Unveiling the Molecular Interplay of VPS34 Inhibition by Novel Drug Targets: A Promising Approach for Tailored Cancer Therapeutics

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Abstract
Many cancer cells utilize autophagy to survive chemotherapy. Understanding autophagy mechanisms in the context of cancer therapy is important. Vacuolar protein sorting 34 (VPS34) is a key regulator in the initiation of autophagosome formation. Current studies are exploring autophagic inhibitors as potential supplements to heighten the responsiveness of cancer cells to established treatments. However, many of these inhibitors lack specificity and have off-target effects. To date, there is no FDA-approved VPS34 antagonist. RD-I-53 is a highly selective inhibitor of VPS34. The binding mode of RD-I-53 to human VPS34 is not known, thus we aim to crystallize VPS34 bound to RD-I-53 to determine RD-I-53's binding mode through X-Ray crystallography. This will strengthen structure-based drug design efforts. The TELSAM crystallization chaperone has been shown to increase crystallization propensity, speed crystallization rate and lower the required protein concentration. We theorize that TELSAM can crystallize proteins up to 60 kDa. However, many important drug targets are larger than 60 kDa. We hypothesize that TELSAM may be able to crystallize the 68 kDa VPS34 catalytic domain. We crystallized VPS34 alone or as a fusion to the 1TEL and 5TEL variants of the TELSAM crystallization chaperone, using a variety of linkers. VPS34 crystallizes readily but the crystals give limited resolution unless RD-I-53 is bound. We show that different constructs of 1TEL-VPS34 rapidly form crystals. The optimization of these crystals as well as the structure of RD-I-53 and its derivatives bound to VPS34 will be discussed. These results will allow us to rationally optimize RD-I-53, leading to new cancer treatment.