, since it provides a rationale to combine OSU-2S with other genotoxic agents in HCC therapy.