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Supplemental Data

The Extracellular Matrix Protein TGFBI Induces Microtubule Stabilization and Sensitizes Ovarian Cancers to Paclitaxel

Ahmed Ashour Ahmed, Anthony D. Mills, Ashraf E. K. Ibrahim, Jillian Temple, Cherie Blenkiron, Maria Vias, Charlie E. Massie, N. Gopalakrishna Iyer, Adam McGeoch, Robin Crawford, Barbara Nicke, Julian Downward, Charles Swanton, Stephen D. Bell, Helena M. Earl, Ronald A. Laskey, Carlos Caldas, and James D. Brenton

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Microarrays

cDNA microarray experiments were carried out as previously described (Ahmed et al., 2004). In brief, total RNA (50 μg) was used for reverse transcription and indirect labelling with Cy3 and Cy5 dyes (Amersham) using random hexamers as previously described (Richter et al., 2002) before hybridization to cDNA expression microarrays (Cancer Research UK DNA Microarray Facility). Scanning was performed using the ScanArray 4000 (Perkin Elmer). Segmentation was performed using QuantArray (Perkin Elmer) software using the histogram method. Expression profiling of patient material was performed using Human Genome U133A 2.0 Arrays (Affymetrix) according to the manufacturer's instructions.

Plasmids and transfection

Oligos A810 5′-GGTAACGGCCAGTACACGC-3′ and K1318 5′-TTAAAGACCAGCTGGCCTC-3′ were designed using the siRNA Design Tool (OligoEngine) and cloned into the pSUPER.retro.puro plasmid (OligoEngine) to generate plasmids pA810 and pK1318. Clones were verified by sequencing and stable transfections were performed using Lipofectamine 2000 (Invitrogen Life Technologies) followed by puromycin selection. Transient knock-down experiments were performed by transfection with a pool of 4 individual siRNA duplexes targeting *TGFBI* (siGenome Smart pool, Dharmacon), or individual duplexes, using Lipofectamine 2000 (Invitrogen) according to the manufacturers' instructions. Either fluorescent-labeled non-targeting siRNAs (SiGLO, RISC-Free siRNA, Dharmacon) or siControl non-targeting siRNA pool (Dharmacon) were used as controls. FAK knock-down was achieved using On-targetplus duplexes against *PTK2* (Dharmacon). Transfection was performed with 2.7 μg of each duplex using the nucleofector kit V (Amaxa Biosystems). The plasmid pbig-wt was a kind gift of Dr Daniel F. Schorderet (Morand et al., 2003). The N19RhoA construct was a kind gift from Dr Shin-Ichi Ohnuma.

Cloning and expression of TGFBI

The *TGFBI* cDNA was amplified by PCR from the cDNA clone MGC:3646 (IMAGE:2958878) using *TGFBI* specific primers forward 5′-CCCAAGCTTGCCGCCACCATGGCGCTCTTCGTGCGG-3′ and reverse 5′-CCCAAGCTTATGCTTCATCCTCTCTAATAACTTTTG-3′ and cloned into the HindIII site of the PCS2+mt plasmid (Invitrogen) in frame with the myc coding sequence (pCSMT-TGFBI). To generate the his-myc tagged rTGFBI (AAA07) construct *TGFBI* cDNA was amplified using *TGFBI* specific primers forward 5′-CCGCTCGAGGCCGCCACCATGGCGCTCTTCGTGC-3′ and reverse 5′-CCCCCGCGGATGCTTCATCCTCTCTAATAAC-3′ and cloned into the XhoI and SacII sites of pcDNA4/myc-his B (Invitrogen).

Real-time PCR

Total RNA was isolated from primary tumours and cancer cell lines using Trizol reagent (Gibco BRL) and 1 μg was reverse transcribed with the Reverse Transcription Kit (Applied Biosystems). PCR was carried out using SYBR Green PCR Master Mix (Applied Biosystems) and an ABI 7900 Sequence Detection System (Applied Biosystems). *TGFBI* was amplified using forward 5′-AGCCCTGCCACCAAGAGAA-3′ and reverse 5′-CTCCGCTAACCAGGATTTCATC-3′ primers. The specificity of the PCR products was confirmed by melting curve analysis and amplification was normalized against *GAPD* amplified using forward 5′-GCAAATTCCATGGCACCGT-3′ and reverse 5′-TCGCCCCACTTGATTTTGG-3′ primers. Expression ratios relative to SKOV-3TR expression were calculated as previously described (Pfaffl, 2001) and $\log_2$ transformed for statistical comparison.

Immunoblot analysis

Antibodies

Anti-TGFBI antibodies were either obtained from Santa Cruz (for immuno-blotting of cultured media and immuno-cytochemistry) or were a kind gift from Dr Ching Yuan (for blots of protein lysates and immunohistochemistry). Other antibodies used were against cleaved caspase 3 (Chemicon), alpha tubulin (Sigma), gamma tubulin (Sigma), cyclin B1 (Vision Biosystems), BubR1 (Abcam), detyrosinated tubulin (Glu-tubulin, [Chemicon]), myc (Cancer Research UK), gm130 (BD Transduction), alphaVbeta3 (Chemicon), FAK (Abcam) and FAK-P397 (Abcam). Secondary anti rabbit and anti-mouse antibodies were either horseradish peroxidase-tagged (DakCytomation) or fluorescine, texas red or Cy5 tagged (Amersham Biosciences)

Conditioned medium

Cells were grown in T75 flasks until confluence and conditioned medium was cleared by centrifugation at $10,000 \times g$ for 30 min and either used directly for immunoblotting or fractionated as previously described (Billings et al., 2002). In brief, cleared medium was put onto strong cation exchange columns (Amersham Biosciences), the column was washed with 10 ml of 50 mM phosphate, pH 6.5, 50 mM NaCl, and bound proteins were eluted with a linear NaCl gradient (0.05–1 M) in the same buffer. Aliquots from each fraction were analyzed by immunoblotting. TGFBI was maximally eluted in fractions 9–12 (out of 20 fractions); these were subsequently used in comparison between different cell types.

Western blot analysis

Aliquots of cell lysates containing equal protein mass or culture medium were resolved on SDS-polyacrylamide gel electrophoresis, transferred to Immobilon-P membranes (Millipore) and probed with primary antibody at the recommended dilutions followed by the relevant secondary antibodies conjugated with horseradish peroxidase in a 1:10,000 dilution. After washing, proteins were detected by chemiluminescence (ECL, Amersham).

Estimation of Glu-tubulin formation

Cells were plated at a density of 1×10^5 per well in 24-well plates, serum starved for 24 hr and treated with the indicated concentrations of paclitaxel for 1 hr. Protein lysates were collected in 150 µl of protein lysis buffer at room tempeature. 20 µl of lysates were denatured using 5× Laemmli buffer and boiling at 110 °C for 3 minutes before being resolved on SDS-polyacrylamide gel electrophoresis. Transfer was performed to Immobilon-FL membranes (Millipore) follwed by blocking using 5% milk for 1 hr and probing using rabbit anti-Glu-tubulin antibody and mouse anti-alpha tubulin antibody overnight. Fluorescein-tagged anti-rabbit and Cy5-tagged anti-mouse secondary antibodies were applied for 4 hr for detection. Membranes were scanned using the Typhoon Trio Scanner (GE Healthcare) to measure the fluorescence intensity volumes for individual bands at appropriate wavelengths using the ImageQuant V2005 software (GE Healthcare) and the ratio of fluorescein over Cy5 values were estimated. Glu-tubulin formation was expressed as fold increase over the baseline of no paclitaxel treatment.

Estimation of soluble tubulin fraction

Cells were lysed in a microtubule stabilizing buffer containing 20 mmol/l Tris-HCL (pH 6.8), 0.14 mol/l NaCl, 0.5% NP40, 1 mmol/l MgCl2, 2 mmol/l EGTA and 4 µg/ml paclitaxel as previously described (Minotti

et al., 1991). The insoluble tubulin fraction was separated using centrifugation at maximum speed for 10 minutes at 4 °C and the resulting pellet was resuspended using 1× Laemmli buffer. Equal volumes of the soluble and insoluble fractions were loaded for western blotting as described above using Immobilon-FL membranes (Millipore). Membranes were probed using anti-alpha tubulin antibody which was detected using Cy5-tagged anti-mouse secondary antibodies. Intensity volumes were quantified as above.

Estimation of paclitaxel uptake

Cells were plated into Lab-Tek 8-well chamber slides and cultured overnight. Cells were washed with PBS twice before the addition of 100 µL of TubulinTracker Green (Invitrogen, Oregon Green 488-taxol) at the recommended dilution in PBS with addition of Hoechst 33342 (Sigma) and with or without verapamil at 3 µM. After 30 minutes the fluorescent taxol mix was washed off with PBS and images immediately collected on an Olympus ScanR system (Olympus Soft Imaging Solutions, OSIS) using a 20x lens. 25 images were collected per sample. Images were subsequently processed using the ScanR Analysis software (OSIS) and the cytoplasmic concentration of retained fluorescent taxol was estimated in a ring around each nucleus.

Time-lapse imaging

1×10^5 cells were plated on cover glass chambers (Lab-tech). Images were obtained using 40× lens on a Nikon Eclipse TE 2000-E microsope (Nikon) in an incubator at 37 °C and humidified 5% CO_2. Images were collected every minute for 16 hr using the NIS-Elements AR V 2.3 imaging software (Laboratory Imaging). The time from the start of prometaphase, as evidenced by nuclear membrane breakdown and chromatin condensation, to the start of telophase, as evidenced by the start of the constriction ring formation, was scored for individual cells.

Cell cycle progression

Hela cells were seeded into 24-well plates at a density of 1×10^5 cells per well. Cells were transfected using either TGFBI siRNA or non-targeting siRNA controls. Knock-down of TGFBI was confirmed by western blotting 48 hr following transfection. 24 hr following transfection double thymidine block and release (first block for 19 hr, release for 9 hr and second block for 16 hr) was performed using 2.5 µM of thymidine (Sigma). Cells were harvested after synchronization and every 3 hr for 24 hr, stained using propidium iodide (sigma) staining and analyzed using flow cytometry.

Synthesis of rTGFBI

The human embryonic kidney 293T-EBNA cells (Invitrogen) were cultured in DMEM supplemented with 10% (v/v) bovine calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin and transfected with the AAA07 construct (2.5 µg/cm^2 tissue culture dish) using lipofectamine (Invitrogen). After 24 hr, the media were changed to serum-free DMEM. Conditioned media was then collected 2–3 days later. Myc-His-tagged TGFBI was captured using Nickel agarose columns (Sigma) using the AKTAprime machine (Amersham Biosciences). Bound TGFBI was eluted using 250 mM imidazole. TGFBI was eluted in 15 fractions and was examined on SDS-PAGE gels by Coomassie blue staining or Western blotting using a mouse monoclonal anti-myc (Cancer Research UK) or mouse anti-his (Roche). Captured rTGFBI was desalted using PD-10 columns (Amersham Biosciences). Purified rTGFBI was quantified using the the BCA kit (Pierce Biotechnology, Inc).

rTGFBI coating and cell adhesion

Tissue culture plastic surface coating was performed as previously described (Billings et al., 2002). For adhesion assays, medium-binding polystyrene 96-well plates (Corning) were coated with rTGFBI , fibronectin (Chemicon) or polylysine at 20 μg/ml in PBS or PBS alone overnight at 4 °C. Plates were washed with PBS and blocked using 2% milk at 37 °C for 1 hr. Cells were trypsinized and counted, and trypsin was neutralized using full media. Cells were plated into wells (5×10^4 cells/well) and incubated for 1 hr at 37 °C and then washed twice using PBS. To quantify cell adhesion, cells were stained using the CyQuant cell proliferation assay kit (Invitrogen) according to the manufacturer's instructions. To test the effect of integrin blocking on adhesion, suspension cells were incubated with the mouse monoclonal blocking antibody anti-alphaVbeta3 (Chemicon) for up to 1 hr (1 in 100 dilution) at 37 °C before seeding. For testing the effect of rTGFBI on Glu-tubulin formation using immuno-fluorescence or on Glu-tubulin formation and FAK phosphorylation using western blotting, Lab-Tek II glass chambers (Nalge Nunc International) or 6-well plates (Corning), respectively, were used for coating as above. For testing the effect of rTGFBI on paclitaxel-induced caspase activation, white flat-bottom 384-well plates (Corning) were used for coating.

Immunohistochemistry

Paraffin-embedded blocks were sectioned at 5 μm thickness. Antigen retrieval was achieved by microwave boiling in a preheated citric acid buffer solution. Primary antibody against TGFBI was a kind gift of Dr Ching Yuan and was used at 1 in 500 dilution. Antibody detection was performed using streptavidin-biotin labeling. Visualization was achieved using two methods; di-amino benzidine chromagen method (Dako) or the EnVision method (Dako) following the manufactures instructions. All slides were reviewed blinded to outcome data. All slides were stained in duplicates on two separate occasions and were scored separately to assess reproducibility.

Immunofluorescence, image collection and processing

Sub-confluent cells growing on cover slips or 20 μm thick frozen-sectioned tissue samples on uncoated slides were fixed immediately using 4% paraformaldehyde in PBS for three to five minutes. Processing, blocking and washing were performed as described previously (Mills et al., 2000). Primary antibodies were incubated at recommended dilutions for 2 hr at 37 °C. Primary antibodies were detected using FITC or Texas Red conjugated anti-mouse or anti-rabbit secondary antibodies (Amersham Biosciences) as appropriate for 1 hr at 37 °C. DNA in nuclei was stained using Hoechst 33258 (Sigma). Images were collected using a BioRad Radiance Lasersharp 2000 software on a Nikon TE2000-U with a 60× planapo NA 1.4 lens. Three-dimensional reconstruction of Z-stack images was performed using Volocity 3.0 software (Improvision).

Cambridge Translational Cancer Research Ovarian Study 01 (CTCR–OV01)

Recruitment

Study recruitment was from January 2002 to December 2004. Patients with histologically confirmed advanced (Stages III and IV) epithelial ovarian cancer with a WHO performance status of 0 or 1 were eligible to participate. Exclusion criteria were (1) Non-epithelial ovarian tumours (2) Patients who had received prior chemotherapy or radiotherapy (3) Patients who were not fit to receive paclitaxel treatment. The study was approved by the Cambridge Local Research Ethics Committee (LREC). All patients gave written informed consent prior to participation.

Procedures

All patients with suspected ovarian cancer (Raised CA 125 and a complex pelvic mass in a perimenopausal woman) were radiologically staged using CT scan and a chest x-ray. Patients with evidence of intra-abdominal metastasis and/or malignant pleural effusion were approached for entry to the study. Tissue biopsy was obtained either under radiological control (core needle biopsy) or via laparoscopic surgery (punch biopsy). Patients with histologically confirmed epithelial ovarian cancer were randomized to receive either three cycles of carboplatin (AUC 7) or paclitaxel (175 mg/m^2). Patients with stable or responding disease had interval debulking surgery. Following surgery, patients received three cycles of combination chemotherapy (carboplatin and paclitaxel) followed by a further three cycles of the drug that they did not initiate treatment on (Figure S6A).

Response evaluation

For the purpose of comparing *TGFBI* expression between paclitaxel-resistant and paclitaxel sensitive patients, CA 125 response to the initial three cycles of single agent treatment was evaluated. CA 125 (marker) response was defined as previously described (Rustin et al., 1996; Bridgewater et al., 1999). In brief, paclitaxel-sensitive patients were defined as those who show either (1) a 50% decrease in serum CA 125 level and the sample showing the 50% decrease was confirmed by a susequent sample or (2) a 75% decrease in any sample compared to the pre-treatment sample.

Statistical methods

All statistical analyses were conducted using the R environment (Ihaka and Gentleman, 1996) and the R packages "Statistics for Microarray Analysis" (Dudoit et al., 2002). For cDNA microarray analysis all spots from each microarray were included in the analysis. Data normalization was performed using scaled loess normalization and differential genes were identified using an empirical Bayes method for analysing replicated microarray data (Lönnstedt and Speed, 2002).

Affymetrix microarrays were analyzed using the R packages affy and affy-PLM (Ihaka and Gentleman, 1996). Background subtraction and normalization was performed as previously described (Li and Wong, 2001). Filtering of genes that either had more than 30% absent calls or that did not show significant variation in gene expression across arrays was performed as described (Simon, 2004). Correlation of the expression of *TGFBI* with ECM-related genes was estimated using Pearson's correlation coefficients.

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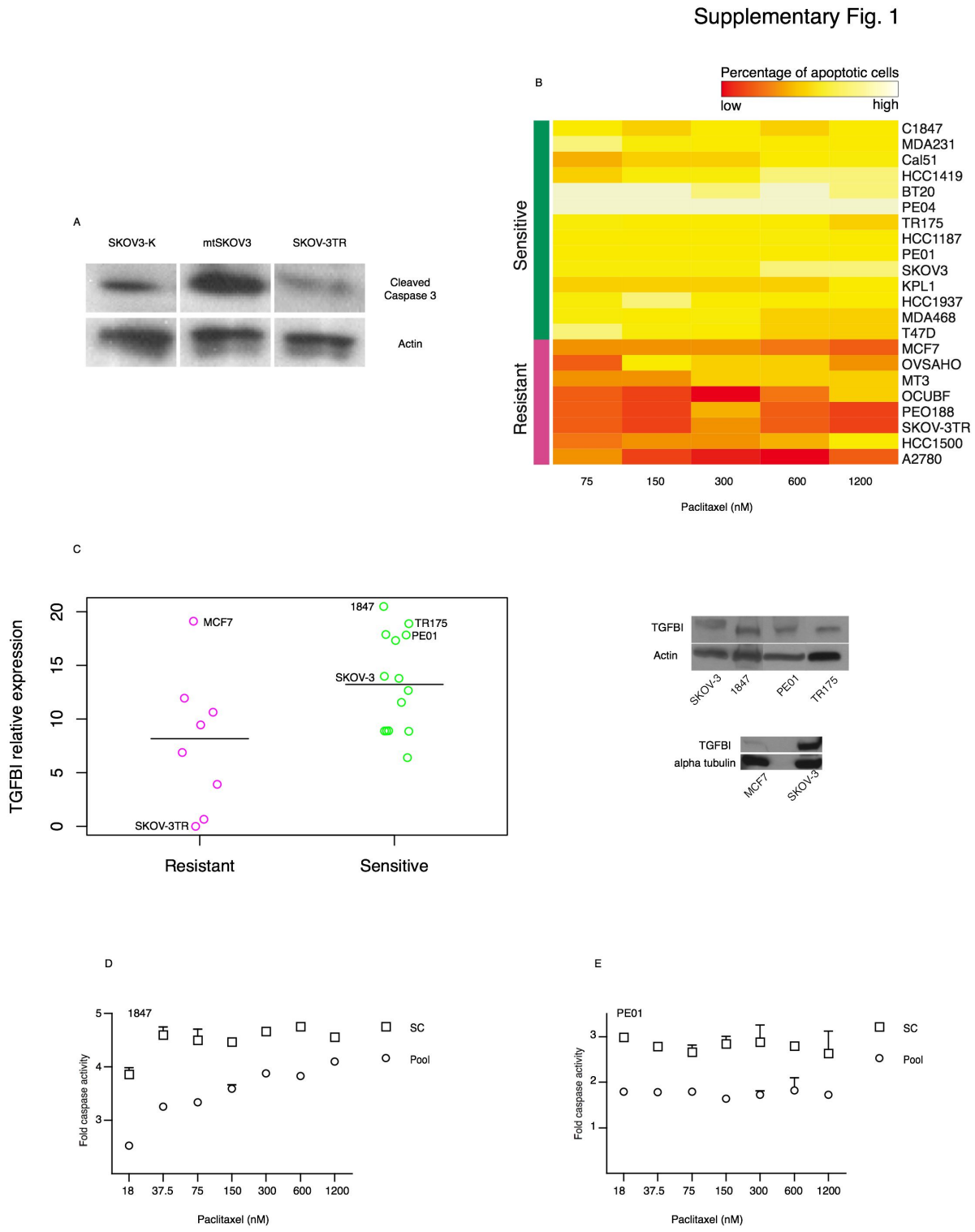


Figure 1: (A) Western-blot analysis of cleaved caspase-3 following 24 hr of paclitaxel treatment at 300 nM. (B) Early apoptosis was measured after 48 hr of paclitaxel exposure at the indicated concentrations by flow cytometry of cells stained with FITC-annexin V and 7-AAD. The average percentages of early apoptosis of at least two independent replicates are expressed as colour intensity (see bar at top right corner). Cells are clustered into resistant (magenta vertical bar) or sensitive (green vertical bar) using K-means clustering analysis based on the similarity of the percentages of apoptosis across all paclitaxel concentrations. (C) Expression of *TGFBI* measured by real-time PCR. Sensitive cell lines (green), resistant cell lines (magenta). Horizontal bars indicate median ratios. Also shown is a western blot confirmation of high expression of TGFBI in sensitive cells. Note that the paclitaxel-resistant MCF7 cell line shown by real-time PCR to over-express *TGFBI* was found to under-express TGFBI protein. (D–E) 48 hr following transfection using either a pool of 4 siRNA oligos targeting *TGFBI* or non-targetting scrambled controls (sc), 1847 cells (D) or PE01 cells (E) were treated with paclitaxel for 48 hr and caspase 3/7 activation was estimated. Data shown represent mean ±s.d..

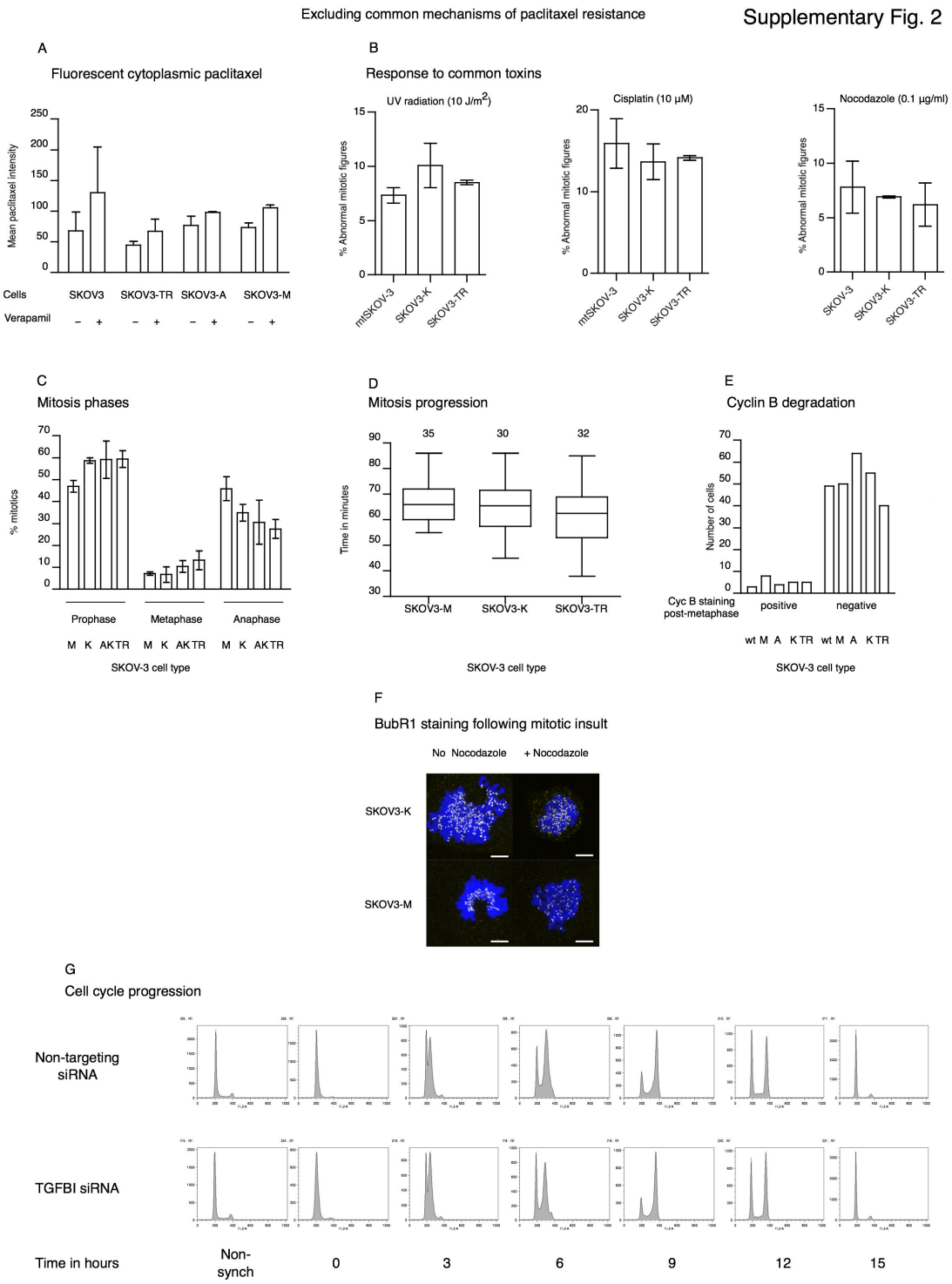


Figure 2: (A) Cells were exposed to a fluorescent derivative of paclitaxel for 30 minutes before the cytoplasmic intensity values of paclitaxel were measured. Shown is the mean intensity values of at least 700 cells measured in two independent experiments. (B) Barplots represent the percentage of early apoptotic cells measured 48 hr following UV radiation, cisplatin or nocodazole at the indicated concentrations using FITC-annexin V and 7-AAD staining. (C) Non-synchronous populations of cells were stained using immuno-fluorescence (IF) for DNA and alpha tubulin. Shown are the percentages of different mitotic phases. In (A–C) data represent mean ±s.d. of at least two independent experiments. (D) Individual cells were followed using time-lapse microscopy by obtaining images every one minute and the duration of progression through mitosis was estimated from the start of prophase to the beginning of telophase. Number of cells followed for each cell line is shown above the box plots. (E) Non-synchronous populations of cells were stained using IF for cyclin B1, DNA and alpha tubulin. Bars represent the number of cells with positive or negative staining for cyclin B1 following metaphase. (F) IF of cells in mitosis either spontaneously or following 0.1 μg/ml of nocodazole for 24 hr. Yellow, BubR1; blue, DNA. Scale bars, 5 μm. (G) 24 hr following transfection using either pool of 4 siRNA oligos targeting *TGFBI* or non-targetting siRNAs, Hela cells were blocked in G1 using double thymidine block and release. Shown are the flow cytometry DNA profiles of cells at indicated time points following release from thymidine block.

Supplementary Fig. 3

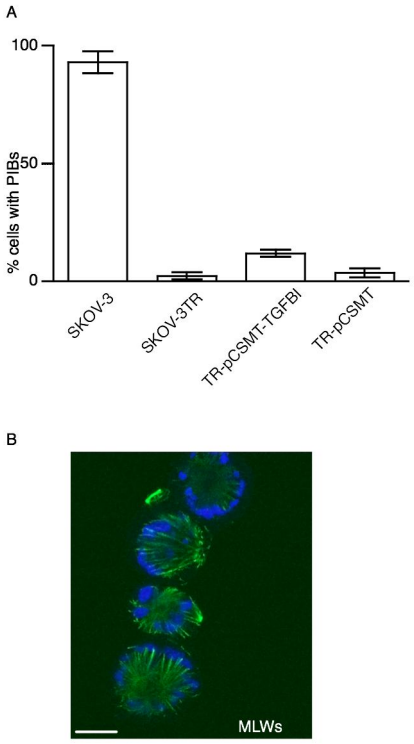


Figure 3: (A) Barplot represents the mean percentages of cells showing PIBs ±s.d. (B) Immunofluorescence staining of paclitaxel-treated SKOV-3 cells showing four mitosis-like wheels (MLWs). Green, alpha tubulin; blue, DNA. Scale bar, 10 μm.

Supplementary Fig. 4

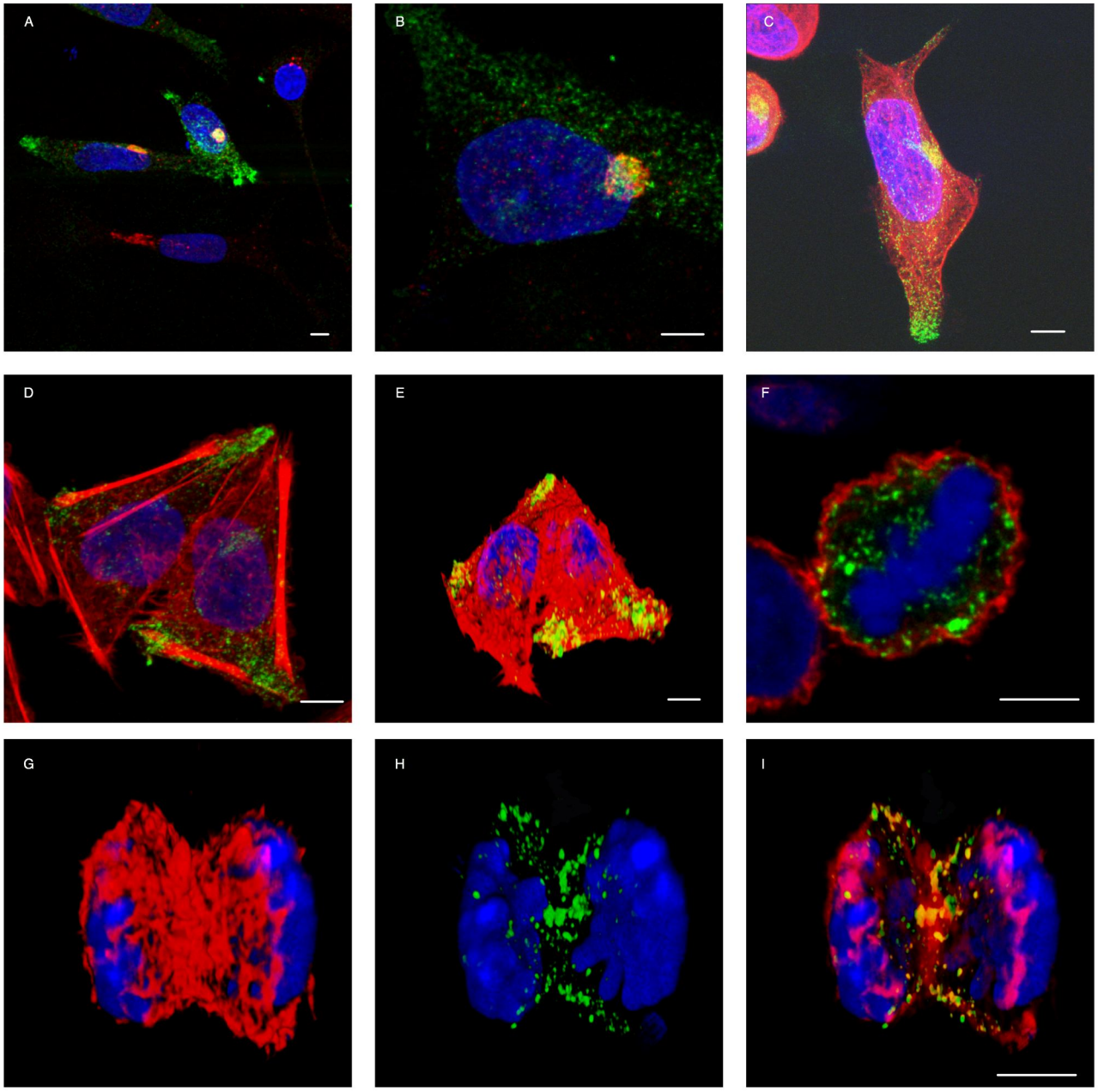


Figure 4: (A) SKOV–3 cells transfected with a Myc-tagged *TGFBI* (green) showing its localization at Golgi, which was labelled using a gm130 antibody in red, and at the end of cellular protrusions. (B) Myc-tagged *TGFBI* co-localizes with anti-gm130 antibodies indicating Golgi localization. (C) SKOV–3 cells transfected using a GFP-tagged *TGFBI* and stained using anti-tubulin antibodies (red) and Hoechst 33258 (blue) showing a similar localization pattern to the myc-tagged version. (D) Myc-tagged *TGFBI* (green) transfected into SKOV–3 cells and counter-stained with rhodamine-tagged phalloidin for actin staining. (E) A three-dimensional reconstruction of the image in (D). (F) Myc-tagged TGFBI showing a random dispersion around the cytoplasm in a metaphase SKOV–3 cell. In cytokinesis, myc-tagged TGFBI (green) preferentially localizes intermingled with actin fibres (red) at the contraction ring; G, H and I shows actin staining (red), myc-tagged TGFBI (green) or merge, respectively. Scale bars, 5 μm.

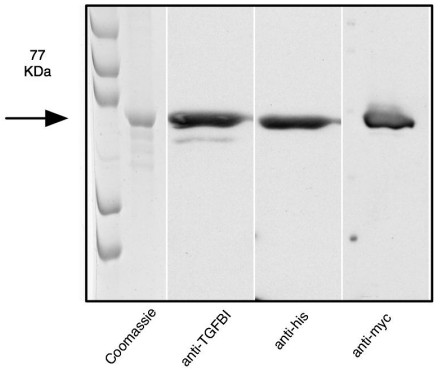
Legend for supplementary figure 5.

(A) Coomassie stain and immunoblots of rTGFBI protein purified from culture media of 293T-EBNA cells following their transfection with a plasmid containing his-myc-tagged *TGFBI* (AAA07). (B) Wells of 96-well plates were coated with rTGFBI or fibronectin at (20 μg/ml) in PBS or PBS alone overnight and then blocked using 2% milk. SKOV3 cells were either pretreated with anti-alphaVbeta3 blocking antibody for 1 hr or not before they were plated on coated wells for 90 minutes. The number of adherent cells was estimated using fluorescence labeling of DNA (CyQuant assay). Shown is the mean ±s.d. of fluorescence intensity values as an estimate of adherent cells. (C) Anti Glu-tubulin and anti phosphorylated FAK (P397) immuno-blots of NIH 3T3 fibroblasts that were either treated with paclitaxel or plated on rTGFBI, fibronectin or Polylysine coated plates for 90 minutes. All cells were suspended before treatment. (D) 48 hr following transfection using either a dominant negative form of Rho A (N19Rho) or empty vector NIH 3T3 cells were plated on rTGFBI, fibronectin or polylysine coated plates for 90 minutes and the percentage of Glu-tubulin positive cells was estimated using immunofluorescence. (E) Cells were serum starved for 24 hours then treated with paclitaxel in serum-free medium (SFM) at 0, 4, 16, 75, 300 and 1200 nM concentrations for one hour before lysates were collected for fluorescence immuno-blotting using anti-Glu-tubulin and anti-alpha tubulin. Bars represent the fold increase in Glu-tubulin fluorescence intensity values normalized for alpha tubulin intensity values. (F) Cells were either pretreated with 50 μg/ml of rTGFBI in SFM or SFM alone for 2 hr followed by paclitaxel treatment for 1 hr, washing and incubation in full media for 48 hr. Shown is the percentage of apoptotic cells measured by FITC-annexin V and 7-AAD staining. K+rTGFBI+anti-alphaVbeta3; SKOV3-K cells pretreated with anti-alphaVbeta3 in SFM before treatment with rTGFBI and paclitaxel, K+rTGFBI+FAK-KD; SKOV3-K cells were transfected with siRNA targeting FAK 48 hr prior to rTGFBI and paclitaxel treatment, K+rTGFBI+C3-toxin; SKOV3-K cells were pretreated with the Rho A inhibitor, C3 toxin, in SFM for 4 hr before the application rTGFBI and paclitaxel. Bar plots show mean ±s.d. (G) 48 hr following transfection using either individual siRNAs (a–c) targeting FAK or non-targeting scrambled controls (sc), SKOV-3 cells were treated with paclitaxel at the indicated concentrations for 48 hr. Shown are the percentages of early apoptotic cells as measured by FITC-annexin V and 7-AAD staining. (H)Percentage of apoptotic SKOV-3 cells that were either pretreated with the cell permeable Rho A inhibitor C3 toxin in serum-free medium (SFM) or SFM alone for 4 hr before the exposure to paclitaxel. Bar plots show mean ±s.d..

Supplementary Fig. 5

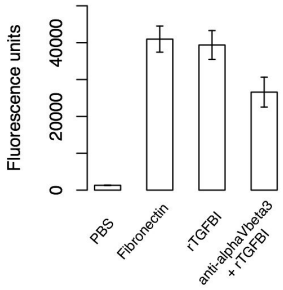
A

Recombinant TGFBI (rTGFBI) protein



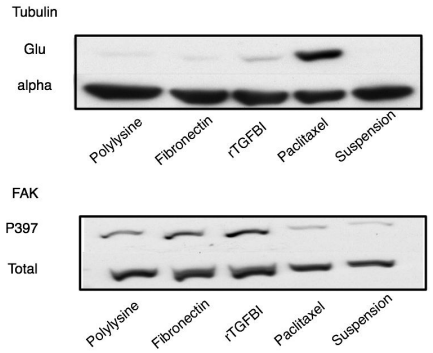
B

rTGFBI promotes integrin-mediated adhesion



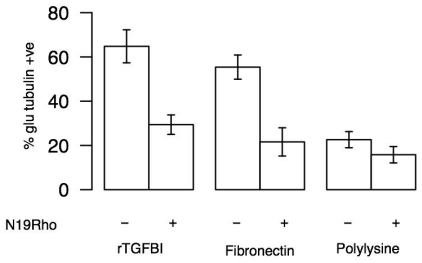
C

rTGFBI induces Glu-tubulin formation and FAK phosphorylation in NIH 3T3 cell line



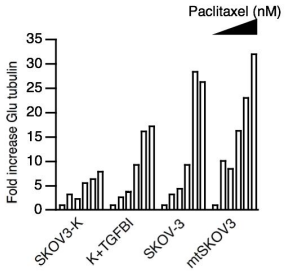
D

rTGFBI induction of Glu-tubulin formation is Rho A dependent



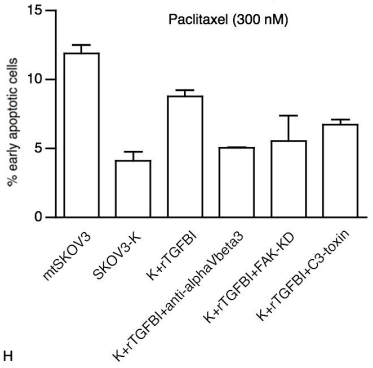
E

Glu tubulin formation



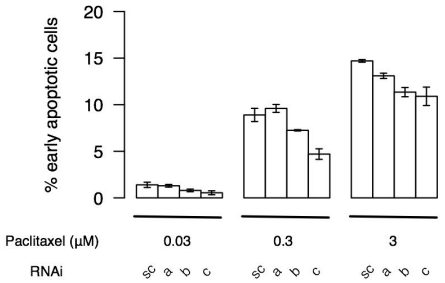
F

Effect of soluble rTGFBI on paclitaxel response



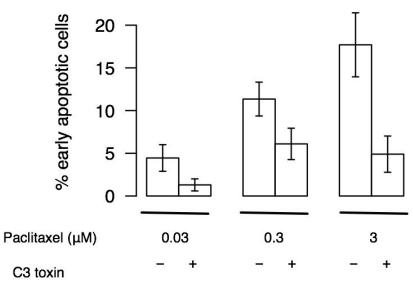
G

Effect of FAK KD on paclitaxel response



H

Effect of Rho inhibition on paclitaxel response



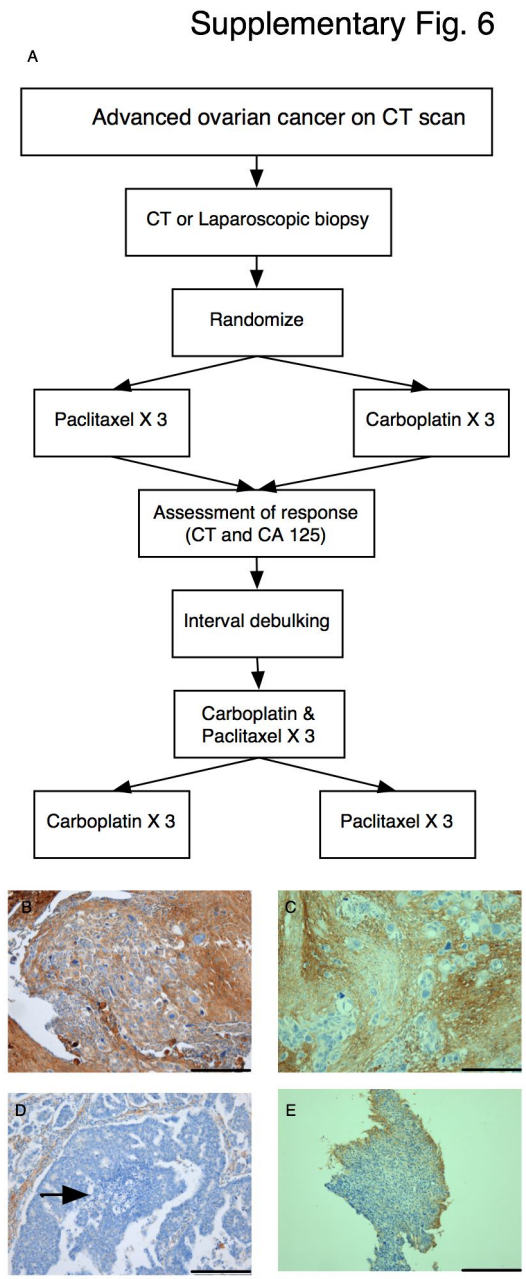


Figure 5: (A) Design of CTCR-OV01 study. (B and C) Tissue samples following paclitaxel treatment showing positive immunohistochemical staining for TGFBI. Note the large cells with lobulated nuclei consistent with prior paclitaxel treatment. (D) Cells with weak TGFBI staining do not show the paclitaxel-induced morphological changes seen in A and B. Multinuclear cells are are not seen in areas of necrosis (arrow). (E) Core biopsy of the case shown in Figure 6F taken before paclitaxel treatment does not show lobulated or multinuclear cells. Scale bars, 200 μm.

Clone ID	Gene name	Log2 ratio	Lods
80631	TGFBI	-2.3251550	5.86547646
840511	VIM	-2.2894248	10.21644042
840511	VIM	-1.6404403	1.25986066
782811	HMGIY	-1.4500556	4.10013254
782811	HMGIY	-1.4151135	6.28274455
669485	EGFR	-1.3785150	6.18581554
743230	MEN1	-1.2499368	4.58421085
in179598	EGFR	-1.1545722	3.71501116
123474	SCD	-1.0237492	2.73473165
23831	ALDOC	-0.9146455	0.88458697
213607	ALDOB	-0.8756128	0.50001628
240961	DSP	-0.7408213	0.78378040
814961	USP5	-0.7150271	0.21808801
815861	PSMD3	0.7801543	0.15551078
937020	CRADD	0.7880153	0.69482461
1470060	TUBA3	0.8859644	0.49478687
884655	GARS	0.9381060	0.75031644
1325605	D4S234E	0.9409464	0.28595809
378461	SPP1	1.0081185	0.38728827
1492147	RPS4X	1.0138637	0.03261918
2531717	WIT-1	1.0310540	0.92019785
789376	TXNRD1	1.1243582	1.76598086
72778	CASP7	1.2189559	4.50004758
882522	ASS	1.2680519	4.11129341
712604	712604	1.4349148	0.92469075
1493527	ASNS	1.5279838	1.12562143
223350	CP	1.9380333	4.86713591
323238	GRO1	2.4693347	2.97985482
324437	GRO1	2.5281610	7.98603941
196992	AKR1C1	2.6769308	7.04380815
1473304	AKR1C3	2.7153086	4.62321325
in635178	SOD2	3.2625134	9.73491271

Table 1: Differentially expressed genes in SKOV-3TR compared to SKOV-3 cell lines. Log2 ratio; the log2 ratio of the expression of a gene in SKOV3-TR over SKOV-3. Minus values indicate under-expression in SKOV-3TR. Lods; the log probability of differential expression. Lod scores more than 0 indicate probable significant differential expression.

Gene symbol	Affymetrix ID	correlation
LOX	215446_s_at	0.92
P4HA2	202733_at	0.896
FN1	211719_x_at	0.889
FN1	210495_x_at	0.887
THBS2	203083_at	0.883
FN1	212464_s_at	0.881
FN1	216442_x_at	0.879
CD163	215049_x_at	0.873
CD163	203645_s_at	0.869
PLOD2	202620_s_at	0.866
CD14	201743_at	0.852
IFI30	201422_at	0.847
CLIC4	201560_at	0.845
ANXA1	201012_at	0.84
NNMT	202237_at	0.834
CA12	215867_x_at	0.831
SLC16A3	202856_s_at	0.829
COL5A1	212488_at	0.829
NNMT	202238_s_at	0.825
CSPG2	204620_s_at	0.824
IQGAP1	200791_s_at	0.819
LOX	204298_s_at	0.818
FCGR2A	203561_at	0.818
VIM	201426_s_at	0.818
CSPG2	221731_x_at	0.817
C2	203052_at	0.815
DAB2	201279_s_at	0.813
CTSB	213274_s_at	0.813
CTSB	200839_s_at	0.811
TIMP1	201666_at	0.811
FCER1G	204232_at	0.81
LXN	218729_at	0.809
BNIP3L	221479_s_at	0.806
COL5A1	203325_s_at	0.802
SPP1	209875_s_at	0.802
CTSB	200838_at	0.801
COL5A1	212489_at	0.801
PRG1	201858_s_at	0.801
LAPTM5	201720_s_at	0.801
RARRES2	209496_at	0.801

Table 2: Transcripts that were highly correlated (Pearson correlation coefficient > 0.8) in expression to *TGFBI* in the CTCR-OV01 study