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Expression of TGF-ß signaling proteins in normal placenta and gestational trophoblastic disease

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Summary. The transforming growth factor ß (TGF-ß) is a vital regulator of placental development and functions. TGF-ß exerts several modulatory effects on trophoblast cells, such as inhibition of proliferation and invasiveness, and stimulation of differentiation by inducing multinucleated cell formation. In this study, we determine the expression patterns of TGF-ß signaling molecules in normal trophoblast, various hydatidiform mole types and choriocarcinoma.

A total of 132 cases, including 51 normal placenta (20 first trimester, 11 second trimester, and 20 third trimester) and 81 gestational trophoblastic diseases (17 choriocarcinoma, and 64 hydatidiform moles: 39 complete, 6 partial, and 19 invasive) were immunohistochemically analyzed with anti-TGF ß1/2, TGF-ß receptor type I (TßRI), TßRII, Smad 2/3, and Smad 4 antibodies on paraffin blocks. In the case of normal placenta, maximal levels of all TGF-ß signaling molecules were observed in villous trophoblast in the first trimester, which decreased with gestational age. Expression of all the TGF-ß signaling proteins except Smad2/3, was significantly enhanced in various moles, relative to normal trophoblast. Moreover, TGF-ß signaling molecules were significantly downregulated in choriocarcinoma, compared to moles. In particular, TßRI and Smad2/3 levels were lower in choriocarcinoma than normal villous trophoblast (TßRI: $p<0.025$, Smad2/3: $p<0.001$). In conclusion, the TGF-ß signaling pathway plays an important role in the pathogenesis and progression of gestational trophoblastic disease, and may thus be employed as a potential therapeutic target and a diagnostic biomarker.

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Introduction

The TGF-ß superfamily comprises a large group of extracellular growth factors with key roles in various biological processes, including cell growth, proliferation and differentiation, angiogenesis, apoptosis, and extracellular matrix remodeling (Massague, 1998). TGF-ß is the most potent autocrine negative regulator of proliferation in the majority of epithelial tissues (Massague, 1998). Loss of TGF-ß responsiveness is a crucial event in the development and progression of epithelial malignancies (Filmus and Kerbel, 1990). Binding of TGF-ß to its receptor type II (TßRII) induces phosphorylation of the receptor, in turn phosphorylating TGF-ß receptor type I (TßRI). This stimulates the translocation of downstream Smad proteins from the cytoplasm to the nucleus, where they function as transcriptional regulators (Massague, 1998).

Smad proteins in humans are divided into three general groups: (i) the receptor-activated including Smad2 and Smad3; (ii) the common mediator including Smad4; and (iii) the inhibitory including Smad6 and Smad7. Following receptor-induced activation, phosphorylated Smad2 and Smad3 oligomerize with Smad4 to form the Smad complex, which translocates to the nucleus and binds nuclear DNA, leading to transcriptional activation of the target gene (Massague, 1998).

The placenta performs vital functions as a gas, nutrient and waste exchange barrier between the maternal circulation and the developing fetus. TGF-ß plays a potential role in reproduction (Ingman and Robertson, 2002) as an important factor that moderates

trophoblast invasion into the maternal uterus (Graham and Lala, 1991), and controls differentiation, proliferation, migration and invasiveness of normal human trophoblast cells (Irving and Lala, 1995; Morrish et al., 1998; Caniggia et al., 1999). Additionally, disruption of the TGF-ß signaling pathway may be an important defect in malignant trophoblast lesions such as choriocarcinoma (Xu et al., 2001).

While the functional importance of a TGF-ß signaling pathway in malignant neoplasms of placenta (such as choriocarcinoma) has been established in vitro, no studies have been performed on the expression of TGF-ß signaling molecules in gestational trophoblastic disease tissue, including premalignant and malignant trophoblastic lesions (Tse et al., 2002; Xu et al., 2002, 2003; Rama et al., 2003).

In this study, we conduct a comprehensive and systematic immunohistochemical analysis of the expression patterns of TGF-ß signaling proteins in normal placenta and gestational trophoblastic diseases. Significant alterations in the levels of TGF-ß signaling proteins are observed in premalignant and malignant gestational trophoblastic lesions.

Materials and methods

Patients, tissue samples and reagents

We investigated 132 cases of normal placenta and gestational trophoblastic disease obtained from the surgical pathology files maintained at the Department of Pathology of the Chungbuk National University Hospital and the Samsung Medical Center. The selected cases comprised 17 choriocarcinoma, 64 hydatidiform moles (39 complete, 6 partial, and 19 invasive mole), and 51 normal placenta (20 first trimester, 11 second trimester, and 20 third trimester). The gestational age of moles ranges from 5 to 17 weeks and the mean gestational age is 9.97 weeks. Most molar cases belong to the first trimester and early second trimester. The criteria for defining the invasive moles is the invasion of the villi into the myometrium without intervening decidual tissue as well as the presence of molar villi admixed with proliferating cytotrophoblast and syncytiotrophoblast. Permission from the Ethics Committee was granted from the institutional review board of Chungbuk National University Hospital and the Samsung Medical Center.

Tissue microarray slides were employed to facilitate detection. To prepare for these slides, we punched tissue columns (3.0 mm in diameter) from the original blocks, and inserted them into new paraffin blocks (each containing 30 holes). Consequently, serially sectioned slides were produced. Each microarray tissue slide (1x3 inches) held 30 specimens, allowing simultaneous analysis with minimum variations in the staining process. All specimens were round in shape and 3.0 mm in diameter, thereby providing a sufficient amount of tissue for histopathologic analysis.

Archival materials were routinely fixed in 10% neutral buffered formalin, and embedded in paraffin. Sections (4 µM) were prepared on silane-coated slides (Sigma, St Louis, MO, USA). Immunostaining kits were purchased from DAKO Inc. (Glostrup, Denmark) and Nichirei Inc. (Tokyo, Japan).

Immunohistochemical staining

Tissue sections in microslides were deparaffinized with xylene, hydrated in serial dilutions of alcohol, and immersed in 3% H_2O_2 to quench endogenous peroxidase activity. Sections were microwaved in 40 mM borate buffer (pH 8.2) supplemented with 1 mM EDTA and 1 mM NaCl for 20 min for antigen retrieval (Kim et al., 2004a, b), and incubated with various primary antibodies for 60 min (anti-TGF-ß1/2, anti-TGF-ß RI, anti-TGF-ß RII , anti-Smad 2/3, and anti-Smad 4). The dilution ratios and optimal retrieval buffers of each antibody are summarized in Table 1. Following three successive rinses in washing buffer, sections were incubated with dextran polymer conjugated to peroxidase and goat anti-rabbit Ab (DAKO, Envision plus), and a polymer kit recognizing goat Ab (Nichirei, Histofine kit) for an additional 20 min at room temperature. Slides were washed, and the chromogen developed for 5 min with liquid 3,3'-diaminobenzidine (DiNonA, Seoul, South Korea). Subsequently, slides were counterstained with Meyer's hematoxylin, dehydrated, and mounted with Canada balsam for examination. We used distilled water with 0.1% Tween-20 as rinsing solution (Kim et al., 2003).

Table 1. Antibodies and retrieval buffers.

	Clonality of antibody	Company	Catalogue Number	Dilution ratio	Retrieval buffer
TGF-ß 1/2	Rabbit polyclonal	Santa-Cruz	sc-146	1:120	Borate buffer
TßR-I	Rabbit polyclonal	Santa-Cruz	sc-398	1:80	Borate buffer
TßR-II	Mouse monoclonal	Santa-Cruz	sc-17792	1:30	Borate buffer
Smad 2/3	Goat polyclonal	Santa-Cruz	sc-6033	1:40	Borate buffer
Smad 4	Mouse monoclonal	Santa-Cruz	sc-7966	1:150	Borate buffer

TßR: TGF-ß receptor.

Evaluation of results of immunohistochemical staining data

We applied the scoring method of Sinicrope et al. (1995) for evaluating both the intensity of immunohistochemical staining and the proportion of stained epithelial cells (cytosolic and nuclear stainings were independently analyzed) (Sinicrope et al., 1995). The staining intensity was subclassified as (i) weak, (ii) moderate, or (iii) strong. Positive cells were quantified as a percentage of the total number of epithelial cells, and assigned to one of five categories (0, <5%; 1, 5-25%; 2, 26-50%; 3, 51-75%; and 4, >75%). The percentage of tumor cell positivity and staining intensity were multiplied in order to generate the immunoreactive score (IS) for each of the tumor specimens. Each lesion was separately examined, and scored by two pathologists (X.Y.H & S.H.K). Discrepancies in scores were discussed to obtain a consensus.

Statistical analysis

Statistical analyses were conducted using Fisher's exact tests, Pearson's χ^2 tests, ANOVA, Mann-Whitney, Tukey's HSD, and Duncan's test (as a post hoc test). *P* values less than 0.05 were regarded as statistically significant. All statistical analyses were performed using SPSS software (SPSS, Chicago, USA).

Results

The expressional patterns of the TGF-ß signaling proteins in normal placenta and various gestational trophoblastic diseases, including choriocarcinoma and invasive/partial/complete moles, are depicted in Table 2 and Figure 1. The average intensity (mean of IS (immunoreactivity score)) of immunostaining was analyzed.

TGF-ß1/2 expression

TGF-ß1/2 levels in the chorionic villi of normal placenta were variable depending on the trimesters. Specifically, expression was relatively strong in the first trimester (IS: 2.50±1.25) but very low in the second (IS: 0.90±0.74) and third trimesters (IS: 0.50±0.69) (Table 2, 3). In the first trimester, similar levels of TGF-ß1/2 were observed in the cytoplasm of both syncytiotrophoblasts and cytotrophoblasts (Fig. 1). In the gestational trophoblastic diseases, except choriocarcinoma, TGF-ß1/2 expression was generally upregulated. The protein level in a complete mole (IS: 3.50±1.99) was higher than that in normal placenta ($p<0.025$) (Tables 2 and 3). In choriocarcinoma, TGF-ß1/2 expression was markedly decreased (IS: 1.53±1.33) to a lower level than that in complete and invasive moles (IS: 3.32±1.49) (complete/invasive mole vs choriocarcinoma; $p<0.001$) (Tables 2, 3).

TGF-ß receptor I expression

The TßRI expression pattern in chorionic villi of normal placenta was similar to that of TGF-ß1/2, i.e., variable, depending on the gestational age. Again, TßRI expression was relatively strong in the first trimester (IS: 2.35±1.95) but weak in the second (IS: 0.30±0.48) and third trimesters (IS: 0.15±0.49) (Tables 2 and 3). In the first trimester, the TbRI was detected in the cytoplasm of syncytiotrophoblasts and at higher levels in cytotrophoblasts (Fig. 1). Expression of TbRI in gestational trophoblastic disease was analogous to that of TGF-ß1/2. Specifically, TßRI expression clearly decreased in choriocarcinoma (IS: 1.24±0.90), but significantly increased in other molar lesions (complete/partial/invasive mole, IS: 3.11±1.56 / 4.33±1.86 / 4.11±1.70 respectively), compared to normal placenta and choriocarcinoma (1st trimester vs partial/invasive mole; $p<0.05$, choriocarcinoma vs complete/partial/invasive mole; $p<0.025$) (Tables 2 and 3). Moreover, TbRI expression in choriocarcinoma (IS: 1.24±0.90) was lower than that in the first trimester trophoblast (IS: 2.35±1.95), with statistical significance ($p<0.025$).

3. TGF-ß receptor II expression

Expression of TßRII in chorionic villi of normal placenta was predominant in the first trimester (IS:

Table 2. The mean of IS of TGF signaling molecules in gestational trophoblastic disease and normal placenta.

Diagnosis	TGF-ß1		TßRI		TßRII		Smad 2/3 (N)		Smad 2/3 (C)		Smad 4 (N)		Smad 4(C)	
1st trimester trophoblast	2.50±1.25		2.35±1.95		3.26±1.56		0.94±0.75		3.71±2.05		2.85±1.73		0.75±1.07	
2nd trimester trophoblast	0.90±0.74		0.30±0.48		0.60±0.84		0.18±0.40		1.27±0.47		0.10±0.32		0.10±0.32	
3rd trimester trophoblast	0.50±0.69	$P < 0.001$	0.15±0.49	$P < 0.001$	0.25±0.44	$P < 0.001$	0.00±0.22	$P < 0.001$	0.55±0.69	$P < 0.001$	0.15±0.67	$P < 0.001$	0.00±0.22	$P < 0.001$
Complete mole	3.50±1.99		3.11±1.56		4.87±1.47		0.51±0.69		3.54±1.71		3.87±1.84		1.67±1.44	
Partial mole	3.00±1.41		4.33±1.86		4.60±1.67		1.00±0.71		4.20±1.92		4.20±1.30		2.20±1.79	
Invasive mole	3.32±1.49		4.11±1.70		3.79±1.23		0.67±0.91		4.33±1.91		4.11±2.81		1.28±1.81	
Chorio-carcinoma	1.53±1.33		1.24±0.90		3.79±1.72		0.00±0.25		1.50±1.63		2.79±1.76		1.21±1.42	

Abbreviations: IS, Immunostaining Score; TßR, TGF-ß receptor. Smad2/3(N), nuclear expression of Smad2.3; Smad2/3(C), cytoplasmic expression of Smad2.3; Smad4(N), nuclear expression of Smad4; Smad4(C), cytoplasmic expression of Smad4.

3.26±1.56), but very weak in the second (IS: 0.60±0.84) and third trimesters (IS: 0.25±0.44) (Tables 2 and 3). In the first trimester, equivalent levels of TßRII were observed in the cytoplasm of both syncytiotrophoblasts and cytotrophoblasts (Fig. 1). In villous trophoblasts of various molar lesions, stronger TßRII expression was

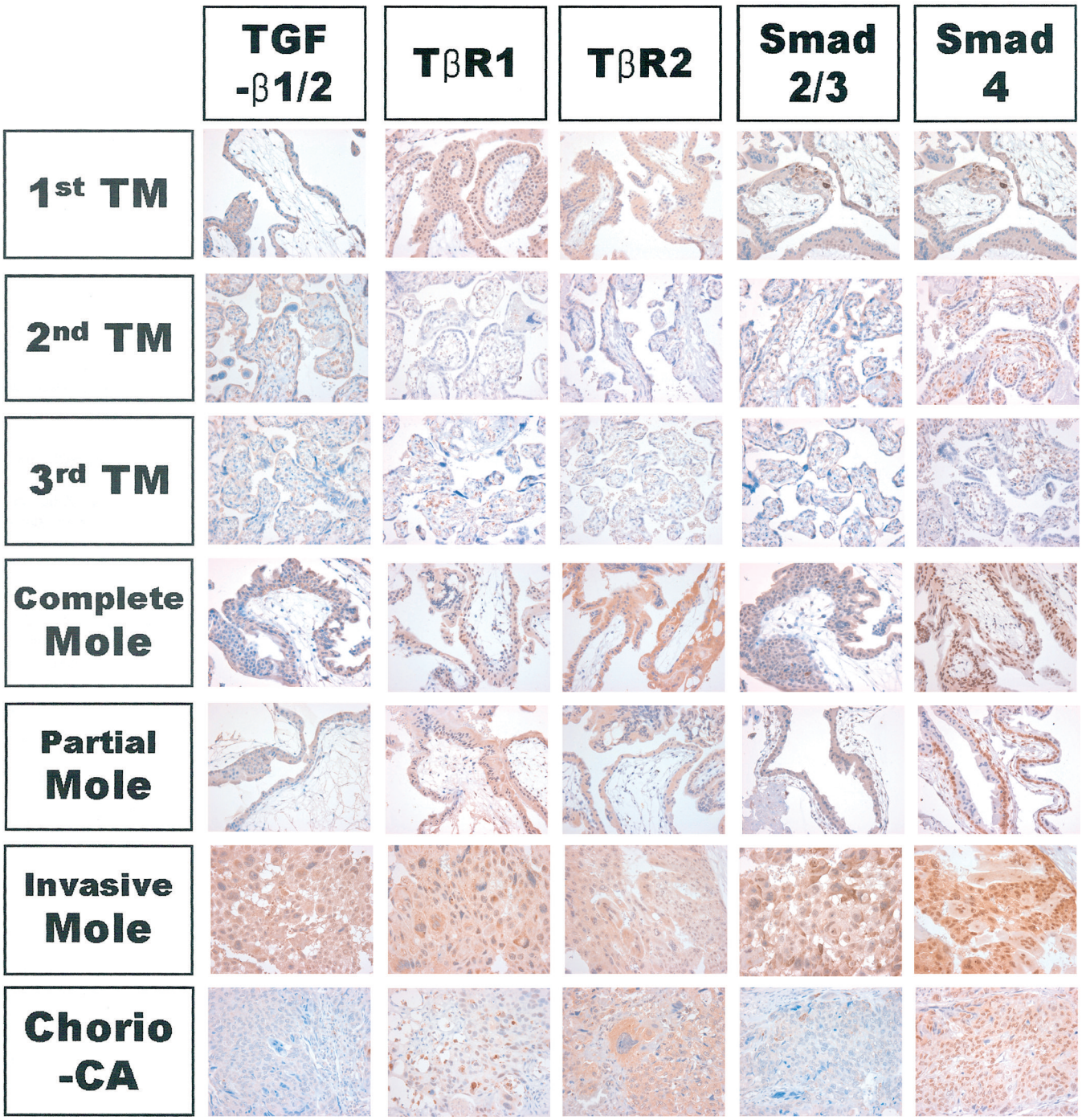

Fig. 1. Immunostaining of the TGF-ß signaling molecules in the normal placenta, and premalignant and malignant tumors of placenta. 1st TM, First trimester placenta; 2nd TM, Second Trimester placenta; 3rd TM, Thrid Trimester placenta; ChorioCA, Choriocarcinoma; TßR, TGFß receptor; Smad2/3(N), nuclear expression of Smad2.3; Smad2/3(C), cytoplasmic expression of Smad2.3; Smad4(N), nuclear expression of Smad4; Smad4(C), cytoplasmic expression of Smad4.

observed. The level in complete/partial moles (IS: 4.87±1.47 / 4.60±1.67 respectively) was significantly higher than that in the first trimester in normal placenta (IS: 3.26±1.56) (p<0.05). However, TbRII expression in choriocarcinoma (IS: 3.79±1.72) was markedly decreased, compared to other precursor lesions (choriocarcinoma vs complete mole; p<0.025) (Tables 2 and 3). No differences in TßRII expressional levels were evident between the choriocarcinoma (IS: 3.79±1.72) and first trimester trophoblasts (IS: 3.26±1.56) (Tables 2 and 3).

Smad2/3 expression

Smad2/3 and Smad4 were expressed in both the cytoplasm and nucleus in contrast to the cytoplasmic localization of other TGF-ß signaling proteins. Cytoplasmic expression of Smad2/3 was restricted to the villous trophoblast in the first trimester (IS: 3.71±2.05), and was very low in second (IS: 1.27±0.47) and third trimesters (IS: 0.55±0.69), with significant differences (p<0.001) (Tables 2 and 3). In the first trimester, similar levels of cytoplasmic Smad2/3 were observed in both syncytiotrophoblasts and cytotrophoblasts (Fig. 1). Smad2/3 in the cytoplasm was generally upregulated in the various molar lesions, except choriocarcinomas analogous to the expression patterns of TGF-ß signaling proteins (Tables 2 and 3). The cytoplasmic expression of Smad2/3 in choriocarcinoma was characteristically lower (IS: 1.50±1.63) than that in all molar lesions (complete/partial/invasive mole, IS: 3.54±1.71 / 4.20±1.92 / 4.33±1.91 respectively) and first trimester trophoblast (IS: 3.71±2.05) (p<0.001) (Tables 2 and 3).

The nuclear expression of Smad2/3 was generally weak in normal placenta, molar lesions, and choriocarcinoma. In the first trimester chorionic villi, the nuclear expression of Smad2/3 was limited to the cytotrophoblast (Fig. 1). Villous trophoblasts of the various moles displayed weak nuclear expression of Smad2/3 (Fig. 1). In choriocarcinomas, no nuclear expression was detected with a few exceptions (Tables 2 and 3).

Smad4 expression

The cytoplasmic expression of Smad4 was generally low throughout in contrast to Smad2/3, which displays high levels in the cytoplasm and low levels in the nucleus. Weak cytoplasmic expression of Smad4 was observed in both villous cytotrophoblasts and trophoblasts in the first trimester (IS: 0.75±1.07) (Fig. 1, Tables 2 and 3). In villous trophoblasts of the complete

Table 3. Summary of results

	1st TM	2nd TM	3rd TM	Complete mole	Partial mole	Invasive mole	Chorio -CA
TGF-ß1/2	+	±	±	++	++	++	+
TßRI	+	±	±	++	+++	+++	±
TßRII	++	±	±	++++	++++	+++	+++
Smad2/3(C)	+++	±	±	+++	+++	+++	±
Smad2/3(N)	±	-	-	±	±	±	-
Smad4(C)	±	-	-	+	+	±	±
Smad4(N)	++	-	-	+++	+++	+++	++

TGF-ß1/2	1st TM vs 2nd & 3rd TM: p<0.01 1st TM vs complete mole: p<0.025 Complete/invasive mole vs choriocarcinoma: p<0.001
TßRI	1st TM vs 2nd & 3rd TM: p<0.001 1st TM vs partial/invasive mole: p<0.05 Complete/partial/invasive mole vs choriocarcinoma: p<0.025 1st TM vs choriocarcinoma: p<0.025
TßRII	1st TM vs 2nd & 3rd TM: p<0.001 1st TM vs complete/partial mole: p<0.05 Complete mole vs choriocarcinoma: p<0.025
Smad2/3(C)	1st TM vs 2nd & 3rd TM: p<0.01 1st TM vs chroicocarinoma: p<0.001 Complete/partial/invasive mole vs choriocarcinoma: p<0.001
Smad2/3(N)	1st TM vs 2nd & 3rd TM: p<0.001 1st TM vs complete mole: p<0.025 Complete/partial/invasive mole vs choriocarcinoma: p<0.025 1st TM vs choriocarcinoma: p<0.001
Smad4(C)	1st TM vs complete/partial mole: p<0.05
Smad4(N)	1st TM vs 2nd & 3rd TM: p<0.001 1st TM vs complete/invasive mole: p<0.025 Invasive mole vs choriocarcinoma: p<0.05

There is no statistical significance in other combinations. Criteria; IS 0 – 0.4 : - IS 0.4-1.5 : ± IS 1.5-2.5 : + IS 2.5-3.5 : ++ IS 3.5-4.5 : +++ IS >4.5 : ++++
Abbreviations: 1st TM, First Trimester placenta; 2nd TM, Second Trimester placenta; 3rd TM, Thrid Trimester placenta; ChorioCA, Choriocarcinoma; TßR, TGF-ß receptor; Smad2/3(N), nuclear expression of Smad2.3; Smad2/3(C), cytoplasmic expression of Smad2.3; Smad4(N), nuclear expression of Smad4; Smad4(C), cytoplasmic expression of Smad4.

and partial moles, the cytoplasmic Smad4 was upregulated (comlete/partial mole, IS: 1.67±1.44 / 2.20±1.79 respectively) to a higher level than that in normal placental trophoblast (1st trimester vs complete/partial mole; $p<0.05$) (Tables 2 and 3).

In the first trimester, nuclear expression of Smad4 in the normal placenta was mainly observed in the cytotrophoblast, with significantly weaker staining in the syncytiotrophoblast (Fig. 1). However, expression was weak or absent in the second and the third trimesters (Tables 2 and 3). In the various molar lesions, nuclear Smad4 was increased (complete/invasive mole, IS: 3.87±1.84 / 4.11±2.81 respectively) to a higher level than that in the first trimester trophoblast (IS: 2.85±1.73) (1st trimester vs complete/invasive moles; $p<0.025$) (Tables 2 and 3). Smad4 expression in choriocarcinoma (IS: 2.79±1.76) was significantly lower than that in invasive mole (IS: 4.11±2.81) analogous to other TGF-ß signaling proteins (choriocarcinoma vs invasive mole; $p<0.05$) (Tables 2 and 3).

Discussion

In this study, we characterize the expression patterns of TGF-ß signaling molecules in normal placenta and various gestational trophoblastic diseases, using immunohistochemical techniques. Our results (summarized in Table 3) indicate that the expression of TGF-ß signaling proteins is extensively altered over the whole normal villous trophoblast-mole-choriocarcinoma sequence of placenta.

To our knowledge, this is the first systematic study on the expression of TGF-ß signaling proteins in molar lesions and choriocarcinomas of the placenta. Expression patterns of TGF-ß signaling proteins are characteristic. Specifically, TGF-ß signaling proteins are decreased in the villous trophoblast of second and third trimester placenta and upregulated in molar lesions but downregulated in choriocarcinoma. In particular, expression of TßRI and Smad2/3, essential mediators of the TGF-ß signaling pathway, is distinctively lower in choriocarcinoma. These results suggest that the TGF-ß signaling pathway is functionally enhanced in molar lesions and inactive in choriocarcinomas.

Several immunohistochemical studies have reported on TGF-ß expression in normal placenta. However, the results do not provide enough evidence of characteristic expression patterns. No study has been reported on TGF-ß expression in molar and choriocarcinoma tissues, apart from some choriocarcinoma-derived cell line-based studies (Xu et al., 2001). Immunohistochemistry and RT-PCR studies by Lysiak et al. (1995), Vuckovic et al. (1992), Ando et al. (1998), Selick et al. (1994), and Graham et al. (1992) disclose expression of TGF-ß 1/2 in the villous trophoblast. Interestingly, Simpson et al.'s report (Simpson et al., 2002) showed negative staining for TGF-ß1/2 in immunohistochemical and Western blotting analyses, but the presence of TGF-ß1 and 2 mRNA by RT-PCR analysis of the same sample. All former studies were performed on a small scale and covered only part of the whole gestational age. The general consensus of these reports is that expression of the TGF-ß1/2 on the villous trophoblast is established and a number of studies demonstrate a decrease in expression in full-term villi (Graham et al., 1992; Vuckovic et al., 1992), consistent with our data.

There is limited information on the expression of other TGF-ß signaling proteins in the tissue of normal placenta, molar lesions, and choriocarcinoma. Most earlier studies were performed using tumorigenic (choriocarcinoma) or non-tumorigenic cell lines. TßRI & TßRII protein expression was reported in trophoblast-enriched primary cultures (Mitchell et al., 1992), and the respective mRNA transcripts were observed in first trimester villous tissue by RT-PCR (Ando et al., 1998). Smad2/3/4 mRNA was detected in normal placental trophoblast cell lines obtained from first trimester human placenta, using Northern blot analysis (Wu et al., 2001). Moreover, Smad2/3/4 mRNA and protein were observed in HTR-8, a normal extravillous trophoblast cell line (Xu et al., 2001). In the choriocarcinoma-derived cell lines, JAR and JEG-3, TGF-ß1 and TGF-ß3 but not TGF-2 transcript were observed. This finding is in marked contrast to normal and premalignant cell lines, HTR-8 and RSVT2/C respectively, which express all three isoforms of TGF-ß (Xu et al., 2001).

While TßRI & TßRII transcripts were evident in both JAR and JEG-3 cell lines, protein levels were not assessed in the former study. All non-malignant and malignant cell lines expressed equivalent levels of Smad2,4,6, and 7 mRNA. However, Smad3 was expressed exclusively in two non-malignant cell lines, HTR-8 and RSVT2/C (Xu et al., 2001). Western blot analysis further confirmed that Smad3 protein was absent in the JAR and JEG-3, but present in the non-malignant cell lines. Smad4 expression was high in premalignant RSCT2/C cells, but very low in JAR and JEG-3 cells (Xu et al., 2001), consistent with our data.

The TGF-ß factors were among the first identified regulators of invasive trophoblast differentiation, since they inhibit proliferation of the first trimester cytotrophoblast (Graham and Lala, 1991). Furthermore, TGF-ß exerts anti-invasive effects on trophoblasts by increasing tissue inhibitors of metalloproteinase (TIMPs) and upregulating plasminogen activator inhibitor-1 (PAI-1), which blocks uPA activity (Graham and Lala, 1991; Graham et al., 1997). The TGF-ß stimulates trophoblast differentiation by inducing the formation of multinucleated cells (Graham et al., 1992). The functionality of Smad proteins has been investigated in HTR-8 and choriocarcinoma cell lines, JEG-3 and JAR (Pollheimer and Knofler, 2005). Upon TGF-ß treatment of HTR-8 cells, Smad3 is phosphorylated and translocates to the nucleus (Xu et al., 2001). Ectopic expression of the Smad3 gene in Smad3-deficient JAR cells restores TGF-ß dependent PAI-1 and TIMP-1 levels but fails to reduce invasion in vitro. This finding suggests that other mechanisms contribute to the

refractoriness of malignant trophoblast cells to both the anti-proliferative and anti-invasive effects of TGF-ß (Xu et al., 2002, 2003). The functional integrity of the Smad proteins was further confirmed in the JEG-3 cells. Expression of Smad2 and Smad4 enhanced the TGF-ß - stimulated luciferase reporter levels, whereas Smad7 inhibited reporter activity (Wu et al., 2001).

About 5-20% of the complete hydatidiform moles proceed to invasive moles or choriocarcinoma. Partial hydatidiform moles can also transform into choriocarcinoma (Seckl et al., 2000). The key steps in the molecular pathogenesis and progression of gestational trophoblastic disease remain to be established. Our data show that all TGF-ß signaling proteins, except Smad2/3, are upregulated in moles, compared to normal villous trophoblasts. Moreover, TGF-ß signaling proteins are characteristically downregulated in choriocarcinoma. This dramatic rise and fall of the elements of the TGF-ß signaling pathway in the normal villous trophoblast-hydatidiform mole-choriocarcinoma sequence is distinctive, and suggests that the TGF-ß signaling is significantly involved in the progression of gestational trophoblastic disease. This finding is, in part, consistent with a previous study by our group demonstrating that Smad2/3 and Smad4 are upregulated in gastric adenoma and downregulated in gastric adenocarcinoma (awaiting submission). While the functional implication of increased TGF-ß signaling molecules in hydatidiform moles remains to be clarified, it may be related to the anti-proliferative activity of TGF-ß signaling. There are numerous proliferation-promoting stimuli associated with the genetic and epigenetic defects of hydatidiform moles. In this molar lesion, strong activation of TGF-ß signaling is required to counterbalance pro-proliferative signals. However, in choriocarcinoma, TGF-ß signaling is disrupted, thus leading to the proliferation and invasion of malignant trophoblast without restriction.

In summary, we demonstrate that expression of the TGF-ß signaling proteins is considerably enhanced in various types of hydatidiform moles, and downregulated in choriocarcinoma. The TGF-ß signaling pathway plays an important role in the pathogenesis and progression of gestational trophoblastic disease, and may thus be exploited as a potential therapeutic target and diagnostic biomarker.

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