CHAPTER 8

Pro-Invasive Molecular Cross-Signaling between Cancer Cells and Myofibroblasts

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Abstract

Cancer cell invasion necessitates the participation of host cells. One of the cell types that stimulates invasion of colon and other cancer cells is the myofibroblast, as evidenced from the histology of cancer and from coculture experiments. Cancer cells produce transforming growth factor-β (TGF-β) and TGF-β converts fibroblasts into pro-invasive myofibroblasts. In the in vitro system with human cancer cell lines and freshly isolated stromal cells, the pro-invasive activity of myofibroblasts is due to the combined action of Hepatocyte growth factor/scatter factor (HGF/SF) and tenasin-C, two molecules known to promote invasion in clinical tumors and their experimental surrogates. The myofibroblasts are themselves invasive and this activity is stimulated by TGF-β. N-cadherin is implicated in the invasion response of myofibroblasts. The question now is which of the multiple factors present in the tumor ecosystem is responsible for the pro-invasive switch that turns a benign tumor into a malignant one.

Host Cells Participate at Cancer Cell Invasion

Epithelial tumors consist of cancer cells and host cells. The presence of host cells is considered as a reaction against the aberrant behavior of the cancer cells, the latter being at the origin of the tumor. This host reaction was understood, originally, as a mechanical and immunological defense against the cancer cells and was described by the pathologist as desmoplasia, inflammation and neoangiogenesis with accumulation of stromal fibroblasts, leukocytes and endothelial cells respectively. Today, there is growing evidence that tumor-infiltrated host cells are recruited by the cancer cells and diverted by the latter to contribute to their malignant progression rather than to protect the host. New blood and lymph vessels, immunocytes and inflammatory cells as well as stromal fibroblasts don’t inhibit but rather stimulate cancer invasion and metastasis, in line with Paget’s “seed” and “soil” hypothesis. Next to recruitment by cancer cells, alternative scenarios for the participation of cancer cells and host cells at tumor progression should be considered. Both cell types may undergo concomitant but independent alterations. Using serial analysis of gene expression (SAGE) during breast carcinogenesis, Allinen et al. found in epithelial, myoepithelial, myofibroblastic, leukocytic and endothelial cell types extensive changes in the expression of genes that encode secreted proteins and receptors. Genetic alterations, however, were found only in the epithelial cancer cells. Others have shown genetic

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mutations in stromal cells, examples being SMAD4, LKB1, HPP1.\textsuperscript{7,9} Moreover, changes in the epithelial compartment might be secondary to alterations of the stroma; a phenomenon called landscaper defect.\textsuperscript{10}

Regardless the sequence of events, we will assume, here, that there exists a continuous cross-signaling between the epithelial and the stromal compartment in normal and in pathological situations and we will discuss some of the critical alterations that lead to invasion. In view of the present symposium our host cell of interest is the myofibroblast. This name was used to describe, in experimental granulation tissue of Wistar rats, fibroblastic cells with a smooth muscle cell-like morphology, a strongly developed microfilamentous apparatus and a contractile phenotype.\textsuperscript{11,12} The most reliable molecular myofibroblast marker is α-smooth muscle actin (α-SMA).

**Myofibroblasts Stimulate Invasion**

Colon cancer cells PROb, a cell line derived from a chemically induced BDIX rat tumor, produced upon subcutaneous injection into syngeneic rats ulcerating tumors that were poorly differentiated and invaded into skin and muscle with isolated cancer cells in the stroma.\textsuperscript{13} These invasive cancers were rich in myofibroblasts, that were positive for α-SMA and localized mainly at the front of invasion. PROb cells harvested from routine cell culture, however, failed to invade when confronted in vitro with collagen, Matrigel or embryonic chick heart tissue. By contrast, freshly dissociated tumor cell suspensions, containing PROb cells and tumor-associated stromal cells, were invasive in all three in vitro assays. The role of the stromal myofibroblasts in invasion was confirmed by experiments in which PROb cells were mixed with cells of the established myofibroblast cell line DHD-FIB that was isolated from a similar BDIX rat colon tumor. The histology of invasion in vitro and in vivo gave the impression that solitary PROb cancer cells were dragged by massively invading myofibroblasts. The pro-invasive activity of myofibroblasts was shown also by De Wever et al\textsuperscript{14} using human colon cancer cells from established cell lines and stromal cells isolated from surgical colon cancer fragments or from normal mucosa at some distance from the tumor. Explants from such fragments yielded myofibroblasts and fibroblasts in case of tumor and normal mucosa respectively. In 48-hour cultures, the colon cancer cells invaded into the collagen only when myofibroblasts but not fibroblasts were added to the collagen. The pro-invasive activity was found also with conditioned media from myofibroblast but not from fibroblast cultures instead of the cells themselves. This activity of myofibroblasts is in line with others' experiments in vitro and in vivo; it is compatible with observations on human colon and other cancers.

Myofibroblasts are present in the stroma of many malignant tumors and they are frequently localized at the front of invasion.\textsuperscript{4} Their presence has been positively correlated with poor prognosis.\textsuperscript{15} That myofibroblasts may participate at the transition from the noninvasive toward the invasive phenotype is compatible with their appearance in benign lesions that have a high risk of progression toward invasive cancer. In CIN (cervical intraepithelial neoplasia), α-SMA-positive stromal cells were considered as a sign of imminent invasion.\textsuperscript{16,17} Similarly, α-SMA-positive pericryptal fibroblasts were rare in low risk tubular adenomas but abundant in villous adenomas and in FAP (familial adenomatous polyposis) hyperplasia, both carrying high risk of malignant progression.\textsuperscript{18}

In animal experiments, fragments of CC531 rat colon adenocarcinoma produced encapsulated noninvasive tumors when transplanted into the subcutaneous connective tissue. When the transplantation was done into experimentally induced granulation tissue, the tumors were invasive with dispersed strands of cancer cells protruding into the surrounding matrix.\textsuperscript{19} This observation has served the development of fruitful models for the analysis of invasion in vivo.\textsuperscript{1} Since the granulation tissue contained macrophages, other inflammatory cells and numerous capillaries next to myofibroblasts, the latter could not be held exclusively responsible for the induction of invasion. More direct evidence was obtained from the formation of invasive cancers upon subcutaneous coinjection of human colon cancer cells HCT-8 with myofibroblasts