Elucidating the role of TGFβ-induced Dact1 in Wnt signaling suppression

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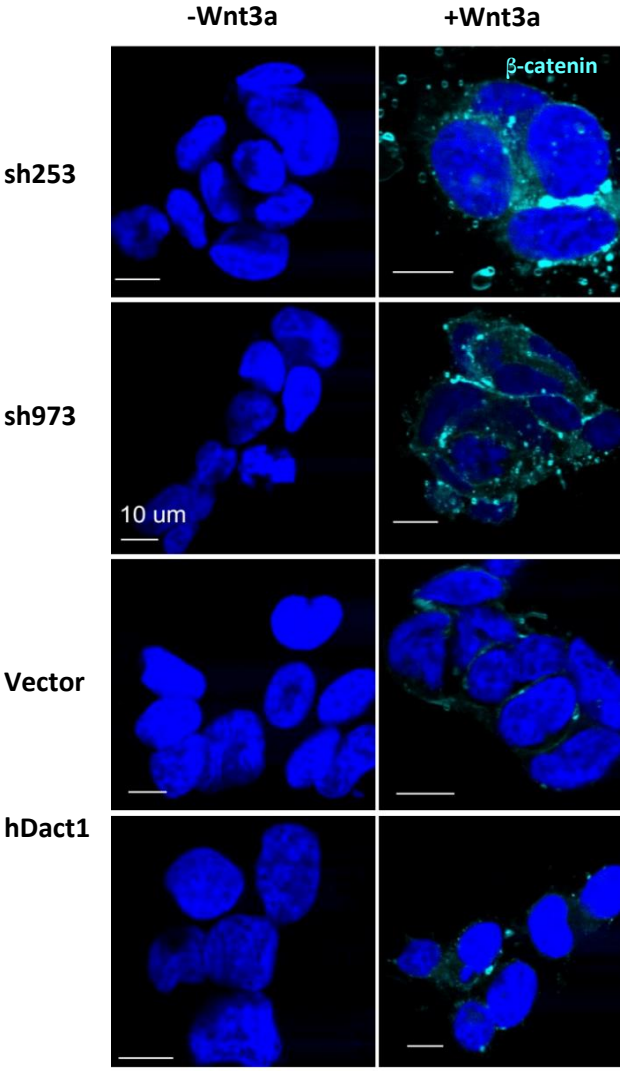
Background

Since its initial discovery in 2002[3], competing hypotheses have emerged to report the function of Dishevelled Binding Antagonist of Beta-Catenin 1 (Dact1). One group of researchers has proposed that Dact1 binds to Disheveled in order to stabilize the associated Beta-catenin destruction complex of the Wnt signaling pathway[3], while another has suggested that Dact1 antagonizes Wnt signaling through the promotion of Disheveled degradation[4]. Meanwhile, our lab has recently shown that Dact1 acts as a TGFbeta-induced suppressor of Wnt signaling within the context of cancer[2]. This lead, which both identified Dact1 as a promoter of bone metastasis and challenged existing reports for Dact1 function, warranted further investigation into the fundamental role of and mechanism for Dact1 mediation in cross-talk between the TGFβ/Smad and Wnt/Beta-catenin signaling pathways. An outstanding gap in knowledge includes the effects of Dact1 activity in non-cancer models. Thus, I sought to replicate work previously conducted using cancer models in a normal cell line to expose any differences in phenotypic properties.

Results

Confocal microscopy images revealed that Dact1 knockdown cells (sh253 and sh973) expressed higher levels of total Beta-catenin, a downstream effector of Wnt signaling, 24 hours after Wnt3a treatment when compared to their overexpression and empty vector counterparts (Figure 2). These results are consistent with the claim that Dact1 functions as a repressor of the Wnt signaling pathway.



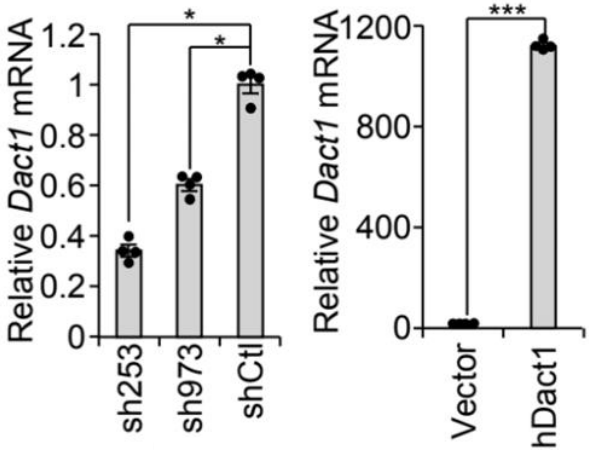


Figure 1: shRNA-mediated down of Dact1 was evaluated by qPCR analysis. Expression levels were normalized to internal control GAPDH.

Figure 2: Immunofluorescence after 24-hour treatment with Wnt3a-conditioned media. Cells were probed with anti-Beta-catenin (cyan) and Hoechst (blue).

Methods and Materials

Cell culture

Human fetal lung fibroblasts (HFL1) were maintained in Ham's F-12 media supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin, 10 μg ml^{-1} insulin, and 20 ng ml^{-1} epidermal growth factor. These cells were routinely verified to be negative for mycoplasma contamination by qPCR analysis.

Establishment of stable cell lines

HFL1 cells were transduced with either an empty vector, Dact1-expressing construct (referred to as hDact1 in figures), or Dact1-targeting short hairpin RNA from Sigma (sh253 and sh973 for short) and 8 μg ml^{-1} polybrene for 12 hours, followed by selection and co-culture with 1 μg/mL of puromycin. Knockdown vectors were purchased from Sigma, while the Dact1 overexpression construct was cloned by a senior lab member.

Immunofluorescence

Cells were grown on glass cover slips in 6-well plates to ≤ 70% confluence before being fixed with 10% buffered formalin for 10 minutes at 4º C, permeabilized with 0.1% Tween 20 in Dulbecco's 1X PBS (PBS-T), and blocked in 5% goat serum for 1 hour at room temperature, sequentially. Samples were then incubated with a 1:100 dilution of total Beta-catenin primary antibody (Cell Signaling Technology #8480) for 48 hours at 4º C in a humidity chamber. The following day, these samples were washed several times with PBS-T and incubated with both Alexa Fluor 647 goat anti-rabbit secondary antibody (Invitrogen) and Hoechst stain (Invitrogen) at 1:1000 dilutions. The applied solution was, again, washed away with PBS-T. And, finally, the slides of interest were mounted with ProLong Gold Antifade media (Invitrogen) and visualized with confocal microscopy. Wnt3a and control-conditioned media used in this experiment were generated by collecting 72-h-

conditioned media from L cells with or without Wnt3a expression (ATCC, CRL-2647 and CRL-2648) followed by filtration at 0.45μm.

RNA isolation, reverse transcription, and quantitative real-time PCR

RNA was extracted from cell samples grown to 70% confluence with the RNeasy Mini Kit (Qiagen) and reverse transcribed using the Superscript IV First-Strand Synthesis System (Invitrogen). Meanwhile, quantitative real-time PCR (qtRT-PCR) assays were performed using the PowerUp SYBR Green Master Mix according to the manufacturer's instructions and with the appropriate primers (Table 1). Relative DACT1 transcript quantification was determined using the standard curve method.

Table 1: Quantitative RT-PCR primers

Gene	Species	Forward sequence	Reverse sequence
Dact1	human	GACGAGCAGAGCAATTACACC	ACCGTTTGAATGGGCAGA
GAPDH	human	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

Discussion

These experiments provide further evidence to suggest that Dact1 is an effective suppressor of Wnt signaling, thereby clarifying the protein's fundamental function despite conflicting reports. Intriguingly, Beta-catenin imaging also revealed a similar intracellular localization pattern to that observed for Dact1. Further investigation should seek to answer whether the Dact1-associated puncta colocalize with those of Beta-catenin in order to illuminate a probable mechanism for Dact1-mediated repression of Wnt signaling.

References

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