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ORIGINAL PAPER

Effects of TGFβ1 on gene expression in the HP75 human pituitary tumor cell line identified by gene expression profiling

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Abstract The pathogenesis of pituitary adenomas and many of the genes influencing growth of these tumors are unknown. TGFβ is known to inhibit proliferation of cultured anterior pituitary cells and anterior pituitary tumors, but the signal transduction pathways involved in the inhibition of growth are unclear. We treated the human HP75 pituitary cell line with 10^{-9} M TGFβ1 for 4, 24, and 96 h and performed global gene expression profiling by Affymetrix GeneChip microarray analysis. Quantitative PCR validation of specific genes involved in the TGFβ1-induced regulation of pituitary cell growth was also done. Of the 15,000 genes queried, there were 37 genes up-regulated and 48 genes down-regulated twofold or more after 4 h of TGFβ1 treatment. There were 121 genes up-regulated and 109 genes down-regulated twofold or more after 24 h of TGFβ1 treatment and 112 genes up-regulated and 43 genes down-regulated twofold or more after 96 h of TGFβ1 treatment. Galectin-3 (Gal-3) protein was decreased by TGFβ1 treatment and several genes which interacted with Gal-3 including RUNX1 and WNT5B were up-regulated after TGFβ1 treatment. SOX4 was also up-regulated by TGFβ1 treatment. SMAD3, which is directly involved in the TGFβ signal transduction pathway, was down-regulated by TGFβ1 treatment. These findings highlight the diverse gene networks and pathways through which TGFβ operates in its effects on pituitary tumor cells.

Keywords Pituitary tumor cells · TGFβ1 · RNA profiling · HP75 cells

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Introduction

Transforming growth factor beta (TGFβ) plays an essential role in many cellular processes including embryonic development, cell growth, differentiation, motility, and apoptosis [1–6]. TGFβ plays an important but complex role in tumor development. TGFβ results in growth inhibition in non-transformed epithelial cells, but epithelial cells may become resistant to TGFβ-mediated growth inhibition [7].

TGFβ1 has been shown to inhibit the growth of rodent [8, 9] and human pituitary cells [10, 11]. The effects of TGFβ on pituitary cell growth is complex and involves not only various TGFβ receptors, but also interaction with cell cycle proteins such as p27 and other molecules such as dopamine and dopamine receptors [7–11].

The sequencing of the human genome and the advent of microarray technology have made it possible to investigate the complexities of pituitary cell growth on a more global scale rather than examining simply one TGFβ-responsive target at a time. Such a global approach should provide insight into many interactions that occur in different pathways during proliferation or apopotosis of pituitary tumor cells.

In this study, the HP75 cell line, developed in our laboratory [12], was treated with TGFβ1 for different times up to 96 h, and gene expression profiling was done with the Affymetrix Human GeneChip HU133A, which contains oligonucleotide probes for approximately 15,000 characterized human genes from the human genome. Our findings show that TGFβ1 has a significant effect by regulating genes in various pathways maximally at 24-h with slightly fewer up- and down-regulated genes after 4 and 96 h of treatment.

Materials and methods

Cell line

The HP75 cell line, which was developed from a non-functioning human pituitary adenoma, was used in these studies. Cells were cultured as previously described [12].

HP75 cells were transferred to media with 2% serum and treated with 10^{-9} M TGFβ1 (R & D Inc., Minneapolis, MN) for 4, 24, and 96 h. Cells were harvested aliquots used for cell counts, and the remainder of the cells frozen at −70°C until used. Three independent experiments were performed. Each experiment was placed on a separate chip and analyzed separately. The control experiment was from the zero time pretreatment.

RNA extraction

Total RNA was extracted with a TRIzol reagent (Invitrogen, Carlsbad, CA) as previously reported [10, 11]. The RNA was re-suspended in Tris–EDTA buffer and purified with the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Integrity of the RNA was verified using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). A total of 8 μg of total RNA for each sample was used for the Affymetrix assay.

Gene array sample preparation, hybridization, and scanning

The purified cDNA was used as a template for in vitro transcription reaction for the synthesis of biotinylated complementary RNA (cRNA) using RNA transcript labeling reagent (Affymetrix). Labeled cRNAs were then fragmented and hybridized onto the Affymetrix GeneChip HU133A oligonucleotide array. Briefly, appropriate amounts of fragmented cRNA and control oligonucleotide B2 were added along with control cRNA (BioB, BioC, and BioD), herring sperm DNA, and bovine serum albumin to the hybridization buffer. The hybridization mixture was heated to 99°C for 5 min followed by incubation at 45°C for 5 min before injecting the sample into the GeneChip. Hybridization was then performed at 45°C for 16 h and then mixing on a rotisserie at 60 rpm. After hybridization, the solution was removed and arrays were washed and stained with streptavidin–phycoerythrin (Molecular Probes, Portland, OR).

After washing and staining, probe arrays were scanned using the Affymetrix Microarray Suite 5.0 and confocal scanner. The quality of the fragmented biotin-labeled cRNA in each experiment was evaluated before hybridizing onto HUU133a expression array by both Agilent Bioanalyzer and hybridizing onto a Test-3 microarray as a measure of quality control. Hybridization and confocal scanning were performed at an institutional microarray core facility.

Gene array data analysis

GeneSpring 7.3 data analysis software (Agilent Technologies, Santa Clara, CA) was used for microarray data analysis. Affymetrix probe expression values were normalized using GCRMA algorithm. A gene was identified as differentially expressed in sample groups being compared if each of the following conditions were satisfied. (1) Fold change was equal or greater than the cut-off value (twofold). (2) A Benjamin and Hochberg False Discovery Rate resulting from ANOVA test was 0.01 or less.

Standard curve cDNA synthesis

Each cDNA standard was generated for RT-qPCR assay as previously described [13, 14]. Briefly, 1 μg of previously extracted total RNA from a normal pituitary was reverse transcribed using the Stratagene First-Strand RT-PCR Kit according to the manufacturer's instruction. Conventional PCR was performed for each primer set using 2 μl of the cDNA from the RT reaction. Each product resolved as a single band on an agarose gel and was excised and purified. The PCR product for each gene (RUNX1, SOX4, WNT5B, and SMAD3) was then ligated into the pGEM-T vector using the pGEM-T Easy Vector System (Promega) and transformed into XL-1 Blue Competent Cells (Stratagene). Transformed cells were cultured overnight in LB medium containing ampicillin (100 μg/ml) and the plasmid was extracted and purified using the Qiafilter Plasmid Midi Kit (Qiagen). The plasmid was digested with *Eco*RI followed by agarose gel electrophoresis. The insert was excised from the gel and purified, and the concentration determined by OD readings. The resulting purified cDNA standard was stored at −70°C in single-use aliquots until used.

Reverse transcriptase-real time quantitative PCR (RT-qPCR)

RT-qPCR was performed by the LightCycler System (Roche) using the FastStart DNA Master SYBR Green I Kit (Roche) as previously reported [13, 14]. Each PCR reaction contained a total volume of 20 µl and the qPCR cycling conditions were performed according to the manufacturer's instruction; 2 µl of a 1:10 dilution of template cDNA, 0.5 mM each primer, and 4 mM $MgCl_2$ were used for each primer set unless otherwise stated. GAPDH was amplified as the internal housekeeping standard. The standard curve samples, ranging from 10^2 to 10^7 copies, were included in the same run as the experimental samples. A negative control in which water was substituted for cDNA was included in each run, and the sample identity was confirmed for each gene by the expected PCR product size on the agarose gel electrophoresis. The primers used for the RT-qPCR along with the annealing temperatures and product sizes are summarized in Table 1 [15, 16].

Relative quantification

To normalize the expression level of each experimental sample, the target copy number was divided by the GAPDH housekeeping copy number resulting in a relative ratio. A minimum of three independent experiments were performed for each gene studied. Results were expressed as the mean ± SEM.

Western blotting

Western blotting was done using proteins extracted from three separate experiments of TGFβ1-treated HP75 cells as previously described [10, 11] with antibodies to Gal-3 (1:500) (Vector Labs, Burlingame, CA), SMAD3 (Novus, Littleton, CO, 1:1000), SOX4 (Novus, 1:1000) and RUNX1 (Calbiochem, San Diego, CA, 1:20). Densitometric analyses of the Western blot films were done with a Bio-Rad Quantity One (Belmont, CA) and the densitometry units (OD) were calculated relative to β-actin for each lane as previously reported [11].

Results

Cell proliferation

Treatment of HP75 pituitary cells for 96 h with TGFβ resulted in a significant inhibition of cell proliferation (Fig. 1).

RNA profiling

The expression profiles of the untreated HP75 pituitary cells were compared to TGFβ1 treated cells after 4, 24, and 96 h using the Affymetrix GeneChip HU133A. After 4 h of treatment with TGFβ1, there were 37 genes up-regulated twofold or greater and 48 genes down-regulated twofold or more (Table 2). After 24 h of treatment with TGFβ1, there were 121 genes up-regulated and 109 genes down-regulated twofold or more (Table 3). TGFβ1 treatment for 96 h resulted in 112 genes up-regulated and 43 genes down-regulated twofold or more.

These differentially expressed genes were functionally categorized on the basis of known or inferred biological functions of their protein products using GeneSpring 7.3 software. Table 3 summarizes the functional clustering of the identified genes with known functions as well as other genes. There were more genes with known functions in the groups after 24 h of TGFβ1 treatment followed by 96 h of treatment. The 4 h of treatment group had the least number of genes that were significantly up- or down-regulated by TGFβ1 treatment.

Identification of candidate genes

Because of the large number of putative candidate genes generated by DNA microarray analysis after treatment with TGFβ1 for various periods of time, we selected a small number of genes related to TGFβ1 signaling and regulation

Table 1 RT-qPCR primer sequences

Gene	Forward sequence (5′ → 3′)	Reverse sequence (5′ → 3′)	Size (bp)	Annealing temperature (°C)	References
GAPDH	aaggtgaaggtcggagtcaacg	gttgtcatggatgaccttggcc	495	61	[13]
RUNX1	agacatcggcagaaactaga	ccaggtattggtaggactga	253	59	[14]
SMAD3	agaagacggggcagctggac	gacatcggattcggggatag	511	64	[15]
SOX4	aagaaacgaaaaggacagacgaag	tgccatcaacaacaacatcaataa	249	64	–[a]
WNT5B	aggagtttgtggatgcccg	gtggcatttgcaggctacgtc	143	60	–[a]

[a] Primers synthesized in our laboratory and the PCR products were checked by sequencing

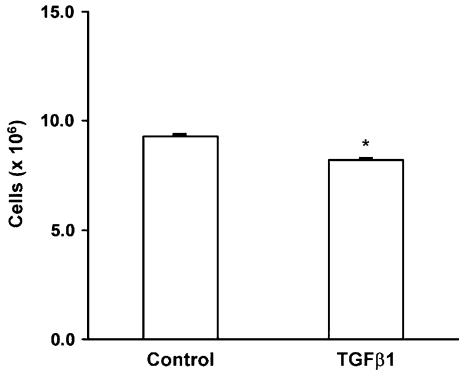

Fig. 1 Effects of TGFβ1 on cell proliferation in HP75 cells. Cells were seeded in T75 flasks in the presence of 1×10^{-9} M TGFβ1 for 96 h. Cells were harvested and enumerated with a hemocytometer. There was a significant decrease in the cells treated with TGFβ1 compared to controls. Results are the mean of three independent experiments ($^*P < 0.05$)

that may be important in pituitary cell growth to validate by RT-qPCR. Genes linked to Gal-3, which were previously shown to be inhibited by TGFβ1 [11] and were important for growth of some types of pituitary tumors including RUNX1, which was one of the genes over-expressed twofold or more after TGFβ1 treatment. RUNX1 was up-regulated 2.5-fold after 4 h of TGFβ1 treatment (Table 4). In addition, WNT5B was also selected because it has been implicated in Gal-3 signaling [17]. WNT5B was up-regulated 3.0-fold after 4 h of TGFβ1 treatment. Genes linked directly to TGFβ1 signaling including SMAD3, which were down-regulated after 4 h TGFβ1 treatment, were also selected. SMAD3 was down-regulated after 24 and 96 h of TGFβ1 treatment. SOX4, which was up-regulated 9.3-fold after TGFβ1 treatment for 96 h (Table 4), was included because of the possible overlap with TGFβ1 signaling.

Validation studies

RT-real time quantitative PCR (RT-qPCR) for RUNX1 showed a 2.85-fold increase in expression after 96 h of TGFβ1 treatment (Fig. 2a). WNT5B showed a 2.85-fold increase in expression level (Fig. 2b). SOX4 showed a 10-fold increase in expression level (Fig. 2c), while SMAD3 showed a 2.3-fold down-regulation after TGFβ1 treatment for 96 h (Fig. 2d).

Western blot analysis with anti-Gal-3 antibody showed a decrease in Gal-3 after 96 h of TGFβ1 treatment as previously reported [11] (Fig. 3a and b). RUNX1 protein treatment levels were similar in the control and TGFβ1 treated cells 96 h after treatment (Fig. 3c and d). SOX4 protein was increased twofold after TGFβ1 treatment for 96 h (Fig. 3e and f), while SMAD3 protein showed a decreased expression in HP75 cells after 96 h of treatment with TGFβ1 (Fig. 3g and h).

Discussion

The factors regulating pituitary cell growth are not well understood. Because pituitary carcinoma, defined by metastatic disease, is very uncommon, signaling pathways that inhibit the proliferation of pituitary cells are probably important for the regulation of pituitary tumor growth. TGFβ1 has been shown to have an inhibitory effect on proliferation of pituitary cells [8, 10]. Based on these previous observations, we examined the genes that were regulated by TGFβ1 in a well characterized human pituitary tumor cell line [12] to determine the pathways involved in TGFβ1 regulation of pituitary tumor cell growth inhibition.

RNA profiling showed that there was a time-dependent change in the genes that were up-regulated and down-regulated by TGFβ1 treatment in cultured HP75 pituitary tumor cells. The maximum number of genes that were changed significantly was seen after 24 h of treatment followed by 96 h of treatment. After 4 h of treatment, a smaller but significant number of genes were up-regulated and down-regulated in this pituitary tumor cell line. It is important to emphasize that we could not distinguish between primary and secondary changes in gene levels, especially after longer periods of TGFβ1 treatment.

Because of the treatment with TGFβ1 which inhibited cell growth and an interest in TGFβ signaling, we selected a small group of genes which appeared to interact in TGFβ signaling pathway. RNA profiling and RT-qPCR analysis showed that RUNX1 was up-regulated by TGFβ1 treatment after 24 and 96 h of treatment. Our recent studies have shown that RUNX1 protein binds the Gal-3 promoter and leads to the up-regulation of Gal-3 expression (unpublished data). Because TGFβ1 inhibits Gal-3 expression in pituitary cells, we examined the effects of TGFβ1 or Gal-3 expression and found that it was decreased. RUNX1 proteins are also important in TGFβ signaling pathway. RUNX1 may be functioning in a negative feedback manner as a result of the effect of the TGFβ1 on decreasing Gal-3 protein levels. Although RUNX1 mRNA was increased after 4, 24 and 96 h of TGFβ1 protein treatment, RUNX1 protein was not significantly increased after 96 h of TGFβ1 treatment. It is possible that a longer period of treatment was needed to see an effect on RUNX1 proteins, since the highest levels of changes in the mRNAs were seen after 24 h of TGFβ1 treatment.

WNT5B mRNA was increased after 24 and 96 h of TGFβ1 treatment. There was a good agreement between the RNA profiling and RT-qPCR analysis. Recent studies have shown that Wnt signaling is important for signal transduction in pituitary cells [18, 19]. Gal-3 is also a key

Table 2 TGFβ1 up- and down-regulated genes in HP75 pituitary cells at 4 h

Fold change	Gene symbol	Description	Genbank
18.55	CRLF1	Cytokine receptor-like factor 1	NM_004750
12.37	THBS1	Thrombospondin 1	NM_003246
8.429	TMEPAI	Transmembrane, prostate androgen induced RNA	NM_020182
5.689	AMIGO2	*Homo sapiens* BAC clone GS1-99H8 from 12 complete sequence	AC004010
5.606	DACT1	Dapper homolog 1, antagonist of beta-catenin (*xenopus*)	NM_016651
5.199	MSC	Musculin (activated B-cell factor-1)	AF060154
4.808	EDN1	Endothelin 1	NM_001955
4.24	PCDH9	Transcribed sequence with strong similarity to protein ref: NP_065136.1 (*H. sapiens*) protocadherin 9 precursor; cadherin superfamily protein VR4-11 (*Homo sapiens*)	AI524125
4.117	ANGPTL4	Angiopoietin-like 4	NM_016109
3.601	SGNE1	Secretory granule, neuroendocrine protein 1 (7B2 protein)	NM_003020
3.575	SKIL	SKI-like	BF725121
3.545	SERPINE1	Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	AL574210
3.49	LTBP2	Latent transforming growth factor beta binding protein 2	NM_000428
3.428	INHBA	Inhibin, beta A (activin A, activin AB alpha polypeptide)	M13436
3.353	GADD45B	Growth arrest and DNA-damage-inducible, beta	AF087853
3.217	CSPG2	Chondroitin sulfate proteoglycan 2 (versican)	BF218922
3.081	F2RL1	601659282R1 NIH_MGC_70 *Homo sapiens* cDNA clone IMAGE: 3895653 3′, mRNA sequence	BE965369
3.079	NQO1	NAD(P)H dehydrogenase, quinone 1	NM_000903
3.046	CSPG2	Chondroitin sulfate proteoglycan 2 (versican)	NM_004385
3.009	WNT5B	Wingless-type MMTV integration site family, member 5B	NM_030775
2.774	ADAM19	A disintegrin and metalloproteinase domain 19 (meltrin beta)	Y13786
2.575	CHST11	Carbohydrate (chondroitin 4) sulfotransferase 11	NM_018413
2.564	COL5A1	Transcribed sequence with strong similarity to protein ref: NP_003874.2 (*H. sapiens*) histone deacetylase 3 (*Homo sapiens*)	N30339
2.553	SLC26A2	Solute carrier family 26 (sulfate transporter), member 2	AI025519
2.526	RUNX1	Runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)	D43968
2.519	CTGF	Connective tissue growth factor	M92934
2.505	ASE-1	CD3-epsilon-associated protein; antisense to ERCC-1	NM_012099
2.391	ARNTL	Aryl hydrocarbon receptor nuclear translocator-like	AB000812
2.296	C5orf13	Chromosome 5 open reading frame 13	NM_004772
2.289	TPM1	Tropomyosin 1 (alpha)	Z24727
2.275	GREM1	Cysteine knot superfamily 1, BMP antagonist 1	AF154054
2.25	VDR	Vitamin D (1,25- dihydroxyvitamin D3) receptor	AA454701
2.232	PRKAR1B	wj75g12.x1 NCI_CGAP_Lu19 *Homo sapiens* cDNA clone IMAGE: 2408710 3′ similar to gb: M65066 CAMP-DEPENDENT PROTEIN KINASE TYPE I-ALPHA REGULATORY (HUMAN); mRNA sequence	AI814660
2.16	TNC	Tenascin C (hexabrachion)	BF434846
2.134	GREM1	Cysteine knot superfamily 1, BMP antagonist 1	NM_013372
2.037	LMCD1	LIM and cysteine-rich domains 1	NM_014583
2.029	CHST3	Carbohydrate (chondroitin 6) sulfotransferase 3	AB017915
0.497	IFI27	Interferon, alpha-inducible protein 27	NM_005532
0.492	UGCG	UDP-glucose ceramide glucosyltransferase	AI378044
0.478		Clone 24889 mRNA sequence	AI566082
0.477	ISG20	Interferon stimulated gene 20 kDa	U88964

Table 2 continued

Fold change	Gene symbol	Description	Genbank
0.47	IL1B	Interleukin 1, beta	NM_000576
0.47	ICAM1	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	NM_000201
0.466	RIS1	Ras-induced senescence 1	BF062629
0.464	SLC2A14	Solute carrier family 2 (facilitated glucose transporter), member 14	AL110298
0.464	RGS17	Regulator of G-protein signaling 17	NM_012419
0.456	FLJ10134	Hypothetical protein FLJ10134	NM_018004
0.441	CA12	Carbonic anhydrase XII	BC000278
0.436	OPTN	AV757675 BM *Homo sapiens* cDNA clone BMFAVB12 5′, mRNA sequence	AV757675
0.436	AK3	Adenylate kinase 3	AI653169
0.426	LDLR	Low density lipoprotein receptor (familial hypercholesterolemia)	S70123
0.425	PBEF1	Pre-B-cell colony enhancing factor 1	NM_005746
0.423	NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	AI078167
0.419	ADM	Adrenomedullin	NM_001124
0.406	SLC2A3	Solute carrier family 2 (facilitated glucose transporter), member 3	AI631159
0.398	RTN1	Reticulin 1	BC000314
0.394	PBEF1	Pre-B-cell colony enhancing factor 1	BF575514
0.393	PELI1	Pellino homolog 1 (*Drosophila*)	NM_020651
0.39	GALNT12	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 12 (GalNAc-T12)	NM_024642
0.389	KLF4	Kruppel-like factor 4 (gut)	BF514079
0.388	OAS2	2′-5′-oligoadenylate synthetase 2, 69/71 kDa	NM_016817
0.378	LDLR	Low density lipoprotein receptor (familial hypercholesterolemia)	NM_000527
0.373	CEBPD	KIAA0146 protein	NM_005195
0.368	CA12	Carbonic anhydrase XII	BC001012
0.366	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	NM_006079
0.357	MAFB	V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	NM_005461
0.356	H1F0	H1 histone family, member 0	BC000145
0.351	IL1R1	Interleukin 1 receptor, type I	NM_000877
0.350	RAI3	Retinoic acid induced 3	AA156240
0.349	EBI3	Epstein-Barr virus induced gene 3	NM_005755
0.337	KYNU	Kynureninase (L-kynurenine hydrolase)	D55639
0.334	IFRG28	28 kD interferon responsive protein	NM_022147
0.324	ISG20	Interferon stimulated gene 20 kDa	NM_002201
0.295	ADARB1	Adenosine deaminase, RNA-specific, B1 (RED1 homolog rat)	NM_015833
0.292	CDH5	Cadherin 5, type 2, VE-cadherin (vascular epithelium)	NM_001795
0.283	GCH1	GTP cyclohydrolase 1 (dopa-responsive dystonia)	NM_000161
0.221	DKFZP434F0318	Hypothetical protein DKFZp434F0318	NM_030817
0.219	ZFP36	Zinc finger protein 36, C3H type, homolog (mouse)	NM_003407
0.212	PTX3	Pentaxin-related gene, rapidly induced by IL-1 beta	NM_002852
0.201	CXCL2	Chemokine (C–X–C motif) ligand 2	M57731
0.16	DUSP6	Dual specificity phosphatase 6	BC005047
0.131	CCL2	Chemokine (C–C motif) ligand 2	S69738
0.128	NFIB	Nuclear factor I/B	AI186739
0.0931	KIBRA	KIBRA protein	AK001727
0.0676	CSF3	Colony stimulating factor 3 (granulocyte)	NM_000759

Table 3 TGFβ1 up- and down-regulated genes in HP75 pituitary cells by categories

Fold change			Gene symbol	Description	Genbank
4 h	24 h	96 h			
Apoptosis					
	10.43		APLP1	Amyloid beta (A4) precursor-like protein 1	U48437
	8.043		CGB	Chorionic gonadotropin, beta polypeptide	NM_000737
3.43	4.308	4.84	INHBA	Inhibin, beta A (activin A, activin AB alpha polypeptide)	NM_002192
3.35	2.574	2.53	GADD45B	Growth arrest and DNA-damage-inducible, beta	NM_015675
	2.441		TGFB1	Transforming growth factor, beta 1 (Camurati-Engelmann disease)	BC000125
	0.468		TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	NM_006290
	0.466		BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3	NM_004052
	0.423		ELOVL2	Catenin (cadherin-associated protein), alpha-like 1	BF508639
	0.348		AHR	Aryl hydrocarbon receptor	NM_001621
Cell adhesion					
	10.43		APLP1	Amyloid beta (A4) precursor-like protein 1	U48437
	9.809	18.69	MFAP4	Microfibrillar-associated protein 4	R72286
	8.719	12.82	THBS1	Thrombospondin 1	NM_003246
12.37	6.048		CSPG2	Chondroitin sulfate proteoglycan 2 (versican)	R94644
3.217	5.839	5.39	COL5A1	Transcribed sequence with strong similarity to protein ref: NP_003874.2 (*H. sapiens*) histone deacetylase 3 (*Homo sapiens*)	N30339
	5.227	5.67	NEDD9	Neuronal precursor cell-expressed developmentally down-regulated gene	AL136139
	5.226	5.52	CSPG2	Chondroitin sulfate proteoglycan 2 (versican)	BF218922
	4.747	6.069	COL11A1	Collagen, type XI, alpha 1	J04177
	4.625		AEBP1	AE binding protein 1	NM_001129
	4.29		FN1	Fibronectin 1	AJ276395
	4.078		COL15A1	Collagen, type XV, alpha 1	NM_001855
	3.589	4.43	POSTN	Periostin, osteoblast specific factor	D13665
2.16	3.469	4.48	TNC	Tenascin C (hexabrachion)	NM_002160
	2.915	2.89	COL16A1	Collagen, type XVI, alpha 1	NM_001856
	2.693		CDH2	Cadherin 2, type 1, N-cadherin (neuronal)	NM_001792
	2.623		NEDD9	Neural precursor cell expressed, developmentally down-regulated 9	U64317
	2.544		COL18A1	Collagen, type XVIII, alpha 1	AF018081
	2.522		DSG2	Desmoglein 2	BF031829
	2.481	2.45	FN1	Fibronectin 1	BC005858
	2.32		ITGB5	Integrin, beta 5	NM_002213
	2.25		EMILIN1	Elastin microfibril interfacer 1	NM_007046
	2.22		CDH2	Cadherin 2, type 1, N-cadherin (neuronal)	M34064
	0.31		ITGBL1	β integrin related protein 10 β integrin EGF-like repeat domains	AL359052
0.131	0.303		CCL2	Chemokine (C–C motif) ligand 2	S69738
	0.102	0.26	ITGBL1	Integrin, beta-like 1 (with EGF-like repeat domains)	NM_004791
Cytoskeleton					
	5.227	5.67	NEDD9	Neuronal precursor cell-expressed developmentally down-regulated gene	AL136139
	2.522		DSG2	Desmoglein 2	BF031829
	2.488	2.07	MYO10	Myosin X	NM_012334
	0.481		PCLO	Piccolo (presynaptic cytomatrix protein)	AB011131
	0.06		PPL	Periplakin	NM_002705

Table 3 continued

Fold change			Gene symbol	Description	Genbank
4 h	24 h	96 h			
Metabolism					
	2.29	2.29	SIAT4B	Beta-galactoside alpha 2,6-sialyltansferase 4β	AI088162
	0.466	0.47	GBE1	Glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme, Andersen disease, glycogen storage disease type IV)	NM_000158
	0.465	0.46	SLC39A8	Solute carrier family 39 (zinc transporter), member 8	NM_022154
	0.418	0.42	OAS1	2′,5′-oligoadenylate synthetase 1, 40/46 kDa	NM_002534
	0.412	0.41	GMDS	GDP-mannose 4,6-dehydratase	NM_001500
0.44	0.403	0.40	CA12	Carbonic anhydrase XII	BC000278
	0.388	0.39	ANXA1	Annexin A1	NM_000700
	0.38	0.38	PPAP2B	Phosphatidic acid phosphatase type 2B	AB000889
	0.376		CA12	Carbonic anhydrase XII	NM_001218
	0.304	0.30	PPARG	Peroxisome proliferative activated receptor, gamma	NM_015869
	0.279	0.28	PTGES	Prostaglandin E synthase	AF010316
	0.254	0.25	ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	D45421
Secretion					
3.49	6.658	7.99	LTBP2	Latent transforming growth factor beta binding protein 2	NM_000428
3.43	4.308	4.83	INHBA	Inhibin, beta A (activin A, activin AB alpha polypeptide)	NM_002192
	0.413		SCG2	Secretogranin II (chromogranin C)	NM_003469
Signaling					
8.42	12.42	14.25	TMEPAI	Transmembrane, prostate androgen-induced RNA	NM_020182
	8.043		CGB	Chorionic gonadotropin, beta polypeptide	NM_000737
3.60	6.658	7.80	LTBP2	Latent transforming growth factor beta binding protein 2	NM_000428
3.60	5.24	6.07	SGNE1	Secretory granule, neuroendocrine protein 1 (7B2 protein)	NM_003020
	4.978		FGFR3	Fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	NM_000142
3.43	4.308	4.84	INHBA	Inhibin, beta A (activin A, activin AB alpha polypeptide)	NM_002192
	3.936		PTHLH	Parathyroid hormone-like hormone	BC005961
3.08	3.521		F2RL1	601659282R1 NIH_MGC_70 Homo sapiens cDNA clone IMAGE: 3895653 3′, mRNA sequence	BE965369
3.01	2.598	2.23	WNT5B	Wingless-type MMTV integration site family, member 5B	NM_030775
	2.578		PDGFA	Platelet-derived growth factor alpha polypeptide	NM_002607
	2.574		GJA1	Gap junction protein, alpha 1, 43 kDa (connexin 43)	NM_000165
	2.563		IL11	Interleukin 11	M57765
	2.51		F2RL1	Coagulation factor II (thrombin) receptor-like 1	NM_005242
	2.509	2.29	BAI2	Brain-specific angiogenesis inhibitor 2	NM_001703
	2.488		MYO10	Myosin X	NM_012334
	2.441	2.07	TGFB1	Transforming growth factor, beta 1 (Camurati-Engelmann disease)	BC000125
	2.333		ADCY7	Adenylate cyclase 7	NM_001114
	2.32		ITGB5	Integrin, beta 5	NM_002213
	2.166		FURIN	Furin (paired basic amino acid cleaving enzyme)	NM_002569
	2.1	2.47	FZD7	Frizzled homolog 7 (Drosophila)	NM_003507
	0.475		TGFBR3	Transforming growth factor, beta receptor III (betaglycan, 300 kDa)	NM_003243
0.20	0.458		CXCL2	Chemokine (C–X–C motif) ligand 1 (melanoma growth stimulating activity, alpha)	NM_001511

Table 3 continued

Fold change			Gene symbol	Description	Genbank
4 h	24 h	96 h			
	0.443		MME	Membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)	NM_007287
	0.427		MCART1		AL138752
	0.405	0.37	MME	Membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)	AI433463
	0.399		RGS4	AL514445 *Homo sapiens* regulator of G-protein signaling 9	AL514445
	0.365		EDG2	Endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2	AW269335
	0.32		CXCL2	Chemokine (C–X–C motif) ligand 2	M57731
0.131	0.303		CCL2	chemokine (C-C motif) ligand 2	S69738
	0.278	0.32	STC1	Stanniocalcin 1	U46768
	0.27		ADM	Adrenomedullin	NM_001124
	0.254		ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	D45421
	0.237		CXCL3	Chemokine (C–X–C motif) ligand 3	NM_002090
	0.178	0.16	ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	L35594
0.068	0.145		CSF3	Colony stimulating factor 3 (granulocyte)	NM_000759
	0.454	0.077	SMAD3	Mothers against deca penta plegic homology 3	U76622
Others					
18.55	96.59	84.49	CRLF1	Cytokine receptor-like factor 1	NM_004750
	11.48		NALP1	NACHT, leucine rich repeat and PYD containing 1	AF310105
	11.43		NRP2	Neuropilin 2	AF022859
	8.879	9.53	SOX4	SRY (sex determining region Y)-box 4	BG528420
2.52	8.522	2.92	RUNX1	Runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)	U19601
	6.98		PCDH1	Protocadherin 1 (cadherin-like 1)	NM_002587
	6.734	5.32	PLXND1	Plexin D1	AL575403
	6.654	5.22	BGN	Biglycan	NM_001711
	6.225		SOX4	SRY (sex determining region Y)-box 4	AL136179
	5.455		MSC	Musculin (activated B-cell factor-1)	AF060154
	5.34		NOX4	NADPH oxidase 4	NM_016931
	4.633		KCNG1	Potassium voltage-gated channel, subfamily G, member 1	AI332979
	4.38		ADAM19	A disintegrin and metalloproteinase domain 19 (meltrin beta)	Y13786
	4.271		NR2F6	Nuclear receptor subfamily 2, group F, member 6	BF000629
5.69	4.132	5.94	AMIGO2	Amphoterin-induced gene 2	AC004010
	3.864	5.72	DACT1	Dapper homolog 1, antagonist of beta-catenin (*xenopus*)	NM_016651
	3.844		HS3ST3A1	Heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1	NM_006042
	3.67		TCF8	Transcription factor 8 (represses interleukin 2 expression)/// transcription factor 8 (represses interleukin 2 expression)	NM_030751
	3.565		PODXL	Podocalyxin-like	NM_005397
2.28	3.419	2.36	TPM1	Tropomyosin 1 (alpha)	NM_000366
	3.411		TCF7	Transcription factor 7 (T-cell specific, HMG-box)	AW027359
	3.409		LAPTM5	Lysosomal-associated multispanning membrane protein-5	NM_006762
3.57	3.349		SKIL	SKI-like	BF725121
	3.334		MRC2	Mannose receptor, C type 2	AB014609
	3.246		IL27RA	Class I cytokine receptor	AI983115

Table 3 continued

Fold change			Gene symbol	Description	Genbank
4 h	24 h	96 h			
	3.097		LTBP3	Latent transforming growth factor beta binding protein 3	NM_021070
	3.035		ANGPTL2	Angiopoietin-like 2	AI074333
3.54	2.954	2.18	SERPINE1	Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	AL574210
	2.91		ARK5	KIAA0537 gene product	NM_014840
	2.835		KIAA0779	KIAA0779 protein	AB018322
	2.687		NRP2	Neuropilin 2	AA295257
	2.656		JUNB	Jun B proto-oncogene	NM_002229
	2.641	3.16	RRAD	Ras-related associated with diabetes	NM_004165
	2.64		RASA4	DNA directed RNA polymerase II polypeptide J-related gene	AB011110
	2.582		PTK7	PTK7 protein tyrosine kinase 7	NM_002821
	2.56		FLJ10357	Hypothetical protein FLJ10357	R42449
	2.555		GSDML	Gasdermin-like	NM_018530
	2.534			–	Y15916
	2.4		IVNS1ABP	Influenza virus NS1A binding protein	AF205218
	2.382		COL4A2	Collagen, type IV, alpha 2	AA909035
	2.354		IVNS1ABP	Influenza virus NS1A binding protein	NM_006469
	2.352		JARID2	Jumonji homolog (mouse)	NM_004973
	2.344		SERPINE2	Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	AL541302
	2.308		MUM1	Melanoma ubiquitous mutated protein	NM_016473
	2.243		COL1A2	Collagen, type I, alpha 2	AA788711
	2.237		MAGED4	Melanoma antigen, family D, 4///melanoma antigen, family D, 4	NM_030801
	2.236		ARFGAP1	ADP-ribosylation factor GTPase activating protein 1	NM_018209
	2.235		FLJ10901	Hypothetical protein FLJ10901	NM_018265
	2.232		JMJD3	KIAA0346 protein	AB002344
2.29	2.218		C5orf13	Chromosome 5 open reading frame 13	NM_004772
	2.215		COL1A1	Collagen, type I, alpha 1	AI743621
	2.207		RALGDS	Ral guanine nucleotide dissociation stimulator	AI421559
	2.206		ITM2C	Integral membrane protein 2C///integral membrane protein 2C	NM_030926
	2.19		PLXND1	Plexin D1	AB014520
	2.189		PLCG1	Phospholipase C, gamma 1 (formerly subtype 148)	AL022394
	2.187		EMS1	*Homo sapiens* transcribed sequence with weak similarity to protein ref: NP_060312.1 (*H. sapiens*) hypothetical protein FLJ20489 (*Homo sapiens*)	AU155105
	2.175	2.48	NALP1	NACHT, leucine rich repeat and PYD containing 1	AF229061
	2.167		DNM1	Dynamin 1	AF035321
	2.143		PRSS11	Protease, serine, 11 (IGF binding)	NM_002775
	2.14		PTPRK	Protein tyrosine phosphatase, receptor type, K	NM_002844
	2.135		BMP1	Bone morphogenetic protein 1	NM_001199
	2.133		MGC3047	Hypothetical protein MGC3047	AW888223
	2.122		GALNT10	UDP-*N*-acetyl-alpha-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase 10 (GalNAc-T10)	NM_024564
	2.094		ABCA2	ATP-binding cassette, sub-family A (ABC1), member 2	AL162060
	2.091	2.48	MMP2	Matrix metalloproteinase 2 (gelatinase A, 72 kDa gelatinase, 72 kDa type IV collagenase)	NM_004530

Table 3 continued

Fold change			Gene symbol	Description	Genbank
4 h	24 h	96 h			
	2.083		IL27RA	Class I cytokine receptor	NM_004843
	2.079		FSTL3	Follistatin-like 3 (secreted glycoprotein)	NM_005860
	0.499		PBEF1	Pre-B-cell colony-enhancing factor	BF575514
	0.499		JUN	V-jun sarcoma virus 17 oncogene homolog (avian)	NM_002228
	0.498		PNMA2	Paraneoplastic antigen MA2	AB020690
	0.497		STX3A	Syntaxin 3A	AJ002077
	0.497		SMC2L1	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	NM_006444
	0.495		NDRG1	*N*-myc downstream regulated gene 1	NM_006096
	0.489		BF	B-factor, properdin	NM_001710
	0.484		MX2	Myxovirus (influenza virus) resistance 2 (mouse)	NM_002463
	0.481		SLC43A3	Likely ortholog of mouse embryonic epithelial gene 1	AI630178
	0.48		BHMT2	Betaine-homocysteine methyltransferase 2	NM_017614
	0.48		IFIT5	Interferon-induced protein with tetratricopeptide repeats 5	NM_012420
	0.479		TM4SF1	Transmembrane 4 superfamily member 1	AI346835
	0.476		FLJ12118	Hypothetical protein FLJ12118	NM_024537
	0.476		PHF15	PHD finger protein 15	AI735639
	0.475	0.273	FLI1	Friend leukemia virus integration 1	M93255
	0.474		C21orf91	Chromosome 21 open reading frame 91	NM_017447
	0.469		NMI	*N*-myc (and STAT) interactor	NM_004688
	0.468		COL13A1	Collagen, type XIII, alpha 1	NM_005203
	0.463		PBEF1	Pre-B-cell colony-enhancing factor	NM_005746
	0.457		ASS	Argininosuccinate synthetase	NM_000050
	0.455		PCAF	p300/CBP-associated factor	AV727449
	0.455		GLDC	Glycine dehydrogenase (decarboxylating; glycine decarboxylase, glycine cleavage system protein P)	NM_000170
	0.454	0.273	SMAD3	DKFZP586N0721 protein	BF971416
	0.452		NEDD4L	Neural precursor cell expressed, developmentally down-regulated 4-like	AB007899
	0.446		IFIT5	Interferon-induced protein with tetratricopeptide repeats 5	N47725
	0.445		MRPL33	Mitochondrial ribosomal protein L33	NM_004891
	0.443		PDE4DIP	Phosphodiesterase 4D interacting protein (myomegalin)	AB007923
	0.443		FLJ21940	FLJ21940 protein	AW975818
	0.442		IFIT4	Interferon-induced protein with tetratricopeptide repeats 4	NM_001549
	0.434		DUSP5	Dual specificity phosphatase 5	U16996
	0.432		DKFZP586A0522	DKFZP586A0522 protein	NM_014033
	0.429		GBP1	Guanylate binding protein 1, interferon-inducible, 67 kDa	NM_002053
	0.427		CA12	Hypothetical protein FLJ20151	BF752277
	0.426		EMP1	Epithelial membrane protein 1	NM_001423
	0.42		CA12	Hypothetical protein FLJ20151	BC001012
	0.415		SLC43A3	Likely ortholog of mouse embryonic epithelial gene 1	BC003163
	0.414		GPNMB	Glycoprotein (transmembrane) nmb	NM_002510
	0.411		CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	AU144855
	0.402		FER1L3	Fer-1-like 3, myoferlin (*C. elegans*)	AF207990
	0.4		PHLDA1	Pleckstrin homology-like domain, family A, member 1	AA576961
	0.393		PGRMC2	Progesterone receptor membrane component 2	NM_006320
0.49	0.388		UGCG	UDP-glucose ceramide glucosyltransferase	AI378044
	0.384		CAV1	Caveolin 1, caveolae protein, 22 kDa	AU147399

Table 3 continued

Fold change			Gene symbol	Description	Genbank
4 h	24 h	96 h			
	0.383		NAV3	Neuron navigator 3	NM_014903
	0.368		FLI1	Friend leukemia virus integration 1	NM_002017
	0.36		COL13A1	Collagen, type XIII, alpha 1	M33653
	0.354		NEF3	Neurofilament 3 (150 kDa medium)	NM_005382
	0.353		CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	AU154504
	0.351		HSXIAPAF1	XIAP associated factor-1	NM_017523
	0.347		NEFL	Neurofilament, light polypeptide 68kDa	BF055311
	0.34		IFIT1	Interferon-induced protein with tetratricopeptide repeats 1	NM_001548
	0.339		EMP1	Epithelial membrane protein 1	BF445047
	0.336		PHLDA1	Pleckstrin homology-like domain, family A, member 1	NM_007350
	0.336		TFPI	Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	BF511231
	0.328		CAV1	Caveolin 1, caveolae protein, 22kDa	NM_001753
	0.327		IGFBP3	Insulin-like growth factor binding protein 3	BF340228
	0.302		IL1R1	Interleukin 1 receptor, type I	NM_000877
	0.289		CUGBP2	CUG triplet repeat, RNA binding protein 2	NM_006561
0.46	0.284		RIS1	Ras-induced senescence 1	BF062629
0.357	0.281		MAFB	V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	NM_005461
	0.279		PI3	Protease inhibitor 3, skin-derived (SKALP)	NM_002638
0.337	0.274	0.205	KYNU	Kynureninase (L-kynurenine hydrolase)	NM_003937
	0.271		PHLDA1	Pleckstrin homology-like domain, family A, member 1	AI795908
	0.268		MEST	Mesoderm specific transcript homolog (mouse)	NM_002402
0.366	0.264	0.378	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	NM_006079
	0.252		EVI2B	Ecotropic viral integration site 2B///ecotropic viral integration site 2B	BC005926
	0.249		IFI35	Interferon-induced protein 35	BC001356
	0.222		CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	NM_000104
	0.216		RAI3	Retinoic acid induced 3	AA156240
	0.204		GAS1	Growth arrest-specific 1	NM_002048
	0.193		NEFL	Neurofilament, light polypeptide 68 kDa	AL537457
	0.133		VNN3	Vanin 3	NM_018399
0.212	0.0535		PTX3	Pentaxin-related gene, rapidly induced by IL-1 beta	NM_002852

Table 4 Effects of TGFβ1 treatment in HP75 pituitary cells on genes related to Gal-3 and TGFβ signaling pathway which were up- and down-regulated and validated by RT-qPCR

Gene	Fold change after TGFβ1 treatment-DNA array			RT-qPCR
	4 h	24 h	96 h	96 h
RUNX1	2.52	8.52	2.92	(2.85)
WNT5B	3.01	2.60	2.23	(2.85)
SOX4	–	6.225	9.53	(10.0)
SMAD3	–	0.454	0.273	0.44

regulator in the Wnt/β-catenin signaling pathway. β-catenin is an integral part of the Wnt signaling pathway and has been identified in human pituitary tumors [20]. Recent studies have shown that the Wnt signaling pathway is important for Gal-3 expression [17], and Gal-3 has a stimulatory effect on the cell growth in some pituitary tumors [11].

TGFβ1 treatment resulted in increased expression of SOX4 in the HP75 pituitary cell line. Several Sox mRNAs including L-Sox5, Sox6, and Sox9 were noted to be increased during fracture healing and early callous

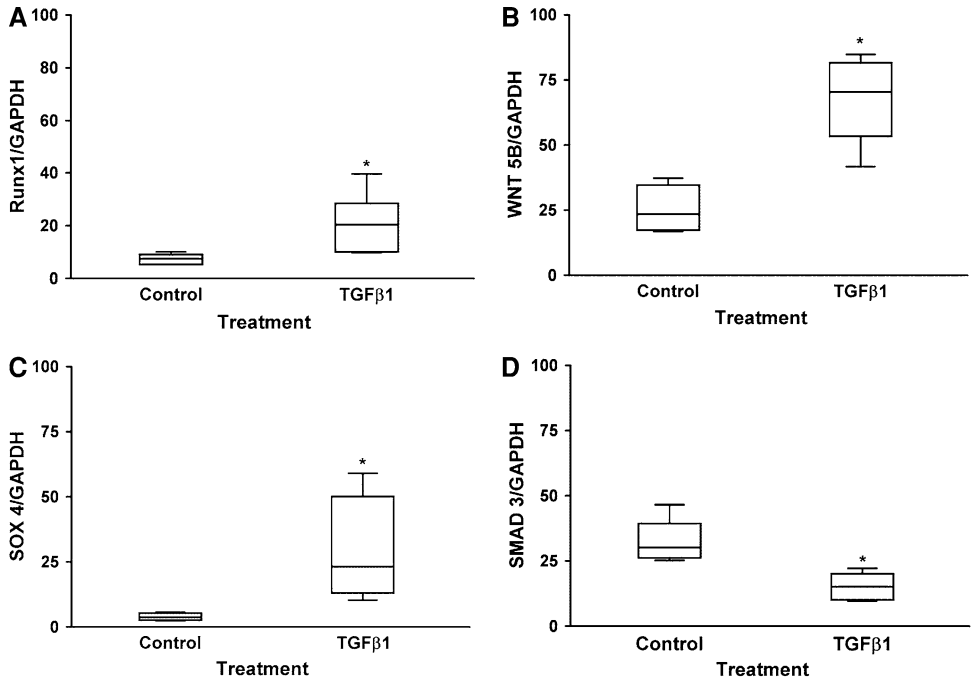

Fig. 2 RT-qPCR graph showing mRNA expression levels of RUNX1 WNT5B, SOX4, and SMAD3 in control and TGFβ1-treated in HP75 pituitary cells. The whiskers represent the maximum and minimum values, the boxes represent the 25th and 75th percentile ranges of values, and the bars represent the median value. Samples were from three independent experiments. (**a**) There was a 2.8-fold higher level of expression of RUNX1 mRNA after TGFβ1 treatment. (**b**) RT-qPCR analysis of WNT5B showed a 2.8-fold increase in WNT5B mRNA levels after TGFβ1 treatment. (**c**) RT-qPCR analysis of SOX4 showed a 10-fold increase in SOX4 mRNA levels after TGFβ1 treatment. (**d**) RT-qPCR analysis of SMAD3 showed a 2.3-fold decrease in SMAD3 mRNA levels after TGFβ1 treatment

formation especially after treatment with bone morphogenetic protein (BMP), a member of the TGFβ family [21]. SMAD3, another protein that was increased by TGFβ1 treatment in pituitary cells, was reported to enhance the transcriptional activity of Sox9 and the expression of α1 collagen gene [22]. In the C3H10T1/2 cell line, Sox9 was up-regulated by BMP2 in a dose-dependent manner [23]. There are few studies exploring the regulatory role of SOX4 in pituitary cells, so the marked decrease in Sox 4 after TGFβ1 treatment should be examined further.

SMAD3 mRNA was decreased after 24 and 96 h of treatment with TGFβ1 in the HP75 pituitary cells. Both the qRT-PCR and Western blot data showed a decrease in SMAD3 mRNA and protein, respectively. The role of Smad signaling in TGFβ action has been well characterized [1–5]. Using a genomic approach, Cao et al. identified downstream targets that were regulated by TGFβ/SMAD3 [24]. They observed that among the apoptotic genes, caspase-3 was induced in rat intestinal epithelial cells whose growth was inhibited by TGFβ [24]. In the current study, caspase-3 was not detected as an apoptotic gene that was significantly regulated by TGFβ1 treatment (Table 3). However, other apoptotic genes included BNIP3, a BCL2 interacting protein; TNFAIP3, a tumor necrosis factor alpha-induced protein were both down-regulated by TGFβ1 treatment for 24 h. Earlier studies from our laboratory showed that TGFβ1 treatment increased apoptosis in HP75 cells [25]. More recent studies have shown that HIF-1 alpha protects HP75 cells from hypoxia-induced apoptosis [26]. Other studies have shown that following TGFβ induction, both the Smad and p38 MAPK pathways converge at the Runx2 gene to control mesenchymal precursor cell differentiation during osteoblast differentiation [27]. These observations provide a link between TGFβ, Smad, and Runx proteins [25]. Kretschmer et al. [28] used gene expression profiling in the HaCaT keratinocyte cell line to show that inhibition of SMAD3 expression was sufficient to interfere with TGFβ-induced cell cycle arrest [28]. The inhibitory effect of TGFβ1 leading to decreased expression of SMAD3 could be a compensatory response to the effects of TGFβ induced cell cycle arrest in pituitary tumor cells.

The aryl hydrocarbon receptor which interacts in its latent form with the aryl hydrocarbon receptor interacting protein (AIP) for cytoplasmic retention of the receptor [27] was down-regulated in the HP75 cell line after 24 h of TGFβ1 treatment. AIP has been shown to be associated with genetic mutations in familial GH tumors [29].

In summary, RNA profiling of a human pituitary cell line, HP75 after 4, 24 and 96 h of treatment with TGFβ1 showed a that the largest number of genes were up- and

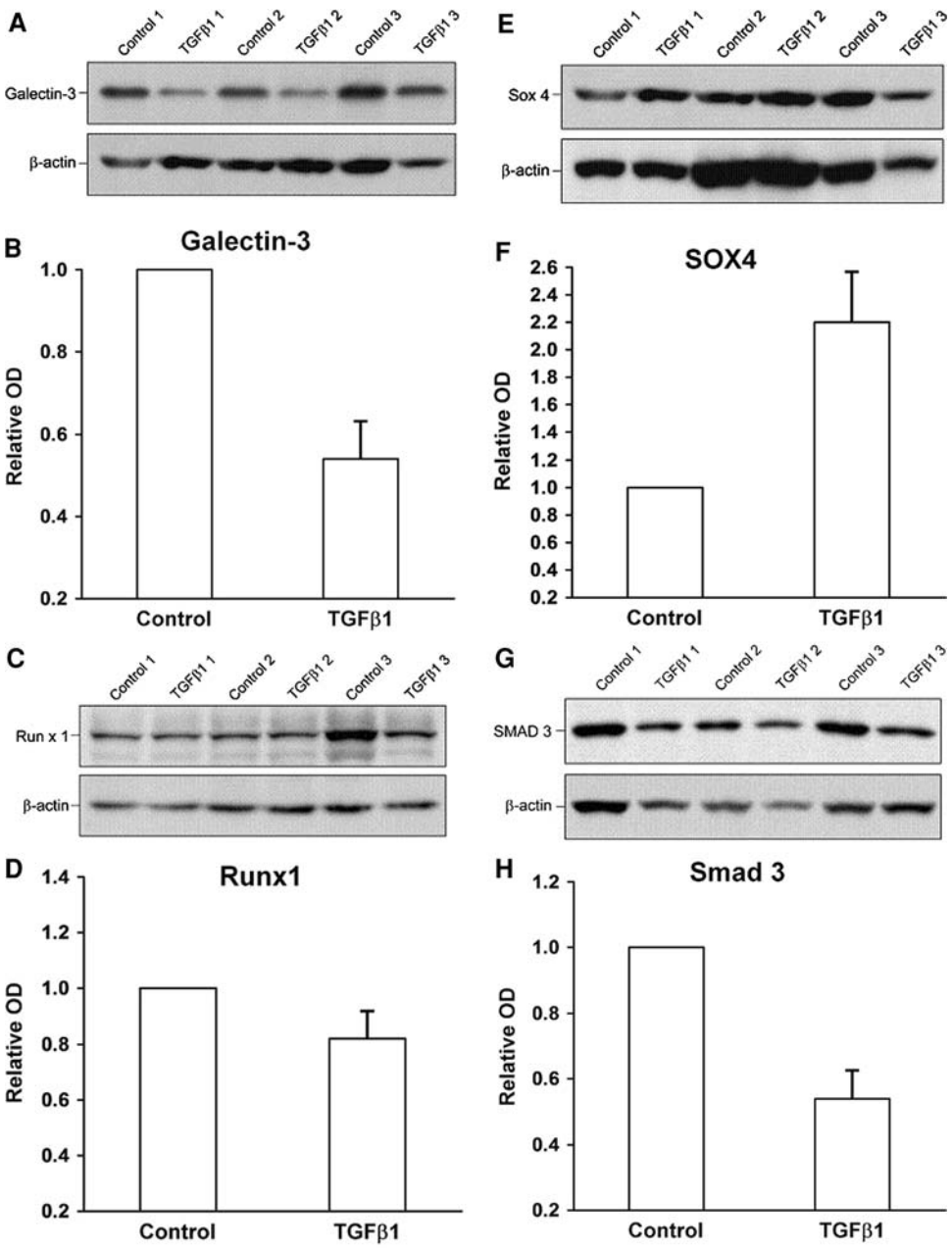

Fig. 3 Western blot analyzing changes in protein levels after TGFβ1 treatment. Twenty-five micrograms of total proteins was used in each lane. β-actin was used to check for protein loading. Densitometric analysis was expressed as relative densitometry units comparing TGFβ1-treated cells to controls for the mean of all three experiments. Three independent experiments were analyzed. (**a**) Galectin-3 levels were decreased after TGFβ1 treatment. (**b**) Densitometric analysis showed a twofold decrease in Gal-3 after TGFβ1 treatment. (**c**) RUNX1 levels were unchanged after TGFβ1 treatment. (**d**) Densitometric analysis showed similar levels in RUNX1 protein. (**e**) SOX4 levels were increased after TGFβ1 treatment. (**f**) Densitometric analysis showing a 2.2-fold increase in SOX4 protein. (**g**) SMAD3 protein levels were decreased after TGFβ1 treatment. (**h**) Densitometric analysis showed a twofold decrease in SMAD3 after TGFβ1 treatment

down-regulated after 24 h of treatment. Validation of specific genes by RT-qPCR and Western blotting showed diverse gene networks and pathways through which TGFβ operates in its effects on pituitary tumor cells.

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References

1. J. Massague, Ann. Rev. Biochem. **67**, 753–791 (1998)
2. J. Massague, J. Seoane, D. Wotton, Genes Dev. **19**, 2783–2810 (2005)
3. R. Derynck, Y.E. Zhang, Nature **425**, 577–584 (2003)
4. H.L. Moses, E.L. Branum, J.A. Proper, R.A. Robinson, Cancer Res. **41**, 2842–2848 (1981)
5. P.M. Siegel, J. Massague, Nat. Rev. Cancer **3**, 807–821 (2003)
6. B. Bierie, H.L. Moses, Cytokine Growth Factor Rev. **17**, 29–40 (2006)
7. B. Bierie, H.L. Moses, Nat. Rev. Cancer **6**, 506–520 (2006)
8. X. Qian, L. Jin, J.P. Grande, R.V. Lloyd, Endocrinology **137**, 3051–3060 (1996)
9. D.K. Sarkar, K. Chaturvedi, S. Oomizu, N.I. Boyadjieva, C.P. Chen, Endocrinology **146**, 4179–4188 (2005)
10. L. Jin, X. Qian, E. Kulig, N. Sanno, B.W. Scheithauer, K. Kovacs, W.F. Young Jr., R.V. Lloyd, Am. J. Pathol. **151**, 509–519 (1997)
11. D. Riss, L. Jin, X. Qian, J. Bayliss, B.W. Scheithauer, W.F. Young Jr., S. Vidal, K. Kovacs, A. Raz, R.V. Lloyd, Cancer Res. **63**, 2251–2255 (2003)

12. L. Jin, E.J. Kulig, X. Qian, B.W. Scheithauer, N.L. Eberhardt, R.V. Lloyd, Endocr. Pathol. **9**, 169–184 (1998)
13. I. Kobayashi, L. Jin, K.H. Ruebel, J.M. Bayliss, O. Hidehiro, R.V. Lloyd, Endocrine **22**, 285–292 (2003)
14. K.H. Ruebel, A.A. Leontovich, L. Jin, G.A. Stilling, H. Zhang, X. Qian, N. Nakamura, B.W. Scheithauer, K. Kovacs, R.V. Lloyd, Endocrine **24**, 141–146 (2004)
15. J. Dang, T. Inukai, H. Kurosawa, K. Goi, T. Inaba, N.T. Lenny, J.R. Downing, S. Stifani, A.T. Look, Mol. Cell Biol. **21**, 5935–5945 (2001)
16. C. Kjellman, S.P. Olofsson, O. Hansson, T. Von Schantz, M. Lindvall, I. Nilsson, L.G. Salford, H.O. Sjogren, B. Widegren, Int. J. Cancer **89**, 251–258 (2000)
17. T. Shimura, Y. Takenaka, T. Fukumori, S. Tsutsumi, K. Okada, V. Hogan, A. Kikuchi, H. Kuwano, A. Raz, Cancer Res. **65**, 3535–3537 (2005)
18. C.S. Moreno, C.O. Evans, X. Zhan, M. Okor, D.M. Desiderio, N.M. Oyesiku, Cancer Res. **65**, 10214–10222 (2005)
19. K.B. Cha, K.R. Douglas, M.A. Potok, H. Liang, S.N. Jones, S.A. Camper, Mech. Dev. **121**, 183–194 (2004)
20. V. Tziortzioti, K.H. Ruebel, T. Kuroki, L. Jin, B.W. Scheithauer, R.V. Lloyd, Endocr. Pathol. **12**, 125–136 (2001)
21. H. Uusitalo, A. Hiltunen, M. Ahonen, T.J. Gao, V. Lefebvre, V. Harley, V.M. Kahari, E. Vuorio, J. Bone Miner. Res. **16**, 1837–1845 (2001)
22. T. Furumatsu, M. Tsuda, N. Taniguchi, Y. Tajima, H. Asahara, J. Biol. Chem. **280**, 8343–8350 (2005)
23. B.K. Zehentner, C. Dony, H. Burtscher, J. Bone Miner. Res. **14**, 1734–1741 (1999)
24. Y. Cao, L. Chen, W. Zhang, Y. Liu, H.T. Papaconstantinou, C.R. Bush, C.M. Townsend Jr., E.A. Thompson, T.C. Ko, Am. J. Physiol. Gastrointest. Liver Physiol. **292**, G28–G38 (2007)
25. E. Kulig, L. Jin, X. Qian, E. Horvath, K. Kovacs, L. Stefaneanu, B.W. Scheithauer, R.V. Lloyd, Am. J. Pathol. **154**, 767–774 (1999)
26. D. Yoshida, K. Kim, M. Noha, A. Teramoto, J. Neurooncol. **78**, 217–225 (2006)
27. K.S. Lee, S.H. Hong, S.C. Bae, Oncogene **21**, 7156–7163 (2002)
28. A. Kretschmer, K. Moepert, S. Dames, M. Sternberger, J. Kaufmann, A. Klippel, Oncogene **22**, 6748–6763 (2003)
29. O. Vienmaa, M. Georgitsi, R. Lehtonen, P. Vahtenisto, A. Kokko, A. Raitila, K. Tuppurainen, T.M. Ebeling, P.I. Salmela, R. Paschke, E. Gundogdus, E. De Menis, M.J. Makinen, V. Launonen, A. Karhu, L.A. Altonen, Science **312**, 1228–1230 (2006)