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Role of Stromal Fibroblasts in Cancer: Promoting or Impeding?

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Abstract
The basement membrane, immune cells, capillaries, fibroblasts and extracellular matrix (ECM) constitute the tumour stroma, commonly referred to as the 'reactive stroma'. The fibroblasts from the initial stages of a tumour, as the main constituents of the reactive stroma, present a different phenotype from the normal fibroblasts and play a crucial role in tumour progression. This review presents the differences between normal and tumour stromal fibroblasts and analyzes the molecular mechanisms (which involve growth factors, ECM components, matrix metalloproteinases, integrins and cell adhesion molecules) in the complex interactions between stromal fibroblasts and tumour cells. To date, several examples of heterotypic interactions between tumour stromal fibroblasts and tumour cells have supported the hypothesis that the tumour stroma promotes the growth of the tumour mass, as well as invasion and metastasis. However, it remains possible that the stroma acts essentially as a local modulator to impede tumorigenesis at an early stage and that the desmoplastic response is a host defence reaction designed to confine the developing tumour. The latter hypothesis has largely been neglected. The review aims to give a broader view on the role of stromal fibroblasts in tumour growth, invasion and metastasis.

Normal and Reactive Stroma

A tumour consists of far more than a collection of homogenous cancer cells, it also includes the 'stroma', an extracellular and cellular tissue framework that surrounds and interacts with the cancer cells [1]. The composition of tumour stroma can vary significantly between tumour types and between different locations, creating structural heterogeneity. This suggests that stroma formation depends on a complex set of interactions between cancer cells, non-malignant cells and the extracellular matrix in a particular tissue.

In general, stroma accompanies a tumour from its initial stages (carcinoma in situ), and is characterized by modifications in the non-epithelial cell types that secrete extracellular matrix (ECM) proteins and growth factors [2–5]. The tumour stroma is constituted by the basement membrane, immune cells, capillaries, fibroblasts and ECM [6–11]. The stroma has similarities to that observed during wound healing, and it is commonly referred to as 'reactive stroma' [8, 9]. The influence of the 'reactive stroma' on cancer cells and vice versa is still debated, but

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there is evidence that active communication occurs between the two through the basement membrane barrier [8–10]. It is still not fully understood what molecular cues determine whether the tumour remains contained in the basement membrane or becomes invasive by degrading the membrane [10]. This creates a huge interest in understanding the differences between normal stroma and 'reactive tumour stroma'. Normal stroma in most organs contains a minimal number of fibroblasts in association with a physiological ECM [7]. Reactive stroma is associated with an increased number of fibroblasts expressing high amounts of α-smooth muscle actin (α-SMA) [11], enhanced capillary density, type I and VI collagen, laminin, entactin, heparan-sulfate proteoglycans and fibrin deposition [11].

Normal Fibroblasts

Fibroblasts were first described in the late 19th century, based on their location and their microscopic appearance [12]. They are the non-vascular, non-epithelial and non-inflammatory cells of the connective tissue [7, 13], and originate from differentiated mesenchymal cells. The fibroblast is the cell responsible for the production of the collagen fibers. Usually the young active secretory cell is referred to as the fibroblast, whereas the older non-secretory cell is called the fibrocyte. In the active spindle-shaped fibroblast, the nucleus contains prominent (usually 1–3) nucleoli and is surrounded by abundant, slightly basophilic cytoplasm. The thinner spindle-shaped fibrocyte has an ovoid flattened nucleus with scanty chromatin, no nucleolus and a faint cytoplasm [14].

Fibroblasts play a crucial role in the deposition of the ECM, the regulation of epithelial differentiation and the down-regulation of inflammation by their involvement in the process of wound healing [13, 15, 16]. As far as the deposition of the ECM is concerned, fibroblasts synthesize many of its constituents, such as type I, III and V collagen and fibronectin and they contribute to the formation of the basement membrane, by secreting collagen type VI and laminin [17]. In addition, they play a crucial role in the maintenance of ECM homeostasis, by regulating its turnover, through the production of the ECM-degrading proteases, called matrix metalloproteinases (MMPs) [18]. Primary cancers secrete MMPs, which break down the basement membrane, allowing direct invasion of underlying tissue and into blood vessels [17]. Very recently, Yana and colleagues [19] suggested that cooperation between stroma-derived MMP2 and tumour-derived membrane type 1-MMP may play a role in tumour invasion and proliferation via remodeling of the tumour-associated basement membrane. Hence, MMPs are considered to be major key-players in the proteolytic cleavage of ECM components, such as collagen and elastin [19]. The tissue inhibitors of MMPs regulate MMP activity [20]. Several previous studies showed that MMPs and their tissue inhibitors were frequently overexpressed in head and neck squamous cell carcinomas, pointing to a crucial role for these proteins in the progression of cancer [21]. In addition, the MMPs have been shown to be involved in the early stages of tumor growth [22]. For example, when MCF-7 human breast cancer cells are injected into nude mice, they form tumours inefficiently and with a long latency. Co-injection of MCF-7 cells with fibroblasts significantly enhances the efficiency of tumor formation and decreases the latency with which tumours form [23].

Apart from maintaining ECM homeostasis, fibroblasts are responsible for the maintenance of adjacent epithelia, through the secretion of growth factors and through direct mesenchymal-epithelial interactions. Under normal circumstances, growth factors, such as transforming growth factor β (TGF-β) and fibroblast growth factor that are secreted during these interactions, enhance the intercellular induction of apoptosis. This induction of apoptosis is mainly regulated by 4 different reactive oxygen species-mediated pathways (NO/peroxynitrite pathway, HOCl pathway, consumption of NO and nitrylchloride pathway). In this case intercellular induction of apoptosis eliminates transformed cells, unless transformed cells establish resistance against reactive oxygen species-mediated intercellular signalling [24]. A cross talk between neighbouring fibroblasts and transformed/tumour cells is then established, giving rise to tumour progression, a situation that needs to be investigated, since it has been underestimated in the past [24].

Reactive Stromal Fibroblasts

As previously mentioned, fibroblasts are not the main constituent of normal stroma, they are surrounded by a physiological ECM and usually lack markers that indicate other lineages. However, fibroblasts at a tumour stroma site are markedly abundant and despite everything we know of their typical characteristics, these tend to express a different phenotype [25, 26]. The reason for their development, as well as their function in a tumour, remains poorly defined and understood at the molecular level [27].

Table 1. Summary of primary activities and principal source of major growth factors in tumorigenesis

Growth factors	Principal source	Primary activities
VEGF	Thrombocytes in peripheral blood, platelets	Increases vascular permeability, plays a role in the earliest stages of the desmoplasia
PDGF	Platelets, endothelial cells, placenta	Promotes proliferation of connective tissue, glial and smooth muscle cells
EGF	Submaxillary gland, Brunner's gland	Promotes proliferation of mesenchymal, glial and epithelial cells
TGF-α	Common in transformed cells	May be important for normal wound healing
TGF-β	Activated Th_1 cells and NK cells	Anti-inflammatory (suppresses cytokine production and class II MHC expression), promotes wound healing, inhibits macrophage and lymphocyte proliferation
FGF	Wide range of cells, protein is associated with the ECM	Promotes proliferation of many cells, inhibits some stem cells, induces mesoderm to form in early embryos
IGF-I	Primarily liver	Promotes proliferation of many cell types
IGF-II	Variety of cells	Promotes proliferation of many cell types primarily of foetal origin

EGF = Epidermal GF; FGF = fibroblast GF; IGF = insulin-like GF; MHC = major histocompatability complex; NK = natural killer; TGF = transforming GF; Th_1 = T helper 1.

Another characteristic of the heterogeneity of fibroblasts is that tumour stromal fibroblasts cultured in vitro differ from normal fibroblasts regarding their morphology and growth patterns [25, 26, 28–31]. It is worth noting that during invasion, the basement membrane is degraded and the activated stroma, which contains activated fibroblasts (also called myofibroblasts), inflammatory infiltrate and newly formed capillaries, comes into direct contact with the tumour cells [7, 32, 33]. This neo-vascularisation causes enhanced vessel permeability, which generates increased fibroblast proliferation and alignment, causing increase of ECM stiffness [34–36]. The fibroblasts differentiate into contractile myofibroblasts, which develop stress fibers composed of actin and myosin and they de novo express α-smooth muscle actin (α-SMA) [32]. In this case there is expansion of the tumour stroma and increased deposition of ECM, creating a dense fibrotic capsule around the tumour [32]. Such an increased ECM deposition in tumours is known as 'desmoplasia' and is similar to changes that are observed during tissue fibrosis [7, 32–36].

Stroma Fibroblast-Tumour Interactions in Tumour Growth, Invasion and Metastasis

The interactions between the stroma and tumour involve several growth factors. These are set out below.

VEGF

Angiogenesis is mainly controlled by vascular endothelial growth factor (VEGF), its related proteins (VEGFs B, C and D) and platelet-derived growth factor (PDGF), which are potent and highly specific mitogens for endothelial cells [37–39]. Therefore, in addition to recruiting new vessels to already-established tumors, the VEGFs may play a role in the earliest stages of the desmoplasia due to their ability to increase vascular permeability. Indeed, early in the generation of the desmoplastic stroma, fibrin clots, composed predominantly of fibrin and fibrinogen, are observed [40]. In breast cancers, VEGF plays a major role in angiogenesis since it is highly expressed in ductal carcinomas and, to a lesser extent, in lobular carcinomas. In both tumor types, VEGF expression is detected in the epithelial compartment whilst being almost absent in stromal cells [41] (table 1).

TGF-β and PDGF

Another two epithelium-produced growth factors, TGF-β and PDGF have been either shown or speculated to generate the desmoplastic stroma [42–45] (table 1). TGF-β specifically is a morphogenic factor that plays a key role in a wide range of biological processes, such as cell proliferation, development, wound healing, inflammation and angiogenesis. Through its variable roles, it seems that it is crucial in the process of desmoplasia and the development of epithelial tumours. This concept was

Table 2. Summary of primary activities and principal source of interleukins in infection

Inter-leukins	Principal source	Primary Activity
TNF-α	Macrophages	Decrease the surface expression of Thy-1 and increase α-SMA levels
IL-2	Activated Th_1 cells, NK cells	Proliferation of B cells and activated T cells, NK functions
IL-4	Th_2 and mast cells	B cell proliferation, eosinophil and mast cell growth and function, IgE and class II MHC expression on B cells, inhibition of monokine production
IL-6	Activated Th_2 cells, APCs, other somatic cells such as hepatocytes and adipocytes	Acute phase response, B cell proliferation, thrombopoiesis, synergistic with IL-1 and TNF on T cells
IL-8	Macrophages, other somatic cells	Chemoattractant for neutrophils and T cells
IL-11	Stromal cells	Synergisitc haematopoietic and thrombopoietic effects
IL-12	B cells, macrophages	Proliferation of NK cells, INF-γ production, promotes cell-mediated immune functions
IL-13	Th_2 cells, B cells, macrophages	Stimulates growth and proliferation of B cells, inhibits production of macrophage inflammatory cytokines
IL-14	T cells and malignant B cells	Regulates the growth and proliferation of B cells
IL-15	Virus infected macrophages	Induces production of NK cells
IL-16	Eosinophils, CD8+ T cells, lymphocytes, epithelial cells	Chemoattractant for CD4+ cells
IL-17	Subsets of T cells	Increases production of inflammatory cytokines, angiogenesis
IL-18	Macrophages	Increases NK cell activity, induces production of INF-γ

INF = Interferon; MHC = major histocompatability complex; NK = natural killer.

tested in a recent study using conditional inactivation of TGF-β signaling pathway [42]. This hypothesis was highlighted by generating mice, the fibroblasts from which were unable to respond to TGF-β, due to fibroblast-specific inactivation of the TGF-β type II receptor ($Tgfbr2^{fspKO}$) [42]. Bhowmick et al. [43] observed that these mice developed aggressive cancers derived from the prostate and stomach epithelium. It is interesting that in the other tissues, such as skin, esophagus, lung, kidney and liver, there was no phenotypic difference between the $Tgfbr2^{fspKO}$ mice and wild types [42]. The authors proposed that it was the result of other TGF-β-related proteins that signal through alternate receptors. In the study from Bhowmick at al. [43] it was shown that normal, presenescent fibroblasts or prostate stromal cells, co-transplanted with prostate carcinoma cells into nude mice, reduced tumour latency and accelerated tumour growth. When their TGF-β signaling was blocked, the fibroblasts and stromal cells still stimulated tumour initiation, but no longer supported tumour growth as control cells did [43]. These findings point to functional differences between fibroblasts from different organs and support the concept that fibroblast heterogeneity can differentially condition a tumor microenvironment to promote tumor growth [44].

PDGF plays an important role in the activation of fibroblasts and tumour progression. PDGF is a potential mitogen and chemoattractant for mesenchymal cells, functioning as homo- or heterodimer of PDGF-A and B polypeptides (PDGF-AA, PDGF-AB or PDGF-BB). It has been shown that generation of a PDGF autocrine signalling loop in human cells by introduction of PDGF into cells expressing PDGF receptors, especially fibroblasts and glioblastoma cells, causes their tumourigenic conversion [45]. Such autocrine growth stimulation, however, does not explain the activities of PDGF in carcinomas. PDGFs A and B are upregulated in a number of breast epithelial tumor cell lines that do not themselves express PDGF receptors, suggesting that PDGF contributes to the growth of tumor cells through a paracrine mechanism [46–48]. Proof of this notion has come from studies in which PDGF-β was overexpressed in either melanoma cells [49] or keratinocytes [50] that lack PDGF receptors. Heterotypic signalling of PDGF from epithelial cells to

normal fibroblasts results in the activation of the stromal environment and promotion of tumorigenesis. PDGF has been shown to be essential for the desmoplastic response in a human breast cancer xenograft model [51].

While both TGF-β and PDGF appear to play critical roles in desmoplasia, both promote this stromal response by quite different mechanisms. PDGF is mitogenic for primary fibroblasts, whereas TGF-β is inhibitory. In addition, PDGF does not induce the differentiation of fibroblasts into myofibroblasts in culture conditions, whereas TGF-β1 does [52]. It has been hypothesized that the induction of desmoplasia by PDGF may occur indirectly through macrophages, which are highly chemotactic for PDGF [53, 54]. Macrophages recruited to the tumour may secrete TGF-β1, leading to the development of the reactive stroma. It is unclear whether the desmoplasia caused by epithelium-produced PDGF is mediated by direct heterotypic signalling to fibroblasts, by indirect signaling to macrophages, or perhaps through both routes. Hence, it is also unclear whether blocking PDGF signalling in a defined fibroblast subset would prevent tumour progression or not.

Cytokines

The proliferative response of epithelial cells seems also to be affected by the production of several cytokines, which are secreted proteins that mediate and regulate immunity, inflammation and haematopoiesis. These must be produced de novo in response to an immune stimulus and they act at very low concentrations. Recently, Hagood et al. [55] showed that exposure of normal human lung fibroblasts to inflammatory cytokines, such as interleukins-1β (IL-1β) and tumour necrosis factor-α (TNF-α) decreased the surface expression of Thy-1 (a cell surface glycoprotein belonging to the immunoglobulin-like superfamily) and increased α-SMA levels (table 2). IL-1 is a mediator of fibroblast activation and it is interesting to speculate that decreased Thy-1 expression on human lung fibroblasts provoked by IL-β may facilitate tumorigenesis in an inflammatory environment by promoting the tumour-associated fibroblast phenotype [56] (table 2). In addition to the role of IL-β, Lejeune et al. [57] have noticed that in malignant disease, high dose local administration of TNF selectively destroys tumour blood vessels, but when chronically produced, TNF may act as an endogenous tumour promoter, contributing to the tissue remodelling and stromal development, necessary for tumour growth and spread [57]. The diversity of the TNF's importance in fibroblast activation is obvious, as a recent study showed that TNF-α alone decreased the basal α-SMA expression [58]. While the physiological significance of these observations remains unknown, the inflammatory state of a tissue seems to support cancer progression.

Alteration of ECM and Cell Adhesion by Tumour-Stroma Interactions

ECM, MMPs and Integrins

In parallel with the above-mentioned factors, ECM in several ways modulates cell differentiation, morphology and proliferation, acting as a substrate to which cells can adhere and as a reservoir of growth factors. The ECM is a complex structural entity of the basement membrane separating the epithelium and the underlying tissue, surrounding and supporting cells. It is composed of three major classes of biomolecules. These biomolecules are structural proteins (collagen and elastin), specialized proteins (fibrilin, fibronectin and elastin) and proteoglycans, composed of a protein core to which long chains of repeating disaccharide units called glycosaminoglycans are attached. The ECM is connected to the nucleus by a network of protein molecules that include transmembrane adhesion proteins, the cytoskeleton and the nuclear matrix. It is one of the environmental factors, together with hormones, that communicate with the cell nucleus, modifying nuclear structures and leading to selective gene expression. It has been proved that the matrix networks are different between normal and tumour cells, and modification of ECM induces alterations in the composition of the nuclear matrix [30, 59].

As MMPs break down the basement membrane, allowing direct invasion of underlying cancer cells and into blood vessels, intravascular solutes of the ECM, such as laminins, are thought to play an important role in allowing cancer cells circulating in the peripheral blood to elude the immune surveillance mechanism and to attach and adhere at the site of metastasis [60]. Saito and Kameoka [60] found that colorectal cancer patients are often laminin-positive, and that the laminin-positive rate tends to increase as the disease stage progresses and as patients develop hepatic metastases. Apart from laminin being a highly active ECM protein involved in tumour progression and metastases, several other proteins play a crucial role. Among these ECM components, fibronectin and tenascin represent predominantly adhesive fibrous proteins, which play a fundamental role in the signalling process between cells and in the control of the activity of other secreted proteins, such as growth factors, proteases

and protease inhibitors. Bento et al. [61] concluded that fibronectin and tenascin are both expressed in pleomorphic adenomas, although tenascin shows a profound expression in all types of stroma present in these adenomas of both major and minor salivary glands. Furthermore, previous studies by Ramos et al. [62] showed the neo-expression of αvβ6 integrin (one of tenascin's integrin receptors) at the invading front of oral cancer tumour nests and identified tenascin-C, a ligand for the αvβ6 complex, in the reactive stroma immediately surrounding the tumour cells. At the same type of cancer, Thomas et al. [63] showed that αvβ6 integrin promotes invasion of squamous carcinoma cells through up-regulation of MMP-9. On the contrary, in normal oral mucosa very little tenascin-C and no αvβ6 could be detected [59, 64]. Finally, Yang and colleagues [65] managed to support the importance of versican, another ECM proteoglycan, in cancer cell biology and specifically in the complex pathways of apoptosis regulation. This particular group had shown that overexpression of V1 versican isoform in cultured fibroblasts (V1 cells) increases both proliferation and apoptotic resistance. Unexpectedly, however, V1 cells were shown to have high resting levels of p53 and murine double minute-2 proteins, correlating with apoptotic sensitivity [65].

The above information shows the close connection between so many molecules, functioning in the interactions of tumour stroma and tumour cells. Integrins belong to a large group of transmembrane proteins (around 24) that are formed from 18α and 8β subunits, each with distinct ligand binding and signaling properties [66]. With their extracellular domain, integrins can bind to different ECM molecules, as mentioned above. Their intracellular domains connect directly or indirectly to the actin cytoskeleton, thus linking the cytoskeleton to the ECM. They also serve as bi-directional signalling receptors, inducing changes in protein activities or gene expression in response to ligand binding, while also modulating adhesive affinity on the cell surface in response to changes in cellular physiology [66]. Many of their functions are restricted to particular cell types and may be altered upon transformation, affecting migration, proliferation and invasive growth in vivo [67].

Cell Adhesion Molecules

Both in health and in many types of inflammatory conditions, adhesive (cell-to-cell and cell-to-matrix) interactions are dynamic and regulated during tissue development and homeostasis. Cadherins are major adhesion molecules, which in combination with catenins are involved in the development and maintenance of all solid tissues, both normal and malignant. A major form of their regulation occurs at the level of cadherin gene expression, which influences the strength of adhesion [68]. The type of cadherin expressed determines the specificity and the properties of cell interactions [69]. As mentioned above, in tumours, cancer cells are surrounded by the tumour stroma, arising mainly from tumour-associated fibroblasts. In this case heterocellular interactions between epithelial cancer cells and tumour-associated fibroblasts are observed and hence, heterotypic adhesion complexes are created upon cell-to-cell contact. It was observed by Omelchenko et al. [69], that when the edge of an epithelial cell comes into contact with the surface of a fibroblast, the epithelial cell reorganizes its actin cytoskeleton and forms transient cadherin-associated contacts. Interestingly, the local organization of the actin cytoskeleton in the epithelial cell results in a small fibroblast-like lamella that protrudes over the top surface of the fibroblast [69]. N-cadherin (neural cadherin) is a major representative of heterotypic adhesion molecules and it is associated with a heightened invasive potential in cancer. Overexpression of N-cadherin in breast carcinoma correlates with invasiveness, as a result of N-cadherin-mediated interactions between cancer and stromal cells [70]. The phenotype of breast cancer cell lines was found to undergo dedifferentiation from epithelial to mesenchymal phenotype, as a result of N-cadherin transfection without the loss of E-cadherin (epithelial cadherin) expression [70]. In parallel to the heterotypic cell-to-cell interactions, intracellular interactions are also observed between tumour cells. E- and P-cadherins have a prominent role in the epithelial differentiation. More specifically, breast E-cadherin is expressed in normal adults in luminal epithelial cells, whereas expression of P-cadherin is confined to myoepithelial cells [71, 72]. Temporary downregulation of E-cadherin was found in budding lobules invading the stroma of mouse breast tissue [73]. Changes in the normal expression pattern of the E-cadherin/catenin complex have been found in various human cancers. In breast cancer, partial or total loss of E-cadherin expression correlates with loss of differentiation characteristics, acquisition of invasiveness, increased tumour grade, metastatic behaviour and poor prognosis [74]. The restoration of E-cadherin in human melanoma cell lines caused sensitization against drug-induced apoptosis [75]. Particularly, the release of mitochondrial cytochrome c was increased in response to staurosporine and activation of caspases 3 and 8 was elevated [76]. Similarly, DNA fragmentation, serving as a marker for ad-

vanced apoptosis, was amplified in cells transduced with E-cadherin [77]. In squamous epithelial cells, expression of N-cadherin produced a scattered phenotype with an epithelial-to-mesenchymal transition in association with a reduction in E- and P-cadherins [78]. In N-cadherin transfected breast cancer cells, N-cadherin promoted motility and invasion, but the reduction in the expression of E-cadherin did not affect metastasis or invasion [77]. These findings indicate that N-cadherin, functioning as an adhesion molecule, may be more important than E-cadherin for metastasis and invasion. Several growth factors and cytokines may modulate expression of cadherins and their function in tumour-stroma interactions. Detailed knowledge of their action could lead to important conclusions concerning the role of active stroma in tumour progression.

Stroma Fibroblasts Act as 'Sentinel' Cells in Inflammatory Processes and Tissue Damage

As previously mentioned, fibroblasts are ubiquitous cells that provide mechanical strength to tissues by providing a supporting framework of ECM [79–81]. However, not all fibroblasts are the same and their properties may change if they are called to play a specific role (e.g. in wound healing, in developmental processes or in a tumour) [80–82]. They are capable of producing various paracrine immune modulators, such as peptide growth factors, cytokines, chemokines and low molecular weight inflammatory mediators [82]. It has also been shown recently that thymic fibroblasts play an important role in early T cell development [81]. There is also growing evidence that even within a single tissue, fibroblasts exist in subsets of cells, much like macrophages and dendritic cells [83]. In addition, fibroblasts regulate the behaviour of haematopoietic cells that infiltrate damaged tissue through interactions between CD40 and its ligand [84]. The engagement of CD40 on fibroblasts from a diverse range of tissues leads to the activation of the nuclear factor (NF)-κB family of transcription factors, causing the fibroblasts to synthesize high levels of IL-6, IL-8, cyclooxygenase-2 and the polysaccharide hyaluronan [85]. For these reasons, fibroblasts are considered as 'sentinel' cells in inflammation processes and in tissue damage and injury [82]. They are not passive players in the immune system, but are actively involved in immunomodulation. In order for the inflammatory lesion to be resolved, dead or redundant cells that were first recruited and proliferated during the active inflammation must be removed. The clearance of the unwanted cells at the end of the inflammatory response is performed through apoptosis and subsequent phagocytosis of the dead cells, by macrophages and fibroblasts mimicking characteristics of macrophage. In chronic inflammation, the clearance phase becomes disordered, leading to persistence of the inflammatory infiltrate, tissue hyperplasia and finally tissue destruction and scarring. Continued stimulation of the fibroblasts without eradication of the infectious agent may lead to persistent colonization and chronic inflammation. This situation is often seen at an advanced tumour stage, where stromal cells seem to be highly abundant, surrounding a deranged, hyperplastic and malignant tissue colony. It is obvious that when tissue homeostasis is chronically perturbed, interactions between innate and adaptive immune cells, as well as between these cells and stromal fibroblasts, can be disturbed [86].

Given that the immune system is designed to eradicate damaged cells or tissues, it is highly controversial why inflammation potentiates cancer development rather than protects against it. This question has been largely neglected and few researchers have considered that at least at an early stage of a tumour, stromal fibroblasts may act as immune surveillors against cancer cell proliferation.

At a progressive stage of a tumour, the state where immune cells and stromal fibroblasts should start resolving the inflammatory lesion caused by cancer cells becomes overwhelmed. At that state, the characteristics of chronic inflammation are quite obvious and the tumour has already managed to escape immune surveillance and to adapt the microenvironment to its needs. To date, this scenario has been described only in early in vitro experiments [87]. At an in vivo situation, the suggested phenomenon would be impossible to see, as it would not be possible to locate tumours that have been destroyed at an early stage by stromal fibroblasts. This hypothesis needs further investigation through a series of in vitro experiments that would prove whether this is plausible or not and would investigate the mechanisms that lead to the transition from a normal immune response to a chronic inflammatory situation, where fibroblasts are supportive rather than detrimental to the tumour cells.

The Effect of Cell-Cell Ratio and Interactions

It has been shown that in co-cultures of HeLa cells with normal human fibroblasts, cell-cell ratio and feeding frequency can be monitored in a way that the growth of fibroblasts can flourish to the point where the latter

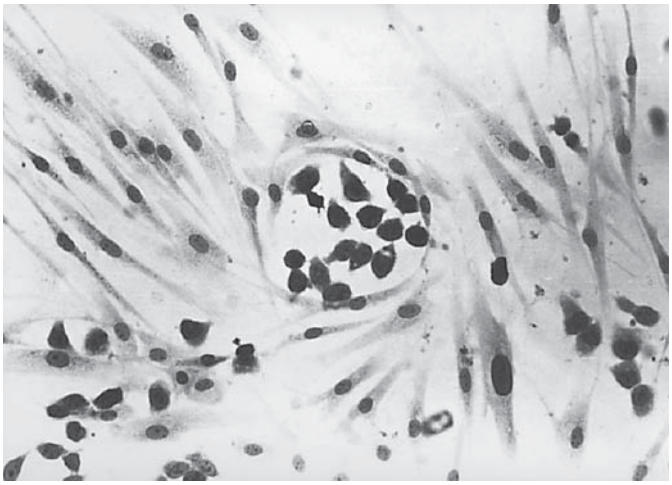


Fig. 1. Light microscopy of 4 days co-culture of HeLa cells with human fibroblasts. The fibroblasts were isolated from a female's breast adenocarcinoma. The cells are stained with Giemsa and show tropism of fibroblasts towards HeLa colonies. Magnification ×400. Image from ref. 90, reproduced with permission.

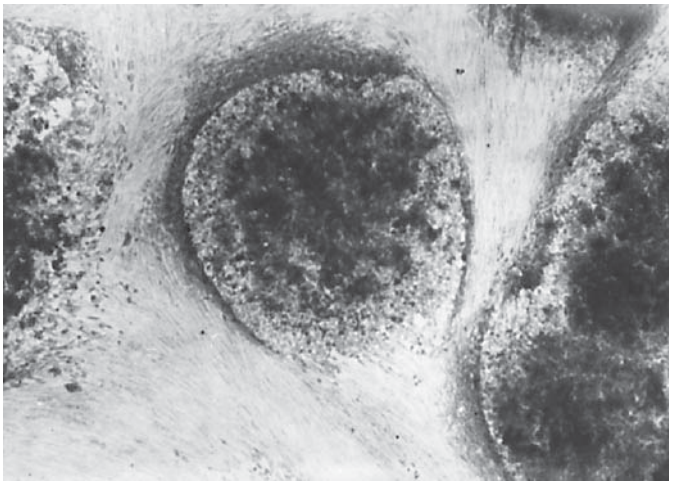
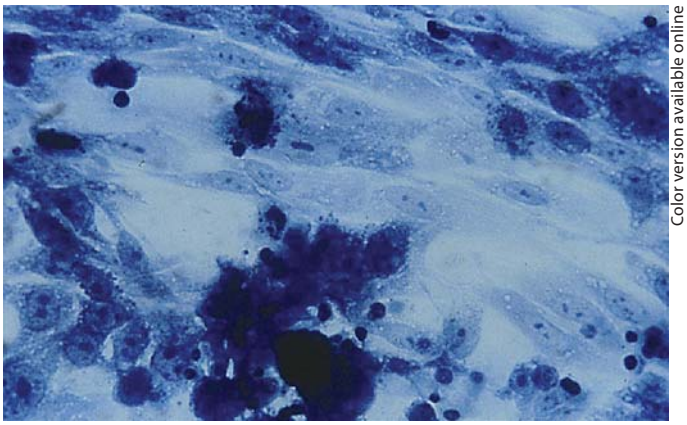


Fig. 2. Light microscopy of 12 days co-culture of HeLa cells with normal human fibroblasts (G-EP), at 1:20 ratio. The cells show the characteristic dense growth bow of fibroblasts that surround the HeLa colonies. Magnification ×60. For details see reference 88.



Color version available online

Fig. 3. Light microscopy of 15 days co-culture of HeLa cells with human fibroblasts (G-EP) 88. The cells are stained with Giemsa. Extensive nuclear fragmentation and cellular disintegration of HeLa cells is shown. Magnification ×800.

cells can attack and destroy whole cancer cell colonies (fig. 1–3) [87, 88]. In addition to the previous findings, Cornil et al. [89] showed that the growth of melanoma cells isolated from early lesions was repressed by dermal fibroblasts in co-cultures. On the contrary, the growth of metastatically competent melanoma cells was actually stimulated in the presence of these fibroblasts [89]. These experiments suggested that at an early stage of a tumour, stromal fibroblasts may act as immune modulators by leading cancer cells to apoptosis and possibly by phagocytosing these dead or redundant cells. In vitro, a remarkable tropism of wandering normal or tumour-derived human fibroblasts towards cancer cells, as well as a profound increase of the proliferation rate of fibroblasts surrounding cancer cell colonies has been observed (fig. 1, 2) [25, 87, 88, 90].

Additionally, the 2 interacting cell types seem to actively communicate through cellular junctions and exchange small molecules, such as nucleotides, but never DNA [90]. Finally, it would be worth noticing the recent observation made by Rubin in 2008 [91], who focused on the effect of cell-to-cell interactions in conditionally determining suppression and selection of the neoplastic phenotype. According to Rubin, experiments in cell culture showed that confluent normal fibroblasts suppress growth of contacting transformed fibroblasts, and that normal keratinocytes similarly suppress tumor formation by adjacent papilloma cells [91].

Under these circumstances, a variety of questions arise from the observations made on the tumour stroma and on the interactions between the latter and the tumour cells. Initially, it is important to understand the mechanisms that lead to the activation of tumour stroma and, more precisely, to the conversion and/or recruitment

of fibroblasts that exhibit the activated myofibroblastic phenotype. The answer to this question necessitates the study of the factors involved in the reaction of inflammation. Such mechanisms/factors involved in the recruitment of activated fibroblasts include vascular permeability (angiogenesis by VEGF), protease inhibitors and growth factors, such as TGF-β, PDGF and insulin-like growth factor I and II, microvessel injuries, direct cell-to-cell contacts and the involvement of cytokines (chemokines, interleukins, interferon, etc.), ECM proteins (laminin, fibronectin, collagen I, heparan sulfate, etc. [92]), MMPs and adhesion molecules (cadherins, catenins and selectins) [93–95]. The function of several molecules mentioned above, such as E-, N- and P-cadherins [94, 95], E-selectin, integrins (such as β1 integrin) and MMPs depend on a low molecular weight GTPase family, called Ras-homologous (Rho) proteins [96, 97]. These proteins play a pivotal role in the regulation of numerous cellular functions associated with inflammation, malignant transformation and metastasis.

Stromal Fibroblasts in Cancer: Promoting or Impeding?

The lack of precise knowledge concerning the mechanisms of fibroblast activation creates another major question, which is directly related to the role they play in tumour invasion and metastasis. Hence, further research is necessary to investigate whether fibroblasts are inducers of cancer progression and invasion, or whether they can protect neoplastic lesions from becoming invasive carcinomas at an early stage of tumour development. These questions remain largely unanswered, but data from co-culture and reconstitution experiments indicate that fibroblasts have a prominent role in defining the rate and extent of cancer progression [3, 4, 87, 88, 91]. Early co-culture experiments with embryonic or differentiated fibroblasts illustrated an inhibiting effect on tumour growth [98, 99]. Co-cultivation of human fibroblasts and HeLa demonstrated an exceptional tropism and affinity between the 2 cell types that leads to specific growth patterns depending on the cell density, feeding frequency and cell-cell ratio in the initial mixed inoculum [88, 90]. Human fibroblasts caused extensive nuclear fragmentation and cellular disintegration of HeLa cells in vitro after co-culture for periods longer than 10 days, without subculturing, and with medium replacement every 2 days (fig. 3) [88]. Basal-cell carcinoma cells grown in association with stromal cells demonstrated an apparent loss of their malignant properties [100]. A recent study showed the potential of immunotherapy for neuroblastoma using syngeneic fibroblasts transfected with IL-2 and IL-12. Treatment of established tumours with 3 intratumoral doses of transfected fibroblasts showed a significant therapeutic effect with reduced growth or complete eradication of tumours in 90% of mice, associated with extensive leukocyte infiltration. Thus, it was concluded that fibroblasts co-transfected with IL-2 and IL-12 mediate therapeutic effects against established disease and are capable of generating immunological memory [101].

On the contrary, a variety of other experiments showed a growth-stimulating effect [3], in particular when tumour-derived fibroblasts were used in co-culture experiments [102, 103]. Haviv [104] also demonstrated that the interaction of fibroblasts with epithelial cells plays an initial role in recruiting inflammatory cells and provokes immune surveillance. In 2 recent studies, it was stated that there is an awareness that signals provided by the reactive stroma, and specifically myofibroblasts, can induce the genetic alterations that underlie tumour formation, can stimulate tumour growth and progression and can dictate both therapeutic response and ultimate clinical outcome [105, 106]. Finally, based on recent data related to the effects of cellular senescence on stromal-tumour interactions, a hypothesis has been raised according to which, senescent cells, due to their functional alterations, can create a permissive environment for the development of epithelial cancer cells. Hence, it is worthy of notice that senescent fibroblasts seem to stimulate specifically the growth of immortalized and cancer epithelial cells, indicating that aging of stromal cells may be one of the factors leading to carcinogenesis [107].

Thus far, there are numerous examples of heterotypic interactions between tumour stromal fibroblasts and tumour cells that have supported the hypothesis that the tumour stroma in human carcinomas promotes the growth of the tumour mass, as well as invasion and metastasis [2, 3]. However, it remains possible that the stroma acts essentially to impede tumorigenesis and that the desmoplastic response is a host defence reaction designed to confine the developing tumour. Pathologists have long noted that desmoplasia is associated with relatively early-stage lesions and that it is much less apparent in highly invasive, advanced tumours [108]. To date, this alternative model has been given little attention. The role of stromal fibroblasts in regulating early tumour inflammation needs to be investigated further,

since unfortunately, the possibility that tumour stroma might act in defence against tumours has been largely neglected [87, 88].

The role of tumour stroma in the invasion and metastasis of cancer lesions still remains unclear. The experimental findings so far have not elucidated the function of the tumour stroma and the role of stroma-to-cancer cell interactions. Hence, in-depth investigation of the various factors involved in these interactions is justified.

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