Li et al: TGF-β induces PTHrP degradation in HCC

Supplemental Methods

Metabolic Labeling

HuH-7 cells were grown in 10-cm dishes (3.2 x 10^5 cells/dish) in complete medium overnight and starved for 4 h in methionine-free labeling medium (1X DMEM containing 500 μg/ml L-cysteine, 4 mM L-glutamine, and 0.1% FBS) (3 ml/dish). The cells were then treated with or without TGF-β in the presence or absence of lactacystin, a proteasomal inhibitor. Metabolic labeling was performed by the addition of L-[^{35}S]methionine (Perkin-Elmer) at a final concentration of 50 μCi/ml for 24 h. Whole cell lysates were prepared followed by immunoprecipitation with an anti-PTHrP antibody and separation on a 4-12% Bis-Tris gel. The gel was then fixed in 10% Acetic Acid and 25% Methanol for 30 min and soaked in 1% Glycerol and 5% PEG 8000 for 30 min. Finally, the gel was dried at 80°C for 1 h under vacuum. Autoradiography was performed for 4 days using the Kodak MR film.

Supplemental Figure 1. TGF-β promotes *de novo* synthesis of the PTHrP protein. HuH-7 cells grown in 10-cm dishes were starved for 4 h in methionine-free labeling medium. The cells were then treated with or without TGF-β in the presence or absence of lactacystin. At the same time, the cells were metabolically labeled with L-[^{35}S]methionine at a final concentration of 50 μCi/ml for 24 h. Whole cell lysates

were prepared followed by immunoprecipitation, PAGE gel separation, and autoradiography.