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Dishevelled-DEP domain interacting protein (DDIP) inhibits Wnt signaling by promoting TCF4 degradation and disrupting the TCF4/β-catenin complex

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ABSTRACT

The TCF4/β-catenin complex, the executor of canonical Wnt/β-catenin signaling, is regulated by a variety of factors. Among these, Dishevelled (Dvl) is a critical regulator that releases β-catenin from degradation and stabilizes TCF4/β-catenin complex. Here, we report that DDIP (*D*ishevelled-*D*EP domain *I*nteracting *P*rotein, also named as Spats1, spermatogenesis associated, serine-rich 1), a novel protein that interacts with Dvl, regulates Wnt signaling. We provide evidence that DDIP suppresses Lef-1 luciferase reporter activity stimulated by Wnt1, Dvl2 or β-catenin, interacts with the TCF4/β-catenin complex, and disrupts the interaction of TCF4 and β-catenin by promoting TCF4 degradation through the proteasome pathway. Our results indicate that DDIP is a negative regulator of the canonical Wnt signaling.

1. Introduction

Wnt signal is critical in many aspects of metazoan development, including cell proliferation, differentiation, and stem cell maintenance. Aberration of Wnt signaling is associated with a variety of diseases including cancers, typeIIdiabetes, and osteoporosis [1–4]. Wnt signaling is propagated on the cell surface when Wnt ligands, a family of secreted glycolipoproteins, bind to their receptor complex of Frizzled and LRP5/6. Wnt signal is transferred to cytoplasm and branches into distinct pathways through the scaffolding protein Dishevelled (Dvl). Three pathways including the canonical Wnt/β-catenin, the non canonical planar cell polarity (PCP), and the non canonical Wnt/calcium signaling pathways [4,5] have been established. Among these, the canonical Wnt/β-catenin signaling pathway has been studied for almost 30 years. The major components of this pathway involved in the Axin/GSK/β-catenin axis. In the absence of Wnt signal, free β-catenin is degraded by a complex consisting of glycogen synthase kinase (GSK) 3β, the tumor suppressor adenomatous polyposis coli (APC) and the Axin scaffolding protein. Under quiescent circumstance, many co-repressors including Groucho, CBP and CtBP [6–8] bind tightly to TCF4, a major player to initiate transcription of Wnt target genes, to silence the transcriptional activity of TCF4 [8]. When cells receive Wnt signals, Axin is recruited to cytoplasm membrane by Dvl, which destructs the GSK3/APC/Axin complex and blocks the degradation of β-catenin. Consequently, β-catenin accumulates, and translocates into nucleus, where it forms an activated complex with TCF4 by replacing co-repressors and initiates the transcription of Wnt target gene [1,4]. Many genes related to tumor growth are transcriptionally initiated by this canonical Wnt/β-catenin pathway.

The canonical Wnt/β-catenin pathway is regulated at both cytoplamic and nuclear levels. In the nucleus, several factors have been identified to regulate the formation of activated complex TCF4/β-catenin [9]. These regulators are grouped into co-activators including APPL1/2 [10], Dvl and c-Jun [11,12], Bcl9/legless and Pygopus [13], and "destructors" such as HDAC1/2 [14], APC [15], and ICAT [16]. The co-activators stabilize the association of TCF4 and β-catenin, while the destructors destroy the TCF4/β-catenin complex by either promoting β-catenin degradation or competing with β-catenin. Under certain circumstance, dominant expression of the regulators maintains the Wnt signaling activated or inhibited.

Dvl family protein, which serves as a hub for the Wnt signal transduction from membrane to cytoplasm, has three homologues (Dvl1, Dvl2 and Dvl3) in human and mouse, and shares three

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conserved domains: DIX (*Di*shevelled, A*x*in), PDZ (*P*ostsynaptic density 95, *D*iscs Large, *Z*onula occludens-1) and DEP (*Di*shevlled, *E*gl-10, *P*leckstrin) [17,18]. In an attempt to clarify the role of Dvl in Wnt signaling pathway, we screened the 11.5-day mouse embryo cDNA library with a bait of the DEP domain and C-terminal of Dvl2, and identified a novel protein that binds to the DEP domain. We

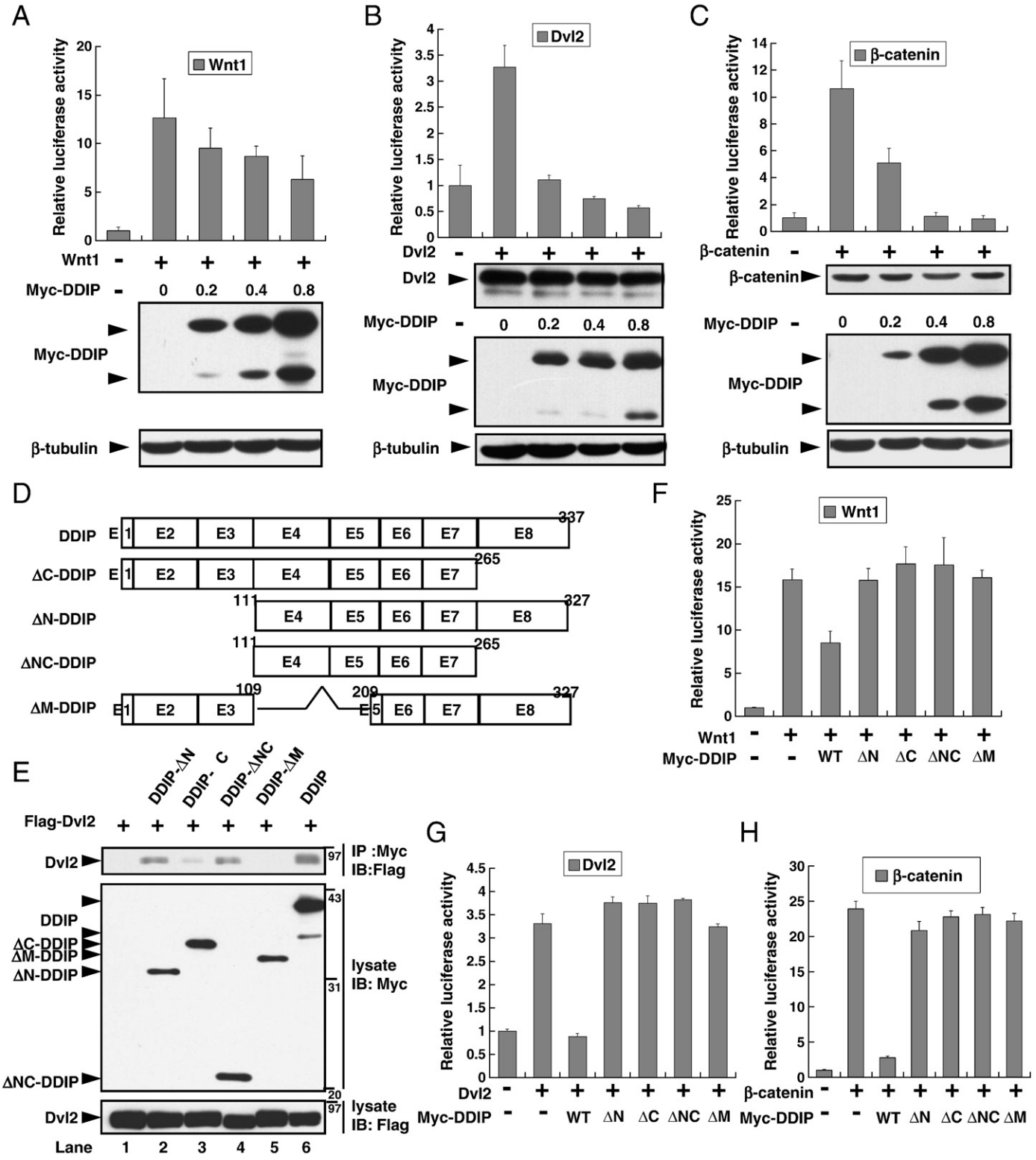

Fig. 1. DDIP inhibits Wnt signaling stimulated by Wnt1 (A), Dvl2 (B), or β-catenin (C) in a dose-dependent manner, but no specific region is responsible for the inhibition (F–H). HEK293T cells in 24-well plate were transfected with 1 μl mixture containing 10 ng Lef-1, 100 ng Lef-1-Luc and 5 ng pRL-TK, and 0.1 μg Wnt1 (A, F) or 0.1 μg Dvl2 (B, G) or 0.1 μg β-catenin (C, H), and either different doses of DDIP (A–C) or 0.4 μg truncated mutants of DDIP (F–H). Error bars indicate S.D. of duplicated data in one experiment, and the results were repeated three times. The protein levels are showed at the bottom in each figure. (D) A schematic diagram of the DDIP protein and its truncated mutants used in this experiment. Boxes with numbers represent exon of DDIP and the thin broken line represents an internal deletion. (E) The central part of DDIP is essential for the interaction between DDIP and Dvl2. Myc-tagged truncated mutants of DDIP were co-expressed with Flag-Dvl2 in HEK293T cells cultured in 60 mm dishes, and cell lysates were immunoprecipitated with an anti-Myc antibody and precipitates were analyzed by Western blot with an anti-Flag antibody. Aliquots of cell lysates were also examined by Western blot with anti-Flag or anti-Myc antibodies.