Targeting STAT3 Signaling as a Therapeutic Approach for Cancer Treatment

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Signal transducer and activator of transcription (STAT3) is a key mediator of tumorigenesis that is constitutively activated in the majority of human cancers with transient activation in normal tissues making it an attractive target for selective cancer therapy. The aberrant activation of STAT3 correlates with poor prognosis and chemoresistance. Targeting STAT3 has been challenging and despite the huge efforts to develop STAT3 inhibitors, no STAT3-targeted therapies have yet been approved. A representative compound was used to design a Proteolysis Targeting Chimera (PROTAC) that can induce proteasome-dependent degradation of STAT3. PROTACs are emerging as promising tools for the degradation of the protein-of-interest (POI) especially undruggable proteins like STAT3. They are bifunctional molecules that consist of POI ligand, E3 ligase ligand and a separating linker and function by recruiting ligases to the POI for consequent degradation. The first step in our approach was to optimize for the linker length and composition. We have designed a series of PROTACs with different linkers that show a decrease in the STAT3 protein expression in different cell lines. Different linkers displayed different activities in terms of killing the cancer cells and reducing the protein expression levels. Next, we wanted to validate the mechanism of action of these compounds by blocking the degradation using proteasome inhibitors (e.g. MG132, bortezomib). The compounds however, displayed similar activity with and without the presence of a proteasome inhibitor. These results have led to further optimization of the compounds. We are currently optimizing for other E3 ligase ligands and for the position of the linker connection to the ligand. Our second approach for targeting STAT3 involves a phenotypic cell-based screen in a pair of wildtype (WT) and STAT3 knockout (KO) cancer cell lines. Around 3,000 compounds from our in-house library were screened for differential activity in the pair of cells lines using a colony formation assay (CFA). Confirmed hits were tested for their effect on phospho-STAT3 (pSTAT3) protein expression and two hits were found to reduce its expression in a dose-dependent manner. The hits were found to decrease the pSTAT3 protein expression with no decrease in the upstream kinases. Analogues displayed similar differential activity in the cells and reduced pSTAT3 protein expression. However, the compounds required further optimization due to solubility issues. The optimization of these compounds is in progress in order to help us further validate them for their activity.

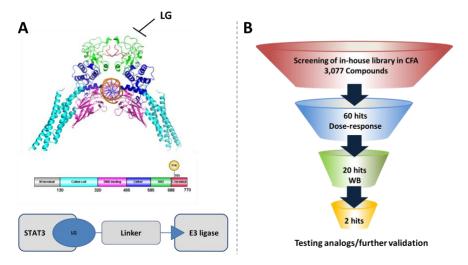


Figure 1. (A) STAT3 crystal structure (top) – the design of STAT3 PROTAC (bottom). (B) Testing funnel for the phenotypic screening approach.