Significance of *TGFBR3* allelic loss in the deregulation of TGFβ signaling in primary human endometrial carcinomas

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Abstract. Downregulation of betaglycan (β-glycan) [transforming growth factor β receptor type III (TGFβR3)], which belongs to co-receptors of the TGFβ pathway, occurs in a broad spectrum of primary human malignancies. However, in the case of endometrial cancer (EC), the mechanisms responsible for genetic alterations are still unknown. Therefore, we investigated allelic imbalance at the *TGFBR3* locus (1p33-p32) in the context of β-glycan mRNA and protein expression, as a possible genetic event determining β-glycan deregulation in EC patients. Study of β-glycan allelic imbalance in 48 primary human ECs was performed with the use of three different microsatellite markers, spanned within or in direct proximity to the *TGFBR3* locus. Real-time PCR and western blotting were used for β-glycan mRNA and protein quantification methods, respectively. Altogether, 25 of 39 (64%) informative cases and 25 of 48 (52%) of all specimens showed allelic imbalance in at least one microsatellite marker, concomitantly with decrease at both the β-glycan transcript and protein levels. Interestingly, 54% (15/28), 36% (8/22) and 35% (7/20) of informative ECs displayed allelic loss in D1S188, D1S435 and D1S1588 microsatellite markers, respectively. It is worth pointing out that 5 out of 39 (13%) informative cases showed loss of heterozygosity (LOH) at two microsatellite markers. Microsatellite instability (MSI) was found in two markers, but to a very strictly limited extent. None of the clinicoprognostic features was found to be of significance. Our results suggest that LOH in the *TGFBR3* locus may be one of the mechanisms responsible for loss of β-glycan expression. No correlation of LOH at the *TGFBR3* locus with clinicopathological parameters suggests that allelic imbalance may be an early genetic event during neoplastic transformation of human endometrium.

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Introduction

Betaglycan (β-glycan) [transforming growth factor β receptor type III (TGFβR3)] belongs to the membrane-bound accessory receptors involved in signal initiation and propagation in cellular pathway, activated by transforming growth factor-β types (TGFβs) (1). Biochemically, this receptor is a proteoglycan encoded by 225,660 bp *TGFBR3*, which is located on chromosome 1p33-p32 (NCBI reference sequence: NG_027757.1). It has two promoters but in the majority of tissues, the proximal type dominates over a distal one. *TGFBR3* is divided into 18 exons and encodes a protein of 851 amino acids (2). The molecular weight of β-glycan protein is approximately 300 kDa, due to heparin and chondroitin sulphate modifications (3-5). It is ubiquitously expressed in almost all cell types, in approximately 200,000 particles/cell, and forms the non-covalently linked homodimers (6).

Although, structural analysis of β-glycan revealed lack of any well-known signaling motif in its amino-acid sequence, it plays a vital role in signal mediation in the TGFβ pathway. Studies on TGFβ binding caused by β-glycan demonstrated its affinity to TGFβ isoforms and inhibin A, BMP-4 and -7, as well as to GDF-5. β-glycan shows the highest effect on TGFβ2-induced signal initiation, which itself binds poorly to the TGFβ type II receptor (TGFβRII). This suggests the particular role of β-glycan in TGFβ2 signaling (7-10). Signal mediation via β-glycan is based on TGFβ factor binding by its extracellular domain, with simultaneous ligand concentration on the cell surface and complex formation with TGFβRII receptor. As a consequence, the β-glycan cytoplasmic region promotes interaction of intracellular domains of heterodimeric TGFβRII and I receptors, as well as trans-phosphorylation of TGFβRI by TGFβRII. After activation of TGFβRII and TGFβRI receptors, β-glycan dissociates from this complex and phosphorylated TGFβRI receptor propagate signal downstream to the cellular TGFβ effector, Smad proteins (11). In addition to the above-mentioned mechanism of action, in physiological conditions, the β-glycan extracellular domain undergoes proteolytic cleavage (ectodomain shedding) by metalloproteases (MT1-MMP and MT3-MMP) and plasmin, resulting in the sequestration of a soluble form of β-glycan (sol-β-glycan) in the extracellular matrix (ECM). Sol-β-glycan is thought to modulate the signal induced by TGFβ factors, competing with

TGFβ receptors for their ligands. Sol-β-glycan functions as an antagonist of TGFβ signaling, suggesting a potential role as an antitumor agent in future therapies (12-17). Another proposed mechanism of signaling orchestration in the TGFβ pathway is the steric effect, which is a result of β-glycan modification by residues of glycosaminoglycans (GAG). Oncogenic Ki-*ras* seems to be involved in post-translational GAG attachment, resulting in increased responsiveness to TGFβ proliferative stimuli and downregulation of p21 in colon cancer (18). Mythreye and Blobe (19) reported that GAG modifications are necessary for inhibition of cell migration. Compared to controls, *TGFBR3*ΔGAG mutants displayed increased migratory properties, as shown either in ovarian cancer (Ovca429) or in normal ovarian surface epithelial (NOSE007) cell lines. The GAG chains inhibit TGFβ induced signaling by preventing formation of TGFβRII-TGFβRI complexes, as confirmed in an LLC-PK_1 model (20).

Some studies suggest an unquestioned role of alterations in the TGFβ signaling pathway in many human diseases, in particular cancer (10,21-23). A large number of factors activating the TGFβ cascade result in the regulation of opposed processes, what is known as the pleiotropic effect on cells. TGFβs inhibit cancer development and progression early in neoplastic transformation, whereas they contribute to acquisition of a metastatic phenotype in more advanced clinical stages (11). Our recent data clearly demonstrated downregulation of β-glycan mRNA and its relationship with clinical and pathological parameters (24,25). It suggests that in the case of ECs, TGFβ deregulation may be a result of impaired TGFβ2 signaling, which is caused by β-glycan. However, a literature search (Medline® database) has revealed that there are no studies evaluating the role of allelic loss of β-glycan (TGFβR3) in primary human ECs.

The results obtained are the basis for the search of potential molecular mechanisms responsible for the β-glycan decline in primary human ECs. The aim of our present study was to evaluate loss of heterozygosity (LOH) as a potential mechanism responsible for downregulation of β-glycan in primary human ECs. We also correlated the prevalence of allelic loss with clinical and pathological variables of uterine malignancies.

Materials and methods

Patient material. Tissue samples from women having undergone surgery for primary ECs were collected in the Second Department of Gynecology, Lublin Medical University, Lublin, Poland, between 2010 and 2014. The study group consisted of 48 EC specimens and matched 48 normal tissue samples. None of the patients had received hormonal therapy, radiation therapy or chemotherapy before surgery. The mean age of the patients was 62 years, ranging from 46 to 81 years. At surgery, tissue specimens were immediately subdivided into two portions; one was fixed in buffered formalin (pH 7.4) for routine pathological examination, and the other was stored at -70˚C until further analysis. The clinical stage was assigned according to a recently established FIGO classification system (26). World Health Organization classification was applied to determine the pathological grading. Myometrial and lymph node invasion and VSI (vascular space invasion) were evaluated as well. Clinicopathological variables of the EC samples are depicted in Table I. The study cohort was subdivided into two age groups: the first group consisted of women <60 years of age (n=21; 44%), the second group of women ≥60 years (n=27; 56%). The Independent Ethics Committee of the Lublin Medical University, Lublin, Poland approved the tissue collection and subsequent experiments, and all the women enrolled provided their informed consent.

Table I. Clinicopathological parameters of the patients with endometrial cancer.

Clinicopathological parameters	No. of patients (%)
Patients' age (years)	
<60	21 (44)
≥60	27 (56)
Clinical stage[a]	
I	22 (46)
II	15 (31)
III	7 (15)
IV	4 (8)
Histological grade[b]	
G1	9 (19)
G2	34 (71)
G3	5 (10)
Depth of myometrial invasion	
<1/2	21 (44)
>1/2	27 (56)
Vascular space invasion	
Not present	37 (77)
Present	11 (23)
Lymph node invasion	
Not present	36 (75)
Present	1 (2)
Not assessed	11 (23)

[a]International Federation of Gynecology and Obstetrics staging system. [b]World Health Organization classification system.

RNA isolation and real-time polymerase chain reaction (PCR). Total RNA was extracted according to a modified Chomczynski and Sacchi protocol. Afterwards, RNA (1 μg) was retro-transcribed using RevertAid™ H Minus First Strand cDNA Synthesis kit (Fermentas, Canada) according to manufacturer's recommendation. Real-time PCR was performed using TaqMan® probes (Life Technologies, Carlsbad, CA, USA), and in line with the protocol on Mastercycler® Epgradient S Realplex (Eppendorf, Hamburg, Germany). *GAPDH* (*glyceraldehyde 3-phosphate dehydrogenase*) served as an internal control. The catalogue numbers of probes were: Hs00234259_m1 for *TGFBR3* (β-glycan) and Hs99999905_m1 for GAPDH. The relative expression level was normalized to GAPDH, and calculated using the following equation: $2^{-\Delta Ct}$ x 1,000.

Western blotting. Tissue samples were homogenized in lysis buffer containing 0.25 M sucrose, 50 mM Tris-HCl (pH 7.4),

Table II. *TGFBR3* microsatellite markers and PCR conditions.

Marker	Chromosomal localization	Repeat motif and product sizes	Annealing temperature	Primer sequences	Dye
D1S188	1p31	$(CA)_n$ 149-173 bp	64˚C	F: 5'-AACCAATCAAGGTGCCTGCA-3' R: 5'-TCCCCTAGTGTCCTGGCAG-3'	- FAM
D1S435	1p31	$(CA)_n$ 157-177 bp	62˚C	F: 5'-GGTTATTAGGCATGATAAGGG-3' R: 5'-ACGCTGTCTCTGACAAGAAA-3'	- FAM
D1S1588	1p33-p32	$(AAT)_n$ 118-139 bp	58.5˚C	F: 5'-CTGGTCCCATAGCTAGTAAACG-3' R: 5'-ATGAGGTCCCCATTTACCAT-3'	- TET

F, forward; R, reverse.

5 mM $MgCl_2$, 0.5% Triton X-100 and 1 mM PMSF. For each sample, protein concentration was evaluated by Lowry protocol (27). The proteins (30 μg/well) were separated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (7.5%) and transferred onto Immobilon-P membranes (Millipore, Billerica, MA, USA) using the semi-dry system. The membranes were incubated overnight at 4˚C with primary antibodies after prior blocking with 5% dry non-fat milk. Following extensive washing with Tris-buffered saline with 0.1% Tween-20, the membranes were incubated with horseradish peroxidase-conjugated (HRP) secondary antibodies for 1 h and visualized with Novex HRP Chromogenic Substrate-TMB (Invitrogen Inc., Carlsbad, CA, USA). β-actin served as loading control. After TMB visualization, blots were incubated at 50˚C for 45 min in stripping buffer containing 2% SDS, 62.5 mM Tris-HCl (pH 6.8), 0.8% β-mercaptoethanol, rinsed with running water, and rehydrated with methanol. Immune-identification of β-actin was carried out. Quantitative analysis was performed by measuring IOD by GelProAnalyzer v. 3.0 for Windows™ software (Media Cybernetics, Baltimore, MD, USA). The following commercially available antibodies were applied: primary: rabbit polyclonal anti-β-glycan antibodies (ab97459; Abcam, Cambridge, UK) against protein fragment corresponding to a region within amino acids 88-274 of human β-glycan (dilution: 1:1,000), goat polyclonal anti-actin antibodies (sc-1616, Santa Cruz Biotechnology, Santa Cruz, CA, USA) against carboxy-terminus of actin of human origin (dilution: 1:1,000); secondary: goat anti-rabbit polyclonal antibodies (A9169, Sigma-Aldrich, Schnelldorf, Germany) against whole molecule (dilution: 1/20,000), rabbit anti-goat polyclonal antibodies (A8919, Sigma-Aldrich) against whole molecule (dilution: 1/5,000).

DNA isolation and PCR. Total genomic DNA was isolated according to the phenol/chloroform protocol. Briefly, tissue was minced and homogenized with denaturing solution consisting of 10 mM EDTA, 10 mM Tris/HCl (pH 8.0), 0.5% SDS. Following overnight incubation at 55˚C with 20 μl of proteinase K (10 mg/ml), an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) mixture was added to the samples. After centrifugation, the aqueous phase was precipitated with an equal volume of isopropanol. DNA pellet was washed twice with 70% ethanol, re-suspended in TE buffer and stored at -70˚C for further analysis. The quality and quantity of DNA was estimated spectrophotometrically with BioPhotometer Plus (Eppendorf, Hamburg, Germany).

PCR was performed in Applied Biosystems 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). Primers used in the reaction were fluorescently labeled (Sigma-Aldrich). Table II presents the *TGFBR3* microsatellite markers and PCR conditions applied. Briefly, the PCR reaction was carried out in a total volume of 12.5 μl and the mixture consisted of 1X PCR buffer [10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin] dedicated for the JumpStart™ *Taq* DNA polymerase (Sigma-Aldrich), 5 ng of genomic DNA, 0.5 μM of each primer, 0.2 mM of each dNTP and 0.625 *U* of JumpStart™ *Taq* DNA polymerase. Sequences of primers were adopted from the National Center for Biotechnology Information database-NCBI (www.ncbi.nlm.nih.gov).

LOH analysis. After PCR, the samples were mixed with solution containing deionized formamide, GeneScan-350 TAMRA (Life Technologies) dye size standard, and loading buffer (blue dextran, EDTA). They were denatured, chilled on ice, and separated in 5% Long Ranger (BioWhittaker Molecular Applications, Rockland, ME, USA) containing 6 M urea. PCR products were analyzed using DNA Sequencer ABI PRISM 377 (Applied Biosystems). Allele lengths were determined with GeneScan v. 3.1.2 and Genotyper v. 2.5 software (Applied Biosystems). Amplification of microsatellite markers yielded one or two allele peaks, depending upon whether the individual is homozygous (non-informative cases) or heterozygous (informative cases) for that marker. Loss of heterozygosity (LOH) was defined when one allelic band from tumor DNA disappeared completely or when the signal intensity (allelic ratio ≤0.5) was reduced <50% in the tumor DNA compared with the paired normal DNA pattern. Allele ratios were calculated only for informative cases according to the following formula: T_1:T_2/N_1:N_2, where T_1 and N_1 are the values for shorter length allele product peak of tumor and normal sample, and T_2 and N_2 are the values for longer length allele product peak of tumor and normal sample, respectively (28). All samples were analyzed in replicates.

Statistical analysis. Statistical tests were performed applying GraphPad Prism v. 5.00 software for Windows (GraphPad Software, Inc., La Jolla, CA, USA). Data are presented as mean ± standard error of mean (SEM). P-values were

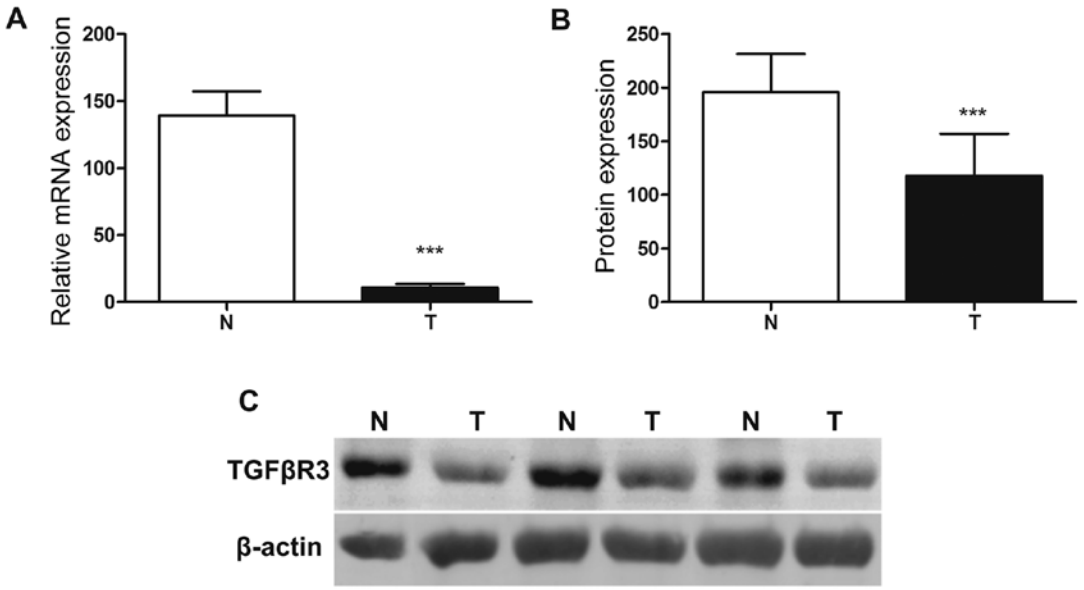

Figure 1. β-glycan downregulation in EC samples compared to normal tissue. (A) *TGFBR3* mRNA expression level in normal (N) and endometrial cancer (T) tissues as assessed by real-time PCR. (B) β-glycan protein expression in normal (N) and endometrial cancer (T) tissues as assessed by western blotting. (C) Immunoblots of β-glycan in normal and cancer samples. β-actin served as a control for the amount of protein loading. Antibodies are described in Materials and methods. Data are presented as mean ± SEM. P-values were estimated by the paired Student's t-test. ***P<0.001.

Table III. Frequency of the loss of heterozygosity (LOH) in the endometrial cancer samples.

Marker	Informative cases/total cases	LOH-positive cases (%)
D1S188	28/48	15 (54)
D1S435	22/48	8 (36)
D1S1588	20/48	7 (35)

calculated using two-tailed paired Student's t-test. The statistical analysis of allelic loss included the assessment of the association between the prevalence of LOH and clinical and pathological variables using Fisher's two-tailed exact test. P<0.05 was considered to indicate a statistically significant difference.

Results

β-glycan expression pattern. Real-time PCR and western blotting were used for β-glycan mRNA and protein quantification methods, respectively. *TGFBR3* expression was found to be significantly downregulated in the EC samples compared to that noted in the normal tissues (P<0.001) (Fig. 1A). Moreover, β-glycan mRNA decline corresponded to its protein decrease, as demonstrated in all EC samples studied. The decrease of β-glycan protein expression in ECs was highly significant (P<0.001) (Fig. 1B). Examples of immunoblots of β-glycan in normal and EC samples in relation to actin (serving as an internal control) are shown in Fig. 1C.

β-glycan allelic loss. To examine whether or not allelic loss is a *bona fide* mechanism clearly responsible for decreased β-glycan expression in human ECs, molecular analysis was performed with the use of three different microsatellite markers: D1S188, D1S435 and D1S1588. These markers are spanned within or in direct proximity to the *TGFBR3* locus (Table II). Loss of heterozygosity is a chromosomal event defined as a direct loss of one allele or as an intensity reduction >50% in EC samples compared to the corresponding normal tissue (Fig. 2A). It is worth pointing out that 25 of 39 (64%) informative cases and 25 of 48 (52%) uterine cancer specimens studied revealed allelic imbalance in at least one microsatellite marker evaluated. Altogether, 54% (15/28), 36% (8/22) and 35% (7/20) of informative ECs revealed an allelic loss in D1S188, D1S435 and D1S1588, respectively (Fig. 2B and Table III). It is worth pointing out that 5 out of 39 (13%) informative cases showed LOH at two microsatellite markers. Microsatellite instability (MSI) was found in only two markers, but to a very strictly limited extent (in two cases - #6 and #32 and in case #27 in the D1S188 and D1S1588 microsatellite markers, respectively) (Fig. 2B).

Correlation between β-glycan LOH and clinicopathological variables of ECs. The correlation between LOH in the *TGFBR3* locus and clinical and pathological variables of EC samples is presented in Table IV. None of the clinicoprognostic features, including clinical staging, histological grading, myometrial invasion, VSI and age at diagnosis, was found to be of significance. Moreover, additional analysis of LOH occurrence in ECs in relation to combined clinicopathological variables, i.e., FIGO stage/myometrial invasion, histological grade/myometrial invasion, FIGO stage/age at diagnosis, histological grade/age at diagnosis did not reveal any significant differences (data not shown). Therefore, the presence of LOH in the *TGFBR3* locus did not appear to determine the malignancy of the sporadic uterine neoplasms, and probably is an early event during endometrial transformation in humans.

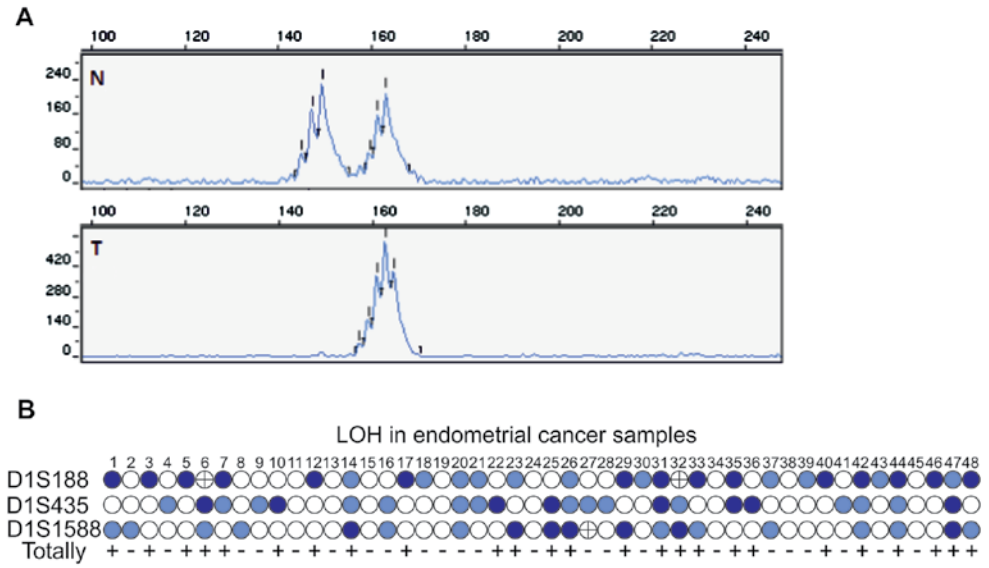

Figure 2. β-glycan loss of heterozygosity (LOH) as analyzed in ECs. (A) A representative LOH in the D1S188 microsatellite marker in cancerous (T) as compared to normal (N) tissue is presented. (B) frequencies of LOH in the *TGFBR3* locus. Dark blue circles, loss of heterozygosity; light blue circles, maintenance of heterozygosity; white circles, non-informative cases (both normal and cancer samples displayed homozygous status); crossed circles, microsatellite instability.

Table IV. Loss of heterozygosity (LOH) in clinicopathological features of EC patients in relation to allelic loss at the *TGFBR3* locus using microsatellite markers D1S188, D1S435 and D1S1588.

	D1S188				D1S435				D1S1588			
Clinicopathological parameters	I 28	N 13	LOH 15	P-value	I 22	N 14	LOH 8	P-value	I 20	N 13	LOH 7	P-value
FIGO stage												
I	9	4	5	0.85	11	7	4	0.49	8	5	3	0.70
II	12	6	6		6	5	1		7	4	3	
III	4	1	3		2	1	1		3	3	0	
IV	3	2	1		3	1	2		2	1	1	
Histological grade												
G1	6	2	4	0.41	6	5	1	0.58	3	1	2	0.33
G2	20	9	11		13	7	6		15	11	4	
G3	2	2	0		3	2	1		2	1	1	
Myometrial invasion												
<1/2	11	5	6	>0.99	12	7	5	0.67	9	6	3	>0.99
>1/2	17	8	9		10	7	3		11	7	4	
Vascular space invasion												
Not present	21	10	11	>0.99	17	11	6	0.61	16	10	6	>0.99
Present	7	3	4		5	3	2		4	3	1	
Age (years) at diagnosis												
<60	11	5	6	>0.99	9	7	2	0.38	7	6	1	0.33
≥60	17	8	9		13	7	6		13	7	6	

I, informative cases; N, heterozygous cases without LOH and MSI. Statistical analysis, Fisher's two-tailed exact test.

Discussion

EC is one of the leading causes of cancer-related mortality among females with approximately 320,000 new cases and 76,000 deaths worldwide in 2012. In Poland, 5426 new cases were diagnosed in 2011 (29). A significantly higher incidence rate is observed in developed countries in contrast to less developed ones. An increase in the EC incidence rate should

be expected in the near future due to the intensified aging of human societies (30).

Other studies suggest that cancer development and progression appears to be associated with alterations in the TGFβ signaling cascade (31). Disturbed signal mediation in the TGFβ pathway triggers its development from a tumor suppressor, early in neoplastic transformation, to a cancer-promoting and -metastatic agent in advanced clinical stages of the disease (32). A particular role in neoplastic transformation has been reported in the case of β-glycan, which acts as a tumor inhibitory agent suppressing cancer cell migration, invasion, proliferation and angiogenesis (6,11). β-glycan downregulation is responsible for impaired ligand presentation to TGFβ canonical receptors - TGFβRII and TGFβRI (33). Loss of β-glycan expression results in impaired signaling driven by TGFβ2 isoform, as TGFβ2 possesses the highest and exclusive affinity to β-glycan (33-35). Besides TGFβ2 isoform signal mediation, β-glycan reduction may favor development of an immunotolerant tumor microenvironment as an immune suppressor and stimulator of Treg cells (36). Furthermore, involvement of β-glycan in cancer development through non-canonical TGFβ signaling pathways cannot be excluded. Significant alterations in β-glycan expression have been reported in several human neoplasms originating from different tissues, such as breast, endometrial, ovarian, pancreatic, prostate, bladder, liver, lung and renal carcinomas (24,37-46). According to previous studies, chromosome 1p exhibits a meaningful allelic imbalance in a number of primary human malignancies (47).

Our current results are in line with those previously published by Florio *et al* (48), who reported a significant downregulation of β-glycan expression with concomitant decrease in the inhibin α-subunit in the case of EC. Current results concerning β-glycan protein expression are not in line with those published previously by our group (24), where ELISA assay was performed. Presently, protein expression was analyzed by western blotting as a more specific and acknowledged method. Moreover, we applied different primary antibodies which corresponded to a region within amino acids 88-74 of the fully processed and mature human form of β-glycan molecule. Previous antibodies directed against β-glycan C-terminus may lead to immunodetection of non-functionally synthesized β-glycan particles with abrogated trafficking to the cell membrane and might also cross-react with endoglin due to the sequence similarities.

The data of our study indicate LOH in the *TGFBR3* region located at chromosome 1p, and its association with a decrease in β-glycan in sporadic human ECs. These results suggest that allelic loss at the *TGFBR3* region may be the mechanism through which EC cells escape from TGFβ-mediated suppression. Similar results have been achieved in studies on allelic imbalance in different human neoplasms, in particular in those derived from hormone-dependent tissues (38,39,41,42). In breast carcinomas, the mechanisms responsible for downregulation of β-glycan included LOH in microsatellite markers (D1S1588 and D1S188), in half of the samples analyzed (38). The study of the D1S1588, D1S2804 and D1S435 microsatellite markers in prostate carcinomas showed LOH in 37.5% of samples studied (41). Comparable results of allelic imbalance in the β-glycan locus have been achieved in investigations of non-small cell lung carcinomas, where LOH was reported in 38.5% of cases, at least in one microsatellite marker (D1S1588, D1S188 or D1S2804) (42). Despite the high percentage of LOH-positive cases in the *TGFBR3* locus in our study (52% of the samples investigated; 64% of informative cases), other mechanisms causing mRNA and protein downregulation in ECs cannot be excluded.

Studies on cancer cell lines revealed that loss of β-glycan expression could be due to altered epigenetic regulation, as observed in prostate and ovarian tumor cell lines, where β-glycan expression was restored after treatment with methyltransferase and histone deacethylase inhibitors (39,41). Indirect epigenetic regulation was reported in bladder urothelioma and renal cell carcinoma (23,49). B-glycan expression in these tumor types appeared to be positively controlled by the GATA3 transcription factor (23,49). Increased methylation of GATA3 was found to result in a decline in β-glycan at the transcriptomic level (49). The molecular mechanisms determining β-glycan expression alterations appear to involve other mechanisms as well, since neither genetic aberrations nor allelic imbalance were found to be associated with β-glycan expression in hepatocellular carcinoma (44). Transcriptional regulation is suggested to play an exclusive role. Moreover, the lack of a statistical significance of LOH occurrence and single or combined clinicopathological parameters of EC samples strongly supports the hypothesis that β-glycan allelic imbalance may be an early genomic event during endometrial neoplastic transformation that does not determine cancer aggressiveness.

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References

1. Cheifetz S, Andres JL and Massagué J: The transforming growth factor-beta receptor type III is a membrane proteoglycan. Domain structure of the receptor. J Biol Chem 263: 16984-16991, 1988.
2. Hempel N, How T, Dong M, Murphy SK, Fields TA and Blobe GC: Loss of betaglycan expression in ovarian cancer: Role in motility and invasion. Cancer Res 67: 5231-5238, 2007.
3. Wang XF, Lin HY, Ng-Eaton E, Downward J, Lodish HF and Weinberg RA: Expression cloning and characterization of the TGF-beta type III receptor. Cell 67: 797-805, 1991.
4. López-Casillas F, Cheifetz S, Doody J, Andres JL, Lane WS and Massagué J: Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-beta receptor system. Cell 67: 785-795, 1991.
5. Morén A, Ichijo H and Miyazono K: Molecular cloning and characterization of the human and porcine transforming growth factor-beta type III receptors. Biochem Biophys Res Commun 189: 356-362, 1992.
6. Gatza CE, Oh SY and Blobe GC: Roles for the type III TGF-beta receptor in human cancer. Cell Signal 22: 1163-1174, 2010.
7. López-Casillas F, Payne HM, Andres JL and Massagué J: Betaglycan can act as a dual modulator of TGF-beta access to signaling receptors: Mapping of ligand binding and GAG attachment sites. J Cell Biol 124: 557-568, 1994.
8. Esparza-Lopez J, Montiel JL, Vilchis-Landeros MM, Okadome T, Miyazono K and López-Casillas F: Ligand binding and functional properties of betaglycan, a co-receptor of the transforming growth factor-beta superfamily. Specialized binding regions for transforming growth factor-beta and inhibin A. J Biol Chem 276: 14588-14596, 2001.
9. Kirkbride KC, Ray BN and Blobe GC: Cell-surface co-receptors: Emerging roles in signaling and human disease. Trends Biochem Sci 30: 611-621, 2005.

10. Bernabeu C, Lopez-Novoa JM and Quintanilla M: The emerging role of TGF-beta superfamily coreceptors in cancer. Biochim Biophys Acta 1792: 954-973, 2009.
11. Bilandzic M and Stenvers KL: Betaglycan: A multifunctional accessory. Mol Cell Endocrinol 339: 180-189, 2011.
12. Andres JL, Stanley K, Cheifetz S and Massagué J: Membrane-anchored and soluble forms of betaglycan, a polymorphic proteoglycan that binds transforming growth factor-beta. J Cell Biol 109: 3137-3145, 1989.
13. Bandyopadhyay A, Zhu Y, Cibull ML, Bao L, Chen C and Sun L: A soluble transforming growth factor β type III receptor suppresses tumorigenicity and metastasis of human breast cancer MDA-MB-231 cells. Cancer Res 59: 5041-5046, 1999.
14. Cheung HK, Mei J and Xu RJ: Quantification of soluble beta-glycan in porcine milk. Asia Pac J Clin Nutr 12: S61, 2003.
15. Velasco-Loyden G, Arribas J and López-Casillas F: The shedding of betaglycan is regulated by pervanadate and mediated by membrane type matrix metalloprotease-1. J Biol Chem 279: 7721-7733, 2004.
16. Bandyopadhyay A, Wang L, López-Casillas F, Mendoza V, Yeh IT and Sun L: Systemic administration of a soluble beta-glycan suppresses tumor growth, angiogenesis, and matrix metalloproteinase-9 expression in a human xenograft model of prostate cancer. Prostate 63: 81-90, 2005.
17. Juárez P, Vilchis-Landeros MM, Ponce-Coria J, Mendoza V, Hernández-Pando R, Bobadilla NA and López-Casillas F: Soluble betaglycan reduces renal damage progression in db/db mice. Am J Physiol Renal Physiol 292: F321-F329, 2007.
18. Yan Z, Deng X and Friedman E: Oncogenic Ki-*ras* confers a more aggressive colon cancer phenotype through modification of transforming growth factor-beta receptor III. J Biol Chem 276: 1555-1563, 2001.
19. Mythreye K and Blobe GC: The type III TGF-beta receptor regulates epithelial and cancer cell migration through beta-arrestin2-mediated activation of Cdc42. Proc Natl Acad Sci USA 106: 8221-8226, 2009.
20. Eickelberg O, Centrella M, Reiss M, Kashgarian M and Wells RG: Betaglycan inhibits TGF-beta signaling by preventing type I-type II receptor complex formation. Glycosaminoglycan modifications alter betaglycan function. J Biol Chem 277: 823-829, 2002.
21. Gordon KJ, Dong M, Chislock EM, Fields TA and Blobe GC: Loss of type III transforming growth factor beta receptor expression increases motility and invasiveness associated with epithelial to mesenchymal transition during pancreatic cancer progression. Carcinogenesis 29: 252-262, 2008.
22. Antony ML, Nair R, Sebastian P and Karunagaran D: Changes in expression, and/or mutations in TGF-β receptors (TGF-β RI and TGF-β RII) and Smad 4 in human ovarian tumors. J Cancer Res Clin Oncol 136: 351-361, 2010.
23. Liu XL, Xiao K, Xue B, Yang D, Lei Z, Shan Y and Zhang HT: Dual role of *TGFBR3* in bladder cancer. Oncol Rep 30: 130-138, 2013.
24. Zakrzewski PK, Mokrosiński J, Cygankiewicz AI, Semczuk A, Rechberger T, Skomra D and Krajewska WM: Dysregulation of betaglycan expression in primary human endometrial carcinomas. Cancer Invest 29: 137-144, 2011.
25. Semczuk A, Zakrzewski PK, Forma E, Cygankiewicz AI, Semczuk-Sikora A, Bryś M, Rechberger T and Krajewska WM: TGFβ-pathway is down-regulated in a uterine carcinosarcoma: A case study. Pathol Res Pract 209: 740-744, 2013.
26. Pecorelli S: Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet 105: 103-104, 2009.
27. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275, 1951.
28. Cawkwell L, Bell SM, Lewis FA, Dixon MF, Taylor GR and Quirke P: Rapid detection of allele loss in colorectal tumours using microsatellites and fluorescent DNA technology. Br J Cancer 67: 1262-1267, 1993.
29. Didkowska J, Wojciechowska U, Zatoński W. Cancer in Poland in 2011. National M. Sklodowska-Curie Cancer Center; Warsaw, pp13-21, 2001.
30. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D and Bray F: Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer 49: 1374-1403, 2013.
31. Drabsch Y and ten Dijke P: TGF-β signalling and its role in cancer progression and metastasis. Cancer Metastasis Rev 31: 553-568, 2012.
32. Principe DR, Doll JA, Bauer J, Jung B, Munshi HG, Bartholin L, Pasche B, Lee C and Grippo PJ: TGF-β: Duality of function between tumor prevention and carcinogenesis. J Natl Cancer Inst 106: djt369, 2014.
33. Blobe GC, Schiemann WP, Pepin MC, Beauchemin M, Moustakas A, Lodish HF and O'Connor-McCourt MD: Functional roles for the cytoplasmic domain of the type III transforming growth factor β receptor in regulating transforming growth factor β signaling. J Biol Chem 276: 24627-24637, 2001.
34. Guo X and Wang XF: Signaling cross-talk between TGF-β/BMP and other pathways. Cell Res 19: 71-88, 2009.
35. Zhang YE: Non-Smad pathways in TGF-β signaling. Cell Res 19: 128-139, 2009.
36. Hanks BA, Holtzhausen A, Evans KS, Jamieson R, Gimpel P, Campbell OM, Hector-Greene M, Sun L, Tewari A, George A, *et al*: Type III TGF-β receptor downregulation generates an immunotolerant tumor microenvironment. J Clin Invest 123: 3925-3940, 2013.
37. Copland JA, Luxon BA, Ajani L, Maity T, Campagnaro E, Guo H, LeGrand SN, Tamboli P and Wood CG: Genomic profiling identifies alterations in TGFbeta signaling through loss of TGFbeta receptor expression in human renal cell carcinogenesis and progression. Oncogene 22: 8053-8062, 2003.
38. Dong M, How T, Kirkbride KC, Gordon KJ, Lee JD, Hempel N, Kelly P, Moeller BJ, Marks JR and Blobe GC: The type III TGF-beta receptor suppresses breast cancer progression. J Clin Invest 117: 206-217, 2007.
39. Hempel N, How T, Cooper SJ, Green TR, Dong M, Copland JA, Wood CG and Blobe GC: Expression of the type III TGF-beta receptor is negatively regulated by TGF-beta. Carcinogenesis 29: 905-912, 2008.
40. Sharifi N, Hurt EM, Kawasaki BT and Farrar WL: *TGFBR3* loss and consequences in prostate cancer. Prostate 67: 301-311, 2007.
41. Turley RS, Finger EC, Hempel N, How T, Fields TA and Blobe GC: The type III transforming growth factor-beta receptor as a novel tumor suppressor gene in prostate cancer. Cancer Res 67: 1090-1098, 2007.
42. Finger EC, Turley RS, Dong M, How T, Fields TA and Blobe GC: TbetaRIII suppresses non-small cell lung cancer invasiveness and tumorigenicity. Carcinogenesis 29: 528-535, 2008.
43. Gordon KJ and Blobe GC: Role of transforming growth factor-beta superfamily signaling pathways in human disease. Biochim Biophys Acta 1782: 197-228, 2008.
44. Bae HJ, Eun JW, Noh JH, Kim JK, Jung KH, Xie HJ, Park WS, Lee JY and Nam SW: Downregulation of transforming growth factor β receptor type III in hepatocellular carcinoma is not directly associated with genetic alterations or loss of heterozygosity. Oncol Rep 22: 475-480, 2009.
45. Bilandzic M, Chu S, Farnworth PG, Harrison C, Nicholls P, Wang Y, Escalona RM, Fuller PJ, Findlay JK and Stenvers KL: Loss of betaglycan contributes to the malignant properties of human granulosa tumor cells. Mol Endocrinol 23: 539-548, 2009.
46. Liu XL, Xue BX, Lei Z, Yang DR, Zhang QC, Shan YX and Zhang HT: *TGFBR3* co-downregulated with GATA3 is associated with methylation of the GATA3 gene in bladder urothelial carcinoma. Anat Rec (Hoboken) 296: 1717-1723, 2013.
47. Ragnarsson G, Eiriksdottir G, Johannsdottir JT, Jonasson JG, Egilsson V and Ingvarsson S: Loss of heterozygosity at chromosome 1p in different solid human tumours: Association with survival. Br J Cancer 79: 1468-1474, 1999.
48. Florio P, Ciarmela P, Reis FM, Toti P, Galleri L, Santopietro R, Tiso E, Tosi P and Petraglia F: Inhibin α-subunit and the inhibin coreceptor betaglycan are downregulated in endometrial carcinoma. Eur J Endocrinol 152: 277-284, 2005.
49. Cooper SJ, Zou H, Legrand SN, Marlow LA, von Roemeling CA, Radisky DC, Wu KJ, Hempel N, Margulis V, Tun HW, *et al*: Loss of type III transforming growth factor-beta receptor expression is due to methylation silencing of the transcription factor GATA3 in renal cell carcinoma. Oncogene 29: 2905-2915, 2010.