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TUMORS: WOUNDS THAT DO NOT HEAL

Similarities between Tumor Stroma Generation and Wound Healing

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SOLID tumors are composed of two discrete but interdependent compartments: the malignant cells themselves and the stroma that they induce and in which they are dispersed.[1,2] In tumors of epithelial-cell origin — carcinomas — a basement membrane is often interposed between the tumor cells and the stroma, but in other types of tumors, malignant cells directly abut on or intermingle with stromal elements.[1,3]

An appreciation of tumor stroma is essential to an understanding of the biology of tumor growth; all solid tumors, regardless of their site of origin, require stroma if they are to grow beyond a minimal size of 1 to 2 mm.[4] The stroma provides the vascular supply that tumors require for nourishment, gas exchange, and waste disposal, and it may also limit the influx of inflammatory cells, thus providing a barrier to immunologic rejection.[5,6] There is a basis for the hope that future approaches to cancer treatment may be directed at preventing the generation of tumor stroma, thereby depriving tumors of the essential support services necessary for survival and growth. Of more immediate relevance to the clinician is the importance of stroma in tumor diagnosis. The scirrhous (from Greek *skirrhos*, "hard") character that enables the examining physician to palpate certain tumors (e.g., breast carcinomas) is largely attributable to a high proportion of collagenous stroma (Fig. 1).

In the past few years, tumor stroma — its composition, its function, and the steps by which it is generated — has become better understood.[6-15] Some remarkable similarities between the generation of tumor stroma and the healing of wounds have been uncovered. My purpose here is to review the pathogenesis of tumor stroma generation and present evidence that tumors behave in the body like wounds and, in fact, induce their stroma by activating the host's wound-healing response. I will suggest that tumor stroma generation is wound healing gone awry.

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COMPOSITION OF TUMOR STROMA

Tumor stroma is composed of many diverse elements.[1,2,4,13,14] These have traditionally been grouped in three main categories: new blood vessels, inflammatory cells (primarily lymphocytes and macrophages),[15] and connective tissue. The last-named includes matrix components — such as fibronectin, interstitial collagens, elastin, and glycosaminoglycans — and the cells responsible for matrix synthesis, including fibroblasts, myofibroblasts, and histiocytes. More recently, a fourth major component of tumor stroma has been recognized: the fibrin-gel matrix. Fibrin gel traps extravasated plasma proteins and water in the interstitium[5,6,16] and forms a provisional matrix that is subsequently replaced by mature stroma.

Although the same elements compose all tumor stroma, the quantity of stroma often varies strikingly from one tumor to another. At one extreme are desmoplastic tumors, such as common carcinomas of the breast (Fig. 1), stomach, and pancreas, in which the stroma may account for over 90 percent of the total tumor mass. At the other extreme are tumors such as malignant melanomas and medullary carcinomas, which possess only minimal stromata. The large differences in stromal content that distinguish various tumors cannot be attributed primarily to differences in the amount of blood-vessel or inflammatory-cell content. Angiogenesis, though essential for the growth of all solid tumors, represents only a small fraction of total tumor volume. Infiltration by inflammatory cells varies markedly among different tumors, and with some exceptions (e.g., Hodgkin's disease), seldom accounts for more than a few percent of the tumor mass. Instead, major differences in the stromal content of tumors largely reflect differences in the amount of connective tissue and fibrin gel. The pathogenetic relations that link the extravascular deposition of fibrin to the formation of new connective tissue are a major theme of this review.

EVENTS OF WOUND HEALING

For the purposes of comparison, I will briefly describe the process of normal wound healing. Local tissue injury results in the leakage of plasma or, more commonly, hemorrhage from damaged blood vessels.[17,18] Extravasated plasma or blood clots rapidly upon contact with tissue procoagulants, primarily tissue factor.[19] The initial clot is a gel that consists of fibrin, fibronectin, and platelets and that entraps plasma and blood cells. Platelets are important in wound

healing because they contribute soluble clotting factors,[20] a surface for prothrombinase assembly,[21] and mitogens and chemoattractants for connective-tissue cells.[20,22,23]

The clot that fills the wound serves as a provisional stroma into which inflammatory cells migrate and in which they ingest debris and degrade the gel locally.[8,17,23] New blood capillaries and fibroblasts follow close behind. Fibroblasts synthesize and deposit the same matrix components that make up tumor connective tissue — fibronectin, interstitial collagens, and glycosaminoglycans. The result is a cellular, edematous, and highly vascular tissue that retains remnants of the original fibrin–fibronectin gel matrix. This tissue is called "granulation tissue" — an old clinical term that accurately describes the granular appearance of the young connective tissue that fills open wounds.[1,17] Eventually, granulation tissue is remodeled, as blood vessels are resorbed and fibroblasts disappear.[1,8] The end result is a scar composed largely of dense collagen, with occasional, widely dispersed fibrocytes and blood vessels.

Fibrin: A Missing Link in Tumor Stroma Generation

Until recently, an important structural component of tumor stroma — the clotting protein fibrin — had been almost entirely overlooked.[6] This is surprising, given the long historical association between cancer and clotting.[24-27] More than a century ago, Trousseau recognized that abnormal hemostasis, which classically manifests as migratory thrombophlebitis, was the harbinger of underlying cancer (Trousseau's sign). Subsequent sporadic reports[28-31] presented histologic and immunologic evidence that fibrinogen or fibrin was deposited in solid tumors. However, these reports were received skeptically. The mere presence of fibrin in tumors does not by itself indicate that it has a role in the biologic development of tumors. For example, fibrin might just be an epiphenomenon associated with focal zones of tumor necrosis or an artifact from clotting that occurred during the removal or transplantation of tumors. Moreover, the experimental data were potentially flawed. The identification of fibrin by routine histologic studies is problematic, and before the development of monoclonal antibodies, immunologic methods could not reliably distinguish fibrinogen from fibrin. Furthermore, nearly all the heteroantiserums developed against fibrinogen also contain antibodies to fibronectin, a protein that is now also known to be a component of tumor stroma.

Recent experiments have dispelled these serious objections. Artifactual coagulation related to tumor manipulation or tissue necrosis has been circumvented.[5,9] Antiserums of proved specificity and monoclonal antibodies[32] have been used with immunoperoxidase methods to localize fibrin precisely in tumors (Fig. 2) and to distinguish it from both fibrinogen and fibronectin. Together, these data have established that fibrin is a regular stromal component of both autochthonous and transplantable tumors.[6,33,34]

Still more recent work has defined the biochemical nature of tumor-associated fibrin. The term "fibrin" does not denote a single entity but can refer to any of several derivatives of the circulating plasma protein fibrinogen.[35] Plasma fibrinogen is a 340,000-dalton hexamer composed of three pairs of nonidentical polypeptide chains (A alpha, B beta, and gamma) covalently linked in a dimeric structure by disulfide bonds.[35] When thrombin clots fibrinogen, it cleaves in sequence fibrinopeptides A and B from the A alpha and B beta chains, respectively, thereby generating soluble fibrin monomers that join spontaneously to form fibrin polymers that are not covalently linked. Only with the further action of factor XIIIa,[36] a transglutaminase that is itself activated by thrombin, does fibrin become covalently cross-linked through its gamma and alpha chains to yield the urea-insoluble molecule most commonly referred to as "fibrin." Extravasated plasma fibronectin is also incorporated into the fibrin clot by the action of factor XIIIa.[37]

Experiments using differential solubility, radiolabeled tracers, polyacrylamide-gel electrophoresis, and the Western blot technique have now demonstrated

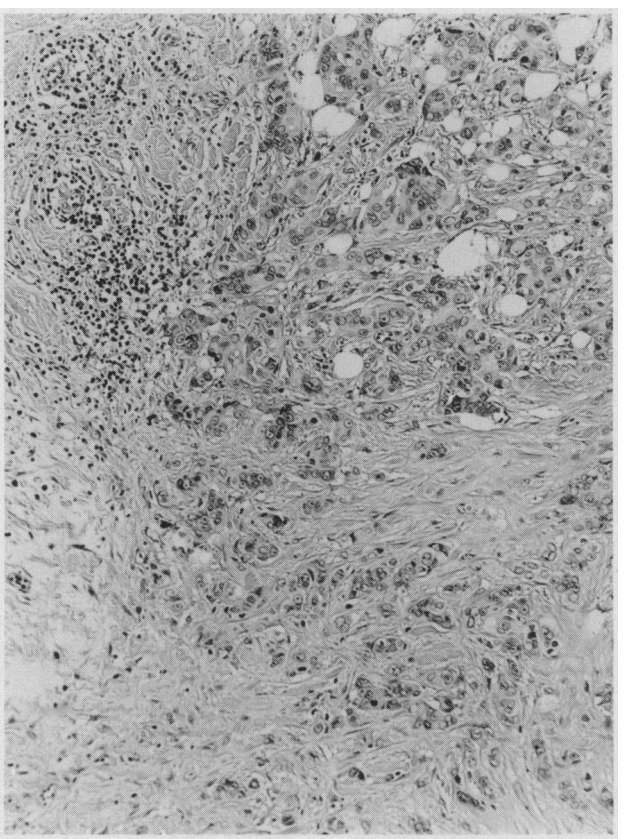

Figure 1. Typical Human Ductal Breast Carcinoma with Extensive Desmoplasia (Hematoxylin–Eosin, ×63).

Lymphocytes at the edge of the tumor (upper left) do not penetrate the fibrous stroma.

that the bulk of fibrin associated with tumors is covalently cross-linked — an important parallel with healing wounds, in which the deposition of cross-linked fibrin is also a central early event.

Regulation of Fibrin Deposition in Solid Tumors

Except for well-differentiated liver cancers, tumor cells do not synthesize fibrinogen or fibrin. Rather, tumor fibrin comes from extravasation and extravascular clotting of plasma fibrinogen.[5,6] For fibrinogen to extravasate, the microvasculature's normally low permeability to plasma proteins must increase substantially. In addition, a mechanism must exist for clotting extravasated fibrinogen. A third regulatory step, that of fibrin degradation, may also be anticipated, because tumor cells are rich in fibrinolysins.[38] The pathogenesis of tumor-associated fibrin deposition thus requires an understanding of how microvascular permeability, extravascular coagulation, and fibrinolysis are regulated. Recent work has established that tumor cells have an important role in each of these three steps (Fig. 3).

Increased Permeability of the Tumor Microvasculature to Plasma Proteins

Permeability to plasma proteins is strikingly enhanced in tumor microvasculature.[5,6,9-12,39,40] Radiolabeled fibrinogen, for example, enters tumors five times more rapidly than it enters control tissues.[9] Vasoactive inflammatory mediators cannot account for the hyperpermeability of tumor vessels,[6,10,11] but a satisfactory general explanation was recently proposed.[10,39]

Tumor cells of guinea pig, mouse, rat, hamster, and human origin all secrete the peptide vascular permeability factor, which causes normal blood vessels to leak plasma proteins.[39] Concentrations of vascular permeability factor are particularly high in tumor ascites, suggesting that this peptide has an important role in the accumulation of malignant fluid in body cavities. The peptide has been purified 10,000-fold from animal and human tumor sources and has a molecular weight of 34,000 to 42,000. It is active at picogram levels, and on a molar basis is over 1000 times as effective as histamine in enhancing vascular permeability.

Like histamine, vascular permeability factor induces the separation of endothelial cells in venules without injuring cells and does not provoke the accumulation of inflammatory cells. Studies of its mechanism of action are incomplete; however, the peptide does not act through basophils, mast cells, or any other classic mediator of increased vascular permeability.[6,10,11] At present, a specific rabbit antibody is the only known inhibitor of vascular permeability factor.[39]

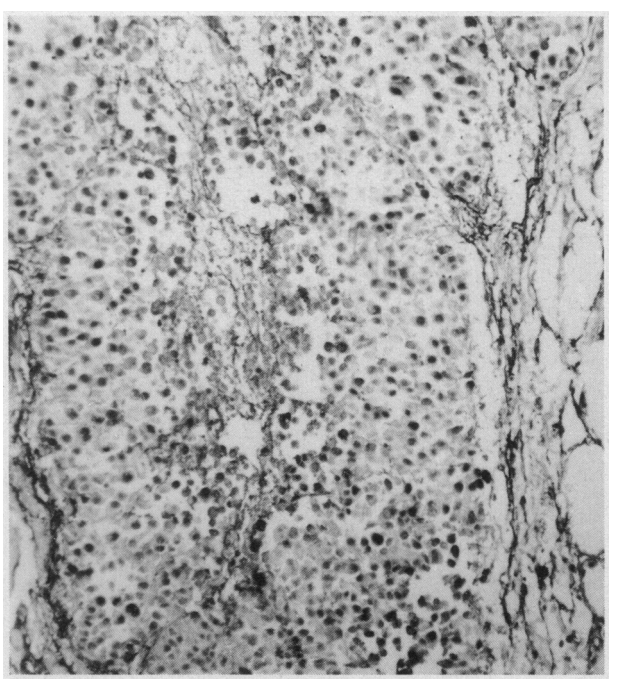

Figure 2. Fibrin in the Line 10 Bile-Duct Carcinoma Demonstrated with the Immunoperoxidase Technique (Hematoxylin–Eosin, ×128).

Even in this relatively fibrin-poor tumor, fibrin is readily identified as the darkly staining fibrils that surround the clumps of tumor cells. The monoclonal antibody that was used distinguishes fibrin from fibrinogen and was the generous gift of Dr. Gary Matsueda.[32]

Coagulation of Extravasated Plasma Fibrinogen

Fibrinogen that leaks from tumor blood vessels is rapidly clotted and transglutaminated to cross-linked fibrin. Tumor-cell procoagulants are probably important in this process.[7,24,25,27,41-44] The tumor procoagulant that has been documented most carefully is tissue factor, a phospholipoprotein cofactor that initiates the extrinsic pathway of the coagulation cascade.[19,24,25,27,41] Tissue factor increases the activity of factor VIIa by several orders of magnitude, thus greatly enhancing the catalysis of factor X to Xa.[19] Living tumor cells as well as tumor-cell homogenates express substantial tissue-factor activity.[24,25,27,41] In addition, many tumor cells release tissue factor into ascites fluid and into tissue-culture medium in the form of nanometer-sized vesicles that are shed from the plasma membrane of the tumor cell.[7]

Not all the procoagulant activity expressed by tumor cells or by shed tumor vesicles can be attributed to tissue factor, however. A variety of tumor cells are also active later in the coagulation cascade, at the level of prothrombinase generation.[42] Prothrombinase, the enzyme that cleaves prothrombin to form thrombin, is a complex of factors Va, Xa, and prothrombin, with an appropriate surface in the presence of calcium ions.[21] Critical to this association is a phospholipid surface that binds factor Va with high affinity. Tumor cells and their shed vesicles provide such a surface. Both bind factor Va avidly (K_d [dissociation constant] = 4×10^{-10} M) and promote efficient prothrom-

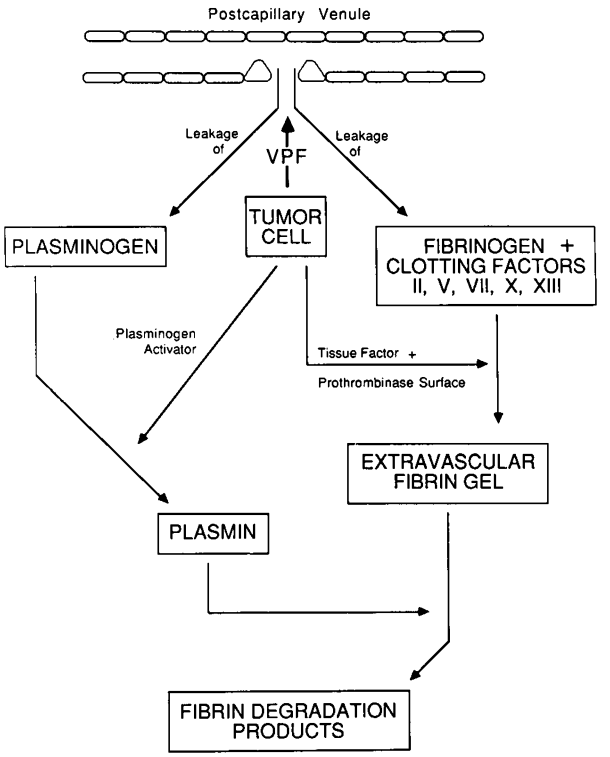

Figure 3. Tumor–Host Cell Interactions That Regulate Fibrinogen Influx, Extravascular Coagulation, Fibrin Deposition, and Fibrin Turnover in Solid Tumors.
VPF denotes vascular permeability factor.

binase assembly.[42] This is important, because activation of the coagulation pathway at any earlier step — for example, by tissue factor — would be ineffective without a functional prothrombinase capable of completing the cascade, generating thrombin from prothrombin and thence fibrin from fibrinogen. Several other coagulation-initiating activities have been ascribed to tumor cells,[43,44] but their role in vivo remains to be established.[6,11,24]

Although tumor procoagulants have attracted much interest, the capacity to effect coagulation in tissues is not a property unique to malignant cells. In several normal tissues, an increase in microvascular permeability sufficient to cause extravasation of plasma proteins leads to the same rapid extravascular deposition of cross-linked fibrin that occurs in tumors.[12] In these tissues, prominent niduses of fibrin emanate from the surfaces of normal tissue fibrocytes and histiocytes. This finding becomes less surprising when one recalls that the fundamental purpose of the clotting system is to staunch bleeding and thereby minimize blood loss after injury. To perform this function effectively, normal peripheral tissues would need procoagulants to clot any extravasated blood or plasma rapidly. Contrary to what many believe,[25,27,43] the capacity to induce coagulation should not be regarded as a newly acquired property of malignant cells but as a fundamental property shared by many cells that populate normal tissues, and one that is often retained by cells after they undergo malignant transformation.

To summarize, the extent of increased microvascular permeability, rather than the availability of tissue procoagulants, is the rate-limiting step that governs coagulation in both normal tissues and tumors.[12]

Fibrinolysis and Net Fibrin Accumulation

Immunohistochemical studies demonstrate large differences in fibrin content among various tumors.[5,8,33,34] These differences reflect differences in the local balance between fibrin deposition and degradation. The amount of fibrin in a tumor at any moment is determined by integrating the rates of fibrinogen influx, extravascular clotting, and fibrinolysis. Because of the persistent leakage of fibrinogen from hyperpermeable tumor vessels and its rapid coagulation and cross-linking in the extravascular space, fibrin is deposited continuously in tumors. However, much of it is rapidly degraded, so that the net accumulation of fibrin is very much less than would be predicted from the rates of fibrinogen leakage and initial clotting. Fibrin catabolism has been estimated at 90 percent or more of the rate of fibrinogen influx.[9]

The extensive fibrinolysis associated with tumors is generally attributed to tumor-secreted plasminogen activators[38] — highly selective proteases that catalyze the conversion of the plasma zymogen plasminogen to the actively fibrinolytic protease plasmin. To degrade tumor fibrin effectively, adequate amounts of substrate (plasminogen) must reach tumor tissues. Thus, like extravascular clotting, fibrinolysis also requires that tumor blood vessels be hyperpermeable to plasma proteins. Other enzymes, such as tumor-secreted cathepsins, may also have a role in fibrin degradation.[45]

In the few experimental tumors studied thus far, fibrinogen influx and initial clotting rates have been found to be similar, even in tumors that differ widely in fibrin content. In contrast, the net amount of tumor fibrin correlated inversely with the amount of plasminogen activators secreted by the tumor cells.[46] At least for these tumors, therefore, the large differences observed in fibrin accumulation probably reflect differences in fibrinolysis.

The amount and distribution of tumor-associated fibrin are remarkably constant for any given tumor[6,11] and, in the case of transplantable tumors, remain constant over several transplant generations. Furthermore, the amount and distribution of fibrin correlate with the amount and distribution of mature stroma that subsequently replaces it; that is, tumors with an abundant provisional fibrin matrix subsequently accumulate abundant mature collagenous stroma, suggesting a pathogenetic relation between these events (see below).

Although all tumors we have studied thus far possess at least some fibrin and connective-tissue stroma, the degree of tumor malignancy does not correlate with the amount of either. Equally aggressive or equally indolent tumors may possess widely different

amounts of fibrin matrix or collagenous stroma. Thus, the desmoplastic phenotype does not by itself guarantee tumor "success," and any advantage conferred by desmoplasia can be achieved by other means in tumors that possess only limited amounts of connective-tissue stroma. Parenthetically, it has also been difficult to correlate prognosis with the accumulation of inflammatory cells, despite the widespread belief that this major category of tumor stroma is important in limiting tumor growth.[15]

The Transformation of Provisional Fibrin–Fibronectin Matrix into Mature Collagenous Tumor Stroma

The fibrin–fibronectin gel that invests tumors is transformed over time into the vascular and collagenous matrix that constitutes mature tumor stroma. This transformation is one of the most important single events in tumor development; indeed, tumor angiogenesis, a process that has rightfully received considerable attention,[4] is really only one component of this metamorphosis.

The transformation of a provisional fibrin–fibronectin matrix into mature tumor stroma requires several sets of ordered cellular events. Monocytes enter the area and differentiate into macrophages.[18] Local fibroblasts and endothelial cells replicate.[17,18] Macrophages, blood vessels, and fibroblasts migrate into the fibrin–fibronectin gel. In the course of these events, the provisional fibrin–fibronectin matrix is degraded and progressively replaced with newly synthesized mature stroma.

Cell Accumulation, Activation, and Migration

Histologic evidence suggests that stroma regulates the access of inflammatory cells to tumors.[5,6,8,15] In many autochthonous and transplantable tumors, inflammatory cells, particularly lymphocytes, are confined largely to the tumor–host interface and do not penetrate mature tumor stroma (Fig. 1) or provisional matrix[5,6,15] to any important extent. The minority of tumors that are extensively infiltrated by inflammatory cells are often those with minimal connective-tissue stroma; examples include medullary carcinoma of the breast and Hodgkin's disease.

Recent studies of macrophage migration in fibrin gels offer some explanation for these histologic findings.[47] Depending on fibrin and thrombin concentrations and on the extent of cross-linking, fibrin matrices can either enhance or inhibit macrophage migration. At low concentrations (e.g., 1 mg per milliliter), cross-linked fibrin affords a preferred substrate for macrophage migration; however, macrophages find the higher fibrin concentrations measured in some tumors (e.g., more than 3 mg per milliliter) virtually impenetrable. Additional variables are also likely to affect inflammatory-cell migration through fibrin matrices. These include the pore size and elasticity of the gel, secretion or expression of plasminogen activators or other fibrinolysins by inflammatory cells, and interactions between gel fibrils and surface receptors for fibrin or fibronectin on macrophages and fibroblasts.[47,48]

The factors responsible for stimulating the migration of inflammatory cells, fibroblasts, and endothelial cells into tumors and wounds are not yet well understood. A large number of candidates have been identified. These include components of the gel matrix itself, degradation fragments of that matrix, and tumor-cell and lymphocyte products. For example, fibrinogen is reported to be chemotactic for endothelial cells,[49] and its plasmin-degradation products are thought to be chemotactic for several types of inflammatory cells.[50] Fibrin gels disrupt endothelial cells in tissue culture[51,52] and, when implanted in vivo, induce the influx of macrophages, new blood vessels, and fibroblasts.[5] A surprisingly large number of factors likely to be present in tumors and wounds are reportedly chemotactic for fibroblasts. These include lymphokines, a complement fragment, native collagens of Types I through V, fibronectin, certain proteolytic digestion fragments of collagen and fibronectin, and platelet-derived and tumor-derived growth factors.[22,53] Further work will be needed to sort out the respective roles and relative importance of all these factors in the stimulation and regulation of cell migration and division. At present, there are too many potential mediators for comfort.

Angiogenesis and Synthesis of Granulation Tissue and Mature Stroma

The ingrowth of new blood vessels and fibroblasts and the synthesis of the components of connective-tissue matrix together transform the fibrin–fibronectin stroma into a highly cellular, highly vascularized tissue that closely resembles the granulation tissue of healing wounds[5,8,17] (Fig. 4). Subsequently, this tumor granulation tissue acquires more collagen and loses its cellularity and vascularity — a process that closely parallels the later stages of wound healing.

Fibrin itself has a role in these events. Collagen is often deposited initially in intimate association with preexisting fibrin.[34] Moreover, as noted above, when cross-linked fibrin gels were implanted in laboratory animals, they were promptly infiltrated by macrophages, new blood vessels, and fibroblasts and were transformed into typical granulation tissue.[5] Other gels (e.g., collagen) induced no such response. Thus, once fibrin is deposited in tissues, angiogenesis and the formation of collagen stroma proceed autonomously, without the need for additional stimuli such as tumor cells or platelets.

Recently, the structural proteins that accumulate in tumor and wound stroma have been analyzed immunohistochemically (Table 1).[3,8,13,14] Fibrin staining, at first substantial in both, declines more rapidly and completely in normally healing wounds than in tumors. Staining for fibronectin also increases initially in both wounds and tumors but then declines precipitously in the former, although it persists in the

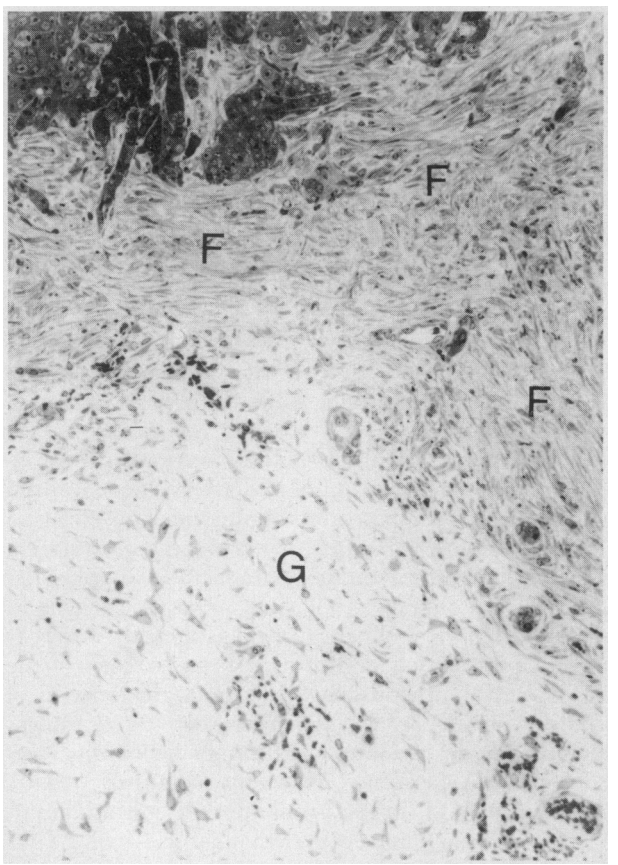

Figure 4. Generation of Mature Stroma in the Scirrhous Line 1 Bile-Duct Carcinoma Growing in a Syngeneic Strain 2 Guinea Pig Host.

The provisional matrix has been largely replaced by edematous, vascular granulation tissue (G) that continues to stain strongly for both fibrin and fibronectin by immunohistochemical methods (not shown). Granulation tissue, in turn, is being replaced by mature fibrous connective-tissue stroma (F) in zones adjacent to tumor cells (top). The figure is a 1-μm Epon section (Giemsa stain, ×250).

latter. Deposits of Types I and III collagen progressively increase in both processes. Thus, mature tumor stroma, like that of healed wounds, is composed largely of interstitial collagens.

Further investigation revealed an additional striking analogy between these processes. The proportions of Types I and III collagen in different areas of a single tumor were found to correlate with stromal maturity.[54] Older, sclerotic tumor stroma contained Type I collagen almost exclusively, whereas less mature stroma contained relatively more Type III. An identical progression from Type III to Type I collagen has been reported in healing wounds.[55,56]

Identification of the cell or cells responsible for synthesizing the individual components of tumor stroma has not been finally resolved. The majority opinion has long held that the collagen and other structural proteins that compose tumor stroma are products of benign fibroblasts that become associated with desmoplastic tumors.[1,2,8,13,57] On the other hand, almost all nucleated mammalian cells can synthesize one or more types of collagen.[58] Generally, malignant cells retain the ability to synthesize the collagen type or types associated with their cell of origin, though often in reduced amounts. Therefore, some have argued that tumor cells themselves synthesize desmoplastic stroma.[8]

This controversy entered a new phase when it was realized that collagens of Types I and III — and, more recently, Type V[59] — composed the bulk of tumor-stroma interstitium, whereas Type IV collagen was confined to basement membranes such as those that envelop blood vessels and epithelial tumor cells.[3,8,14] A number of benign and malignant epithelial cells synthesize Type IV collagen,[13,14,59-61] and in at least two instances, these same tumor cells were found not to make Types I, III, and V.[13,59] Conversely, fibroblasts taken from several sources synthesize collagen Types I and III and, to a lesser extent, Type V, but not Type IV. I am persuaded, therefore, that benign fibroblast cells of the host are generally responsible for synthesizing the several types of collagen that compose the desmoplastic stroma of carcinomas, whereas epithelial tumor cells synthesize primarily the Type IV collagen deposited in their basement membranes. However, there may be exceptions to this rule. At least some benign and malignant epithelial cells do synthesize interstitial collagens in tissue culture.[60,62] To discover whether they also do so in vivo will require the use of newer methods, such as in situ hybridization, that allow identification of the cellular sources of individual collagen types. Also, of course, interstitial collagens are the expected product of tumors derived from connective-tissue cells, such as sarcomas.[58]

Similarities and Differences between Tumor Stroma Generation and Wound Healing

Several important similarities between wound healing and the generation of tumor stroma are summarized in Table 2. Some additional important comparisons between these processes are discussed below.

Table 1. Structural Proteins That Compose the Stroma of Tumors and Wounds, as Determined by Immunofluorescence.*

	Time after Tumor Transplantation or Wounding			Normal Skin
	days			
	1–4	5–7	15–21	
Line 1 bile-duct carcinoma				
Fibrin	4+	3–4+	2+	—
Fibronectin	2+	3+	4+	—
Collagen, Types I and III	tr	4+	4+	—
Healing skin wound				
Fibrin	4+	1–2+	tr	0
Fibronectin	4+	3+	tr	tr
Collagen, Types I and III	0	4+	4+	4+

*Data drawn in part from Dvorak et al.[8] Deposits were graded on a scale of 0 to 4+; tr denotes trace.

First, wounds and consequently the fibrin deposited in them result from an external agency (e.g., injury or a surgeon's knife), whereas tumor cells induce local fibrin gels by secreting vascular permeability factor, which renders nearby vessels hyperpermeable to plasma proteins.[10,39] Therefore, the generation of tumor stroma and wound healing are initiated by different mechanisms. In both, however, plasma spills into the extravascular space, thereby triggering the clotting cascade and its sequelae.

Second, platelets participate in the hemostasis and the generation of provisional matrix that initiate the wound-healing process.[1,22] Platelets may also be important at later stages of wound healing, because they produce platelet-derived growth factor, a potent mitogen and chemoattractant for fibroblasts.[20,22] In contrast, platelets have not been found outside of blood vessels in solid tumors. However, tumor cells themselves perform some of the functions ascribed to platelets in healing wounds. Among these are the expression of procoagulant activity, including the provision of a platelet-like surface suitable for generating prothrombinase.[21,42] In addition, many tumor cells secrete transforming growth factors that function as fibroblast mitogens and chemoattractants.[63-65] At least one oncogene product has a close homology with platelet-derived growth factor.[22,63-65]

Third, fibrin and — to an even greater extent — fibronectin persist in tumor stroma, whereas both proteins appear only transiently in wounds that heal normally (Table 1).[8,66,67] This difference is probably explained by the fact that tumors produce vascular permeability factor constitutively.[39] The result is protracted vessel leakage and continuing clotting of extravasated fibrinogen and fibronectin. In wounds, on the other hand, normal vascular permeability is restored within a few days after injury, and prolonged leakage of fibrinogen or fibronectin is thereby prevented.

Table 2. Similarities between Tumor Stroma Generation and Wound Healing.*

Initiation by leakage of plasma proteins from hyperpermeable or injured blood vessels, often accompanied in wounds by spillage of platelets and other blood cells.
Extravascular clotting primarily by the tissue-factor pathway; factor XIII cross-linking of fibrin and extravasated plasma fibronectin to form a fibrin–fibronectin gel that serves as a provisional matrix.
Transformation of the fibrin–fibronectin provisional matrix into mature stroma.
Replication and immigration of inflammatory cells (particularly macrophages), fibroblasts, and new capillaries.
Degradation of fibrin–fibronectin gel matrix.
Synthesis by fibroblasts of fibronectin, interstitial collagens, and glycosaminoglycans.
Synthesis of additional interstitial collagen, resorption of capillaries, and disappearance of many fibroblasts; the result is dense, relatively acellular, poorly vascularized, and hypoxic connective tissue (desmoplasia or scar).

*An additional similarity not directly related to stroma formation also deserves mention. In the case of healing wounds (for example, a laceration of the skin), epithelial cells are activated to divide and to migrate to cover the breach; they also synthesize products peculiar to basement membranes, such as Type IV collagen and laminin.[8,17,23] Tumor cells engage in very similar activities, but they are less tightly regulated; that is, malignant cells also divide (not necessarily any faster than healing benign epithelium), migrate (invade), and at least in the case of tumors of epithelial origin (carcinomas), also synthesize Type IV collagen and laminin.[3,8,13,17]

Fourth, the abundant fibrin deposited around some tumors may serve as a cocoon that hinders lymphocytes, macrophages, and other inflammatory cells from reaching tumors.[5,8,15,47] In wounds, however, inflammatory cells readily infiltrate the fibrin–fibronectin provisional matrix. This difference may also be attributed to the protracted hyperpermeability of blood vessels in tumors, which results in local fibrin concentrations sufficient to impede the immigration of inflammatory cells.[47] In contrast, at the lower concentrations found in healing wounds, fibrin provides a matrix that actually facilitates cell migration.

Furthermore, routine histologic sections of desmoplastic tumors reveal abundant dense collagen but relatively little fibrin or granulation tissue. This observation has been used to argue that fibrin and granulation tissue are not intermediate products in desmoplasia,[14] and therefore that desmoplasia is not a type of wound healing. However, this argument is flawed. The reliable demonstration of fibrin in tumors requires the use of special methods, such as immunohistochemical studies (Fig. 2) or electron microscopy[5,33,34]; unless such techniques are employed, tumor fibrin may easily be missed. Certainly, successive stages of stromal maturation — fibrin gel, granulation tissue, and finally, mature collagenous connective tissue — can be observed in single biopsies of the same tumor (Fig. 4).[5,8] It must be emphasized that in any sequence of linked reactions such as those that make up tumor stroma generation, intermediate products will accumulate only when their rates of generation and degradation are imbalanced. If the rate of degradation equals or exceeds that of formation, the product will not accumulate. Thus, as intermediate products, fibrin and granulation tissue will be visualized only when their rates of generation exceed the rates of their progression to further stages along the pathway of stromal maturation.

In addition, tumors and wounds are both hypoxic tissues.[68,69] Tumor hypoxia results from the insertion of a fibrinous and later a collagenous matrix that separates tumor cells from blood vessels; also, the angiogenesis that follows is characteristically suboptimal, providing tumors with less blood than that available to adjacent normal tissues.[4] Wounds are hypoxic for similar reasons. Blood vessels are damaged and fibrin is deposited at the time of injury. As with tumors, repair leads to poorly vascularized scar tissue.

Finally, a word should be said about the amounts of connective-tissue stroma that accumulate in tumors and wounds. I suggested above that the amount of collagenous stroma deposited in tumors was related to the net amount of fibrin laid down earlier as provisional stroma and that the net deposition of fibrin was, in turn, determined by a balance between fibrin-gel deposition and fibrinolysis. Desmoplasia thus reflects

an imbalance in which large amounts of provisional fibrin stroma accumulate, presumably because of limited fibrinolysis.[9] Pathologic overproduction of collagen may also occur in wound healing, resulting in hypertrophic scars and keloids. The pathogenesis of these entities is poorly understood.[70] Perhaps keloids, like desmoplastic tumors, result from an imbalance between clotting and fibrinolysis, so that excessive fibrin accumulates and is subsequently transformed into scar tissue.

Conclusions

Wound healing and the generation of tumor stroma share many important properties. Both begin with the spillage of plasma proteins, including fibrinogen, fibronectin, and plasminogen. In both processes, extravasated fibrinogen is clotted and cross-linked to itself and to fibronectin, inserting into the tissues an insoluble, water-holding gel. In both processes, this extravascular fibrin–fibronectin clot serves as a provisional stroma, providing a matrix for the immigration of macrophages, fibroblasts, and new capillaries. In both, the fibrin–fibronectin gel is degraded and transformed into granulation tissue and eventually into dense, relatively acellular collagen.

The recognized differences between tumor stroma generation and wound healing are minor and can be attributed primarily to the distinct mechanisms that initiate each. In most wounds, extravascular fibrin gel is laid down for only a limited interval after injury. In contrast, tumors constitutively secrete a vascular permeability factor that renders local blood vessels permeable to plasma proteins for protracted periods. The result is persistent extravasation of fibrinogen and fibronectin around tumors and the continuous generation of new provisional matrix. Fibrin and subsequently mature collagenous stroma provide a cocoon for tumors that may protect them from host inflammatory cells, particularly the lymphocytes and macrophages that are thought to be responsible for host defense. A second obvious difference, the participation of platelets in wounds but not in tumors, may be less important, because tumor cells themselves perform several critical functions of platelets; these include the capacity to express procoagulants and to secrete factors that are chemotactic and mitogenic for both inflammatory and connective-tissue cells.

Viewing wound healing as a paradigm for the generation of tumor stroma makes considerable biologic sense. Without linking tumor stroma generation to some fundamental host process, one is forced to postulate that the body responds to tumors with a unique mechanism, some deus ex machina whose sole function is to generate tumor stroma. This perspective should also encourage the development of new therapeutic strategies that aim to limit tumor growth by interfering with stroma generation. Like the scar tissue of healing wounds, mature tumor stroma is composed of poorly vascularized, hypoxic connective tissue that is poorly adapted to support rapidly growing, actively metabolizing tumor cells. With so tenuous a blood supply, it is not surprising that tumors commonly undergo extensive ischemic necrosis in the absence of any treatment. It follows that even slight therapeutic disruptions of stroma generation could profoundly affect tumor growth and survival.

In summary, I argue that successful tumors — that is, tumors that grow progressively in the host — are obligate parasites. They have developed the capacity to preempt and subvert the wound-healing response of the host as a means to acquire the stroma they need to grow and expand. They mimic wounds by depositing an extravascular fibrin–fibronectin gel. Such gels, in tumors as at sites of local injury, signal the host to marshal the wound-healing response. This response is stereotyped and similar in both tumors and wounds. In tumors, however, the fibrin–fibronectin matrix signal that evokes the wound-healing response is not self-limited; it continues to operate and new gel is continuously deposited. Thus, tumors appear to the host in the guise of wounds or, more correctly, of an unending series of wounds that continually initiate healing but never heal completely.

Discussion

Dr. Jeffrey Flier: Could you say something further about the analogy between vascular permeability factor and histamine? Does vascular permeability factor have other actions similar to that of histamine? Is it a peptide analogue of something that binds to the histamine receptor?

Dr. Dvorak: Vascular permeability factor is a large peptide that does not cause anaphylactic symptoms after systemic injection and is not inhibited by either type H_1 or H_2 antihistamines.[39] Therefore, it seems unlikely that this factor is closely related to histamine. Vascular permeability factor is also distinct from other vasoactive mediators commonly encountered in inflammation, including clotting factor XIIa, bradykinin, serotonin, prostaglandins, leukotrienes, and acetyl glyceryl ether phosphorylcholine.

Dr. Howard Frank: You have shown us a lovely series of steps in the progression of tumor stroma to scar. I am also told that scars sometimes lead to tumors. Could you comment on this?

Dr. Dvorak: There has been a belief that certain cancers develop in relation to preexisting scars. "Scar cancers" have been described as tumors (for the most part adenocarcinomas) that arise in the peripheral lung adjacent to suspected sites of scarring related to earlier infection or infarction. It has been assumed that such tumors arise from atypical epithelium that regenerated after tissue injury. Others, however, have challenged this notion and have argued that the "scar tissue" found in association with such tumors is actually a desmoplastic response of the host to the tumor, not a preexisting scar in which cancer arose. This ar-

gument is supported by biochemical analyses,[54,71] which reveal a relative increase of Type III collagen in such tumors. This has been taken as evidence that the "scar" is composed of immature connective tissue and is part of an ongoing process. In contrast, areas of fibrosed lung tissue at some distance from tumor expressed a "mature" collagen pattern with an increase in Types I and V collagens and a relative decrease in Type III collagen. These findings reinforce the main thrust of my lecture — that the capacity to initiate the wound-healing response is a fundamental property of tumors and that tumors are wounds that ultimately don't heal.

A PHYSICIAN: What is the difference between a solid tumor and an ascites tumor, and why doesn't ascites fluid clot?

DR. DVORAK: You have posed a very important question. Solid tumors consist of groupings of malignant cells interspersed in a stroma that they themselves induce. In contrast, a very different growth pattern is commonly observed when the same tumor cells enter serous body cavities, such as the peritoneum or pleural space. Here, malignant cells proliferate in suspension in the apparent absence of any organized stroma. Unless tumor cells form implants on the peritoneal wall (and thus form solid tumors) or unless gross hemorrhage occurs, fibrin is not deposited in ascites tumors. Tumor ascites fluid clots poorly or not at all. Thus, fibrinogen or fibrin metabolism apparently represents a central point of difference between solid and ascites forms of tumor growth. The reasons for this difference have not yet been worked out. Obvious possibilities are the failure of intact fibrinogen to enter body cavities or the rapid catabolism of fibrinogen when it does. Consistent with the latter are reports of soluble products of fibrin degradation in tumor ascites fluids.[72]

DR. FLIER: Is there any evidence that people or animals with extensive tumors have an increase in vascular permeability apart from the local tumor site?

DR. DVORAK: Albumin is reportedly cleared from the plasma of patients with advanced cancer at a rate about twice that of normal subjects.[73] However, this finding does not distinguish between a generalized or systemic increase in microvascular permeability and one that is confined to local sites of tumor growth. We have rarely detected the activity of vascular permeability factor in the blood of animals with advanced cancer. Generally, however, the increase in microvascular permeability is sharply confined to tumors; normal tissues even a few millimeters away exhibit normal vascular permeability.

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