

# The Role of Cancer Stem Cells and MicroRNAs in the Development and Progression of Pancreatic Cancer

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## Abstract

Pancreatic Cancer is one of the most malignant cancers in existence today. Due to its rapid ability to spread, it has resulted in poor prognosis. While the aggressive nature of the cancer is not fully understood, current research has suggested the role of microRNAs (miRNAs) and cancer stem cells (CSCs) in pancreatic cancer aggressiveness. The miRNAs are small RNA molecules that post-transcriptionally modify the expression of multiple genes by targeting different messenger RNAs (mRNAs), and miRNAs has been found to be associated with CSCs that are capable of undergoing self-renewal, cell growth and differentiation, leading to the development and maintenance of tumor aggressiveness. The connection between miRNAs and CSCs can be seen because the targets of certain miRNAs are associated with the expression of CSCs markers, and thus deregulation in the expression of both miRNAs and mRNAs in the CSCs are important for developing targeted therapies especially because such targeting may allow drug resistant cells such as CSCs to become resistive to conventional chemotherapeutics. This chapter will provide a comprehensive review on the role of some selected miRNAs in the context of CSCs and pancreatic cancer aggressiveness.

**Keywords:** Cancer stem cells; MicroRNA; Pancreatic cancer

## Introduction

As cancer research progresses, there has recently been a dramatic increase in the amount of research being conducted with regards to microRNAs (miRNAs). The reason for this newly found popularity is that miRNAs have been found to be correlated with tumor aggressiveness of human cancers. Essentially, miRNAs are a class of small, non-coding molecules that regulate genes at the post-transcriptional level [1]. Such regulation in turn leads to the regulation of different molecular and cellular aspects within cancer cells, such as cell differentiation, apoptosis, and metastasis. For details on the biogenesis of miRNAs, please refer to earlier published report [2]. The significance of miRNAs is due to the fact that they are able to target multiple signaling pathways within the body, and thus have a profound impact upon many cellular processes [3]. There have also been attempts to profile miRNAs into different categories based on their many biological properties. Two such categories are tumor suppressing miRNAs and oncogenic miRNAs, which will be discussed in greater detail within this article. Based on the amount of miRNAs present in cells, these classifications are now also being used to identify patients as cancerous or not [4].

The progression of cancer research is also gaining momentum with respect to the study of cancer stem cells (CSCs). CSCs are the cells that are capable to self-replicate as well as have the unique ability to retain their cellular differentiation function into heterogeneous cell population [5]. Under normal condition, once the stem cells are differentiated, they are "matured" and are able to more effectively maintain the growth of the tissue in which they are found. More specifically, CSCs are cancerous cells found within tumors that have the ability to self-replicate, hence the addition of the words "stem cells" to their name. CSCs have most of the same general properties as normal and regular stem cells. However, different cell markers such as CD44 and CD133 can be found on the surface of CSCs, making it easier for researchers to identify the presence and characterization of CSCs from a tumor [5]. Evidence has suggested that CSCs plays fundamental roles in the transition of cells from epithelial to mesenchymal [6]. Furthermore, epithelial to mesenchymal transition (EMT) has been noted as a key

factor in the progression and spread of cancer. For most part, EMT signals a more aggressive onset of the disease. CSCs are thought to be involved in the recurrence of tumors due to their ability to initiate the growth of tumor cells, as well as their ability to regenerate without additional help [7]. Thus, metastasis of tumor cells in general is believed to be the result of increased number of CSCs. Consequently, resistivity to different treatments of drugs has also been partially attributed to the growth and multiplication of CSCs [6].

## The Role of MicroRNAs (miRNAs) and Cancer Stem Cells (CSCs) in Cancer

As advancement in the field of cancer research continues, it has become increasingly obvious that miRNAs as well as CSCs are both important factors to be considered. The up-regulation or down-regulation of different miRNAs has been shown to correlate with the progression of different diseases, including numerous types of cancers. The miRNAs are key regulators in a variety of different biological pathways within the body, making their effects widespread and amplified. The regulation of different pathways by miRNAs depends on the manner in which the expression of their target genes is affected. Stem cells become cancer stem cells (CSCs) when their pathways that are regulated by the miRNAs are altered, causing them to become more tumorigenic [8,9]. The miRNAs also have the ability to silence or express hundreds of genes, while at the same time one gene can be

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targeted by multiple miRNAs [10]. As a result, miRNAs are able to regulate proliferation, apoptosis, and most importantly in the context of this article, the renewal and differentiation of stem cells [11]. Changes in miRNAs has allowed some miRNAs to function as oncogenes, by most prominently enhancing EMT and metastasis, while others act as tumor suppressors, by suppressing proliferation and aiding in the induction of apoptosis.

## The Role of Cancer Stem Cells (CSCs) and MicroRNAs (miRNAs) in Pancreatic Cancer

Pancreatic Cancer is one of the least promising cancers in terms of long-term survival. In fact, the American Cancer Society has determined that it is the fourth leading cause of cancer related death in the United States [12]. With such a negative prospect, it has been proven difficult to conduct research in improving the survival of patients diagnosed with pancreatic cancer. Most treatments have only been temporarily successful, as pancreatic cancer patients frequently relapse within a short time [13]. The most common type of pancreatic cancer is called pancreatic ductal adenocarcinoma (PDAC), thus it is also one of the most studied types of pancreatic cancer [14]. There have been many speculated causes for relapse, including that of the role of CSCs. The CSCs have been thought to be one of the causes for rapid metastasis of pancreatic cancer. Because such cells are difficult to maintain and develop targeted therapy, there seems to be reduced enthusiasm in the realm of effective and long lasting pancreatic cancer treatments; however, it does opens newer avenues for further cutting-edge research. In fact, CSCs are also widely known as “tumor initiating cells” [15]. However, recently a miRNA signature specifically designed for pancreatic cancer has been created. The signature includes the up-regulation of a variety of miRNAs, including miRNA-155, miRNA-21, and miRNA-221, all of which will be further discussed in this article [15]. By utilizing this signature, researchers have been able to see which miRNAs are also able to regulate the CSCs through different mechanisms in order to mediate the spread of the pancreatic cancer [16]. Moreover, monitoring the expression of different miRNAs in cells will aid in the differentiation between PDAC cells from normal pancreatic cells.

### Key microRNAs (miRNAs) in pancreatic cancer

This chapter will elaborate upon the roles of specific types of miRNAs found to have a prominent impact on the field of pancreatic cancer research. The following miRNAs are classified based on their roles in pancreatic cancer as oncogenes or tumor suppressors as shown in table 1. While not each of them have been found to have a connection with CSCs, evidence is still mounting in favor of elucidating if such a connection exist for all miRNAs.

### Oncogenic microRNAs (miRNAs) in pancreatic cancer

**miRNA-155:** The miRNA-155 has been widely classified as an oncogenic miRNA. It is generally found in large quantities in

MicroRNAs (miRNAs)	Oncogenic/Tumor Suppressor	Targets
miR-155	Oncogenic	TP53INP1
miR-21	Oncogenic	Ras, Notch, PDCD4, TIMP3
miR-221	Oncogenic	CDKN1B/p27, CDKN1C/p57
Let-7 family	Tumor Suppressor	KRAS, EMT
miR-200 family	Tumor Suppressor	ZEB1, PTEN
miR-34a/b/c	Tumor Suppressor	P53, Bcl-2, Notch

**Table 1:** Oncogenic or Tumor Suppressive microRNAs and their Targets.

experimental data obtained from various cancer tissues and is one of the most differentially expressed miRNAs in the affected tissues [17]. Due to this classification, it is thought to be involved in promoting pancreatic cancer. Furthermore, miRNA-155 has the potential to be a significant biomarker for the early onset of pancreatic cancer because of the drastic amounts of the miRNA found in patients with solid tumors including pancreatic cancer [17]. One well-researched target of miRNA-155 is Tumor Suppressor Protein 53 (p53)-induced nuclear protein 1 (TP53INP1) [17,18]. TP53INP1 is a pro-apoptotic protein that interacts with p53 when triggered by stress [18]. However, due to the elevated amount of miRNA-155 in pancreatic cancer cells, studies have suggested that it can suppress the expression of TP53INP1 and therefore inhibit apoptosis of cancerous cells [18]. Support for this theory was made after the finding of a region in the TP53INP1 3' untranslated region that was highly complementary to miRNA-155 [18]. This study thus suggests that miRNA-155 can function by eliminating the expression of various anti-tumoral proteins, such as TP53INP1 [19]. Furthermore, when TP53INP1 was introduced into MiaPaCa2 cell lines in one particular study, not only did pancreatic tumor growth decreased, but also the tumor spread into other areas was found to be much reduced [18].

**miRNA-21:** Another miRNA known to promote pancreatic tumors is miRNA-21. Multiple studies have shown that miRNA-21 is overexpressed in pancreatic cancer cells, implying that the action should be taken to find a method by which one could decrease its expression as a possibility for improving patient prognosis. Ras, another oncogene, is one of the targets of miRNA-21 [20]. Although the mechanism of this targeting process is not yet fully understood, studies have illustrated that the expression of miRNA-21 has a direct correlation with the expression of Ras and its GTPase activity [20]. Furthermore, increased expression of Ras frequently plays a critical role in the development and subsequent progression of pancreatic CSCs. CDF, a compound synthesized from curcumin, has been found to decrease the expression of miRNA-21 in pancreatic cancer cells, thus CDF was also able to decrease the Ras and its GTPase activity [21]. This has resulted in CDF being tested as a potential treatment option, as it has also demonstrated that it can significantly decrease the number of CSCs both *in vitro* and *in vivo* [20].

On a similar note, miRNA-21 was also found to enhance tumor progression in breast cancer cells by activating EMT and attenuating CSC characteristics [22]. This provides a starting point for the hypothesis that miRNA-21 may also trigger EMT in pancreatic cancer cells. One study did actually depict that miRNA-21 expression in pancreatic cells was increased due to Notch-1, which was responsible for EMT and a CSC-like phenotype [23]. Overexpression of Notch-1 in turn lead to the overexpression of miRNA-21, thus allowing for the CSCs to undergo self-renewal, causing the aggressive nature of the tumor to continue to increase as well as to reduce susceptibility to various drugs. Other targets of miRNA-21 include programmed cell death 4 (PDCD4) and tissue inhibitor of metalloproteinase (TIMP3). These targets function as tumor suppressors and their expression is decreased in the presence of excess miRNA-21, further promoting tumor development and pancreatic ductal adenocarcinoma progression [24].

**miRNA-221:** Another important miRNA that is overexpressed in pancreatic cancer cells is miRNA-221. Aberrant expression of miRNA-221 has coincided with a malignant cancerous phenotype, increasing metastasis and tumor formation. Prime targets of miRNA-221 are CDKN1B/p27 and CDKN1C/p57, both of which function as regulatory proteins in the cell cycle [25,26]. There functions

are inhibited in the presence of miRNA-221, subsequently causing proliferation of pancreatic cancer cells with suppression of Cyclin-dependent kinase inhibitors that inhibit the cell cycle [26]. In turn, this causes the cell cycle to continue unregulated, increasing the amount of cancer cells and their CSC-like behavior of self-replication.

However, a potential treatment option that has been researched includes utilizing antisense oligonucleotides of miRNA-221 to regulate the negative effects of overexpression of the miRNA itself. When anti-miRNA-221 was transfected into not only PDAC, but also into human hepatocellular carcinoma cells, the results were the same in that p27 and p57 were both up-regulated [26,27]. This confirmed the argument that p27 and p57 are the legitimate targets of miRNA-221, while also illuminating possible therapeutic options. The anti-miRNAs were able to continue regulation of the cell cycle, allowing cancer cells to arrest in the G1 phase before DNA synthesis and self-replication of the cell ensued, thus decreasing CSC-like characteristics [27]. On the contrary, apoptosis was induced allowing proliferation and metastasis to subside [27]. Additionally, antisense inhibition of miRNA-221 increased the sensitivity of PDAC cells to gemcitabine, a currently employed chemotherapy drug [25,27]. Therefore, utilization of our knowledge of p27 and p57 as targets of miRNA-221 has allowed us to understand that the knockdown of miRNA-221 using anti-miRNAs can allow for reduced expression of the miRNA and increased regulation of the cell cycle in cancer. This also regulates the CSC-like features of the pancreatic cancer cells, which are induced by the miR-221, and to decrease the overall replication of the cells and therefore prevent further spreading of the malignant cells into other regions of the body.

### Tumor-suppressing microRNAs (miRNAs) in pancreatic cancer

**The let-7 family:** The let-7 family of miRNAs is well known for their tumor suppressing ability and their relative loss of expression has been found in pancreatic cancer cells among other types of cancers. The oncogenic protein K-RAS is one of the target of let-7 that has been extensively investigated [28-31]. K-RAS and several other oncogenes are inhibited by let-7, contributing to the classification of let-7 as a tumor suppressor. Constitutive unregulated expression of mutated K-RAS proteins resulting from decreased expression of let-7 miRNA enhances the spreading of the malignant pancreatic cancer phenotype [31]. Let-7 has also been recognized to control CSCs by inducing cell differentiation and restraining self-replication [29]. Furthermore, an inverse correlation between let-7 miRNA and EMT has been discovered whereby down-regulation of let-7 miRNA has been shown to promote EMT [29,32], suggesting that strategies by which let-7 could be up-regulated would become a useful tool of the treatment of pancreatic and other human malignancies with loss of let-7 expression.

A variety of natural agents have been investigated with regards to let-7 miRNA expression and reversing the EMT phenotype. For example, BioResponse-formulated 3,3'-diindolylmethane (B-DIM) and G2535 (isoflavone mixed with genistein) are two novel natural agents that have recently been tested in pancreatic cancer cells [29]. When B-DIM and G2535 were separately introduced into pancreatic cancer cells, the expression of let-7 miRNAs was found to be increased [29]. This suggests that the tumor suppressing abilities of let-7 miRNAs could be achieved by reversing the EMT phenotype, which will help in decreasing cell proliferation and tumor formation. While the majority of research supports such positive outcomes associated with the re-introduction of let-7 miRNAs into tumor cells, there are studies in which let-7 has been shown to inhibit cell proliferation, yet this strategy failed to restrain PDAC tumor growth and progression [28].

Moreover, when tested with gemcitabine-resistant pancreatic cancer cells, both B-DIM and G2535 demonstrated an increased ability to sensitize the cells to the effects of the drugs, decreasing metastasis and self-replication [29]. Additionally, CDF, a previously discussed agent, was also found to re-express let-7 miRNAs in pancreatic cancer cells, indicating that CDF also has the ability to regulate let-7 expression [30]. Therefore, all three agents, B-DIM, G2535, and CDF could possibly be useful strategy in the direction towards their clinical utility or even synthesizing additional novel anti-cancer drugs.

**The miRNA-200 family:** Another family of miRNAs that tends to be down-regulated in pancreatic cancer cells is miRNA-200. Its lack of expression in PDAC suggests that it has tumor suppressing abilities. The presence of relatively higher amounts of miRNA-200 has been found to be associated with the expression of E-cadherin, which is also a well-known tumor suppressor gene and indication of a more positive prognosis [33]. Also, miRNA-200 has been deemed as a family with stemness inhibiting properties, contributing to its potential use in therapies directed against the spread of CSCs [34].

Two types of proteins have been found to correlate with the expression of miRNA-200 family. Because decreased levels of miRNA-200 contribute to the activation of EMT and increased metastasis and invasion of cancerous cells, it is important to understand the role that ZEB1 and PTEN play in pancreatic cancer. ZEB1 directly suppresses transcription of certain members in the miRNA-200 family, fostering tumorigenicity and EMT [34,35]. Yet the inverse relationship existing between ZEB1 and the miRNA-200 family functions in both directions such that forced re-introduction of different miRNAs in the miRNA-200 family suppresses the expression of ZEB1 allowing for the reversal of EMT [35]. On the other hand, PTEN is a tumor suppressor gene similar to members of the miRNA-200 family targeting gene, and thus higher expression of miR-200 family and PTEN, and the loss of ZEB1 could be biologically important. However, other miRNAs, such as miRNA-21, also target PTEN [36], suggesting the deregulation of miRNAs and their target genes could be context dependent and specific to a tumor type. Several studies have utilized CDF to test potential therapeutic effects showing that treatment of cells with CDF could allow for the re-expression of members of the miRNA-200 family, repression of miRNA-21, and up-regulation of PTEN activity, consequently could result in positive prognosis [21,36]. This relationship between the different miRNAs, proteins, and newly synthesized drugs illuminates the complex and intricate mechanisms at play in the progression of pancreatic cancer.

Additionally, two other natural agents discussed previously (B-DIM and G2535) could also up-regulate miRNA-200. The same study done for certain let-7 miRNAs was also done with particular miRNA-200s, with a similar outcome. Up-regulation of the tumor suppressing miRNAs allowed increased chemo-sensitivity to gemcitabine to pancreatic cancer cells that was previously resistant [29]. Moreover, EMT phenotype was reversed consistent with deregulation of different EMT markers, such as reduction in vimentin expression and increased expression of E-cadherin [29].

**miRNA-34a/b/c:** Similar to the two miRNAs discussed above, the miRNA-34 family has also been classified as tumor suppressors and showed little or no expression in pancreatic cancer. Studies have shown an association with the expression of miRNA-34 family and the ability to arrest the cell cycle in G1, induction of apoptosis, and increased sensitivity to chemotherapy [37,38]. P53, recognized for its tumor suppressing abilities, is generally mutated or deficient in a variety of cancers. The CpG methylation of miRNA-34 occurs in the early stages

of tumor formation and hastens tumorigenesis by suppressing p53 [37]. However, a recent study has found that the introduction of miRNA-34 can potentially reestablish p53 expression in pancreatic cancer cells, inhibiting invasion and tumor formation [38].

Additionally, miRNA-34 may possibly take part in the regulation of CSCs. The miR-34 is able to target both Bcl-2 and Notch genes, which are involved in self-renewal and differentiation, the traits that is associated with CSCs [38]. Down-regulation of Notch and Bcl-2 by restoration of miRNA-34 in one study showed 80% decrease in the CD44+/CD133+ tumor initiating CSCs [38]. Furthermore, miRNA-34 provides a prime example of the benefit of miRNAs as a potential therapeutic target; however strategies by which one could re-express miR-34 expression remains to be fully investigated. The forced up-regulation of miRNA-34 in pancreatic cancer cells not only increases the function of p53, but at the same time suppresses the stemness like characteristics of cancer cells. Both of these roles cause suppression of the tumor and restrain it spreading. If one miRNA is able to regulate these two processes, it is obvious that future research with other miRNAs is necessary in order to discover other innovative approaches showing how different mechanisms within the body can be beneficially maintained and used to our advantage toward clinical application.

### The impact of miRNAs and CSCs in pancreatic cancer

As the study of miRNAs and their relationship to CSCs continues, our understanding of the mechanisms in which these two molecules work separately, and also together, can aid in the therapeutic advancement of pancreatic cancer. The miRNAs are able to regulate CSCs by binding to important regulators of stem cell pluripotency, such as Nanog and Sox2 [16]. Various stem cell surface markers, such as CD44, can also serve as alternative targets to miRNAs, suggesting that increased understanding of the specific role of each miRNA and its targets in a given tumor would aid in the development of novel therapeutic agents. Since the role of miRNAs is very crucial in the regulation of genes in human malignancies, deregulation of miRNAs could become novel strategies for the elimination of CSCs or the reversal of EMT phenotype, which will be useful for achieving better treatment outcome in pancreatic cancer patients. Identification of CSCs and their level of differentiation are also beneficial to patient diagnosis and prognosis, not only in pancreatic cancer, but in other types of cancers as well.

Many patients have been shown to be resistant to conventional chemotherapeutic agents and recent evidence has illustrated that this could possibly be due to the proliferation of CSCs, down-regulation of tumor suppressing miRNAs, and up-regulation of oncogenic miRNAs. In addition, the role of EMT with treatment failure has also been documented especially due to the absence of E-cadherin expression and increased expression of vimentin, ZEB1, and ZEB2 associated with tumor aggressiveness. Emerging studies have shown some remarkable progress in the development of novel strategies for overcoming therapeutic resistance. For examples, we have discussed in this article that CDF, B-DIM, and G2535 along with other natural agents could gain popularity as adjunct to conventional therapeutics for the treatment of human malignancies especially pancreatic cancer with better treatment outcome, therefore further research is warranted in order for our dream for the cure of cancer [39].

### Conclusion

Pancreatic cancer continues to be one of the most deadly cancers to date; therefore the field of research must continue to develop newer strategy for eliminating pancreatic tumors. To that end, targeting specific

miRNAs especially in cancer cells that are resistant to conventional therapeutics such as the EMT phenotypic cells or the CSCs by novel approach would become attractive in the future. We believe that natural agents or synthetic novel agents derived from natural agents could be useful for the deregulation of miRNAs and their targets for achieving better treatment outcome in patients diagnosed with pancreatic cancer. However, further in-depth studies are warranted in the field of miRNAs, EMT and CSC not only for mechanistic understanding but also for the development of treatment strategies to win the battle against pancreatic cancer.

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