Development of Expression Systems for Production of Recombinant Human Wnt Signaling Antagonists for Functional Analysis

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Wnt proteins comprise a large family of secreted signaling glycoproteins that regulate body-axis specification, morphogenesis, tumor development, metastasis, cell differentiation, senescence and death. Dysregulation of Wnt pathways has been implicated in many types of cancer, including different types of leukemia. Activation and repression of Wnt signaling pathways are determined by interactions between these proteins and different membrane receptors and secreted modulators, such as DKK1 and SFRP2, which can play a role on tumor promotion or suppression depending on the cellular context. In this project, we developed mammalian expression systems for DKK1 and SFRP2 in order to obtain these proteins for functional studies and inhibition assays. The cDNAs encoding human DKK1 and SFRP2 proteins were subcloned into the pIRES2-EGFP vector with a C-terminus His-tag. This vector allows simultaneous expression of the protein of interest and GFP from a single bicistronic mRNA transcript, with EGFP serving as reference for expression efficiency. The two cDNAs were also subcloned into a modified version of the pcDNA3.1 vector, containing two expression cassettes: one for the antagonists and one for EGFP. HEK293T cells were transfected with lipofectamine and analyzed by fluorescence microscopy. Transient SFRP2 expression from both plasmids and DKK1 from pIRES2-EGFP were tested 24, 48, 72 and 96h after transfection. Purifications were performed by IMAC using Ni-NTA Superflow resin (Qiagen) or an automated chromatography system with a His-Trap-FF crude column (GE Healthcare). Expression levels of both plasmids were compared by SDS-PAGE and Western Blot. Both proteins were successfully expressed in HEK293T cells and the highest expression levels were obtained 72h after transfection with the bicistroninc vector pIRES2-EGFP. SFRP2 has been partially purified and optimization of the purification protocols for both proteins is in progress. We concluded that the expression systems were efficient to produce the amounts of recombinant proteins required for the planned functional studies.

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