MOLECULAR BASIS OF ANGIOGENESIS IN OVARIAN CANCER

INTRODUCTION

Epithelial ovarian cancer (EOC) is the most common malignancy of the female genital tract in Western countries: 1–2% of all women develop EOC at some time during their lives. Most women with EOC present with peritoneal spread, the principal cause of morbidity and mortality. Owing to the paucity of symptoms and their insidious onset, most patients present with advanced disease, and five year survival rates are around 20%. At present, EOC has by far the worst prognosis of all gynecological cancers and is responsible for half the deaths caused by female genital tract malignancy.

The conventional therapeutic protocols have so far disappointing results. There is a need for new
therapeutic drugs, and the ones, which might target the process of neo-angiogenesis are an interesting prospect. In this light understanding the molecular basis of angiogenesis in ovarian cancer becomes a priority.

**TUMORS AND THEIR VASCULATURE: GROWTH IS ANGIOGENESIS-DEPENDENT**

Angiogenesis, the development of new blood vessels from the existing vasculature, is an essential component of solid tumour growth and metastasis. Several angiogenic factors are expressed by many tumours, suggesting that tumours promote their own vascularization by activating the endothelium of the host.

Embryonic development, reproductive functions (including ovarian cycling), wound healing, rheumatoid arthritis, and tumorigenesis are all proliferative processes that are crucially dependent on the development of a new vascular supply. Angiogenesis is the stimulation of growth of new vascular endothelial cells and the development of new blood vessels (1).

In the absence of blood vessels tumour expansion cannot proceed beyond 1–2 mm³ because tumor proliferation is severely limited by nutrient supply too, and waste removal from the tumor into the surrounding microenvironment. Therefore, angiogenesis is a crucial phenomenon associated with the progression of solid tumours and promotes metastases in many malignant diseases, including EOC (2).

The formation of the vascular stroma plays an important role in the pathophysiology of malignancy(3). In the absence of vascular support tumors become necrotic, and individual tumor cells undergo apoptosis. The onset of angiogenesis marks a phase of rapid proliferation, local invasion, and ultimately metastasis, although angiogenesis can also play a role in pre-malignant lesions .

Vascularization is a prerequisite for tumor cells to spread by shedding into the circulation. The newly formed, immature and leaky vessels aid the process of metastasis because their basement membranes are discontinuous, allowing greater accessibility for stray tumor cells.

**VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)**

In last years, much progress has been made in the identification of positive and negative regulators of angiogenesis. Most notably, vascular endothelial growth factor (VEGF) is widely distributed and has been shown to play a coordinated role in endothelial cell proliferation and assembly of the vessel wall in a variety of normal and abnormal circumstances (4). There are now five members of the VEGF family, in addition to four members of the angiopoietin family and at least one member of the ephrin family of regulators (5); they must all work in a complementary and coordinated manner to form functional vessels.

In addition, many other growth factors that are not vascular endothelium specific are also required for blood vessel formation, such as members of the platelet derived growth factor and transforming growth factor (TGF) families; these factors also have crucial roles in many other systems.

By virtue of its permeability inducing properties a central role for VEGF/VPF in tumour stroma generation has been suggested (6). In addition, VEGF can act as a specific mitogen for a variety of endothelial cells in vitro and as an angiogenic molecule in vivo. VEGF is a potent and very specific mitogen for vascular endothelial cells. It stimulates the full cascade of events required for angiogenesis both in vitro and in vivo, and greatly augments the permeability of the existing microvessels.(7). It is a potent multifunctional cytokine that exerts several potentially independent actions on the vascular endothelium, including endothelial mitogenesis, permeability, vascular tonus, the production of vasoactive molecules, and the stimulation of monocytes chemotaxis. VEGF also functions as a potent pro-survival (anti-apoptotic) factor for endothelial cells in newly formed vessels, and this may be one of its most important functions.

Several studies have now shown that VEGF is over expressed in a variety of tumors including those of the breast, ovary, bladder, vulva, uterus and cervix. (8). VEGF values are often raised, and blocking its activity, for example, by specific neutralizing antibodies to VEGF or to VEGF receptors (VEGFR) expressed by “activated” endothelial cells, can inhibit experimental tumor growth in vivo, but not in vitro. Thus, tumor cells can “feed” (induce) new blood vessels by producing VEGF which, in turn, can nourish the tumor cells, an insidious and self perpetuating paracrine loop. The possibility of therapeutic disruption of this loop has stimulated an intense search in the biotechnological and pharmaceutical industries, in addition to academic centers, for agents such as anti-VEGF antibodies, VEGF–toxin conjugates, aptamers, and small molecule VEGFR antagonists.

Recent observations have identified a group of several growth factors, the VEGF family, which interact with
different receptors to induce endothelial mitogenesis. The most important member of the group is VEGF itself (VEGF-A). In addition to VEGF, the family currently includes: VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factors PI GF-1 and PI GF-2.

The various members of the VEGF family have overlapping abilities to interact with a set of cell surface receptors that trigger responses to these factors. These receptors are involved in initiating signal transduction cascades in response to the VEGF and PIGF proteins. They comprise a family of closely related receptor tyrosine kinases consisting of three members now termed VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR-2 mediates the major growth and permeability actions of VEGF, whereas VEGFR-1 may have a negative role, either by acting as a decoy receptor or by suppressing signalling through VEGFR-2. VEGFR-3 may be important for blood vessel development, but is unique among the VEG receptors because it is also expressed on lymphatic vessels, and may therefore have an important role in lymphangiogenesis.(9)

Another factor that has been shown to stimulate angiogenesis is platelet derived endothelial cell growth factor (PD-ECGF). Originally isolated from platelets, this protein promotes cell growth and chemotaxis in endothelial cells in vivo and angiogenesis in vitro. It has been found in various tissues such as the placenta, lung, endometrium, and ovary, in addition to certain cancer tissues. The enzyme thymidine phosphorylase (TP), which catalyses the reversible phosphorylation of thymidine to thymidine deoxyribose 1-phosphate, has been shown to be homologous to PD-ECGF.

Angiopoietin 1 (Ang-1) and Ang-2 are growth factors that are ligands for the “ties”, a family of receptor tyrosine kinases that are selectively expressed within the vascular endothelium, as are the VEGF receptors. Although both Ang-1 and Ang-2 bind tie-2, Ang-1 functions as an agonist whereas Ang-2 behaves as an antagonist at this receptor (10). Indeed, Ang-2 can cause the regression of newly formed vessels by stimulating endothelial cell apoptosis, unless VEGF is present, in which case the two collaborate to promote angiogenesis.

Numerous ephrin ligands (for example, ephrin A1, B1, and B2) bind to the Eph receptor tyrosine kinases; these comprise the largest known family of growth factor receptors and include EphA2, EphB2, EphB3, and EphB4.26 Recent knockout studies have suggested key roles for ephrin B2 and its EphB4 receptor during vascular development. Mouse embryos lacking ephrin B2 and EphB4 suffer fatal defects in early angiogenic remodelling that are somewhat reminiscent of those seen in mice lacking Ang-1 or tie-2.

In adult settings of angiogenesis, as in tumours or in the female reproductive system, the endothelium of new vessels strongly re-expresses ephrin B2, suggesting that ephrin B2 may be important in these angiogenic settings. The various members of this family appear to regulate the interactions between arterial and venous endothelial cells, as reviewed by other authors.(11)

The expression of this factors is largely restricted to steroidogenic glands, such as ovary, testis, adrenal cortex, and placenta. Although these proteins show no structural homology with the VEGF family, they display several striking biological similarities to VEGF: they induce endothelial proliferation and migration, they have the ability to induce fenestration in capillary endothelial cells derived from endocrine glands, and they are regulated by hypoxia (12).

The process of angiogenesis in adult neovascularisation, including tumour formation, is currently understood as follows (13): angiogenesis is primarily mediated by VEGF, which drives endothelial cell proliferation, migration, and tube formation. Subsequently, Ang-1, in physiological situations, leads to vessel maturation and stabilization. However, such stabilized vessels can be destabilized by Ang-2 and, in the presence of VEGF, a new round of angiogenesis can begin; in the absence of VEGF, vessel regression would ensue. The balance of at least two biological systems (VEGF–VEGFR and Ang–tie) along with the natural angiogenic inhibitors regulate the outcomes of vessel formation and vessel regression, and these complexities must be taken into account when designing and developing anti-angiogenic agents. Until recently, vascularization of malignant tumors was considered the exclusive result of directed capillary ingrowth (endothelial sprouting). However, recent advances have been made in identifying the processes involved in angiogenesis and vascular remodeling.

Tumor vasculature is not necessarily derived from endothelial cell sprouting, instead, cancer tissue can acquire its vasculature by co-option of pre-existing vessels, intussusceptive microvascular growth and postnatal vasculogenesis

Consequently, the simple model of an invading capillary sprout has been deemed insufficient to describe the entire spectrum of morphogenic and molecular events required to form a neo-vascular network. Before discussing the different ways a tumor is vascularized, we should emphasize that these mechanisms are not mutually exclusive; in fact, in most cases they are
interlinked, participating concurrently in physiological as well as in pathological angiogenesis. Although the various types of cancer vascularization share some molecular features and may be controlled in part by similar sets of regulatory factors, a considerable variety of differences also exists. Although the molecular regulation of endothelial sprouting has been extensively studied and reviewed in the literature, the morphogenic and molecular events associated with alternative cancer vascularization mechanisms are less understood.

The best-known mechanism by which tumors promote their own vascularization is inducing new capillary buds from pre-existing host tissue capillaries. The first description of this process dates back to the 1970s, when Ausprunk and Folkman suggested the following sequence or tumor-induced capillary sprouting:

1) The basement membrane is locally degraded on the side of the dilated peritumoral postcapillary venule situated closest to the angiogenic stimulus, interendothelial contacts are weakened, and endothelial cells (ECs) emigrate into the connective tissue, towards the angiogenic stimuli.

2) There is formation of a solid cord by ECs succeeding one another in a bipolar fashion.

3) Lumen formation occurs by cell-body curving of a single EC or by participation of more ECs in parallel with the synthesis of the new basement membrane and the recruitment of pericytes/mural cells.

The main disadvantages of this model are its inability to identify the nature and origin of the stimulus necessary for lumen formation and the assumption that dedifferentiation and redifferentiation take place during the same process, manifest in the loss and regaining of luminal-basal EC polarity. Furthermore, although it has been well established that the stimulus necessary for lumen formation comes from the developing basement membrane, according to this model, basement membrane deposition occurs after lumen formation. In the early 1990s, a different sprouting model was describe. This model suggests a three-stage sequence to explain ultrastructural changes during tumor-induced endothelial sprouting:

1) There is structural alteration of the basement membrane characterized by the loss of electron density (gel-sol transition) over the entire circumference of the dilated “mother vessel” (although basement membrane components such as laminin and collagen IV can still be detected by imuno-histochemistry).

2) Further migration of ECs, which are arranged in parallel, maintaining their basal-luminal polarity and forming a slit-like lumen, takes place continuously with the lumen of the mother vessel and sealed by intact inter-endothelial junctions. Basement membrane of low electron density is deposited continuously by the polarized ECs while only the very tip of the growing capillary bud is free of basement membrane material.

3) Proliferating pericytes of the mother vessel migrate along the basement membrane of the capillary bud, resulting in complete pericyte coverage of the new vessel. In parallel, the appearance of electron-dense basement membrane around the maturing capillary buds (sol-gel transition) can be observed. According to the above model, no stimulus is necessary for the induction of lumen formation, because ECs do not lose their polarity during the process.

These complexe mechanisms are yet to be completely understood, but they represent the starting point for new and more effective therapy for ovarian cancer.

The high mortality rate of ovarian cancer results predominantly from the occult progression of the tumour within the peritoneal cavity, with the initial diagnosis usually only being made at an advanced stage. Modifications in chemotherapy and/or surgery are unlikely, in the near future, to improve the poor prognosis associated with this disease. An improved understanding of the mechanisms regulating the growth of EOC cells may eventually lead to techniques that facilitate early diagnosis, establish the prognosis, or determine the response to treatment. Eventually, it may even be possible to design effective target treatments that will work by interfering with the biochemical processes that govern the growth of EOC cells.
References: