



The Contribution of Long Noncoding RNAs Inpancreatic Adenocarcinoma; in Silico Analysis



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Submission: May 11, 2018; **Published:** May 31, 2018

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Abstract

Long noncoding RNAs (lncRNAs) are lengthy noncoding transcripts with important roles in regulation variety of cellular and molecular pathways in the cell like cell proliferation, apoptosis and differentiation. Therefore, it is not surprising if their altered expression connected with human diseases especially cancers. Here, we investigated the expression levels of 192 approved lncRNAs in pancreatic adenocarcinoma using available cancer biportals. Compared to other lncRNAs, the expression levels of lncRNAsGAS5, NORAD, XIST, ZFAS1 and PVT1 were altered in more patients. Additionally, these five genes were upregulated in tumorous tissues with z-score more than 3. These finding indicates that these lncRNAs are promising gene targets for experimental studies on pancreatic adenocarcinoma which may eventually lead to find a novel biomarker for this cancer.

Keywords: Long noncoding RNA; Cancer; Pancreatic adenocarcinoma; GAS5; NORAD; XIST; ZFAS1; PVT1

Introduction

Long noncoding RNAs (lncRNAs) are lengthy transcripts with no capacity for protein production [1]. Increasing body of evidences have been demonstrated that lncRNAs play an important role in regulation of variety of cellular and molecular pathways in the cell including cell growth, proliferation, apoptosis and differentiation [2,3]. Therefore, it is not unexpected if their aberrant expression was attributed with human tumors. The contribution of lncRNAs in progression and invasion of human cancers including breast, gastric, hepatocellular carcinoma and colorectal adenocarcinoma [4] was subject of variety of studies. Meanwhile, valuable feature of lncRNAs like their tissue-specific expression and the long-term stability in body fluids like serum, urine, saliva, breast milk and cerebrospinal fluids, has attracted the cancer researchers to focus more and more on these noncoding molecules as novel marker of human cancers [5]. Pancreatic adenocarcinoma is the fourth cause of cancer-related death worldwide and can progress due to the presence of some risk factors like cigarette smoking, obesity or exposure to chemicals like beta-naphthylamine and benzidine [6]. As the pancreas plays vital role in production of digestive enzymes and hormone, diagnosis and treatment of pancreatic lesions is essential

[7]. The involvement of lncRNAs in pancreatic adenocarcinoma is recently proposed by some studies, however, the need for further experiments is felt [8]. Owing to this fact, this study was aimed to analyze the expression levels of all approved lncRNAs in pancreatic adenocarcinoma tissues using available cancer bioportal. This study proposes which lncRNAs are the best candidates for experimental researches on pancreatic adenocarcinoma.

Material and Methods

To find the association of lncRNAs with pancreatic adenocarcinoma, 192 approved lncRNAs were extracted from HGNC (www.genenames.org). All the studies lncRNAs were then subjected into the cBioPortal dataset [9] and their alteration was analyzed in 179 samples of pancreatic adenocarcinoma (TCGA, provisional) whose RNA-seq v2 data was available. The lncRNAs which were altered in $\geq 10\%$ of the patients were considered as candidate genes for further analysis. The expression level of these candidate lncRNAs were also examined in the studies tumorous samples. Using cBioPortal, the copy number variations including gene amplification or deletion were also examined in candidate lncRNAs to see if the

altered expression arises from such genetic mutation. Using the lncR Inter dataset, the interaction of our candidate lncRNAs with DNA, RNA, transcription factors were evaluated [10]. The tendency of these candidate lncRNAs toward co-occurrence was examined using cBio Portal getaways. The contribution of candidate lncRNAs with pathologic parameters of the tumor samples like neoplasm disease stage, neoplasm histological grade, neoplasm histological

type, primary tumor site and tumor anatomic site was also examined and analyzed in R using the cgdsr and cbaf [11]. The contribution of altered expression of candidate lncRNAs with patients' overall survival was studied using Kaplan-Meier estimate. The Log-Rank Test P-Value <0.05 was considered as statistically significant. All the statistics were performed in R package.

Results

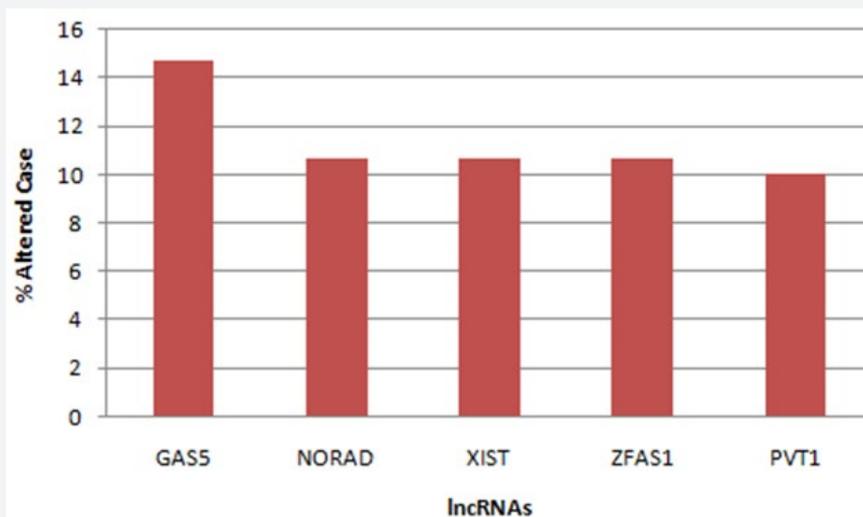


Figure 1: The lncRNAs with maximum frequency of % altered cases. The data was extracted from cBioPortal database.

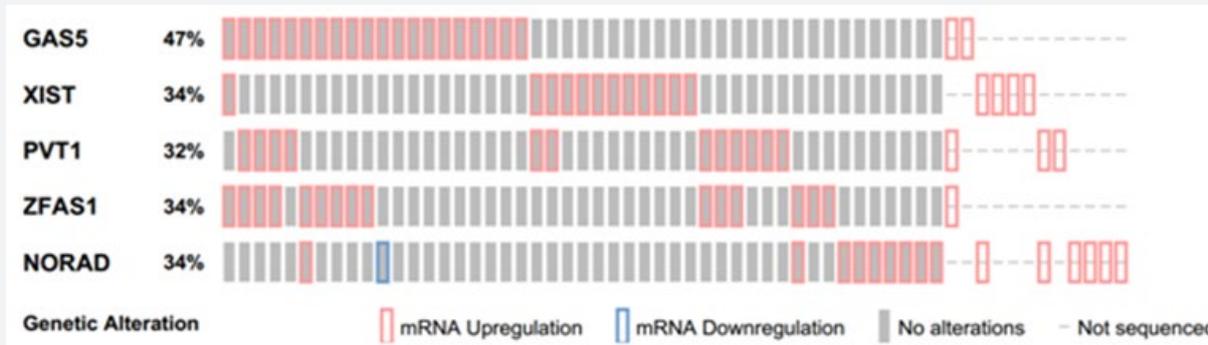


Figure 2: The oncoprint showing the percent of contribution of each lncRNAs in cases with alteration at least in one of the lncRNAs GAS5, NORAD, XIST, ZFAS1 and PVT1. The data extracted from cBioPortal Data set.

From 192 lncRNAs incorporated in this analysis, 59 lncRNAs have been altered at least in one patient. As shown in Figure 1, the lncRNAs GAS5, NORAD, XIST, ZFAS1 and PVT1 were altered in more than 10% of patients. Totally, 39% of the pancreatic adenocarcinoma cases altered for expression of at least one of these five lncRNAs and GAS5 was the gene which allocated the maximum number of cases with alteration in its expression level (Figure 2). We also found these five lncRNAs have been upregulated in cancer tissues with the Z-score more than three (Figure 3) in comparison with normal group. It was found that gene amplification was observed in 75%, 20% and 7% of cases who were upregulated for the lncRNAs PVT1,

GAS5 and ZFAS1 respectively (Figure 4). As shown in Table 1, these lncRNAs interact with variety of gene sequence, transcripts and protein. We also found that GAS5, PVT1 and ZFAS1 have tendency toward co-occurrence (P-value<0.05) (Table 2). In spite of NORAD, It was found that the expression level of all candidate lncRNAs was more upregulated in high grade tumor (G4) in comparison with low grade (G1) one although this value was statistically significant only in GAS5 (P-value=0.03) (Figure 5). In case of NORAD, we found that this lncRNA was downregulated in G4 grade tumor and increased expression was recorded in low grade samples (Figure 5).

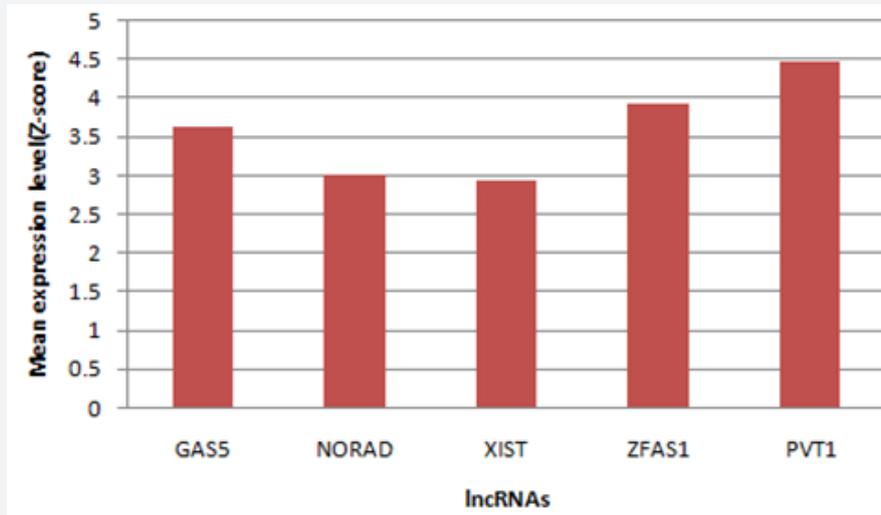


Figure 3: Mean expression levels of candidate lncRNAs GAS5, NORAD, XIST, ZFAS1 and PVT1 in pancreatic adenocarcinoma. The data was extracted from cBioPortal database.

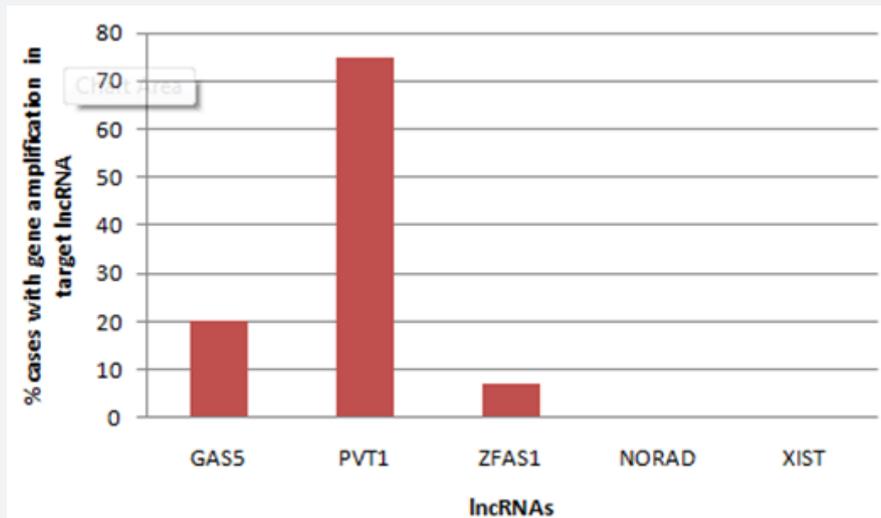


Figure 4: Gene amplification was observed in some of the cases with upregulation of GAS5, NORAD, XIST, ZFAS1 and PVT1.

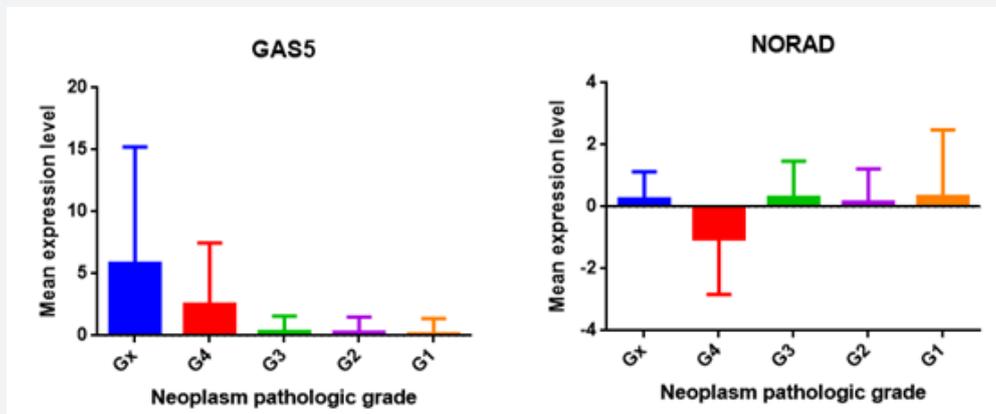


Figure 5: The association of lncRNA expression level with pancreatic adenocarcinoma grade.

Table 1: Interaction mode of candidate lncRNAs with cellular DNA, RNA and proteins. Data has been recruited from lncRInter database.

lncRNA	Interacting Partner	Interaction Class	Interaction Mode
PVT1	EZH2	RNA-Protein	Binding
	NOP2	RNA-Protein	Binding
	TP53	DNA-TF	Binding
	MYC	DNA-TF	Binding
	MYCN	DNA-TF	Binding
	MYC	RNA-TF	Binding
GAS5	YBX1	RNA-TF	Binding
	MIR21	RNA-RNA	Binding
	ANXA2	RNA-Protein	Regulation
	TNFSF10	RNA-Protein	Regulation
	AGO2	RNA-Protein	Binding
	NR3C1	DNA-TF	Binding
	SCTR	DNA-Protein	Binding
	CDK6	RNA-Protein	Regulation
XIST	YY1	RNA-TF	Binding
	SP1	RNA-TF	Binding
	TBP	RNA-TF	Binding
	MIR152	RNA-RNA	Binding
	JPX	RNA-RNA	Regulation
	TSIX	RNA-RNA	Regulation
	BRCA1	RNA-Protein	Binding
	EZH2	RNA-Protein	Binding
	SUZ12	RNA-Protein	Binding
	SRSF1	RNA-Protein	Binding
	NXF1	RNA-Protein	Binding
	BRCA1	RNA-Protein	Binding
HNRNPU	DNA-Protein	Binding	
ZFAS1	MIR150	RNA-RNA	Binding

Table 2: The mutual exclusivity analysis of GAS5, NORAD, XIST, ZFAS1 and PVT1 among 179 pancreatic adenocarcinoma. The data was extracted from cBioPortal.

Gene A	Gene B	Log Odd Ratio	P-value	Tendency
GAS5	ZFAS1	>3	<0.001	Co-occurrence
PVT1	ZFAS1	2.7	<0.001	Co-occurrence
GAS5	PVT1	1.46	0.0232	Co-occurrence

Discussion

Here is the first report in which the contribution of lncRNAs with pancreatic adenocarcinoma was examined using available cancer bioportals. One hundred eighty two approved lncRNAs were considered in this study. The expression levels of these lncRNAs in pancreatic adenocarcinoma tissues as well as the percent of

cases with alteration in lncRNAs were calculated separately. The lncRNAs with acceptable values in both analyses were considered as candidate lncRNAs. We found that lncRNA GAS5, NORAD, XIST, ZFAS1 and PVT1 were the genes with good score in both analyses and upregulated in tumorous tissues. In case of lncRNAs PVT1, GAS5 and ZFAS1, we found that this upregulation may partly due to gene amplification of the corresponding genes in some cases.

We also found that GAS5, ZFAS1 and PVT1 have tendency toward co-occurrence which may due to presence of same regulator or contribution in same biological processes [3]. Our analysis showed that our lncRNAs interact with variety of cellular genes, RNAs and protein which some of these target partner including MYC, P53, miR-21, EZH2 play an important role in cell proliferation, growth, apoptosis and repair. Except NORAD lncRNA, all the other lncRNAs were upregulated in high grade samples showing the probable role of these genes in tumor invasion. However, NORAD was suppressed in high grade samples which propose this gene act in initiation step of pancreatic adenocarcinoma. The association of lncRNAs with pancreatic cancer has been recently reported in some studies [12]. Among which the role of lncRNAs MALAT1 [13], HOTAIR [14], MEG3 [15] and HOTTIP [16] in cell growth and progression of pancreatic cancer has been well- established. Similar to our obtained results, in a study by [17], they found that lncRNA PVT1 has been reported to be upregulated in pancreatic cancer cells and connected with poor prognosis in these patients. Zhao et al. [18] showed that PVT1 can promote the pancreatic cancer metastasis through an interaction with miR-448. The lncRNA NORAD has been recently reported in pancreatic cancer cell metastasis through the induction of EMT signaling pathways [19]. The oncogenic potential of XIST lncRNA has been demonstrated in pancreatic cancer which seem due to the interaction of this lncRNAs with miRNAs like miR-133 and miR-34a [20,21]. However, mentioned study are limited to 1-2 studies and more experimental works is urgent to validate the involvement all of these lncRNAs in pancreatic cancer progression. In case of lncRNAs GAS5 and ZFAS1 no experimental works has been published until now. Regarding to above mentioned data, we strongly suggest that lncRNAs GAS5, NORAD, XIST, ZFAS1 and PVT1 are good choices for starting experimental project focus on lncRNA-associated pancreatic cancer which may open a new window I discovery of novel biomarker for this current cancer.

Acknowledgment

This study was affiliated to Ahvaz Jundishapur University of medical sciences. The authors are so thankful from all the colleagues whose comments improved the quality of this manuscript.

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DOI: [10.19080/JTMP.2018.03.555606](https://doi.org/10.19080/JTMP.2018.03.555606)

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