Materials

NuPAGE gels, molecular weight markers, and the CyQUANT cell proliferation and adhesion assay kit were obtained from Invitrogen (San Diego, CA). Recombinant human activin-A was generated using a stable activin-A-expressing cell line generously provided by Dr. J. Mather (Genentech, Inc.) and was purified by Wolfgang Fischer (Peptide Biology Laboratory, Salk institute). Recombinant mouse Nodal, human TGF-β1, human activin-B, mouse Cripto, and anti-E-Cadherin (1:500) were purchased from R&D Systems (Minneapolis, MN). Protein A- and G-agarose and the PI3K (LY294002) and MEK kinase (PD98059) inhibitors were purchased from Calbiochem (San Diego, CA). ^{125}I-Cripto was prepared using the chloramine T method as described previously (Vaughan & Vale, 1993). Polyclonal anti-Cripto antibody (6900) was previously described and used at a 1:500 dilution (Shani et al., 2008). Goat IgG, anti-GRP78 (N-20) and anti-phospho-tyrosine (PY99) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and used at 1:200 dilution for Western blots. Anti-phospho-Smad2 (1:500), anti-Smad2/3 (1:500), anti-pan-actin (1:1000), anti-phospho-Akt (1:500), anti-Akt (1:500), anti-phospho-Erk1/2 (1:500), anti-Erk1/2 (1:200), anti-phospho-Src (Y416) (1:500), and anti-Src (1:500) were purchased from Cell Signaling Technologies, Inc. (Danvers, MA). Anti-HA (1:200), anti-M2 Flag (1:500) and anti-Flag (M2) agarose were purchased from Sigma (St. Louis, MO). Horseradish peroxidase-linked anti-mouse (1:10000), anti-goat (1:10000), anti-

rabbit IgG (1:10000), 3,3',5'5-tetramethlbenzidine substrate, chemiluminescent substrate (Supersignal^{TM}), and the BCA protein assay kit were obtained from Pierce (Rockford, IL).

Expression constructs

The wild-type Cripto-Flag expression constructs have previously been described (Gray et al., 2006). GRP78-HA and Δ19-68 GRP78-HA constructs were made using standard PCR techniques (Harrison et al., 2003). The lentiviral expression (Miyoshi et al., 1998) and shRNA (Singer et al., 2005) vectors for Cripto (Gray et al., 2006) and GRP78 (Shani et al., 2008) have been previously described.

Confocal Microscopy

Cells were first blocked in 5%FCS/PBS (buffer A) at room temperature for 15-30 min, and then stained in buffer A containing rabbit anti-Cripto (6900), goat anti-GRP78 (N-20, Santa Cruz Biotechnology, Santa Cruz, CA), and mouse anti-E-cadherin (36/E-Cadherin, BD Biosciences, San Jose, CA) at 10^0C for 30 min. Following washing, cells were stained with anti-rabbit, anti-goat, and anti-mouse fluorescently-conjugated secondary antibodies in buffer A at 10^0C for 30 min. Cells were washed and fixed with 4% paraformaldehyde at room temperature for 15 min. Coverslips were then mounted in the presence of DAPI (4', 6'-diamidino-2-phenylindole), and analzed by confocal microscopy using a Leica TCS SP2 AOBS confocal microscope (Leica, Wetzlar, Germany).

SUPPLEMENTAL REFERENCES

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