Growth factors and ovarian cancer

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Introduction

Ovarian cancer is the most common cause of death from gynaecological malignancy developing in about 1 woman in 70 and killing 1 in 100. Annually, it accounts for about 5000 new cases in the UK and 24 000 in the USA, with approximately 3500 and 13 500 deaths respectively in the same period. The growth and progression of this disease is driven by a variety of regulators including growth factors, hormones and cytokines.

Polypeptide growth factors are an important class of signalling molecules which bind to cell surface receptors and initiate intracellular signalling cascades resulting in the activation or repression of specific genes. These events generally result in cell division, but in some cases may lead to growth inhibition. Growth factors (together with cytokines and hormones) regulate and control the growth of normal cells by activating these pathways, when appropriate, for limited periods of time.

In malignant cells, growth factor pathways are commonly dysregulated by oncogene activation or tumour suppressor gene inactivation, leading to continuous signalling. The majority of oncogene products are components of these pathways and include growth factors, growth factor receptors, second messengers and the transcription factors at the ends of these pathways. The changes produced by many oncogenic activations result in a permanent switching on of these pathways without the need for added growth factor and without the ability to ‘switch-off’. Other mechanisms can also lead to an increased dependency on growth factors in cancer cells. Overexpression of a growth factor or its receptor is commonly observed in a tumour, resulting in an increased contribution from that pathway. Malignant cells may produce their own growth factors in an autocrine manner, resulting in autonomy from their local environment and allowing independent growth (Sporn & Roberts 1985). All of these mechanisms have been shown to operate in ovarian cancer cells.

Ovarian cancer cells both express and respond to many types of growth factor. This review will concentrate on those growth factor families which have been most strongly associated with the growth and progression of this disease (Fig. 1).

Growth factor families

Epidermal growth factor-related peptides

Of all the growth factor families, the epidermal growth factor (EGF)-related peptides have been the most extensively studied in ovarian cancer. EGF, a 6 kDa protein, and the related factors transforming growth factor (TGF)-α and amphiregulin bind to, and activate, the EGF receptor. All three factors have been identified in ovarian tumours and cultured ovarian carcinoma cells. TGF-α is reported to be present in 50-100%, EGF in 28-71% and amphiregulin in 18% of malignant ovarian tumours (Kommoss et al. 1990, Morishige et al. 1991, Owens et al. 1991b, Kohler et al. 1992, Stromberg et al. 1994). TGF-α is detected in the sera of 62% of women with ovarian cancers compared with 28% with benign ovarian tumours and 11% of normal female controls (Chien et al. 1997). Similarly, TGF-α is found in the urine of 79% of ovarian cancer patients compared with 17% of patients with benign tumours and 23% of controls (Feldkamper et al. 1994).

In experimental systems, TGF-α and EGF stimulate the growth of ovarian cancer cell lines in vitro, indicating that these factors are mitogenic for this disease (Morishige et al. 1991, Rodriguez et al. 1991, Scambia et al. 1991, Crew et al. 1992, Zhou & Leung 1992). Rat ovarian epithelial cells which have gained the ability to grow in anchorage independent assays often show an increased responsiveness to EGF, suggesting an involvement in tumour progression (Salazar et al. 1995). Antibodies directed against either TGF-α or the EGF receptor can inhibit the proliferation of ovarian cancer cells which both produce TGF-α and possess the EGF receptor, a result suggesting that autocrine growth regulation via a TGF-α/EGF receptor loop is operational in these cells (Kurachi et al. 1991, Morishige et al. 1991, Jindal et al. 1994).

The EGF receptor, a 170 kDa glycosylated membrane-spanning protein, is present in between 33 and 75% of primary ovarian tumours and has been detected by both ligand binding (Bauknecht et al. 1988, Battaglia et al. 1989, Morishige et al. 1991, Owens et al. 1991a, Henzen-Logmans et al. 1992) and immunohistochemical techniques (Berchuck et al. 1991, Morishige et al. 1991, Henzen-Logmans et al. 1992, Owens et al. 1992). Levels of EGF receptor appear to be higher in malignant than
benign tumours or normal ovary, suggesting a possible biological role in malignant progression (Berns et al. 1992, Owens & Leake 1993). Consistent with this, many studies have shown that its presence relates to poor prognosis in malignant tumours (Bauknecht et al. 1988, Battaglia et al. 1989, Berchuck et al. 1991, Scambia et al. 1992).

The EGF receptor (c-erbB-1) is a member of the type I tyrosine kinase growth factor receptor family and shares structural similarities with c-erbB-2, c-erbB-3 and c-erbB-4. The c-erbB-2 (HER-2/neu) protein is a 185 kDa transmembrane protein that is overexpressed in 20-30% of ovarian tumours, primarily as a result of gene amplification (Slamon et al. 1989, Berchuck et al. 1990a). Like the EGF receptor, increased expression of c-erbB-2 is associated with poor survival. In a study of patients undergoing exploration for gynaecological malignancy, biopsies of normal peritoneum revealed a significantly higher median c-erbB-2 expression in patients with ovarian cancer than in patients with benign disease suggestive of altered expression in loco-regional tissues of the peritoneum, perhaps via a paracrine mechanism (Jennings et al. 1994).

The c-erbB-3 receptor is present in the majority of ovarian tumours with 89% of malignant, 100% of borderline and 61% of benign tumours reported positive by immunohistochemical staining (Simpson et al. 1995a). Overexpression seems to be more strongly associated with borderline and early invasive lesions and also with increased grade of differentiation (Simpson et al. 1995a, Rajkumar et al. 1996). The c-erbB-4 receptor shows a more limited expression pattern in this disease, being identified in only 34% of primary ovarian cancers (Langdon et al. 1998). Expression was associated with serous histology, advanced stage and poor survival. Both c-erbB-3 and c-erbB-4 are activated by the heregulin family of growth factors (Holmes et al. 1992) and both heregulin-α and heregulin-β are mitogenic for many ovarian cancer cell lines (Gilmour et al. 1998). Expression of mRNA for a constant region of heregulin is found in 80% of ovarian cancer cell lines indicating the potential of autocrine regulation via heregulin/c-erbB-3 or c-erbB-4 pathways (Gilmour et al. 1998).

Ligand-induced activation of the c-erbB receptors encourages receptor dimerization which in turn initiates a signalling cascade via the ras/mitogen activated protein

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**Figure 1** The network of growth factor interactions in human ovarian cancer. TGF, transforming growth factor; EGF, epidermal growth factor; IGF, insulin-like growth factor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; PDECGF, platelet-derived endothelial cell growth factor; VEGF, vascular endothelial growth factor.
(MAP) kinase pathway, resulting in transcriptional activation. Not only do receptors of the same type produce dimers (homodimerization), but different members of the type I kinase family can interact (heterodimerization), for example experimental evidence demonstrating interactions between the EGF receptor and c-erbB-2 has been obtained in ovarian cancer lines (Marth et al. 1992). Multiple expression of c-erbB receptors is significantly higher in malignant than in borderline or benign ovarian tumours and the formation of ligand-induced c-erbB heterodimers may confer a selective advantage on cells expressing more than one receptor (Simpson et al. 1995b).

The family of type I kinase receptors clearly represents possible targets for therapy and a number of approaches are currently under consideration. Experimental studies using ovarian cancer model systems have demonstrated that targeting the EGF receptor by antibody blockade, antisense knockout of the mRNA or tyrosine kinase inhibition can effectively inhibit growth of ovarian cancer cells which possess this receptor (Simpson et al. 1996, 1998a,b). Combination approaches which target these receptors together with cytotoxic agents are also under consideration, for example the use of the combination of cisplatin and antibodies targeting c-erbB-2. Co-administration of antibody with drug markedly enhanced the cytotoxicity of cisplatin against ovarian cancer models that overexpress this receptor (Hancock et al. 1991). In addition to the antibody blocking a potentially mitogenic signalling pathway, it also appears to enhance the effects of cisplatin in resistant cells, indicating a possible application in chemo-resistant disease (Langton-Webster et al. 1994, Pietras et al. 1994).

Transforming growth factor-β superfamily

The transforming growth factor-β family of polypeptide growth factors are involved in cell growth regulation, tissue remodelling, angiogenesis and immune suppression (Roberts & Sporn 1990). Three forms of TGF-β have been identified in human systems, namely TGF-β1, TGF-β2 and TGF-β3, and these exist as homodimeric chains of between 111 and 113 amino acids, with molecular masses of 25 kDa. These growth factors interact with cell surface serine-threonine kinase linked receptors which mediate their regulatory effects (Massague 1992, Wrana et al. 1994). The TGF-β isoforms bind directly to the TGF-β II receptor, whereupon the type I receptor is recruited into the complex, becomes phosphorylated and, in turn, propagates the signal to downstream substrates (Massague 1992).

TGF-β peptides have been shown to inhibit the growth of normal epithelial ovarian cultures and also the growth of most (95%) ovarian cancer cultures obtained from ascites (Hurteau et al. 1994). The growth of approximately 50% of immortalized ovarian carcinoma cell lines is also inhibited by TGF-β (Berchuck et al. 1990b, 1992, Marth et al. 1990, Bartlett et al. 1992, Jozan et al. 1992). It has been proposed that TGF-β may be an important regulator of normal ovarian epithelium and autocrine growth inhibition may be lost in many ovarian cancer cell lines, perhaps as an early step in the development of some ovarian cancers. Certain ovarian cancers that are growth inhibited by TGF-β are also more prone to undergo apoptosis than normal ovarian epithelial cells (Havrilesky et al. 1995).

In primary ovarian cancer, mRNA for the three isoforms, TGF-β1, TGF-β2 and TGF-β3 has been detected in 46, 66 and 66% respectively of malignant tumours, the predominant pattern of expression being either dual or triple co-expression (Bartlett et al. 1997). The TGF-β II receptor was present in over 90% of samples. Patterns of expression were similar between malignant, borderline and benign tumours. TGF-β3 was associated with advanced stage and reduced survival, suggesting that perhaps the influence of this factor on angiogenesis and other features of tumour progression is more significant than direct inhibitory effects on growth (Bartlett et al. 1997). In support of this, an association between TGF-β expression and features of angiogenesis in ovarian tumours has recently been identified (Nakanishi et al. 1997).

Two other members of the TGF-β superfamily, inhibin and Mullerian inhibiting substance (MIS) have also been studied in ovarian cancer. Inhibin is a polypeptide produced by the granulosa cells of the ovary; its function is to inhibit follicle-stimulating hormone (FSH) secretion by the pituitary gland. Inhibin is produced by all granulosa cell tumours and a positive serum level has been proposed as a marker for this subtype of ovarian cancer in postmenopausal women (Lappohn et al. 1989). Inhibin has also been investigated in two studies of epithelial ovarian cancer (Blakkaer et al. 1993, Cooke et al. 1995). In the first, sera levels of inhibin were elevated in 9 of 29 cases and in the second, in 14 of 24 cases. In the latter study, the survival time of the women with elevated levels of inhibin was 5 times longer than that for women not producing inhibin; FSH levels were also significantly lower in the inhibin-producing patients. These data would be consistent with inhibin acting as a physiological defense mechanism to reduce elevated gonadotrophin levels.

Like inhibin, MIS shares homology with TGF-β at the C-terminal domain (Cate et al. 1986). In the male embryo, MIS causes regression of Mullerian duct tissues that would otherwise develop into the Fallopian tubes, the uterus and upper vagina. Given its normal physiological role, it has been investigated for antitumour efficacy in ovarian tumour models. A limited degree of activity has been demonstrated against ovarian cancer cells grown in...

**Insulin-like growth factors**
The insulin-like growth factors, IGF-I and IGF-II, are an important pair of mitogenic growth factors which show close structural similarity to insulin (Barreca & Minuto 1989). IGF-II is considered the major IGF mitogen in foetal growth, while IGF-I is the more important from birth onwards. The structures of the IGFs are sufficiently similar to insulin that they can influence metabolic activity via the insulin receptor and exert their mitogenic activities via IGF receptors, the IGF type I receptor being the major mediator of IGF activities. These receptors belong to the type II receptor tyrosine kinase class. The IGFs bind to specific carrier proteins, the IGF binding proteins (IGFBPs), when circulating in extracellular fluids (Shimasaki & Ling 1991).

The IGFBPs have important roles in the normal ovary and exert intra-ovarian control in the replication and differentiation processes of folliculogenesis (Adashi et al. 1985, Giordano et al. 1992). In these processes they synergize with gonadotrophins and interact with both thecal and granulosa cells in autocrine and paracrine pathways.

The IGFs, their receptors (insulin, type I and type II receptors) and members of the IGFBP family (IGFBP-2, -3, -4, -5, -6) have been identified in a number of ovarian tumours (Foekens et al. 1990a, b, Beck et al. 1994, Van Dam et al. 1994, Weigang et al. 1994) and in ovarian cancer cell line models (Yee et al. 1991, Krywicki et al. 1993, Resnicoff et al. 1993, Hofmann et al. 1994, Bartlett et al. 1995). IGF-I and insulin, when added to these cell lines stimulate growth and, since both the peptide and receptors are co-expressed, this provides the potential for autocrine control. Consistent with this view, a DNA antisense oligonucleotide targeted to the mRNA for the IGF-I receptor (leading to its degradation) produced growth inhibition in an ovarian cancer cell line (Resnicoff et al. 1993).

A recent study has demonstrated that IGFBP-2 levels are high in the sera of patients with epithelial ovarian cancer, providing a possible tumour marker (Karasik et al. 1994, Flyvberg et al. 1997). Levels of this binding protein are also elevated in malignant ovarian cyst fluid (Karasik et al. 1994). IGFBP-2 mRNA is increased 2- to 30-fold in malignant compared with benign tumours and is also correlated with the aggressiveness of the tumour, being higher in invasive tumours than in those with borderline pathology (Kavet et al. 1996).

**Endothelins**
The endothelins (ETs) comprise a family of three 21 amino acid peptides (ET-1, ET-2 and ET-3) which interact with two populations of receptors, ET_A and ET_B. Although the endothelins were originally recognised as potent vasoconstrictors produced by vascular endothelial cells, both ET-1 and ET-3 have been identified in ovarian cancer cells together with both types of receptor (Bagnato et al. 1995, Moraitis et al. 1997). Addition of ET-1 and ET-2 produced growth stimulation in ovarian cancer cell lines while use of receptor-specific antagonists or receptor-targeted antisense oligonucleotides produced growth inhibition, suggesting the presence of autocrine growth regulation.

**Platelet-derived growth factor**
Platelet-derived growth factor (PDGF) expression is found in 75% of primary ovarian tumours but its expression is undetectable in benign tumours or normal ovaries (Sariban et al. 1988, Henriksen et al. 1993, Versnel et al. 1994). Expression of both the PDGF A- and B-chains has been identified in ovarian cancer cell lines (Versnel et al. 1994). Ovarian cancer patients with tumours expressing the PDGF receptor (a type III receptor tyrosine kinase) demonstrated an overall shorter survival time compared with those whose tumours did not express the receptor (Henriksen et al. 1993). A similar correlation was found in patients with stage III cancer. Only the α form of the receptor was found and the β form could not be detected. The concomitant expression of PDGF and its receptor is related to progression and is suggestive of a functional role of PDGF via autocrine growth stimulation.

**Fibroblast growth factors**
The fibroblast growth factor (FGF) family consists of seven FGF peptides and five receptors (members of the type IV receptor tyrosine kinase family) which possess varying affinities for each ligand. Basic fibroblast growth factor (bFGF) and its receptor are both expressed in ovarian cancer cells while addition of bFGF to cultured cells produces growth stimulation, suggesting that the factor can act in an autocrine manner (Di Blasio et al. 1993, Crickard et al. 1994). Suramin, a known FGF inhibitor, inhibited the proliferation of ovarian cancer cell lines in a manner consistent with the levels of expression of factor and receptor (Crickard et al. 1994). Amplification of several FGF receptors, including FGFR1 (the flg oncogene), FGFR3 and FGFR4, have been demonstrated in ovarian tumours as has one of the FGF ligands, the oncogene int-2. The FGFs stimulate not only mitogenesis but also angiogenesis, which is required for tumour growth beyond about 2 mm.

**Other growth factors**
Other factors produced by ovarian cancer cells, such as platelet-derived endothelial cell growth factor (PDECGF)
and vascular endothelial growth factor (VEGF) are potent angiogenic factors. PDECGF is produced by ovarian cancer cells and a recent study has indicated that increased expression of the factor is associated with areas of high blood velocity in malignant tumours (Reynolds et al. 1994). Malignant tumours express greater quantities of this factor than do benign tumours or normal ovaries (Reynolds et al. 1994, Fujimoto et al. 1998).

VEGF has also been shown to be overexpressed in ovarian carcinomas and is co-expressed with its receptor (Boocock et al. 1995). The factor is produced by tumour cells and accumulates in the stromal matrix. Overexpression of VEGF by tumour cells could, therefore, facilitate growth and invasion, not only indirectly via its effects on endothelial cells, but also directly via tumour cell receptors. High expression is associated with poor survival in both advanced (Hartenbach et al. 1997, Yamamoto et al. 1997) and early stage (Paley et al. 1997) disease.

Endocrine regulation of growth factors in ovarian cancer

While many of these growth factors are likely to be operating under autocrine and paracrine controls, evidence has also been obtained to indicate that endocrine regulation of growth factors may be present in ovarian cancer cells. In breast cancer, a number of growth factors are regulated by oestrogen and these have been proposed to mediate its mitogenic effects (Lippman et al. 1987). These growth factors include TGF-α and IGF-I. Oestrogen-stimulated growth has been demonstrated in several ovarian cancer cell lines and these are characterized by possessing an oestrogen receptor (ER) content greater than 30 fmol/mg protein (Langdon et al. 1990, 1993, 1994). In an ER-positive ovarian cancer model, 17β-oestradiol (E₂) increased levels of TGF-α mRNA (Nash et al. 1989) and this was reflected in increased TGF-α protein secretion (Simpson et al. 1998a). Furthermore, concentrations of EGF receptors are reduced after treatment with E₂. The oestrogen-mediated growth effects could be partially reversed by an antibody targeted to the EGF receptor indicating a participation of this receptor in the oestrogen response (Simpson et al. 1998a). Addition of oestrogen to malignant or benign ovarian tumour tissue increases the release of EGF/TGF-α (Ridderheim et al. 1994). Higher concentrations of TGF-α are found in ER-positive/progesterone receptor-positive primary ovarian tumours, consistent with possible oestrogen regulation (Leake et al. 1994).

Although IGF-I levels appear to be unchanged by E₂, several IGFBPs are modulated in an ER-positive model (Krywicki et al. 1993). Therefore, concentrations of IGFBP-3 are decreased by E₂ while those of IGFBP-5 are increased; other IGFBPs are unaffected. In malignant ovarian cysts oestradiol, IGF-I and IGFBP-2 levels are high, suggesting that regulation of IGF-I and oestradiol might be interactive (Karasik et al. 1994).

Conclusion

It is clear from the above that many growth factor families are involved in the growth and progression of ovarian cancer. This disease is clinically heterogeneous with regard to its patterns of spread and its long term outcome, and some of this variation may be explained by the types and levels of growth factors and their receptors present within the primary tumour and its metastases. Within any single tumour, multiple growth factors are likely to be acting, many in an autocrine manner and some via paracrine and endocrine mechanisms. The expression of growth factors and their receptors may have applications in screening, diagnosis, assessment of prognosis and even monitoring follow-up if the growth factor or its receptor is shed into sera or urine. The observed associations of growth factors and receptors with important clinical features such as survival emphasises their important biological role in this disease. If there is sufficient dependency on a particular pathway this could provide an attractive target for therapeutic intervention. Currently, there is great interest in developing new therapeutic approaches targeting growth factor pathways with many entering clinical study for the first time; hopefully, ovarian cancer will be one of many cancer types amenable to such approaches.

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