Symposium: Endocrine aspects of follicular and oocyte growth

Role of transforming growth factor β in ovarian surface epithelium biology and ovarian cancer

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Abstract

Ovarian cancers arise out of the ovarian surface epithelium (OSE), which is the single layer of epithelial cells covering the ovary. These cells go through repeated cycles of proliferation with the growth and rupture of ovarian follicles. One growth factor involved in the regulation of OSE is transforming growth factor β (TGFβ). The different isoforms of TGFβ (TGFβ1, TGFβ2 and TGFβ3) and its receptor are all present in both OSE and the underlying ovarian surface stroma. The levels of the TGFβ isoforms and receptors are regulated independently of each other in these different ovarian tissues. Observations suggest the existence of multiple autocrine/paracrine TGFβ signalling loops. TGFβ acts to inhibit proliferation of normal OSE and early stage ovarian carcinomas. Conversely, in later stage ovarian cancer the inhibitory actions of TGFβ on epithelial proliferation have been overcome, while TGFβ is able to promote malignant neoplastic behaviours. The regulation of TGFβ signalling by ovarian steroid hormones may be one mechanism by which the OSE responds to cyclic changes in the underlying follicles.

Keywords: *ovary, ovarian cancer, ovarian surface epithelium (OSE), transforming growth factor β*

Introduction

Ovarian cancer is the most lethal of the gynaecological cancers, causing an estimated 13,900 deaths per year in 2002 in the USA (ACS, 2002). Ovarian cancers almost always arise out of the ovarian surface epithelium (OSE), which is the single layer of epithelial cells covering the ovary. These cells go through repeated cycles of proliferation with the growth and rupture of ovarian follicles in order to maintain an epithelial covering over the ovary. There is some correlation between the number of ovulations that a woman experiences and the onset of ovarian cancer. This has led to the idea that the repeated cycles of proliferation and wound healing experienced by the OSE put these cells at risk for transformation and the development of ovarian carcinoma (Murdoch, 1996). The balance between cell proliferation,

apoptosis and quiescence must be tightly controlled in normal OSE and be responsive to the changes in adjacent follicles that occur with the reproductive cycle. One growth factor involved in the regulation of OSE is transforming growth factor β (TGFβ). The autocrine and paracrine signalling roles played by TGFβ in surface epithelium and the underlying ovarian stroma that regulate growth of both OSE and ovarian cancer are the subject of this review.

The TGFβ superfamily of growth factors comprises a large group of related molecules that bind to receptors with serine/threonine kinase activity. In addition to TGFβ, the family includes bone morphogenic proteins (BMP), growth differentiation factors (GDF) and Müllerian inhibitory substance (MIS) (Massague, 1990). TGFβ was originally identified and named as a factor capable of transforming cells in culture to a neoplastic phenotype (de Larco and Todaro, 1978). It has been subsequently characterized as a strong suppressor of growth for epithelial cells (Coffey, 1988; Roberts, 1990). Three major isoforms of TGFβ are found in mammals: TGFβ1, TGFβ2 and TGFβ3 (Massague, 1990). Knockout experiments indicate that the different isoforms are responsible for different physiological functions (Shull *et al.*, 1992; Proetzel *et al.*, 1995; Sanford *et al.*, 1997). TGFβ1, TGFβ2 and TGFβ3 can be differentially expressed in various tissues both during development and in adults (Li and Brooks, 1997; Gupta *et al.*, 1998; Itoh *et al.*, 1998; Chang *et al.*, 1999; Cupp *et al.*, 1999).

TGFβs are secreted from cells as an inactive pro-cytokine and bound in the extracellular matrix with latent transforming growth factor beta binding proteins (LTBP). Enzymatic cleavage of the pro-cytokine releases active TGFβ (Gleizes *et al.*, 1997). All active TGFβ isoforms bind to the same receptor complex. The binding of a TGFβ ligand to TGFβRII activates this receptor subtype (Massague, 1990; Lin *et al.*, 1992; Hu *et al.*, 1998). When activated, TGFβRII heterodimerizes with TGFβRI and then TGFβRI is phosphorylated. Phosphorylated TGFβRI in turn phosphorylates SMAD as effectors of an intracellular signalling pathway. SMAD protein complexes, especially SMAD 2 or 3 with 4, induce changes in transcription of target genes (Miyazono *et al.*, 2001). An example of a target gene is the cyclin dependent kinase inhibitor $p15^{Ink4B}$, which, when activated, leads to growth arrest (Feng *et al.*, 2000; Derynck *et al.*, 2001). When TGFβ is present, the Myc protein repressor is removed from the p15 promoter and the SMAD 2/3/4 complex can interact with the transcription factor Sp1 and DNA in the promoter region and activate p15 transcription (Feng *et al.*, 2000, 2002; Seoane *et al.*, 2001). Other genes whose expression is modulated, either directly or indirectly, by TGFβ include cyclin dependent kinase inhibitor *p21*, tyrosine phosphatase inhibitor *Cdc25A* (Massague *et al.*, 2000), anti-apoptotic *Bcl-2* (Choi *et al.*, 2001) and kit ligand (KL) (Ismail *et al.*, 1999).

Localization of TGFβ and receptors in the ovary

Studies have reported differential TGFβ isoform expression in different compartments of the ovary. In the quail, TGFβ2 is the predominant isoform in stromal/interstitial cells (Van Nassauw *et al.*, 1996). In the hamster TGFβ1 is found in pre-pubertal and adult ovarian interstitium (Roy and Hughes, 1994; Roy *et al.*, 1992). Both TGFβ1 and TGFβ2 were localized to the interstitial cells of the rat ovary (Teerds and Dorrington, 1995). In human ovaries, Chegini and Flanders report that only TGFβ1 is expressed in the stroma, while TGFβ2 is expressed in theca cells of the follicles (Chegini and Flanders, 1992). Secretion of TGFβ by theca cells has also been seen in cattle (Skinner *et al.*, 1987). Human ovaries have also been reported to express TGFβRII in the stromal/interstitial compartment (Roy, 1998). However, the portion of the ovarian stroma that is probably most important for regulation of the OSE is the surface stroma. The stromal cells near the surface of the ovary are morphologically distinct in their pattern and orientation compared with stroma deeper within the ovary (Vigne *et al.*, 1994). In cattle ovaries immunocytochemical analysis and measurements of mRNA levels indicate that TGFβ2, TGFβ3 and TGFβRII are present at higher levels in surface stroma than in deeper stroma (Nilsson *et al.*, 2001). TGFβ1 is also present in surface stroma at levels similar to underlying stroma (Nilsson *et al.*, 2001). Cultured cells from bovine surface stroma have been shown to secrete a substance with TGFβ-like activity (Vigne *et al.*, 1994). In human ovarian surface stroma, all TGFβ isoforms are expressed, as are LTBP, but levels of TGFβRI and TGFβRII expression are low (Henriksen *et al.*, 1995).

The OSE has been shown to express all the components of the TGFβ signalling system: TGFβ1, TGFβ2, TGFβ3, LTBP, TGFβRI and TGFβRII (Henriksen *et al.*, 1995; Nilsson *et al.*, 2001). In bovine OSE the level of expression of TGFβ3 appears higher than that of TGFβ1 or TGFβ2 (Nilsson *et al.*, 2001), while in human OSE the expression of TGFβ1 is comparatively low (Henriksen *et al.*, 1995). Taken together, these data indicate that all the components are available for autocrine and paracrine TGFβ signalling events to occur in the OSE and underlying stroma. The observation that the different isoforms of TGFβ and components of the signalling system can be regulated independently of each other in OSE and stroma suggests that specific TGFβ signalling events are important for the regulation of these tissues.

Actions of TGFβ on OSE

The TGFβ are well known as inhibitors of epithelial proliferation (Coffey *et al.*, 1988; Roberts *et al.*, 1990). Several studies have confirmed that OSE proliferation *in vitro* is inhibited by TGFβ (Berchuck *et al.*, 1992; Ismail *et al.*, 1999; Choi *et al.*, 2001). In addition, TGFβ can inhibit the growth of OSE stimulated to proliferate with transforming growth factor α (TGFα) or epidermal growth factor (EGF) (Vigne *et al.*, 1994; Nilsson *et al.*, 2001).

TGFβs can also affect the ability of OSE to express the growth factor kit ligand (KL). In rat OSE, TGFβ treatment inhibits KL production (Ismail *et al.*, 1999). Since the OSE has no c-kit receptor for KL, but the underlying stroma does, TGFβ may regulate this paracrine signalling pathway from OSE to surface stroma. Conversely, TGFβ treatment of bovine OSE has been shown to stimulate KL and keratinocyte growth factor (KGF) mRNA expression (Nilsson *et al.*, 2001).

One characteristic of neoplastic cells is their ability to invade and grow into an underlying substrate. This capacity can be assayed *in vitro* by measuring the penetration of proliferating

cells into soft agar. Even normal epithelial cells show some invasive behaviour in these assays. However, invasiveness is not a desirable behaviour of OSE cells *in vivo*. TGFβ has been shown to decrease the invasive behaviour of OSE (Rodriguez *et al.*, 2001). The actions of TGFβ on normal OSE are thus to inhibit proliferation, regulate secretion of growth factors and decrease invasiveness. These actions can be thought of as being antagonistic to neoplastic cell behaviours, and TGFβ may play a role in keeping OSE activity within normal bounds.

Actions of TGFβ on tumours

The components of TGFβ signalling are present not only in OSE and stroma but also in the ovarian carcinomas that arise from OSE (Bartlett *et al.*, 1997; Bristow *et al.*, 1999; Jindal *et al.*, 1995). Sometimes specific isoforms or other components are up-regulated in tumours (Gordinier *et al.*, 1999). TGFβ1 and LTBP-1 were found to be increased in more malignant tumours (Higashi *et al.*, 2001). TGFβ receptor levels also have been found to be increased in some tumours (Jindal, 1995; Bristow, 1999). Just as TGFβ can inhibit proliferation of OSE, TGFβ can inhibit cell proliferation of early-stage ovarian carcinoma (Arteaga *et al.*, 1988; Berchuck *et al.*, 1992; Cui *et al.*, 1996; Akhurst and Derynck, 2001; Choi *et al.*, 2001). TGFβ has also been shown to increase apoptosis in early stage tumours by decreasing expression of the anti-apoptotic factor Bcl-2 (Chow *et al.*, 1996).

However, TGFβ acts paradoxically on later-stage, more malignant ovarian cancer cells to either promote tumour growth (Akhurst and Derynck, 2001), or to inhibit the growth to a much lesser degree than with earlier stage cancers (Berchuck *et al.*, 1992; Tsao *et al.*, 1995). TGFβ can also act to promote malignant characteristics in tumours, such as invasiveness and de-differentiation (Cui *et al.*, 1996; Portella *et al.*, 1998; Thiery and Chopin, 1999). How is it that TGFβ can inhibit OSE and early stage carcinoma growth, and also promote growth and malignancy in later stage tumours? Some mechanisms have been investigated. In many tumours the cell cycle genes downstream of TGFβ signalling which would normally be inhibited become constitutively activated. These cell cycle genes have then escaped the inhibitory control of TGFβ signalling (Linardopoulos *et al.*, 1995; Kretzschmar *et al.*, 1999; Kretzschmar, 2000). TGFβ has been shown to promote tumour vascularization (Pertovaara *et al.*, 1994). TGFβ has also been shown to promote tumour invasiveness by increasing matrix metalloproteases (MMP) (Rodriguez *et al.*, 2001) and decreasing tissue inhibitors of matrix metalloproteases (TIMP) (Hagedorn *et al.*, 2001). Similarly, TGFβ increases expression of the invasiveness marker tenascin-c by stroma (Wilson *et al.*, 1999). TGFβ has been shown to decrease the body's anti-tumour immune response. TGFβ decreased the activities of NK cells so as to increase the growth and metastasis of breast cancer cells in nude mice (Arteaga *et al.*, 1993). TGFβ was also able to inhibit neutrophil-mediated rejection of a carcinoma (Chen *et al.*, 1998), and has been demonstrated to suppress the cytotoxic function and activation of T-lymphocytes (Vanky *et al.*, 1997). All these actions of TGFβ would tend to promote tumour activity in later-stage, more malignant ovarian cancers.

TGFβ signalling may regulate OSE in response to ovarian steroids

The OSE must proliferate, become quiescent or even undergo apoptosis depending on the activity of the nearby ovarian follicles. When a follicle grows, the OSE must proliferate to maintain a layer over the ovary. When a follicle ovulates, the OSE must first separate and regress and then rapidly proliferate to cover the developing corpus luteum (CL). Follicle growth, ovulation and CL formation are associated with changes in ovarian steroid levels. Since the OSE responds to TGFβ regulation, it is possible that one way for OSE to be responsive to follicular growth and ovulation is if TGFβ levels

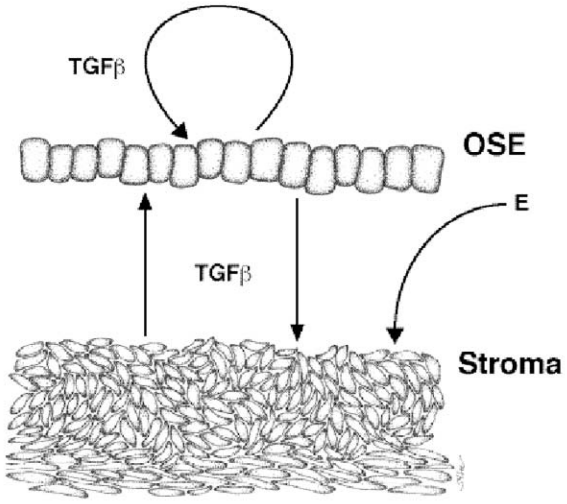

Figure 1. TGFβ signalling pathways affecting normal OSE are illustrated. A diagram of the surface of the ovary is shown, indicating the ovarian surface epithelium (OSE) and the underlying surface stromal cells (Stroma). The OSE and stroma are shown separated from each other to clarify the signalling pathways. Autocrine and paracrine TGFβ signalling interactions are indicated by arrows. In a normal ovary, the effect of TGFβ signalling is primarily to inhibit OSE proliferation. Ovarian steroids such as oestrogen (E) may act to regulate TGFβ expression.

are regulated by ovarian steroids. There is some limited evidence to support this model of ovarian steroids affecting TGFβ expression, which in turn affects OSE activity. In Rhesus macaques treated with oral progestins the expression of TGFβ1 in OSE decreased, while the expression of TGFβ2 and/or TGFβ3 increased. This was associated with an increase in OSE apoptosis (Rodriguez *et al.*, 2002). Perhaps this is one method for progesterone from a newly forming CL to prevent over-proliferation of the OSE following the burst of cell division that occurs when the OSE grows to cover the ovulation wound.

Preliminary data from this laboratory suggest that TGFβ3 expression in bovine surface stroma is regulated by oestrogen concentrations, while at the same time neither OSE nor surface stroma proliferation is directly affected by oestrogen (Nilsson, 2002; unpublished). This again would support a model whereby ovarian steroids regulate TGFβ. This TGFβ may act in a paracrine manner to regulate OSE. Additional research is needed to characterize further the methods by which OSE responds to cyclic changes in the ovary.

It should be noted that the cells of the surface stroma are also likely to be responsive to TGFβ signalling (**Figure 1**). Thecal/interstitial cells have been shown to increase apoptosis in response to TGFβ treatment (Foghi *et al.*, 1997). The surface stroma itself expresses TGFβ receptors (Henriksen *et al.*, 1995; Roy and Kole, 1998; Nilsson *et al.*, 2001). All these data on the localization of TGFβs and their receptors, and the actions of TGFβ signalling on stroma, OSE and ovarian cancers, suggest the existence of multiple autocrine/paracrine TGFβ signalling loops. These signalling pathways are present in normal OSE, in ovarian surface stroma, and in ovarian cancer. In the case of ovarian cancer the inhibitory actions of TGFβ on epithelial proliferation can be overcome, while the ability of TGFβ to promote malignant neoplastic behaviour often remains. Normal OSE proliferative activity depends on a combined input of these TGFβ signalling loops and input from other stimulatory growth factor signalling pathways. The regulation of TGFβ signalling by ovarian steroid hormones may be one mechanism by which the OSE responds to cyclic changes in the underlying follicles. Further research into how TGFβ signalling affects OSE and ovarian cancers may reveal therapeutic targets for future cancer treatments.

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