δ-Catenin increases the stability of EGFR by decreasing c-Cbl interaction and enhances EGFR/Erk1/2 signaling in prostate cancer

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1. c-Cbl increases the stability of δ-catenin.

We also investigated the effect of c-Cbl on δ-catenin in the presence and absence of EGF. CWR22Rv-1 cells were transfected with the plasmids shown in Figure S1. The cells were serum starved for 1 day and then treated with 100 ng/ml EGF for the time periods shown in Figure S1 (A). The harvested cells were subjected to Western blotting with the following antibodies: anti-δ-catenin, anti-c-Cbl, anti-p-Erk, and anti-Erk antibody. Interestingly, we observed that overexpression of c-Cbl increased the δ-catenin protein level in the absence or presence of EGF.

To further demonstrate the effect of δ-catenin on c-Cbl protein levels, we transfected CWR22Rv-1 cells with c-Cbl-HA and δ-catenin in a dose dependent manner. The transfected cells were serum starved for 1 day and then treated with 100 ng/ml EGF for the time periods shown in Figure S1 (B). The cells were harvested and Western blotting was carried out. We found that overexpression of δ-catenin had no effect on the stability of c-Cbl.
Figure S1. Effect of c-Cbl on δ-catenin and vice versa. (A) c-Cbl increased δ-catenin stability in the presence and absence of EGF. (B) δ-Catenin had no effect on c-Cbl in the presence or absence of EGF. These experiments were repeated three times.

2. Overexpression of δ-catenin can slightly increase cell viability in prostate cancer cell.

To check the effect of δ-catenin on cell viability of prostate cancer cell, we performed MTS cell proliferation assay. RV/C and RV/δ cells were cultured in 96 well for 24 h. After this, each well were treated with 10μM and 20μM of paclitaxel (Sigma #T7191) for next 24 h. Then, MTS solution (Promega #G3580) was added for 2 h and cell viability was checked. We observed that the cell
viability was slightly higher for δ-catenin overexpressing cell compared to control. However, we could not find any statistically significant difference on cell viability between RV/δ and RV/C.

Furthermore, we also checked the expression of cleaved PARP (Indicator of apoptosis) in RV/δ and RV/C cells. Both cells were treated with 0µM, 10µM and 20µM paclitaxel for 24 h and then cleaved PARP expression was examined by immunoblotting with anti-cleaved PARP antibody. We observed that treatment with paclitaxel increased the levels of cleaved PARP regardless of the presence or absence of δ-catenin.

Taken together, these data suggest that cells overexpressing δ-catenin do not show dramatic resistance to drug used for cancer therapy.
Figure S2. δ-Catenin overexpression can slightly increase cell viability compared to control in prostate cancer cell. However, we could not find any statistically significant difference on cell viability between RV/δ and RV/C. (B) Paclitaxel increased the levels of cleaved PARP regardless of the presence or absence of δ-catenin. (C) Quantitation of cleaved PARP as shown in Figure S2 (B).
Effect of δ-catenin on EGFR phosphorylation.

Phosphorylation of Tyr1045 creates a major docking site for c-Cbl, an adaptor protein that leads to receptor ubiquitination and degradation following EGFR activation (Grøvdal et al., 2004). Our proposed model for δ-catenin-mediated stabilization of EGFR suggests δ-catenin competes with c-Cbl to bind directly to EGFR, hence inhibiting c-Cbl-mediated downregulation of EGFR. If our proposed model is true, δ-catenin should inhibit the ubiquitination and degradation of p-EGFR 1045, thus increasing the ratio of p-EGFR 1045 to total EGFR in δ-catenin-stabilized cells. Control cells and δ-catenin-stabilized cells were serum-starved for 24hrs and treated with EGF for 0, 2, 5, 15, 30 and 60 mins respectively. The cells were harvested, and western blot was performed to analyse the proportion of p-EGFR 1045 and total EGFR. The graph below shows the ratio of p-EGFR 1045 to total EGFR in controlled Rv-1 cells versus δ-catenin-stabilized Rv-1 cells. In δ-catenin-stabilized cells, the ratio of p-EGFR Y1045 to EGFR was found to be higher than in control cells during different times of EGF treatment as indicated in the figure. This finding supports our proposed model on δ-catenin-mediated stabilization of EGFR.

c-Cbl is also known to bind with EGFR indirectly through Grb2 adaptor protein. The GRB2 adaptor protein binds activated EGFR at phospho-Tyr1068 (Grøvdal et al., 2004). Our findings show δ-catenin does not affect the indirect binding between c-Cbl and EGFR via Grb2 (Figure 5A). This suggests that there should be no effect of δ-catenin on the proportion of phospho-EGFR 1068 and EGFR in cells. To investigate the effect of δ-catenin to the ratio of p-EGFR 1068 to total EGFR, control cells and δ-catenin-stabilized cells were serum-starved for 24hrs and treated with EGF for 0, 2, 5, 15, 30 and 60mins respectively. The cells were harvested, and western blot was performed. The graph below shows the ratio of p-EGFR 1045 to total EGFR in controlled Rv-1 cells versus δ-catenin-stabilized Rv-1 cells. In δ-catenin-stabilized cells, the ratio of p-EGFR to total EGFR was found to be similar to in control cells during different times of EGF treatment as indicated in the figure. This finding supports our proposed model that claims δ-catenin decreases the direct binding between c-Cbl and EGFR at phosphorylated Tyr1045. However, it has no effect on the indirect binding of c-Cbl to EGFR via Grb2.
Figure S3. Effect of δ-catenin on phosphorylation of EGFR Y1045 and EGFR Y1068.