HHS Public Access

Author manuscript

Cancer Metastasis Rev. Author manuscript; available in PMC 2015 June 22.

Published in final edited form as:

Cancer Metastasis Rev. 2009 June ; 28(0): 177–183. doi:10.1007/s10555-008-9175-2.

Tumor stroma derived biomarkers in cancer

Malin Sund[1,2] and **Raghu Kalluri**[1]

[1]Division for Matrix Biology CLS 11087, Beth Israel Deaconess Medical Center and Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215, USA

[2]Department of Surgery, Umea University, Umea, Sweden

Abstract

In recent years the importance of the tumor stroma for the development, promotion and invasion of cancer is becoming increasingly clear. Besides a malignantly transformed cancer cell, tumors also contains many other cell types, including endothelial cells, fibroblasts and cells of the immune system. These cells together with the cancer cells produce the sum extracellular matrix (ECM) of the tumor. The ECM and the non-malignant cells of the tumor are defined as the “tumor stroma”. Just as the malignant cell itself can be the source of substances that can be used as biomarkers of cancer, the tumor stroma contains factors that potentially can be used as biomarkers when treating patients with cancer. In this review we will discuss the role of the tumor stroma as a source of new cancer biomarkers. This concept highlights a novel view of cancer and treats them as organized organs. Additionally, this further stresses the importance of including factors related to the tumor stroma into the diagnostic and therapeutic equation of cancer.

Keywords

Cancer; Biomarker; Stroma; Collagen

Introduction

The traditional view of cancer as a group of malignantly transformed cells has been greatly revised in recent years. The importance of other cell types of the tumor, the tumor vasculature, the immune system as well as the extracellular matrix (ECM) in the development of cancer from a single transformed cell has been clearly shown in many experimental studies as reviewed by Kalluri & Zeisberg [1]. It is also becoming evident that there are many in situ cancers that never progress into an invasive cancer, most likely due to host-derived factors that prevent this development. This fact has been termed, “cancer without disease”, thereby highlighting the fact that a transformed cell per se is not enough to cause cancer, but that this process requires the recruitment of a tumor microenvironment permissive of further tumor growth [2]. The initial local growth of a tumor, the subsequent spreading of the malignant cells into the vasculature and/or lymphatic system, and finally the establishment of a distant metastasis are all processes in which host-derived factors are highly involved [1]. The microenvironment surrounding the malignant cells is called the

Contact: Raghu Kalluri, rkalluri@bidmc.harvard.edu.

tumor stroma, and consists of the other non-malignant cells of a tumor, as well as the tumor ECM with a multitude of growth factors embedded in the ECM, affecting the growth of the tumor. Although the stromal cells appear to be non-malignant in the sense of genetic mutations, they do exhibit epigenetic changes, which affect their behavior and protein expression [1, 3, 4].

In histological analysis of the primary tumor many of these stromal factors have been found to correlate with prognosis of disease, a well-known example of this being that primary tumors with high vascularity indicate poor prognosis for many cancer types. This is related to the activation of angiogenesis, which is a crucial process in cancer progression and spreading [5, 6]. Another emerging stromal factor of great importance is the finding of activated fibroblasts and macrophages—CAF's (cancer-associated fibroblasts) and TAM's (tumor-associated macrophages)—in the tumor [1]. These stromal factors have in recent studies exhibited significant prognostic value [7]. Therefore, stromal factors can provide important additional knowledge, and potentially make it possible to distinguish two tumors of the same size and in the same location. A malignant tumor requiring more aggressive treatment, versus one that can be considered cured after radical surgical removal.

In oncology today there is a discrepancy between the vast knowledge about the biology of the cancer in an experimental setting, and translation of this knowledge into information that can be used in clinical decision-making. Although the concept of dormant tumors is well established, biomarkers which can determine the ability of dormant tumors to convert into malignant cancers are unknown [8]. Although tremendous developments in tumor imaging through sophisticated equipment and methods of modern radiology, there still are detection limits which prevent us from identifying microscopic spread of the disease [8]. There is a need for biomarkers that would be easily detectable from biological samples collected with minimally invasive methods and of a low cost. Many currently used biomarkers, such as PSA, CEA, CA-125, CA-19, are substances expressed by the cancer cells themselves. Although many of these are widely used and of tremendous value in clinical a setting, others are less specific and therefore the interpretation of the levels of these in relation to disease progress or in evaluating the risk for disease relapse is a difficult task. The tumor stroma is continuously remodeled during tumor progression, and many factors related to the stroma can be found in the blood and tissue of patients with cancer. Therefore, the potential use of such substances as tumor biomarkers, together with the cancer cell derived biomarkers, needs to be evaluated.

In this review we will discuss the role of the tumor stroma in cancer development and in the process of metastasis. Our focus will be on the major alterations that metastasis induces on the stroma and ECM. We will discuss recent evidence of how such changes can be used to aid clinical decision making to determine prognosis and prediction of treatment efficacy. Finally, our aim is to discuss whether the stroma could be a source of a new generation of tumor biomarkers. We will use the example of stromal derived endostatin, an endogenous anti-angiogenic substance cleaved from the ECM molecule type XVIII collagen [9, 10].

Tumor stroma

The traditional focus in cancer research has been on the malignantly transformed cell, but in recent years there has been increasing interest in the role of the tumor stroma in the subsequent tumor development and progression. The stroma consists of the non-malignant cells of a tumor, the vasculature and its cells, the activated fibroblasts, macrophages and other immune cells (Fig. 1) [1]. It is well known that ECM-producing cells are activated in cancer and this leads to a phenomenon known as tumor desmoplasia, which appears to be important for tumor progression. The reactive stroma of a tumor is associated with larger number of ECM producing fibroblasts, higher vascularity and increased production of ECM products such as collagens [1].

The cancer cell and stroma both modulate the ECM of a tumor by secreting tumor-associated proteases, which subsequently break down proteins of the ECM such as collagens, proteoglycans, etc. (Fig. 1). This remodeling also releases substances sequestered in the ECM, such as vascular endothelial growth factor (VEGF), which further influences tumor progression [11, 12]. Additionally, many cleavage products from ECM proteins have properties that affect tumor progression. A well-known example of this is the anti-angiogenic activity of endostatin, tumstatin, canstatin, arresten and hexastatin—all substances cleaved from the basement membrane (BM) proteins types XVIII and IV collagens during tumor growth [13]. Desmoplastic reaction has been seen as a defensive mechanism to confine the tumor and to prevent further tumor growth [1, 9, 14–17]. However, the emerging data shows that it is an active process, participating in several aspects of tumor progression such as, angiogenesis, invasion and metastasis [1, 18].

Circulating stroma derived substances

The requirement of a circulating tumor biomarker is that it should be elevated in patients with cancer, and reflective of tumor phenotype and size. The marker should normalize when the tumor is removed and it should increase again when the disease relapses. Most importantly the marker should be easy to monitor (Fig. 2).

All tumors use blood supply to grow [5, 6]. It is likely in cancer, both in the case of a local tumor burden and in metastatic disease, tumor specific markers are found in the vascular compartment. Biomarkers can potentially be derived from the cancer cells and from the stromal compartment. Using an experimental colorectal cancer model (APCmin mice) Hung et al employed plasma proteomics to identify differences in circulating proteins between mice with tumors and healthy controls [19]. They used mass-spectroscopy to identify proteins in total plasma proteome and the plasma glycoproteome. They observe significant portion of proteins found in the samples from the tumor bearing mice with potential stromal origin [19]. The authors speculate that such stromal markers could be used in conjunction with cancer cell derived markers, such as CEA, to achieve a higher level of sensitivity and specificity [19]. This finding illustrates that the stroma could be a source for novel circulating biomarkers.

ECM and the stroma of a primary carcinoma

Normal epithelia is always associated with a basement membrane (BM)—a highly specialized ECM. The BM contains many ECM proteins, the main components being type IV collagen, laminin, types XV and XVIII collagens, perlecan and nidogen [13]. Many of these proteins as well as domains cleaved from them an effect on tumor progression. The cleaved fragments of BM proteins inhibit angiogenesis suppressing tumor growth [9, 13–17]. By definition a tumor becomes invasive once it invades the BM and can enter the underlying normal stromal zone. Prior to invasion of BM, the tumor is referred to as an in situ carcinoma. However, before the BM is broken down by cancer cells, they appear to influence the stroma and activating it [4]. Activation of stromal cells such as fibroblasts, is observed in the context of in situ carcinoma. Both cancer cells as well as stroma cells express proteases that break down the BM barrier. Upon which, the cancer cells and activated stromal cells can collectively influence tumor progression [4]. Is it possible to use activation and breakdown of the ECM as new prognostic and predictive biomarkers? This needs to be determined.

Although the importance of the stroma in cancer cell progression has been well studied in several experimental studies, the clinical applications of these findings are still largely untested. In breast cancer research vascularization and vascular invasion have been shown to be factors critical for prognosis. Both of these features are independent prognostic variables and finding of vascular invasion, as well as a high degree of vascularization indicate a worse prognosis [20–23]. The latter reflects the angiogenic activity within the primary tumor [24]. By studying the desmoplastic reaction in the stroma through an analysis of a fibrotic focus (FF) in the tumor, it has been established that this is an useful histological prognostic parameter for patients with breast cancer [24]. Similar findings have been observed for patients with colon, pancreatic and lung cancer [24]. There is likely association between the presence of FF and the presence of intratumoral hypoxia leading to increased angiogenesis and lymphangiogenesis [24].

The importance of other cells types of the stroma for prognosis of cancer outcome, was recently shown by Finak et al. In this study a gene array based on expression patterns of cells from the stromal compartment from 53 primary breast cancers were used to generate a stroma derived prognostic predictor (SDPP) [7]. By using this assay the authors found that breast cancer patients could be divided into three distinct and well defined categories; first a group with good prognosis, second a group with quick relapse of disease and worse prognosis, and third an intermediate group [7]. When examining the difference in gene expression pattern between these groups, it was found that patients within the first group shared an activation of immune response genes, whereas the patients in the second group display an upregulation on several genes related to hypoxia and angiogenesis [7]. This highly interesting finding indicates that these two groups might require quite different treatment strategies. The authors speculate that the first group might have a positive response to a cancer vaccine, whereas the second group might be most suitable for anti-angiogenic treatment strategies. The most striking finding however was that the SDPP was independent from all traditional prognostic factors such as tumor size, grade and age [7]. This means that the SDPP might be able to actually pick out more aggressive tumors based

on the surrounding stromal reaction to the cancer cell mass. This finding further underlines the importance of the stroma in tumor progression and also points to potential implications for future therapeutic strategies. Similar studies and findings on the prognostic importance of the stroma have been described in other breast cancer studies and cancer of the head- and neck [25, 26].

Stroma and the metastasis

Although it is systemic spread of cancer in the process of metastasis that leads to the death of most cancer patients [27], the process of metastasis has traditionally received less focus in cancer research. Since Paget's hypothesis of the soil and seed it has been evident that the development of metastases from a primary tumor is not a random process simply explained by the pattern of circulation [28, 29]. Metastasis appears to localize in a permissive soil. The process of metastasis requires many distinct steps such as the loss of cell adhesion, increased motility and invasiveness, intravasation and survival in the circulation, extravasation into the future site of metastasis, and finally the colonization of this distant site [27–29].

Once the malignant cells encounter the normal tissue stroma at the site of metastasis, it is vital that it can orchestrate the generation of a stroma permissive for the subsequent organization of the metastasis. Whether this is achieved by the cancer cell it self, or by this cell together with cells from the stromal compartment of the primary tumor and/or by activating stems cells from the bone marrow, is unknown. However, there are recent findings of the importance of mesenchymal stem cells in the process of conferring increased metastatic potential to cancer cells. Karnoub et al have shown that cancer cells stimulate secretion of the chemokine RANTES from the mesenchymal stem cells [30]. This chemokine then acts in a paracrine fashion on the cancer cells, leading to a higher metastatic potential through increased motility and invasiveness in a reversible fashion [30]. It is believed that this organization of the metastasis is a dual process, in which there is an initiation of a premetastatic niche that facilitates the initial survival of metastatic cells. This is subsequently followed by an upregulation of genes necessary to effectively colonize the new site [27]. Not all seeded cells will develop into metastases, which is illustrated by the fact that in many cancers, tumor cells can be found in locations such as the bone marrow years before the development of a metastasis [31]. This finding also suggests that the cancer cell may require the development of metastasis, and that the metastatic phenotype most likely depends on continuing interaction with stromal cells [32].

There is a multitude of factors that limit the process of metastasis, such as the physical barrier by the ECM and BM, the effect of endogenous angiogenesis inhibitors in preventing the formation of a tumor vasculature, the limited availability of nutrients and oxygen and the tumor suppressive effect of the immune system [1, 27]. Nevertheless, some cancer cells overcome all these barriers and successfully metastasize to distant sites.

Endostatin—a stroma derived tumor biomarker?

Endostatin is the C-terminal fragment of the BM protein type XVIII collagen. This protein fragment has been studied extensively due to its anti-angiogenic activity in numerous experimental studies [9, 33–35]. Endostatin has also been studied as a cancer therapeutic in

phase I and II clinical trials for patients with metastatic cancer [36, 37]. In these trials endostatin was well tolerated, although the efficacy was disappointingly low compared to that seen in the experimental studies. Stable disease and regression of disease was observed in many cases [36, 37]. Partly, this can be explained by the fact that the studies were conducted on patients with advanced metastatic cancer as well as of very mixed cancer origin. Recently, endostatin has re-entered the clinic in a modified form that renders it more stable [38]. This modified version is now used in certain countries for the treatment of lung and gastric cancer [39, 40].

Endostatin expression by tumor tissue has been studied in several different cancer types [41–44]. It is important to remember that there currently are no antibodies specific for the cleaved endostatin fragment, and that staining in tissues therefore can originate either from the endostatin domain still attached to the parent molecule type XVIII collagen or from the cleaved endostatin fragment. The typical pattern for many cancers is the diffuse expression pattern of endostatin in the tumor stroma as well as in the tumor vasculature, when compared to the distinct BM pattern in normal tissues (Fig. 3) [43]. Most likely the diffuse staining pattern is related to the degree of BM breakdown and therefore an indication of the invasiveness of the tumor. High expression of endostatin indicates poor prognosis, likely reflecting a large tumor burden and active stromal remodeling, although for certain cancer types, the opposite has been noted.

Endostatin is cleaved from the parent molecule by several tumor-associated proteases, such as matrix metalloproteases (MMPs), cathepsin-L and elastase [45–47]. It is also known that endostatin can physiologically be found in the circulation, although different levels have been described, partly due to the fact that endostatin is taken up by the platelets, leading to a difference in plasma and serum concentrations [48]. It has been shown for many cancer types that patients present increased circulating endostatin levels [49–56]. This has been attributed to the effect of tumor load i.e. the larger the tumor the more ECM remodeling and subsequently endostatin release. Although increased endostatin levels also have been observed in certain other pathological conditions. The level of endostatin has been related to various outcomes, and in general a high endostatin level is a marker for worse prognosis. Although endostatin is important in the process of angiogenesis, there are few studies in which the endostatin level was analyzed in the context of it being a predictive marker for outcome of anti-angiogenic treatment.

Compared to the number of studies with endostatin as a prognostic marker, there are far less studies in which this molecule is evaluated in the context of response to cancer treatment. It was recently shown for pancreatic cancer that patients with initially increased endostatin levels normalize these in response to surgical treatment or intraperitoenal chemotherapy [43]. Most likely this is due to a cure or a temporary reduction in tumor burden. This might suggest mean that an increase in tumor load would lead to higher endostatin levels, and if this is the case it is of interest whether this can be observed before other clinical signs of relapse. Endostatin has been discussed here as a potential stroma derived tumor biomarker, but naturally there are multiple such molecules that should be characterized in the context of their role as potential new tumor biomarkers.

Conclusions

Although the importance of the tumor stroma for cancer development and progression is well established in experimental studies, the clinical applications of such findings still remain limited. The stromal compartment contains an abundance of prognostic information, which should be carefully evaluated in clinical samples and hopefully in the future might aid us in designing appropriate treatments for cancer patients. The stroma is a source of many potential new tumor biomarkers and such biomarkers used together with established cancer cell specific biomarkers, maybe be useful in evaluating metastatic potential of a given tumor.

Acknowledgments

This study was partially funded by a research grant from NIH DK 55001 (RK), DK62987 (RK), AA13913, DK61688, CA125550, and research funds from the Beth Israel Deaconess Medical Center for the Division of Matrix Biology. MS was funded by the Swedish Society for Medical Research (SSMF) and the Swedish Society for Medicine.

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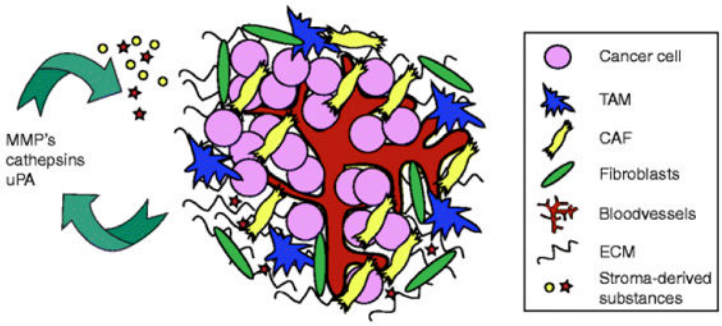

Figure 1.

The tumor stroma. Cancer is much more than just a group of malignant cells and should be viewed more as an organ containing many different stromal cells such as fibroblasts, immune cells and the cells of the vasculature. Both stromal and cancer cells produce proteases that continuously remodel the ECM of the tumor. The ECM remodeling leads to release of substances sequestered in the ECM, as well as bio-active cleavage fragments from ECM proteins such as collagens.

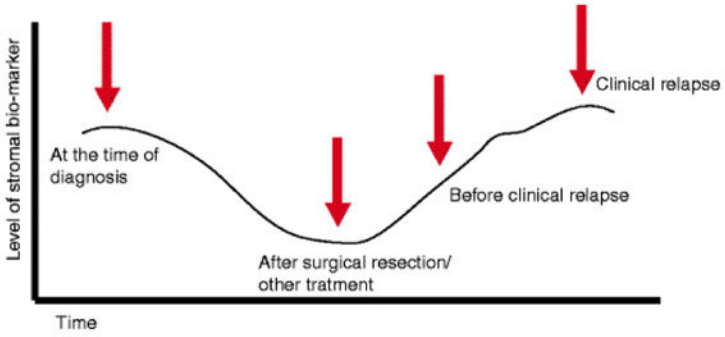

Figure 2.

A model for a stroma derived tumor biomarker and their potential utility. During tumor remodeling process, the stroma may release substances into circulation and they could potentially serve as a novel class of tumor biomarkers. Circulating levels would decrease with successful removal/treatment of the tumor and increase again when disease relapses. Additionally the expression pattern of a stromal biomarker in the primary tumor or metastasis can be of importance in predicting disease progression and outcome of the therapy.

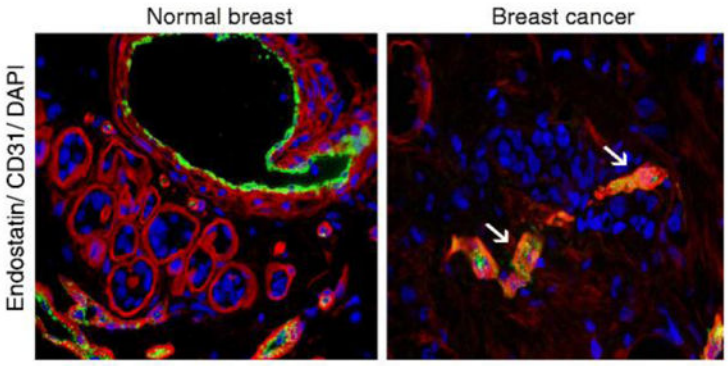

Figure 3.

Expression pattern of type XVIII collagen/endostatin in human breast cancer. a In normal breast tissue the type XVIII collagen/endostatin signal (in red) is located in the BM underlining the ductal epithelium as well as the BM of blood vessels. b In cancer the BM signal is lost from the epithelium and diffuse signal is now seen in the tumor stroma and co-localizing with the endothelial marker CD31 (in green) in the tumor vessels. Cell nuclei are stained with DAPI (in blue).