

Long non-coding RNA in pancreatic adenocarcinoma and pancreatic neuroendocrine tumors

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Abstract

Interest in non-coding regions of DNA has been increasing since the mapping of the human genome revealed that human DNA contains far fewer genes encoding proteins than previously expected. However, analysis of the derivatives of DNA transcription (transcriptomics) revealed that the majority of the genetic material is transcribed into non-coding RNA (ncRNA), indicating that these molecules probably provide the functional diversity and complexity of the physiology of the human body that cannot be attributed to the proteins. Of these ncRNA, long ncRNA (lncRNA) have a length greater than 200 nucleotides and share many common components with the coding messenger RNA (mRNA): They are transcribed by RNA polymerase II, comprised of multiple exons and subjected to normal RNA splicing giving RNA products of several kilobases. Scientific data reveal the regulatory role of lncRNA in the control of gene expression during cell development and homeostasis. However, to date, very few lncRNAs have been characterized in depth, and lncRNAs are thought to have a wide range of functions in cellular and developmental processes. These molecules will have the possibility to be used as biomarkers and contribute to the development of targeted therapies. Concerning pancreatic cancer, there are limited data in the literature that correlate the growth of these tumors with deregulation of various lncRNA. We herein review the literature regarding the role of lncRNA as a diagnostic and prognostic biomarker and possible therapeutic target in the neoplasms of the pancreas, particularly pancreatic adenocarcinoma and pancreatic neuroendocrine tumors.

Keywords Long non-coding RNA, pancreatic ductal adenocarcinoma, pancreatic neuroendocrine tumors, pancreatic tumors

Ann Gastroenterol 2017; 30 (6): 622-628

Introduction

According to the World Health Organization classification, the pancreas gives rise to several malignant and benign neoplasms. Ductal adenocarcinoma, which represents the most common

type of exocrine carcinoma, accounts for approximately 85% of pancreatic tumors, while tumors derived from the endocrine pancreas, arising from the cells of the islets, represent 5% [1].

Pancreatic ductal cell adenocarcinoma (PDAC) is showing an increasing incidence in the developed countries [2-4]. Factors associated with the higher incidence of PDAC are smoking, obesity, diet, diabetes mellitus, chronic pancreatitis, and genetic predisposition in approximately 5-10% of patients [2,5-8]. Genes found to be involved in the majority of adenocarcinomas are *KRAS*, *CDKN2A*, *TP53*, and *SMAD4* [6]. Surgical resection seems to be the method of choice for a possible cure; however, only 10-20% of patients have potentially resectable pancreatic cancer (PC) at the time of diagnosis [9,10]. Following surgery, adjuvant chemotherapy with gemcitabine or 5-fluorouracil is recommended [10,11]. FOLFIRINOX and protein-bound paclitaxel (nab-paclitaxel) chemotherapy was found to be more effective than gemcitabine, but with notable side effects [12]. Several ongoing late-phase trials are currently evaluating a number of therapeutic targets (inhibition of growth factor receptors: Epidermal growth factor receptor, platelet-derived growth factor receptors, vascular endothelial growth factor receptor, insulin-like growth factor receptor-1, tyrosine kinase inhibitors, MEK1/2, mTOR blockade and PI3K and HER2-neu

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Conflict of Interest: None

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Received 25 March 2017; accepted 5 July 2017; published online 4 August 2017

DOI: <https://doi.org/10.20524/aog.2017.0185>

pathway inhibitors); their results will become available over the next 1-2 years [13].

Pancreatic neuroendocrine tumors (PNET) occur in two distinct epidemiologic groups: Sporadic tumors and the tumors that affect kindreds with the multiple endocrine neoplasia type 1 syndrome (MEN1) in a pattern of autosomal dominant inheritance [14]. Gastrinoma and insulinoma are the most frequent lesions, with equal incidences [15], while nonfunctional tumors account for 14-48% of neoplasms of the endocrine pancreas [16,17]. No well-established environmental factors are associated with the development of neoplasms of the endocrine pancreas [18]. Genes frequently mutated in these patients include *DAXX*, *mTOR* and *ATRX* [19]. Other than surgery for functioning tumors [20,21], the somatostatin analogs, such as octreotide, can reduce the excessive production of these hormones [22]. Targeted therapy with everolimus or sunitinib can control symptoms and decelerate the progression of the disease [19,23].

Materials and methods

An extensive English-language literature search was conducted using PubMed to identify original studies and review articles, using as keywords “pancreatic tumors”, “prognostic factors”, “biomarkers”, “pancreatic adenocarcinoma”, “pancreatic neuroendocrine tumors”, and “long non-coding RNA”.

Discussion

Non-coding RNAs (ncRNAs)

NcRNA can be classified into two groups according to their size: The group of short ncRNA, which are less than 200 nucleotides (nt) in length and include micro RNA (miRNA), piwi-interacting RNA (piRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), and telomere-associated RN [24]; and the other group of lncRNA, which contain 200 nt or more [25]. Based on their proximity to protein-coding genes, lncRNA are classified into five types: Sense, antisense, bidirectional, intronic and intergenic [26,27].

Long ncRNA (lncRNA)

Initially, lncRNA was considered to be “transcriptional noise” without any biological function. However, nowadays it has been demonstrated that lncRNA, interacting with DNA, RNA and transcription factors, participates in a series of biological processes, such as DNA methylation, histone modification, and chromatin remodeling. In this way, it manages the expression status of target genes controlling the cell cycle [28-30], the induction of angiogenesis, the promotion of metastasis, and the evasion of tumor suppressors [31,32,49,50], making them important “players” in the diagnosis and treatment of malignant tumors [33,34].

Different studies have reported that the primary sequences of lncRNA show very little conservation, in contrast with their secondary and tertiary structures, which are highly conserved and might be potentially related to their biological functions [35-37]. Recent studies have shown that a variety of lncRNA can be used as tumor markers [38-40].

All lncRNA execute their functions through five main means: As signals, decoys, guides, enhancers, or scaffolds [41]. In these ways lncRNA regulates the progress of various biologic processes and the expression of various genes controlling the homeostasis of the cell cycle.

lncRNAs and PDAC

In recent years, the regulatory roles of lncRNA in the etiology of several diseases have attracted increasing interest. The strongest relation at present is with cancer [42,43]. Altered expression of lncRNA has been documented in different human cancers, increasing the interest in their role as biomarkers for diagnosis and prognosis, as well as potential therapeutic targets for these types of diseases [44,45]. Abnormal lncRNA expression in many cancers might be a major cause of oncogenesis. These cancers can be distinguished according to their altered lncRNA expression [25,46].

PDAC is known for its aggressiveness and lack of effective therapeutic options. Thus, there is an urgent need to explore novel approaches to the diagnosis and treatment of this dismal disease.

Genetic alterations associated with PC progression include events such as mutations in K-RAS (approx. 90%) and overexpression of HER-2/neu. Inactivation of the p16/CDKN2A (approx. 75%) tumor suppressor gene often occurs at later stages, followed by loss of p53 (approx. 65%), SMAD4 deregulation (approx. 50%), and BRCA2 signaling pathways that facilitate deregulation of cell-cycle control, invasion and metastasis. Despite these findings, the alterations of oncogenes and tumor suppressor genes have not yet led to solutions for therapeutic interventions [47].

In recent years, it has been proposed that epigenetic events may also play an important role in the development and maintenance of PC. lncRNAs are already known as important epigenetic regulators that have a role in diverse cellular processes including cell proliferation, development, differentiation, apoptosis and therefore oncogenesis [48].

Of the lncRNAs normally expressed on pancreatic tissue, some were significantly upregulated and others significantly downregulated in PDAC tissue samples.

H19

H19 is a maternally expressed and paternally imprinted 2.3kb gene; it resides close to the telomeric region of chromosome 11p15.5 and is mainly localized in cytoplasm [49]. *H19* is known to respond to various stress conditions, such as reduced p53 and hypoxia-inducing epithelial-to-mesenchymal transition (EMT), cell invasion and extravasation/migration, by activation mechanisms of cell survival [50].

According to a model suggested by Zipori *et al* [51,52], p53, a well-known genome keeper, interplays with *H19* in cellular homeostasis. p53 is an *H19* suppressor. During stress conditions prorogated by downregulation of p53, cells can respond to this by upregulation of *H19*.

Another common stress condition in tumors is hypoxia. In addition to the abnormal vascularization commonly affecting their essential nutrient and gas exchange [53], the high proliferation rate of cancer cells impedes their access to normal blood supply, prorogating hypoxia [54] that induces *H19* transcription [55] when p53 is null or impaired [56]. Hypoxia-inducible factor-1 α (HIF-1 α) mediates *H19* induction during hypoxia when p53 is impaired, possibly because of the loss of p53's inhibitory effect on HIF-1 α . Nuclear localization of p53 is essential for *H19* inhibition [56]. *H19* promotes direct tumor metastasis by upregulation of EMT, favoring the dedifferentiation of epithelial cells that become able to disseminate and migrate through the extracellular matrix (ECM), ensuring the intravasation into blood vessels that will carry them to a distal site. Once extravasated to the secondary site, the tumor cell re-differentiates, through the reverse mechanism of mesenchymal-to-epithelial transition, and then proliferates and colonizes, becoming a secondary tumor [57]. In one study, it was found that *H19* was overexpressed in PDAC compared with adjacent normal tissues. Upregulation of *H19* expression was also notable in primary tumors that subsequently metastasized, compared to those that did not [58]. In the same study, downregulation of *H19* impaired PDAC progression. *H19* has been demonstrated to promote PDAC cell invasion and migration, mainly by increasing HMGA2-mediated EMT through antagonizing let-7 [58]. The role of *H19* in the oncogenesis and metastasis of PDAC is schematically described in Fig. 1.

A phase I/IIa study in patients with unresectable PC investigated the efficacy of intratumoral administration of the plasmid diphtheria toxin A (DTA)-*H19* composed of the *H19* gene regulatory sequences. DTA-*H19* is a double-stranded DNA plasmid that carries the gene for the DTA chain under the regulation of the *H19* promoter sequence. This plasmid embodies a targeted therapy approach, in that the plasmid enters all dividing cells, but the DTA expression is triggered by the presence of *H19* transcription factors found only in tumor cells, thus destroying the tumor without affecting normal cells [59].

Additionally, studies have demonstrated that the efficacy of PC treatment should be improved by DNA-based therapy controlled by *H19* gene sequences, either alone or in combination with gemcitabine [60].

LncRNA HOXA transcript at the distal tip (HOTTIP)

The HOTTIP lncRNA, located at the 5' end of the HOXA cluster, was recently functionally characterized [61]. The activity of HOTTIP in the regulation of proliferation, invasion, and chemoresistance of PC is a result of its interaction with the WDR5/MLL complex, which enhances histone H3 lysine 4 trimethylation to activate the expression of multiple 5' HOXA genes [61].

It has been demonstrated that targeted silencing of HOTTIP impairs the mechanisms of proliferation, invasion,

and EMT capability. In the same study, HOTTIP was found to be upregulated in most PDAC tissues compared with adjacent non-tumor tissues [62]. Moreover, HOTTIP seems to have a prognostic value for gemcitabine chemoresistance in PC cells. Furthermore, HOXA13, a gene also involved in the progression of PDAC, is located in contiguity with HOTTIP and is one of its significant targets. In a study that investigated chronic pancreatitis tissues, 27 lncRNA, including HOTTIP, were upregulated more than 10-fold in PDAC compared with these chronic pancreatitis tissues [62].

From the five splice variants of HOTTIP, HOTTIP-005 expression in a normal cell line and six PC cell lines was examined [63]. All PC cell lines had higher HOTTIP-005 expression compared with the normal cells. Univariate analysis of overall survival revealed that TNM stage, early recurrence and HOTTIP-005 level expression were independent prognostic indicators for the overall survival of patients with PC; the median time of survival in the high- and low-level HOTTIP-005 expression subgroups was 14 and 45 months, respectively. These results indicate that increased HOTTIP-005 expression is a poor prognostic factor for patients with PC [63].

Metastasis associated lung adenocarcinoma transcript 1 (MALAT1)

MALAT1 is more than 8000 nt in length and localized on chromosome 11q13 [64].

It is significantly over expressed in many cancer types [65-67] and has been shown to promote tumor cell proliferation, invasion and metastasis through polycomb repressive complex 2 (PRC2) [68,85], the Wnt pathway [69], the ERK/MAPK pathway [70] and the SFPQ/PTBP2 complex [71], which is involved in tumor growth. MALAT1 can also facilitate EMT by downregulating the expression of E-cadherin and upregulating the expression of N-cadherin and fibronectin to enhance transforming growth factor- β -induced EMT [72]. Thus, high

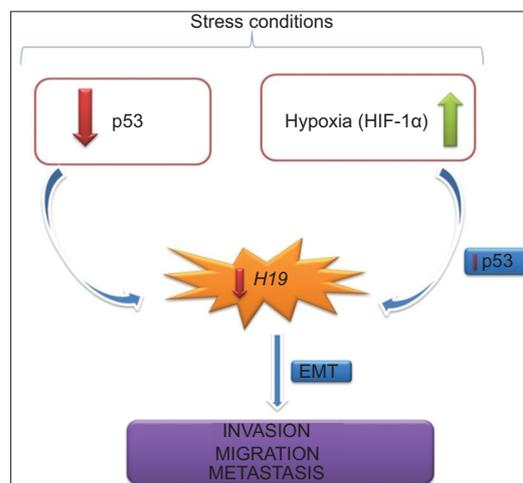


Figure 1 Role of *H19* in pancreatic adenocarcinoma. HIF-1 α , hypoxia-inducible factor-1 α ; EMT, epithelial-to-mesenchymal transition

levels of MALAT1 expression may be related to tumor prognosis, indicating its potential use as a biomarker of cancer.

Another analysis indicated that overexpression of MALAT1, additionally to the tumor location and nerve invasion, was an independent predictive factor for overall survival in patients with PDAC. The level of MALAT1 was significantly higher in PDAC compared to the adjacent normal pancreatic tissues. Furthermore, MALAT1 expression level showed significant correlation with TNM stage. Higher MALAT1 expression was associated with poorer disease-free survival in patients with PC [73]. In contrast to the previous study by Liu *et al* [73], another study did not confirm the MALAT1 upregulation in PDAC [74]. A meta-analysis carried out in 2016 found that in PC there is a significant association between MALAT1 overexpression and TNM stage, as well as with distant metastasis and cancer prognosis but not with lymph node metastasis [75].

HOX antisense intergenic RNA (HOTAIR)

HOTAIR is a lncRNA localized on chromosome 12q12.13 at a boundary in the HOXC gene cluster. The activity of HOTAIR is due to its interaction with PRC2 (EZH2, SUZ12 and EED), which enhances H3K27 trimethylation to decrease the expression of multiple genes involved in cell proliferation and metastasis [76]. Studies showed that HOTAIR is more highly expressed in advanced tumors compared to early-stage tumors and is overexpressed in pancreatic tumors compared to the normal pancreas [77-79]. Targeted silencing of HOTAIR in PC cells decreased cell proliferation, inhibited cell-cycle progression, and induced apoptosis [76].

HOTAIR is also highly expressed in the metastatic sites of cancer that have poor outcomes in patients undergoing chemotherapy or radiotherapy [80]. In another study, HOTAIR was more highly expressed in locally advanced tumors (T₃) compared with tumors only detected in the pancreas (T₂), and more highly expressed in tumors involving regional lymph nodes (N₁) compared with tumors localized only in the pancreas (N₀) [78]. In this same study, a survival analysis showed that low HOTAIR expression (<85%) was associated with significantly increased overall survival compared to high HOTAIR expression in patients with PC. HOTAIR levels and N stage are strongly correlated with overall patient survival [78].

A study by Chang's group uncovered a new mechanism whereby HOTAIR leads to transcriptional silencing of a distant chromosomal region through epigenetic regulation [81]. Enforced HOTAIR expression in epithelial cancer cells was found to increase invasive and metastatic abilities and reprogram the PRC2 occupancy pattern to resemble embryonic fibroblasts [45].

AF339813

AF339813 is regulated positively by NUF2. A study that investigated NUF2 expression in cancer tissues from patients with PC found it to be significantly higher than that in adjacent normal tissues. It was also demonstrated that knockdown of AF339813 in PC cells significantly reduced cell proliferation and promoted apoptosis [82].

Growth arrest-specific 5 (Gas5)

The expression level of gas5 was found to be significantly decreased in PC compared with normal tissues. Overexpression of gas5 in these cells inhibits cell proliferation, whereas gas5 inhibition induces a significant decrease in the G₀/G₁ phase and an increase in the S phase. It was also demonstrated that gas5 negatively regulates CDK6 (cyclin-dependent kinase 6) expression *in vitro* and *in vivo*. Knockdown of CDK6 partially reduce gas5-induced cell proliferation [83].

ENST00000480739

A study of 35 PDAC noted that lncRNA ENST00000480739 expression was remarkably lower in PDAC tissues than in adjacent normal tissues. Results revealed that ENST00000480739 expression was negatively correlated with tumor size and with lymph node involvement by modulating HIF-1 α . No significant correlation between ENST00000480739 expression and age, sex, diameter or degree of differentiation was proved [84].

Highly upregulated in liver cancer (HULC)

HULC is a cancer-related lncRNA, residing on chromosome 6p24.3 and located in the cytoplasm. Overexpression of HULC is observed in a group of advanced staged and metastatic pancreatic tumors. This expression profile is related to the promotion of cell proliferation *in vitro* [85].

LncRNAs and PNET

The most common location of NETs is in the intestines, pancreas, and lung; they can be classified as functional (hormone secreting) or nonfunctional (non-hormone secreting) NETs [86]. There are few studies in the literature relating to ncRNA and neuroendocrine malignancies. Data from a study by Roldo *et al* showed that a common pattern of miRNA expression distinguishes any tumor type from normal pancreas, suggesting that this set of miRNA might be involved in pancreatic tumor genesis. Specifically, the expression of miR-103 and miR-107, together with a lack of expression of miR-155, characterize neoplastic but not normal pancreatic tissue. miR-204 is primarily expressed in insulinomas and correlates with the immunohistochemical expression of insulin, while overexpression of miR-21 is strongly associated with both a high Ki67 proliferation index and the presence of liver metastasis [87].

There are only scanty data in the literature concerning the role of lncRNA in PNET. The most studied correlation concerns the MEN1 gene-encoding "menin" protein in PNET, but how this initiates tumorigenesis is not yet well understood. In a study by Modali *et al* [88], it was found that lncRNA Meg3 (maternally expressed gene) has tumor-suppressor activity in PNET cells. Menin produced in PNET activates Meg3. Meg3 overexpression in insulinoma cells delays cell proliferation by downregulation of the expression of the proto-oncogene c-Met and these cells show significantly reduced

cell migration and invasion. It was also found that Meg3 and c-MET levels are reciprocally correlated, not only with MEN1-associated PNET, but also with human sporadic insulinomas.

These observations provide a strong basis for the investigation of Meg3 activation and c-MET suppression as novel therapeutic approaches for the treatment of pancreatic neuroendocrine tumors and possibly for the prevention of metastasis.

LncRNA as biomarkers and therapeutic target in PC

An ideal biomarker is defined as the marker that offers the advantages of early detection, high specificity, sufficient sensitivity, robustness and minimal invasion. Many lncRNA are emerging as new candidate biomarkers of cancer, having regulatory functions in both oncogenic and tumor-suppressive pathways, such as the p53, MYC, and NF- κ B pathways [89,90]. The concept of lncRNA as disease markers is strongly related to the discovery that lncRNA demonstrate extreme molecular stability. Additionally, as opposed to mRNA, the lncRNA are functional molecules and their expression level may be an improved marker of the disease. Furthermore, the highly specific expression patterns of lncRNA can be used for accurate disease diagnostics and classification [91]. To date, there is no specific biomarker for the early diagnosis of PC. CA 19-9 is used as biomarker in general clinical practice, although its level has no utility as a screening tool in asymptomatic patients. Even in symptomatic patients where there is suspicion of PC, elevated CA 19-9 is a poor predictor, with a predictive value of 0.5-0.9% [92].

The importance of lncRNA as a regulatory molecule of the cell cycle makes it an attractive therapeutic target; thus, there is a need to find methods for modulating lncRNA. For this reason, short DNA sequences complementary to the RNA of interest, called allele-specific oligonucleotides (ASO), have been recruited. These oligonucleotides work by hybridizing to the RNA, which then blocks the action of the RNA. So far, two ASO have been approved as drugs by the Food and Drug Administration (FDA) of the United States: Fomivirsen, which is used to treat cytomegalovirus retinitis [93], and mipomersen, which is used to treat homozygous familial hypercholesterolemia [94]. ASO act by entering the nucleus and knocking down nuclear lncRNA [95].

A major challenge to working on lncRNA is that the molecular mechanisms underlying their functions are not yet fully understood. Further insight into the biological significance and functioning of lncRNA will require additional studies to be conducted, which may lead to the discovery of yet more mechanisms of action. A further confounding factor in our understanding of lncRNA is that lncRNA may have more than one mechanism of action to confer transcriptional activation or repression of their target genes. The characteristics of deregulated lncRNA described in PDAC and PNET are summarized in Table 1.

Concluding remarks

Expansion of our knowledge of the role of lncRNA in oncogenesis will help improve the diagnosis and develop

Table 1 Deregulated lncRNA in PDAC and PNET

lncRNA	Deregulation	Neoplasm	Sample origin
H19	up	PDAC	Pancreatic tissue
HOTTIP	up	PDAC	Pancreatic tissue
MALAT1	up	PDAC	Pancreatic tissue
HOTAIR	up	PDAC	Pancreatic tissue
AF339813	up	PDAC	Pancreatic tissue
Gas5	down	PDAC	Pancreatic tissue
ENST00000480739	down	PDAC	Pancreatic tissue
HULC	up	PDAC	Pancreatic tissue
Meg3	up	PNET	Pancreatic tissue

lncRNA, long non-coding RNA; PDAC, pancreatic ductal cell adenocarcinoma; PNET, pancreatic neuroendocrine tumors

therapeutic options for PDAC and PNET. Taking into account the limited number of studies regarding the role of lncRNA in these neoplasms, more studies will be needed in the future to demonstrate the importance of lncRNA in tumor initiation, invasion and metastasis, as well as their utility as therapeutic targets. Moreover, considering that until now lncRNA have been detected in pathological pancreatic tissues, their investigation in "easy access" materials like blood in the future will make them even more attractive and less invasive biomarkers. The potential therapeutic options for modulating lncRNA include the form of ASO, as well as other technologies that may arise. lncRNA-based therapies could become an important strategy deserving consideration.

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