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Review Article

Tumour-induced Angiogenesis: A Review

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Angiogenesis, the formation of new blood vessels, has become a broad subject and is a very active area for current research. This paper describes the main biological events involved in angiogenesis and their importance in cancer progression. In the first section, a fundamental overview of tumour biology is presented. In the second section, the biology of healthy blood vessels is described and, in the third section, the mechanisms of cell migration and proliferation, which are crucial to angiogenesis, are discussed. In the fourth section, a detailed account of tumour-induced angiogenesis is given, whilst the pro- and anti-angiogenic factors involved are reviewed in the fifth section. Finally, the processes of tumour invasion and metastasis are examined in the sixth section.

Keywords: Tumour biology; Angiogenesis; Invasion and metastasis

FUNDAMENTALS OF TUMOUR BIOLOGY

The most common cause of primary tumours is the genetic mutation of one or more cells, resulting in uncontrolled proliferation. The mutated cells have a proliferative advantage over neighbouring, healthy cells and are able to form a growing mass. The reason for this advantage is not necessarily an increase in the proliferation rate, but may, in some cases, be a decrease in the cell death rate (King, 1996). For example, one of the key functions of tumour7 suppressor genes such as p53 is to induce apoptosis (programmed cell death) in damaged cells (Santini *et al.*, 2000). Loss of p53, the most commonly mutated gene in human tumour cells (Santini *et al.*, 2000), thus allows propagation of damaged DNA (King, 1996).

If the mutated cells remain contained within a single cluster, with a well defined boundary separating them from neighbouring normal cells, the tumour is said to be benign, and surgical removal will often provide a complete cure. However, if the tumour cells are inter-mixed with normal cells and attempt to invade the surrounding tissue, the growth ceases to be contained and the tumour is described as malignant (Alberts *et al.*, 1994). Figure 1 shows schematically the difference between a benign and a malignant tumour; only a malignant tumour constitutes a cancer. In this case, surgery is not guaranteed to be successful because the tumour does not possess a well defined boundary and the presence of a single mutated cell could be enough to regenerate the tumour colony (King, 1996). Departure of mutated cells from the primary site represents the transition from *in situ* to invasive growth and is a key event in cancer progression. Subsequent entry of tumour cells into the bloodstream or lymphatic system allows access to remote parts of the body and may lead to the formation of secondary tumours (metastases) (Schirrmacher, 1985), making treatment very difficult.

Cancers are categorised by the cell type from which they arise. Those arising from epithelial cells (cells covering the external surface of the body and lining internal cavities) are called carcinomas and are by far the most common form of cancer. Those arising from muscle cells or connective tissue are called sarcomas, whilst cancers arising from haemopoietic cells (precursors of all blood cells) are called leukaemias.

Tumours are additionally classified by their tissue of origin (for example, carcinomas may originate in the breast, skin, lung, colon and so on) and cancers originating in different tissue or cell types generally behave very differently. Indeed, it is often said that cancer is not, in reality, one disease, but a class of different diseases with the common features of excessive cell proliferation and tissue invasion (King, 1996). It is the combination of these features that makes cancer so dangerous: a single mutated cell which does not have a proliferative disorder is

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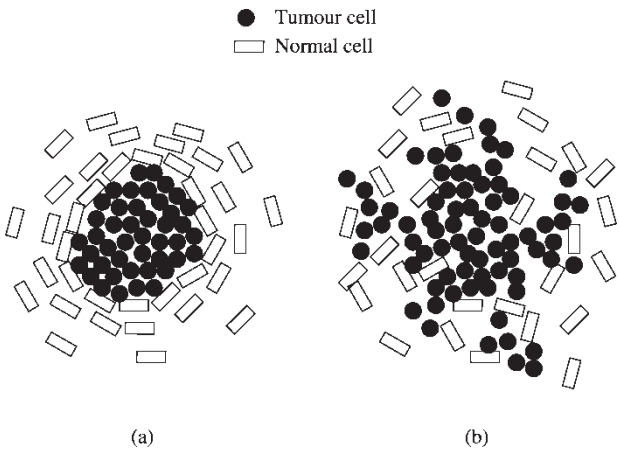

FIGURE 1 A schematic diagram of: (a) a benign tumour, (b) a malignant tumour.

harmless; likewise, a population of abnormally proliferating cells that does not invade surrounding tissue is easily treatable (Alberts *et al.*, 1994).

Tumour cells typically form a contiguous growing cluster, which is reliant on passive diffusion for the supply of oxygen and nutrients and the removal of waste products (Sutherland, 1988). The tumour's need for nutrients grows in proportion to its volume, but its ability to absorb diffusing substances from the surrounding tissue is proportional to its surface area. This imposes a maximum size to which the tumour can grow before it experiences nutrient deficiency: some of the tumour cells (usually those towards the centre of the tumour, where nutrient levels are at their lowest) will not have sufficient nutrients to continue to proliferate and will become quiescent. If the nutrient supply is not improved, necrosis (cell death caused by insufficient nutrition or injury) will set in, leading to the development of a necrotic core of dead cells (Sutherland, 1986). The tumour thus develops a three-layer structure: a necrotic core, surrounded by a layer of quiescent cells, which is in turn surrounded by a thin proliferating rim (Folkman and Hochberg, 1973).

The existence of the quiescent layer presents a problem for treatments, such as chemotherapy, that are based on the intravenous administration of an agent that is toxic to proliferating cells (King, 1996). The proliferating rim may be eradicated, but the cells underneath will not be affected and will emerge from quiescence to become proliferating cells (Sutherland, 1988). Moreover, use of an agent that is also effective against quiescent cells may not be an improvement because these cells do not have good access to substances in the circulation (this is the very reason they are quiescent) and most of the drug will be absorbed by the proliferating cells (King, 1996). Continued administration of such drugs is not possible due to the side-effects, so there is nothing to prevent quiescent cells re-establishing themselves as a viable proliferating rim (Sutherland, 1988).

A tumour may persist in a diffusion-limited state, usually not more than 2 mm in diameter (Folkman, 1971), with cell proliferation balanced by cell death, for many months or years. It rarely causes significant damage in this dormant phase, and often goes undetected.

A tumour may, however, emerge from dormancy by inducing the growth of new blood vessels, a process termed angiogenesis, or neovascularisation (Folkman, 1971) (see the 'Tumour-induced Angiogenesis' section). This process allows the tumour to progress from the avascular (lacking blood vessels) to the vascular (possessing a blood supply) state. There are a large number of pro-angiogenic and anti-angiogenic factors, some of which are produced by the tumour, some of which are produced by host cells in response to the tumour, and some of which are present in normal tissue (Carmeliet and Jain, 2000). It is a shifting of the balance from the anti- to the pro-angiogenic factors (the so-called 'angiogenic switch') that causes the transition from the dormant to the angiogenic phase (Hanahan and Folkman, 1996). This switch is a highly complex process, which is not yet fully understood, but hypoxia (oxygen deficiency) in the tumour is thought to be an important factor, stimulating production of pro-angiogenic molecules by the tumour cells (Shweiki *et al.*, 1992).

Angiogenesis greatly improves the tumour's blood supply, providing it with an almost unlimited supply of oxygen and nutrients and a system for the removal of waste products, thus permitting rapid growth (Muthukkaruppan *et al.*, 1982). In addition, the proximity of large numbers of blood vessels increases the likelihood of tumour cells entering the bloodstream and being transported to remote parts of the body (Schirrmacher, 1985). This is very dangerous as they can then establish secondary tumours, making successful clinical intervention much more difficult. The more malignant the tumour, the greater its angiogenic potential. Highly malignant tumours are able to induce robust angiogenesis almost indefinitely and, if not successfully treated, will certainly prove fatal (Paweletz and Kneirim, 1989). Figure 2 shows a schematic diagram of a vascularised tumour.

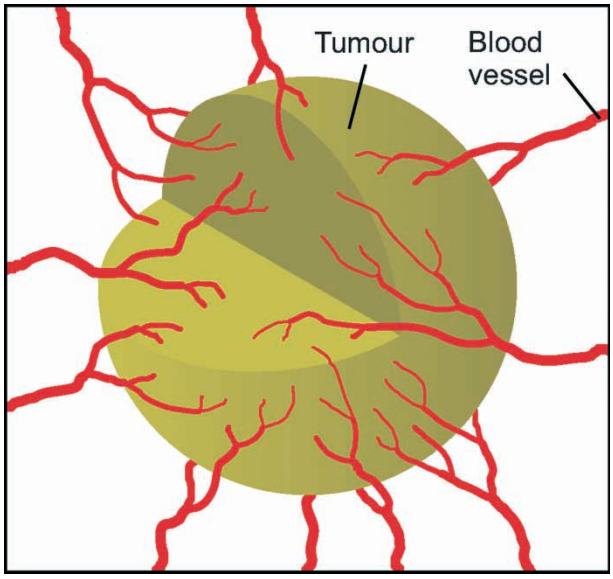

FIGURE 2 A schematic diagram of a vascularised tumour.

Angiogenesis provides the crucial link between the avascular and vascular states and, as such, is a key event for sustained tumour growth and cancer progression (Folkman, 1971). This has raised hope of finding a cancer therapy based on anti-angiogenesis, keeping the tumour in the avascular state, in which it is usually harmless.

BIOLOGY OF THE HEALTHY VASCULATURE

The most essential component of blood vessels is the endothelial cell (EC). Every vessel, from the aorta down to the smallest capillaries, consists of a monolayer of EC (called the endothelium), arranged in a mosaic pattern around a central lumen, through which blood can flow (Fig. 3a). In the smallest vessels, a cross-section of the endothelium may consist of a single EC, which has wrapped around to form a lumen (Fig. 3b). The endothelium controls the passage of nutrients, white blood cells and other materials between the bloodstream and the tissues (Alberts *et al.*, 1994). The healthy endothelium represents a highly stable population of cells: cell–cell connections are tight and the cell turnover period is measured in months or years (Han and Liu, 1999).

Outside the endothelium is an extracellular lining called the basement membrane, separating the EC from the surrounding connective tissue. This is composed of protein fibres, mainly laminin and collagen (Alberts *et al.*, 1994), and may also contain peri-endothelial support cells. These are pericytes in the microvasculature (capillaries) and smooth muscle cells in larger vessels. The basement membrane serves as a scaffold on which the EC rest and helps to maintain the endothelium in its quiescent state (Paweletz and Kneirim, 1989). Cell–cell contacts and cell–basement membrane contacts, mediated by adhesion molecules (such as cadherins and integrins, respectively), are extremely important and loss of either or both can lead to local destabilisation of the endothelium and EC apoptosis (Lobov *et al.*, 2002). The peri-endothelial cells play a particularly important role in maintaining blood vessels in the stable state, and may be involved in the regulation of blood flow (Hirschi and D'Amore, 1996).

Larger vessels have a thick wall of smooth muscle outside the basement membrane, whereas capillaries consist only of the endothelium, basement membrane and pericytes. We are primarily concerned with capillaries (the microvasculature), as opposed to larger vessels, since it is the former that are involved in angiogenesis; the latter can form only via remodelling of the microvasculature following endothelial branching and tube formation.

Separating the vessel from the functional tissue of an organ (the parenchyme) is a layer of connective tissue (the stroma). This is composed of stromal cells, principally fibroblasts, which secrete a matrix of extracellular protein fibres, such as collagen and fibronectin (Alberts *et al.*, 1994).

The formation of blood vessels can be divided into two separate processes. Vasculogenesis is the *in situ* differentiation of endothelial cells from haemangioblasts (precursors of EC) and their subsequent organisation into a primitive vascular network. Angiogenesis is the sprouting, splitting and remodelling of existing vessels (Han and Liu, 1999). Vasculogenesis is confined to early embryonic development and is responsible for the formation of the primary vasculature, including the main vessels of the heart and lungs (Patan, 2000). Angiogenesis subsequently extends the circulation into previously avascular regions by the controlled migration and proliferation of EC (Risau, 1997).

CELL PROLIFERATION AND MIGRATION

In quiescent endothelia, EC proliferation and migration are minimal, but it is vital that EC retain the ability to perform these functions relatively rapidly should the need arise, for example, in case of tissue damage. In particular, EC proliferation and migration are crucial for angiogenesis. The following is a brief description of the mechanisms by which cells achieve these two activities.

Proliferation

Cells reproduce by duplicating their contents and then splitting into two; this process is part of the cell cycle,

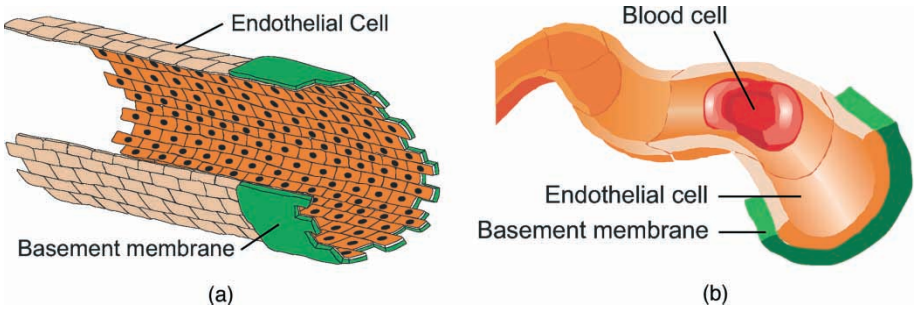

FIGURE 3 The endothelium consists of the mosaic arrangement of a monolayer of endothelial cells around a central lumen: (a) a large vessel, (b) a capillary.

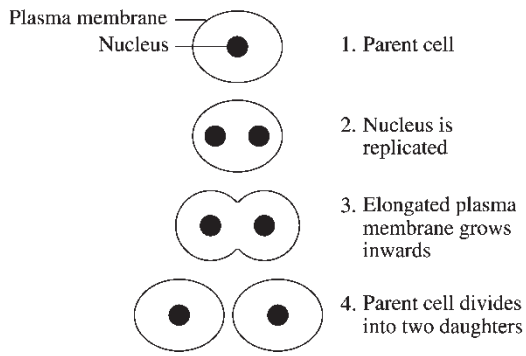

FIGURE 4 The process of mitosis (the *M*-phase) by which a cell divides.

common to almost all cells. The cell cycle comprises four main phases. In the *S*-phase, the cell's DNA is replicated, whilst the physical division of the nucleus and then the cell itself occur during the *M*-phase, or mitosis phase (see Fig. 4). Between these two phases are two 'gap' phases, G_1 and G_2, during which the cell grows (see Fig. 5). In the G_1 phase, the cell may pause its progress in the cycle by entering a resting state, called the G_0-phase, where it can remain indefinitely (Alberts *et al.*, 1994). From here, the cell can either re-emerge into the *S*-phase to begin the process of reproduction, or undergo apoptosis.

Apoptosis is distinct from necrosis as a mechanism for cell death. The latter, which is characterised by cell inflammation and rupture and is common in the interior of solid tumours (Sutherland, 1986), is the result of adverse environmental conditions, such as high pressure, causing physical injury to the cell, or hypoxia (King, 1996). The former, which is characterised by DNA fragmentation and cell shrinkage (Santini *et al.*, 2000) and is sometimes called 'cell suicide', is part of the normal cell cycle: cells will undergo programmed cell death when age, health or condition dictate. In particular, cells check for DNA damage prior to entering the DNA replication phase (*S*-phase) and will undergo apoptosis if damage is detected (Santini *et al.*, 2000). Tumour suppressor genes, such as p53, play a crucial role in inducing cell cycle arrest in damaged cells. It is the loss of such genes that contributes to the uncontrolled proliferation of mutated cells that is associated with cancer (King, 1996).

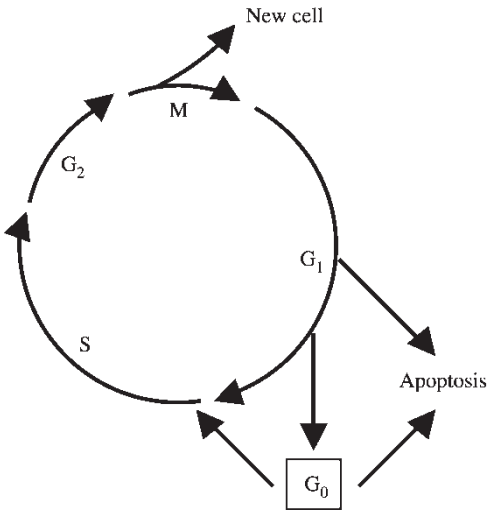

FIGURE 5 The cell cycle: *M* is the mitosis phase, *S* is the DNA replication phase, G_1 and G_2 are gap phases and G_0 is a resting phase.

There are many factors affecting an EC's decision on whether to enter and when to emerge from the G_0-phase, and whether to undergo apoptosis; the key external signalling molecules will be discussed in the 'Pro- and Anti-angiogenic Factors' section. Tight cell–cell and cell–basement membrane contacts, and the presence of certain survival factors, help to keep the EC in the G_0 phase (Santini *et al.*, 2000). In the absence of these, the cell's decision on whether or not to re-enter the *S*-phase is governed by the presence or absence of a mitogenic signal (Liu *et al.*, 2000).

Migration

The movement of a cell in response to an external stimulus is called taxis: for example, phototaxis is movement in response to light. One particularly important mechanism for cell movement is chemotaxis, movement in response to a gradient of chemical concentration. A classic example is *Dictyostelium discoideum*, a type of amoeba which lives on the forest floor. When their food supply is exhausted, these bacteria secrete a chemical called cyclic AMP. The *Dictyostelium* migrate up spatial gradients of cyclic AMP concentration by chemotaxis and aggregate to form a fruiting body (Alberts *et al.*, 1994).

A chemotactic response occurs when receptors on one side of a cell detect a different chemical concentration to receptors on the other side of the cell. If the chemical in question is a chemoattractant for that cell, the cell will extend tiny protrusions, called pseudopodia, on the side of the higher concentration. These attach to the underlying substratum, via cell–matrix adhesion molecules such as integrins, and are then used to pull the cell in that direction, enabling it to migrate up the concentration gradient (see Fig. 6a).

There are a finite number of receptors on the cell surface. When a receptor binds a molecule of chemoattractant, it is internalised to transmit the signal to the cell interior, and then recycled to the cell surface. This process takes time, so if the local concentration of the attractant is high and a large proportion of the receptors becomes internalised, there will be a significant reduction in the number of free receptors on the cell surface. This can lead to a loss of the ability to detect the local concentration gradient (Dahlquist *et al.*, 1972). Therefore, the chemotactic response to a concentration *gradient* may decrease as the concentration *level* increases (Lauffenburger and Zigmond, 1981) (see Fig. 6b).

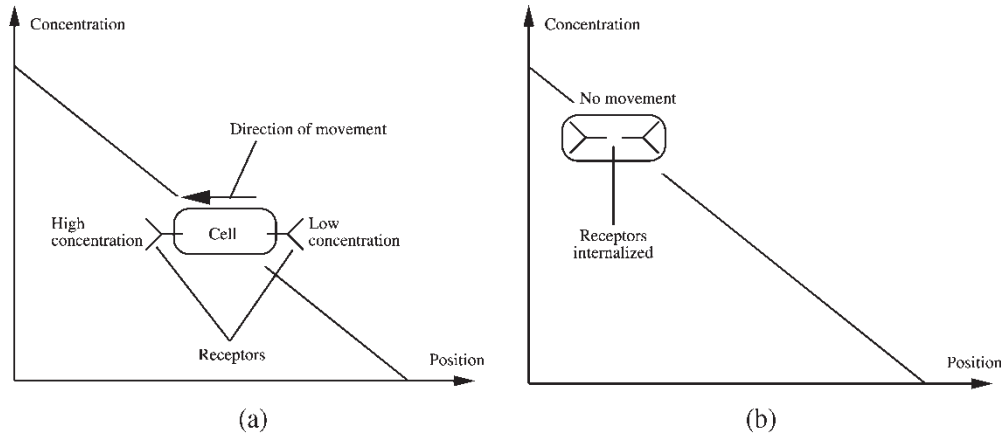

FIGURE 6 The chemotactic response of a cell to a spatial gradient of chemical concentration: (a) at medium concentration levels, (b) at high concentration levels.

In contrast to chemotaxis, which is a directional response to a concentration gradient, chemokinesis is a non-directional response to a concentration level. Thus, a chemokinetic agent increases the *rate* of cell movement, but does not affect the *direction* of movement. In the absence of any directional stimulus, chemokinesis will simply increase the random, diffusive motion of a cell, accelerating the process of Brownian motion.

Another important mechanism of cell migration is haptotaxis, or movement along an adhesive gradient (Carter, 1965). All cell movement takes place via the attachment of pseudopodia to some underlying substratum, usually protein fibres, via adhesion molecules (primarily integrins) on the cell surface (Bökel and Brown, 2002). However, the substratum is not usually homogeneous and variations in its density can affect cellular adhesion and hence migration. Cells may, therefore, exhibit a preference for areas of the substratum to which they can better adhere (Nicosia *et al.*, 1993).

Thus, in addition to responding to concentration gradients of diffusible chemicals by chemotaxis, cells can also respond to gradients of adhesive molecules, such as collagen and fibronectin. The specific role of haptotaxis in angiogenesis is discussed in the 'Tumour-induced Angiogenesis' section.

TUMOUR-INDUCED ANGIOGENESIS

Angiogenesis is absolutely essential for embryonic growth and tissue growth and repair. Nevertheless, its occurrence is highly restricted and, in the healthy adult, angiogenesis is confined to the female reproductive cycle (Reynolds *et al.*, 1992). Angiogenesis can, however, be induced under certain pathological conditions, such as rheumatoid arthritis (Walsh, 1999), wound healing (Hunt *et al.*, 1984), diabetic retinopathy (Sharp, 1995) and solid tumour growth (Folkman, 1971). The following is a detailed description of tumour-induced angiogenesis, but the key features are common to all physiological and pathological angiogenesis. Figure 7 shows a diagram of the key events of angiogenesis.

Historical Overview

When it was first suggested by Folkman (1971) that the growth of a tumour beyond a diameter of approximately 2 mm is dependent on its ability to recruit new blood vessels, it was not known how this process might take place, nor how the tumour might induce it. It was postulated that the tumour secretes some diffusible substance, named tumour angiogenesis factor (TAF), which would stimulate the growth of new capillaries.

Interest in the subject of angiogenesis increased and experimental models, allowing the *in vivo* formation of new blood vessels to be observed directly, were developed. These most commonly involve the implantation of tumour cells into the mouse or rabbit cornea (an ideal tissue because of its transparent and avascular nature) and observing angiogenic outgrowth from the limbal vessel (see, for example, Ausprunk and Folkman, 1977; Muthukkaruppan and Auerbach, 1979; Sholley *et al.*, 1984 and Fig. 8).

The first direct evidence for Folkman's hypothesis came when basic fibroblast growth factor (bFGF) was shown by Shing *et al.* (1984) to be capable of inducing an angiogenic response *in vitro*. Subsequently, many other angiogenic growth factors have been isolated (Folkman and Klagsbrun, 1987), including vascular endothelial growth factor (VEGF) (Leung *et al.*, 1989), initially termed vascular permeability factor, which acts specifically on EC (Shweiki *et al.*, 1992) and is often observed at elevated levels in tumours (Klagsbrun and D'Amore, 1996). Thus, Folkman's TAF turned out not to be a single substance, but a range of different factors, more of which are still coming to light.

Several naturally occurring angiogenic inhibitors have also been discovered, such as interferon-α/β (Dvorak and Gresser, 1989), thrombospondin-1 (Good *et al.*, 1990),

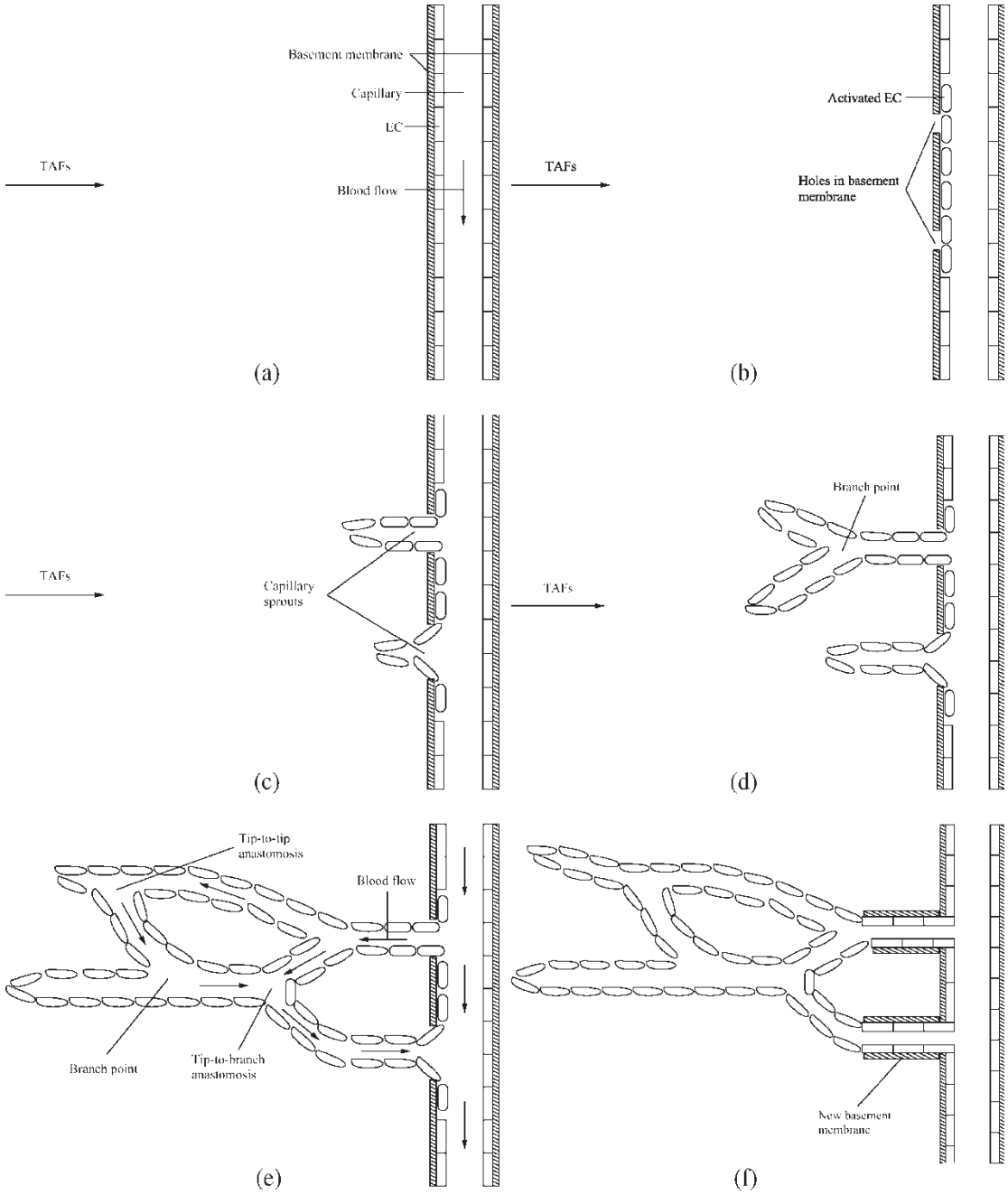

FIGURE 7 The key events of angiogenesis: (a) a quiescent capillary receives TAFs secreted by a nearby tumour, (b) the EC produce proteases, which degrade the basement membrane, (c) EC move out of the parent vessel, forming new capillary sprouts, (d) EC proliferation begins and capillary branching is observed, (e) anastomosis creates closed loops and circulation can begin in the new capillaries, (f) the new vessels start to undergo maturation, involving deactivation of the EC and formation of a new basement membrane.

angiostatin (O'Reilly *et al.*, 1994) and endostatin (O'Reilly *et al.*, 1997). This has given new impetus to research into tumour angiogenesis and the search for anti-angiogenic cancer therapies.

In recent years, the angiopoietins, angiopoietin-1 (Ang-1) (Davis *et al.*, 1996) and angiopoietin-2 (Ang-2) (Maisonpierre *et al.*, 1997), have emerged as important regulators of angiogenesis. The subtle interplay between these two EC-specific factors is crucial in governing the transition between quiescence and angiogenic growth.

The reader may now begin to appreciate the complexity of angiogenesis as a biological process, governed by numerous pro- and anti-angiogenic factors (see the 'Pro- and Anti-angiogenic Factors' section). The mechanism of action of each of these factors is different, as are their origin and the stimuli for their production. They interact with each other, with tumour cells, EC and immune cells, and with the extra-cellular matrix (ECM) to induce an apparently orderly formation of new vessels. The intricacies of this process are far from being fully understood and

FIGURE 8 An experimental model for studying angiogenesis in the mouse cornea: the response of the limbal vessel to an implant of angiogenic growth factors. Image taken from Kubo *et al.* (2002).

tumour angiogenesis is very much an active area for biological research.

Emergence from the Dormant Phase and the Angiogenic Switch

As described in the 'Fundamentals of Tumour Biology' section, an avascular tumour is reliant on passive diffusion for the supply of its oxygen and nutrients and the removal of its waste products. This imposes a limiting size of approximately 2 mm to which it can grow (Folkman, 1971); once it has reached this size, the tumour is described as dormant. Hypoxic tumour cells are known to produce growth factors, including VEGF (Shweiki *et al.*, 1992); they may also produce certain endogenous inhibitors of angiogenesis, such as transforming growth factor-beta (TGF-β) (Bikfalvi, 1995). Moreover, macrophages (cells of the immune system), which congregate in the region of the abnormal growth, respond to the presence of the tumour and its secretions by producing both pro- and anti-angiogenic substances (Bingle *et al.*, 2002). These molecules diffuse through the tissue and will be detected by the EC of proximal capillaries.

Initially, the inhibitors outweigh the growth factors and the EC remain quiescent. However, if the tumour is capable of producing enough growth factors and/or suppressing the expression of inhibitors, it may succeed in flipping the 'angiogenic switch' in favour of new growth (Hanahan and Folkman, 1996).

Initiation of Angiogenesis

On receiving a net angiogenic stimulus, EC in capillaries near the tumour become activated: they loosen the normally tight contacts with adjacent cells (Papetti and Herman, 2002) and secrete proteolytic enzymes (or proteases), whose collective effect is to degrade extracellular tissue (Pepper *et al.*, 1990). There are a large number of such enzymes, which may be broadly divided into matrix metalloproteases (MMPs) and the plasminogen activator (PA)/plasmin system (Pepper, 2001). The MMPs are capable of digesting different protein types and may be subdivided accordingly into collagenases, gelatinases, stromelysins, matrelysins and membrane-type MMPs (Vihinen and Kahari, 2002). PAs activate the widely expressed, but inactive substance, plasminogen, into the broad-spectrum protease, plasmin (Pepper *et al.*, 1992).

Both of these families of proteases have an associated class of inhibitors. MMPs are inhibited by tissue inhibitors of metalloproteases (TIMPs) (Jiang *et al.*, 2002). PAs are inhibited by plasminogen activator inhibitor (PAI), which is also expressed by fibroblasts and activated EC (Pepper *et al.*, 1992).

The first target of the proteases produced by the EC is the basement membrane (Pepper, 1997) (Fig. 7b). When this has been sufficiently degraded, the EC are able to move through the gap in the basement membrane and into the ECM. Neighbouring EC move in to fill the gap and may subsequently follow the leading cells into the ECM (Paweletz and Kneirim, 1989).

The first function of the angiogenic growth factors, therefore, is to stimulate the production of proteases by EC (Pepper, 1997). This is a key step in the angiogenic cascade because, in the absence of proteolytic activity, the EC are hemmed in by the basement membrane and will be unable to escape from the existing capillary (parent vessel) (Cavallaro and Christofori, 2000).

Endothelial Cell Migration, Proliferation and Tube Formation

Following extravasation, the EC continue to secrete proteolytic enzymes, which also degrade the ECM (Burke and DeNardo, 2001). This is necessary to create a pathway along which the cells can move (Pepper, 2001), and may also release growth factors, such as VEGF, that have been sequestered in the matrix, thus augmenting the angiogenic signal (Hirschi and D'Amore, 1996). They continue to move away from the parent vessel and towards the tumour (Ausprunk and Folkman, 1977), thus forming small sprouts (Fig. 7c). More EC are recruited from the parent vessel, elongating the new sprouts. These sprouts may initially take the form of solid strands of cells, but the EC subsequently form a central lumen, thereby creating the necessary structure for a new blood vessel (Pepper, 1997).

In addition to the angiogenic balance between growth factors and inhibitors, there is a proteolytic balance between proteases and protease inhibitors (Pepper, 2001). A certain amount of proteolysis is necessary to degrade the basement membrane and ECM, allowing EC to move out of the parent capillary and facilitating migration towards the tumour. However, excessive proteolysis is incompatible with angiogenesis because EC migration and tube

formation are dependent on the cells' ability to attach to the underlying substratum (Pepper *et al.*, 1992). The secretion of proteases must therefore be precisely regulated. The anti-proteolytic factor, PAI, may play an important role in preventing excessive matrix degradation (Pepper, 2001).

EC migration is governed mainly by a chemotactic response to concentration gradients of diffusible growth factors produced by the tumour, which create a potent directional stimulus (Papetti and Herman, 2002). Thus the second key function of the angiogenic growth factors is to induce directed EC migration towards the tumour (Paweletz and Kneirim, 1989).

Haptotaxis, cell movement in response to an adhesive gradient, also plays a role. The effect of haptotaxis, however, is more complicated, and not fully understood, because the EC are continually modifying the adhesive properties of their micro-environment via proteolysis (Pepper, 2001) and the synthesis of new ECM components (Birdwell *et al.*, 1978). Perhaps the most important substance involved in cell–matrix adhesion is the ECM component, fibronectin. *In vitro* experimental observations have demonstrated that fibronectin can promote EC migration chemokinetically (i.e. by increasing random, diffusive movement) (Yamada and Olden, 1978; Nicosia *et al.*, 1993), and can induce the directional migration of EC up a fibronectin concentration gradient (Bowersox and Sorgente, 1982; Maier *et al.*, 1999). However, the situation *in vivo* may not be so straightforward. Some degradation of the ECM is needed to facilitate migration, so although EC may move preferentially up a fibronectin gradient at relatively low concentration levels, their progress may be impeded if the concentration becomes too high. Furthermore, it is possible that by-products of fibronectin proteolysis act as chemoattractants, thus effectively stimulating EC migration towards regions of *low* fibronectin concentration (Nicosia *et al.*, 1993). The effects of haptotaxis are, therefore, far from clear. One possibility is that, at high concentration levels, EC will migrate down a fibronectin concentration gradient to enable them to move through the ECM, whereas, at low concentration levels, EC will exhibit their natural tendency to migrate up a fibronectin concentration gradient to a region of higher cell–matrix adhesion.

In quiescent endothelia, the turnover of EC is very slow, typically measured in months or years (Han and Liu, 1999). For a short period following extravasation, the low mitosis levels continue: the initial response is entirely migratory rather than proliferative (Ausprunk and Folkman, 1977). Nevertheless, after this initial period of migration, rapid EC proliferation begins a short distance behind the sprout tips, increasing the rate of sprout elongation (Paweletz and Kneirim, 1989). In experimental studies using irradiated EC (which are incapable of dividing) exposed to an angiogenic stimulus, the initial response is unaffected and a primitive network of capillary sprouts forms, but the growth stops after a few days and angiogenesis is not completed (Sholley *et al.*, 1984). EC proliferation is therefore necessary for vascularisation to take place, and its stimulation is the third and final key function of the angiogenic growth factors (Han and Liu, 1999).

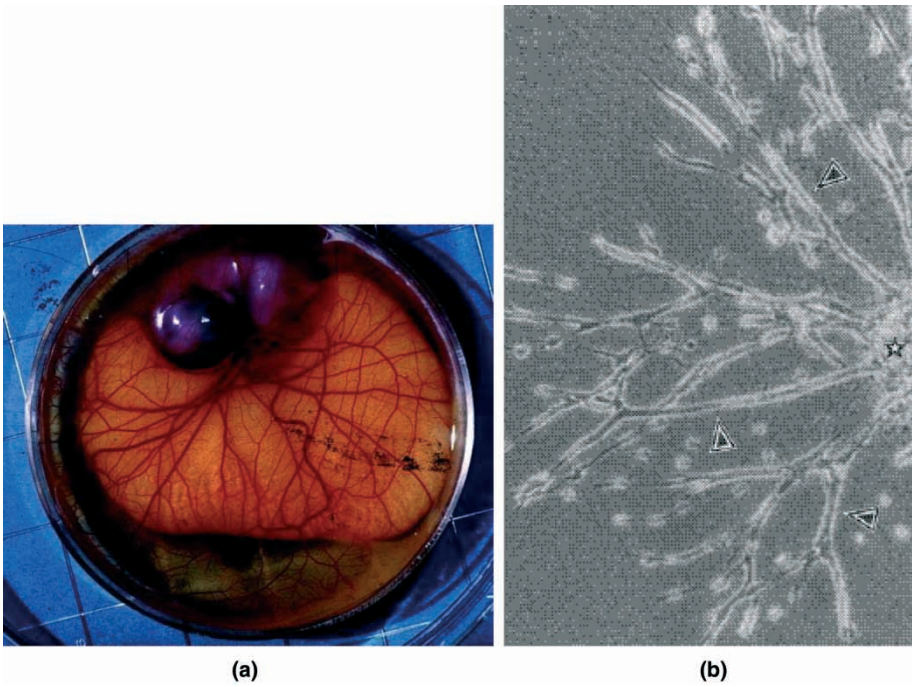

FIGURE 9 Experimental capillary networks formed by angiogenesis: (a) in the chorioallantoic membrane (Auerbach *et al.*, 2003), (b) in an *in vitro* collagen gel (Vernon and Sage, 1999).

Sprouts are seen to branch, adding to the number of migrating tips (Fig. 7d). The sprouts begin by growing approximately parallel to each other but, at a certain distance from the parent vessel, begin to incline towards other sprouts. This leads to the formation of closed loops (anastomoses), which are necessary for circulation to begin in the new vessels (Fig. 7e) (Paweletz and Kneirim, 1989). This is a crucial event in the formation of a functional vascular network (such as those shown in Fig. 9), but the precise stimulus for the change of sprout direction and anastomosis is unknown.

In some cases, branching and looping become much more pronounced as the sprouts approach the tumour, producing a dense, highly fused network, with a massive number of sprout tips. This has been termed the brush-border effect (Muthukkaruppan *et al.*, 1982; Sholley *et al.*, 1984) and its causes are poorly understood. One possibility is that the higher concentrations of angiogenic factors experienced near the tumour stimulate an increase in EC proliferation and/or vessel branching. Saturation of receptors on the EC surface (see the 'Migration' section) may also have an effect, rendering the EC unable to detect the concentration gradients of the growth factors. If this does occur, it is a transient phenomenon: the receptors eventually recover, allowing the EC to continue to migrate towards the tumour. The capillaries thus reach and penetrate the tumour, vastly improving its blood supply and allowing rapid growth.

The Vascular Phase

In physiological angiogenesis, once the target tissue has been vascularised, the expression of angiogenic growth factors ceases. EC migration, proliferation and proteolysis then come to a halt and the newly formed vessels undergo a maturation process (Kraling *et al.*, 1999). Tight cell–cell connections are re-established in the endothelium and the EC secrete proteins, such as laminin and collagen, to form a continuous basement membrane (Fig. 7f) (Paweletz and Kneirim, 1989). Finally, peri-endothelial support cells (primarily pericytes in the microvasculature) are recruited (Loughna and Sato, 2001) and the new vessels become part of the quiescent vascular system.

This maturation process does not usually occur in tumour-induced angiogenesis. Despite the fact that capillaries penetrate the edge of the tumour, supplying it with oxygen, there are still hypoxic regions within the tumour and these continue to produce angiogenic factors (Sutherland, 1986). In addition, as the newly vascularised areas of the tumour grow, they outstrip their own blood supply and develop hypoxic areas themselves (Holash *et al.*, 1999). The angiogenic switch thus remains turned on and new capillaries continue to grow, extending the blood supply throughout the now rapidly growing and highly heterogeneous tumour.

However, continued angiogenesis simply fuels further tumour growth, which in turn demands an improved blood supply. In a highly malignant tumour, the demand for new blood vessels will never be satisfied (Paweletz and Kneirim, 1989).

The tumour-related capillaries are not usually able to form mature, stable vessels with a continuous basement membrane, because of the continued production of angiogenic factors (Papetti and Herman, 2002). The new vasculature is irregular, leaky and tortuous (Hashizume *et al.*, 2000) and is constantly being remodelled: some areas of the network regress; some areas undergo robust new angiogenesis, providing a blood supply for previously avascular regions (Vajkoczy *et al.*, 2002).

PRO- AND ANTI-ANGIOGENIC FACTORS

There are a large number of pro- and anti-angiogenic factors involved in the angiogenic switch. A summary of the more important angiogenic activators and inhibitors is given in Table I, although this list is by no means exhaustive. Note that some of the factors have both pro- and anti-angiogenic functions and it is not uncommon for a substance to be pro-angiogenic in some circumstances, but anti-angiogenic in others. The following is a more detailed discussion of some of the key factors involved in angiogenesis.

Vascular Endothelial Growth Factor (VEGF)

VEGF is the best characterised angiogenic factor (Leung *et al.*, 1989; Yancopoulos *et al.*, 2000) and it has become clear that it is the main driving force behind, not only tumour angiogenesis, but all blood vessel formation (Klagsbrun and D'Amore, 1996). The three key activities of EC in angiogenesis are secretion of proteases, migration and proliferation (see the 'Tumour-induced Angiogenesis' section). VEGF is capable of inducing all three of these (Klagsbrun and D'Amore, 1996; Ferrara, 2000; Papetti and Herman, 2002) and acts specifically on EC (VEGF receptors are expressed almost exclusively by EC) (Shweiki *et al.*, 1992). It is also a survival factor for EC, inhibiting apoptosis (Liu *et al.*, 2000).

Perturbation of the genes encoding VEGF (or its EC receptors, Flt-1 and Flk-1) causes severe disruption of vasculogenesis (the embryonic process by which the main vessels of the circulatory system are formed by the *in situ* differentiation of haemangioblasts) (Patan, 2000). This results in an almost complete absence of a vasculature and early embryonic lethality (Carmeliet *et al.*, 1996). Early post-natal inactivation of VEGF is also lethal, but inactivation is less harmful in the adult, suggesting that VEGF is critical during growth, but is not required for the maintenance of the adult vasculature (Yancopoulos *et al.*, 2000). In cancer patients, high levels of VEGF expression are associated with a poor prognosis (Rosen, 2002).

VEGF was initially termed vascular permeability factor because it induces loosening of EC contacts,

TABLE I Angiogenic activators and inhibitors

Factor	Expression	Activating effects	Inhibiting effects	References
Ang-1	Widely expressed in normal tissue (by pericytes) and tumour tissue (by cancer cells)	Stimulation of EC tube formation; inhibition of EC apoptosis; maturation of new vessels; EC chemoattractant	Maintenance of quiescent endothelium	Davis *et al.* (1996), Jones (1997), Stratmann *et al.* (1998)
Ang-2	Secreted by activated EC	Loosening of cell–cell and cell–matrix contacts	Blocking of Ang-1 signalling pathway	Jones (1997), Maisonpierre *et al.* (1997), Stratmann *et al.* (1998)
Angiostatin	By-product of plasminogen proteolysis		Inhibition of EC migration, proliferation, proteolysis and tube formation	Moser *et al.* (2002), O'Reilly *et al.* (1994), Stack *et al.* (1999)
bFGF	Widely expressed in normal and tumour tissue	Stimulation of EC chemotaxis, proliferation and PA expression		Han and Liu (1999), Presta *et al.* (1992), Shing *et al.* (1984)
Endostatin	By-product of collagen proteolysis		Inhibition of EC migration, proliferation and tube formation	O'Reilly *et al.* (1997), Sim *et al.* (2000)
IF-α/β, ILs	Secreted by immune cells		Inhibition of EC migration and proliferation; downregulation of VEGF and bFGF	Carmeliet and Jain (2000), Dvorak and Gresser (1989), Maier *et al.* (1999)
MMPs	Secreted by tumour cells and activated EC	BM and ECM degradation, facilitating cell migration	Generation of angiostatin/endostatin	Vihinen and Kahari (2002)
PAs	Secreted by activated EC	Activation of plasminogen into plasmin	Generation of angiostatin/endostatin	Pepper (2001), Pepper *et al.* (1992)
PAI	Secreted by fibroblasts and activated EC	Inhibition of angiostatin generation; protection against excess proteolysis	Inhibition of PA-mediated proteolysis and of EC migration	Pepper (2001), Pepper *et al.* (1992)
PDGF	Secreted by platelets, activated EC and macrophages	Stimulation of EC strand formation; recruitment of SMC and pericytes		Hirschi and D'Amore (1996), Papetti and Herman (2002), Uemura *et al.* (2002)
Plasmin	Formed by activation of plasminogen by PA	BM and ECM degradation, facilitating cell migration	Generation of angiostatin/endostatin	Pepper (2001), Stack *et al.* (1999)
TGF-β	Widely expressed in normal and tumour tissue; activated by plasmin	Stimulation of EC cord formation, PA expression and ECM synthesis	Inhibition of EC migration and proliferation; stimulation of PAI expression	Bikfalvi (1995), Mandriota *et al.* (1996)
TIMPs	Present in normal tissue	Inhibition of angiostatin generation	Inhibition of proteolysis by MMPs and EC migration	Jiang *et al.* (2002), Vihinen and Kahari (2002)
TNF-α	Secreted by activated macrophages	Stimulation of EC strand formation	Inhibition of EC proliferation and migration	Maier *et al.* (1999), Papetti and Herman (2002)
TSP-1	Secreted by fibroblasts, EC, SMC, macrophages and tumour cells		Inhibition of EC migration, proliferation, tube formation and ECM synthesis	Good *et al.* (1990), Han and Liu (1999)
VEGF	Secreted by hypoxic tumour cells and macrophages	Stimulation of EC chemotaxis, proliferation, protease expression, survival, differentiation and permeability		Ferrara (2000), Klagsbrun and D'Amore (1996), Leung *et al.* (1989)

Ang: angiopoietin; bFGF: basic fibroblast growth factor; BM: basement membrane; IF: interferon; IL: interleukin; MMP: matrix metalloprotease; PA: plasminogen activator; PAI: plasminogen activator inhibitor; PDGF: platelet-derived growth factor; SMC: smooth muscle cells; TGF: transforming growth factor; TIMP: tissue inhibitor of metalloproteases; TNF: tumour necrosis factor; TSP: thrombospondin; VEGF: vascular endothelial growth factor.

causing vessel leakiness (Klagsbrun and D'Amore, 1996). This may be due to downregulation of cell–cell adhesion molecules, such as vascular endothelial cadherin (Wright *et al.*, 2002). VEGF can also upregulate cell–substrate adhesion molecules, such as integrins (Senger *et al.*, 1997; Rupp and Little, 2001), thus shifting the adhesive balance from cell–cell adhesion (characteristic of a quiescent phenotype) towards cell–matrix adhesion (characteristic of an invasive phenotype). This idea is supported by experimental evidence (Vernon and Sage, 1999) that high concentrations of VEGF favour matrix invasion by single EC, as opposed to multicellular sprouts.

VEGF is so potent an angiogenic activator that its expression must be precisely controlled both spatially and temporally for angiogenesis to proceed correctly. Overexpression of VEGF results in a hyperfused and hyperpermeable vascular network with vessels forming in usually avascular areas (Klagsbrun and D'Amore, 1996; Han and Liu, 1999).

Hypoxic tumour cells express large amounts of VEGF (Shweiki *et al.*, 1992). In addition, the presence of a tumour can stimulate, directly or indirectly, the production of VEGF by host cells, such as macrophages (Bingle *et al.*, 2002). In contrast to the precisely regulated expression levels seen in vasculogenesis and physiological angiogenesis, tumour-induced angiogenesis is characterised by the excess production of VEGF for an indefinite period of time. This is the main reason that the tumour-associated neovasculature is often tortuous, leaky and hyperfused (Hashizume *et al.*, 2000).

Basic Fibroblast Growth Factor (bFGF)

Unlike VEGF, basic fibroblast growth factor (bFGF) acts on a variety of cell types, including smooth muscle cells, pericytes and fibroblasts, as well as EC (Han and Liu, 1999). In common with VEGF, it is a potent EC chemoattractant and mitogen (Presta *et al.*, 1992; Bikfalvi, 1995), and is widely expressed by hypoxic tumour cells. There is evidence (Vernon and Sage, 1999) that bFGF and VEGF can act synergistically (i.e. the two factors together elicit a much greater response than either factor alone).

bFGF upregulates expression of both PA and PAI (see "Initiation of Angiogenesis" Section) by EC, but its net effect is to increase proteolytic degradation (Liotta *et al.*, 1991; Pepper *et al.*, 1992). It can also increase cell–matrix adhesion, thus increasing the potential for invasion of the ECM by EC (Presta *et al.*, 1992).

Transforming Growth Factor Beta (TGF-β)

In common with bFGF, transforming growth factor beta (TGF-β) is a multi-functional signalling molecule, which can be expressed by, and act on, a variety of cell populations, such as EC, peri-endothelial support cells, fibroblasts and tumour cells (Bikfalvi, 1995). It is expressed in a latent form, and needs to be activated by the proteolytic enzyme, plasmin, before it can bind to the TGF-β receptor (Mandriota *et al.*, 1996). Although TGF-β is usually classified as an anti-angiogenic factor, it can have pro-angiogenic effects under certain circumstances. For example, low concentrations of TGF-β have been shown to stimulate EC cord formation (Bikfalvi, 1995) and TGF-β induces expression of proteases by EC (Pepper *et al.*, 1992).

Unlike bFGF and VEGF, however, TGF-β stimulates the expression of protease inhibitors in excess of proteases, and thus has a net anti-proteolytic effect (Liotta *et al.*, 1991). This raises the possibility of a self-regulating mechanism for proteolysis. Activated EC secrete PA, leading to the generation of plasmin. This, in addition to degrading the ECM, activates latent TGF-β. PAI expression is thereby increased, thus limiting the amount of plasmin generated (Mandriota *et al.*, 1996). Such a mechanism may be one way in which the required proteolytic balance is achieved, allowing sufficient proteolytic activity to facilitate EC migration, but preventing excessive degradation (Pepper, 2001).

In addition, TGF-β can inhibit EC migration and proliferation (Paweletz and Kneirim, 1989; Vernon and Sage, 1999). TGF-β may play a role in vessel maturation, by inducing EC to revert to the quiescent phenotype and stimulating synthesis of a new basement membrane (Carmeliet, 2000). Disruption of TGF-β signalling causes defects in EC-pericyte interactions, resulting in abnormal vascular development (Ramsauer and D'Amore, 2002).

Platelet-derived Growth Factor (PDGF)

PDGF is expressed by activated EC, whilst its receptors are expressed principally by peri-endothelial support cells and their precursors (such as fibroblasts) (Uemura *et al.*, 2002), although micro-capillary EC may also express PDGF receptors (Hirschi and D'Amore, 1996). PDGF is a chemoattractant and mitogen for support cells (Carmeliet, 2000) and is thought to play an important role in their recruitment to nascent capillaries (Benjamin *et al.*, 1998; Loughna and Sato, 2001). PDGF can also induce differentiation of fibroblasts to a pericyte-like phenotype (Hirschi and D'Amore, 1996). Blocking PDGF signalling during angiogenesis disrupts pericyte recruitment, resulting in leaky, immature vessels (Ramsauer and D'Amore, 2002).

Contact of EC with pericytes leads to activation of latent TGF-β (Hirschi and D'Amore, 1996). This, together with pericyte-derived Ang-1 (see the 'The Angiopoietins' section) promotes vessel maturation and a return to the quiescent phenotype (Benjamin *et al.*, 1998; Uemura *et al.*, 2002). PDGF expression by EC subsequently decreases and it is not thought that PDGF is required for the maintenance of EC-pericyte interactions in the quiescent vasculature, or for pericyte

survival (Uemura *et al.*, 2002). The EC and pericytes both contribute to the synthesis of a new basement membrane (Hirschi and D'Amore, 1996), completing the process of vessel maturation.

The Angiopoietins

Two members of the recently discovered angiopoietin family, Ang-1 (Davis *et al.*, 1996) and Ang-2 (Maisonpierre *et al.*, 1997), have been found to be important regulators of angiogenesis. In particular, they are key players in the angiogenic balance between quiescence and activation of the endothelium.

The angiopoietins are ligands for the EC-specific receptor tyrosine kinase, Tie-2. Ang-1 is widely expressed throughout the tissues (Maisonpierre *et al.*, 1997) and is thought to play a stabilizing role, maintaining cell–cell interactions (Suri *et al.*, 1996), inhibiting apoptosis (Liu *et al.*, 2000; Harfouche *et al.*, 2002) and mediating interactions between the EC and the basement membrane (Witzenbichler *et al.*, 1998). In angiogenesis, Ang-1 is necessary for the maturation of newly formed vessels (Ashara *et al.*, 1998). For example, in embryos deprived of Ang-1 (or of the Tie-2 receptor), vasculogenesis proceeds normally, but the angiogenic remodelling and stabilisation of the vessels is severely perturbed (Suri *et al.*, 1996). Vessels formed in response to VEGF, in the absence of Ang-1, are leaky and inflamed (Thurston *et al.*, 2000; Thurston, 2002).

Ang-1 is secreted by peri-endothelial support cells (Sundberg *et al.*, 2002), the recruitment of which is an important stage in the formation of new blood vessels. Although new vessels can grow in the absence of such cells, their subsequent remodelling is severely disrupted, resulting in leaky and poorly organised vessels. It has been shown that normal vascular remodelling can be restored by administration of Ang-1 independently of support cells, suggesting that Ang-1 acts directly on EC, reducing vessel permeability and promoting vascular integrity (Uemura *et al.*, 2002).

Some tumour cells can also express Ang-1 (Stratmann *et al.*, 1998), which is capable of inducing angiogenic sprouting and chemotactic migration of EC *in vitro* (Koblizek *et al.*, 1998), but does not stimulate proliferation (Witzenbichler *et al.*, 1998). It is possible that the uniform expression pattern of Ang-1 observed under normal physiological conditions is responsible for vessel stabilisation, whereas increased expression of Ang-1 by tumour cells may generate an Ang-1 gradient, providing an additional chemotactic stimulus (Lauren *et al.*, 1998).

In the vascular endothelium, Ang-2 is a natural antagonist for Ang-1: it binds to the Tie-2 receptor, but does not activate it, thus blocking the normal effects of Ang-1 (Maisonpierre *et al.*, 1997). Over-expression of Ang-2 leads to similar defects as knockout of the genes encoding Ang-1 (Loughna and Sato, 2001). In the presence of Ang-2, vessels therefore become destabilised: cell–cell and cell–matrix connections are loosened and both the basement membrane and the peri-endothelial support cells become disassociated from the endothelium (Hanahan, 1997; Zagzag *et al.*, 1999). If VEGF is also present, the EC begin to form sprouts from the existing vessel and angiogenesis follows. However, in the absence of VEGF, the EC undergo apoptosis and vessel regression is observed (Holash *et al.*, 1999; Acker *et al.*, 2001). Unlike Ang-1, Ang-2 is not widely expressed under normal physiological conditions: its spatial and temporal expression are tightly regulated and it is expressed by EC only in localised regions of vascular remodelling (Maisonpierre *et al.*, 1997). The precise stimulus for Ang-2 expression is unclear, although it is known that EC in areas of angiogenic growth express Ang-2, possibly in response to angiogenic growth factors and/or hypoxia (Oh *et al.*, 1999; Yuan *et al.*, 2000). It has been suggested that Ang-2 is expressed specifically at the tips of growing capillaries (Maisonpierre *et al.*, 1997; Acker *et al.*, 2001). This would maintain vessel plasticity at the leading edge of the capillary network, but allow vessel maturation to take place away from the capillary tips. Ang-2 is neither chemotactic nor mitogenic for EC (Witzenbichler *et al.*, 1998).

The quiescent state of the healthy endothelium is maintained by Ang-1, with the vast majority of cells in the G_0-phase (resting phase) (see the 'Proliferation' section) at a given point in time. Localised expression of Ang-2 appears to stimulate an exit from the G_0-phase, allowing remodelling. Co-expression of VEGF stimulates re-entry into the cell division cycle, resulting in proliferation. In the absence of a mitogenic signal from VEGF, however, EC are more likely to undergo apoptosis, leading to vessel regression (see Fig. 10).

The existence of such an agonist–antagonist relationship allows the Tie-2 signalling pathway to be regulated with a high degree of spatial and temporal precision. Simply switching off expression of Ang-1 would be followed by a delay while residual ligand clears, whereas the ability to express an antagonist, Ang-2, allows instant blocking of the Tie-2 receptor (Jones, 1997).

It appears that the angiopoietins do not participate in initial vasculogenic development, but play critical roles in angiogenic outgrowth, remodelling and maturation (Maisonpierre *et al.*, 1997). Moreover, the Ang/Tie-2 signalling pathway is important in pathological angiogenesis. For example, it has been observed (Holash *et al.*, 1999) that in a tumour which had co-opted host blood vessels, the vessels underwent regression, leading to a secondarily avascular tumour. The tumour was subsequently rescued, however, by a large angiogenic response at its periphery. It was proposed that the reason for the vessel regression was the autocrine expression of Ang-2 by the EC of the co-opted vessels, combined with an absence of VEGF from the well vascularised tumour. Regression then led to hypoxia within the tumour, inducing marked VEGF expression. This, combined with the destabilising effect of Ang-2, stimulated robust angiogenesis at the tumour edge.

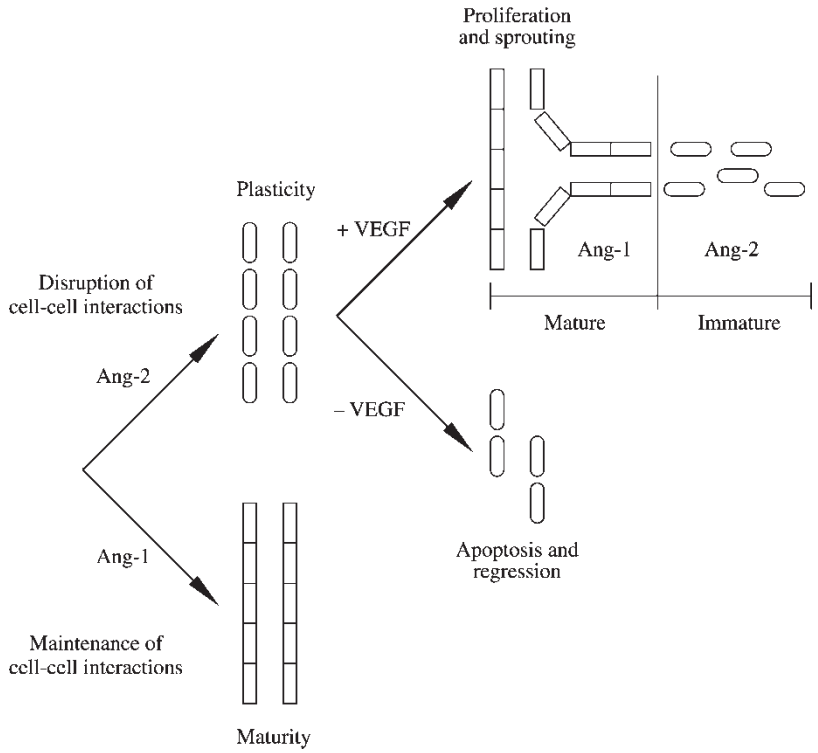

FIGURE 10 Regulation of EC behaviour by the angiopoietins and VEGF.

Angiostatin

One of the more promising anti-angiogenic molecules is angiostatin. O'Reilly *et al.* (1994) discovered angiostatin during an attempt to understand the observation that surgical removal of a primary tumour is often followed by the rapid growth of previously dormant and undetectable metastases (Cavallaro and Christofori, 2000). The hypothesis was that production by the primary tumour of pro-angiogenic factors locally outweighs production of angiogenic inhibitors, angiostatin in particular, resulting in the angiogenic response required by the tumour. The longer half-life of angiostatin, however, allows it to circulate and reach the vascular bed of a metastasis in excess of angiogenic stimulators, and thus inhibit secondary tumour growth. Removal of the primary tumour cuts off the source of angiostatin and angiogenesis at secondary tumours can then proceed unchecked, leading to rapid growth.

Subsequent research has established that angiostatin is generated by proteolytic cleavage of plasminogen (Gately *et al.*, 1996, 1997) and that it can induce EC apoptosis and inhibit EC migration and tube formation (Claesson-Welsh *et al.*, 1998; Lucas *et al.*, 1998) as well as proliferation. It has been hypothesised (Stack *et al.*, 1999) that the mechanism by which angiostatin reduces the invasive capacity of EC (and cancer cells) is via inhibition of protease production. This would also account for the inhibition of EC tube formation, whilst the induction of apoptosis could account for the observed reduction in EC proliferation (Soff, 2000). Angiostatin may also reduce the binding to, and activation of, EC receptors by VEGF (Moser *et al.*, 2002).

Angiostatin thus acts both by blocking the stimulatory effects of pro-angiogenic factors (Moser *et al.*, 2002) and by direct negative effects on EC survival (Cavallaro and Christofori, 2000). Angiostatin has been shown to inhibit tumour growth in a variety of cancers (Kirsch *et al.*, 2000) and is currently undergoing clinical trials as a potential anti-angiogenic cancer therapy (Burke and DeNardo, 2001; Cao, 2001).

The discovery that angiostatin is a by-product of plasminogen proteolysis has forced the traditionally held view—that proteolysis is a pro-angiogenic event—to be reconsidered. It also goes some way to explaining the disappointing performance of protease inhibitors, such as TIMPs, in anti-angiogenic trials (Jiang *et al.*, 2002). For example, it has been shown (Pozzi *et al.*, 2002) that inhibiting the expression of matrix metalloprotease-9 (MMP-9) *in vitro* can block generation of angiostatin, leading to increased tumour vascularisation and growth. Conversely, increased levels of MMP-9 resulted in a significant reduction of EC proliferation.

INVASION AND METASTASIS

The development of a network of capillaries in close proximity to the tumour massively increases the likelihood of tumour cells entering the bloodstream (Schirrmacher, 1985). Indeed, some of the newly formed tumour vessels may lack a continuous endothelium and tumour cells may,

therefore, be in direct contact with the vessel lumen (McDonald and Foss, 2000). This is relatively rare, however, and an invading tumour cell will more commonly have to perform a sequence of actions in order to gain access to the circulation.

Firstly, the cancer cell must either secrete proteases, or induce host cells to do so, in order to degrade the surrounding tissue and enable matrix invasion. This process of detachment from the primary tumour mass distinguishes *in situ* carcinoma, which is easily treatable, from more advanced cancers and is one of the hallmarks of a malignant tumour (King, 1996).

The invading cell must then traverse connective tissue to a nearby capillary. Depending on the maturity of that capillary, the cancer cell may additionally have to degrade the vessel's basement membrane before entering the lumen (Adatia *et al.*, 1992). Once in the circulation, the cell must evade immune surveillance and lodge in the microvasculature of a distant organ. Finally, the cell must once again degrade the basement membrane to allow it to leave the circulatory system (extravasate) and begin to proliferate in the new environment, establishing a secondary tumour colony (Saaristo *et al.*, 2000). Proliferation at the secondary site is initially confined to within 1 mm of the blood vessel and further growth is angiogenesis-dependent (King, 1996). Typically, many cancer cells will begin this process, leaving the primary site and locally invading the ECM, but few will succeed in entering the circulation, and only a tiny minority will complete the metastatic cascade to establish secondary tumours (Kirsch *et al.*, 2000).

The process of tissue invasion by cancer cells is strikingly similar to that of angiogenesis, with the fundamental components of proteolysis, migration and proliferation common to both processes. The major difference between the invading tumour cell and the angiogenic EC is that the tumour cell is unregulated in these three properties, whereas the EC reverts to a quiescent phenotype as soon as the external angiogenic stimulus is removed (Liotta *et al.*, 1991). In particular, tissue degradation via the secretion of proteases is once again vital. Protease inhibitors (such as TIMPs) have been shown to reduce tumour, invasion (although, under some circumstances, they can promote cancer progression via a reduction in the level of the angiogenic inhibitors, angiostatin and endostatin—see the 'Pro- and Anti-Angiogenic Factors' section) (Jiang *et al.*, 2002; Pozzi *et al.*, 2002). Angiostatin itself may additionally be capable of inhibiting cancer cell invasion, independently of EC, via inhibition of proteolytic enzymes (Stack *et al.*, 1999; Moser *et al.*, 2002).

The factors governing tumour cell migration are not fully understood, though cell–matrix interactions are important and haptotaxis may play a role (Liotta *et al.*, 1983; McCarthy and Furcht, 1984). Chemotaxis in response to a gradient of, for example, oxygen concentration or the concentration of some proteolytic fragment, may also be involved (Schirrmacher, 1985; Adatia *et al.*, 1992; King, 1996).

SUMMARY

- Angiogenesis, the growth of new blood vessels, is essential for the growth of solid tumours beyond approximately 2 mm in diameter. Without angiogenesis, the tumour remains in the avascular phase and poses little danger to the host.
- The new vessels form by the controlled migration and proliferation of EC from an existing vessel, stimulated by growth factors produced by the tumour. The best characterised growth factor is VEGF, but there are many other important factors, including bFGF, TGF-β and PDGF.
- The three most important activities of EC in angiogenesis are secretion of proteolytic enzymes, migration towards the tumour and proliferation. EC migration is controlled primarily by chemotaxis in response to tumour-derived growth factors; haptotaxis and chemokinesis may also play important roles. Angiogenic sprouting can occur in the absence of EC proliferation, but sustained angiogenesis and the formation of a functional capillary network cannot.
- The angiopoietins are important angiogenic regulators: Ang-1 stabilises vessels; Ang-2 destabilises them. Ang-2 can thus lead to either angiogenic growth or vessel regression, depending on whether a VEGF signal is detected.
- Angiostatin, which is formed by the proteolysis of plasminogen, can inhibit angiogenesis.
- The processes of angiogenesis and tumour cell invasion of host tissue have important similarities.
- Key references: Folkman (1971), Paweletz and Kneirim (1989), Alberts *et al.* (1994), Han and Liu (1999) and Yancopoulos *et al.* (2000).

References

Acker, T., Beck, H. and Plate, K.H. (2001) "Cell type specific expression of vascular endothelial growth factor and angiopoietin-1 and -2 suggests an important role of astrocytes in cerebellar vascularisation", *Mech. Dev.* **108**, 45–57.

Adatia, R., Poggi, L., Thompson, E.W., Gallo, R.C., Fassina, G.F. and Albini, A. (1992) "Assessment of angiogenic potential—the use of AIDS-KS cell supernatants as an *in vitro* model", In: Steiner, R., Weisz, P.B. and Langer, R., eds, Angiogenesis: Key Principles—Science–Technology–Medicine (Birkhauser Verlag, Basel), pp 321–326.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J.D. (1994) The Molecular Biology of the Cell, 3rd Ed. (Garland, New York).

Ashara, T., Chen, D., Takahashi, T., Fujikawa, K., Kearney, M., Magner, M., Yancopoulos, G.D. and Isner, J.M. (1998) "Tie-2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularisation", *Circ. Res.* **83**, 233–240.

Auerbach, R., Lewis, R., Shinners, B., Kubai, L. and Akhtar, N. (2003) "Angiogenesis assays: a critical overview", *Clin. Chem.* **49**, 32–40.

Ausprunk, D.H. and Folkman, J. (1977) "Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumour angiogenesis", *Microvasc. Res.* **14**, 53–65.

Benjamin, L.E., Hemo, I. and Keshet, E. (1998) "A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF", *Development* **125**, 1591–1598.

Bikfalvi, A. (1995) "Significance of angiogenesis in tumour progression and metastasis", *Eur. J. Cancer* **31**, 1101–1104.

Bingle, L., Brown, N.J. and Lewis, C.E. (2002) "The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies", *J. Pathol.* **196**, 254–265.

Birdwell, C.R., Gospodarowicz, D. and Nicolson, G.L. (1978) "Identification, localisation and role of fibronectin in cultured bovine endothelial cells", *Proc. Natl Acad. Sci. USA* **75**, 3273–3277.

Bökel, C. and Brown, N.H. (2002) "Integrins in development: moving on, responding to, and sticking to the extracellular matrix", *Dev. Cell* **3**, 311–321.

Bowersox, J.C. and Sorgente, N. (1982) "Chemotaxis of aortic endothelial cells in response to fibronectin", *Cancer Res.* **42**, 2547–2551.

Burke, P.A. and DeNardo, S.J. (2001) "Antiangiogenic agents and their promising potential in combined therapy", *Crit. Rev. Oncol. Haematol.* **39**, 155–171.

Cao, Y. (2001) "Endogenous angiogenesis inhibitors and their therapeutic implications", *Int. J. Biochem. Cell Biol.* **33**, 357–369.

Carmeliet, P. (2000) "Mechanisms of angiogenesis and arteriogenesis", *Nat. Med.* **6**, 389–395.

Carmeliet, P. and Jain, R.K. (2000) "Angiogenesis in cancer and other diseases", *Nature* **407**, 249–257.

Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M., Fahrig, M., Vandenhoeck, A., Harpal, K., Eberhardt, C., Declercq, C., Pawling, J. and Moons, L. (1996) "Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele", *Nature* **380**, 435–439.

Carter, S.B. (1965) "Principles of cell motility: the direction of cell movement and cancer invasion", *Nature* **208**, 1183–1187.

Cavallaro, U. and Christofori, G. (2000) "Molecular mechanisms of tumour angiogenesis and tumour progression", *J. Neuro-Oncol.* **50**, 63–70.

Claesson-Welsh, L., Welsh, M., Ito, N., Anand-Apte, B., Soker, S., Zetter, B., O'Reilly, M. and Folkman, J. (1998) "Angiostatin induces endothelial cell apoptosis and activation of focal adhesion kinase independently of the integrin-binding motif RGD", *Proc. Natl Acad. Sci. USA* **95**, 5579–5583.

Dahlquist, F.W., Lovely, P. and Koshland, D.E. (1972) "Quantitative analysis of bacterial migration in chemotaxis", *Nat. New Biol.* **236**, 120–123.

Davis, S., Aldrich, T.H., Jones, P.F., Acheson, A., Compton, D.L., Jain, V., Ryan, T.E., Bruno, J., Radziejewski, C., Maisonpierre, P.C. and Yancopoulos, G.D. (1996) "Isolation of angiopoietin-1, a ligand for the Tie-2 receptor, by secretion-trap expression cloning", *Cell* **87**, 1161–1169.

Dvorak, H.F. and Gresser, I. (1989) "Microvascular injury in pathogenesis of interferon-induced necrosis of subcutaneous tumours in mice", *J. Natl Cancer Inst.* **81**, 497–502.

Ferrara, N. (2000) "Vascular endothelial growth factor and the regulation of angiogenesis", *Recent Prog. Horm. Res.* **55**, 15–36.

Folkman, J. (1971) "Tumour angiogenesis: therapeutic implications", *N. Engl. J. Med.* **285**, 1182–1186.

Folkman, J. and Hochberg, M. (1973) "Self-regulation of growth in three dimensions", *J. Exp. Med.* **138**, 745–753.

Folkman, J. and Klagsbrun, M. (1987) "Angiogenic factors", *Science* **235**, 442–447.

Gately, S., Twardowski, P., Stack, M.S., Patrick, M., Boggio, L., Cundiff, D.L., Schnaper, H.W., Madison, L., Volpert, O., Bouck, N., Enghild, J., Kwaan, H.C. and Soff, G.A. (1996) "Human prostate carcinoma cells express enzymatic activity that converts human plasminogen to the angiogenesis inhibitor, angiostatin", *Cancer Res.* **56**, 4887–4890.

Gately, S., Twardowski, P., Stack, M.S., Cundiff, D.L., Grella, D., Castellino, F.J., Enghild, J., Kwaan, H.C., Lee, F., Kramer, R.A., Volpert, O., Bouck, N. and Soff, G.A. (1997) "The mechanism of cancer-mediated conversion of plasminogen to the angiogenesis inhibitor angiostatin", *Proc. Natl Acad. Sci. USA* **94**, 10868–10872.

Good, D.J., Polverini, P.J., Rastinejad, F., LeBeau, M.M., Lemons, R.S., Frazier, W.A. and Bouck, N.P. (1990) "A tumour suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin", *Proc. Natl Acad. Sci. USA* **87**, 6624–6628.

Han, Z.C. and Liu, Y. (1999) "Angiogenesis: state of the art", *Int. J. Haematol.* **70**, 68–82.

Hanahan, D. (1997) "Signalling vascular morphogenesis and maintenance", *Science* **277**, 48–50.

Hanahan, D. and Folkman, J. (1996) "Patterns and emerging mechanisms of the angiogenic switch during tumourigenesis", *Cell* **86**, 353–364.

Harfouche, R., Hassessian, H.M., Guo, Y., Faivre, V., Srikant, C.B., Yancopoulos, G.D. and Hussain, S.N.A. (2002) "Mechanisms which mediate the antiapoptotic effects of angiopoietin-1 on endothelial cells", *Microvasc. Res.* **64**, 135–147.

Hashizume, H., Baluk, P., Morikawa, S., McLean, J.W., Thurston, G., Roberge, S., Jain, R.K. and McDonald, D.M. (2000) "Openings between defective endothelial cells explain tumour vessel leakiness", *Am. J. Pathol.* **156**, 1363–1380.

Hirschi, K.K. and D'Amore, P.A. (1996) "Pericytes in the microvasculature", *Cardiovasc. Res.* **32**, 687–698.

Holash, J., Wiegand, S.J. and Yancopoulos, G.D. (1999) "New model of tumour angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF", *Oncogene* **18**, 5356–5362.

Hunt, T.K., Knighton, D.R., Thakral, K.K., Goodson, W.H. and Andrews, W.S. (1984) "Studies on inflammation and wound healing: angiogenesis and collagen synthesis stimulated *in vivo* by resident and activated macrophages", *Surgery* **96**, 48–54.

Jiang, Y., Goldberg, I.D. and Shi, Y.E. (2002) "Complex roles of tissue inhibitors of metalloproteinases in cancer", *Oncogene* **21**, 2245–2252.

Jones, P.F. (1997) "Tied up (or down?) with angiopoietins", *Angiogenesis* **1**, 38–44.

Kim Lee Sim, B., MacDonald, N.J. and Gubish, E.R. (2000) "Angiostatin and endostatin: endogenous inhibitors of tumour growth", *Cancer Metastasis Rev.* **19**, 181–190.

King, R.J.B. (1996) Cancer Biology (Longman, Essex).

Kirsch, M., Schackert, G. and Black, P.M. (2000) "Angiogenesis, metastasis and endogenous inhibition", *J. Neuro-Oncol.* **50**, 173–180.

Klagsbrun, M. and D'Amore, P.A. (1996) "Vascular endothelial growth factor and its receptors", *Cytokine Growth Factor Rev.* **7**, 259–270.

Koblizek, T.I., Weiss, C., Yancopoulos, G.D., Deutsch, U. and Risau, W. (1998) "Angiopoietin-1 induces sprouting angiogenesis *in vitro*", *Curr. Biol.* **8**, 529–532.

Kraling, B.M., Wiederschain, D.G., Boehm, T., Rehn, M., Mulliken, J.B. and Moses, M.A. (1999) "The role of matrix metalloprotease activity in the maturation of human capillary endothelial cells *in vitro*", *J. Cell Sci.* **112**, 1599–1609.

Kubo, H., Cao, R., Bräkenhielm, E., Mäkinen, T., Cao, Y. and Alitalo, K. (2002) "Blockade of vascular endothelial growth factor receptor-3 signalling inhibits fibroblast growth factor-2 induced lymphangiogenesis", *Proc. Natl Acad. Sci. USA* **99**, 8868–8873.

Lauffenburger, D.A. and Zigmond, S.H. (1981) "Chemotactic factor concentration gradients in chemotaxis assay systems", *J. Immunol. Methods* **40**, 45–60.

Lauren, J., Gunji, Y. and Alitalo, K. (1998) "Is angiopoietin-2 necessary for the initiation of tumour angiogenesis?", *Am. J. Pathol.* **153**, 1333–1339.

Leung, D.W., Cachianes, G., Kuang, W.J., Goeddel, D.V. and Ferrara, N. (1989) "Vascular endothelial growth factor is a secreted angiogenic mitogen", *Science* **246**, 1306–1309.

Liotta, L.A., Rao, C.N. and Sanford, H.B. (1983) "Tumour invasion and the extracellular matrix", *Lab. Investig.* **49**, 636–649.

Liotta, L.A., Steeg, P.S. and Stetler-Stevenson, W.G. (1991) "Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation", *Cell* **64**, 327–336.

Liu, W., Ahmad, S.A., Reinmuth, N., Shaheen, R.M., Jung, Y.D., Fan, F. and Ellis, L.M. (2000) "Endothelial cell survival and apoptosis in the tumour vasculature", *Apoptosis* **5**, 323–328.

Lobov, I.B., Brooks, P.C. and Lang, R.A. (2002) "Angiopoietin-2 displays vascular endothelial growth factor-dependent modulation of capillary structure and endothelial cell survival *in vivo*", *Proc. Natl Acad. Sci. USA* **99**, 11205–11210.

Loughna, S. and Sato, T.N. (2001) "Angiopoietin and Tie signalling pathways in vascular development", *Matrix Biol.* **20**, 319–325.

Lucas, R., Holmgren, L., Garcia, I., Jimenez, B., Mandriota, S.J., Borlat, F., Sim, B.K.L., Wu, Z., Grau, G.E., Shing, Y., Soff, G.A., Bouck, N. and Pepper, M.S. (1998) "Multiple forms of angiostatin induce apoptosis in endothelial cells", *Blood* **92**, 4730–4741.

Maier, J.A.M., Morelli, D., Lazzerini, D., Menard, S., Colnaghi, M.I. and Balsari, A. (1999) "Inhibition of fibronectin-activated migration of microvascular endothelial cells by interleukin-1α, tumour necrosis factor-α and interferon-γ", *Cytokine* **11**, 134–139.

Maisonpierre, P.C., Suri, C., Jones, P.F., Bartunkova, S., Wiegand, S.J., Radziejewski, C., Compton, D., McClain, J., Aldrich, T.H., Papandopoulos, N., Daly, T.J., Davis, S. and Sato, T.N. (1997) "Angiopoietin-2, a natural antagonist for Tie-2 that disrupts *in vivo* angiogenesis", *Science* **277**, 55–60.

Mandriota, S.J., Menoud, P.A. and Pepper, M.S. (1996) "Transforming growth factor-β1 down-regulates vascular endothelial growth factor receptor-2/Flk-1 expression in vascular endothelial cells", *J. Biol. Chem.* **271**, 11500–11505.

McCarthy, J.B. and Furcht, L.T. (1984) "Laminin and fibronectin promote the haptotactic migration of B16 mouse melanoma cells *in vitro*", *J. Cell Biol.* **98**, 1474–1480.

McDonald, D.M. and Foss, A.J.E. (2000) "Endothelial cells of tumour vessels: abnormal but not absent", *Canc. Metastasis Rev.* **19**, 109–120.

Moser, T.L., Stack, M.S., Wahl, M.L. and Pizzo, S.V. (2002) "The mechanism of action of angiostatin: can you teach an old dog new tricks?", *Thromb. Haemostasis* **87**, 394–401.

Muthukkaruppan, V.R. and Auerbach, R. (1979) "Angiogenesis in the mouse cornea", *Science* **205**, 1416–1418.

Muthukkaruppan, V.R., Kubai, L. and Auerbach, R. (1982) "Tumour-induced neovascularisation in the mouse eye", *J. Natl Cancer Inst.* **69**, 699–708.

Nicosia, R.F., Bonanno, E. and Smith, M. (1993) "Fibronectin promotes the elongation of microvessels during angiogenesis *in vitro*", *J. Cell Physiol.* **154**, 654–661.

O'Reilly, M.S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, R.A., Moses, M., Lane, W.S., Cao, Y., Sage, E.H. and Folkman, J. (1994) "Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma", *Cell* **79**, 315–328.

O'Reilly, M.S., Boehm, T., Shing, Y., Fukai, N., Vasios, G., Lane, W.S., Flynn, E., Birkhead, J.R., Olsen, B.R. and Folkman, J. (1997) "Endostatin: an endogenous inhibitor of angiogenesis and tumour growth", *Cell* **88**, 277–285.

Oh, H., Takagi, H., Suzuma, K., Otani, A., Matsumura, M. and Honda, Y. (1999) "Hypoxia and vascular endothelial growth factor selectively upregulate angiopoietin-2 in bovine microvascular endothelial cells", *J. Biol. Chem.* **274**, 15732–15739.

Papetti, M. and Herman, I.M. (2002) "Mechanisms of normal and tumour-derived angiogenesis", *Am. J. Physiol. Cell Physiol.* **282**, C947–C970.

Patan, S. (2000) "Vasculogenesis and angiogenesis as mechanisms of vascular network formation, growth and remodelling", *J. Neuro-Oncol.* **50**, 1–15.

Paweletz, N. and Kneirim, M. (1989) "Tumour-related angiogenesis", *Crit. Rev. Oncol. Haematol.* **9**, 197–242.

Pepper, M.S. (1997) "Manipulating angiogenesis", *Arterio. Thromb. Vasc. Biol.* **17**, 605–619.

Pepper, M.S. (2001) "Extracellular proteolysis and angiogenesis", *Thromb. Haemostasis* **86**, 346–355.

Pepper, M.S., Belin, D., Montesano, R., Orci, L. and Vassalli, J.D. (1990) "Transforming growth factor-β1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells *in vitro*", *J. Cell Biol.* **111**, 743–755.

Pepper, M.S., Bassalli, J.D., Orci, L. and Montesano, R. (1992) "Proteolytic balance and capillary morphogenesis *in vitro*", In: Steiner, R., Weisz, P.B. and Langer, R., eds, Angiogenesis: Key Principles–Science–Technology–Medicine (Birkhauser Verlag, Basel), pp 137–145.

Pozzi, A., LeVine, W.F. and Gardner, H.A. (2002) "Low plasma levels of matrix metalloproteinase-9 permit increased tumour angiogenesis", *Oncogene* **21**, 272–281.

Presta, M., Rusnati, M., Urbinati, C., Tanghetti, E., Statuto, M., Pozzi, A., Gualandris, A. and Ragnotti, G. (1992) "Basic fibroblast growth factor bound to cell substrate promotes cell adhesion, proliferation, and protease production in cultured endothelial cells", In: Steiner, R., Weisz, P.B. and Langer, R., eds, Angiogenesis: Key Principles–Science–Technology–Medicine (Birkhauser Verlag, Basel), pp 205–209.

Ramsauer, M. and D'Amore, P.A. (2002) "Getting Tie(2)d up in angiogenesis", *J. Clin. Investig.* **110**, 1615–1617.

Reynolds, L.P., Killilea, S.D. and Redmer, D.A. (1992) "Angiogenesis in the female reproductive cycle", *FASEB J.* **6**, 886–892.

Risau, V. (1997) "Mechanisms of angiogenesis", *Nature* **386**, 671–674.

Rosen, L.S. (2002) "Clinical experience with angiogenesis signalling inhibitors: focus on vascular endothelial growth factor blockers", *Cancer Control* **9**, 36–44.

Rupp, P.A. and Little, C.D. (2001) "Integrins in vascular development", *Circ. Res.* **89**, 566–572.

Saaristo, A., Karpanen, T. and Alitalo, K. (2000) "Mechanisms of angiogenesis and their use in the inhibition of tumour growth and metastasis", *Oncogene* **19**, 6122–6129.

Santini, M.T., Rainaldi, G. and Indovina, P.L. (2000) "Apoptosis, cell adhesion and the extracellular matrix in the three-dimensional growth of multicellular tumour spheroids", *Crit. Rev. Oncol. Haematol.* **36**, 75–87.

Schirrmacher, V. (1985) "Cancer metastasis: experimental approaches, theoretical concepts and impacts for treatment strategies", *Adv. Cancer Res.* **43**, 1–73.

Senger, D.R., Claffey, K.P., Benes, J.E., Perruzzi, C.A., Sergiou, A.P. and Detmar, M. (1997) "Angiogenesis promoted by vascular endothelial growth factor: regulation through $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins", *Proc. Natl Acad. Sci. USA* **94**, 13612–13617.

Sharp, P.S. (1995) "The role of growth factors in the development of diabetic retinopathy", *Metabolismo* **44**(Suppl. 4), 72–75.

Shing, Y., Folkman, J., Sullivan, R., Butterfield, C., Murray, J. and Klagsbrun, M. (1984) "Heparin affinity: purification of a tumour-derived capillary endothelial cell growth factor", *Science* **223**, 1296–1299.

Sholley, M.M., Ferguson, G.P., Seibel, H.R., Montour, J.L. and Wilson, J.D. (1984) "Mechanisms of neovascularisation", *Lab. Investig.* **51**, 624–634.

Shweiki, D., Itin, A., Soffer, D. and Keshet, E. (1992) "Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis", *Nature* **359**, 843–845.

Soff, G.A. (2000) "Angiostatin and angiostatin-related proteins", *Cancer Metastasis Rev.* **19**, 97–107.

Stack, M.S., Gately, S., Bafetti, L.M., Enghild, J.J. and Soff, G.A. (1999) "Angiostatin inhibits endothelial and melanoma cellular invasion by blocking matrix-enhanced plasminogen activation", *Biochem. J.* **340**, 77–84.

Stratmann, A., Risau, W. and Plate, K.H. (1998) "Cell type-specific expression of angiopoietin-1 and angiopoietin-2 suggests a role in glioblastoma angiogenesis", *Am. J. Pathol.* **153**, 1459–1466.

Sundberg, C., Kowanetz, M., Brown, L.F., Detmar, M. and Dvorak, H.F. (2002) "Stable expression of angiopoietin-1 and other markers by cultured pericytes: phenotypic similarities to a subpopulation of cells in maturing vessels during later stages of angiogenesis *in vivo*", *Lab. Investig.* **82**, 387–401.

Suri, C., Jones, P.F., Patan, S., Bartunkova, S., Maisonpierre, P.C., Davis, S., Sato, T.N. and Yancopoulos, G.D. (1996) "Requisite role of angiopoietin-1, a ligand for the Tie-2 receptor, during embryonic angiogenesis", *Cell* **87**, 1171–1180.

Sutherland, R.M. (1986) "Importance of critical metabolites and cellular interactions in the biology of microregions of tumours", *Cancer* **58**, 1668–1680.

Sutherland, R.M. (1988) "Cell and environment interactions in tumour microregions: the multicell spheroid model", *Science* **240**, 177–184.

Thurston, G. (2002) "Complementary actions of vascular endothelial growth factor and angiopoietin-1 on blood vessel growth and leakage", *J. Anat.* **200**, 575–580.

Thurston, G., Rudge, J.S., Ioffe, E., Zhou, H., Ross, L., Croll, S.D., Glazer, N., Holash, J., McDonald, D.M. and Yancopoulos, G.D. (2000) "Angiopoietin-1 protects the adult vasculature against plasma leakage", *Nature Med.* **6**, 460–463.

Uemura, A., Ogawa, M., Hirashima, M., Fujiwara, T., Koyama, S., Takagi, H., Honda, Y., Wiegand, S.J., Yancopoulos, G.D. and Nishikawa, S.I. (2002) "Recombinant angiopoietin-1 restores higher-order architecture of growing blood vessels in mice in the absence of mural cells", *J. Clin. Investig.* **110**, 1619–1628.

Vajkoczy, P., Farhadi, M., Gaumann, A., Heidenreich, R., Erber, R., Wunder, A., Tonn, J.C., Menger, M.D. and Breier, G. (2002) "Microtumour growth initiates angiogenic sprouting with simultaneous expression of VEGF, VEGF receptor-2 and angiopoietin-2", *J. Clin. Investig.* **109**, 777–785.

Vernon, R.B. and Sage, E.H. (1999) "A novel, quantitative model for study of endothelial cell migration and sprout formation within three-dimensional collagen matrices", *Microvasc. Res.* **57**, 118–133.

Vihinen, P. and Kahari, V. (2002) "Matrix metalloproteinases in cancer: prognostic markers and therapeutic targets", *Int. J. Cancer* **99**, 157–166.

Walsh, D.A. (1999) "Angiogenesis and arthritis", *Rheumatology* **38**, 103–112.

Witzenbichler, B., Maisonpierre, P.C., Jones, P., Yancopoulos, G.D. and Isner, J.M. (1998) "Chemotactic properties of angiopoietin-1 and -2, ligands for the endothelialspecific receptor tyrosine kinase Tie-2", *J. Biol. Chem.* **273**, 18514–18521.

Wright, T.J., Leach, L., Shaw, P.E. and Jones, P. (2002) "Dynamics of vascular endothelial-cadherin and β-catenin localisation by vascular endothelial growth factor-induced angiogenesis in human umbilical vein cells", *Exp. Cell. Res.* **280**, 159–168.

Yamada, K.M. and Olden, K. (1978) "Fibronectins—adhesive glycoproteins of cell surface and blood", *Nature* **275**, 179–184.

Yancopoulos, G.D., Davis, S., Gale, N.W., Rudge, J.S., Wiegand, S.J. and Holash, J. (2000) "Vascular-specific growth factors and blood vessel formation", *Nature* **407**, 242–249.

Yuan, H.T., Yang, S.P. and Woolf, A.S. (2000) "Hypoxia up-regulates angiopoietin-2, a Tie-2 ligand, in mouse mesangial cells", *Kidney Int.* **58**, 1912–1919.

Zagzag, D., Hooper, A., Friedlander, D.R., Chan, W., Holash, J., Wiegand, S.J., Yancopoulos, G.D. and Grumet, M. (1999) "*In situ* expression of angiopoietins in astrocytomas identifies angiopoietin-2 as an early marker of tumour angiogenesis", *Exp. Neurol.* **159**, 391–400.