INTERACTION BETWEEN WNT PATHWAY AND ORPHAN NUCLEAR RECEPTORS

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Introduction: The canonical Wnt pathway controls gene expression by protecting cytosolic free beta-catenin from degradation. Free beta-catenin migrates to the nucleus where it regulates gene expression. The identity of nuclear factors that regulate the expression of Wnt-responsive genes is only partially known. Recently, a number of putative regulators of the Wnt pathway has been identified in Drosophila by genomewide RNA interference (RNAi) screening (1). We decided to examine the regulation of Wnt signalling by nuclear orphan receptors using PC12 cells transfected with a reporter gene controlled by Wnt-responsive elements (TCF). Here, we report data obtained with an orphan nuclear receptor named "ONR" (the real identity of the nuclear receptor cannot be revealed at this stage). *Materials and Methods*: PC12 cells were cultured in Dulbecco's modified Eagle's medium supplemented with glutamine 2mM and 10% serum. Transfection and reporter assays were carried out essentially as described (2). *Results*: In co-transfection experiments, the human "ONR" expression construct potently activated Wnt-responsive gene expression to a much greater extent than Wnt7a itself. Surprisingly, "ONR" did not synergize with Wnt in activating TCF-luciferase activity. In contrast, Wnt7a reduced the stimulation of the Wnt-responsive reporter by "ONR" in a concentration-dependent fashion. To examine whether the activation of TCF-luciferase expression by "ONR" was modulated by the canonical Wnt pathway we co-transfected the cells with Dickkopf-1 (Dkk1). Dkk1 is a secreted glycoprotein that inhibits the canonical Wnt pathway by interacting with the Wnt co-receptor, low-density lipoprotein receptor-related protein type 5/6 (LRP5/6). Nevertheless, Dkk-1 mimicked the action of Wnt7a in attenuating the activation of gene expression produced by "ONR", and, even more surprising, the inhibitory actions of Wnt7a and Dkk1 were additive. These data suggest that "ONR" activates TCF-mediated transcription via a mechanism which is dependent upon components of the canonical Wnt pathway, and specifically by those affected by Dkk1. *Conclusions*: These results suggest that "ONR" and Wnts interact in regulating gene expression, although the underlying mechanism is unknown. This interaction is relevant because abnormalities in Wnt signalling have been associated to human pathology .

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