

Non-coding RNAs in pancreatic cancer: challenges and opportunities for clinical application

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Abstract

Background Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with a dismal prognosis for which new therapeutic strategies are desperately needed. Non-coding RNAs (ncRNAs), especially microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), may yield new therapeutic concepts for the treatment of PDAC. A vast number of miRNAs, including the well-studied miR-21, miR-155 and miR-34, has been shown to regulate PDAC growth, invasion and metastasis in vitro and in vivo by targeting members of key signaling pathways. In addition, other miRNAs and lncRNAs, such as HOTTIP and MALAT-1, have been shown to influence the malignant behavior of PDAC cells.

Methods Here, we discuss several ncRNAs that may be used either as new therapeutic agents or as targets of new therapeutic agents. Furthermore, we discuss the problem of proper delivery of nucleotide-based agents and novel methods that may be used to circumvent this problem.

Conclusions Although the number of reports addressing the role of ncRNAs in PDAC virtually grows by the day, there are still many steps to be taken before the application of ncRNA-based therapies will become reality in clinical practice.

Keywords Pancreatic cancer · Targeted therapy · microRNA · Long non-coding RNA · Non-coding RNA · Gene therapy · Digestive-tract cancer · Chemotherapy

1 Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with a poor prognosis. Each year more than 40,000 PDAC-related deaths occur in the European Union, making it the fourth most common cause of cancer death in men and women [1]. The main reasons for its devastating character are the late occurrence of clinical symptoms and the intrinsic malignant nature of the disease [2]. Since curative treatment in the form of radical surgery is only possible in a minority of patients, novel therapeutic options are urgently needed [2]. Common genetic alterations include mutations in the *KRAS* (>90 %), *TP53* (> 50 %), *SMAD4* (> 60 %) and *CDKN2A* (> 80 %) genes [2–4]. Moreover, studies in murine models have shown that *TP53* and *KRAS* mutations, when occurring in a rapid sequence, underlie the development of PDAC in 75 % of the cases [5]. The introduction of the FOLFIRINOX and Gemcitabine plus Nab-Paclitaxel chemotherapy regimens has resulted in a successful prolongation of the survival of PDAC patients, but with frequent and sometimes considerable adverse effects, such as severe rashes [6]. Even though preliminary data show efficacy of small molecule agents such as the tyrosine-kinase inhibitor Erlotinib, successful targeted therapies for the treatment of PDAC have yet to be developed [6].

Non-coding RNAs (ncRNAs) are RNA molecules that are involved in translation, DNA replication, RNA splicing and epigenetic regulation. They are not translated into proteins [7–11]. ncRNAs comprise transfer RNA (tRNA), ribosomal RNA (rRNA), small interfering RNA (siRNA), Piwi-interacting RNA (piRNA), microRNA (miRNA), Y-RNA

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and small Cajal body-specific RNA (scaRNA). As a group, ncRNAs are believed to be ancient relics of evolution, whereas in modern animals and plants, different mechanisms by which ncRNAs regulate gene expression and DNA replication have evolved. Within the human genome, genes encoding ncRNAs are more than five times as numerous as those encoding proteins. ncRNAs may be transcribed in intergenic regions or regions overlapping with genes encoding proteins [12]. Moreover, mutations in ncRNA genes may underlie disease development, analogous to mutations in protein coding genes [13]. Interestingly, some chemotherapeutic agents, such as Metformin or Vorinostat, are believed to function at least partly through modifying the expression of ncRNAs [14, 15]. Especially miRNAs and lncRNAs have shown therapeutic potential, as they can be introduced into cells via viral-vector transfection or mechanical transfection, conceptually resembling ‘classical’ gene therapy. Delivery of therapeutics to the proper site is important, and methods have been reported that provide the possibility of local application. Of these, endoscopic ultrasound-guided fine-needle injection (EUS-FNI) or computer-tomography (CT)-guided delivery of therapeutic nucleotides have proven to be successful in PDAC-patients [16].

1.1 MicroRNA characteristics

MicroRNAs (miRNAs) represent a subgroup of 19 to 25 nucleotides-long ncRNAs that post-transcriptionally regulate gene expression by a series of steps referred to as RNA interference [17, 18]. miRNAs are evolutionarily conserved and constitute an important part of epigenetic gene regulation, affecting up to 60 % of all mammalian genes, and have been subject to intensive research in recent years [19–25]. From an evolutionary perspective, miRNAs may have facilitated the development of higher complexities in organisms and concomitant biologic functions, making life as we know it now possible [26]. The biogenesis of miRNAs is achieved by the processing of RNAs transcribed by RNA polymerase II (RNAP-II) [27] and the processing of hairpin-shaped pre-miRNAs to miRNAs by the enzymes Drosha and Dicer in the nucleus and the cytoplasm, respectively [28–30]. Subsequently, single stranded miRNA molecules associate with argonaute proteins to form the RNA-induced silencing complex (RISC), which then binds to complementary messenger RNA (mRNA) to either block translation initiation or to initiate mRNA degradation [31]. RISC and its associated miRNAs usually bind to the 3′ untranslated region (3′-UTR) of the complementary mRNA [31]. Perfect complementary binding of a miRNA with its target mRNA leads to mRNA cleavage and degradation [32]. In mammals, miRNA binding to its target mRNA is mostly imperfect. This imperfect binding leads to a blockade of translation of the target mRNA [33]. However, the seed region of a miRNA, which represents a 7

nucleotide long region (nucleotides 2–8) in the 3′-UTR, still needs to bind perfectly to its mRNA target in order to block translation. Some mRNAs may have multiple seed regions complementary to a variety of miRNAs [34]. Hence, a single mammalian miRNA may inhibit the expression of hundreds of genes, while a single gene may undergo translational inhibition by numerous miRNAs [34]. This notion may provide a rationale for the development of future therapeutic strategies, as agents with multiple targets may act synergistically and, as such, may preclude the development of therapy resistance. Related to their function, miRNAs that promote aggressiveness in tumors are termed “onco-miRs”, whereas miRNAs that have the opposite effects are termed “tumor-suppressor-miRs”. Onco-miRs may inhibit the translation of tumor suppressor genes such as *PTEN*, while tumor-suppressor-miRs may inhibit the translation of oncogenes such as *KRAS* [35]. Also, the expression of onco-miRs and tumor-suppressor-miRs itself is subject to specific regulatory mechanisms. It has for example been found that *KRAS* enhances the expression of onco-miR miR-21 while, conversely, the expression of *KRAS* is reduced by miR-217 [35, 36]. Thus, teleologically, loss of miR-217 function may increase the expression of miR-21 through increased *KRAS* signaling. This notion adds another layer of complexity to the traditional concept of tumor-suppressor genes and oncogenes. A recent study revealed that at least 500 miRNAs are aberrantly expressed in PDAC [37]. Next to PDAC, the role of some of these miRNAs has also been investigated in the two major precursors of PDAC, i.e., pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasm (IPMN) [38–40].

1.2 Long non-coding RNA characteristics

Long non-coding RNAs (lncRNAs) are transcripts defined by a length of over 200 nucleotides that are involved in the regulation of gene transcription, post-transcriptional gene regulation and epigenetic gene regulation [41–43]. lncRNAs are transcribed by RNAP-II, poly-adenylated and spliced similar to mRNAs, but are not translated into protein [44]. lncRNAs represent a heterogeneous group of RNAs that can be divided into different subgroups. From a functional perspective, lncRNAs may be assigned to archetypical categories, i.e., signaling, decoying, scaffolding and guidance, although most lncRNAs are multifunctional [45]. Signaling lncRNAs are RNAs that regulate gene expression intrinsically, i.e., by binding to target genes or regulatory elements. A prime example is the X-chromosomal inactivation-related lncRNA Xist. In female mammals, Xist is expressed from the inactive X-chromosome and covers it to repress gene expression [46]. Subsequent gene silencing and guidance of chromatin modifying enzymes to target locations on the X-chromosome is, however, conducted by distinct RNA sequences, thereby assigning it to both categories of lncRNA function [47].

Decoy lncRNAs affect gene expression in an indirect manner by binding to RNA-binding proteins (RBPs). Decoy lncRNAs consequently titrate away proteins such as transcription factors and chromatin modifying enzymes [45]. Examples of decoy lncRNAs are metastasis-associated MALAT-1 and mTOR pathway-associated Gas5 [45, 48]. Scaffolding lncRNAs serve as platforms for complexes of different effector molecules, analogous to the function of scaffolding proteins [45]. A good example of a scaffolding lncRNA is HOTAIR, which has been implicated in pancreatic carcinogenesis and acts by forming a complex with chromatin modifying enzymes [49]. Regarding their association with genomic regions, lncRNAs may be divided into five classes: promoter-associated lncRNAs (pRNAs), antisense and sense gene body-associated lncRNAs (gsRNAs), enhancer-associated lncRNAs (eRNAs) and intervening lncRNAs (lincRNAs) [50]. In recent years, lncRNAs have been shown to play essential roles in human diseases, such as cancer, endocrine disorders (e.g. diabetes mellitus) and cardiovascular diseases [51–53]. lncRNAs have also been found to be involved in the pathophysiology of PDAC [37] and some of them appear to show potential for diagnostic and/or RNA-based gene therapeutic purposes.

2 ncRNA-based therapy of PDAC

In the search for putative novel therapeutic targets, miRNAs have been studied intensively in recent years. On the one hand as drug targets in the form of miRNA antagonists such as antisense oligonucleotides (ASOs), and on the other hand as miRNA mimics [39, 54] or miRNA-encoding plasmid cDNA (pcDNA) that can restore miRNA or lncRNA function [55]. ASOs are single-stranded RNA molecules that share the same sequence as their target mRNA or ncRNA, but in a reversed order. Binding to their complementary RNA leads to degradation of ASO-mRNA or ASO-ncRNA complexes by RNase H [56]. ASOs that target miRNAs are usually called anti-miRs or antagoniRs. miRNA mimics are synthetic double-stranded RNA molecules that are homologous to endogenous miRNAs. Similar to miRNAs, lncRNAs have been targeted experimentally, both in vitro and in vivo. It has been found that over-expression of lncRNAs can be achieved by introducing gene-carrying pcDNAs in target cells. lncRNA silencing can, on the other hand, be brought about using siRNAs or shRNAs. Also, methods to target lncRNAs with ASOs have been developed. The latter confers the advantage of nuclear inhibition of lncRNAs rather than cytoplasmic inhibition [57]. A notable exception is lncRNA H19, which is central to a different therapeutic approach and is discussed in more detail under 2.2.8 [58]. Here, emphasis is put on well-investigated ncRNAs in PDAC, some of which have been successfully

tested in vitro and in vivo and may serve as candidates for RNA-based gene therapy.

2.1 Potential therapeutic miRNAs

2.1.1 *KRAS*-related miRNAs

A number of miRNAs has been shown to regulate *KRAS* expression in PDAC. This is an interesting finding since *KRAS* is mutated in >90 % of PDAC precursor lesions and PDACs (see above) and, as such, is thought to be one of the driving oncogenes in pancreatic carcinogenesis [2, 59]. Inhibition of the MAPK/ERK-pathway, of which *KRAS* is an upstream member, has shown anti-oncogenic effects in PDAC cells [60]. Mutant *KRAS*, in turn, has shown to regulate miRNAs in PDAC-derived cell lines, as well as in human Nestin-expressing cells (HPNE) derived from non-neoplastic epithelium (NNE) of pancreatic ducts harboring a *KRAS* mutation [61]. *KRAS* is a known target of tumor-suppressor-miRs that are deregulated in PDACs. These include miR-217, miR-206, miR-143, miR-145 and Let-7 [35, 55, 62, 63]. It has been shown that transfection with miR-217, which is lowly expressed in PDAC cells, significantly down-regulates *KRAS* expression and upregulates phosphorylated-AKT1 levels in PDAC-derived cell lines. Additionally, in vitro reduced anchorage-independent colony formation and in vivo reduced tumor growth in xenograft models has been reported [35]. Active *KRAS* exerts its oncogenic function through up-regulation and repression of miRNAs. miR-31 is a recently discovered *KRAS*-regulated miRNA that represses *RASA1* in a MAPK-dependent manner, leading to increased RhoA-GTPase activity. It has been reported that exogenous expression of miR-31 leads to increased proliferation, migration and invasion of PDAC-derived cells [64]. miR-143 and miR-145, which are down-regulated in the presence of activated *KRAS*, have been found to lead to inhibition of proliferation and to mediate the induction of apoptosis in PDAC-derived cells in vitro and in vivo [61, 63, 65]. Kent et al. observed down-regulation of miR-143 and miR-145 in PDAC-derived cells and showed that viral-mediated delivery of miR-143 and miR-145 resulted in decreased anchorage-independent growth [61]. *KRAS*-mediated repression of miR-143 and miR-145 has also been observed in other tumor types, such as colorectal carcinoma (CRC) [66–68]. It has also been found that miR-206 re-expression has inhibitory effects, not only on tumor growth and tumor vascularization in vitro and in vivo, but also on the motility and invasive behavior of PDAC cells. These effects are achieved at least in part through the inhibition of pro-inflammatory and pro-angiogenic genes downstream of the NF- κ B pathway, such as those encoding chemokine (C-X-C-motif) ligand 1 (CXCL1), granulocyte colony-stimulating factor-2 (CSF2) and vascular endothelial growth

factor A and B (VEGFA, VEGFB), but they may also be achieved through direct inhibition of *KRAS* mRNA itself [62].

2.1.2 miR-155

Activating *KRAS*-mutations may also lead to increased expression of onco-miR miR-155, mediated by MAPK and NF- κ B signaling, thereby promoting oxidative stress responses by inactivation of the tumor-suppressive transcription factor Foxo3a [69]. miR-155 has been found to be over-expressed in most PDAC cases and to correlate with a poor survival of PDAC patients [70]. During pancreatic carcinogenesis, miR-155 has been found to become over-expressed with a gradually increasing frequency during the progression of PanINs, the most important precursor lesions of PDAC, with highest expression levels in PanIN-3 lesions [39]. miR-155 is a known inhibitor of TP53INP1, which mediates apoptosis upon DNA damage by promoting p53 activation [71]. It is also involved in the creation of a tumor-growth promoting microenvironment by transforming normal pancreatic fibroblasts into cancer-associated fibroblasts (CAFs) that allow PDAC cell invasion, and by promoting tumor-angiogenesis [72]. Anti-miR-155 has been shown to reduce the invasive behavior and migration of PDAC cells by down-regulation of the signal transducer and activator of transcription 3 (STAT3), which can be activated through various signaling pathways, such as the EGF, Il-6 and interferon-related pathways [73, 74]. Hence, miR-155 may serve as a promising target for experimental PDAC therapy, as it is a well-investigated miRNA that has important implications both in early pancreatic carcinogenesis and in advanced stages of PDAC. Research aimed at the development of *KRAS*-targeting strategies still awaits satisfactory results. The failure so far to develop agents that target *KRAS* may be due to the complex crystallographic structure of the *KRAS* protein [6], a problem that may be circumvented by miRNA-based approaches.

2.1.3 miR-21

miR-21 is one of the most extensively investigated miRNAs in malignant tumors, and has been found to be involved in many oncogenic pathways [75–77]. miR-21 has also been investigated in PDAC, and it has been shown that its expression level correlates well with several prognostic parameters and with resistance to Gemcitabine [78–81]. In PDAC it has been found that miR-21 exerts its oncogenic function through up-regulation of Bcl-2 [82], inhibition of PTEN and RECK [83], FasL [80], p85 α [84] and PDCD4 [85]. It has also been found to be up-regulated by *KRAS* signaling, Gemcitabine-induced histone-deacetylation of the *MIR21* promoter [36] and by hypoxia [86]. Consequently, miR-21 is a strong enhancer of PI3K-AKT signaling, thus promoting the growth,

invasion and migration of PDAC cells [87]. Moreover, miR-21 is involved in creating a tumor-promoting environment by the recruitment of CAFs, which promotes the invasion and metastasis of PDAC cells [88]. Several studies have shown that inhibition of miR-21 via ASOs leads to decreased invasion and metastasis [79], decreased proliferation and increased apoptosis [83] and Gemcitabine sensitivity in PDAC cells [79, 81, 83, 89]. In one study, mice inoculated with tumor cells were treated with anti-miR-21 after palpable tumor formation. Twelve days after intra-tumor injection the control group showed fast progressing tumors, whereas the group of mice treated with anti-miR-21 showed little to no progression [89]. A combined use of anti-miR-21 and the receptor tyrosine-kinase inhibitor Sunitinib led to a synergistic decrease of cell viability, which was more pronounced than with a combination of Sunitinib and anti-miR-221, another highly up-regulated miRNA in PDAC [90, 91]. In view of current data, targeting of miR-21 appears a valid option in the treatment of PDAC, as it is upregulated in most PDACs and interferes with essential malignancy-associated signaling pathways.

2.1.4 miR-34

Recently, a number of studies has revealed an important tumor-suppressive activity of miR-34 in PDAC through synergistic interaction with the p53 pathway. This holds true for all three known homologues of miR-34, referred to as miR-34a-c, respectively. Although these homologues all share a seed sequence, their expression levels vary strongly among different tissues, suggesting tissue-specific roles [92]. It has been shown that miR-34a and b can inhibit PDAC growth and viability, although different mechanisms underlying these inhibitions have been reported. Insight into the role of miR-34c is still lacking. It has been reported that miR-34a exerts its function partly by inhibition of target genes encoding Bcl-2, Notch-1 and Notch-2 [93, 94], while miR-34b also inhibits the expression of SMAD3, thereby repressing proliferative signaling through the TGF- β pathway [95]. It has also been found that p53 wild-type PDAC-derived cell lines show considerably increased apoptotic rates when transfected with miR-34 mimics [96]. Interestingly, inhibition of tumor growth has also been seen in p53-deficient PDAC-derived cell lines, suggesting the occurrence of p53-independent interactions of miR-34 [94, 96]. Other investigators found that treatment with the demethylating agent 5-Aza-dC and the HDAC inhibitor Vorinostat resulted in re-expression of miR-34a and a consecutive up-regulation of p53. In addition, it was found that this treatment may lead to an increased expression of the downstream signaling molecules p21, p27 and PUMA, while cell proliferation, cell cycle progression and EMT (epithelial to mesenchymal transition) were inhibited. Additionally, it was found that stem cell characteristics were reduced [15].

Vorinostat is already in clinical use for the treatment of refractory cutaneous T-cell-lymphoma and has an acceptable adverse effects profile, which is probably due to its selective targeting of tumor cells [97]. Also, a successful safety study on PDAC patients has been performed [98]. The estrogenic isoflavone Genistein has been shown to inhibit PDAC growth and to induce apoptosis by enhancing the expression of miR-34a [93]. Genistein has little to no side effects [99], rendering it into an interesting therapeutic option. Clearly, the miR-34 family of miRNAs has therapeutic potential for PDAC, as the p53 pathway is commonly altered in these tumors. Moreover, miR-34 may also exert its tumor-suppressive function via p53-independent pathways, such as the TGF- β pathway. Since at least one of these pathways is altered in most PDACs, miR-34 may be suitable for a broad-scale application [2]. Since the targeted delivery of miRNAs is still challenging (see below), the use of agents such as Vorinostat may support the development of new targeted therapies. As miR-34 is among the most strongly dysregulated miRNAs in different malignancies such as hepatocellular carcinoma (HCC) or leukemia [100, 101], efforts have already been made to utilize miR-34 mimics for tumor therapy. MRX34, which will be discussed in detail later in this article, is a miR-34-mimic-based therapeutic that is currently evaluated for the treatment of unresectable HCCs and has shown promising results in murine models [102]. Apart from miR-34, miR-148 has also been found to be efficacious in PDAC, in part via Bcl-2 inhibition, leading to significant reduction in tumor growth and invasive behavior both in vitro and in vivo [103]. A similar mode of action has been reported for miR-345, although only one study so far has dealt with PDAC [104].

2.1.5 TGF- β -related miRNAs

Other tumor-suppressor-miRs that have been reported to target the TGF- β pathway are miR-483 and miR-367. miR-483-3p has been shown to inhibit PDAC growth through down-regulation of SMAD4, leading to decreased growth-inhibitory signaling of the TGF- β pathway [105]. The *SMAD4* gene has been found to be mutated in >60 % of the PDAC cases (see above). Therefore, it is assumed that tumors that do not exhibit *SMAD4* mutations may over-express miR-483-3p in a higher proportion, although this has so far not been documented. Interestingly, the *SMAD4* gene status poorly correlates with its mRNA-level, suggesting that post-transcriptional regulation by miRNAs may be at work [106]. It has been found that miR-367 targets the inhibitory SMAD protein SMAD7, leading to an increased in vitro PDAC-derived cell growth [107]. As yet, however, data on the exact role of SMADs, including SMAD7, in the pathobiology of PDAC are still conflicting, although it has been suggested that down-regulation of SMAD4 and SMAD7 is essential [106]. Fact is, nevertheless,

that the TGF- β pathway is highly deregulated in PDACs, and that it may represent a pivotal target for targeted therapy.

2.1.6 MUC4-related miRNAs

MUC4 is a membranous high-molecular weight glycoprotein that enhances the malignant behavior of PDAC cells by repressing apoptosis and immune modulation, and by aggravating chemoresistance. It has been shown that MUC4 expression is regulated by miRNAs [108–111]. MUC4 and the epidermal-growth factor receptor-2 (HER-2/neu) are proven targets of miR-150, and they have been found to interact closely to promote HER-2 activation. This interaction leads to an increased stability of the HER-2/neu receptor, thus enhancing downstream signaling and tumor cell proliferation [111, 112]. Restoration of miR-150 expression has led to decreased growth and invasiveness of PDAC-derived cell lines in in vitro studies. The effect could mainly be attributed to repression of MUC4 expression by miR-150, although other targets of miR-150 have also been identified [111]. Another miRNA that targets MUC4 is miR-219-1-3p. It was found that miR-219-1-3p over-expression in PDAC-derived cell lines successfully reduced activation of the AKT and MAPK/ERK pathways through inhibition of MUC4 expression. Furthermore, it was found that injection of miR-219-1-3p mimics reduced tumor growth and MUC4 expression in a murine PDAC xenograft model [113]. The use of miRNAs that target MUC4 seems promising, as MUC4 is a molecule involved in oncogenic pathways essential for PDAC, such as the MAPK/ERK and AKT signaling pathways. Moreover, MUC4 and HER-2 have been found to be over-expressed in most PDAC cases [2], suggesting that targeting these molecules with miRNAs might be considered for the majority of patients.

2.1.7 miR-200

MiR-200, comprising miR-200a-c and miR-141, is known to exhibit tumor suppressive properties and is currently being evaluated as therapeutic agent in a variety of tumors [114–116]. MiR-200 exerts its tumor suppressive action by inhibiting key steps in the EMT cascade, thereby inducing epithelial characteristics in tumor cells [117]. Conversely, miR-200 promotes MET (mesenchymal to epithelial transition), the last step of metastasis in which migratory tumor cells form stable colonies [118]. The expression of miR-200a has been found to be markedly decreased in PDAC cells, especially in its cancer stem-cells (CSCs). Moreover, it has been found that a low expression of miR-200a correlates with EMT-related traits such as a high expression of Vimentin and a low expression of E-cadherin [119]. It has also been found that re-expression of miR-200a using miR-200a-mimics can restore epithelial traits in PDAC CSCs in vitro,

while decreasing their invasive and migratory behavior [119]. Ma et al. showed that miR-200c mimics, when transfected into PDAC cells, exhibited a negative effect on their metabolism with a subsequent decrease in survival, invasive behavior and colony forming capacity [120]. Furthermore, subpopulations of CSCs typically expressing the stem-cell markers CD44 and CD24, showed a pronounced dose-dependent decrease in cell viability upon Gemcitabine treatment [120].

2.1.8 miR-141

Another important member of the mir-200 family is miR-141, which exerts its function through inhibition of the Yes-associated protein-1 (YAP1), the transmembrane-4-L-6-family-1 (TM4SF1) protein and the mitogen-activated protein kinase isoform 4 (MAP4K4) [121–123]. A low miR-141 expression is frequently found in PDAC and has been shown to correlate with an impaired overall survival and advanced clinic-pathological features in PDAC patients [123, 124]. In vitro functional studies have shown that re-expression of miR-141 using miR-141 mimics results in a reduced growth and colony forming capacity and an increased apoptosis, effects that are largely attributable to YAP1 inhibition [124]. Lijian et al. showed that miR-141 mimics led to suppressed PDAC cell migration and invasion, although no effect on cell growth or apoptosis was observed [122]. Zhao et al. found that in vitro miR-141 over-expression may lead to G1-phase cell cycle arrest and induction of apoptosis in conjunction with down-regulation of cyclin D1 and Bcl-2 expression. The colony forming and invasive capacities of the respective cells were markedly inhibited. In addition, it was found in murine xenograft models that miR-141 can inhibit in vivo tumor cell growth [123].

2.1.9 miR-375

miR-375 is a miRNA that is expressed especially in pancreatic islet cells under conditions that are necessary for an appropriate development of the endocrine pancreas and the regulation of glucose-dependent insulin secretion [125, 126]. miR-375 has been found to be down-regulated in PDAC cells and to function via the targeting of pyruvate dehydrogenase kinase-1 (PDK-1), Insulin-Like Growth Factor Binding Protein 5 (IGFBP5) and Caveolin-1 [127, 128]. It has also been found that miR-375 signaling leads to reduced activation of the AKT pathway in PDAC cells [128]. Furthermore, over-expression of miR-375 has repeatedly been shown to inhibit PDAC cell growth and to induce apoptosis [127–129]. Zhou et al. found that miR-375 over-expression may lead to a significant increase of cells in the G1/G0-phase of the cell cycle, a reduced cell proliferation and an increased apoptotic rate [128, 129]. The same group showed that miR-375 over-expression may be effective in suppressing tumor growth in xenograft models.

It was found that after 1 month mice with solid PDAC tumors that were injected with miR-375 mimics showed significantly smaller tumor volumes than the non-injected controls [130].

2.1.10 miR-221

miR-221 is one of the most strongly deregulated miRNAs in PDACs and PanINs, and has been found to be over-expressed in other tumor entities as well [127, 131, 132]. In PDAC, it effectively enhances tumor viability and resistance to Gemcitabine [83, 133]. It has been found that miR-221 exerts its oncogenic function in PDAC through down-regulation of a number of target genes, including *PTEN*, *p27 (kip1)*, *p57 (kip2)*, *PUMA* and tissue inhibitor of metalloproteinases-2 (*TIMP-2*) [83, 133, 134]. This mode of action may explain its enhancing effects on proliferation, apoptosis and invasive behavior. Xu et al. found that administration of miR-221 mimics inhibited apoptosis and promoted cell proliferation and invasion in vitro, while an anti-miR-221 exhibited opposite effects. These alterations were found to be accompanied by a significant up-regulation of the invasion-associated matrix-metalloproteinases -2 and -9 (MMP-2 and MMP-9) as a result of TIMP-2 inhibition. TIMP-2 is crucial for maintaining a proper ECM-homeostasis that affects the invasive capacities of PDAC cells, and is a target of miR-221 [133]. It has been shown that inhibition of miR-221, using an anti-miR-221, leads to a slow growth, a shortened survival and an up-regulation of tumor-suppressive molecules in PDAC cells in vitro, while miR-221 mimics were found to induce opposite effects [127, 134]. Basu et al. showed in a mouse model that miR-221 expression is triggered by overactive KRAS, i.e., activating *KRAS* mutations, indicating that KRAS acts as an upstream regulator of miR-221 [127]. Tumor suppressor-miRs and onco-miRs that may be employed for targeted PDAC therapy are listed in Tables 1 and 2, respectively. In Fig. 1 the putative functions of these miRNAs in PDAC are depicted.

2.2 Potential therapeutic lncRNAs

In comparison to miRNAs, there is a paucity of literature on lncRNAs in PDAC but, nevertheless, pivotal roles of lncRNAs in this tumor entity have been delineated as well [240]. A recent next generation-based sequencing study revealed that at least 319 lncRNAs may be deregulated during pancreatic carcinogenesis [241]. In addition, it has been shown that lncRNA deregulation constitutes an important aspect of the metastatic process [242]. The affected lncRNAs often appear to be involved in (de)regulation of the MAPK pathway [242], indicating that these ncRNAs may contribute to PDAC metastasis formation by promoting uncontrolled growth. In Table 3 lncRNAs that may potentially be useful

Table 1 Potential therapeutic tumor-suppressor-miRs

microRNA	Verified target genes in PDAC	Prognostic influence ^{a,b}	Investigated In vitro/in vivo ^c	Chemo-resistance	References
Let-7	<i>HMGAI, HMGA2, KRAS</i>	–	In vivo	inhibited	[55, 135–138]
miR-15	<i>BMI1, BCLXL, FGFR2</i>	–	In vitro	–	[139, 140]
miR-20	<i>STAT3</i>	–	In vitro	–	[141]
miR-29	<i>RAN</i>	–	In vitro	–	[142, 143]
miR-34	<i>BCL2, CDK6, SIRT1, NOTCH1, SMAD3</i>	SR, LNM, M	In vivo	inhibited	[15, 93–96]
miR-96	<i>NUAK1, HERG1, GPC1</i>	SR, D, TS	In vivo	–	[144–147]
miR-99	<i>MTOR</i>	–	In vitro	–	[148]
miR-100	<i>FGFR3</i>	–	In vivo	inhibited	[149]
miR-101	<i>HMGA2</i>	–	In vivo	–	[150, 151]
miR-107	<i>CDK6</i>	–	In vitro	–	[152]
miR-124	<i>RAC1</i>	SR	In vitro	–	[153]
miR-126	<i>ADAM9</i>	–	In vitro	–	[154]
miR-130	<i>STAT3</i>	SR	In vivo	–	[155]
miR-132	<i>ARHGAP32</i>	–	In vitro	–	[156]
miR-135	<i>BMI1</i>	–	In vitro	–	[157]
miR-138	<i>VIM</i>	–	In vitro	Inhibited	[158]
miR-140	<i>PPP1R13L, MMP2, MMP9</i>	–	In vitro	–	[159]
miR-141	<i>TM4SF1, YAP1, MAP4K4</i>	SR, LNM	In vivo	inhibited	[122–124]
miR-142	<i>HSP70</i>	–	In vitro	–	[160]
miR-143	<i>COX2, KRAS, GEF1, GEF2, IGF1R, RREB1</i>	–	In vivo	–	[61, 161, 162]
miR-145	<i>NEDD9, MUC13, KRAS, RREB1</i>	–	In vivo	inhibited	[61, 163, 164]
miR-146	<i>EGFR, MMP16, IRAK1</i>	–	In vivo	–	[165–167]
miR-148	<i>DNMT1, CCKBR, BCL2, AMPKα1, CDC25B</i>	SR, LNM, M, TS	In vivo	inhibited	[103, 168–171]
miR-150	<i>MYB, IGF1R, MUC4</i>	–	In vitro	–	[111, 172, 173]
miR-152	<i>DNMT1</i>	–	In vitro	–	[168]
miR-183	<i>BMI1</i>	SR	In vitro	–	[174]
miR-191	<i>USP10</i>	–	In vitro	–	[175]
miR-193	<i>STMN1, UPAR</i>	–	In vitro	–	[176]
miR-198	<i>PBX1</i>	SR	In vivo	–	[177]
miR-200	<i>MUC4, MUC16, PTEN, EP300</i>	SR	In vitro	inhibited	[119, 120, 135, 178–180]
miR-203	<i>CAV1, BIRC5</i>	SR	In vivo	–	[181–183]
miR-205	<i>E2F2, E2F5, ERBB3, HRAS</i>	–	In vivo	inhibited	[184]
miR-206	<i>KRAS, ANXA2</i>	–	In vivo	–	[185, 186]
miR-211	<i>RRM2</i>	SR	In vitro	Inhibited	[187]
miR-216	<i>BECN1, JAK2</i>	–	In vivo	–	[188, 189]
miR-217	<i>KRAS</i>	–	In vivo	–	[35]
miR-218	<i>ROBO1, UGT8</i>	–	In vitro	–	[190, 191]
miR-219	<i>MUC4</i>	–	In vivo	–	[113]
miR-326	–	–	In vitro	–	[192]
miR-335	<i>OCT4</i>	–	In vitro	–	[193]
miR-345	<i>BCL2</i>	–	In vitro	–	[104]
miR-375	<i>PDK1, IGFBP5, CAV1</i>	LNM	In vitro	–	[127–130]
miR-410	<i>AGTR1</i>	–	In vitro	–	[194]
miR-491	<i>BCL2L1, TP53</i>	–	In vitro	–	[195]
miR-494	<i>MYC, SIRT1, FOXM1</i>	–	In vitro	inhibited	[196, 197]
miR-497	<i>FGFR1, FGF2, IGF1R</i>	SR	In vivo	inhibited	[198, 199]
miR-506	<i>PIM3</i>	–	In vitro	–	[200]
miR-520	<i>ABCG2</i>	–	In vitro	–	[141]

Table 1 (continued)

microRNA	Verified target genes in PDAC	Prognostic influence ^{a,b}	Investigated In vitro/in vivo ^c	Chemo-resistance	References
miR-545	<i>DDX58</i>	SR	In vitro	–	[201]
miR-548	–	–	In vitro	inhibited	[202]
miR-615	<i>IGF2</i>	–	In vivo	–	[203]
miR-630	<i>IGF1R</i>	–	In vitro	–	[172]
miR-655	<i>ZEB1, TGFBR2</i>	–	In vitro	–	[204]
miR-802	<i>TCF4</i>	–	In vitro	–	[205]
miR-1181	<i>SOX3, STAT3</i>	SR	In vivo	–	[206]
miR-1247	<i>NRP1, NRP2</i>	SR	In vivo	–	[207]

^a Prognostic influence of microRNA expression in tumor samples

^b SR Survival rate, LNM Lymph-node metastasis, M Metastasis, TS Tumor size, D Differentiation

^c In studies listed with in vivo studies, in vitro results are always implied

for PDAC therapy are listed, whereas in Fig. 2 the functions and effects of these lncRNAs are depicted.

2.2.1 *Gas5*

Growth arrest-specific 5 (*Gas5*) is a lncRNA that functions by negatively regulating cell cycle progression. It is expressed at a low level in several tumor entities such as B-cell lymphoma and cervical cancer [243–245]. *Gas5* is a downstream member of the mammalian target of rapamycin (mTOR) pathway, which is currently subject of intense research in the field of targeted therapy [48]. It has been found that in prostate cancer mTOR inhibition leads to up-regulation of *Gas5* while, in reverse, silencing of *Gas5* reduces the effect of mTOR inhibition [246]. In PDAC, *Gas5* expression is reduced compared to normal pancreatic ductal epithelium. Lu et al. showed that inoculation of *Gas5* pcDNA-containing vectors leads to a significant in vitro reduction of G1/S-phase cells in the PDAC-derived cell lines PANC1 and BxPC3 by negatively regulating cyclin-dependent kinase 6 (CDK6) expression [247]. Based on these observations, reintroduction of *Gas5* may therapeutically be beneficial. In addition, *Gas5* may have potential as a biomarker for clinical response in mTOR-targeted therapy in PDAC as well as in other tumors due to the close relationship between mTOR signaling and *Gas5* expression. As such, *Gas5* may facilitate the selection of patients that are eligible for mTOR-targeted therapy and, additionally, for monitoring the efficacy of mTOR-targeted therapy in PDAC patients.

2.2.2 *MALAT-1*

The >8000 nucleotides-long intronic ncRNA (lincRNA) metastasis-associated lung adenocarcinoma transcript-1 (*MALAT-1*) is a highly conserved lncRNA with oncogenic properties that was first detected in non-small cell lung cancer (NSCLC) [248]. *MALAT-1* is a lincRNA with a hitherto

poorly understood function. It is up-regulated in PDAC and especially in PDAC-CSCs [249] and is located predominantly in the nucleus. It has been shown that *MALAT-1* expression correlates with various clinicopathologic features and a poor clinical outcome in PDAC patients [249]. Recent work has revealed a beneficial effect of *MALAT-1* inhibition in PDAC through different molecular mechanisms. Silencing of *MALAT-1* leads to decreased cellular growth via a p21-mediated cell cycle arrest at the G1/S-phase transition and an increased p53-dependent apoptosis [250]. In addition, *MALAT-1* silencing has been found to result in decreased migration and down-regulation of the expression of EMT-associated mesenchymal markers [250]. *MALAT-1* silencing has also been found to decrease tumor vascularization and to increase sensitivity to Gemcitabine [251]. These observations provide promising outlooks for an assessment of *MALAT-1* as therapeutic target in PDAC. Additionally, it certainly has potential as a prognostic biomarker and as an indicator of metastasis and chemoresistance in PDAC patients.

2.2.3 *HULC*

Highly up-regulated in liver cancer (*HULC*) is a cytoplasmic 482 nucleotides-long lncRNA. It was first described in HCC where it regulates the expression of multiple genes associated with the malignant behavior of tumor cells [252]. It has been found to be up-regulated in the presence of Hepatitis B protein x (HBx) and to promote HBV-mediated liver cirrhosis. In PDAC, *HULC* expression has been found to correlate significantly with a reduced overall survival and time to recurrence. In addition, *HULC* expression turned out to be highly sensitive and specific in distinguishing cancer tissue from healthy tissue. Silencing of *HULC* has been demonstrated to result in a decreased viability and proliferation of PDAC cells by blocking the G1/S-phase transition [253]. Thus, *HULC* may

Table 2 Potential therapeutic onco-miRs

microRNA	Verified target genes in PDAC	Prognostic influence ^{a,b}	Investigated in vitro/in vivo ^c	Chemo-resistance	References
miR-10a	<i>HOXB1, HOXB3, HOXA1, KLF4, SDC1, NFI</i>	–	In vitro	–	[208, 209]
miR-10b	<i>TIP30</i>	SR, M	In vitro	–	[210, 211]
miR-17-5p	<i>BIM</i>	SR	In vitro	promoted	[212, 213]
miR-21	<i>BCL2, FASLG, PDCD4, PTEN, RECK</i>	SR, R	In vivo	promoted	[79–83, 85, 88, 89, 214–216]
miR-27	<i>SPRY2</i>	–	In vitro	–	[217]
miR-31	<i>RASA1</i>	–	In vitro	–	[64]
miR-92a	<i>DUSP10</i>	–	In vitro	–	[208, 218]
miR-95	–	–	In vivo	–	[219]
miR-106	<i>TIMP-2</i>	–	In vitro	–	[220]
miR-153	<i>SNAI1</i>	SR	In vitro	–	[221]
miR-155	<i>TP53INP1, SELIL, FOXO3, SOCS1</i>	–	In vitro	promoted	[71–73]
miR-181	<i>CYLD</i>	–	In vitro	promoted	[222]
miR-186	<i>NR5A2</i>	SR, LNM	In vitro	–	[192]
miR-191	<i>USP10</i>	SR, M, LNM	In vitro	promoted	[175, 223]
miR-192	<i>SIP1</i>	–	In vitro	–	[224]
miR-194	<i>DACHI</i>	–	In vitro	–	[225]
miR-196a	<i>NFKBIA, ING5</i>	–	In vitro	–	[226, 227]
miR-197	<i>CTNND1</i>	–	In vitro	–	[228]
miR-208	–	–	In vitro	–	[229]
miR-221/222	<i>TIMP2, CDKN1B, PTEN, CDKN1C, PUMA</i>	SR	In vitro	promoted	[83, 90, 127, 133, 134, 230]
miR-223	<i>FBW7</i>	–	In vitro	promoted	[231, 232]
miR-301a	<i>NKRF, BIM</i>	–	In vivo	promoted	[233, 234]
miR-301b	<i>CDH1</i>	–	In vitro	–	[235]
miR-365	<i>SHC1, BAX</i>	–	In vitro	promoted	[236]
miR-367	<i>SMAD7</i>	SR	In vivo	–	[107]
miR-371	<i>ING1</i>	SR	In vivo	–	[237]
miR-424	<i>SOCS6</i>	–	In vitro	–	[238]
miR-483	<i>SMAD4</i>	–	In vitro	–	[105]
miR-1246	<i>CCNG2</i>	SR	In vivo	promoted	[239]

^a Prognostic influence of microRNA expression in tumor samples

^b SR Survival rate, LNM Lymph-node metastasis, M Metastasis, TS Tumor size, D Differentiation

^c In studies listed with in vivo studies, in vitro results are always implied

be used to monitor patients after curative surgery and, in addition, may serve as a potential therapeutic target.

2.2.4 HOTAIR

HOX transcript antisense RNA (HOTAIR) is an oncogenic lncRNA and negative prognostic factor in a variety of solid cancers including breast, colon and pancreatic cancer [254]. It has been discovered that increased HOTAIR expression levels correlate with more advanced tumor stages and a short overall-

survival in PDAC patients [255]. HOTAIR acts as a molecular scaffold to coordinate the function of the chromatin modifying enzymes Polycomb Repressive Complex 2 (PRC2) and LSD1 to propagate gene suppression of the HOXD locus. In human embryogenesis, it is expressed predominantly in distal anatomic regions, i.e. the head and tail of the embryo [256]. It has been found that HOTAIR promotes the malignant behavior of PDAC cells by different mechanisms [49]. HOTAIR promotes e.g. the invasive behavior of PDAC cells by regulating the expression of genes such as *GDF15*, although its effects appear to vary

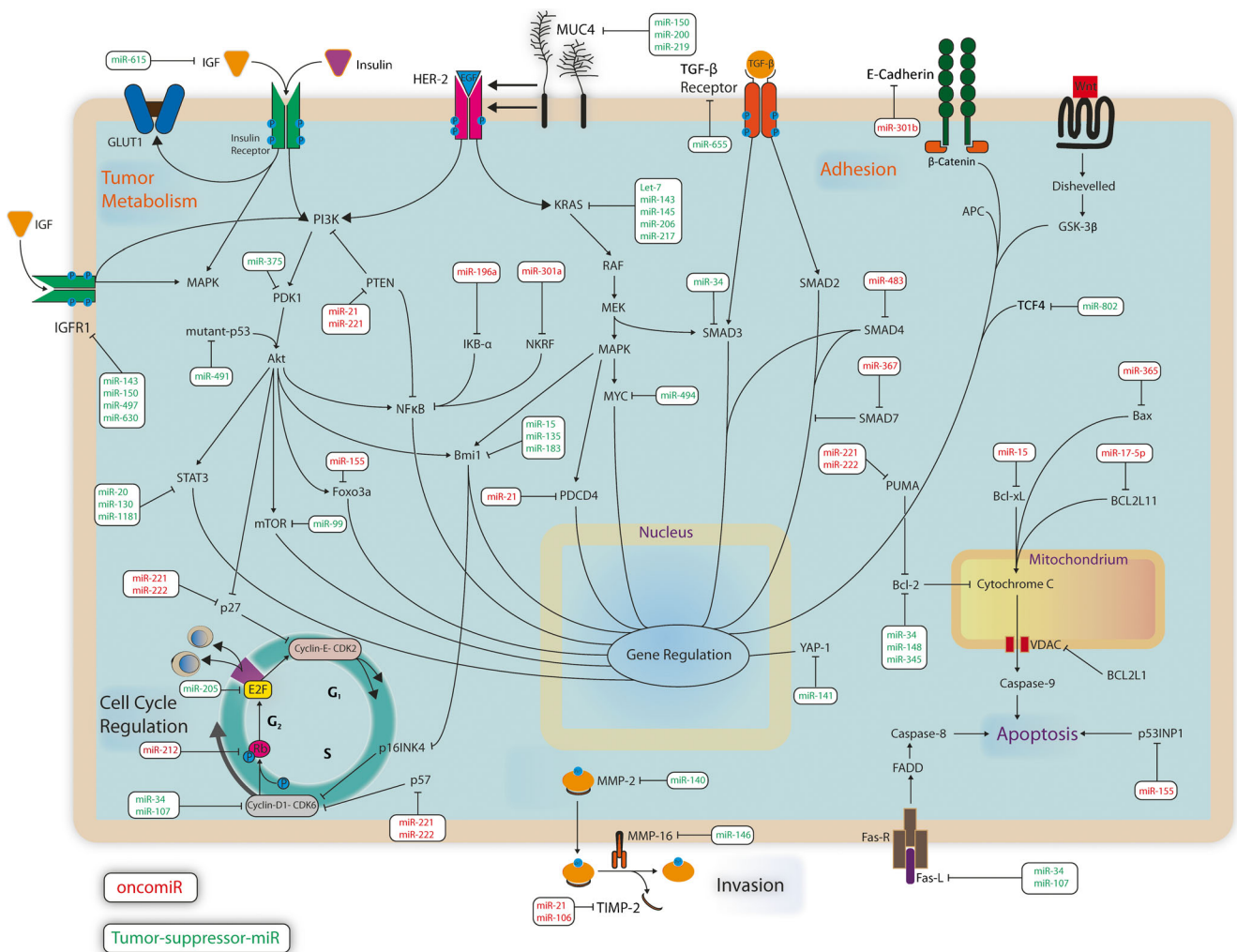


Fig. 1 Figure 1 illustrates the function of hitherto investigated microRNAs (miRNAs) in PDAC. In the PDAC cell, miRNAs are involved in the regulation of important signaling pathways by inhibiting

expression of their target molecules at the mRNA level. miRNAs with oncogenic function are marked in red, whereas miRNAs with tumor suppressive function are marked in green

drastically between primary tumors and tumor-derived cell lines, which may at least partly be explained by varying PRC2 activities [49, 257]. Recent data indicate that HOTAIR inhibition may reinforce the anti-proliferative and pro-apoptotic

effects of radiotherapy, in part by up-regulating Wnt inhibitory factor 1 (WIF-1) [258]. Although HOTAIR may serve as a potential therapeutic target in PDAC, future studies should be aimed at identifying tumors that might benefit most of

Table 3 Potential therapeutic long non-coding RNAs

lncRNAs	Function	Prognostic influence	References
HOTTIP	Epigenetic regulation of <i>HOXA</i> gene locus	negative	[263]
MALAT-1	Regulation of cell-cycle at G2/M-phase transition	negative	[249, 250, 277]
LOC389641	Inhibition of E-cadherin. Upregulation of TRAIL-receptors, promoting tumor growth and metastasis.	negative	[266]
ENST00000480739	Regulation of HIF-1 α levels	negative	[265]
H19	Embryonic/Fetal development	negative	[58, 272]
HOTAIR	Epigenetic regulation of <i>HOXD</i> gene locus	negative	[49]
HULC	Not determined in PDAC	negative	[253]
GAS5	Cell-cycle regulation at G1/S-phase transition	favorable	[247]

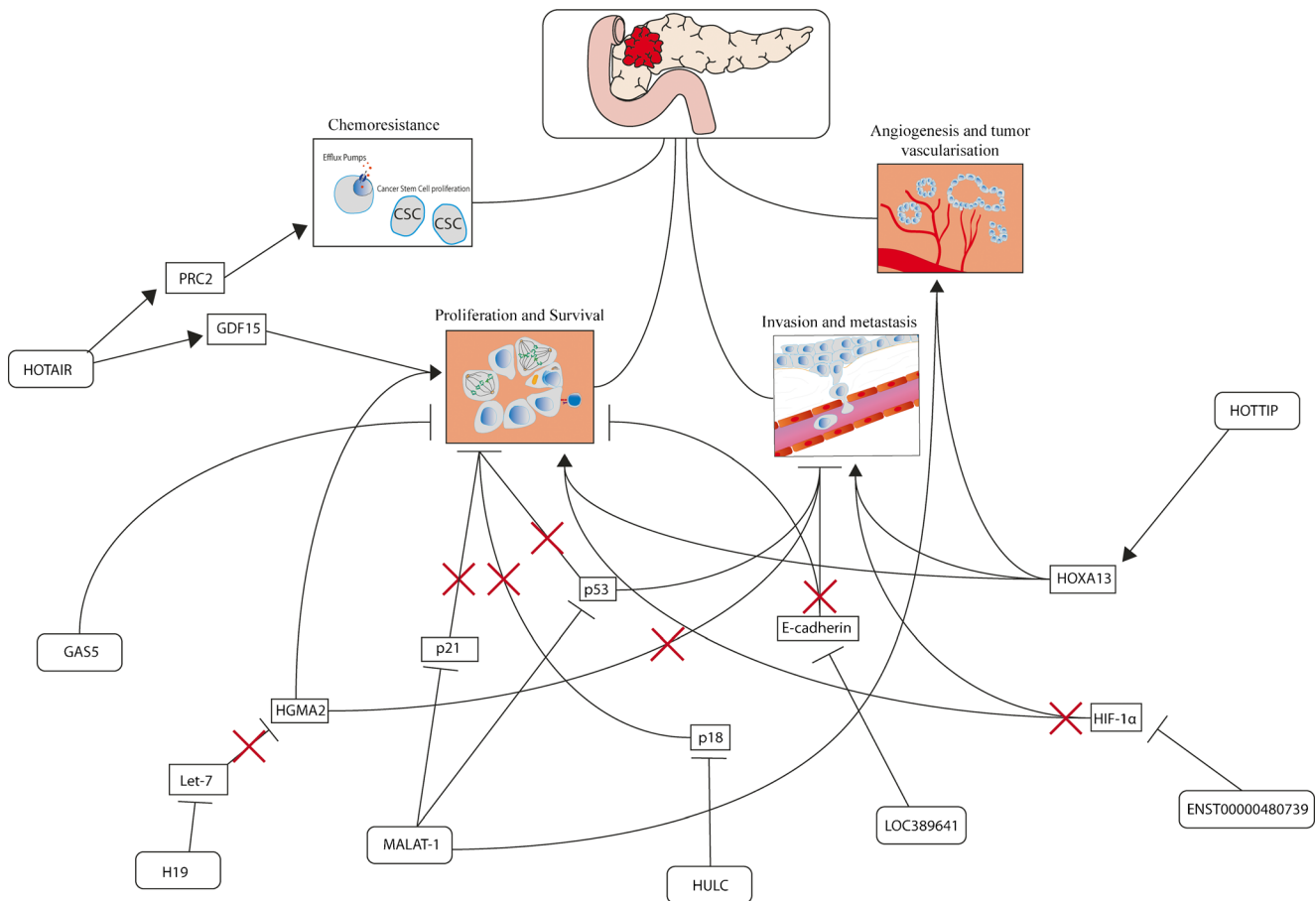


Fig. 2 Functions and potential therapeutic effects of long non-coding RNAs

HOTAIR inhibition. Moreover, the effect of HOTAIR expression on the chemoresistance of PDAC should be evaluated, as recent work has shown that HOTAIR may promote chemoresistance in ovarian cancers [259].

2.2.5 HOTTIP

The lincRNA HOXA transcript at the distal tip (HOTTIP) has been shown to up-regulate HOXA gene expression through interaction with WDR5/MLL and subsequent histone methylation [260, 261]. Recently, HOTTIP expression has been linked to an impaired survival in HCC patients and to overlap with regulation of the HOTAIR gene, although the latter regulates primarily HOXD genes [262]. HOTTIP has been identified as one of the most profoundly up-regulated lncRNAs in PDAC cells compared to non-neoplastic pancreatic duct epithelium [240]. Knock-down of HOTTIP has been found to lead to cell cycle inhibition at the G1/S-phase transition, a decrease in the expression of EMT-associated factors and enhanced sensitivity to Gemcitabine, both in vitro and in vivo. HOTTIP exerts its effects on PDAC cells mainly via up-regulation of HOXA13 expression, but also via up-regulation of Aurora A kinase, which may promote

aneuploidy [263, 264]. HOTTIP over-expression has been associated with a low degree of tumor differentiation, lymphatic metastases and early postoperative recurrences [240]. HOTAIR-targeted therapy may hold promise, especially in early stage cancers. Since it is up-regulated especially in tumors prone to recur early in the postoperative phase, it could be used to select patients who might benefit from aggressive adjuvant chemotherapy. These patients would benefit most from simultaneous HOTTIP targeted therapies, as this may enhance the chemosensitivity of PDAC cells.

2.2.6 ENST00000480739

The recently discovered lncRNA ENST00000480739 has been shown to exhibit tumor-suppressive properties in PDACs, and to be lowly expressed in > 90 % of primary tumor samples as well as in a number of PDAC-derived cell lines. Sun et al. found that a high ENST00000480739 expression negatively correlates with the occurrence of lymph node metastases and positively with an improved clinical outcome, thus considering it as an interesting candidate PDAC biomarker. In addition, low ENST00000480739 expression was found to serve as an independent risk factor for impaired

postoperative survival. Experimental work has shown that ENST00000480739 over-expression results in suppression of tumor cell invasion, both in vitro and in vivo. Its effects were found to be mediated by up-regulation of osteosarcoma amplified-9 (OS-9), which leads to a decrease in hypoxia inducible factor-1-alpha (HIF-1 α) expression levels and a subsequent down-regulation of matrix-metalloproteinase (MMP) expression levels [265]. Because of its anti-invasive effect on PDAC cells, ENST00000480739 may have potential as a therapeutic agent, i.e., its exogenous over-expression may reduce the likelihood of recurrence. Moreover, a low expression after surgery may be considered as an indicator of metastasis and, thus, as a need for re-evaluation of the patient's TNM status.

2.2.7 LOC389641

The newly discovered lncRNA LOC389641 was found to play an important role in PDAC. Statistical analyses revealed that LOC389641 expression closely correlates with the overall survival and the occurrence of lymph node metastasis in PDAC patients [266]. LOC389641 has been found to be over-expressed in primary PDAC tissues and in PDAC-derived cell lines and, in addition, to contribute to PDAC cell proliferation through cell cycle-independent mechanisms. Additional in vitro and in vivo studies have shown that LOC389641 can increase the invasion and migration of PDAC cells. The latter is caused by inhibition of E-cadherin expression and a subsequent induction of EMT in the tumor cells, indicated by increased expression of the mesenchymal marker Vimentin and the EMT-associated transcription factor Snail. In addition, it has been found that LOC389641 can up-regulate the expression of *TNFRSF10A*, a gene encoding a TRAIL-receptor, which is a mediator of metastasis-promoting signals in TRAIL-resistant cancer cells [266]. Targeting LOC389641 may be considered as an actionable approach, since the up-regulation of TRAIL receptors is highly specific for tumor cells [267] and the damage of normal cells, therefore, appears unlikely. Moreover, E-cadherin down-regulation is commonly seen in PDACs, suggesting a broad efficacy of the use of LOC389641 inhibition.

2.2.8 H19

lncRNA H19 is highly expressed in many human tissues during embryogenesis until the time of birth, after which its expression levels drop to practically zero [268, 269]. Expression in adult individuals appears to be confined to cancer cells [269–271]. H19 has been found to be highly expressed in PDAC cells, and seems to be involved in the modulation of their migration, invasion and metastasis. This effect is at least partially due to the antagonistic effects of H19 on let-7 activity, which results in up-regulation of HGMA2 expression

levels and, subsequently, tumorigenesis [272]. Recently, H19 has been subjected to targeted tumor therapy research. The plasmid-based drug BC-819 that utilizes polyethylenimine-coated plasmid DNA (also known as DTA-H19), induces expression of diphtheria toxin (DT) and cell death in the presence of H19-specific transcription factors, which are restricted to malignant tumors in adults [273]. This drug has been shown to be effective in the treatment of human bladder and ovarian cancer in case studies [274, 275]. In a phase I/IIa trial the effects of ultrasound-guided endoscopic or CT-guided injection of BC-819 (see below) were investigated in patients suffering from PDAC. The results were promising with respect to reducing tumor mass, even leading to resectability in one case by tumor shrinkage. Although the trial was not powered to provide statistically significant results, the safety of treatment was confirmed [58]. Currently, a multicenter randomized phase-IIb trial with the aim to assess the effect of BC-819 on progression-free survival is under way [276].

3 Delivery systems for ncRNA-based therapeutics

Whereas many questions about the pharmacokinetic properties of ncRNA-based therapies remain, the major challenges of ncRNA-based therapies lie in the development of reliable delivery systems. Several systemic therapies with miRNA-carrying viral vectors and non-viral agents have been studied in recent years. Current therapeutic delivery methods that utilize ncRNA can be divided into several different categories: chemically modified ncRNAs, viral and non-viral vectors, ncRNA “sponges”, and endoscopic ultrasound and CT-guided injections [230, 278, 279].

3.1 Chemically modified ncRNAs

It has been reported that by adding chemical backbone modifications to a miRNA mimic or anti-miR, its degradation can be inhibited and its in vitro and in vivo activity can be improved [280]. A recent study has shown that miR-155 could be targeted more effectively when a pH-low insertion peptide (pHLIP) was chemically conjugated with anti-miR-155 oligonucleotides, enabling their accumulation specifically in acidic tumor tissues. Using this approach, the authors of this study found that intravenous application of this combined agent led to a significant reduction in tumor volume in mice with solid lymphomas [279]. This seems to be an attractive approach, as it overcomes the problems posed by systemic introduction of unmodified oligonucleotides, which mainly undergo hepatic accumulation and subsequent clearance [56]. Moreover, hypoxia and acidosis are typical characteristics of PDAC that could be used in this concept, whereas they represent barriers for conventional therapeutics [281]. Even though data showing that pHLIP-conjugated ncRNAs indeed

do accumulate in PDAC tissue are lacking, PDAC-imaging data from mouse models with multispectral optoacoustic tomography do suggest this [282].

3.2 Viral and non-viral vectors

The use of viral vectors may have potential for future therapeutic application, as tropism against cells that express certain molecules can be established. Lentiviral, adeno-associated-viral and adenoviral vectors have been used to express ncRNA in target cells. Viruses possess a complex endogenous machinery for gene processing, which makes them highly effective in transferring nucleotides [283]. Disadvantages of viral vectors are their complicated manufacturing process and possible immunologic, hematologic or oncologic side effects. Non-viral vectors have the advantage of permitting a larger size nucleotide-cargo and an uncomplicated large scale production [283]. The non-viral miR-34-mimic-based therapeutic MRX34 is currently under evaluation in a phase-I clinical trial for the treatment of HCC and, as such, represents the first clinical application of miRNA-mimics [102]. MRX34 utilizes the liposomal carrier-system Smarticles™ for delivery into tumor cells, which has shown promising results in both in vitro and in vivo studies [284]. Whether an analogous approach would lead to satisfactory accumulation in PDACs is unlikely, because of the aforementioned hepatic clearance. This obstacle may be bypassed by the use of poly-ethylene-glycol (PEG)-coated liposomes, also called “stealth”-liposomes. Stealth-liposomes can evade degradation in the reticuloendothelial system and remain in circulation for up to 48 h. The PEG-coats of stealth-liposomes can be chemically equipped with functional groups to enable coupling with tumor-specific antibodies and proteins to target tumor cells or other cell types [285, 286]. Another interesting option that has been known for almost a decade are Carbon nanotubes (CNTs). CNTs are small versatile molecules of condensed rings of carbon atoms. It has been shown that CNTs permit the transport of nucleic acids in vitro and in vivo. In addition, they have the advantage of a high transport capacity. They also allow diverse surface modifications, turning them into interesting candidate non-viral vectors. The specific targeting of solid tumors after systemic delivery still remains a challenge [287]. However, modifications that enable a pH-dependent nucleotide-cargo release have been found to result in an adequate accumulation in solid tumors [278].

3.3 ncRNA sponges

Yet another interesting system is the use of a transfectable “miRNA sponge”, as was recently demonstrated by Jung et al. [230]. In this study, the authors simultaneously inhibited five miRNAs, i.e., miR-21, miR-155, miR-10, miR-211 and miR-222, by sequestration into a circular sponge carrying

binding sites for the respective miRNAs, which resulted in a significant reduction in tumor growth and viability [230]. The antiviral drug Miravirsen, which has been successfully tested in humans for the treatment of hepatitis C-virus (HCV), has an analogous function. It binds intracellular miR-122 to inhibit the propagation of viral RNA without toxic side-effects [288]. An equivalent approach is, however, unlikely to work for the treatment of PDAC without further modification, as oligonucleotides are subject to hepatic clearance as outlined above. Therefore, more work is needed to improve the applicability of nucleotide-based therapies, which remains one of the biggest challenges of this research field.

3.4 Endoscopic ultrasound and CT-guided injections

Endoscopic ultrasound-guided fine needle injections (EUS-FNI) for different diagnostic and therapeutic applications in PDAC have been reported [289]. This method involves the use of fine needles attached to an endoscope by which different fluids can be injected into the pancreas during retrograde-cholangiopancreatography (ERCP). The use of EUS-FNI for celiac plexus neurolysis is an upcoming method to treat PDAC-associated pain, whereas also the effect of local application of chemotherapeutics is currently being investigated [289]. CT-guided delivery is performed via a posterior percutaneous access to reach retroperitoneal organs. CT-guided injections have also been used to treat PDAC-associated pain with good results after unsuccessful celiac plexus neurolysis, turning it into a back-up system or alternative method for EUS-FNI [290]. Endoscopic ultrasound-guided or CT-guided injection of BC-819 in PDAC patients has been shown to improve clinical outcomes in case studies, but as yet without statistical benefit [58]. Mouse model data have shown that BC-819 is able to significantly reduce tumor growth and to counteract metastasis in treated mice compared to untreated controls [291]. As yet, however, limitations of this procedure, such as its accuracy or chemoresistance to BC-819, are not well defined. Intraperitoneal injection of miR-26-carrying viral vectors has been proven to successfully induce gene expression in pancreatic beta-cells in murine models [292]. In humans, this approach may provide a simpler, cheaper and less invasive access to the pancreas than EUS-FNI or CT-guided injections, although questions regarding efficacy and side effects can be addressed only by speculation at this point.

4 Perspectives of ncRNA-based therapies in PDAC

Recent research has revealed many ncRNAs that are involved in the pathobiology of various human cancers [293]. Especially miRNAs and lncRNAs have been shown to regulate multiple crucial genes involved in cell cycle progression,

apoptosis, invasion, migration and vascularization, often in concert with ncRNAs regulating multiple genes [34]. In PDAC, several miRNAs and lncRNAs have been found to substantially affect tumor behavior by regulating multiple target genes and pathways. This was exploited by using ncRNA mimics or ASOs, delivered by viral or non-viral vectors, for the design of in vitro and in vivo PDAC therapies. In addition, more subtle methods have been employed to create ncRNA-based therapeutics, such as the experimental agent BC-819 and the chemical conjugation of pHLIP to ncRNAs. It has also been found that drugs such as Metformin and Vorinostat inhibit in vitro PDAC cell growth at least in part via down-regulation of certain key miRNAs [14, 15]. Additional more complicated systems, such as the plasmid-based agent BC-819 that utilizes the abnormally expressed lncRNA H19 in PDAC cells resulting in DT production and cell death, may be promising.

Whether combinations of different ncRNA mimics and ASOs exhibit synergistic effects to positively interfere with tumor biology, or whether simultaneous application of ncRNAs may abrogate or enhance the anti-tumor effects of each other is currently unknown, but it has been shown that a combined approach may be superior [230]. This holds also true for the combined use of therapeutic ncRNAs and conventional chemotherapeutic agents such as Gemcitabine or 5-FU, as also other agents such as the tyrosine-kinase inhibitor Sunitinib [90], although clinical trials are still needed. Ye et al. showed that interactions of miRNAs, mRNAs, lncRNAs and transcription factors encompass an intriguing network of regulation, in which several miRNAs and lncRNAs inhibit each other and/or lead to degradation of mRNA [37], which clearly indicates that the administration of several ncRNAs may result in adverse effects. Further knowledge on the regulation of ncRNAs in PDAC may lead to the identification of pivotal ncRNAs that may serve as the most promising candidates for therapeutic approaches and help to understand the subtleties of PDAC biology in further detail.

An important aspect of personalized cancer therapy is knowledge about the genetic and molecular differences between individual patients that result in different responses to therapy. This holds true especially for PDAC, as only 25 % of the patients respond well to Gemcitabine treatment, and the overall survival is only marginally improved in responders [294]. It has amply been shown that tumor-related miRNAs may be easily identified in blood, stool or saliva [25, 295, 296]. This circumstance may enable us to create patient-specific ncRNA profiles to guide tumor therapy. A recent clinical study has shown that miR-21, miR-100 and miR-99 expression levels in tumor specimens after curative surgery in patients with UICC stage II PDACs may predict the response to adjuvant Gemcitabine therapy [297]. So, the measurement

of miRNA levels may become a useful tool to guide adjuvant chemotherapy, since alternatives to Gemcitabine treatment have proven more efficacious, but also since they carry the risk of more severe side-effects that may not be acceptable.

5 Conclusions

To date, many ncRNAs have been identified that may serve as targets for tumor therapy, but additional information clearly needs to be gathered before clinical application can be considered. The use of currently available viral delivery systems may pose problems, such as adverse effects or treatment failure due to unknown toxicity, host immunity and the risk of genomic insertion and a consequent risk of (secondary) cancer. Although, non-viral delivery systems have shown promising results, it still needs to be established whether systemic application will result in adequate concentrations at the a priori target site. The intravenously applied miR-34-based non-viral therapeutic agent MRX34 is currently undergoing evaluation in a phase I trial, which may be refined so as to target PDAC cells. In case of insufficient efficacy, local application via EUS-FNI or CT-guided injection may be considered. However, systemic therapy should be the primary aim, as most PDACs are incurable by operation due to the frequent presence of metastases at the time of discovery [2].

As yet, little is known about the possibility of resistance to ncRNA-based therapy, and how to choose the right ncRNA as target out of a plethora of candidate ncRNAs. This connects the search for potential ncRNA therapeutic targets with that for ncRNA-based diagnostics. Studies aimed at early-stage recognition of PDAC using miRNAs alone or in combination with CA19-9 have yielded a higher sensitivity and specificity than using CA19-9 alone [298]. In addition, it has been found that a postoperative prognosis can be established precisely through miRNA measurement [299]. The promising miRNA-mimic-based therapeutic agent MRX34 and several lncRNA-based therapeutics are currently being evaluated in clinical studies, but it will require intensive additional research to finally reach systemic therapy. Therefore, we anticipate that the clinical role of ncRNAs in the near future may primarily lie in its diagnostic use, such as the monitoring of response to conventional chemotherapy, and their use as prognostic markers, as they represent stable differentially expressed molecules that are amenable to sensitive detection. Interestingly, recent high throughput studies have revealed that the lncRNAs that are most widely deregulated in PDAC cells have not been thoroughly investigated yet [241]. Therefore, there may still be many of them around of which the biological role and clinical applicability await discovery.

Compliance with ethical standards

Conflict of interest The authors of this article, V.Taucher, J. Haybaeck and H. Mangge declare no conflicts of interest.

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