**SUPPLEMENTARY METHODS**

**Molecular reagents and published assays**

Antibodies against the following proteins were used as indicated: BMP4 (Millipore), pSMAD1/5/8 (Cell Signalling), Tubulin (Sigma), HSP60 (Gift from Dr. N. Hoogenraad, La Trobe University), SMAD4 (Abcam) and SMAD7 (Abcam), isotype immunoglobulin controls (Jackson Labs). Detection of BMP4 in conditioned medium and in mouse serum was achieved by ELISA according to manufacturer’s instructions (R&D Systems). Cell conditioned medium was assessed for BMP4 activity based on a previously published assay for BMP-specific induction of alkaline phosphatase activity in C2C12 promyoblasts ([3](#_ENREF_3)). Western blots were completed using standard methods ([4](#_ENREF_4)). Recombinant BMP4 and Noggin proteins were purchased from R&D Systems. *In vitro* cell proliferation was assessed using the sulforhodamine B assay ([1](#_ENREF_1)). Plasma calcium levels were analysed by the Haematology Department at Peter MacCallum Cancer Centre, from blood drawn via terminal exsanguination.

**Analysis of online gene expression databases**

 For Oncomine analyses, we searched for *BMP4* in human breast cancer vs normal tissue and considered significant down regulation based on a P value of 0.05 and a 2-fold change of expression. The datasets identified are listed in Supplementary Table S1. For BreastMark analysis, we assessed the correlation of BMP4 mRNA (stratified by median expression) to disease-free survival, distant metastasis-free survival (DMFS) and overall survival. For GOBO analyses we evaluated the correlation of BMP4 mRNA expression (median stratified) between molecular subtypes, breast tumour grade and DMFS. Multivariate analysis of DMFS included parameters of tumour grade, tumour size, age, node status and ER-status. Survival correlations were considered significant using a log rank test, *P* value < 0.05. Analysis between groups was assessed by ANOVA and considered significant when *P* < 0.05.

**Genetic engineering of breast cancer cells**

Alteration of gene expression in breast cancer cells was achieved by retroviral or lentiviral gene transduction. Full length mouse *Bmp4* and *Bmp7* were cloned by PCR from mammary tissue into pCI-neo and the native transcript confirmed by sequencing. Both clones were excised from pCI-neo using *EcoRI* and *Sal1* restriction enzymes and sub-cloned into the complementary sites of pBABE retroviral mammalian expression vector. Human BMP4 cDNA clone (in pSPORT) was obtained from the Mammalian Gene Collection (Clone Id: 4399276, Dharmacon) and cloned into pLV-EF1a-IRES-HygroR vector for gene transduction (AddGene). pBABE-puro-FLAG-SMAD7 was a kind gift from Dr. A. Glick (Penn State University, USA). For shRNA vectors, we cloned oligonucleotides specific for the targeted silencing of *Bmp4* or *SMAD7* into either the *BglII*/*HindIII* sites of pRetroSuper or the *XhoI*/*EcoRI* sites of pLMP (Open Biosystems). The oligonucleotides used for the construction of shRNA vectors are described in Supplementary Table S4. As a control, we utilised non-silencing controls that form functional short hairpins but do not target murine genes (Supplementary Table S4). The non-silencing pLMP-control was a gift from Prof. R. Johnstone (Peter MacCallum Cancer Centre, Melbourne, Australia). Constructs were transfected into a 293-based viral packaging cell system and recombinant virus harvested for the transduction of breast cancer cells as described previously ([5](#_ENREF_5)). Transient gene silencing of SMAD7, or non-targeting control, was performed by PEI-based transfection of SMARTpool siRNA purchased from Millennium Science (Australia).

**RNA isolation and real-time RT-PCR**

Total RNA was extracted with TRIzol solution (Invitrogen), according to the manufacturer’s instructions and the integrity of RNA was assessed by spectrophotometry and agarose gel analysis. RNA was converted to cDNA by reverse transcription using MMLV Reverse Transcriptase (Promega) and gene expression analysed by real-time quantitative PCR (qPCR) using SYBR Green I reagents (ABI 7000, Applied Biosystems) using previously established methods ([1](#_ENREF_1)). Oligonucleotides utilised for the analysis of gene expression are listed in Supplementary Table S4. The expression of each gene was calculated based on the cycle threshold (CT), set within the linear range of DNA amplification. The expression (arbitrary units) was calculated as the relative transcript abundance (RTA) by: RTA = 10,000/(2∆CT), where ∆Ct = Ct(gene of interest) - Ct(GAPDH).

**Immunohistochemistry**

Immunohistochemical staining was completed on formalin-fixed, paraffin-embedded tissues. High temperature, high pressure antigen retrieval was achieved using citrate buffer prior to the immunodetection of either BMP4 or SMAD7 as described previously ([6](#_ENREF_6)). For immunocytochemistry, cells (treated with 30ng/mL BMP4 or vehicle) were cultured in glass slide chambers prior to fixation in 4% paraformaldehyde, then blocked in PBS containing 2% bovine serum albumin (BSA) for 30 minutes prior to an overnight incubation with primary antibody at 4°C. After several washes with PBS, the primary antibody was detected with an Alexa488-conjugated secondary antibody (Invitrogen) and visualised by fluorescence microscopy. Cells were enumerated from five random visual fields, from triplicate samples.

**Animal studies**

 4T1.2, 4T07, 168FARN and 66cl4 tumours were established in female Balb/c mice (6–8 weeks) obtained from ARC (Perth, Australia) or Walter and Eliza Hall Institute (WEHI, Australia). For xenograft tumour studies involving MDA-MB-231 and SUM159 tumours; NOD-SCID-Gamma mice were utilized and obtained from ARC or MD Anderson (Department of Experimental Radiation Oncology). All mice were housed with food and water *ad libitum* in the animal facility. Orthotopic tumours were established via injection of cancer cells into the fourth inguinal mammary gland and tumour growth monitored by calliper measurements. For therapeutic studies, rBMP4 or saline as vehicle control, was administered twice-daily to tumour-bearing mice by intraperitoneal injections (10µg/kg rBMP4/injection), beginning the day after tumour cell implantation. The therapeutic dose was based on a previously established strategy in mice that utilised twice-daily dosing to circumvent potential issues with the short half-life of active BMP4 protein ([7](#_ENREF_7)). Metastatic burden was assessed as described previously ([1](#_ENREF_1),[5](#_ENREF_5)) and GFP-based fluorescence imaging using CRi Maestro 2 Multispectral Imaging System. Briefly, genomic DNA was isolated from the whole organ, either lung or spine. The abundance of tumour cells present in the organ was quantitated by real-time PCR assay that detects the levels of a reporter gene (neomycinR or puromycinR) transfected into the cancer cells, relative to the genomic levels of vimentin, present in all cells. Tumour excision was completed on anesthetised mice, under aseptic conditions and their post-surgical health monitored daily. Evidence of tumour recurrence and metastatic disease were monitored concurrently. All animal procedures were conducted with approval from either the Peter MacCallum or Austin Health Animal Ethics Committees.

**Cancer cell isolation from primary tumours**

Epithelial cancer cells were isolated from established orthotopic primary tumours using mild collagenase A digestion and an immuno-magnetic bead-based purification method ([8](#_ENREF_8)).

**Anoikis assay**

 Cells at 70% confluence were lifted, re-suspended in culture medium at a density of 3000 cells/mL in Falcon polystyrene round-bottom tubes and incubated at 37°C with mild agitation. Aliquots from these cultures were taken at multiple time points, seeded into 6-well plates, returned to standard culturing conditions. After seven days, plates were stained with crystal violet solution and the number of colonies enumerated. In experiments utilising exogenous BMP4, the recombinant protein was added to the suspended cultures but was removed during clonogenic expansion. Where siRNA was transfected for gene knockdown, this was performed in cells at 50% confluency, 24 hours before the start of the assay. Approximately 10-fold reduced SMAD7 mRNA expression was confirmed (in 4T1.2 cells stimulated with 100ng/mL rBMP4, 90min) by RT-PCR (data not shown). Samples were analysed in triplicate for each experiment.

**Chromatin immunoprecipitation**

Cells were serum-starved for 24 hours prior to stimulation with 30ng/mL rBMP4 protein. Three hours post treatment, cells were washed twice with ice cold PBS, scraped and pelleted by centrifugation. Cells were lysed for 15 minutes at 4°C in 1mL nuclear extraction buffer (10mM Tris-HCl, pH 8; 140mM NaCl; 0.1% IPEGAL; 1.5mM MgCl2; including a protease inhibitor cocktail (Roche)). Nuclei were recovered by centrifugation (1000rpm, 4°C, 5 minutes), resuspended in 1mL of nuclear extraction buffer (containing 5mM CaCl2, and PMSF), briefly sonicated and digested with micrococcal nuclease for six minutes. The reaction was inhibited with 50mM EDTA, the sample clarified by centrifugation twice and the DNA content quantitated by spectrometry and appropriate genomic digestion verified by gel electrophoresis. Digested DNA (20µg) was diluted in 1mL of nuclear extraction buffer, supplemented with the appropriate antibody and incubated overnight at 4°C with agitation. Protein G magnetic beads (Millipore) (10µL per sample) were washed in PBS, blocked in 1% BSA (supplemented with 100µg/ml herring sperm DNA) for 30 minutes, washed again in PBS and equilibrated in nuclear extraction buffer prior to their addition to the antibody-DNA complex. Beads were incubated for 4 hours at 4°C prior to washing in a series of three stringency buffers (nuclear extraction buffer containing either 75mM, 125mM, and finally 175mM NaCl). Samples were washed twice in each buffer at 4°C for 5 minutes with agitation. Enriched samples were finally resuspended in Tris-EDTA buffer and frozen until analysed by real-time qPCR. Transcription factor binding was quantitated on triplicate samples by real-time qPCR utilising the ∆∆Ct method on input normalised samples ([9](#_ENREF_9)). qPCR was completed essentially as above, using SYBR Green I reagents (Applied Biosystems) and primers shown in Supplementary Table S4. Specificity of this assay with respect to *SMAD4* immunoprecipitation was determined by reduced signals in samples immunoprecipitated with an isotype-matched or with non-conjugated beads. Specificity to the *SMAD7* promoter (-660bp) was determined by lack of *SMAD4* signal in genomic sites distal to the 5’-UTR (+1637bp).

**Patient samples for immunohistochemical analysis**

Breast tumour samples from 535 patients treated at a single institution, Royal Perth Hospital, Western Australia, between January 1996 and December 2001 were analysed ([10](#_ENREF_10)). An invasive component was present in 460 cases, whereas 75 had DCIS alone. All patient records were examined, and relevant data collated in a TMA-specific database. The full dataset included clinicopathological features of the primary tumour, adjuvant treatments received, relapse data including site and subsequent survival data. Mean age at surgery was 53. All patients underwent primary tumour removal either by mastectomy or wide local excision (WLE) with axillary lymph node clearance. Postoperative radiotherapy was given with WLE and to mastectomy patients with large tumours or those with close surgical margins. Axillary radiotherapy was given to those with four or more affected axillary lymph nodes. Eighty-one percent received adjuvant endocrine treatment, 70% of which involved tamoxifen, and 38% received adjuvant chemotherapy either cyclophosphamide, methotrexate and fluorouracil (19%) or an anthracycline-based regimen (19%). Patient follow-up on the invasive cohort was available until last database update on 1st July 2014 with a median follow-up on the subset with assessable invasive cancer of 9.8 years with 5- and 10-year follow-up on 99.0% and 48.0% respectively. The use of patient samples and clinical information in this study was approved by the Human Research Ethics Committee of Royal Perth Hospital.

**Breast cancer tissue microarray and analysis**

The tissue microarray was constructed from archival formalin fixed paraffin embedded tumour blocks. Three to five 1.0 mm core biopsy samples were taken from histopathologically representative areas, the number of cores for a specific case depending on tumour tissue volume present. Slides were immunostained as described above. During scoring, each sample was assessed for epithelial tissue type by a histopathologist (B. Latham) to confirm invasive carcinoma, DCIS or normal epithelium and to subtype invasive histology. Estrogen receptor (ER) and progesterone receptor (PR) status was available from initial histopathological records, as assessed by immunohistochemistry (IHC). Routine HER2 testing was not standard of care until 2000 and so was only available initially on approximately 20% of samples. Extended testing was subsequently carried out by a collaborative group and consisted of both IHC and *in situ* hybridisation analysis as described elsewhere ([10](#_ENREF_10)). TMA slides were scanned to produce digital images for back-up purposes and to capture representative images using the Aperio system (Aperio ePathology Systems, Vista, CA). BMP4 and SMAD7 staining intensity was evaluated across each individual core biopsy by three scorers, a histopathologist (B. Latham) and two medical oncologists (A. Redfern, P. Lau). Scores attributable were; 0 (no staining), 1+ (weak staining), 2+ (moderate staining) and 3+ (strong staining). Where scores differed for a core, a mean score of the two was attributed. Cores varying by more than a single unit were conjointly reviewed and were awarded a consensus score. This was required for less than 1% of samples. Observed agreement between scorers was 91.3%. A mean score of all relevant cores was then recorded for each patient. Scores for normal breast epithelium, DCIS and invasive malignancy were summated separately for each individual. For bivariate analyses, a score of 1.5 or greater was considered as positive for BMP4 expression; and a score of 1.0 or greater considered positive for SMAD7 expression.

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