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SMAD4 Deletion and EGF Co-Operate in Favoring mTOR Activation in PDAC Cells

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Context EGFR overexpression occurs early, while loss of SMAD4 occurs in more advanced PDAC. Loss of SMAD4 alters TGFb1 signalling, associates with a reduced stromal and an increased cancer cell expression of S100A8/A9. **Objective** To ascertain whether the effects on NF-kB, Akt, mTOR and MAPK signalling pathways exerted by S100A8/A9, TGFb1 and EGF depend on SMAD4 deletion and whether they are sensitive to EGF chronic exposure. **Methods** BxPC3 (SMAD4 homozygous deletion) and SMAD4 expressing BxPC3 (BxPC3-SMAD4+; plasmid expression vector) were stimulated with EGF (100 ng/mL), S100A8/A9 (10 nM) and TGFb1 (0.02 ng/mL) alone or combined. Cells were both left untreated or were pre-treated with EGF for 3 days. Total protein lysates were used for the WB analysis of: Akt (Ser473, Thr308), mTOR (Ser2448, Ser2481), NF-kB (p-IkB-a), MAPK (p-p38, pErk 1/2). **Results** In BxPC3-SMAD4+ cells, EGF activated, while TGFb1 and S100A8/A9 inhibited Akt and MAPK. In these cells, S100A8/A9 and EGF stimulated, while TGFb1 inhibited NF-kB. SMAD4 deletion did not affect EGF signalling, reverted TGFb1 and S100A8/A9 effects on Akt, and allowed mTOR activation after TGFb1, S100A8/A9 and EGF treatments. EGF pre-treatment of BxPC3-SMAD4+ caused de-sensitization of NF-kB and MAPK to all stimuli, which inhibited Akt and activated mTOR. In the same conditions loss of SMAD4 caused NF-kB, MAPK and mTOR response to all stimuli. Only in BxPC3-SMAD4+ S100A8/A9 synergized with TGFb1 in inhibiting Akt. **Conclusion** SMAD4 deletion in pancreatic cancer cell and chronic treatment with EGF co-operate in activating pro-proliferative and pro-metastatic pathways when cells are treated with growth factors, inflammatory proteins and TGFb1.