

# Growth factors and ovarian cancer

S P Langdon and J F Smyth

ICRF Medical Oncology Unit, Western General Hospital, Edinburgh, EH4 2XU, UK

(Requests for offprints should be addressed to S Langdon)

## 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).

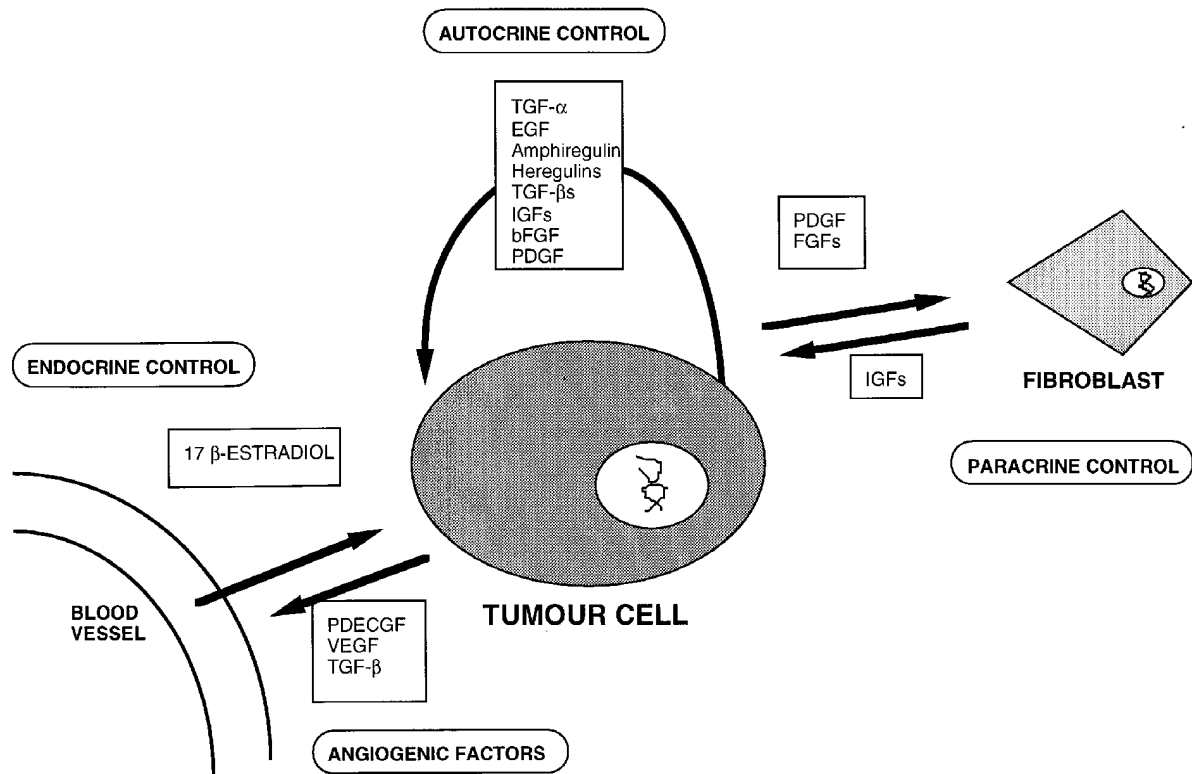
## Growth factor families

### 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)- $\alpha$  and amphiregulin bind to, and activate, the EGF receptor. All three factors have been identified in ovarian tumours and cultured ovarian carcinoma cells. TGF- $\alpha$  is reported to be present in 50-100%, EGF in 28-71% and amphiregulin in 18% of malignant ovarian tumours (Kommos *et al.* 1990, Morishige *et al.* 1991, Owens *et al.* 1991b, Kohler *et al.* 1992, Stromberg *et al.* 1994). TGF- $\alpha$  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- $\alpha$  is found in the urine of 79% of ovarian cancer patients compared with 17% of patients with benign tumours and 23% of controls (Feldkammer *et al.* 1994).

In experimental systems, TGF- $\alpha$  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- $\alpha$  or the EGF receptor can inhibit the proliferation of ovarian cancer cells which both produce TGF- $\alpha$  and possess the EGF receptor, a result suggesting that autocrine growth regulation via a TGF- $\alpha$ /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



**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.

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- $\alpha$  and heregulin- $\beta$  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

(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- $\beta$ superfamily

The transforming growth factor- $\beta$  family of polypeptide growth factors are involved in cell growth regulation, tissue remodelling, angiogenesis and immune suppression (Roberts & Sporn 1990). Three forms of TGF- $\beta$  have been identified in human systems, namely TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3, 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- $\beta$  isoforms bind directly to the TGF- $\beta$  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- $\beta$  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- $\beta$  (Berchuck *et al.* 1990b, 1992, Marth *et al.* 1990, Bartlett *et al.* 1992, Jozan *et al.* 1992). It has been proposed that TGF- $\beta$  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- $\beta$  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- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3 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- $\beta$  II receptor was present in over 90% of samples. Patterns of expression were similar between malignant, borderline and benign tumours. TGF- $\beta$ 3 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- $\beta$  expression and features of angiogenesis in ovarian tumours has recently been identified (Nakanishi *et al.* 1997).

Two other members of the TGF- $\beta$  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 (Blaakaer *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- $\beta$  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

culture and *in vivo* (Donahoe *et al.* 1981, Fuller *et al.* 1982, Wallen *et al.* 1989, Chin *et al.* 1991).

### 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 IGFs 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<sub>A</sub> and ET<sub>B</sub>. 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, Henrikson *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  $\alpha$  form of the receptor was found and the  $\beta$  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 (PDEC GF)

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- $\alpha$  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, 17 $\beta$ -oestradiol (E<sub>2</sub>) increased levels of TGF- $\alpha$  mRNA (Nash *et al.* 1989) and this was reflected in increased TGF- $\alpha$  protein secretion (Simpson *et al.* 1998a). Furthermore, concentrations of EGF receptors are reduced after treatment with E<sub>2</sub>. 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- $\alpha$  (Ridderheim *et al.* 1994). Higher concentrations of TGF- $\alpha$  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 E<sub>2</sub>, several IGFBPs are modulated in an ER-positive model (Krywicky *et al.* 1993). Therefore, concentrations of IGFBP-3 are decreased by E<sub>2</sub> 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.

### References

- Adashi EY, Resnick CE, D'Ercole AJ, Svoboda ME & van Wyk JJ 1985 Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. *Endocrine Reviews* **6** 400-420.
- Bagnato A, Tecce R, Moretti C, Di Castro V, Spergel D & Catt KJ 1995 Autocrine actions of endothelin-1 as a growth factor in human ovarian carcinoma cells. *Clinical Cancer Research* **1** 1059-1066.
- Barreca A & Minuto F 1989 Somatomedins: chemical and functional characteristics of the different molecular forms. *Journal of Endocrine Investigations* **12** 279-293.
- Bartlett JMS, Rabiasz GJ, Scott WN, Langdon SP, Smyth JF & Miller WR 1992 Transforming growth factor- $\beta$  mRNA expression in growth control of human ovarian carcinoma cells. *British Journal of Cancer* **65** 655-660.
- Bartlett JMS, Rabiasz GJ, Scott WN, Langdon SP, Hirst GL, Lee A, Smyth JF & Miller WR 1995 Growth control of human ovarian carcinoma cells by insulin-like growth factors. *Oncology Reports* **2** 857-862.
- Bartlett JMS, Langdon SP, Scott WN, Love S, Miller EP, Katsaros D, Smyth JF & Miller WR 1997 Transforming

- growth factor beta isoform expression in human ovarian tumors. *European Journal of Cancer* **33** 2397-2403.
- Battaglia F, Scambia G & Beneditti Panici P 1989 Epidermal growth factor receptors in gynecologic malignancies. *Gynecology and Obstetric Investigations* **27** 42-44.
- Bauknecht T, Runge M, Schwall M & Pflleiderer A 1988 Occurrence of epidermal growth factor receptors in human adnexal tumors and their prognostic value in advanced ovarian carcinomas. *Gynecologic Oncology* **29** 147-157.
- Beck EP, Russo P, Gliozza B, Jaeger W, Papa V, Wildt L, Pezzino V & Lang N 1994 Identification of insulin and insulin-like growth factor-I (IGF-I) receptors in ovarian cancer tissue. *Gynecologic Oncology* **54** 196-201.
- Berchuck A, Kamel A, Whitaker R, Kerns B, Olt G, Kinney R, Soper JT, Dodge R, Clarke-Pearson DL, Marks P, McKenzie S, Yin S & Bast RC 1990a Overexpression of HER-2/neu is associated with poor survival in advanced ovarian cancer. *Cancer Research* **50** 4087-4091.
- Berchuck A, Olt GJ, Everitt L, Soisson AP, Bast RC & Boyer CM 1990b The role of peptide growth factors in epithelial ovarian cancer. *Obstetrics and Gynecology* **75** 255-262.
- Berchuck A, Rodriguez GC, Kamel A, Dodge RK, Soper JT, Clarke-Pearson DL & Bast RC 1991 Epidermal growth factor expression in normal epithelium and ovarian cancer. *American Journal of Obstetrics and Gynecology* **164** 669-674.
- Berchuck A, Rodriguez GC, Olt GJ, Boente MP, Whitaker RS, Arrick B, Clarke-Pearson DL & Bast RC 1992 Regulation of growth of normal ovarian epithelial cells and ovarian cancer cell lines by transforming growth factor- $\beta$ . *American Journal of Obstetrics and Gynecology* **166** 676-684.
- Berns WMJJ, Klijn JGM, Henzen-Logmans SC, Rodenburg CJ & van der Burg MEL 1992 Receptors for hormones and growth factors and (onco)-gene amplification in human ovarian cancer. *International Journal of Cancer* **52** 218-224.
- Blaakaer J, Micic S, Morris ID, Hording U, Bennett P, Toftager-Larsen K, Djursing H & Bock JE 1993 Immunoreactive inhibin-production in post menopausal women with malignant epithelial ovarian tumors. *European Journal of Obstetrics and Gynecology* **52** 105-110.
- Boocock CA, Charnock-Jones DS, Sharkey AM, McLaren J, Barker PJ, Wright KA, Twentyman PR & Smith SK 1995 Expression of vascular endothelial growth factor and its receptors flt and KDR in ovarian carcinoma. *Journal of the National Cancer Institute* **87** 506-516.
- Cate RL, Mattaliano RJ, Hession C, Tizard R, Farber NM, Cheung A, Ninfa EG, Frey AZ, Gash DJ, Chow EP, Fisher RA, Bertoni JM, Torres G, Wallner BP, Ramachandran KL, Ragin RC, Manganaro TF, MacLaughlin DT & Donahoe PK 1986 Isolation of the bovine and human genes for Mullerian inhibiting substance and expression of the human gene in animal cells. *Cell* **45** 685-698.
- Chien CH, Huang CC, Lin YH, Shen J & Chow SN 1997 Detection of serum transforming growth factor-alpha in patients of primary epithelial ovarian cancers by enzyme immunoassay. *Gynecologic Oncology* **66** 405-410.
- Chin TW, Parry RL & Donahoe PK 1991 Human Mullerian inhibiting substance inhibits tumor growth *in vitro* and *in vivo*. *Cancer Research* **51** 2101-2106.
- Cooke I, O'Brien M, Charnock FM, Groome N & Ganesan TS 1995 Inhibin as a marker for ovarian cancer. *British Journal of Cancer* **71** 1046-1050.
- Crew AJ, Langdon SP, Miller EP & Miller WR 1992 Mitogenic effects of epidermal growth factor and transforming growth factor- $\alpha$  on EGF receptor-positive human ovarian carcinoma cell lines. *European Journal of Cancer* **28** 337-341.
- Crickard K, Gross JL, Crickard U, Yoonessi M, Lele S, Herblin WF & Eidsvoog K 1994 Basic fibroblast growth factor and receptor expression in human ovarian cancer. *Gynecologic Oncology* **55** 277-284.
- Di Blasio AM, Cremonesi L, Viganò P, Ferrari M, Gospodarowicz D, Vignali M & Jaffe RB 1993 Basic fibroblast growth factor and its receptor messenger ribonucleic acid are expressed in human ovarian epithelial neoplasms. *American Journal of Obstetrics and Gynecology* **169** 1517-1523.
- Donahoe PK, Fuller AF, Scully RE, Guy SR & Budzik GP 1981 Mullerian inhibiting substance inhibits growth of a human ovarian cancer in nude mice. *Annals of Surgery* **194** 472-480.
- Feldkamper M, Enderle-Schmitt U, Hackenberg R & Schulz KD 1994 Urinary excretion of growth factors in patients with ovarian cancer. *European Journal of Cancer* **30A** 1851-1858.
- Flyvberg A, Mogenson O, Mogensen B & Nielsen OS 1997 Elevated serum insulin-like growth factor binding protein 2 (IGFBP-2) and decreased IGFBP-3 in epithelial ovarian cancer: correlation with cancer antigen 125 and tumor-associated trypsin inhibitor. *Journal of Clinical Endocrinology and Metabolism* **82** 2308-2313.
- Foekens JA, van Putten WLJ, Portengen H, Rodenburg CJ, Reubi J-C, Berns PMJJ, Henzen-Logmans SC, van der Burg MEL, Alexieva-Figusch J & Klijn JGM 1990a Prognostic value of pS2 protein and receptors for epidermal growth factor (EGF-R), insulin-like growth factor-I (IGF-1-R) and somatostatin (SS-R) in patients with breast and ovarian cancer. *Journal of Steroid Biochemistry and Molecular Biology* **37** 815-821.
- Foekens JA, van Putten W, Portengen H, Rodenburg CJ, Reubi JC, Henzen-Logmans SC, Alexieva-Figusch J & Klijn J 1990b Prognostic value of receptors for epidermal growth-factor (EGF-r), insulin-like growth factor-I (IGF-I-r), and somatostatin (SS-r), and of pS2 protein, in patients with breast and ovarian cancer. *European Journal of Cancer* **26** 154-158.
- Fujimoto J, Ichigo S, Sakaguchi H, Hirose R & Tamaya T 1998 Expression of platelet-derived endothelial cell growth factor (PD-ECGF) and its mRNA in ovarian cancers. *Cancer Letters* **126** 83-88.
- Fuller AF, Guy S, Budzik GP & Donahoe PK 1982 Mullerian inhibiting substance inhibits colony growth of a human ovarian carcinoma cell line. *Journal of Clinical Endocrinology and Metabolism* **54** 1051-1055.
- Gilmour LMR, Macleod KG, Miller WR, Smyth JF & Langdon SP 1998 Mitogenic actions of heregulin isoforms and TGF- $\alpha$  in ovarian cancer models. *British Journal of Cancer* **78** (Suppl 1) 69.
- Giordano G, Barreca A & Minuto F 1992 Growth factors in the ovary. *Journal of Endocrinological Investigations* **15** 689-707.

- Hancock MC, Langton BC, Chan T, Toy P, Monahan JJ, Mischak RP & Shawver LK 1991 A monoclonal antibody against the c-erbB-2 protein enhances the cytotoxicity of cisdiammine-dichloroplatinum against human breast and ovarian tumor cell lines. *Cancer Research* **51** 4575-4580.
- Hartenbach EM, Olson TA, Goswitz JJ, Mohanraj D, Twigg LB, Carson LF & Ramakrishnan S 1997 Vascular endothelial growth factor (VEGF) expression and survival in human epithelial ovarian carcinomas. *Cancer Letters* **121** 169-175.
- Havrilesky LJ, Hurteau A, Whitaker RS, Elbendary A, Wu S, Rodriguez GC, Bast RC & Berchuck A 1995 Regulation of apoptosis in normal and malignant ovarian epithelial cells by transforming growth factor  $\beta$ 1. *Cancer Research* **55** 944-948.
- Henrikson R, Funa K, Wilander E, Backstrom T, Ridderheim M & Oberg K 1993 Expression and prognostic significance of platelet-derived growth factor and its receptors in epithelial ovarian neoplasms. *Cancer Research* **53** 4550-4554.
- Henzen-Logmans SC, Berns EMJJ, Klijn JGM, van der Burg MEL & Foekens JA 1992 Epidermal growth factor receptor in ovarian tumours: correlation of immunohistochemistry with ligand binding assay. *British Journal of Cancer* **66** 1015-1021.
- Hofmann J, Wegmann B, Hackenberg R, Kunzmann R, Schulz K & Havemann K 1994 Production of insulin-like growth factor binding proteins by human ovarian carcinoma cells. *Journal of Cancer Research and Clinical Oncology* **120** 137-142.
- Holmes W, Sliwkowski M, Akita R, Henzel M, Lee J, Park J, Yansura D, Abadi N, Raab H, Lewis G, Shepard H, Kuang W-J, Wood W, Goeddel D & Vandlen R 1992 Identification of heregulin, a specific activator of p185 erbB-2. *Science* **256** 1205-1210.
- Hurteau JA, Rodriguez GC, Whitaker RS, Shah S, Mills G, Bast RC & Berchuck A 1994 Transforming growth factor- $\beta$  inhibits proliferation of human ovarian cancer cells obtained from ascites. *Cancer* **74** 93-99.
- Jennings TS, Dottino PR, Mandeli JP, Segna RA, Kelliher K & Cohen CJ 1994 Growth factor expression in normal peritoneum of patients with gynecologic carcinoma. *Gynecologic Oncology* **55** 190-197.
- Jindal SK, Snoey DM, Lobb DK & Dorrington JH 1994 Transforming growth factor alpha localization and role in surface epithelium of normal human ovaries and in ovarian carcinoma cells. *Gynecologic Oncology* **53** 17-23.
- Jozan S, Guerrin M, Mazars P, Dutaur M, Monsarrat B, Cheutin F, Bugat R, Martel P & Valette A 1992 Transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) inhibits growth of a human ovarian carcinoma cell line (OVCCR1) and is expressed in human ovarian tumors. *International Journal of Cancer* **52** 766-770.
- Karasik A, Menczer J, Pariente C & Kanety H 1994 Insulin-like growth factor-I (IGF-I) and IGF-binding protein-2 are increased in cyst fluids of epithelial ovarian cancer. *Journal of Clinical Endocrinology and Metabolism* **78** 271-276.
- Kavet H, Kattan M, Goldberg I, Kopolovic J, Ravia J, Menczer J & Karasik A 1996 Increased insulin-like growth factor binding protein-2 (IGFBP-2) gene expression and protein production lead to high IGFBP-2 content in malignant ovarian cyst fluid. *British Journal of Cancer* **73** 1069-1073.
- Kohler M, Bauknecht T, Grimm M, Birmelin G, Kommos F & Wagner E 1992 Epidermal growth factor receptor and transforming growth factor alpha expression in human ovarian carcinomas. *European Journal of Cancer* **28A** 1432-1437.
- Kommos F, Wintzer HO, von Kleist S, Kohler M, Walker R, Langton B, van Tran K, Pfliederer A & Bauknecht T 1990 *In situ* distribution of transforming growth factor  $\alpha$  in normal human tissues and in malignant tumours of the ovary. *Journal of Pathology* **162** 223-230.
- Krywicky RF, Figueroa JA, Jackson JG, Kozelsky TW, Shimasaki S, von Hoff DD & Yee D 1993 Regulation of insulin-like growth factor binding proteins in ovarian cancer cells by oestrogen. *European Journal of Cancer* **29A** 2015-2019.
- Kurachi H, Morishige K, Amemiya K, Adachi H, Hirota K, Miyake A & Tanizawa O 1991 Importance of transforming growth factor  $\alpha$ /epidermal growth factor receptor autocrine mechanism in an ovarian cancer cell line *in vivo*. *Cancer Research* **51** 5956-5959.
- Langdon SP, Hawkes MM, Lawrie SS, Hawkins RA, Tesdale AL, Crew AJ, Miller WR & Smyth JF 1990 Oestrogen receptor expression and the effects of oestrogen and tamoxifen on the growth of human ovarian carcinoma cell lines. *British Journal of Cancer* **62** 213-216.
- Langdon SP, Ritchie A, Young K, Crew AJ, Sweeting V, Bramley T, Hillier S, Hawkins RA, Tesdale AL, Smyth JF & Miller WR 1993 Contrasting effects of 17 $\beta$ -estradiol on the growth of human ovarian carcinoma cells *in vitro* and *in vivo*. *International Journal of Cancer* **55** 459-464.
- Langdon SP, Hirst GL, Miller EP, Hawkins RA, Tesdale AL, Smyth JF & Miller WR 1994 The regulation of growth and protein expression by estrogen *in vitro*: a study of 8 human ovarian carcinoma cell lines. *Journal of Steroid Biochemistry and Molecular Biology* **50** 131-135.
- Langdon SP, Macleod KG, Gilmour LMR, Simpson BJB, Miller WR & Smyth JF 1998 Putative roles of c-erbB-3 and c-erbB-4 in ovarian cancer. *Proceedings of the American Association for Cancer Research* **39** 112.
- Langton-Webster BC, Xuan J-A, Brink JR & Saloman DS 1994 Development of resistance to cisplatin is associated with decreased expression of the gp185 c-erbB-2 protein and alterations in growth properties and response to therapy in an ovarian tumor cell line. *Cell Growth and Differentiation* **5** 1367-1372.
- Lappohn RE, Burger HG, Bouma J, Bangah M, Krans M & De Bruijn HWA 1989 Inhibin as a marker for granulosa cell tumours. *New England Journal of Medicine* **321** 790-793.
- Leake R, Barber A, Owens O, Langdon S & Miller B 1994 Growth factors and receptors in ovarian cancer. In *Ovarian Cancer*, vol 3, pp 99-108. Eds F Sharp, P Mason, T Blackett & J Berek. London: Chapman and Hall.
- Lippman ME, Dickson RB, Gelmann EP, Rosen N, Knabbe C, Bates S, Bronzert D, Huff K & Kasid A 1987 Growth regulation of human breast carcinoma occurs through regulated growth factor secretion. *Journal of Cell Biochemistry* **35** 1-16.
- Marth C, Lang T, Koza A, Mayer I & Daxenblicher G 1990 Transforming growth factor- $\beta$  and ovarian carcinoma cells:

- regulation of proliferation and surface antigen expression. *Cancer Letters* **51** 221-225.
- Marth C, Lang T, Cronauer MV, Doppler W, Zeimet AG, Bachmair F, Ullrich A & Daxenbichler G 1992 Epidermal growth factor reduces HER-2 protein level in human ovarian carcinoma cells. *International Journal of Cancer* **52** 311-316.
- Massague J 1992 Receptors for the TGF- $\beta$  family. *Cell* **69** 1067-1070.
- Moraitis S, Langdon SP & Miller WR 1997 Endothelin expression and responsiveness in human ovarian carcinoma cell lines. *European Journal of Cancer* **33** 661-668.
- Morishige K, Kurachi H, Amemiya K, Fujita Y, Yamamoto T, Miyake A & Tanizawa O 1991 Evidence for the involvement of transforming growth factor  $\alpha$  and epidermal growth factor receptor autocrine growth mechanism in primary human ovarian cancers *in vitro*. *Cancer Research* **51** 5322-5328.
- Nakanishi Y, Kodama J, Yoshinouchi M, Tokumo K, Kamimura S, Okuda H & Kudo T 1997 The expression of vascular endothelial growth factor and transforming growth factor-beta associates with angiogenesis in epithelial ovarian cancer. *International Journal of Gynaecological Pathology* **16** 256-262.
- Nash J, Hall L, Ozols R, Young R, Smyth J & Hamilton T 1989 Estrogenic regulation and growth factor expression in human ovarian cancer *in vitro*. *Proceedings of the American Association for Cancer Research* **30** 1189.
- Owens OJ & Leake RE 1993 Epidermal growth factor receptor expression in malignant ovary, benign ovarian tumours and normal ovary: a comparison. *International Journal of Oncology* **2** 321-324.
- Owens OJ, Stewart C, Brown I & Leake RE 1991a Epidermal growth factor receptors (EGFr) in human ovarian cancer. *British Journal of Cancer* **64** 907-910.
- Owens OJ, Stewart C & Leake RE 1991b Growth factors in ovarian cancer. *British Journal of Cancer* **64** 1177-1181.
- Owens OJ, Stewart C, Leake RE & McNichol AM 1992 A comparison of biochemical and immunohistochemical assessment of EGFr expression in ovarian cancer. *Anticancer Research* **12** 1455-1458.
- Paley PJ, Staskus KA, Gebhard K, Mohanraj D, Twigg LB, Carson LF & Ramakrishnan S 1997 Vascular endothelial growth factor expression in early stage ovarian carcinoma. *Cancer* **80** 98-106.
- Pietras RJ, Fendley BM, Chazin VR, Pegram MD, Howell SB & Slamon DJ 1994 Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene* **9** 1829-1838.
- Rajkumar T, Stamp GWH, Hughes CM & Gullick WJ 1996 c-ErbB3 protein expression in ovarian cancer. *Journal of Clinical Pathology: Molecular Pathology* **49** M199-M202.
- Resnicoff M, Ambrose D, Coppola D & Rubin R 1993 Insulin-like growth factor-I and its receptor mediate the autocrine proliferation of human ovarian carcinoma cell lines. *Laboratory Investigations* **69** 756-760.
- Reynolds K, Farzaneh F, Collins WP, Campbell S, Bourne TH, Lawton F, Moghaddam A, Harris AL & Bicknell R 1994 Association of ovarian malignancy with expression of platelet-derived endothelial cell growth factor. *Journal of the National Cancer Institute* **86** 1234-1238.
- Ridderheim M, Stendahl U & Backstrom T 1994 Progesterone and estradiol stimulate release of epidermal growth factor/transforming growth factor alpha by ovarian tumours *in vitro*. *Anticancer Research* **14** 2763-2768.
- Roberts AB & Sporn MB 1990 The transforming growth factor-betas. In *Peptide Growth Factors and Their Receptors*, pp 419-472. Eds MB Sporn & AB Roberts. Heidelberg: Springer Verlag.
- Rodriguez GC, Berchuck A, Whitaker RS, Schlosman D, Clarke-Pearson DL & Bast RC 1991 Epidermal growth factor receptor expression in normal ovarian epithelium and ovarian cancer. 2. Relationship between receptor expression and response to epidermal growth factor. *American Journal of Obstetrics and Gynecology* **164** 745-750.
- Salazar H, Godwin AK, Getts LA, Testa JR, Daly M, Rosenblum N, Hogan M, Ozols RF & Hamilton TC 1995 Spontaneous transformation of the ovarian surface epithelium and the biology of ovarian cancer. In *Ovarian Cancer*, vol 3, pp 145-156. Eds F Sharp, P Mason, T Blackett & J Berek. London: Chapman and Hall.
- Sariban E, Sitaras NM, Antoniades HN, Kufe DW & Pantazis P 1988 Expression of platelet-derived growth factor (PDGF)-related transcripts and synthesis of biologically active PDGF-like proteins by human malignant epithelial cell lines. *Journal of Clinical Investigations* **82** 1157-1164.
- Scambia G, Benedetti-Panici P, Battaglia F, Ferrandina G, Gaggini C & Mancuso S 1991 Presence of epidermal growth factor (EGF) receptor and proliferative response to EGF in six human ovarian carcinoma cell lines. *International Journal of Gynecological Cancer* **1** 253-258.
- Scambia G, Benedetti-Panici P, Battaglia F, Ferrandina G, Baiocchi G, Greggi S, De Vincenzo R & Mancuso 1992 Significance of epidermal growth factor receptor in advanced ovarian cancer. *Journal of Clinical Oncology* **10** 529-535.
- Shimasaki S & Ling N 1991 Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5 and -6). *Progress in Growth Factor Research* **3** 243-266.
- Simpson BJB, Weatherill J, Miller EP, Lessels AM, Langdon SP & Miller WR 1995a c-ErbB-3 protein expression in ovarian tumours. *British Journal of Cancer* **71** 758-762.
- Simpson BJB, Phillips HA, Lessels AM, Langdon SP & Miller WR 1995b c-ErbB growth factor receptors in ovarian tumours. *International Journal of Cancer* **64** 202-206.
- Simpson BJB, Macleod KG, Miller WR & Langdon SP 1996 Antisense oligonucleotide to epidermal growth factor receptor in ovarian cancer. *British Journal of Cancer* **73** Suppl XXVI 62.
- Simpson BJB, Langdon SP, Rabiasz GJ, Macleod KG, Hirst GL, Bartlett JMS, Crew AJ, Hawkins RA, Macineira-Perez PP, Smyth JF & Miller WR 1998a Estrogen regulation of transforming growth factor- $\alpha$  in ovarian cancer. *Journal of Steroid Biochemistry and Molecular Biology* **64** 137-145.
- Simpson BJB, Bartlett JMS, Macleod KG, Rabiasz G, Miller EP, Rae AL, Gordge P, Leake RE, Miller WR, Smyth JF & Langdon SP 1998b Inhibition of TGF- $\alpha$  mediated growth

- effects in ovarian cancer cell lines by a tyrosine kinase inhibitor ZM 252868. *British Journal of Cancer* (In Press).
- Slamon DJ, Godolphin W, Jones LA, Holt J, Wong SG, Keitch DE, Levin WJ, Stuart SG, Udove J, Ullrich A & Press MF 1989 Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244** 707-712.
- Sporn MB & Roberts AB 1985 Autocrine growth factors and cancer. *Nature* **313** 745-747.
- Stromberg K, Johnson GR, O'Connor DM, Sorensen CM, Gullick WJ & Kannan B 1994 Frequent immunohistochemical detection of EGF supergene family members in ovarian carcinogenesis. *International Journal of Gynecological Pathology* **13** 342-347.
- Van Dam PA, Vergote IB, Lowe DG, Watson JV, Van Damme P, Van der Auwera J-C & Shepherd JH 1994 Expression of c-erbB-2, c-myc and c-ras oncoproteins, insulin-like growth factor receptor I, and epidermal growth factor receptor in ovarian carcinoma. *Journal of Clinical Pathology* **47** 914-919.
- Versnel MA, Haarbrink M, Langerak AW, de Laat PAJM, Hagemeyer A, van der Kwast TH, van den Berg-Bakker LAM & Schrier PI 1994 Human ovarian tumours of epithelial origin express PDGF *in vitro* and *in vivo*. *Cancer Genetics and Cytogenetics* **73** 60-64.
- Wallen JW, Cate RL, Kiefer DM, Riemen MW, Martinez D, Hoffman RM, Donahoe PK, Von Hoff DD, Pepinsky B & Oliff A 1989 Minimal antiproliferative effect of mullerian inhibiting substance on gynecological tumor cell lines and tumor explants. *Cancer Research* **49** 2005-2011.
- Weigang B, Nap M, Bittl A & Jaeger W 1994 Immunohistochemical localization of insulin-like growth factor I receptors in benign and malignant tissues of the female genital tract. *Tumor Biology* **15** 236-246.
- Wrana JL, Attisano L, Wieser R, Ventura F & Massague J 1994 Mechanism of activation of the TGF- $\beta$  receptor. *Nature* **370** 341-347.
- Yamamoto S, Konishi I, Mandai M, Kuroda H, Komatsu T, Nanbu K, Sakahara H & Mori T 1997 Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinicopathology and patient survival, and analysis of serum VEGF levels. *British Journal of Cancer* **76** 1221-1227.
- Yee D, Morales FR, Hamilton TC & von Hoff DD 1991 Expression of insulin-like growth factor I, its binding proteins, and its receptor in ovarian cancer. *Cancer Research* **51** 5107-5112.
- Zhou LI & Leung BS 1992 Growth regulation of ovarian cancer cells by epidermal growth factor and transforming growth factor- $\alpha$  and - $\beta$ 1. *Biochimica et Biophysica Acta* **1080** 130-136.