

## Differential effects of dishevelled 2 and 3 on TCF/ LEF- mediated transcriptional activation in wnt-3atreated breast cancer cell line MDA-MB-231

### ABSTRACT

Background: Dishevelled (Dvl) proteins are central mediators of the Wnt signalling pathway. Previous studies have shown that Dvl1, 2 and 3 can differentially mediate the Wnt signalling pathway and act at different points in the signalling cascade depending on the cell lines used. It is unclear whether these differences also occur in MDAMB-231 which is a highly invasive fibroblastic triple negative human breast cancer cell line. It is important to determine the differential roles of dishevelled proteins as they could serve as potential useful therapeutic targets in cancer therapy. Methods: Small interfering RNAs (siRNAs) targeting Dvl1, Dvl2 and Dvl3 in MDAMB-231 were used to silence the expression of Dvl1, 2 and 3. Western Blot and RTqPCR were performed to show the silencing of Dvl2 and Dvl3. The invasive and migratory properties of Dvl2 and Dvl 3 were studied using invasion assay and wound healing assay, respectively. This is followed by TCF/LEF-sensitive luciferase reporter assay to study the involvement of Dvl isoforms in TCF-LEF transcriptional activation in Wnt3-stimulated MDA-MB-231. Finally, Western Blot was performed to detect the expression of phosphorylated  $\beta$ -catenin and total  $\beta$ -catenin in Dvl2- and Dvl3-silenced cells. Results: Reduced migration abilities were seen in Dvl2- silenced cells and a 10% and 12% reduction in invasiveness of MDA-MB-231 transfected with siRNAs against Dvl2 and Dvl3, respectively. There was a decrease in Wnt3a-mediated TCF/ LEF transcriptional activation with Dvl3 siRNA but an increase with Dvl2 siRNA. Reduction in phospho- $\beta$ -catenin levels was seen in Dvl2 but not Dvl3 knockdown. The level of total  $\beta$ -catenin was sustained in both cases. Conclusion: In summary, there is involvement of cell migration on Dvl 2. Human Dvl3 plays a significant role in mediating Wnt3a stimulated TCF/LEF transcriptional activation downstream of canonical Wnt signaling pathway. The level of phosphorylated  $\beta$ -catenin was not changed in Dvl3 silenced cells. MDA-MB231 can be a cell line used to elucidate the differential roles of Dvl 2 and 3. It is important to determine the roles of individual dishevelled proteins and their interacting proteins as they could be potential targets for therapeutic intervention.

**Keyword:** Dishevelled;  $\beta$ -catenin; MDA-MB-231