Mechanism Comparison of Gemcitabine and Dasatinib-Resistant Pancreatic Cancer by Integrating mRNA and miRNA Expression Profiles

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SUMMARY

Background: Pancreatic cancer is one of the most lethal cancers with limited treatment options. Gemcitabine has been the standard drug for patients with advanced pancreatic cancer. Dasatinib is a competitive inhibitor of Src kinase, which has shown promise in treatment of pancreatic cancer. Several studies have revealed the drug resistant mechanism of gemcitabine or dasatinib in human cancers; however, few reports focused on the different mechanisms of gemcitabine and dasatinib resistance in pancreatic cancer. Here, we integrate mRNA and miRNA expression profiles to achieve it.

Methods: Two mRNA expression profiles were downloaded from GEO database. The differentially expressed genes (DEGs) were identified with |fold change| ≥ 2 and p-value ≤ 0.05. Further function of the DEGs were annotated with GO and KEGG pathway enrichment. Finally, the mRNA-miRNA interaction networks were constructed to explore the molecular mechanism.

Results: Results showed that 116 and 238 DEGs were detected in gemcitabine-resistant cell lines and dasatinib-resistant cell lines respectively. Meanwhile, 4 common DEGs were identified in both resistant cell lines, which can clearly divide all cell lines into different sub-groups. KEGG pathway enrichment analysis displayed that the DEGs of both gemcitabine-resistant cell lines and dasatinib-resistant cell lines can map to drug metabolism-cytochrome P450 and metabolism of xenobiotics by cytochrome P450, while DEGs of gemcitabine-resistant cell lines can also map to several metabolism related pathways and dasatinib-resistant cell lines for several cancer related pathways. GO annotation analysis showed that the DEGs of gemcitabine-resistant cell lines and dasatinib-resistant cell lines can also be categorized into drug metabolism. Additionally, the miRNA-mRNA regulation network of gemcitabine-resistant cell lines revealed 16 DEGs were regulated by 6 miRNAs, indicating that these miRNAs may play a key role in gemcitabine treatment of pancreatic cancer.

Conclusions: The difference of gemcitabine resistance in pancreatic cancer were explored by mechanism comparison via the mRNA and miRNA expression profile. These findings support strategies to target molecules and relevant pathways for improving the efficacy of chemotherapy in pancreatic cancer patients.


KEY WORDS

mRNA, DEGs, miRNA, pancreatic

INTRODUCTION

Pancreatic cancer is one of the deadliest cancers with the overall 5-year survival rate less than 5% across the world [1, 2], and it may become the second leading cause of cancer
Pancreatic cancer's tendency to spread silently makes it difficult to diagnose during the early stages, and pancreatic cancer with typical symptoms has reached an advanced stage, suggesting pancreatic cancer has few or no effective therapies [4, 5]. Adjuvant chemotherapy with gemcitabine is an important treatment option to extend life for patients with advanced pancreatic cancer [6]. Unfortunately, most pancreatic cancer patients do not respond well to gemcitabine and even develop chemoresistance [7]. Preclinical studies have supported the antitumoral action of gemcitabine and dasatinib for pancreatic cancer [8, 9]; however, a phase II study reported that single-agent dasatinib does not have clinical activity in patients with metastatic pancreatic cancer [10]. In addition, the molecular mechanism of gemcitabine and/or dasatinib resistance remains unclear. Therefore, it is critical to understand the drug resistant mechanism of gemcitabine and dasatinib to improve treatment of pancreatic cancer.

Gemcitabine resistance is mainly caused by genetic alterations, epigenetic modification and tumor microenvironment. Dysregulated expression of genes involved in gemcitabine transport and metabolism confers chemosensitivity to gemcitabine in pancreatic adenocarcinoma cells, such as nucleoside transporter-1 (hENT1), being responsible for gemcitabine uptake, and ribonucleotide reductase which participates in gemcitabine metabolism [11-13]. The altered expression of genes related to cell survival and apoptosis, such as S100A4, HMGAlA1, BMP, the tyrosine kinases FAK, eSra, and dual specificity protein phosphatase 1 (DUSP1), also promotes chemosensitivity to gemcitabine resistance in pancreatic adenocarcinoma cells [14-19]. Numerous microRNAs, like miRNA-101-3p, miR-125a, and miR-301a-3p, have been shown to be involved in the development of gemcitabine resistance in pancreatic cancer. MicroRNA-101-3p reverses gemcitabine resistance by inhibition of ribonucleotide reductase M1 in pancreatic cancer [13]. MiR-125a regulates chemosensitivity to gemcitabine in human pancreatic cancer cells through targeting A20 [20]. Downregulation of miR-301a-3p sensitizes pancreatic cancer cells to gemcitabine treatment via PTEN [21]. In addition to the aforementioned factors, tumor microenvironment also plays an important role in gemcitabine resistance. Liu et al. confirmed that peroxistin promotes the chemotherapy resistance to gemcitabine in pancreatic cancer [22]. Various reports have tried to elaborate the drug resistant mechanism of gemcitabine in pancreatic cancer; however, only Shen et al. performed microRNA-mRNA regulatory network analysis to uncover the molecular mechanism of gemcitabine-resistant development [23].

Similar to gemcitabine resistance, previous studies on dasatinib resistance focused mainly on genetic and epigenetic levels in human cancers except pancreatic cancer. In chronic myeloid leukemia, one-third of resistant patients on dasatinib have developed mutations in the ABL1 kinase domain [24]. Meanwhile, the overexpression of genes involved in drug transport (ABCB1, ABCG2, MVP, and SLC22A1) is associated with dasatinib resistance in K562 cell lines [25], especially ABCB1, in which overexpression is a key initiating factor of resistance to dasatinib [26]. Moreover, Liu et al. confirmed that niclosamide enhances the sensitivity of chronic myeloid leukemia cells to dasatinib through inhibiting Erk/Mnk1/eIF4E signaling pathway, suggesting the genes participating in the Erk/Mnk1/eIF4E signaling pathway may play a key role in dasatinib resistance [27]. In contrast to chronic myeloid leukemia, dasatinib resistance in lung cancer was related to the signal pathway according to previous studies. Lu et al. showed that the IGFBP2/FAK pathway is causally associated with dasatinib resistance in non-small cell lung cancer cells [28]. Gordian et al. reported that transforming growth factor beta signaling overcomes dasatinib resistance in lung cancer [29]. Beauchamp et al. demonstrated that DDR2 gatekeeper mutation and bypass pathway activated by NF1 loss conferred resistance to dasatinib in lung cancer cell lines [30]. In line with lung cancer, mitogen-activated protein kinase pathway facilitates resistance to the src inhibitor dasatinib in thyroid cancer [31]. Few miRNAs reported have causal relationship with dasatinib resistance. Acute leukemia cells acquire dasatinib resistance via downregulation of miR-217 and upregulation of DNMT3A [32]. MiR-106a-ULK1 signaling pathway promotes dasatinib resistance in lung adenocarcinoma cells via cytoprotective autophagy [33].

Here, to gain further insight into the drug resistant mechanism of gemcitabine and dasatinib in pancreatic cancer, we performed a mechanism comparison of gemcitabine and dasatinib resistance by a comprehensive analysis of mRNA and miRNA expression profiles. Briefly, the significant differentially expressed genes (DEGs) of two mRNA expression datasets were first identified using advanced bioinformatic algorithms. Then, GO and KEGG pathway enrichment analysis were conducted to analyze the function of the DEGs. Finally, the regulatory network between mRNAs and miRNAs was constructed.

MATERIALS AND METHODS

mRNA and miRNA data resources
Various high throughput datasets including microarray and next-generation sequencing have been uploaded to GEO or ArrayExpress databases for public research purposes. To have a comprehensive understanding about drug resistant mechanisms of pancreatic cancer, here we searched the databases and downloaded two representative mRNA expression datasets. One dataset with GEO accession ID GSE35141 and the other one with GEO accession ID GSE59357. The GSE35141 dataset contain three sets of parental and gemcitabine-resistant cell lines (PK-9 and RPK-9, PK-1 and RPK-1, and PK-59 and RPK-59), and each one has a duplicate. GSE59357 has three dasatinib-resistant (MiaPaCa2, Zongjing Chen et al. 2 Clin. Lab. 5/2018
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RESULTS

Identification of DEGs in gemcitabine and dasatinib resistant cell lines

After background correction and normalization, the DEGs in gemcitabine and dasatinib resistant cell lines were figured out using robust empirical bayesian algorithm. Results indicated that a total of 116 and 238 genes were significantly dysregulated in the gemcitabine-resistant cell lines and dasatinib-resistant cell lines. In gemcitabine-resistant cell lines, 68 genes (58.6%) were up-regulated and 48 genes (41.4%) were down-regulated. In dasatinib-resistant cell lines, 64 genes (26.9%) were up-regulated and 174 genes (73.1%) were down-regulated. In addition, 4 DEGs were identified both in gemcitabine and dasatinib-resistant cell lines (Figure 1), and the fold change and adj.pValue were listed in Table 1. Among the four DEGs, MSLN and ADAM19 were down-regulated in the two datasets. While, the expression level of MECOM and ELF5 in gemcitabine-resistant cell lines is opposite to the dasatinib-resistant cell line. Further, the expression pattern of the common DEGs in all cell lines was constructed, and the cell lines were subject to hierarchical clustering. Figure 2 showed that the all cell lines can be clearly classified into different sub-groups except for one dasatinib-sensitive cell line (sensitive.9), which was probably due to large expression value variation.

GO and KEGG pathway annotation

To have a better understanding about the biology function of the DEGs, GO and KEGG pathway functional annotation were conducted using DAVID online tool. KEGG pathway annotation showed that both gemcitabine-resistant cell lines related DEGs and dasatinib-resistant cell lines related DEGs can map to two common pathways, drug metabolism-cytochrome P450 and metabolism of xenobiotics by cytochrome P450 (Figure 3). In addition, DEGs of gemcitabine-resistant cell lines can map to several metabolism related pathways such as tyrosine metabolism and retinol metabolism (Figure 3). While, DEGs of dasatinib-resistant cell lines can map to several cancer related pathways such as the p53 signaling pathway, PI3K-Akt signaling pathway, and so on (Figure 3). Further, GO term comparison of DEGs in gemcitabine-resistant cell lines and dasatinib-resistant cell lines also show that DEGs from the two groups can be enriched into drug metabolism (grey in Figure 4). Apart from the common GO terms, DEGs of dasatinib-resistant cell lines can also be enriched into several unique GO terms such as tissue development, epidermis development, morphogenesis of an epithelium, and so on (Figure 4).

mRNA-miRNA regulation network construction

Various studies have shown that miRNA appears to play an important role in the whole process of cancer development and progression. In Li’s study, six miRNAs, miR-200b, miR-200c, let-7b, let-7c, let-7d, and let-7e, were identified to be significantly down-regulated in gemcitabine-resistant pancreatic cancer cell lines [34]. To explore the regulation mechanism between the miRNAs and DEGs, the interaction network was constructed using CyTargetLinker plugin in Cytoscape. Briefly, target genes of the six miRNAs were predicted based on microCosm, mirTarbase, and Target-Scan databases. Then the common DEGs were screened out from the target genes.

Panc1, SU8686) and three dasatinib-sensitive (Panc0504, Panc0403, Panc1005) pancreatic cancer cell lines, and each cell line has biological triplicates. The GSE 35141 and GSE59357 datasets were generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F and Illumina Human HT-12 V4.0 expression beadchip platform.

Detailed experiment information can be obtained in the previous published papers.

Identification of DEGs in gemcitabine and dasatinib resistant cell lines

To identify the significant DEGs in gemcitabine or dasatinib-resistant pancreatic cancer cell lines, the expression profiles were carefully analyzed using in-house R script. In brief, first the expression values for each probe were subject to background correction. Then normalization and log2 transformation were carried out by applying the GeneChip Robust Multi-array Analysis (GC-RMA) method. Also, control probe sets were removed, and mean expression value was calculated for genes with multiple probes. Finally, DEGs between the resistant pancreatic cancer cell lines and parental cell lines were screened out using Limma package (Linear Models for Microarray Analysis). The criteria were strictly set to adjust p-value \( \leq 0.05 \) and absolute log2 fold change \( \geq 2 \). In addition, common DEGs between the two different drug-resistant cell lines were identified based on venn diagram. An expression pattern of the common DEGs was plotted using heatmap.2 method within the ggplot package.

GO and KEGG pathway annotation

Further, the function of the DEGs were explored by GO and KEGG enrichment analysis using online tools of DAVID (Database forAnnotation, Visualization and Integrated Discovery). The criteria were set to default. GO enrichment analysis of DEGs with diverged expression patterns between gemcitabine and dasatinib-resistant cell lines was performed by using the ClueGO plugin (version 2.3.3) of cytoscape (version 3.4).

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Table 1. The identified 4 common DEGs in GSE35141 and GSE59357.

<table>
<thead>
<tr>
<th>Gene</th>
<th>GSE35141</th>
<th>GSE59357</th>
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<tr>
<td></td>
<td>Fold change</td>
<td>adj-p-value</td>
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<tr>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>ELF5</td>
<td>2.1150437</td>
<td>0.03237</td>
</tr>
</tbody>
</table>

Figure 1. Venn diagram of the DEGs in GSE35141 and GSE59357.

173, 502 and 608 genes in miRTarBase, TargetScan, and MicroCosm database, respectively, and each target should exist in at least two databases. Sixteen genes out of the target genes were also identified in the DEGs of gemcitabine-resistant cell lines. Then the miRNA-mRNA pairings were constructed using Cytoscape (Figure 5). Here we found that numerous genes can be regulated by multiple miRNAs such as NOSTRIN which is the target of hsa-let-7b-5p, hsa-let-7e-5p, hsa-let-7c-5p and hsa-let-7d-5p.

DISCUSSION

In the present study, we conducted a drug resistant mechanism comparison of gemcitabine and dasatinib by a comprehensive analysis of differential gene and microRNA expression profiles. First, by analyzing the DEGs in two datasets, 4 common DEGs, MSLN, ADAM19, MECOM, and ELF5, were identified, and all samples can be clearly classified into different subgroups according to the common DEGs. Then, GO annotation and KEGG pathway enrichment analysis exhibited that the gemcitabine and dasatinib resistance may be associated with metabolism and cancer related pathways, respectively, except the common drug metabolism. Finally, the miRNA-mRNA regulatory network on gemcitabine resistance was constructed based on the differentially expressed genes and six documented miRNAs.

The different mechanism of gemcitabine and dasatinib resistance in pancreatic cancer was unveiled in DEGs. The MSLN gene encodes a precursor protein of shed protein, megakaryocyte potentiating factor (MPF), and membrane bound protein, mesothelin [35], and its overexpression promotes autocrine IL-6/sIL-6R trans-signal- ing to stimulate pancreatic cancer cell proliferation [36]. ADAM19 (a disintegrin and metalloproteinase 19 or adanalysis 19) is a cell surface glycoprotein, endopeptidase, that cleaves extracellular matrix proteins and sheds growth factors and cytokines such as tumor necrosis factor (TNF)-α and TNF-related activation-induced cytokine [37]. The silencing of ADAM19 inhibited cell proliferation, migration, and invasion in non-
Figure 2. Heat map showing the expression pattern of 4 common DEGs in drug-resistant and drug-sensitive cell lines. The x-axis represents samples and y-axis represents genes. The bar on the top indicates the type of sample using red (drug-resistant) and blue (drug-sensitive).

Figure 3. KEGG enrichment results for DEGs of gemcitabine-resistant and dasatinib-resistant cell lines. The dot size represents fold enrichment and the color corresponds to adj-p-value.
small-cell lung cancer, retinoblastoma, and colon cancer [38-40], implying ADAM19 may have similar influence in pancreatic cancer. Previous studies on MSLN and ADAM19 were consistent with our results, which exhibited MSLN and ADAM19 were down-regulated in both datasets. Meanwhile, it also confirmed that MSLN and ADAM19 play the same roles in gemcitabine and dasatinib treatment of pancreatic cancer.

According to our results, MECOM and ELF5 were up-regulated in gemcitabine-resistant cell lines and down-regulated in dasatinib-resistant cell lines, suggesting these genes play a specific role in gemcitabine and dasatinib resistance. The MECOM, also called EVI1 (ectotropic viral integration site 1) gene codes for a zinc finger transcriptional factor that plays an important role in normal development and in oncogenesis [41]. MECOM depletion caused remarkable inhibition of cell growth and migration in pancreatic cancer cells [42], explaining part of gemcitabine resistance. E74-like factor 5 (ELF5) is a transcriptional factor, which regulates diverse cellular biology including later stages of terminal differentiation of keratinocytes [43], trophoblast differentiation [44] and epithelial-mesenchymal transition in tumor cells [45]. Knockdown of ELF5 by shRNA led to increased cell viability in prostate cancer [46], suggesting ELF5 may possess an equal function in pancreatic cancer and its downregulation is involved in dasatinib resistance.

KEGG pathway annotation showed that cytochrome P450 participated in the drug and xenobiotics metabolism pathway of gemcitabine-resistant and dasatinib-resistant cell lines. Cytochrome P450 (CYP) is a super-
family of heme-containing enzymes, which are responsible for the metabolism of drug and xenobiotic compounds in human tissues [47], and inhibitors of CYP enzymes may potentially serve as anticancer agents [48]. This may explain the common molecular mechanism of gemcitabine and dasatinib treatment. Meanwhile, additional metabolism pathways, like tyrosine metabolism and retinol metabolism, indicate that gemcitabine may be involved in the synthesis metabolism of materials in pancreatic cancer, and cancer related pathways enriched in dasatinib resistant cell lines manifest that dasatinib may directly or indirectly participate in development of pancreatic cancer.

The drug resistant mechanism of gemcitabine in pancreatic cancer was further uncovered by miRNA-mRNA regulation network. Regulation network showed that 16 genes can be regulated by multiple miRNAs. For instance, the nitric oxide synthase traffic inducer (NOSTRIN) was the target genes of hsa-let-7b-5p, hsa-let-7e-5p, hsa-let-7c-5p, and hsa-let-7d-5p. NOSTRIN encodes a member of F-BAR proteins, which triggers the translocation of endothelial NO synthase from the plasma membrane to vesicle-like subcellular structures, thereby attenuating ENOS-dependent NO production [49], and NO is associated with the development and progression of pancreatic cancer [50,51]. The overexpression of NOSTRIN suppressed migration and invasion of pancreatic cancer cells and promoted sensitivity to gemcitabine [52]. Apart from NOSTRIN, several genes, MOB1B, DUSP23, PARM1, RIBC1 and others, were also identified in gemcitabine-resistant cell lines.

CONCLUSION

Collectively, our study investigated the difference of gemcitabine resistance in pancreatic cancer by mechanism comparison via the mRNA and miRNA expression profiles. These findings support strategies to target molecules and relevant pathways for improving the efficacy of chemotherapy in pancreatic cancer patients.
Authors’ Contribution:
All authors participated in data collection, data analysis. The manuscript was written by Bicheng Chen.

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Declaration of Interest:
The authors declare that there are no conflicts of interest.

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