Plotnikov et al, Impaired turnover of prolactin receptor contributes to transformation of human breast cells

Supplementary Data

Legends to supplementary figures

Figure 1S: Degradation of Flag-tagged PRLr-WT or PRLr-S349A mutant in MCF10AΔp53 cells was analyzed by cycloheximide chase followed by immunoblotting using anti-Flag antibody (upper panel). Analysis of β-actin levels was used as a loading control (lower panel).

Figure 2S: Representative morphology of indicated MCF10AΔp53 cell lines growth in three-dimensional cultures at the indicated day after plating. Scale bar - 15μM.

Figure 3S: Pictures of H&E stained histological slides were taken at 8 day after indicated MCF10AΔp53 cells injection into the NCRNU-M mice. The pictures were taken using the indicated microscope objectives.

Figure 4S: Effect of anadamide on the growth of T47D cells. The graph represents the number of live cells calculated with trypan blue ± SE at indicated times after the treatment. The differences between groups were statistical significant ($pV < 0.05$) at all indicated times.

Figure 5S: Effect of anandamide on S349-PRLr phosphorylation in -MCF10AΔp53 cells. MCF10ApΔ53 cells expressing flag-tagged PRLr-WT were pretreated with 20mM of methylamine for 2 hours, and then with 10μM of anandamide for indicated

times. The lysates immunoprecipitated with anti-flag M2 agarose, separated on SDS–PAGE and analyzed by immunoblotting with antibodies against pSer349-PRLr, upper panel; and Flag, bottom panel.

Figure 6S: Degradation of Flag-tagged PRLr-WT in MCF10AΔp53 cells after anandamide treatment was analyzed by cycloheximide chase followed by immunoblotting using anti-Flag antibody (upper panel). Analysis of β-actin levels was used as a loading control (lower panel).

Figure 7S: Photos of the tumors derived from the indicated T47D cells at 32 day after cell injection either into animal flank or into the abdominal mammary glands as indicated.