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MicroRNA regulation in cancer-associated fibroblasts

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Abstract

The microenvironment of cancer cells has proven to be a critical component of tumors that strongly influences cancer development and progression into invasive and metastatic disease. Compared to normal tissue, dramatic differences in gene expression occur in multiple cell types that constitute the tumor microenvironment including cancer-associated fibroblasts (CAFs) which are important stromal components of growing tumors. In this review, we present recent advances in understanding how microRNAs are deregulated in cancer-associated fibroblasts (CAFs) and how this affects tumor biology. The microRNA signature of CAFs is discussed with respect to their functional relevance to tumor cells as well as other cell types involved in tumor homeostasis.

Keywords

cancer-associated fibroblasts; microRNA; microenvironment; tumor; CITIM 2011

Introduction

Cells that compose the tumor microenvironment are increasingly recognized as critical components of tumor progression. Co-evolution and “reprogramming” of the stromal compartment is a prerequisite for cancer to progress to advanced stages [1–3]. Crosstalk and interactions between neoplastic cells and their neighboring cells in the microenvironment provide critical factors that can determine whether tumor cells remain dormant or progress into invasive and metastatic cancer [4–7].

Although the cells within the microenvironment do not undergo malignant transformation, significant changes occur in their pattern of gene expression and consequently in their function, that distinguish these cells from their normal counterparts [8–10]. The tumor microenvironment consists of various non-transformed cells including fibroblasts, myofibroblasts, inflammatory immune cells, endothelial cells and bone marrow-derived mesenchymal progenitor cells. These cells are recruited to transformed tumor cells where they undergo “reprogramming” as a result of complex crosstalk with the tumor cells and other components of the microenvironment. This results in critical changes in the production of extracellular matrix proteins, cytokines, growth factors and proteases that promote the development and progression of cancer [1, 11]. This review will focus on changes that occur in microRNA expression in stromal fibroblasts that become reprogrammed to become cancer-associated fibroblasts (CAFs) as tumors develop and how these changes appear critical for the maintenance and progression of cancer. Understanding what changes occur and how they are regulated may provide new avenues for intervention to prevent or treat many cancers.

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Cancer-associated fibroblasts

Cancer-associated fibroblasts comprise one of the most abundant cell types in the stroma of solid tumors [12, 13]. Several studies have demonstrated that normal fibroblasts restrict oncogenic growth of adjacent epithelial neoplastic cells [14–16]. However, at advanced stages of tumor growth the stromal fibroblast population can induce angiogenesis, secrete a plethora of growth factors and enzymes involved in remodeling of the extracellular matrix and suppress host immune responses that normally would inhibit tumor development [8, 17–19]. The degree of activated CAF infiltration in a tumor correlates with higher grades of malignancy and poorer prognostic outcome [20, 21]. It has been shown that CAFs are a heterogeneous population of fibroblastic cells including myofibroblasts expressing alpha-smooth muscle actin (SMA) and other fibroblastic cells that do not express alpha-SMA but nevertheless promote tumor growth [22–24].

Origin of CAFs

A key question in cancer biology is how normal fibroblasts transition into tumor-promoting, but non-transformed CAFs. Studies using mouse xenograft models have demonstrated that 20–40 percent of CAFs originate from the bone marrow [25, 26]. These progenitor cells are recruited to the sites of neoplasia where they trans-differentiate into myofibroblasts and secrete chemokines, including CCL5, which promote tumor cell growth and metastasis [27]. Experimental data also suggests that the growth factor TGF-β secreted by tumor cells may “educate” resident fibroblasts to become CAFs, further triggering an autocrine loop of TGF-β synthesis by CAFs themselves [28–30]. Another recent study using a mouse model of squamous cell carcinoma demonstrated that resident normal fibroblasts are stimulated by immune cells or tumor cells that produce IL-1β to activate an inflammatory signature mediated by the NF-κB pathway [31]. This event initiates a conversion of normal fibroblasts into CAFs and promotes tumor progression by orchestrating pro-angiogenic programs during carcinogenesis.

Interestingly, although CAFs play an important role in supporting tumor growth, they do not display features of transformed cells, i.e. they do not produce tumors when injected into immunocompromised mice. When grown in culture they senesce after 10–15 population doublings and they exhibit a normal karyotype [8, 18]. Mutations in p53 and PTEN genes have been reported in CAFs [32–35], but it is likely that these mutations accumulate as these cells adapt survival mechanisms. This is supported by the fact that most solid tumors create a hypoxic environment. p53 has been shown to play an important role in mediating apoptosis in hypoxic regions. Therefore, tumor hypoxia may act as a selective pressure for the inactivation of p53 in order for these cells to survive and flourish in this hostile, hypoxic environment [36]. In addition, more recent studies of CAFs using array CGH and SNP analysis have shown that genetic alterations are extremely rare [17, 37, 38]. This raises the possibility that the significant changes in the gene expression in CAFs may result from epigenetic modifications. Indeed, altered methylation patterns have been found not only in tumor cells but also in stromal fibroblasts [39–41].

Another important mechanism that may be involved in the activation of fibroblasts and trans-differentiation of progenitor cells to CAFs is post-transcriptional gene regulation by microRNAs. MicroRNAs are small, non-coding RNAs that can regulate hundreds of genes and have proven to play key roles in a variety of processes including cellular differentiation, development, cell motility and senescence, as well as pathological conditions such as oncogenic transformation and inflammation [42].

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MicroRNA properties

MicroRNAs are small 19–25 nucleotides RNA that play a regulatory role in diverse biological processes [43, 44]. They regulate gene expression at the post-transcriptional level by hybridizing to the complementary sites in the 3′UTR of their target genes that result in translational inhibition or mRNA degradation. Most microRNAs are transcribed by RNA polymerase II as long primary RNAs that contain a 5′CAP and 3′ polyA tail. About 50 per cent of all microRNAs are located within the introns of protein coding genes and are released by splicing of the introns after parental mRNA processing. Primary miRNAs, which may be several kilobases long, are further truncated within the nucleus by RNAse III Drosha and its partner DGCR8. Pre-miRNA is then transported to the cytoplasm by Exportin 5 where it is further cleaved by another RNAse III enzyme, Dicer, into a 22 bp double stranded RNA. Subsequently, a guiding strand of the miRNA is loaded into the RNA Induced Silencing Complex (RISC) that binds to the target protein coding mRNAs and represses its activity by either mRNA destabilization in the case of a near-perfect complementarity between the miRNA and mRNA or by translational inhibition in the case of an imperfect match between the miRNA and the target mRNA. Recent data clearly indicate that microRNAs may function as oncogenes or tumor suppressors in a variety of cancers (for recent review see [42]).

Recently, exciting studies have demonstrated that exosomes produced by the shedding of the plasma membrane from tumor and other cells contain functional microRNAs that were internalized by neighboring cells and delivered to the sites of microRNA-mediated mRNA targeting [45]. Exosome-mediated delivery of microRNAs provides a novel mechanism for cell-cell communication that had previously been considered to be mediated only through the secretion of soluble factors like hormones, chemokines and cytokines that are released by one cell type and the signal through interaction with receptors on the neighboring cells. In addition, microRNAs may also be transferred to other cells through circulating blood cells including monocytes and T-cells [46]. If microRNAs can be transferred from CAFs to the other cells within the tumor stroma, this could have profound effects on the biology of the multiple immune cell types as well as endothelial cells. Therefore, we will consider the potential role of miRNAs expressed by CAFs in regulating processes not only in the CAF s but also in other cells within the tumor microenvironment.

MicroRNA signature of CAFs

We investigated the role of microRNAs in CAFs isolated from endometrial cancer or normal tissue and identified 11 microRNAs that were differentially regulated in CAFs (Fig. 1) [47]. Mir31 was the most down-regulated microRNA in CAFs. Further analysis revealed that re-expression of miR-31 in CAFs impairs their ability to stimulate endometrial tumor cell migration and invasion, implying that miR-31 targets gene(s) are responsible for the secretion of soluble factor(s) implicated in tumor cell dissemination. Matched mRNA gene expression profiling of the same CAF samples revealed a reciprocal activation of the homeobox gene SATB2, a chromatin remodeling factor capable of activating or repressing gene expression that is dependent upon the cellular context [48]. Indeed, when we overexpressed SATB2 in fibroblasts derived from normal endometrium, we identified a number of genes that were upregulated and responsible for promoting tumor expansion, metastasis and angiogenesis (Fig2).

Interestingly, two of the microRNAs suppressed in CAFs, miR-31 and miR-148a, were found to act as metastasis suppressors in various tumor types. Screening of a large panel of breast cancer cell lines revealed an inverse correlation between miR-31 expression and metastatic potential of the cells [49]. This *in vitro* observation was then correlated to patient tumor samples where the abundance of miR-31 in primary breast tumors was inversely

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associated with the propensity of tumors to metastasize. Moreover, using mouse xenograft models, it was shown that overexpression of miR-31 impedes multiple steps of the metastatic cascade including invasion, survival in the blood stream and colonization at distant organs [49].

The crosstalk between CAFs and immune cells in the tumor microenvironment has been well established. It is possible that the exchange of microRNAs between these cellular components may be another important means of cell-cell communication. It is important to keep in mind that targeting the expression of a given microRNAin a tumor may affect multiple cell types. Therefore, it is critical to understand how a particular microRNA may affect multiple cellular components within the microenvironment. For instance, recent data about the role of miR-31 in regulatory T cells (Tregs) provide additional insight into a possible function of miR-31 in Treg-mediated supression. Tregs may be partially responsible for the absence of an adequate immune response against tumor cells. miR-31 inhibits expression of the forkhead box P3 (FoxP3) transcription factor that is necessary for active Treg function [50]. Additional experiments are needed to determine whether forced miR-31 expression will reduce Treg suppressive properties and hence augment tumor-specific immunity. Thus, miR-31 may not only be a direct tumor suppressor of tumor cells, but may also inhibit tumorigenesis by improving an immune response against tumor development.

Another microRNA that is downregulated in CAFs is miR-148a. Importantly, miR148 is uniformly silenced in all tumor types that have been studied. MiR-148a is an intergenic microRNA transcribed from its own promoter and its tumor suppressor role is further supported by the discovery that its promoter is silenced by methylation in a variety of tumors [51, 52]. Moreover, downregulation of miR-148a was reported as a metastatic marker [53, 54] and its expression negatively correlated with lymph node positive disease [52].

There is ample evidence that miR-148a is acting as a tumor suppressor in various tumor types, however not much is known about the role of miR-148a in other tumor stromal cells. The available data indicate that miR-148 function is also important for dendritic cells maturation. Dendritic cells, which contribute another important component of the microenvironment, play an important role in tissue remodeling. Some subpopulations of dendritic cells accumulate in solid tumors and induce immune tolerance and suppress antitumor immune responses. Through direct targeting of calcium/calmodulin-dependent protein kinase II (CAMKII), the enzyme required for maturation and function of dendritic cells, miR-148 was shown to inhibit the function of these cells and the secretion of inflammatory cytokines, including IL-6, IL-12 and TNF-α by these cells [55].

The RIP-Tag2 mouse model of pancreatic cancer has proven to be very useful for characterizing the process of multistep tumor development. Tumors initially arise from multiple hyperplastic/dysplastic islets which may undergo an “angiogenic switch” and progress into encapsulated solid tumors, some of which form highly invasive carcinomas and acquire metastatic properties [56]. Each stage of tumor progression has been characterized by the induction or downregulation of a subset of specific microRNAs [54]. Two of the microRNAs, miR-146a and miR-424, that we found up-regulated in CAFs were also induced during the angiogenic switch early in tumor development. Interestingly, treatment of mice bearing RT2 tumors with sunitinib, a potent inhibitor of angiogenesis, for 7 days reversed the expression of miR-424 to normal levels. Also, the same study indicated that miR-148a was the most down-regulated microRNA in metastatic lesions compared to primary tumor cells. miR-148a together with the other miR-148 family members miR-148b and miR-152, constitutes a metastatic signature of pancreatic cancer. Remarkably, the

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majority of microRNAs found differentially expressed at different stages of these mouse neuroendocrine tumors were similarly affected in a number of human tumors implying the generality of these observations.

As discussed previsouly, the origin of CAFs remains under active investigation. It seems most likely that these heterogeneous fibroblast populations that form CAFs transdifferentiate form several progenital or resident host cells within the tumor microenvironment. A recent study using an HPV16-driven mouse model of multistep squamous cell carcinogenesis showed that the carcinoma cells "educate" resident fibroblasts to express proinflammatory genes through activation of NF-kB signaling [31]. This gene signature persisted in CAFs through subsequent carcinoma stages and was present in mouse models of mammary and pancreatic tumors. Inhibition of NF-κB signaling in CAFs resulted in a slower growth of tumor cells co-injected with these fibroblasts compared to control CAFs.

One of the possible mechanisms of NF-kB stimulation in CAFs was secretion of IL-1β by neoplastic cells. Indeed, IL-1β is a potent inducer of NF-κB and neutralizing IL-1β in conditioned media reduced the expression of known NF-κB target genes in fibroblasts [31]. Relevant to this observation, we found two upregulated microRNAs in CAFs that are involved in NF-κB signaling. miR-181b-1 was recently identified as a key player that links inflammation to cellular transformation of MCF10A human mammary epithelial cells [57]. Non-transformed MCF10A cells were generated to express an activated Src oncogene which induces morphological transformation accompanied by increased growth in soft agar, cell invasion, and tumor growth in mouse xenografts. In this model inflammatory genes were activated by STAT3-mediated induction of miR-181b-1 that targets the tumor suppressor CYLD. In turn, CYLD is a deubiquitinating enzyme that negatively regulates NF-κB activity. The positive feedback loop is completed by induction of IL-6 by NF-κB which phosphorylates and activates STAT3 (Fig. 3). Another microRNA upregulated in CAFS, miR-146a, is directly activated by NF-κB through multiple NF-κB binding sites in the promoter region of miR-146 gene [58]. Importantly, miR-146 was shown to target and suppress two key adapter molecules in the NF-κB pathway, IRAK1 and TRAF6, thus limiting NF-κB activation. Therefore, activation of miR-146 by NF-kB constitutes a negative feedback loop. Taken together, it is clear that the inflammatory pathway mediated by NF-κB is under strict control of multiple regulatory factors including microRNAs (Fig. 3).

Future directions

While we have learned much about the miRNA expressions changes associated with the formation of CAFs, much remains to be studied about what role these miRNAs play within CAFs and whether CAFs export miRNAs to influence the biology of tumor cells and other components of the microenvironment. Interactions between tumor epithelial cells and CAFs are critical for development and progression of tumorigenesis. In order to invoke microRNAs as potential therapeutic agents, we need to understand the functions of miRNAs in the various cell types that constitute a tumor and contribute to tumor progression.

Another yet unanswered question is the commonality of microRNA signatures in CAFs and other stromal cells in different tumor types. It is quite possible that microRNA signatures in CAFs may be unique for different types of tumor. It has been shown that miR-15 and -16 are downregulated in CAFs isolated from prostate cancer and that reconstitution of these microRNAs partially reduces the prostate tumor burden in mouse xenograft models [59]. MiR15 and miR-16 were not found to be downregulated in CAFs from endometrial cancer [47]. Similarly, a specific pro-inflammatory gene signature was identified in dermal, mammary and pancreatic CAFs, but not in CAFs isolated from cervical tumors [31].

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Future studies will analyze microRNA expression from additional cellular components of the tumor microenvironment and determine the functional consequences of altering miRNA expression. Since miRNAs in CAFs appear to play an important role in tumor biology, one can imagine interfering with miRNA expression or function to potentially inhibit critical aspects of tumorigenesis that could be translated clinically in the future.

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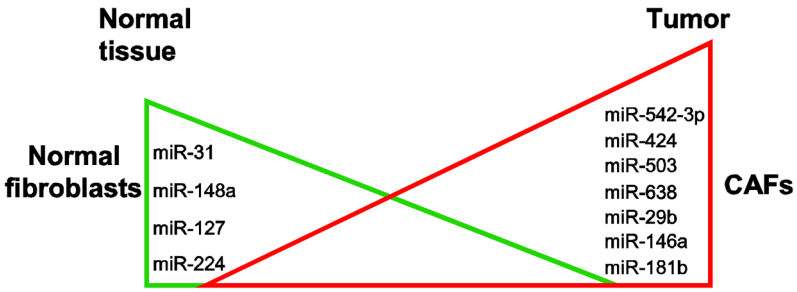

Figure 1.
MicroRNAs differentially expressed in endometrial tumor CAFs vs. fibroblasts from normal endometrium. The green triangle contains microRNAs expressed in normal fibroblasts that are downregulated in CAFs. The red triangle contains microRNAs upregulated in CAFs.

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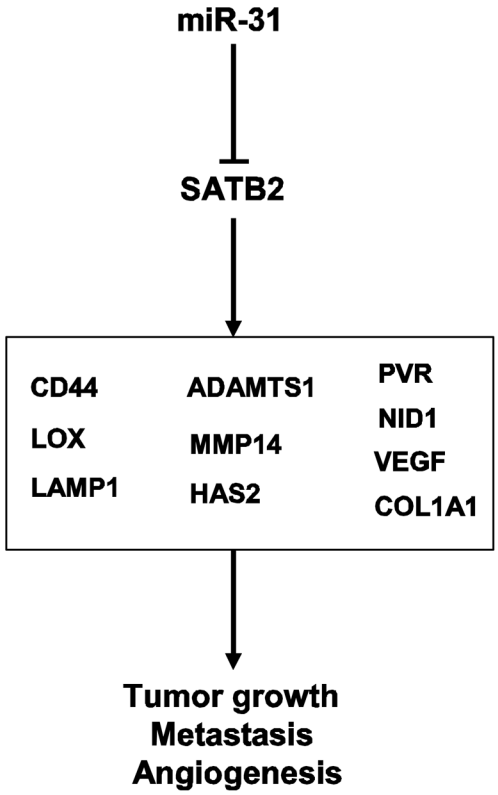

Figure 2.
miR-31 targets the homeobox gene SATB2 that triggers expression of multiple pro-tumorigenic and angiogenic factors.

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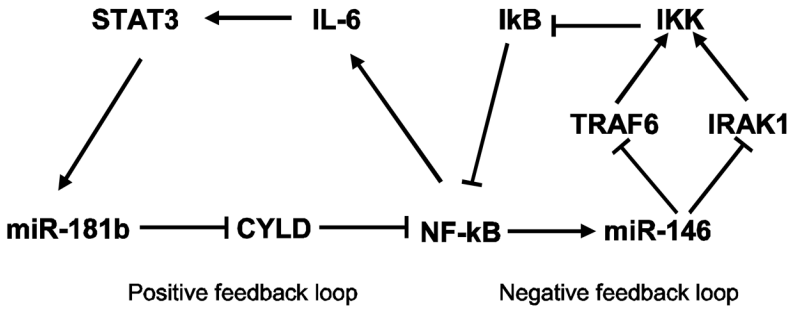

Figure 3.
miR-181b and miR-146a induced in CAFs are involved in the regulation of the NF-kB signaling pathway.