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How the Extracellular Matrix and Macrophages Contribute to Angiogenesis-dependent Diseases

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INTRODUCTION

ANGIOGENESIS, OR new microvessel growth, is one of the most fundamental processes encountered in mammalian organisms [1–4]. The process is driven by a complex array of soluble mediators, matrix molecules and accessory cells that function to fine tune and co-ordinate the response in both time and space. In recent years, the role of the extracellular matrix (ECM) in angiogenesis and the enzyme systems responsible for its continuous remodelling, have received considerable attention [5–9]. In addition to providing a scaffolding during capillary morphogenesis, the ECM, by virtue of its ability to transmit biomechanical forces to cells, has been shown to exert complex local controls on the functions of endothelial cells. Depending on the composition and local activity of proteolytic enzymes, the ECM is able to regulate the availability of soluble angiogenic mediators to endothelial cells and specify the nature and type of interactions with integrin and cellular adhesion molecules [10–16]. Finally, by exerting mechanical forces along the course of developing blood vessels, the ECM is able to alter signalling patterns for cell cycle progression and promote co-ordinated changes in the cellular cytoskeleton and nuclear architecture and function [17–19].

Early studies of the ECM, investigating new capillaries, demonstrated that angiogenesis was dependent on the precise regulation, synthesis and degradation of the ECM [5, 19, 20]. *In vitro* studies of the organisation of new capillaries derived from primary cultures or endothelial cell lines have revealed the importance of the ECM in the morphology, proliferation, cytoskeletal organisation and shape of endothelial cells [21–23]. The ECM transmits a number of important morphogenetic signals to endothelial cells and growing capillaries [5, 6, 9]. Alterations in the secretion of collagen or the deposition and assembly of other ECM proteins has been shown to promote the regression of growing capillaries in several model systems [24–28]. The ECM has been shown to induce changes in gene expression and the secretory phenotype of endothelial cells during capillary morphogenesis. These include, among other changes, alterations in fibronectin, laminin and collagen during tubular reorganisation, initiation of the expression of type I collagen and the upregulation of a secreted protein, acidic and rich in cysteine (SPARC) [24, 29, 30]. It has been suggested that, in cultures of endothelial cells that spontaneously give rise to capillary-like structures, type I collagen is a necessary substrate for the attachment and spreading of endothelial cells during the formation of endothelial tubes. SPARC is thought to play an important role in inducing changes in the cytoskeleton that promote cell rounding and migration and initiate the reorganisation of endothelial cells [24, 29, 30].

The *in vivo* significance of these data is supported by studies in which the systemic or local administration of pharmacological agents that interfere with ECM structure and function also affect capillary morphogenesis. Agents that suppress collagen accumulation or cross-linking, or interfere with ECM metabolism, have been shown to interfere with the induction and maintainence of endothelial cells in a stable tubular network [8, 28]. *In vitro* models, developed to investigate the early events in angiogenesis, have demonstrated the importance of the ECM remodelling in angiogenesis. These studies have revealed that the balanced production of degradative proteinases and their inhibitors is what is required for normal vascular morphogenesis and invasion. Migrating endothelial cells produce type VI collagenase and other members of the matrix metalloproteinase family [31, 32]. Specific inhibitors of type VI collagenase, general metalloproteinase inhibitors and serine proteinase inhibitors block endothelial invasion of the ECM [31–34, 34]. Conditions that tip this balance, leading to suppression of proteolysis by decreasing protease synthesis and secretion, can block angiogenesis. For example, strategies used to block TIMP (tissue inhibitor of metalloproteinase) transcription and translation has been shown to attenuate markedly angiogenesis *in vivo* [35]. Agents that induce angiogenesis, such as bFGF (basic fibroblast growth factor) also work through the ECM by inducing urokinase-type plasminogen activator (uPA) as well as plasminogen activator inhibitor (PAI-1) [34].

The ECM acts locally to modulate the responsiveness of endothelial cells to external factors. Matrix proteins such as laminin, collagen and fibronectin often contain bFGF sequestered in the ECM complexed to heparin sulphate proteoglycans [7, 11]. It has been proposed that during blood vessel injury, bFGF is released as part of a self-repair process whereby bFGF released following injury to blood

vessels may initiate repair by inducing angiogenesis [7, 11]. At another level, the ECM functions as an effective barrier to angiogenesis [8]. Degradation of the ECM by endothelial cells' proteinases is thought to facilitate capillary migration; hence there is considerable interest in collagenase and proteinase inhibitors as anti-angiogenic therapeutic agents [8, 36]. The angiogenic stimulator bFGF induces plasminogen activator (PA) and collagenase production by endothelial cells, suggesting that one of its major functions in angiogenesis is to stimulate ECM remodelling. Alternatively, the inhibitory effects of TGFβ (transforming growth factor-beta) on endothelial growth has been attributed to enhanced ECM production which facilitates quantitative changes in the ECM and inhibits integrin expression [16].

TUMOUR CELLS CAN EVADE OR SUBVERT HOST DEFENCES DESIGNED TO SUPPRESS ANGIOGENESIS

One mechanism by which tumours develop a growth advantage is by inducing angiogenesis. This can be accomplished by several different mechanisms, which are not mutually exclusive. Tumours often loose the ability to produce natural inhibitors of angiogenesis with or without the enhanced production of pro-angiogenic factors. They can recruit host cells, such as macrophages, which contribute a rich array of pro-angiogenic cytokines in an environment already enriched with angiogenic factors. A third mechanism is by subverting host defences that normally guard against unwarranted angiogenesis. A good example of the later mechanism is that which occurs when normal endothelial cell progress toward the malignancy, Kaposi's sarcoma (KS).

KS is a complex mesenchymal neoplasm of suspected vascular endothelial cell origin [37–40]. It presents in several distinct pathological settings, with AIDS-associated KS being the most severe and life-threatening form of the disease. During the course of our early studies of KS carcinogenesis, we found that when HTLV-II conditioned media was added to cultures of human umbilical vein or human dermal microvascular endothelial cells (HDMEC), within 24 h the normal epithelioid cobble stone morphology of endothelial cells changed to a spindle-shaped appearance, with some cells demonstrating prominent dendritic processes. This phenotypic conversion of endothelial cells to a KS tumour phenotype not only involved a change in the morphology of endothelial cells, but also a dramatic upregulation in the expression of cell surface antigens including factor XIIIa, ICAM-1 and several cytokines that are unique to KS cells [37, 41]. Upon removal of KS conditioned media, endothelial cells rapidly reverted to their normal, unstimulated phenotype. As normal endothelial cells proceed through the multistep carcinogenic process to KS, they frequently begin producing elevated levels of scatter factor (SF) and express the SF receptor, the *C-MET* proto-oncogene. SF is a mesenchymal cell-derived pleiotrophic mediator that we have suggested is involved in driving endothelial cells towards malignancy (Figure 1). We later found that the active mediator in KS conditioned media, responsible for inducing the transient phenotypic conversion of HDMEC to KS-like tumour cells, is SF. However, the functional significance of the phenotypic conversions was unclear. To define the biological implications of the KS phenotype further, we initiated studies to define the functional consequence of this transient conversion phenomenon.

When normal HDMEC are exposed for 28–48 h to KS tumour conditioned media, they acquire rapidly the potent ability to stimulate the migration of other normal HDMEC and stimulate neovascularisation in the rat cornea model of angiogenesis (Figure 2). We also found that pure human recombinant SF was as potent as KS conditioned media, if not more so, in inducing HDMEC to express angiogenic activity. The role of SF in the induction of this phenotypic conversion phenomenon was also confirmed using neutralising antibodies to SF, which abrogated most of the angiogenic activity induced by SF or KS conditioned media. Since expression of angiogenic activity is one of the earliest detectable changes that occurs in cells destined to become

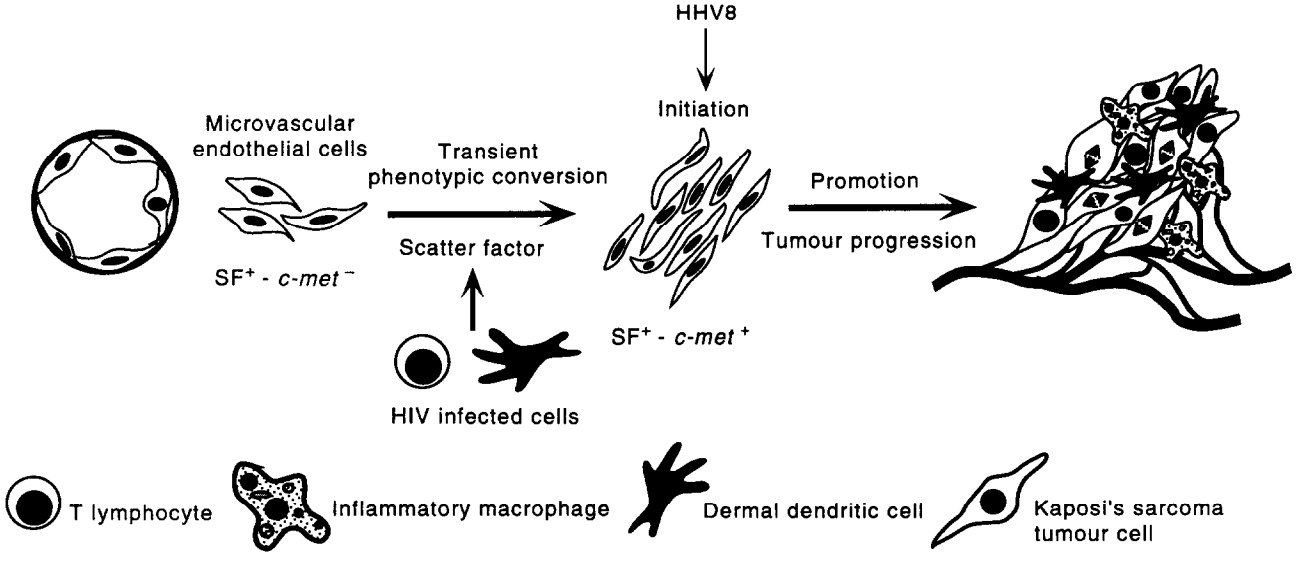

Figure 1. Model system depicting the role of scatter factor (SF) in Kaposi's sarcoma carcinogenesis and the phenotypic conversion of microvascular endothelial cells to KS-like tumour cells.

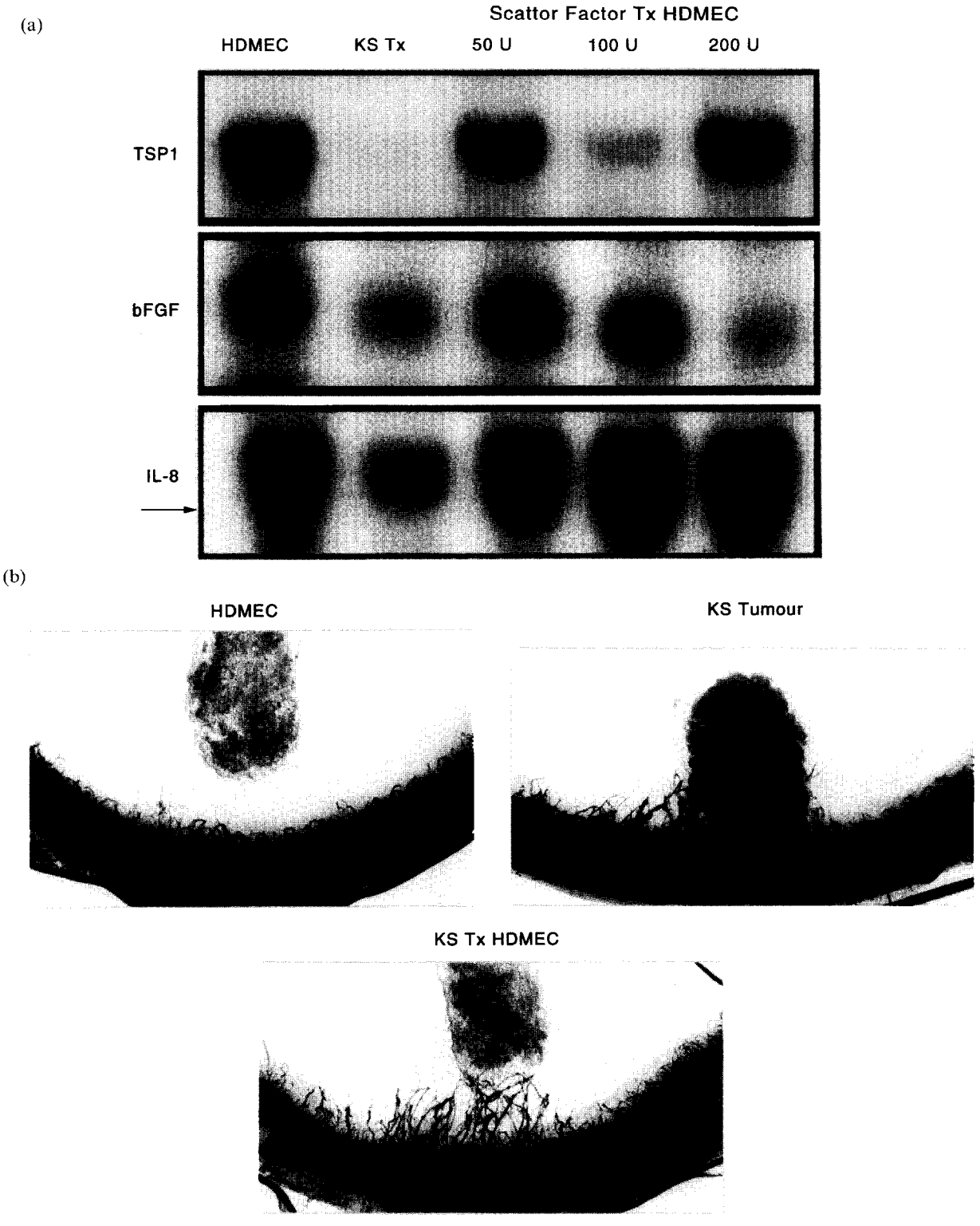

Figure 2. The effect of KS conditioned culture media and human recombinant SF on TSP-1, bFGF and IL-8 RNA expression in human dermal microvascular endothelial cells (HDMEC). (a) shows that both KS conditioned media and pure SF (100 U) are able to suppress expression of TSP-1 RNA, but has little or no effect on bFGF and IL-8 RNA expression. (b) shows angiogenic responses in rat corneas after implanting Hydron pellets containing conditioned culture media from normal HDMEC, KS tumour and HDMEC treated for 48 h with KS conditioned media. Note the absence of detectable angiogenic activity in normal HDMEC and the vigorous responses induced by KS tumour and KS-treated HDMEC.

fully malignant, we speculated that the transient expression of angiogenic activity mimicked this early step in KS carcinogenesis. In other experiments (data not shown), endothelial cells transduced to overexpress SF induced a vigorous angiogenic response in the rat cornea and dermal angiogenesis transplantation into SCID mice, but did not grow as a solid tumour. These results confirmed previous studies which have demonstrated that angiogenesis is necessary, but not sufficient for tumorigenesis.

We then examined the phenotypically converted HDMEC for expression of a selected panel of positive and negative regulators of angiogenesis. Two mediators produced by KS tumour cells and activated endothelial cells, bFGF and IL-8, were not elevated above levels normally produced by unstimulated HDMEC. In contrast, when the level of the angiogenesis inhibitor TSP1 (thrombospondin-1) was examined, an ECM molecule normally made in substantial quantities by endothelial cells, its expression was markedly suppressed. These results suggested that one consequence of this transient phenotypical conversion was the acquisition of angiogenic activity due primarily to suppression of TSP1 expression by endothelial cells. Thus, when inhibitory constraints afforded by TSP-1 are removed, endothelial cells are able to express angiogenic activity without producing significantly elevated levels of pro-angiogenic mediators. These studies also imply that as KS tumours grow, they are able to suppress TSP1 production in adjacent normal endothelial cells and effectively recruit them into the pool of stromal cells capable of augmenting KS angiogenesis.

MACROPHAGES THAT FAIL TO SWITCH FROM A PRO-ANGIOGENIC TO AN ANGIO-INHIBITORY PHENOTYPE CONTRIBUTE TO PATHOLOGICAL ANGIOGENESIS

The angiogenic switch during tumour development, in which tumours lose their ability to produce inhibitors of angiogenesis and thus gain the ability to stimulate angiogenesis, is an emerging paradigm that is only now being validated in other angiogenesis-dependent diseases. This concept also provides a clear explanation of how angiogenesis is most likely regulated in a physiological setting. There is mounting evidence that macrophages, key angiogenesis accessory cells, must also undergo a similar switch, as has been reported during chronic inflammation and wound repair, if they are to participate effectively in the timely ingrowth and regression of capillaries that characterises granulation tissue. In this case, macrophages switch from a pro-angiogenic to angio-inhibitory phenotype, a situation that is the reverse for tumours. Therefore, one might predict that if macrophages fail to undergo this conversion, they could potentially contribute to the unwarranted angiogenesis that is associated with disease processes where they are a frequent participant. There is indirect and direct evidence which suggests that when macrophages fail to produce appropriate levels of the angio-inhibitory ECM, TSP-1, they can contribute to the persistent angiogenic activity encountered in solid tumours and in the skin disease, psoriasis.

Macrophages have been recognised as important angiogenesis effector cells for a number of years [42–44]. They have been shown to participate actively in the initiation and maintenance of wound neovascularisation where they produce a spectrum of soluble mediators capable of stimulating the growth and migration of microvascular endothelial cells. They have been shown to function during the process of tumour angiogenesis to augment both tumour growth and neovascularisation [45, 46]. Also, persistent and unrelating formation of granulation tissue is a feature common to chronic inflammatory diseases, such as rheumatoid arthritis and psoriasis, and these are examples where the destructive effects of persistent granulation tissue and aberrant angiogenesis are due, in large part, to the promiscuous angiogenic activity of macrophages [46].

The macrophage may influence new capillary growth by several different mechanisms. First, macrophages produce factors that act directly to influence angiogenesis-linked endothelial cell functions. *In vitro* studies have shown that macrophages produce in excess of 20 molecules that reportedly influence endothelial cell proliferation, migration and differentiation *in vitro* [42], and are potentially angiogenic *in vivo*. A second mechanism by which macrophages might modulate angiogenesis is by modifying ECM. The composition of the ECM has been shown to influence endothelial cell shape and morphology dramatically, and may profoundly influence new capillary growth [9, 20]. Macrophages can influence the composition of the ECM either through the direct production of ECM components, or through the production of proteases, which effectively alter the structure and composition of the ECM [45]. A third mechanism is by producing substances that suppress angiogenesis. One of these macrophage-derived inhibitors of angiogenesis that has received considerable attention in recent years is the ECM, TSP-1.

TSP-1 is one member of a family of five homologous proteins. It is a 450 kDa disulphide-linked trimer that is composed of three identical chains with a monomeric mass of approximately 140 kDa. Its modular structure, in part, enables it to interact with a variety of extracellular matrix proteins, cell surface and serum proteins and cations. TSP-1 is present in great abundance in the platelet alpha granules and is secreted by a wide variety of epithelial and mesenchymal cells [47–51]. It has been shown to participate in cell–substrate interactions where many cells have been shown to attach, spread and migrate on insoluble TSP-1 [48, 52]. TSP-1 was first implicated as an inhibitor of neovascularisation when an anti-angiogenic hamster protein, whose secretion was controlled by a tumour suppressor gene, was found to have an amino acid sequence similar to human platelet TSP-1 [53]. Authentic TSP-1 was then purified from platelets and shown to block neovascularisation *in vivo* [52]. The role of TSP-1 in the inhibition of angiogenesis is supported by several observations. It is present adjacent to mature quiescent vessels and is absent from actively growing sprouts both *in vivo* [54] and *in vitro* [25]. Haemangiomas, which consists of rapidly proliferating endothelial cells, fail to produce detectable TSP-1 [54]. Antibodies to TSP-1 added to endothelial cell cultures enhance sprouting *in vitro* [25] and endothelial cells in which TSP-1 production has been downregulated by antisense TSP-1 exhibit an accelerated rate of growth, enhanced chemotactic activity and an increase in the number of capillary-like cords [55]. More recently, DiPietro and associates [58] have shown that the addition of antisense TSP-1 oligomers to wounds in the skin of mice results in delayed heal-

ing, suggesting that TSP-1 is just as important in the initiation of the wound response as it is in the organisation phase of wound repair. Also, Polverini and associates [59] have reported that mice with a targeted disruption in TSP-1 show delayed wound organisation, prolonged wound neovascularisation and heightened infiltration by macrophages. Previous investigations have shown that both resting and activated macrophages produce TSP-1. DiPietro and colleagues [60] have reported an approximately 6-fold increase in the steady-state levels of TSP-1 mRNA expression in the murine monocyte line, WEHI-3, when the cells were treated for 24 h with the potent activating agent, lipopolysaccharide (LPS), with peak secretion of TSP-1 protein occurring at 8 h. An examination of TSP-1 knockout mice clearly demonstrated that a deficiency in TSP-1 can have a subtle, yet detectable effect on a physiological function such as wound neovascularisation. Also, the introduction of pure TSP-1 or TSP-1-expressing macrophages from wild-type mice partially corrects the defect in neovascularisation (P.J. Polverini, University of Michigan School of Dentistry, Ann Arbor, Michigan, U.S.A.).

ALTERATIONS IN MACROPHAGE-DERIVED TSP-1 EXPRESSION EFFECTS TUMOUR NEOVASCULARISATION AND VASCULAR PROLIFERATION IN PSORIASIS

Mononuclear phagocytes are a frequent component of the stroma of neoplastic tissues [61]. Interest in cells of the mononuclear phagocyte system, in relation to the growth of neoplasms, stems largely from the observation that tumour associated macrophages (TAM), when appropriately activated, are able to arrest the growth or kill neoplastic and transformed target cells. Macrophages express diverse functions essential for tissue remodelling, inflammation and immunity. Analysis of the functions of tumour associated macrophages (TAM) suggests that these multifunctional cells have the capacity to affect diverse aspects of neoplastic development, including vascularisation, growth rate and metastasis, stroma formation and destruction. There is evidence that, in some neoplasms, including human cancers, macrophages can confer a distinct advantage on tumour growth. These observations emphasise the dual potential of TAM to influence neoplastic growth and progression in opposite directions, with activities that promote tumour growth often prevailing in the absence of therapeutic interventions in many tumours.

The importance of how alterations in the expression of TSP-1 by TAM can affect tumour angiogenesis is exemplified by recent studies by Lingen and associates [62, 63]. These workers have demonstrated that retinoic acid, a chemopreventive agent currently used to reduce the incidence of secondary tumour growth in patients with head and neck squamous cell carcinoma, does so, in part, by inducing tumour cells to produce inhibitors of angiogenesis, and by rendering endothelial cells refractory to pro-angiogenic mediators. In addition, Lingen, Bouck and Polverini (P.J. Polverini, University of Michigan School of Dentistry, Ann Arbor, Michigan, U.S.A.) have found that human squamous cell carcinoma treated with the chemopreventive agent retinoic acid failed to activate macrophages, resulting in a diminished capacity to express angiogenic activity *in vitro* and *in vivo*. Moreover, when these cells were phenotyped for expression of pro-angiogenic and angio-inhibitory molecules, the most dramatic change observed was a marked increase in the level of TSP-1 production. These results suggest that the ability of retinoic acid to reduce the incidence of secondary tumour growths may, in addition to affecting tumour cells and endothelium, interfere with the sustained infiltration and activation of macrophages into tumours, and drive TAM toward an angio-inhibitory phenotype.

The last piece of evidence linking a defect in the acquisition of the angio-inhibitory activity by macrophages to pathological angiogenesis is in the skin disease, psoriasis. Psoriasis is a chronic skin disease, linked to both genetic and environmental triggering factors [64]. It is characterised pathologically by excessive growth of epidermal keratinocytes, inflammation and microvascular proliferation, which is believed to result from a disruption in the complex and reciprocal molecular cross-talk between activated keratinocytes and dermal cells [65] (Figure 3). Several lines of evidence have implicated psoriatic keratinocytes, inflammatory macrophages and dermal dendritic cells in the persistent vascular proliferation that accompanies this disease. Using fresh human psoriatic lesional tissue, which was separated into epidermal and dermal components, the angiogenic potential of the lesion was found by two different groups to

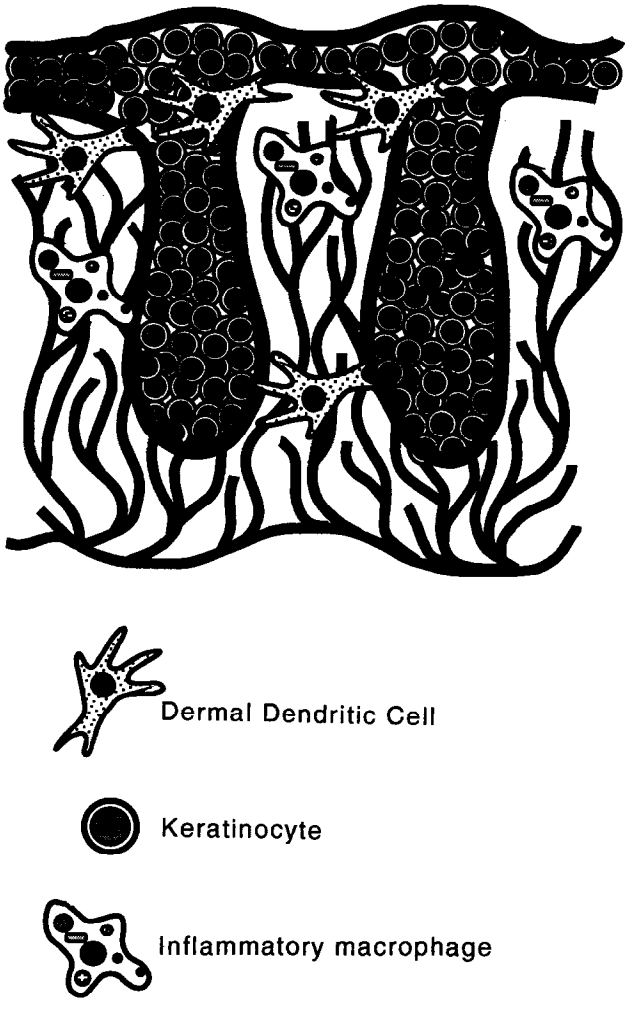

Figure 3. Model depicting the pathogenesis of psoriasis.

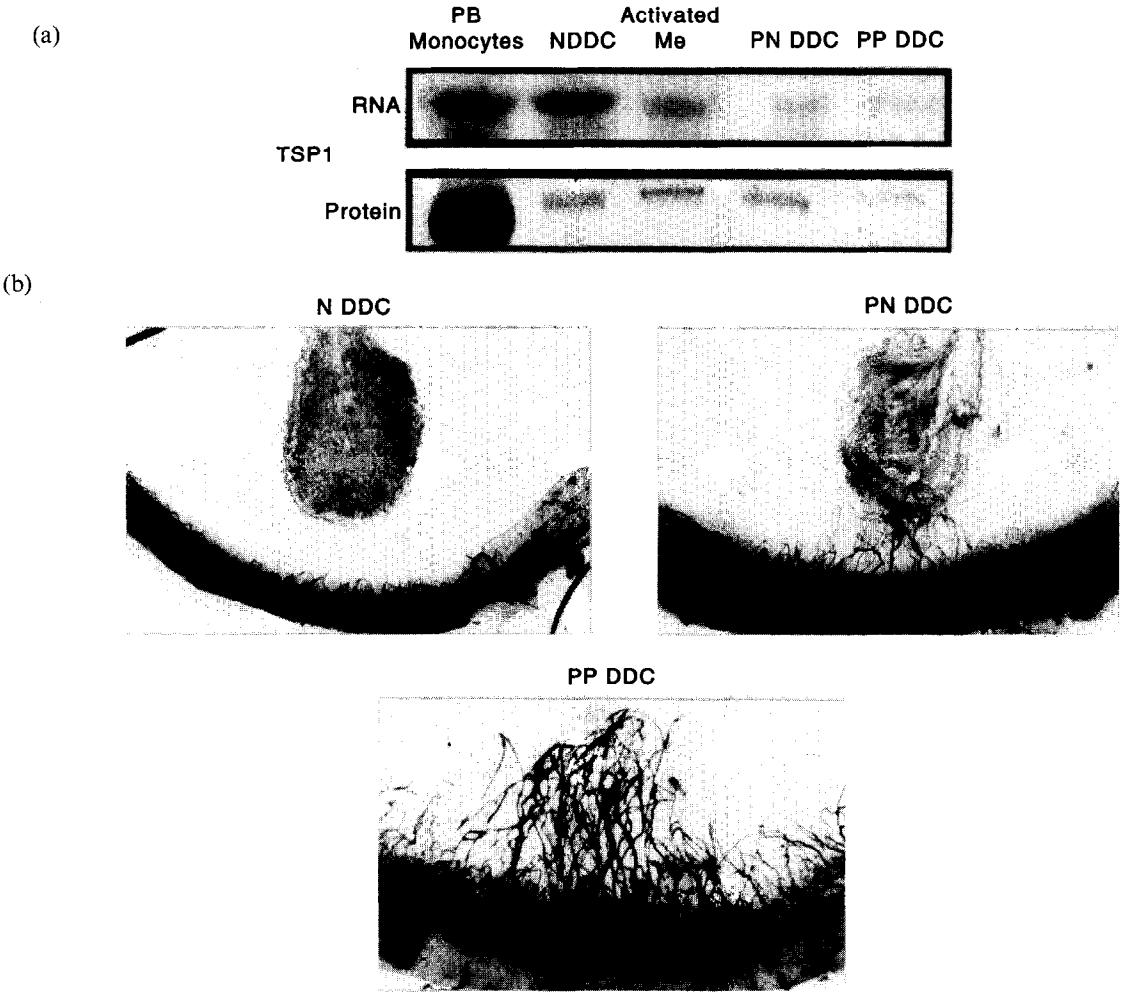

Figure 4. (a) TSP-1 RNA and protein expression in peripheral blood (PB) monocytes, dermal dendritic cells isolated from the skin of a healthy individual (NDDC), from the phorbol activated peripheral blood derived macrophages (activated macrophages), and dermal dendritic cells from the skin of psoriatic patients with active disease (PP DDC) and symptomless skin (PN DDC). Note that in PP DDC, there is little detectable TSP-1 expression. (b) shows angiogenic responses in rat corneas following implantation of Hydron pellets containing 48 h conditioned media from N DDC, PN DDC and PP DDC. Note the positive neovascular response induced by PP DDC and PP DDC conditioned media.

reside in both the dermal and the epidermal compartment [66–68]. Psoriatic keratinocytes are known to produce a variety of pro-angiogenic cytokines such as bFGF, IL-1, TGF-α and IL-8 [65]. In addition to expressing several candidate mediators of angiogenesis, keratinocytes are also known to be a source of the angiogenesis inhibitor, TSP-1 [70]. In an attempt to define further the molecular mechanism underlying this chronic inflammatory skin disease, Nickoloff and associates [69] examined the mechanisms responsible for the deregulated vasoproliferation that characterises psoriasis. These workers showed that psoriatic keratinocytes appeared to have a combined defect in both the overproduction of the pro-angiogenic cytokine, IL-8, and a deficiency in the production of the angiogenesis inhibitor, TSP-1. Previous studies have described differences between normal and psoriatic keratinocytes with respect to their growth response [70] and immunomodulating capacity [65]. It would appear that psoriatic keratinocytes also have a defect in which there is an imbalance in the production of positive and negative angiogenic mediators that governs the orderly growth of the new capillary endothelial cells.

Direct evidence implicating inflammatory macrophages and/or dermal dendritic cells in psoriatic angiogenesis are not as clear. However, Polverini and Nickoloff (P.J. Polverini, University of Michigan School of Dentistry, Ann Arbor, Michigan, U.S.A.) have found that dermal dendritic cells, isolated from psoriatic and symptomless skin, as well as human monocyte-derived macrophages and dendritic cells from normal skin, potently express angiogenic activity when exposed to conditioned media from psoriatic keratinocytes, as one might predict (Figure 4). Interestingly, when TSP-1 levels in these macrophage or dendritic cells were examined and compared with dendritic cells derived from symptomless or normal skin, TSP-1 was virtually undetected (Figure 4). These observations therefore suggest that inflammatory macrophages and dermal dendritic cells can be activated by psoriatic keratinocytes to express angiogenic activity and interfere with their ability to express sufficient

levels of TSP-1 that would enable them to counterbalance their heightened vasoproliferative activity.

CONCLUSION

In summary, capillary growth and differentiation may be controlled in a tissue environment that contains high levels of soluble angiogenic mediators by altering either the composition or function of the ECM. Changes in ECM composition can be induced by soluble factors produced by tumour cells or diseased tissues, such as psoriatic keratinocytes, that alter ECM gene expression, protein synthesis or protein secretion. ECM molecules may have different effects on capillary morphogenesis, depending on the type and number of ligands that are bound to ECM. Alternatively, anti-angiogenic molecules may modulate angiogenesis by physically interfering with the ECM–cell surface interconnections, disrupting focal contacts or by reprogramming cells and driving them toward a pro-angiogenic phenotype. Thus, angiogenesis can be blocked by interfering with either the deposition or degradation of the capillary ECM or by upregulating the expression of soluble mediators or ECM molecules with either pro-angiogenic or angio-inhibitory activity.

1. Folkman J, Cotran RS. Relation of vascular proliferation to tumor growth. *Int Rev Exp Pathol* 1976, **16**, 207–248.
2. Folkman J. Toward an understanding of angiogenesis: search and discovery. *Perspect Biol Med* 1985, **9**, 10–36.
3. Folkman J, Klagsbrun M. Angiogenic factors. *Science* 1987, **235**, 442–447.
4. Polverini PJ. The pathophysiology of angiogenesis. *Crit Rev Oral Biol Med* 1995, **6**, 230–247.
5. Madri JA, Williams SK. Capillary endothelial cell cultures: phenotypic modulation by matrix components. *J Cell Biol* 1983, **97**, 153–165.
6. Ingber DE, Folkman J. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis *in vitro*: role of extracellular matrix. *J Cell Biol* 1989, **109**, 317–330.
7. Klagsbrun M. Regulators of angiogenesis: stimulators, inhibitors, and extracellular matrix. *J Cell Biochem* 1991, **47**, 199–200.
8. Ingber DE. Extracellular matrix as a solid-state regulator in angiogenesis: identification of new targets for anti-cancer therapy. *Cancer Biol* 1992, **3**, 57–63.
9. Ingber D. Extracellular matrix and cell shape: potential control points for inhibition of angiogenesis. *J Cell Biochem* 1991, **47**, 236–241.
10. Ausprunk DH, Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. *Microvasc Res* 1982, **14**, 53–65.
11. Vlodavsky I, Fuks Z, Ashai-Michaeli R, *et al.* Extracellular matrix-resident basic fibroblast growth factor: implications for control of angiogenesis. *J Cell Biochem* 1991, **47**, 167–176.
12. Falcone DJ, McCaffrey TA, Haimovitz-Friedman A, Garcia M. Transforming growth factor Beta 1 stimulates macrophage urokinase expression and release of matrix-bound basic fibroblast growth factor. *J Cell Physiol* 1993, **155**, 595–605.
13. Plopper GE, McNamee HP, Dike LE, Bojanowski K, Ingber DE. Convergence of integrin and growth factor receptor signaling pathways within focal adhesion complex. *Mol Biol Cell* 1995, **6**, 1349–1365.
14. Brooks PC, Stromblad S, Sanders LC, *et al.* Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin αvβ3. *Cell* 1996, **85**, 1–20.
15. Merwin JR, Anderson JM, Kocher O, Van Itallie CM, Madri JA. Transforming growth factor beta-1 modulates extracellular matrix organization and cell–cell junctional complex formation during *in vitro* angiogenesis. *J Cell Physiol* 1990, **142**, 117–128.
16. Frank R, Adelmann-Grill BC, Herrmann K, Haustein UF, Petri JB, Heckmann M. Transforming growth factor-β controls cell–matrix interactions of microvascular dermal endothelial cells by downregulation of integrin expression. *J Invest Dermatol* 1996, **106**, 36–41.
17. Ingber DE, Madri JA, Folkman J. Endothelial growth factors and extracellular matrix regulate DNA synthesis through modulation of cell and nuclear expansion *in vitro*. *Cell Devel Biol* 1987, **23**, 387–394.
18. Singhvi R, Kumar A, Lopez GP, *et al.* Engineering cell shape and function. *Science* 1994, **264**, 696–698.
19. Ingber DE, Prusty D, Sun Z, Betensky H, Wang N. Cell shape, cytoskeletal mechanics, and cell cycle control in angiogenesis. *J Biomechanics* 1995, **28**, 1471–1484.
20. Ingber DE, Folkman J. How does extracellular matrix control capillary morphogenesis. *Cell* 1989, **58**, 803–805.
21. Montesano R. Regulation of angiogenesis *in vitro*. *Eur J Clin Invest* 1992, **22**, 504–515.
22. Montesano R, Orci L, Vassalli P. *In vitro* rapid organization of endothelial cells into capillary-like networks is promoted by collagen matrices. *J Cell Biol* 1983, **97**, 1648–1652.
23. Montesano R, Pepper MS, Belin D, Vassalli JD, Orci L. Phorbol ester induces cultured endothelial cells to invade a fibrin matrix in the presence of fibrinolytic inhibitors. *J Cell Physiol* 1987, **132**, 509–516.
24. Iruela-Arispe ML, Hasselaar, Sage H. Differential expression of extracellular proteins is correlated with angiogenesis *in vitro*. *Lab Invest* 1991, **64**, 174–185.
25. Iruela-Arispe ML, Diglio CA, Sage H. Modulation of extracellular matrix proteins by endothelial cells undergoing angiogenesis *in vitro*. *Arteriosclerosis Thrombosis* 1991, **11**, 805–815.
26. Crum R, Szabo S, Folkman J. A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. *Science* 1985, **230**, 1375–1378.
27. Ingber DE, Madri JA, Folkman J. A possible mechanism for inhibition of angiogenesis by angiostatic steroids: induction of capillary basement membrane dissolution. *Endocrinology* 1986, **119**, 1768–1775.
28. Ingber DE, Folkman J. Inhibition of angiogenesis through inhibition of collagen metabolism. *Lab Invest* 1988, **59**, 44–51.
29. Kubota Y, Kleinman HK, Martin GR, Lawley TJ. Role of laminin and basement membrane in morphological differentiation of human endothelial cells into capillary-like structures. *J Cell Biol* 1988, **107**, 1589–1598.
30. Jaye M, McConathy E, Drohan W, Tong B, Deuel T, Maciag T. Modulation of the sis gene transcript during endothelial cell differentiation *in vitro*. *Science* 1985, **228**, 882–884.
31. Moses MA, Sudhalter J, Langer R. Identification of an inhibitor of neovascularization form cartilage. *Science* 1990, **248**, 1408–1410.
32. Mignatti P, Tusboi R, Robbins E, Rifkin DB. *In vitro* angiogenesis on the human amniotic membrane: requirement for basic fibroblast growth factor-induced proteinases. *J Cell Biol* 1988, **108**, 671–682.
33. Levin EG, Santell N. Association of plasminogen activator inhibitor (PAI 1) with the growth substratum of human endothelial cells. *J Cell Biol* 1987, **105**, 2543–2549.
34. Pepper MS, Belin D, Montesano R, Orci L, Vassalli J-D. Transforming growth factor-beta 1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells *in vitro*. *J Cell Biol* 1990, **111**, 743–755.
35. Johnson MD, Kim H-RC, Chesler L, Tsao-Wu G, Bouck N, Polverini PJ. Inhibition of angiogenesis by tissue inhibitor of metalloproteinase. *J Cell Physiol* 1994, **160**, 194–202.
36. Fisher C, Gilbertson-Beadling S, Powers EA, Petzold G, Poorman R, Mitchell MA. Interstitial collagenase is required for angiogenesis *in vitro*. *Dev Biol* 1994, **162**, 499–510.
37. Polverini PJ, Nickoloff BJ. Role of scatter factor and the c-met protooncogenes in the pathogenesis of AIDS-associated Kaposi's sarcoma. *Adv Cancer Res* 1995, **66**, 235–253.
38. Rutgers JL, Weiczorek R, Bonetti F, *et al.* The expression of endothelial cell surface antigens by AIDS-associated Kaposi's sarcoma: evidence for a vascular endothelial cell origin. *Am J Pathol* 1986, **122**, 493–499.
39. Roth WK, Werner S, Risau W, Remberger K, Hofschneider PH. Cultured, AIDS-related Kaposi's sarcoma cells express en-

dothelial cell markers and are weakly malignant *in vitro*. *Int J Cancer* 1988, **42**, 767–773.
40. Zhang Y-M, Bachmann S, Hemmer C, *et al.* Vascular origin of Kaposi's sarcoma. Expression of leukocyte adhesion molecule-1, thrombomodulin, and tissue factor. *Am J Pathol* 1994, **144**, 51–59.
41. Naidu YM, Rosen EM, Zitnick R, *et al.* Role of scatter factor in the pathogenesis of AIDS-related Kaposi's sarcoma. *Proc Natl Acad Sci USA* 1994, **91**, 5281–5285.
42. Polverini PJ, Cotran RS, Gimbrone Jr MA, Unanue ER. Activated macrophages induce vascular proliferation. *Nature* 1977, **269**, 804–806.
43. Sunderkotter C, Goebeler M, Schultze-Osthoff K, Bhardwaj R, Sorg C. Macrophage-derived angiogenesis factors. *Pharmacol Ther* 1991, **51**, 195–216.
44. DiPietro LA, Polverini PJ. Role of the macrophage in the positive and negative regulation of wound neovascularization. *Behring Inst Mitt* 1993, **92**, 238–247.
45. Sunderkotter C, Steinbrink K, Goebeler M, Bhardwaj R, Sorg C. Macrophages and angiogenesis. *J Leucocyte Biol* 1994, **55**, 410–422.
46. Polverini JJ, Leibovich SJ. Induction of neovascularization *in vivo* and endothelial cell proliferation *in vitro* by tumor-associated macrophages. *Lab Invest* 1984, **51**, 635–642.
47. Polverini PJ. Macrophage-induced angiogenesis: a review. In Sorg C, ed. *Macrophage-Derived Regulatory Factors*. Basel, Karger, 1989, 54–73.
48. Lawler J. The structural and functional properties of thrombospondin. *Blood* 1986, **67**, 1197–1209.
49. Sage H, Bornstein P. Extracellular proteins that modulate cell–matrix interactions. *J Biol Chem* 1991, **266**, 14831–14834.
50. Frazier WA. Thrombospondin: a modular adhesive glycoprotein of platelets and nucleated cells. *J Cell Biol* 1987, **105**, 625–632.
51. Frazier WA. Thrombospondin. *Curr Opin Cell Biol* 1991, **3**, 792–799.
52. Bornstein P. Diversity of function is inherent in matrix proteins: an appraisal of thrombospondin 1. *J Cell Biol* 1995, **130**, 503–506.
53. Good DJ, Polverini PJ, Rastinejad F, *et al.* A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. *Proc Natl Acad Sci USA* 1990, **87**, 6624–6628.
54. Rastinejad F, Polverini PJ, Bouck NP. Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. *Cell* 1989, **56**, 345–355.
55. O'Shea KS, Dixit VM. Unique distribution of the extracellular matrix component thrombospondin in the developing mouse embryo. *J Cell Biol* 1988, **107**, 2737–2748.
56. Sage H, Bornstein P. Endothelial cells from umbilical vein and a hemangioendothelioma secrete basement membrane largely to the exclusion of interstitial procollagens. *Arteriosclerosis* 1982, **2**, 27–36.
57. DiPietro LA, Nebgen DR, Polverini PJ. Downregulation of endothelial cell thrombospondin 1 enhances *in vitro* angiogenesis. *J Vasc Res* 1994, **31**, 178–185.
58. DiPietro LA, Nissen NN, Gamelli RL, Koch AE, Pyle JM, Polverini PJ. Thrombospondin 1 synthesis and function in wound repair. *Am J Pathol* 1996, **148**, 1851–1860.
59. Polverini PJ, DiPietro LA, Dixit VM, Hynes RO, Lawler J. Thrombospondin 1 knockout mice show delayed organization and neovascularization of skin wounds. *FASEB* 1995, **9**, 272a.
60. DiPietro LA, Polverini PJ. Angiogenic macrophages produce the angiogenesis inhibitor thrombospondin 1. *Am J Pathol* 1994, **143**, 678–684.
61. Mantovani A. Biology of disease. Tumor-associated macrophages in neoplastic progression: a paradigm for the *in vitro* function of chemokines. *Lab Invest* 1994, 71, 5–16.
62. Lingen MW, Polverini PJ, Bouck N. Inhibition of squamous cell carcinoma angiogenesis by direct interaction of retinoic acid with endothelial cells. *Lab Invest* 1996, 74, 476–483.
63. Lingen MW, Polverini PJ, Bouck N. Retinoic acid induces cells cultured from oral squamous cell carcinomas to become anti-angiogenic. *Am J Pathol* 1996, **149**, 247–258.
64. Farber EM, Nall ML, Watson W. Natural history of psoriasis in 61 twin pairs. *Arch Dermatol* 1974, **109**, 207–211.
65. Nickoloff BJ. The cytokine network in psoriasis. *Arch Dermatol* 1991, **127**, 871 884.
66. Wolfe Jr JE, Hubler Jr WR. Angiogenesis in psoriasis. In Farber E, Cox A, eds. *Psoriasis: Proceedings of the Second International Symposium*. New York, York Medical Books, 1976, 375–377.
67. Wolfe JE. Angiogenesis in normal and psoriatic skin. *Lab Invest* 1989, **61**, 139–142.
68. Malhotra R, Stenn KS, Fernandez LA, Braverman IM. Angiogenic properties of normal and psoriatic skin associated with epidermis, not dermis. *Lab Invest* 1989, **61**, 162–165.
69. Nickoloff BJ, Mitra RS, Varani J, Dixit VM, Polverini PJ. Aberrant production of interleukin-8 and thrombospondin-1 by psoriatic keratinocytes mediates angiogenesis. *Am J Pathol* 1994, **144**, 820–828.
70. Nickoloff BJ, Riser BL, Mitra RS, Dixit VM, Varani J. Inhibitory effect of gamma interferon on cultured keratinocytes thrombospondin production, distribution, and biological activity. *J Invest Dermatol* 1988, **91**, 213–218.