The Role of the Stroma in Carcinogenesis

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Abstract

This systematic review considers the most recent attitudes and news regarding the influence of the stroma on tumor initiation and progression. It is now widely accepted that tumor stroma plays an active role in carcinogenesis. Many different signaling molecules, ligands and signaling pathways recently have been discovered. This review considers the complexity of interactions between malignant cells and its stroma (cross-talk). The recent advances and better understanding of the tumor-stroma interactions will have important impact on the new and combined therapeutic approaches and modalities.

KEY WORDS: carcinogenesis, tumor stroma, cross-talk, stromal fibroblasts

Introduction

The cancer research has largely been guided by so called a reductionist model which considered the tumor as a disease of cancer cells neglecting all other structures surrounding the tumor cells, named the stroma (1). The stroma consists of the following structures:

- Extracellular matrix
- Blood vessels
- Inflammatory cells
- Fibroblasts (2)

It is well established that there is a reciprocal (mutual) relation between normal epithelium and its stroma. This relation is necessary for the normal development of the tissues and their morphogenesis (3). It is also widely accepted that the tumor formation is caused by accumulation of somatic mutations within epithelial

cells. However, tumor behavior is largely influenced by the stroma factors (microenvironment) (4). Molecular studies confirmed significant differences between the stroma of normal tissues, carcinoma in situ and invasive carcinomas. These alterations are already obvious between normal tissue and carcinoma in situ (5). The most of these alterations are related to the stromal fibroblasts (3, 5). Also, perturbations between epithelium and its extracellular matrix can cause cancer development (2). We emphasize that genetic mutations within tumor cells can cause very important alterations and activation of the stroma. These changes include:

a. Neoangiogenesis (development of new blood vessels)
b. Inflammatory response and activation of inflammatory cells (lymphocytes, mast cells and macrophages)
c. Different expression of extracellular matrix components
d. Increased proliferation of stromal fibroblasts (carcinoma associated fibroblasts)

It is important to underline that a cross talk and reciprocal relations between tumor and its stroma are necessary for tumor formation and its progression. Different ligands and signal pathways between tumor and stromal cells are included in these interactions (6, 7). The communication between tumor cells and stroma is primarily based on a paracrine signals. These paracrine signals include:

- Growth factors
- Chemokines
- Cytokines (8)

The most of these signals stimulate proliferation of the epithelial cells. Only the family of transforming growth factor-β (TGF-β) can take different actions (2, 4, 9). According to experimental studies, multifunctional TGF-β (via its receptors, TβR) can act as both inhibitor and stimulator of proliferation of epithelial cells (10, 11, figure 2B). These antagonistic effects of TGF-β are caused by the heterogeneity of the stromal fibroblasts (2). Also the effects of TGF-β are specific for different cell types (8), see figure 1. Antiproliferative activities of TGF-β are based on cyclin-dependent kinases activation (e.g. p15 which causes G1 arrest of the cell, 11, 12). TGF-β starts to stimulate tumor cells as soon as resistance to its inhibitory effects occurs. This happens due to mutations of the genes involved in the TGF-β signaling pathways (see figure 1). Then, both tumor and stromal cells compensatory produce additional TGF-β resulting in increased invasiveness and metastasis of tumor cells. TGF-β activities are changed since angiogenesis, increased motility and interactions of tumor cells with extracellular matrix are now stimulated. In addition it shows immunosuppressive effects. The resistance of tumor cells on TGF-β activities is extremely important step, not only in tumor development but also in its invasiveness (11). In vivo study on stroma of gastric and prostate cancer showed that TGF-β receptor type 2 inactivation on surface of stromal fibroblasts leads to progression and increased tumor aggressiveness. In both cases inactivation of TGF-β receptors led to accumulation of the tumor stroma (2, 4). TGF-β also shows immunosuppressive potential and plays important role in deposition of extracellular matrix components (11, 13). It has been confirmed that inactivation of TGF-β receptors could cause increased expression of hepatocyte growth factor (HGF). HGF is also important paracrine molecule involved in a cross-talk between tumor and its stroma (2) and it acts via its receptor on the surface of epithelial cells – c-Met, see figure 1. Non-tumors effects of TGF-β as well as its production by other cell types are not the matter of discussion in this paper. We want to point out that many other soluble factors produced by stromal fibroblasts have been discovered. Many of them play important roles in proliferation; apoptosis, angiogenesis and morphogenesis (see Table 1). Fibroblast activation protein (FAP), transmembrane protein on surface of stromal fibroblasts, is one of important molecules involved in cross-talk between stromal fibroblasts and epithelial cells. FAP shows proteolytic activity and its activation is related to increased aggressiveness and tumor expansion in rats. It is partly confirmed by use of specific antibodies which inhibit FAP activities. It results in tumor growth arrest (14). According to another in vivo study human fibroblasts extracted from human breast cancer cell lines induced more rapid tumor growth and invasion of adjacent epithelial cells comparing with the fibroblasts extracted from the normal tissue (15). Basic acting mechanism of stromal fibroblast happens via secretory molecules called stromal cells-derived factor 1 (SDF-1). Besides, tumor fibroblasts support Neoangiogenesis by activation of progenitor endothelial cells. SDF-1 also stimulates tumor growth directly acting on its receptors like CXCR4 (chemokine receptor) whose expression is presented on surface of carcinoma cells (15). It is important to emphasize that tumor cells alone can stimulate stromal fibroblasts and secretion of different signaling molecules which increase tumor aggressiveness. Some other in vivo studies confirmed the importance of stromal fibroblasts in control of tumor cell prolifera-

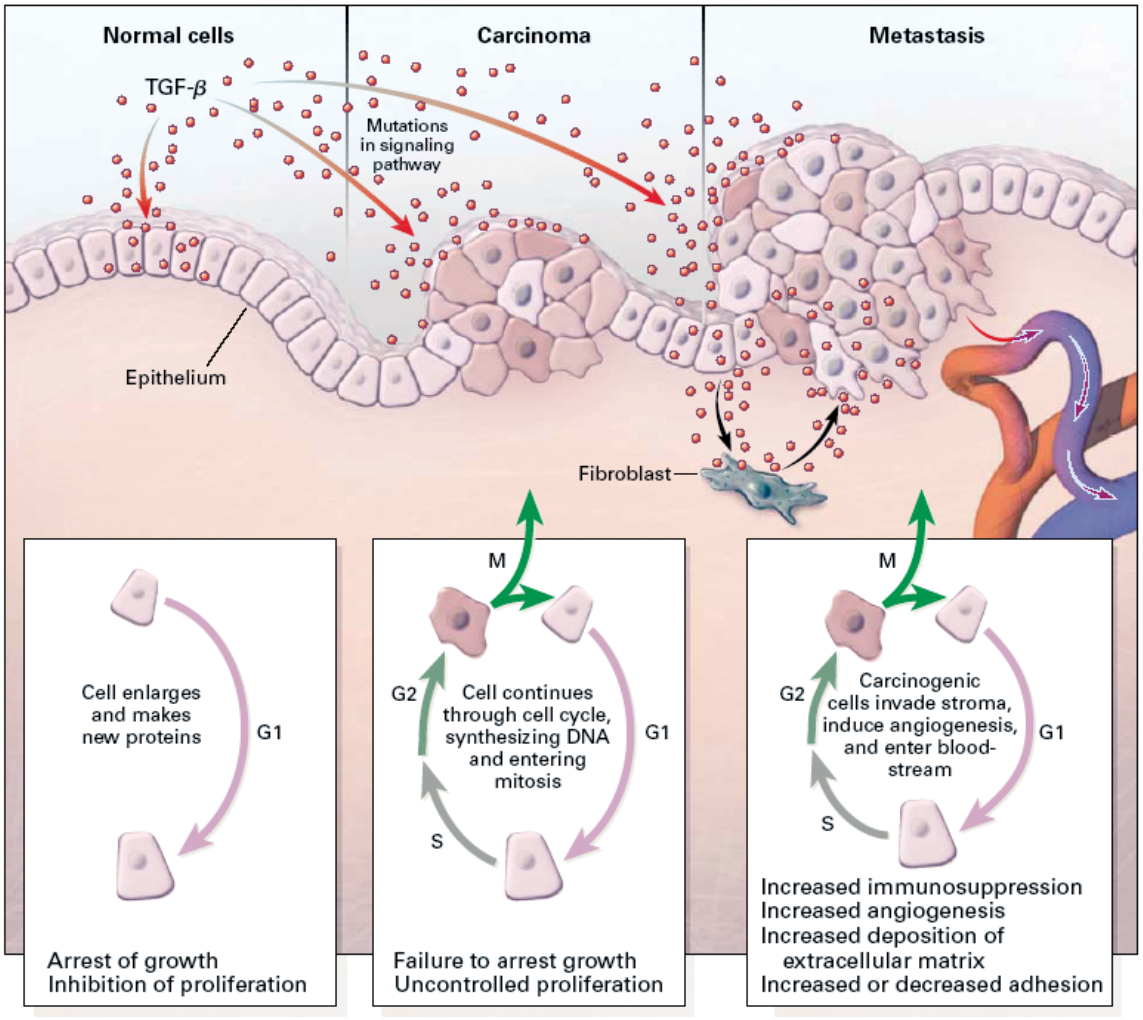

FIGURE 1. The role of TGF-β in carcinogenesis. In normal cells TGF-β acts via signaling pathways which cause cell cycle arrest in G1 phase, thus inhibiting proliferation and stimulating differentiation or apoptosis. During carcinogenesis, the mutations of the genes involved in TGF-β signaling pathways occur. This makes tumor cells resistant on TGF-β activities. It results in increased tumor proliferation and production of TGF-β by stromal fibroblasts. Then TGF-β acts on stromal cells including immunocytes, endothelial cells and smooth muscle cells showing its immunosuppressive and proangiogenic activities. All these activities lead to increased tumor aggressiveness (11).

tion (5, 16). Current methods as gene expression profiling one applied on normal breast tissue and breast cancer tissue confirmed genetic alterations in all cell types within tumor (3). The stromal fibroblasts showed alterations in gene expression of two chemokines – CXCL4 and CXCL12. These chemokines act via their receptors on surface of epithelial cells enabling proliferation, migration and invasion of tumor cells (3). The results of another study which used the same method on malignant melanoma cells confirmed the relation between melanoma cells and stromal fibroblasts (17). This argues that tumor cells stimulate proinflammatory and tumorigenic response in stromal fibroblasts implicating the importance of inflammation in carcinogenesis as earlier approved in some other tumors (e.g. colorectal cancer, hepatocellular carcinoma, gastric carcinoma). Some of the most frequent mutations detected within tumor cells (e.g. TP53 and PTEN) are also detected within stromal fibroblasts (18, 19). Genetic alterations in stromal fibroblasts without previous genetic alterations in epithelial cells may induce tumor development (2). In vivo studies demonstrated that stroma might be carcinogenesis promoter as it happened in the case of exposition to forbol ester compounds or to irradiation (8).

SIGNATURE: CAFs – carcinoma-associated fibroblasts; VEGF – vascular endothelial growth factor; SDF-1α – stromal derived factor 1α; FSP-1 – fibroblast-specific protein-1; HGF – hepatocyte growth factor; MSP – macrophage-stimulating protein; EGF – epidermal growth factor; TGF-α – transforming growth

SOLUBLE FACTOR	CELLS EXPRESSED	RESPONDING CELLS	POSSIBLE ROLE
HGF	Fibroblasts	Epithelial cells	Proliferation (+) Transformation (+) Morphogenesis (+)
INSULIN-LIKE GROWTH FACTORS 1, 2 (IGF-1, 2)	Fibroblasts	Epithelial cells (breast)	Proliferation (+) Apoptosis (-)
TGF-β1, β2, β3	Epithelial cells and Fibroblasts	Epithelial cells and fibroblasts	Apoptosis (+/-) Morphogenesis (+) Proliferation (-)
FIBROBLAST GROWTH FACTOR 2 (FGF)	Fibroblasts	Epithelial cells	Proliferation (+) Transformation (+)
STROMAL CELL-DERIVED FACTOR 1α	Fibroblasts	Epithelial cells (glioblastoma)	Proliferation (+) Transformation (+)
MATRIX-METALLOPROTEINASE TYPE 1 AND 7 (MMP-1, 7)	Fibroblasts	Extracellular matrix and growth factor activation in the stroma affect epithelia	Morphogenesis (+) Proliferation (+/-) Apoptosis (+/-)

TABLE 1. Some of the paracrine molecules involved in a cross-talk between epithelium and stroma (2)

factor-α; TGF-β – transforming growth factor-β; FGF – fibroblast growth factor; IL-6 – interleukin 6; LIF – leukemia inhibitory factor; NGF – nerve growth factor;

THE ROLE OF SENESCENT FIBROBLASTS

The cellular aging (senescence) is important mechanism that suppresses tumor development in vitro (20, 21). Cellular senescence is also thought to contribute to aging. The senescence is based on signal transduction which leads cells to irreversible cell arrest in G1 phase of cell cycle. It disables entry into S phase regardless the mitogen stimuli are present or not (22). The senescence can be activated by exogenous stimuli like irradiation. Exposition of stromal fibroblasts in breast tissue to irradiation stimulates its senescence. It has a multiple effects on adjacent structures: increased pseudopodia formation due to alterations in cytoskeleton, increased degradation of extracellular matrix due to the matrix-metalloproteinases (MMP) secretion and disturbed ductal morphogenesis in breast tissue (23). The senescent cells produce different molecules which can stimulate pre-cancerous cells to proliferate. Although cellular senescence suppresses carcinogenesis early in life, it may promote tumors in aged organisms (24). In vivo studies on mouse pre-cancerous epithelial cells model demonstrated that their exposition to the senescent fibroblasts led to irreversible loss of differentiation, invasion and complete malignant transformation (20). Also, the senescent fibroblasts in the culture of normal epithelial breast cells can cause disruption of alveolar architecture, functional differentiation and morphogenesis. One of the key molecules involved in these processes is matrix-metalloproteinase-3 (MMP-3). It is interesting that both senescent and cancer-associated fibroblasts produce the same paracrine molecules (signals, growth factors) which can stimulate adjacent epithelium proliferation (23). However there are significant differences between two fibroblast types: the tumor fibroblasts are able to induce epithelial carcinogenesis while the senescent fibroblasts are not able to transform normal (non-transformed) epithelial cells into malignant (2, 24). These investigations support the idea that senescent cells contribute to age-related pathology including cancer (20).

Clinical implications

The impact of the stroma has been discussed in many studies. Unfavorable prognostic value of fibrosis in human tumors has been confirmed in the most of them. The influences of the stromal factors on overall survival and disease-free survival have been analyzed. One of the most investigated factors is TGF-β and its responding receptors. TGF-β is involved in colorectal cancer pathogenesis since its presence is detected in malignant cells of colorectal mucosa (25). The presence of TGF-β is more expressed in low-grade carcinomas and its expression is correlated with overall survival and relapse-free survival. However, TGF-β type 2 is related to unfavorable prognosis and it is involved in disease progression (25). Unlike in colorectal cancer, in head and neck tumors, TGF-β did not show any prognostic value (26). In the case of prostate cancer, the higher TGF-β expression is linked to decreased expression of its responding receptors and higher aggressiveness of the prostate cancer. It includes

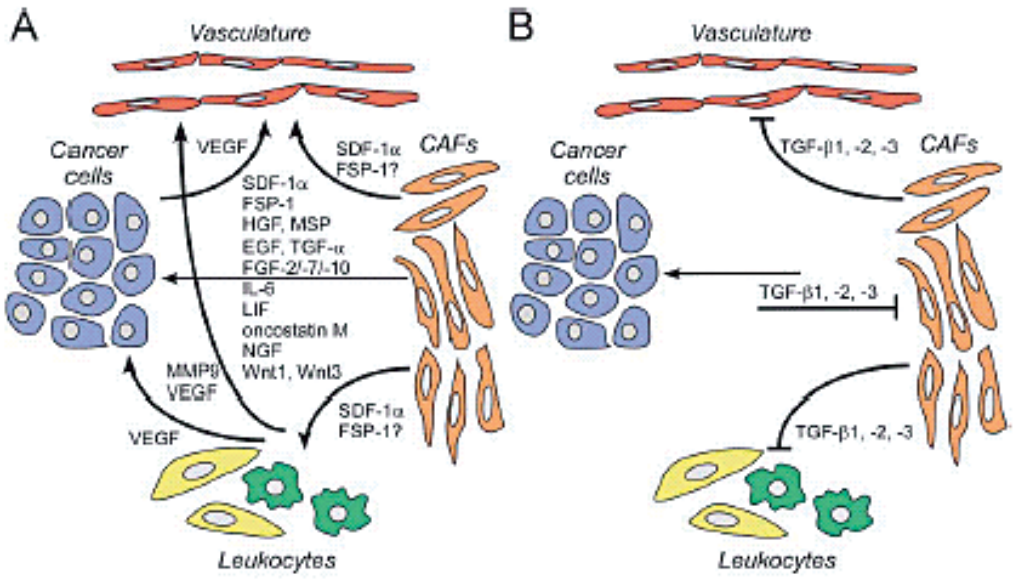

FIGURE 2. Molecular mediators of epithelium-stroma interactions. The cells within tumor tissue communicate during carcinogenesis via growth factors, chemokines and cytokines. A: Different paracrine signals; B: TGF-β is specific mediator since it can show dual effects (inhibitory and stimulating) (8)

higher Gleason score and more frequent extracapsular invasion of prostate cancer (27). CD10 enzyme, a neutral endopeptidase on surface of stromal fibroblasts, has also been investigated. Higher expression of CD10 in basal cell carcinoma correlated positively with aggressiveness and invasiveness of the tumor (28). Expression of CD10 in stroma of the breast cancer revealed that CD10 had been not only the marker of invasiveness but also independent prognostic factor in breast cancer (29). Our study on CD10 in breast cancer did not confirm these results (30). In the case of the gastric cancer, CD10 expression on the surface of stromal fibroblasts positively correlated with tumor and vascular invasion depth as well as with metastasis to the regional lymph nodes (31). The study of Ogawa et al. (32) also confirmed importance of CD10 enzyme in invasion and metastasis of colorectal cancer. In malignant melanoma, higher CD10 expression within both melanoma cells and stromal fibroblasts correlated with invasiveness and metastasis of the tumor cells (33). In breast cancer, the presence of stromal fibroblasts generally has been linked to tumor aggressiveness and to prognosticators related to worse outcome (34). The study clearly demonstrated that secretory molecules produced by stromal fibroblasts correlated with aggressiveness and higher metastatic potential of the tumor. Interestingly the investigators found positive correlation between the presence of the stromal fibroblasts and neoangiogenesis. In vitro studies have already demonstrated that stromal fibroblasts induce neoangiogenesis by production of hypoxia induced vascular growth factor (35). TGF-β signaling pathways have been involved in the potential therapeutic implications. Specific TGF-β inhibitors have been synthesized. Their application significantly decreased the metastatic potential of the primary breast cancer without important side effects. The novel TGF-β inhibitors also opened a question and dilemma: Did such inhibitors induce the tumor development in normal stroma inhibiting (or blocking) TGF-β? Therefore the clinical use in treatment of human tumors of TGF-β inhibitors has been limited so far. The second important therapeutic implication was related to HGF molecule. Specific HGF inhibitors also have been synthesized. These inhibitors were able to decrease invasiveness of the pancreatic cancer since in vitro studies had revealed high expression of HGF in pancreatic cancer. The stroma of pancreatic cancer was exposed to irradiation and it resulted in several mutations including those related to higher HGF activity.

Conclusion

It is clear today that carcinogenesis is not possible without the active role of the stroma (especially in the case of carcinomas). The mutual relation and the complex signaling pathways (cross-talk) between epithelium and stroma are basic mechanism of malignant alteration and progression. The recent advances revealed the importance of the stromal and senescent fibroblasts and their active role in carcinogenesis. Numerous signaling molecules (especially TGF-β), signaling pathways as well as specific gene mutations have been discovered within the stromal cells. Three-dimensional (3D) systems of the cell cultures have been developed to simulate the tumor microenvironment in vivo. These advances enable deeper understanding of the tumor development and treatment modalities. Unfortunately a specific drug has not been developed till now. However research is going on and it probably will result in novel therapeutic approaches based on recent discoveries.

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