Review

MicroRNAs in the Communication between Cancer Associated Fibroblasts (CAF) and Cancer Cells

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Abstract: Tumor microenvironment including cancer-associated fibroblasts (CAF) has developed as an important target for understanding tumor progression, clinical prognosis and treatment responses of cancer. Cancer cells appear to transform normal fibroblasts (NF) into CAFs involving direct cell-cell communication and epigenetic regulations. This review summarizes the current understanding on miR involvement in cancer cell – tumor environment/stroma communication, transformation of NFs into CAFs, their involved targets and signaling pathways in these interactions; and clinical relevance of CAF-related miR expression profiles. There is evidence that miRs have very similar roles in activating hepatic (HSC) and pancreatic stellate cells (PSC) as part of precancerous fibrotic diseases. In summary, deregulated miRs affect various intracellular functional complexes, such as transcriptional factors, extracellular matrix, cytoskeleton, EMT/MET regulation, soluble factors, tyrosine kinase and G-protein signaling, apoptosis and cell cycle & differentiation, but also formation and composition of the extracellular microenvironment. These processes result in the clinical appearance of desmoplasia involving CAFs and fibrosis characterized by deregulated stellate cells. In addition, modulated release of soluble factors can act as (auto)activating feedback loop for transition of NFs into their pathological counterparts. Furthermore, epigenetic communication between CAFs and cancer cells may confer to cancer specific functional readouts and transition of NF into their pathological counterparts. MiR related epigenetic regulation with many similarities should be considered as key factor in development of cancer and fibrosis specific environment.

Keywords: MicroRNA; cancer associated fibroblasts; cell-cell communication; transformation; epigenetic

1. Introduction

Over the years the tumor microenvironment has developed as an important target for understanding tumor progression, clinical prognosis and treatment responses of cancer, such as for chemotherapy and radiation. General understanding of a dynamic relationship between the expanding tumor and the host surrounding tissue is advancing. Cancer-associated fibroblasts (CAF) are a major cellular constituent of this tumor stroma, but little is known how cancer cells transform normal fibroblasts (NF) into CAFs and about the cell-cell communication between cancer cells and CAFs. These cells and their interactions seem to be relevant for various cancer related phenomena, but also for resistance against treatment modalities. [1] For example, CAFs can promote tumorigenesis, growth, invasion and metastasis of cancer, whereas NFs are thought to suppress tumor progression. [2] As one key event CAF activation appears to induce alternative production and secretion of extracellular matrix (ECM) proteins resulting in ECM remodeling and cancer cell invasion. [3] Resulting desmoplasia is not only attributed to CAFs but also to surrounding stellate cells, which are believed to play critical roles in conferring cancer aggressiveness.

Although it is fully accepted that microRNA (miRs) are deregulated in human cancers, it is only at the beginning to elucidate whether miR expression and function in resident fibroblasts of the tumor microenvironment are affected by their interactions with cancer cells. There is increasing evidence that

miRs are involved in the transformation from NFs into CAFs and that vice versa miRs released from CAFs can affect various characteristics of cancer cells. Collectively, published results suggest a crosstalk between CAFs and cancer cells, which in part may grant increased aggressiveness of the tumors. [4]

MiRs are small noncoding RNA molecules that negatively regulate gene expression at a posttranscriptional level. Their target genes are known to affect cell differentiation, adhesion, migration, proliferation, secretion and cell-cell interaction, among others. It has also been proposed that miRs can reprogram various somatic cells to become pluripotent stem cell. [5] MiRs frequently occur in clusters at a short inter-miR distance [6] and are usually combined by a single promotor/ transcription region for all cluster members. They have to be differentiated from miR families where sequence homology is the major determinant. Clustering appears to be an important way of evolutionary spreading of miR genes throughout the human genome and many clusters have a significant degree of evolutionary conservation [7], indicating that miR clustering is important for their biological role due to combined regulation of their expression. [8]

The aim of this review was to summarize the current understanding on a) how miRs are involved in cancer cell – tumor environment/stroma communication, specifically the transformation of NFs into CAFs, their involved targets and signaling pathways in these interactions; and b) whether CAF-related miR expression profiles are clinically relevant. By reviewing the literature we also found evidence that miRs have very similar roles in the activation of hepatic (HSC) and pancreatic stellate cells (PSC) which are also specifically activated fibroblasts being part of precancerous fibrotic diseases, like pancreatic and hepatic fibrosis.

2. Methods

A PubMed search was performed using combinations of the following keywords: miR, microRNA, cancer associated fibroblasts, cancer and fibroblasts, hepatic stellate cells (HSC) or pancreatic stellate cells (PSC). Articles that met the criteria included

- ([mir] OR [miR] OR [microRNA]) AND [cancer] AND ([caf] OR [cancer associated fibroblasts]) = 184;
- ([mir] OR [miR] OR [microRNA]) AND [cancer] AND ([hsc] OR [hepatic stellate cells]) = 64;
- ([mir] OR [miR] OR [microRNA]) AND [cancer] AND ([psc] OR [pancreatic stellate cells]) = 15.

Relevant publications were identified by screening the abstracts and the text of the entire published paper as necessary. Reference lists from relevant articles were also searched for additional literature. Data were extracted from the article, or - if only available – from the abstracts. This method was applied until the beginning of May 2016.

A narrative review of relevant literature was conducted to identify and summarize evidence that miRs play a pivotal role in the communication between cancer cells and environmental fibroblasts. Due to clinical and functional similarities of fibroblasts in cancers and precancerous lesions, pancreatic and hepatic fibrosis were included in this search. Specific focus was given towards cluster organization of the reported miRs.

3. Clinical relevance of miR in CAF

Very few data are available on deregulated miR-expression in CAFs of clinical specimens. Up- and downregulation of miRs has been found comparing normal or tumor-adjacent fibroblasts. For example, in ovarian CAFs, miR-31 and miR-214 were downregulated, whereas miR-155 was upregulated. [5] In endometrial cancer miR-148a [9] and miR-31 [10] were downregulated. MiR-26b was downregulated and miR-92 was upregulated in CAFs from estrogen receptor (ER)-positive breast cancers. [11, 12] Downregulation of miR-200 family members in activated CAFs has been found in breast [3,13], gastric [14] and pancreatic cancer [15]. Interestingly, a reciprocal correlation of miR-200 and its putative target expression (SIP1 and E-cadherin) compared between pancreatic CAFs and

cancer specimens was reported. [15] In addition, miR-21 was frequently upregulated in CAFs in various entities. [16, 17] Moreover, expression of this miR in stellate cells derived from normal pancreas was substantially lower when compared to PSCs or CAF cells. [4] Specific upregulation of miR-409-3p and miR-409-5p was found in prostate cancer stromal tissue specimens [18], whereas miR-15 and miR-16 were downregulated in fibroblasts surrounding prostate tumors [19]. Additional mixed patterns were found by array technologies in breast [9, 13], lung [20] and prostate [21] cancer specimens in very small patient cohorts, but the evidence for reproducible cancer specificity of such signatures is still very limited. These genome-wide screenings provided potentially deregulated miRNAs in CAFs from different entities (upregulated: miR-221-5p, miR-31-3p, miR-221-3p; and reduced: miR-205, miR-200b, miR-200c, miR-141, miR-101, miR-342-3p, let-7g, miR-26b). (Table 1)

Prognostic impact of altered miR expression in CAFs in clinical cohorts has been observed for patients with breast cancer (miR-26b) [11], gastric cancer (miR-106b [22], miR-143 [23], miR-145 [24], miR-200b [14]), esophageal carcinoma (miR-21 [16], miR-27a/b [25]) and pancreatic adenocarcinoma (miR-21 [16]). Furthermore, miR-21 expression in CAFs was elevated in colorectal cancer specimens compared to fibroblasts in colonic polyps [26] and it was associated with decreased overall survival in pancreatic cancer patients who received 5-FU, but not gemcitabine. [17] It has also been reported that miR-27a/b are involved in resistance to chemotherapy in esophageal cancer through miR-27a/b-induced transformation of NFs into CAFs. [25]

Clinical expression data for PSCs have not been published yet, but for HSCs and liver fibrosis several investigations are available. The hepatic contents of miR-21 [27, 28], miR-33a [29] and miR-200b [30] were significantly increased in liver specimens from human patients with liver fibrosis as compared to normal patients. Upregulation was also identified for miR-199a-5p/199a-3p and miR-221/222 in hepatitis C induced liver fibrosis in a fibrosis progression-dependent manner. [31] Members of the miR-17-92 cluster (19a, 19b, 92a) [32], miR-29, miR-133, miR-193 and miR-30c [33, 34] were observed to be specifically downregulated in human liver fibrosis and HSC, while they showed a reciprocal expression pattern after recovery from liver fibrosis. Reduced expression of miR-144 was correlated with elevated HSC-specific expression of transforming growth factor-β1 (TGF-β1) and expression of α-SMA in fibrotic liver tissues. In contrast, miR-200c did not show comparable differences and correlations. [35] Hepatic expression levels of miR-199a, miR-199a*, miR-200a, and miR-200b were positively associated with progression of liver fibrosis. [30, 36]

In liquid biopsies, levels of miR-17-5p [37], miR-21 [27], miR-33a [29] and miR-181b but not miR-181a expression [38] were higher in fibrotic than in normal patients. In addition, miR-133a serum levels were increased in patients with chronic liver disease and indicative for the presence and progression of liver cirrhosis. [34]

Overall, the body of evidence for clinically useful miR expression patterns in CAFs as prognostic or predictive markers is not yet sufficient for any recommendation into clinical applications. In addition, a systematic approach for identification of the relevant miR expression profiles has not been done thoroughly and, therefore an overall picture for patients is not available yet. Most promising candidates for future clinical use appear to be miR-21, miR-31 and the miR-200 family. Furthermore, a number of miRs that are known for their involvement in cancer cells have been identified to also be important in patients derived CAFs suggesting a further look into their functional consequences.

4.   Functional consequences in CAF

Molecular mechanisms leading to transition of tissue-resident fibroblasts into CAFs, circulating bone marrow-derived fibroblast progenitors or mesenchymal stem cells are largely unknown. [21] The spectrum of miRs that have been identified as relevant regulators of this CAF transition and/ or for their interactions with cancer cells is relatively limited. Except for two publications investigating squamous cell esophageal carcinomas [25, 39] all data were derived from adenocarcinomas of different organs. Interestingly, the majority of these miRs belongs to miR clusters (<10kb sequence distance between the members) with functional overlap between the various cluster members. Deregulation of miRs in fibroblasts in general appears to induce as major effects smooth muscle α-

actin (α-SMA) upregulation and modulated secretion of cytokines including: increased IL-6 and CXCL12, activation of the TGF-ß and inhibition of various signaling pathways, such as the PI3K-AKT pathways. [2, 3, 20] Various miRs interfere with multiple cellular functions and therefore be found at different point in the subsequent listing.

An overview about the miRs with altered expression in CAFs is provided in Table 2A+B (extended data are available in Supplemental Table 2

Deregulated miRs in A) CAFs and B) HSC/PSC, consequences on cellular functions and identified target structures. MiRs that occur in clusters are highlighted with blue background

A)

miR with Cluster	Cancer Entities	Regulation of miR in CAFs	Interaction in CAFs	migration	invasion	adhesion	growth	proliferation, progession	differentiation	chemoresistance	apoptosis	methylation	Pathway	Targets
15, 16	PC	↓	α-SMA ↑	↑			↑	↑					p-AKT, p-ERK ↑	Fgf-2 and its receptor Fgfr1 ↑
27a/b	EOC	↑	α-SMA ↑							↑	↓		TGF-ß ↑	
92	BC	↓			↑									
106b	GC	↑	α-SMA ↑	↑	↑		↑						TGF-ß ↑	PTEN
101	BC, LC	↓↑	α-SMA ↑, IL-6 ↑	↓↑	↓↑	↓		↓↑	↓		↑	↓	PI3K-AKT ↓, TGFß ↑	CXCL 12 ↓
143, 145	GC	↑	α-SMA ↑, Collagen Typ III ↑			↑		↑	↑				TGF-β/SMAD signaling ↑	
200a, 200b	BC, LC, PaC, GC	↓↑	α-SMA ↓↑ , IL-6 ↑	↓↑	↓↑	↓↑		↓↑	↓↑		↓		TGF-ß ↑↓	ZEB1, ZEB2 ↑; Flt 1 ↓ ; SIP1
221	BC, PaC	↑	α-SMA ↑, IL-6 ↑	↑	↑					↑			TGF-ß ↑	NF-κB, K-Ras ↑
214	OC	↓	cytokines ↑	↑	↑		↑							CCL5
127	BC	↓						↑					p53/p21 ↑	BCL6 oncogene ↑
133b	PC	↑	α-SMA, IL-6 ↑, Collagen 1A1					↑					TGF-ß ↑	
141, 200c	BC	↓↑	α-SMA ↑, IL-6 ↑,	↑	↑	↑		↑	↑		↑		TGF-ß ↑	Fli-1, TCF12
342	BC	↓	α-SMA ↑ , IL-6 ↑	↑	↑	↑		↑	↑		↑		TGF-ß↑	
365	BC	↓	IL-6 ↑	↑	↑								p38 MAPK ↓	NF-kBp65 ↑
409	PC	↑	α-SMA, EMT ↑, extracelluar vesikel (EV) release ↑				↑	↑						Ras suppressor 1, stromal antigen 2

B)

miR with Cluster	PSC/HSC	Regulation of miR in PSC/HSC	Interaction in cells	Cellular consequences					Pathway	Targets
				migration	growth	development, movement	proliferation, progession, activation	fibrosis, apoptosis		
15, 16	PSC	↓						↑		BCL-2 ↑
27a/b	HSC	↑					↑			retinoid X receptor alpha
17, 19b	HSC	↑↓	Collagen Type I and α-SMA ↑↓, expression of α1(I) and α2(I) procollagen in mRNAs ↑		↓		↓	↑	TGF-ß 1 ↑↓	SMAD7 ↓, TGF-ß 2 rezeptor and SMAD 3, GRB2
101	HSC	↑		↓			↓		TGFß ↓	TβRI/KLF6 ↓
143	PSC	↑			↑	↑			Smad 2/4	p39 MAP kinase & extracell.-signal–regulated kinase
200a, 200b	HSC	↓↑	α-SMA , EMT process ↓	↑	↓		↓↑	↑	Wnt/β-catenin, TGFβ , PI3K/Akt ↑, Hh pathway	FOG2 ↓-regulation, Keap1/Nrf2 ↑, Gli2 ↓
221, 222	HSC, PSC	↑	α1Collagen and α-SMA ↑	↑		↑		↑	Smad 2/5, NF-κB	p40 MAP kinase & extracell.-signal–regulated kinase, Cox-2 protein expression, NF-κB ↑
214	HSC	↓	Collagen Type I ↓					↑		HDAC4 ↑, Cox-2 protein expression, NF-κB ↑
29a	HSC, PSC	↓↑	Collagen Type I	↑	↑	↑		↑	TGF-β1 (SMAD3 dependant) ↑	
34c	HSC	↑	α-SMA ↑							PPARγ ↓
122	HSC	↓	collagen maturation and ECM production ↑				↑			P4HA1 ↓
130b	HSC		ECM ↑				↑			PPARy ↓
144	HSC	↓	α-SMA ↑					↑	TGF-ß1 ↑	

Table 3) showing that various miRs occur up- and downregulated even within the same tumor entity, such as miR-101, miR-200a and miR-200b. It appears quite interesting, that those miRs (except miR-101) belong to the miR-200 family, which is clustered at chromosomes 1 & 12. Similar observations have been summarized recently for the miR23~24~27 clusters in cancer cells. [8]

4.1. EMT/MET Switch

The known functions of miR-200 family members in the EMT/MET switch (targeting ZEB1, ZEB2; E-cadherin expression) can also be found in CAFs. [2] MiR-146b inhibition is sufficient to transactivate NFs into CAFs, which promote EMT transition in breast cancer cells in a paracrine manner. [40] In a similar manner miR-148a targets genes of the WNT family, WNT1 and WNT10b with comparable cellular effects. [9] Furthermore, miR-21 overexpression was associated with enhanced ERK1 signaling and EMT in liver fibrosis via direct suppression of SPRY2 and HNF4α expression. [27] Promotion of tumor induction and EMT was also induced by ectopic expression of miR-409 (member of a very large cluster) in prostate NFs conferring a CAF-like phenotype and leading to the release of miR-409 via extracellular vesicles. This miR also enhanced tumorigenesis through repression of tumor suppressor genes such as Ras suppressor-1 (RSU-1) and stromal antigen 2 (STAG2). [18] Additional targets were observed that are also involved in both processes, including Fli-1 (directly regulated by miR-200c), TCF12 (directly targeted by miR-141) [3], SIP1 (by promoter methylation via miR200a/b) [15] and Flt-1 (directly targeted by miR-200b) [41].

4.2. TGF-β Signaling

A relatively large body of data has been published for miR-21 (chromosome 17q23.2) which is one of the few non-clustered miRs that have been found to be relevant in CAFs. This miR may be an important factor in "activating" NFs into CAFs and its expression is mostly confined to cancer stroma [16, 4]. Via direct targeting miR-21 is a negative regulator of programmed cell death protein 4/activation protein-1 (PDCD4/AP-1) in cancer cells [26] and HSCs [28]. This appears to be based on a miR-21 feedback loop with these signaling pathways promoting fibrogenesis and the TGF-β signaling pathway underlying HSC activation. [28]

MiR-145 is inducible by treatment with TGF-β leading to enhanced α-SMA expression in normal gastric fibroblasts and CAFs. [24] MiR-27a/b-transfected normal fibroblast showed α-SMA expression and increased production of TGF-β as typical characteristics of CAFs. [25] In addition, miR-126 can support TNFα induced TGF-β1 expression. [42] MiR-205 has been observed as most downregulated miR in prostate cancer cells upon CAF stimulation due to direct transcriptional repression by HIF-1α, a known redox-sensitive transcription factor. [57] In addition, miR-127 is the best described member of a large cluster of miRs on chromosome 14 that also act as key modulators of TGF-related cellular senescence by targeting critical regulators of the senescence pathways. This miR is upregulated in senescent fibroblasts and may function as tumor suppressor and inhibitor of breast cancer cell proliferation by modulating the BCL-6 oncogene. [43]

Functional data for members of the three miR-17-92 clusters in cancer are almost lacking. For fibrotic progression, however, various investigations showed the importance of these cluster members for intensive interactions with TGF-β signaling. For example, HSC activation is a pivotal event in initiation and progression of hepatic fibrosis and a major contributor to collagen deposition driven by TGF-β. In animal models miR-19b showed the highest fold-change of the cluster members. Its mimic negatively regulated TGF-β signaling components including decreased TGF-β receptor II (TGF-βRII) (by direct binding) and SMAD3 reduced expression of type I collagen and blockage of TGF-β-induced expression of α1(I) and α2(I) procollagen mRNAs. Similarly, enhanced miR-17 expression was observed in rat liver fibrosis. Its inhibition also led to suppression of HSC proliferation induced by TGF-β1 without affecting cellular apoptosis, but with significant association of type I collagen and α-SMA expression in HSC. [37]

Comparable to the tumor environment expression levels of fibrosis related genes in HSCs were increased by overexpression of miR-200 family members (miR-200a, and 200b). [36] For example, miR-

200a was decreased in TGF-β1-induced HSC activation and induced liver fibrosis. Overexpression of miR-200a in HSCs inhibited α-SMA activity and proliferation. In addition, β-catenin and TGF-β2 were confirmed as two functional downstream targets of miR-200a in the fibrotic framework. [44] Divergent roles of miR-181 cluster members in HSCs have been reported. MiR-181b but not miR-181a could promote HSC proliferation induced by TGF-β1 through regulation of cell cycle (targeting p27Kip1). [38] Similarly, the X-chromosomal miR-221/222 is increased in liver fibrosis models (stimulated by TGF-α or TNF-α). MiR-222 can bind to CDKN1B in HSCs and its induction can be suppressed by NF-κB inhibitor. [31]

Members of the miR-101 family (miR-101a/b) can act as suppressors of TGF signaling by directly targeting TGF- RI and its transcriptional activator Kruppel-like factor 6 (KLF6) during liver fibrogenesis where these miRs are reduced in activated HSCs. Meanwhile, upregulation of TGF-RI/KLF6 was observed in the fibrotic liver. [45]

4.3. Extracellular Matrix, Migration and Invasion

MiR-122 overexpression also led to decreased collagen maturation and ECM production [46] whereas miR-126 [42] and miR-17-92 cluster members [37] induced type 1 collagen expression. Reduced expression of miR-335 during HSC activation promotes their cell migration via targeting tenascin-C and enhanced expression of α-SMA and type 1 collagen. [47] Furthermore, miR-19b blunted the activated HSC phenotype by morphological assessment and decreased α-SMA expression. [32] In HSCs another cluster member, miR-133a, is downregulated by TGF-β with subsequent stimulated expression of collagens. [34] The expression levels of fibrosis related genes (TIMP-1, MMP13, α1-procollagen) in HSCs were increased by overexpression of other members of the miR-214 cluster (miR-199a, 199a*). [36] Moreover, miR-200b appears to enhance expression of matrix metalloproteinase-2 (MMP-2), which may increase the migration of HSCs during liver fibrosis progression. [30] MiR-31 may also mediate liver fibrosis by promoting HSC activation and enhancing MMP-2 expression. Functionally this appears to be mediated via FIH1, a suppressor of hypoxia-inducible factor (HIF-1). [48]

Among others, miR-200 family members were identified as potential direct mediators of NF reprogramming into CAFs and of ECM remodeling. NFs with downregulated miR-200s displayed the traits of activated CAFs, including accelerated migration and invasion. [3] Overexpression of miR-200b can enhance migration and proliferation of fibrotic HSCs; this is accompanied by stimulated phosphorylation of Akt, a downstream effector of phosphatidyl-inositol 3-Kinase (PI3K). Within this PI3K/Akt pathway FOG2 is an additional target of miR-200b that directly binds to p85α inhibiting activation of the signaling. In fibroblasts with amplification of miR–92 expression an enhanced invasive capacity of breast cancer cells was reported. [12] CAFs with downregulated miR-106b could significantly inhibit gastric cancer cell migration and invasion by targeting PTEN. [22] In addition, miR-21 can play a role in expression and activity of MMP2 and HSC activation through PTEN/Akt pathway. [49]

Intercellular communication between ER-positive breast cancer cells and CAFs reduced miR-26b expression in CAF but enhanced cancer cell migration and invasion. Pathway analyses of differentially expressed proteins revealed that glycolysis/TCA cycle and cytoskeletal regulation by Rho GTPases are downstream of miR-26b. In addition, its targets (TNKS1BP1, CPSF7, COL12A1) were identified and their expression in cancer stroma was shown to be significantly associated with breast cancer recurrence. [11] Both, miRNA-150 and miRNA-194 inhibit HSC activation and ECM production, at least in part, via inhibition of c-MyB and Rac-1 expression. [50]

In fibrotic stromal reaction of pancreatic cancer [51] and in liver fibrosis [52] members of the miR-29 cluster (miR-29a) are significantly decreased. This loss influences the activation of CAFs/PSCs/HSCs and is correlated with increase in extracellular matrix (ECM) deposition including type 1 collagen-α1. This profibrogenic phenotype is likely caused by inhibition of HDAC4 function including histone acetylation and mediated by SMAD3 dependent TGF-β1 activation. In contrast to other family members miR-146a (chromosome 5) has been found downregulated in liver fibrotic tissues [53] and in HSCs in response to TGF-β1 or TNF-α stimulation with subsequent α-SMA/type 1

collagen expression and attenuated HSC apoptosis via direct targeting of TIMP-3 [54], Wnt1 and Wnt5a [53]. In vitro, miR-210 was identified as upregulated in and secreted by PSCs inducing activated ERK and Akt, but not hypoxia-inducible factor-1α (HIF-1α) pathway in effector cancer cells. Subsequently, this stimulated migration, expression of vimentin and Snail-1, and blocked membrane-associated expression of β-catenin. [55]

4.4. Soluble Factors

Interactions between CAFs and cancer cells by soluble factors appears to induce most of the transcriptional changes characteristic for patient-derived CAFs. For example, miR-15/miR-16 cluster members (chromosomes 3 & 13) and their correlated growth factor targets, such as fibroblast growth factor-2 (FGF-2) and its receptor FGFR-1, seem to promote tumor expansion and invasiveness through the concurrent activity on stromal and cancer cells. Similarly, miR-483 members act together to target two pro-fibrosis factors, platelet-derived growth factor B (PDGF-B) and TIMP-2, which can suppress activation of HSCs and their overexpression can reduce liver fibrosis in vivo. [56] In the opposite regulatory direction, among a panel of soluble growth factors only TGF-β remarkably increased miR-31 expression in these cells by direct binding of SMAD3 to miR-31's promoter suggesting regulatory counteraction of both molecules. [48] Treatment of HSCs with PDGF-B stimulated a1(I) collagen mRNA synthesis and the protein expression of α-SMA in a miR-21 dependent manner, which both are characteristics of HSC activation and simultaneously increased miR-21 expression. [49] This suggests feedback loops of activation.

Comparably, miR-133b (member of the miR-133 cluster) interferes with paracrine communication of CAFs by interleukin-6 (IL-6) that can promote tumor progression. [21] Mediated by miR-146b (chromosome 10) and by repression of IL-6 secretion the tumor suppressor p16INK4A protein inhibits the pro-carcinogenic effects of breast stromal fibroblasts. In addition, miR-205 blocks tumor-driven activation of surrounding CAFs by reducing pro-inflammatory cytokine secretion and vice versa prevents CAF-induced EMT in cancer cells in vitro. [57] The miR-193b/-365a cluster is also involved in cytokine related cell-cell communication. TGF-β-dependent downregulation has been identified involving Snail-1, an important regulator of extracellular matrix in HSCs [33]. Suppressed NF-κB dependent p65 activation inhibits miR-365 expression with resulting increased IL-6 secretion within the cancer environment. [58]

Furthermore, the miR-reprogrammed normal fibroblasts and patient-derived CAFs shared a large number of upregulated genes highly enriched in chemokines, which are known to be important for CAF function. The most highly upregulated chemokine, CCL5, (C-C motif ligand 5) was found to be a direct target of miR-214. [5] Similarly, estradiol (E2) as another soluble environmental factor and a major determinant of gender-based differences in the development of hepatic fibrosis was modulated by miR-19b in regulation of HSC proliferation via direct interference with GABA(B) receptor GRB2. [59]

The role of other forms of miR-involvement in cell-cell communication has been recently introduced. Pancreatic cancer cells can reprogram adjacent NFs into CAFs by means of secreted microvesicles containing miR-155 likely to be considered as exosomal miR transfer. [60] Comparably, miR-21 release from fibroblasts appears to influence migration and invasion capacity of cancer cells [26].

4.5. Cell Cycle and Proliferation

Downregulated miR-15a/miR-16-1 in CAFs can promote prostate cancer growth, progression and cancer cell survival, proliferation and migration. Their reconstitution impaired tumor supportive capability of these modified stromal cells. [19] In contrast, members of the paralog cluster (miR-15b/miR-16-2) seem to induce apoptosis in activated PSC (targeting Bcl-2 [61]) and HSC (cyclin D1 [62]) and their decreased expression can support pancreatic and liver fibrosis, respectively. Downregulated miR-146a also attenuated HSC apoptosis via direct targeting of SMAD4 [63].

The synthesis pathways of various ECM proteins, especially collagens, is targeted by miRs in fibroblasts and thereby enhancing the fibrotic phenotypes. For example, in vitro and in a mouse fibrosis model miR-122 expression was reduced in activated HSCs supporting their proliferation which appears to be mediated by direct targeting of prolyl 4-hydroxylase subunit alpha-1 (P4HA1). Binding activity of CCAAT/enhancer binding protein alpha (C/EBPα) to the miR-122 promoter region was reduced in these cells. [46] Furthermore, one member of the miR-33 family (miR-33a), which is located in intronic regions within protein-coding genes for Sterol regulatory element-binding proteins (SREBP-2 and SREBP-1), is highly expressed during TGF-β1 induced activation of the PI3K/Akt pathway in HSCs. This appears to result in expression of type 1 collagen (Col1A1) and α-SMA due to direct targeting of peroxisome proliferator activated receptor-alpha (PPARα). [29] Similarly, miR-34a has not been described in CAFs yet, but in activated HSCs and liver fibrosis it was found to be upregulated, regulating a plethora of target proteins involved in the cell cycle, apoptosis, differentiation and cellular development, such as upregulated PPARγ and downregulated α-SMA. [64] PPARγ maintains HSCs in a quiescent state, and its downregulation induces HSC activation. This appears to be related to TGF-β1 induced enhanced expression of various members of the miR-130 precursor family (miR-130a, miR-130b, miR-301a), miR-27b and miR-340 in liver fibrosis. Overexpression of miR-130a and miR-130b enhanced cell proliferation involving Runx3 and upregulation of ECM genes. [65] Such quiescent HSC phenotype can also be stimulated by overexpression of miR23~24~27 cluster members (likely via targeting retinoid X receptor) [18] and miR-146a with decreased cell proliferation [63].

Cellular senescence acts as a barrier to cancer progression, and non-clustered miR-22 is thought to be a potential senescence regulator by inducing growth suppression and acquisition of a senescent phenotype in human normal and cancer cells. Its knockdown in pre-senescent fibroblasts decreased cell size, motility and invasion. This seems to be mediated by direct targeting of CDK6, SIRT1, and Sp1, genes involved in the senescence program. [66] Upregulation of miR-9a and downregulation of its target SIRT1 were observed in fibrotic liver models. Overexpression of SIRT1 could partially abrogate the miR-9a mediated suppression of HSCs proliferation, migration and activation. [67]

A number of other transcription factors have been identified as miR-targets in fibroblasts. For example, miR-200a can regulate the Keap1/Nrf2 pathway in HSCs and hepatic fibrosis through the association of Nrf2 with Keap1, which results in cytoplasmic Nrf2 degradation and interference with induction of genes that encode antioxidant enzymes, such as NQO1. [68] In addition, in CAFs miR-31 is among the most downregulated miRs that directly targets the homeobox gene SATB2, which is responsible for chromatin remodeling and regulation of gene expression as part of CAF transition. [1] However, in PSC upon activation, this miR was upregulated, among others. [69] In HSCs and PSCs reduced miR-126 was observed [69] promoting the inhibition of NF-κB activation by upregulation of IκBα protein expression.

5. MiR-targeted functional readouts

Importantly, the expression of many miRs that are deregulated in fibroblasts associated with pathological conditions appear to be regulated themselves by soluble factors, including TGF-ß as main regulator, but also by other growth factors, IL-6, TNF-α and several chemokines. In addition, few proteins (c/EBPα, SIRT1) were identified that bind to miR promotor regions hereby modulating miR transcription. Furthermore, feedback loops have been described for TGF-α and IL-6 that appear to modulate expression of a large variety of miRs.

Most miR targets that have been found in CAFs/HSCs/PSCs can be grouped into various functional complexes. These include transcriptional factors, extracellular matrix, cytoskeleton, EMT/MET regulation, soluble factors, tyrosine kinase and G-protein signaling, apoptosis and cell cycle & differentiation (Figure 1). However, as known for many miRs also in CAFs and related cell types multiple (N-to-N) relationships can be preferably found resulting in a large network of interactions. Considering the phenomenological appearance of desmoplastic reactions and fibrosis it is not surprising, that many regulatory interactions of identified miRs focus at cytoskeleton, esp. α-SMA,

and ECM, esp. type 1 collagen. The largest number of identified targets belongs to the transcriptional machinery with a high variety of interactions. This appears to be relevant for the transition of NF into their pathological counterparts with subsequent changes in the readout of many cell functions and modulated protein expression profiles in CAFs/HSCs/PSCs and their microenvironment. MiR related epigenetic regulation should therefore be considered as key factor in the development of cancer and fibrosis specific environment and both transitions have many epigenetic similarities. Although many deregulated miRs in CAFs/HSCs/PSCs belong to miR clusters functionally different consequences between clustered and non-clustered miRs cannot be attributed according to the currently available literature. (Table 4) However, due to the fact that about two third of the identified miRs belong to clusters it seems to be likely that conjoint deregulation of clustered miRs might play important roles in transforming NF into their pathological counterparts.

Unexpectedly, the epigenetic cell-cell communication between cancer cells and their surrounding fibroblasts, such as by microvesicles including exosomes, has only been recently investigated in very few studies. [18] They showed miR transfer from CAFs towards cancer cells thereby inducing cancer specific alterations in the target cells, such as modulated proliferation. The opposite direction of epigenetic communication has been reported for the pancreatic cancer microenvironment [60].

In summary, deregulated miRs affect various intracellular functional complexes, but also formation and composition of the extracellular microenvironment. These processes result in the clinical appearance of desmoplasia involving CAFs and fibrosis characterized by deregulated stellate cells. In addition, modulated release of soluble factors can act as (auto)activating feedback loop for transition of NFs into their pathological counterparts. Furthermore, epigenetic communication between CAFs and cancer cells, for example via extracellular microvesicles, may confer to cancer specific functional readouts. (Figure 2)

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Conflicts of Interest: The authors declare no conflict of interest related to this review.

Figures

Figure 1

Currently known miR target structures in CAF/HSC/PSC. Targets are grouped according to main cellular functions, miRs are ordered in clusters (left), unclustered miRs are grouped on the right side. These interactions underline frequent n-to-n-relationships of target regulation.
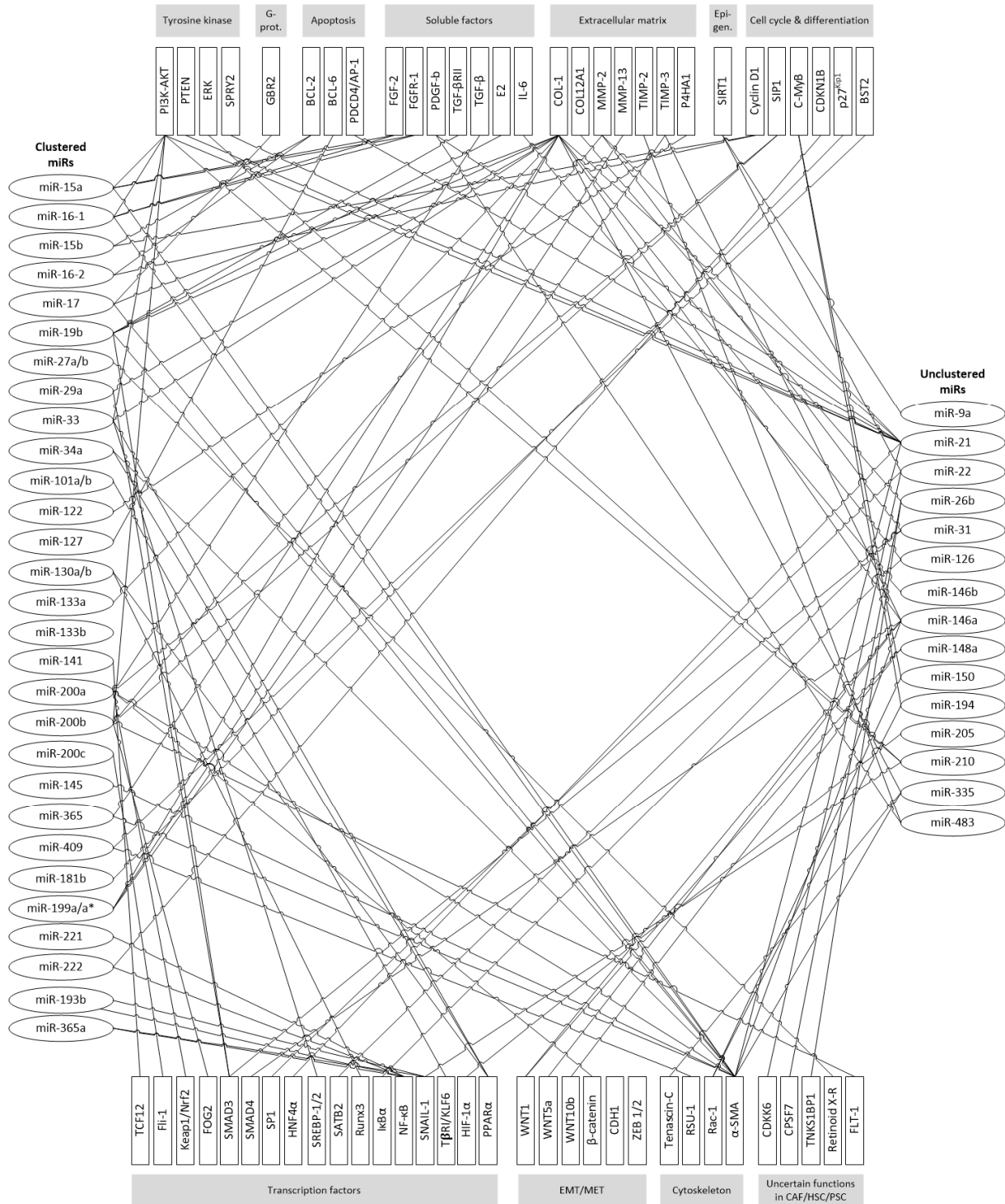
Figure 2

Deregulated miRs affect intracellular functional complexes in fibroblasts and formation of surrounding ECM; thus supporting transition of NFs into their pathological counterparts and development of desmoplasia and fibrosis. Modulation of soluble factors can act as (auto)activating feedback loop. Epigenetic communication between CAFs and cancer cells, such as by extracellular microvesicles, is likely, but rarely investigated. The most important targets affected by miRs within fibroblasts are added in relation to involved cellular functions.

Tables

Table 1

Direction of deregulated miR expression in CAFs in clinical cohorts of various cancer entities.

Cancer entity

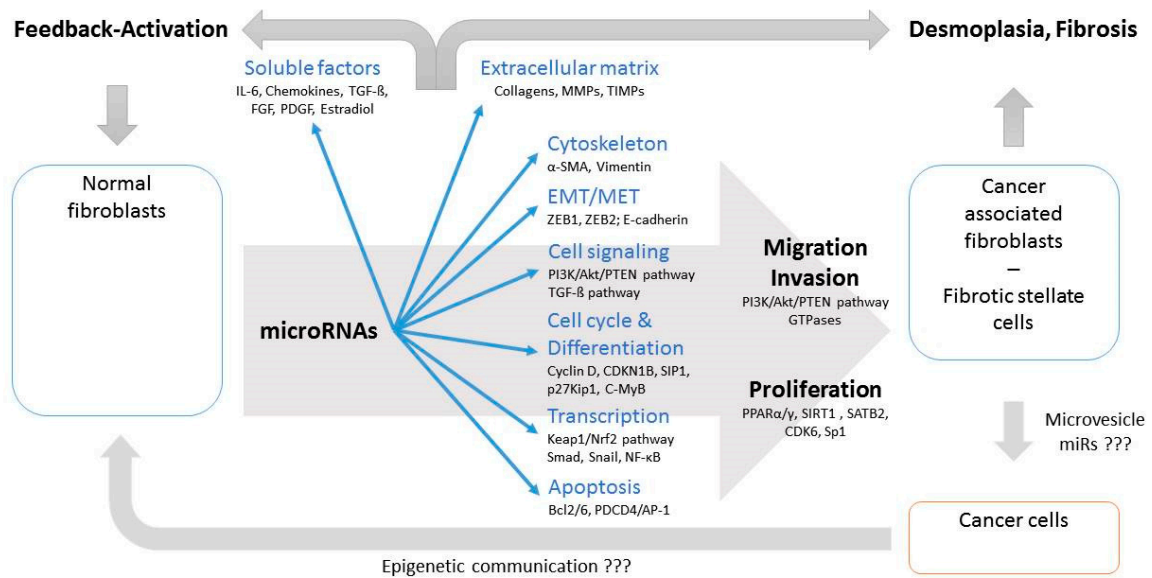|		breast	ovarian	endometrial	esophageal	gastric	colorectal	pancreatic	prostate	mixed entities
upregulated	miR-21				↑		↑	↑		
	miR-31									↑
	miR-92	↑								
	miR-221									↑
	miR-409								↑	
	miR-155		↑							
downregulated	miR-15								↓	
	miR-16								↓	
	miR-26b	↓								
	miR-31		↓	↓						
	miR-101									↓
	miR-106b					↓				
	miR-141	↓								↓
	miR-148a			↓						
	miR-200	↓abc				↓b		↓ab		↓bc
	miR-205									↓
	miR-214		↓							
	miR-342									↓
	miR-let7g									↓

Table 2

Deregulated miRs in A) CAFs and B) HSC/PSC, consequences on cellular functions and identified target structures. MiRs that occur in clusters are highlighted with blue background

A)

miR with Cluster	Cancer Entities	Regulation of miR in CAFs	Interaction in CAFs	migration	invasion	adhesion	growth	proliferation, progession	differentiation	chemoresistance	apoptosis	methylation	Pathway	Targets
15, 16	PC	↓	α-SMA ↑	↑			↑	↑					p-AKT, p-ERK ↑	Fgf-2 and its receptor Fgfr1 ↑
27a/b	EOC	↑	α-SMA ↑							↑	↓		TGF-ß ↑	
92	BC	↓				↑								
106b	GC	↑	α-SMA ↑	↑	↑		↑						TGF-ß ↑	PTEN
101	BC, LC	↓↑	α-SMA ↑, IL-6 ↑	↓↑	↓↑	↓		↓↑	↓		↑	↓	PI3K-AKT ↓, TGFß ↑	CXCL 12 ↓
143, 145	GC	↑	α-SMA ↑, Collagen Typ III ↑		↑		↑	↑					TGF-β/SMAD signaling ↑	
200a, 200b	BC, LC, PaC, GC	↓↑	α-SMA ↓↑ , IL-6 ↑	↓↑	↓↑	↓↑		↓↑	↓↑			↓	TGF-ß ↑↓	ZEB1, ZEB2 ↑; Flt 1↓ ; SIP1
221	BC, PaC	↑	α-SMA ↑, IL-6 ↑	↑	↑					↑			TGF-ß ↑	NF-κB, K-Ras ↑
214	OC	↓	cytokines ↑	↑	↑		↑							CCL5
127	BC	↓						↑					p53/p21 ↑	BCL6 oncogene ↑
133b	PC	↑	α-SMA, IL-6 ↑, Collagen 1A1					↑					TGF-ß ↑	
141, 200c	BC	↓↑	α-SMA ↑, IL-6 ↑,	↑	↑	↑		↑	↑		↑		TGF-ß ↑	Fli-1, TCF12
342	BC	↓	α-SMA ↑ , IL-6 ↑	↑	↑	↑		↑	↑		↑		TGF-ß↑	
365	BC	↓	IL-6 ↑	↑	↑								p38 MAPK ↓	NF-kBp65 ↑
409	PC	↑	α-SMA, EMT ↑, extracelluar vesikel (EV) release ↑				↑	↑						Ras suppressor 1, stromal antigen 2

B)

miR with Cluster	PSC/HSC	Regulation of miR in PSC/HSC	Interaction in cells	migration	growth	development, movement	proliferation, progession, activation	fibrosis, apoptosis	Pathway	Targets
15, 16	PSC	↓						↑		BCL-2 ↑
27a/b	HSC	↑					↑			retinoid X receptor alpha
17, 19b	HSC	↑↓	Collagen Type I and α-SMA ↑↓, expression of α1(I) and α2(I) procollagen in mRNAs ↑		↓		↓	↑	TGF-ß 1 ↑↓	SMAD7 ↓, TGF-ß 2 rezeptor and SMAD 3, GRB2
101	HSC	↑		↓			↓		TGFß ↓	TβRI/KLF6 ↓
143	PSC	↑				↑	↑		Smad 2/4	p39 MAP kinase & extracell.-signal–regulated kinase
200a, 200b	HSC	↓↑	α-SMA , EMT process ↓	↑	↓		↓↑	↑	Wnt/β-catenin, TGFβ , PI3K/Akt ↑, Hh pathway	FOG2 ↓-regulation, Keap1/Nrf2 ↑, Gli2 ↓
221, 222	HSC, PSC	↑	α1Collagen and α-SMA ↑	↑		↑		↑	Smad 2/5, NF-κB	p40 MAP kinase & extracell.-signal–regulated kinase, Cox-2 protein expression, NF-κB ↑
214	HSC	↓	Collagen Type I ↓					↑		HDAC4 ↑, Cox-2 protein expression, NF-κB ↑
29a	HSC, PSC	↓↑	Collagen Type I	↑	↑	↑		↑	TGF-β1 (SMAD3 dependant) ↑	
34c	HSC	↑	α-SMA ↑							PPARγ ↓
122	HSC	↓	collagen maturation and ECM production ↑				↑			P4HA1 ↓
130b	HSC		ECM ↑				↑			PPARγ ↓
144	HSC	↓	α-SMA ↑					↑	TGF-ß1 ↑	

Table 3

Supplemental table

MiRs identified with specific roles in fibroblasts under pathological conditions (CAFs, HSCs, PSCs)
OC (Ovarian Cancer), EC (Endometial Cancer), BC (Breast Cancer), GC (Gastric Cancer), EOC (Esophageal Squamous cell carcionoma), PaC (Pancreatic Carcinoma), CRC (Colorectal Cancer), PC (Prostate Cancer), LC (Lung Cancer)

Cell type	miRNA	Direction of deregulation	Type of validation	Functional effect in NFs turning to CAFs	Interaction in CAFs	Ref.
OC	31 214 155	↓	in vivo: paired samples of CAF (metastasis) and NF; in vitro: NF co-cultured with HeyA8 OC cells	proliferation ↑	cytokines ↑ target: CCL5	5
EC	31	↓	in vitro: paired samples of EC tissue and tumor free tissue; in vivo: mouse xenograft model	migration, invasion of NFs ↑	target: SATB2 ↑	1
	148a	↓	in vitro: paired samples of EC tissue and tumor free tissue		target: WNT10B	9
BC	26b	↓	in vitro: paired samples of BC tissue and tumor free tissue culture models	↓	targets: TNKSIBPI, CPSF7, COL12A1	11
	31	↑	in vitro: BC tissue compared with tumor free tissue	activation ↑	α-SMA, IL-6 ↑, pathway: TGF-ß ↑	13
	221	↑				
	200b	↓				
	200c	↓				
	101	↓				
	141	↓				
	342	↓				
	205, let -7g, 26b	↓				
	127	↓	in vitro: BC cell lines and sets of primary breast tumors and adjacent normal tissues from the same patients	proliferation ↑	p53/p21 ↑ target: BCL6 oncogene ↑	43
	22	↑	in vitro: epithelial and BC cell lines, in vivo: by inducing cellular senescence in a mouse model of breast carcinoma	proliferation ↓	targets: CDK6, SIRT1, and Sp1 genes	66
	92	↓	in vitro: tissue samples and in breast epithelium and stroma during BC progression	expression of miR in FIBs ↓		12
	146b	↓	in vitro and in vivo: tissue samples with and without BC	activation ↑	IL-6 ↑ (p 16 related)	40
	200	↓	in vitro	activation ↑	α-SMA ↑ targets: Fli-1 is directly regulated by miR-200c, TCF12 is directly targeted by miR-141 -> ECM	3

Cell type	miRNA	Direction of deregulation	Type of validation	Functional effect in NFs turning to CAFs	Interaction in CAFs	Ref.
					remodeling ↑	
	365	↓	in vitro, in vivo	↓	IL6 ↑ pathway: p38 MAPK ↓ target: NF-kBp65 ↑	58
GC	**106b**	↑	in vitro: paired samples of GC tissue and tumor free tissue	proliferation ↑, motility ↑	α-SMA ↑, TGF-ß ↑ target: PTEN	22
	200b	↓	in vitro: gastric cancer cell lines compared with CAF; in vivo: mouse xenograft model; clinicopathological: comparing miR-200b expression in 173 patients	activation ↑	α-SMA ↑ targets: ZEB1, ZEB2 ↑ ; repressed E-cadherin expression	14
	149	↓	in vitro: GC cell lines, fibroblasts from tumor tissues and non-tumor tissues, in vivo: mouse xenograft model	activation ↑	IL6 ↑ -> EMT (epithelial-to-mesenchymal transition) ↑ target: PGE2 induces the epigenetic silencing of miR-149 in fibroblasts	70
	145	↑	in vitro: tissue samples and cell lines	activation ↑	α-SMA ↑ pathway: TGF-ß ↑	24
	143	↑	in vitro: tissue samples and cell lines, in situ hybridization	activation ↑	Collagen type III ↑ pathway: TGF-β/SMAD signaling ↑	23
EOC	**27a/b**	↑	in vitro: serum expression of miR, from tissue with and without cancer	activation ↑	α-SMA ↑ pathway: TGF-ß ↑	25
	21	↑	in vitro: samples from patients with esophageal squamous-cell carcinoma, and a co-culture system of normal fibroblasts and esophageal cancer cells	activation ↑		39
PaC	21	↑	in vitro: tumor and tumor free tissue	activation ↑	α-SMA ↑	16
	21	↑	in vitro: tumor and tumor free tissue	activation ↑		17

Cell type	miRNA	Direction of deregulation	Type of validation	Functional effect in NFs turning to CAFs	Interaction in CAFs	Ref.
	222	↑	in vitro co-cultured experiments with: human normal and cancer associated stellate cells, human cancer associated fibroblast cells, human PaC cell lines	activation ↑	α-SMA ↑	4
	221				α-SMA ↑, targets: NF-κB, K-Ras ↑	
	155	↑	in vitro: isolated primary pancreatic fibroblasts from mice co-cultured with pancreatic cancer cell lines	activation ↑ (by microvesicles)	alpha SMA ↑ target: TP53INP1 ↓ by direct targeting of miR-155 and microvesicles	60
	200a, 200b	↑	in vitro: cell lines and tissue samples with and without cancer	activation ↑	target: SIP1 by promoter methylation and retain expression of E-cadherin, regulating EMT	15
CRC	21	↑	in vitro: human cell lines and tissue samples with and without cancer	activation ↑	cytokines ↑, PDCD4 ↓(inversely correlated with miR expression)	26
PC	205	↓	in vitro: cell culture	activation ↑	inflammatory cytokine secretion ↓ target: HIF-1 ↑, EMT ↑	57
	409	↑	in vitro: prostate and bone and in patient tissue samples and cells , in vivo: mouse xenograft	expression ↑	α-SMA, EMT ↑, extracellular vesicle(EV) release ↑	18
	15 16	↓	in vitro: 23 non-neoplastic and tumor tissues samples, in situ hybridization, in vivo: mouse model	↓	α-SMA ↑ targets: Fgf-2 and its receptor Fgfr1	19
	133b	↑	in vitro: human PC cells and human prostate tissue with or without cancer	activation ↑	α-SMA, IL-6 ↑ pathway: TGF-ß ↑	21
LC	**101**	↑	in vitro: human lung cell lines, and human lung tissue with and without cancer	proliferation ↑	pathway: PI3K-AKT ↓ targets: CXCL 12 ↓	20
	200b	↑	in vitro: cell lines	activation ↑	α-SMA ↑ target: Flt 1 ↓	41
PSC	**143**	↑	Rat PSCs were isolated from the pancreas tissue of male Wistar rats. PSCs were activated in vitro by culture in serum-containing medium	activation ↑	interaction with p39 mitogen-activated protein kinase, extracellular-signal–regulated kinase, and SMAD 2/4 pathway	69

Cell type	miRNA	Direction of deregulation	Type of validation	Functional effect in NFs turning to CAFs	Interaction in CAFs	Ref.
	221	↑			interaction with p40 mitogen-activated protein kinase, extracellular-signal–regulated kinase, and SMAD 2/5 pathway	
	31	↑			interaction with p38 mitogen-activated protein kinase, extracellular-signal–regulated kinase, SMAD 2/3 pathway	
	126	↓			interaction with p41 mitogen-activated protein kinase, extracellular-signal–regulated kinase, SMAD 2/6 pathway	
	146a	↓			interaction with p42 mitogen-activated protein kinase, extracellular-signal–regulated kinase, SMAD 2/7 pathway	
	150	↓			interaction with p43 mitogen-activated protein kinase, extracellular-signal–regulated kinase, SMAD 2/8 pathway	
	210	↑	in vitro: Panceatic cancer cells mono-cultured or indirectly co-cultured with PSCs	activation ↑	EMT ↑ pathway: ERK and Akt ↑	55
	15b 16	↓	in vitro: rat PSC	activation ↑	target: BCL-2 ↑	61
	29	↓	in vitro: Immortalized mouse pancreatic stellate (mPSC) cell lines and tissue samples, in vivo: mouse model	activation ↑	ECM ↑ pathway: TGF-β1 (SMAD3 dependent) ↑	51

Cell type	miRNA	Direction of deregulation	Type of validation	Functional effect in NFs turning to CAFs	Interaction in CAFs	Ref.
HSC	335	↓	in vitro: cultured HSC from rats	activation ↑	α-SMA and Collagen Type 1 ↑ target: TNC (tenascin-C) expression ↓	47
	150	↑	in vitro: HSC isolated from sham-operated and bile duct-ligated rats compared with HSC isolated from fibrotic rats	activation ↓	α-SMA and Collagen Type 1 ↓ target: C-myb ↓	50
	194	↑			target: Rac 1 ↓	
	146a	↓	in vitro	activation ↑	α-SMA and Col-1 ↑ targets: Wnt1 and Wnt5a ↑	53
	146a	↓	in vitro: cell culture, in vivo: rat model	activation ↑	α-SMA ↑, TGF-ß ↑ by direct targeting of SMAD4	63
	200a	↓	in vitro: cell culture, in vivo: rat model	activation ↑	α-SMA ↑ pathway: Wnt/β-catenin and TGFβ ↑	44
	200a	↑	no information	activation ↓	α-SMA ↓, EMT process ↓, Gli2 (downstream signaling protein of the Hh pathway) ↓	71
	9a	↑	in vitro: cell culture, in vivo: rsat model	activation ↑	target: SIRT1	67
	17	↑	no information	activation ↑	Collagen Type I and α-SMA ↑, TGF-ß 1 ↑, SMAD7 ↓ (direct target)	37
	33a	↑	in vitro: cell culture and human liver and serum samples	activation ↑	α1 Collagen (Col1A1) and α-SMA ↑, TGF-ß1 ↑, PI3K/Akt pathway target: PPAR-α	29
	200b	↑	in vitro: human hepatic stellate cell line	proliferation, migration ↑	PI3K/Akt through FOG2 ↓-regulation	30
	21	↑	in vitro: human immortalized HSC line	activation ↑	Collagen Type I and α-SMA ↑, PTEN/Akt pathway	49
	130a/b	↑	in vitro: immortalized rat HSC line, in vivo: rat model	activation ↑	ECM ↑ target: PPARγ ↓	65

Cell type	miRNA	Direction of deregulation	Type of validation	Functional effect in NFs turning to CAFs	Interaction in CAFs	Ref.
	30, 193	↓	in vitro: hepatic fibrosis and human tissue samples, in vivo: mouse model	activation ↑	TGF-ß , TGF-β2 and SNAIL1 as potential tagets	33
	144	↓	in vitro: human tissue samples	activation ↑	α-SMA ↑, TGF-ß1 ↑	35
	146a	↓	in vivo: cell culture	activation ↑	TGF-ß1 ↑ target: SMDA4	63
	126	↑	no information	activation ↑	TGF-ß1, IκBα protein expression ↓ pathway: NF-κB	42
	221 222	↑	in vitro: cell culture and liver biopsy specimens, in vivo: mouse model	activation ↑	α1-Collagen and α-SMA ↑	31
	19b (-1/-2)	↓	in vitro: rat HSCs, in vivo: rat model	activation ↑	Type I Collagen ↑, expression of α1(I) and α2(I) procollagen in mRNAs ↑ pathway: TGF-ß ↑ targets: TGF-ß 2 receptor and SMAD 3	32
	101	↑	in vitro: Primary rat and mouse HSCs, in vivo: mouse model	activation ↓	targets: TβRI and KLF6, inhibiting TGFβ signalling pathway	72
	31	↑	in vitro: human tissue samples, in vivo: rat model	activation ↑	TGF-ß ↑	48
	19b (19b-1, 19b-2)	↑	in vitro: tissue samples, in vivo: rat model	activation ↓	target: GRB2	59
	34a/	↑	in vitro: human HSC line, in vivo: rat model	activation ↑	α-SMA ↑ target: PPARγ ↓	64
	21	↑	in vitro: human tissue samples, rat HSC cell line	activation ↑	induces hepatocyte EMT pathway: ERK1 targets: SPRY2, HNF4α	27
	101	↑	in vitro: Primary rat and mouse HSCs, in vivo: mouse model	activation ↓	TGFß signaling ↓ by suppressing TβRI/KLF6 (targets)	45
	181 b	↑	in vitro: human cell line, in vivo: rat model	activation ↑	α-SMA and Type I Collagen ↑ pathway: PI3K/Akt ↑ target: PTEN expression ↓	38

Cell type	miRNA	Direction of deregulation	Type of validation	Functional effect in NFs turning to CAFs	Interaction in CAFs	Ref.
	200a	↑	in vitro: HSC-T6 cell lines, in vivo: rat model	activation ↓	pathway/ targets: Keap1/Nrf2; Nrf2 ↑ inhibits TGF-β1 induced growth	68
	483	↑	in vitro: cell lines, in vivo: mouse model	activation ↓	TGF-ß ↓ targets: TIMP-2 and PDGF-β ↓	56
	21	↑	in vivo: mouse model	activation ↑	TGF-ß, α-SMA ↑ targets: PDCD4/AP-1 ↑	28
	126	↓	in vitro: primary rat HSC, in vivo: rat model	activation ↑	PI3K, p-AKT ↑	73
	29a	↓	in vitro: mice HSC, in vivo: mouse model	activation ↑	alpha SMA ↑ target: HDAC4 ↑	52
	150	↑	in vitro: human cell lines	activation ↓	alpha SMA ↓ targets: Sp1 and Col4A4	74
	133a	↓	in vitro: cell lines, in vivo: mouse model	activation ↑	TGF-ß, collagen ↑	34
HSC	**122**	↓	in vitro: rat HSC, in vivo: rat model	activation ↑	collagen maturation and ECM production ↑ target: P4HA1 ↓, binding activity of C/EBPα to miR-122 promoter	46
HSC	181b	↑	in vitro: serum samples and human cell lines	activation ↑	TGF-ß ↑ target: p27	38
HSC	26a	↓	in vitro: primary rat HSC, in vivo: rat	activation ↑	Collagen Type I , Cox-2 protein expression, NF-κB ↑	54
	29a	↑				
	214	↓				
	146a	↓				
HSC	199a, 199a*	↑	in vitro: human clinical samples, in vivo: mouse model	activation ↑	TGF-ß ↑	36
	200a, 200b	↑				
HSC	16	↓	in vitro: rat HSC	activation ↑	CD1, Bcl-2 ↑	62
HSC	**27a/b**	↑	in vitro: rat HSC	activation ↑	target: retinoid X receptor alpha	75

Table 4

MiR families and clusters that have been identified as important in CAFs/HSCs/PSCs

miR family	chromosomes	members of miR-clusters
101	9	mir-101-precursor-9
101	1	101-1, 3671
34	11	34b, 34c
34	1	34a
33	17	33a, 33b, 6777
29	7	29a, 29b-1
29	1	29b-2, 29c
27	19	23a, 27a, 24-2
27	9	23b, 24-1, 27b, 3074
17	x	18b, 19b-2, 20b, 92a-2, 106a, 363
17	7	25, 93, 106b
17	13	17, 18a, 19a, 19b-1, 20a, 92a-1
15	17	195, 497
15	3	15b, 16-2
15	13	15a, 16-1
8	1	200a, 200b, 429
8	12	141, 200c
365	17	365b, 4725
365	16	193b, 365a
342	14	151b, 342
221	x	221, 222
214	1	199a-2, 214, 3120
181	7	181a-1, 181b-1
181	1	181a-2, 181b-2
154	14	134, 154, 323b, 365, 377, 409, 410, 412, 485, 496, 541, 656
144	17	144, 451a, 451b, 4732
143/145	5	143, 145
133	6	133b, 206
133	18	1-2, 133a-1
133	20	133a-2
130	11	130a
130	22	130b, 301b
127	14	127, 136, 337, 431, 433, 432, 665
122	18	122, 3591

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