INHIBITORY EFFECT OF CRYSTALTINS ON LENS EPITHELIAL TO MESENCHYMAL TRANSITION VIA BLOCKING OF αB -CRYSTALLIN ACTIVITY

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Cataract surgery is one of the most common surgeries in the world, with a little over two million surgeries performed annually in the United States alone. The most common complication is the development of a posterior capsular opacification (PCO), which occurs in over 20% of cases within 2-5 years after surgery. It has been shown that PCOs develop due to residual lens epithelial cells undergoing epithelial to mesenchymal transition (EMT). This can be instigated by TGF-$\beta 2$, a predominant cytokine in the eye. EMT triggers the formation of fibrotic tissues and wrinkles in the posterior capsule. Our goal is to identify a potential agent that can prevent EMT via interactions with αB-crystallin, a promoter of TGF-$\beta 2$ signaling during the development of PCO. In this study, we aim to determine a mechanism by which two drugs, which we dubbed crystaltin-1 and crystaltin-2, affect the EMT process and how they specifically inhibit αB- crystallin's behavior.

To determine whether the drugs inhibit αB-crystallin chaperone activity, we assessed the chaperone activity using alcohol dehydrogenase (ADH) as a client protein in a thermal aggregation assay. The drugs at 20 μM were incubated for four hours with αB-crystallin, before we measured the ADH scattering at 400nm. For cell culture, we used the FHL124 cell line (fetal human epithelial cells) to determine the potency of the drugs in inhibiting the TGF-$\beta 2$ induced EMT. Cells were pre-treated with 2 μM of either drug for four hours, and then incubated with 10 ng/mL of human recombinant TGF-$\beta 2$ for an additional 1-48 hours. Western blots were performed to measure the expression of TGF-$\beta 2$ induced markers, αSMA and αB-crystallin. To assess effects on the TGF-$\beta 2$ signaling pathway, we also measured the phosphorylation of SMAD2, AKT, and ERK.

The ADH aggregation assay revealed that both drugs significantly inhibited αB-crystallin chaperone activity. Western blot data demonstrated that both drugs decreased the expression of αSMA, but not the expression of αB-crystallin; both of which are induced by TGF-$\beta 2$ in FHL124 cells. Crystaltin-2 was more efficacious in inhibiting EMT response than crystaltin-1. In addition, neither drug influenced the phosphorylation of SMAD2, ERK, or AKT.

Our results suggest that both crystaltins inhibit the TGF-$\beta 2$ induced EMT of lens epithelial cells, but they do not alter the expression of αB-crystallin. On the other hand, the drugs inhibit the chaperone activity of αB-crystallin, therefore inhibiting EMT of lens epithelial cells. The lack of change in the phosphorylation of SMAD2, ERK, and AKT suggest that the crystaltins do not influence either the canonical or the non-canonical signaling pathways. Taken together, crystaltin-1 and crystaltin-2 have a strong potential for use in the prevention of PCO.

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