Evaluation of K-ras and p53 expression in pancreatic adenocarcinoma using the cancer genome atlas

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Abstract

Genetic alterations in K-ras and p53 are thought to be critical in pancreatic cancer development and progression. However, K-ras and p53 expression in pancreatic adenocarcinoma have not been systematically examined in The Cancer Genome Atlas (TCGA) Data Portal. Information regarding K-ras and p53 alterations, mRNA expression data, and protein/protein phosphorylation abundance was retrieved from The Cancer Genome Atlas (TCGA) databases, and analyses were performed by the cBioPortal for Cancer Genomics. The mutual exclusivity analysis showed that events in K-ras and p53 were likely to co-occur in pancreatic adenocarcinoma (Log odds ratio = 1.599, P = 0.006). The graphical summary of the mutations showed that there were hotspots for protein activation. In the network analysis, no solid association between K-ras and p53 was observed in pancreatic adenocarcinoma. In the survival analysis, neither K-ras nor p53 were associated with both survival events. As in the data mining study in the TCGA databases, our study provides a new perspective to understand the genetic features of K-ras and p53 in pancreatic adenocarcinoma.

Introduction

Pancreatic cancer (PC) happens when pancreatic cells start to proliferate without control and form a mass. As the most frequent type of PC, pancreatic adenocarcinoma takes the largest proportion 85% of all cases, which is usually equivalent to the expression "pancreatic cancer". The global annual incidence rate for PC is about 8/100,000 persons.[1] Recently it is reported that the treatment is promising in the near future.[2] Furthermore, as the overall 5-year survival rate was only 5%, PC remains one of the most lethal cancers [3,4]. Meanwhile, an overall reduction in cancer-related mortality has occurred among lung, breast, colorectal and prostate cancer over the last few decades.[5]

Nowadays, how to improve the prevention and treatment of pancreatic adenocarcinoma remains a critical issue. Knowing PC’s molecular biology benefits the development of new approaches to advances in its clinical management. The occurrence and evolution of pancreatic adenocarcinoma involves multiple genetic alterations. Genetic alterations K-ras and p53
are considered key to both pancreatic cancer progression.\[6\] The K-ras proto-oncogene encodes a small 21 kDa protein (p21ras). This protein possesses the activity of GTPase, enabling inactivation of cancer cell.\[7\] It is common that genetic deletions or mutations of the p53 tumor suppressor gene exists in pancreatic adenocarcinoma (40–87% of cases). Activation of the p53 tumor suppressor gene causes cell cycle arrest by encoding a nuclear phosphoprotein and binding directly to DNA.\[8\]

Previous studies tried to explore the correlation of these genes’ alterations and clinical features and survival data.\[9–19\] The conclusions on K-ras, p53 mutations and prognosis is inconsistent. Furthermore, few studies have explored the relationship of mutations in K-ras and p53 together. It is important for researchers to make it clear that K-ras and p53 are on different pathways, or are part of a common pathway of inactivation of pancreatic adenocarcinoma. The Cancer Genome Atlas (TCGA) Data Portal contains information on DNA, RNA, proteins and survival status in various cancers.\[20,21\] However, the relationship between K-ras and p53 expression in pancreatic adenocarcinoma was not clear in genes’ alterations and clinical outcomes. This study aimed to assess the genetic alterations of K-ras and p53 and their relationship in pancreatic adenocarcinoma in TCGA data sets. Additionally, we correlate these changes with clinical outcomes.

Materials and methods

Gene expression databases

Information regarding K-ras and p53 alterations, mRNA expression, and protein in pancreatic adenocarcinoma can be obtained from The Cancer Genome Atlas (TCGA) database, an open access database publicly available at http://www.cbioportal.org.\[21,22\]

Before visualizing and analyzing genomic alterations of K-ras and p53 in the TCGA data on pancreatic adenocarcinoma, we selected several options in the web interface of cBioPortal. We selected cancer study “pancreatic adenocarcinoma (TCGA)” and data type priority “Mutation and CNA (DNA copy-number alterations).” For the gene set of interest, terms of “K-ras p53” were entered into the input box. No statements of approval or informed consent were required for our study as we obtained data from an open access database.

Genomic alterations summary

An OncoPrint was used to summarize the genomic alterations of K-ras and p53 through tumor samples. On the table, rows represented genes and columns represented samples. Genomic alterations including mutations, CNA (amplifications and homozygous deletions), and changes in gene expression were summarized by glyphs and color coding. This was a preliminary way to know about the different gene signaling in pancreatic adenocarcinoma. In this section, mutual exclusivity and co-occurrence between K-ras and p53 were analyzed. In mutually exclusive, gene-related events associated with a particular cancer are often mutually exclusive in a group of tumors—that is, only one genetic event is likely to exist in each cancer sample. The other situation is the co-occurrence that multiple genes are altered in the same cancer sample.\[21\] This was a preliminary way to gather information about the different gene signaling in pancreatic adenocarcinoma.

Mutations in K-ras and p53 in pancreatic adenocarcinoma

From the Mutations tab, the location and frequency of all mutations in Pfam protein domains were given. The gray bars meant the whole lengths of the K-ras and p53 proteins and the bottom of each gray bar displayed the number of amino acids. Protein domains were showed by
the green, red, and blue boxes. The locations and frequencies of genes were showed by the lines and dots. Red represented nonsense or frameshift mutations, green represented missense mutations, and black represented in-frame deletions.[23]

Network analysis
The network includes all neighbors of K-ras and p53.[21] Only neighbor genes with the highest alteration frequency (only the 50 neighbors were presented if more than 50 neighbor genes existed) in addition to the query genes were shown. Color coded edges were used to highlight the frequency of gene alteration.

Survival analysis
From survival analysis, overall survival and disease-free survival differences were compared between samples with more than or equal to one alteration of query gene(s) and samples without alteration. This was done if the survival data were available.

Statistics
For correlation analysis, a scatter plot of mRNA expression versus copy-number status or the protein level versus mRNA option in each sample was presented. For survival analysis, Kaplan-Meier plots with a logrank test were performed to compare the overall survival and the disease-free survival of pancreatic adenocarcinoma with at least one alteration or without alteration in query gene(s). Samples with over-expression were identified by a threshold of \( Z > 2 \) (mean expression over 2 SDs). The \( \alpha \) level was set at 0.05. All of the analyses mentioned above were performed in cBioPortal. For the details of original data, see S1 and S2 Datasets.

Results
Genomic alterations summary
From the OncoPrint (Fig 1), 140 (94%) out of 149 cases had an alteration in no less than one of the two genes. Specifically, 91% cases had an alteration in K-ras, most of which were missense mutations. Others included a few amplifications and deep deletions. 70% of the cases had an alteration in p53, consisting mainly of missense mutation and truncating mutation. The mutual exclusivity analysis showed that events in K-ras and p53 were likely to co-occur in pancreatic adenocarcinoma (Log odds ratio = 1.599, \( P = 0.006 \)).

This result illustrated that the gene signaling in pancreatic adenocarcinoma was mediated by the activation of K-ras through missense mutations, or by the inactivation of p53 through truncating mutation and missense mutation.

Mutations in K-ras and p53 in pancreatic adenocarcinoma
The graphical summary of the mutations showed that there were 139 K-ras nonsynonymous mutations in pancreatic adenocarcinoma samples, and 130 of them were G12C/D/R/S/V in the kinase domain (Fig 2). There were 105 P53 nonsynonymous mutations in pancreatic adenocarcinoma samples.
adenocarcinoma samples, 9 of them being R248L/Q/W in the kinase domain (Fig 3), illustrating that these were hotspots for protein activation.

Network analysis

Network view of the K-ras and p53 neighborhood in pancreatic adenocarcinoma was presented in Fig 4. The query genes, K-ras and p53 were depicted with a thick border and neighbor genes were distributing around them. Interestingly, no solid association between K-ras and p53 was observed in pancreatic adenocarcinoma.

Survival analysis

Kaplan-Meier plots was used to compare overall survival and disease free survival in pancreatic adenocarcinoma cases with or without K-ras and p53 over-expression. For the overall survival analysis, mutations simultaneously in p53 and K-ras were found in 54.73% (81/148) of cases and were not related to decreased overall survival (19.65 months versus 19.94 months for those without both mutations, \( P = 0.473 \)) (Fig 5). For the disease free survival analysis, mutations in K-ras and p53 were simultaneously found in 61.74% (71/115) of cases and were not associated with decreased disease free survival (14.45 months versus 16.75 months for those without both mutations, \( P = 0.157 \)) (Fig 6). Similarly, neither K-ras nor p53 were associated with both survival events (Figures not presented).

Discussion

In the current study, we have used The cBioPortal for Cancer Genomics as a tool for exploring, visualizing, and analyzing the biological and clinical features of p53 and K-ras alterations in pancreatic adenocarcinoma cases from TCGA databases. As far as we know, this is the first data mining study to explore the relationship between alterations of K-ras and p53 and patient prognosis in TCGA databases.
Fig 4. Network analysis of the K-ras and p53 neighborhood in pancreatic adenocarcinoma.
https://doi.org/10.1371/journal.pone.0181532.g004

Fig 5. Overall survival analysis.
https://doi.org/10.1371/journal.pone.0181532.g005
Similar to previous reports, \( K-ras \) has high alteration frequency of 47–100% in pancreatic adenocarcinoma.[24–26] \( K-ras \) alterations are also found in benign pancreatic disease and at all stages of pancreatic ductal anaplasia.[27] \( K-ras \) alterations are thought to be an early event in pancreatic carcinogenesis. \( p53 \) has the function of enhancing G1 arrest for DNA damage and reducing damaged DNA to be replicated. In our study, \( p53 \) alterations were found in 70% cases of pancreatic adenocarcinoma, a proportion similar to previous literature which has reported a range of 40–87%.[28,29]

Mutual exclusivity is inferred by a statistical analysis which can reveal a rough relationship between different genes. The network analysis can tell us more about the mechanisms of interaction among the different genes. Our study observed that \( K-ras \) and \( p53 \) were likely to co-occur, and that network analysis found no solid association between them in pancreatic adenocarcinoma. “No solid association” does not mean no association. It can be seen from Fig 4 that \( K-ras \) and \( p53 \) have some association, but that this association is “no solid” when compared with the other 48 neighbor genes. This suggests that \( K-ras \) and \( p53 \) alterations mostly coexist in pancreatic adenocarcinoma, but alterations in these genes are on independent pathways to pancreatic adenocarcinoma and are not in a common way of cumulative gene variation. This is consistent with a previous cohort study.[8] This data mining study cannot draw an initiating cause of pancreatic adenocarcinoma, as only prospective experimental studies can confirm this hypothesis. This is one limitation for our study.

For survival analysis, no association was observed between the two genes and survival events in our study. Coincidentally, most papers published in recent years have failed to prove the association of these two genes’ molecular changes and patient prognosis. Kawesha et al. [30] found that \( K-ras \) mutation alone was not related with survival, but significant differences in survival might exist due to the type of \( K-ras \) mutation. Shin et al.[6] verified that \( K-ras \) mutation alone was related with patients’ survival, and that GAT subtype had the closest relationship with survival among the Korean population. These findings suggest that \( K-ras \) mutation has a different prognosis value of pancreatic adenocarcinoma in different geographic locations and populations. Additionally, the majority of recent published studies have reported no association between \( p53 \) alterations and patient survival,[31–34] while some other studies have
shown that mutations of p53 gene could reduce postoperative survival.[35,36] From our results, mutations in \textit{K-ras} and \textit{p53} were not related with decreased overall survival and disease-free survival. This was due to a small sample size which was not sufficient to confirm explanatory power.

\section*{Conclusions}

In the current study, we used The cBioPortal for Cancer Genomics as a tool for exploring, visualizing, and analyzing the biological and clinical features of \textit{p53} and \textit{K-ras} alterations in pancreatic adenocarcinoma cases from TCGA databases. As far as we know, this is the first data mining study to explore the relationship between alterations of \textit{K-ras} and \textit{p53} and patient prognosis in TCGA databases. Many findings in our research are consistent with previous reports. Interestingly, our study observed that \textit{K-ras} and \textit{p53} alterations mostly coexist in pancreatic adenocarcinoma. Alterations in these genes are on independent pathways to pancreatic adenocarcinoma and are not in a common way of cumulative gene variation. Though neither \textit{K-ras} nor \textit{p53} were associated with both survival events (overall survival and disease free survival) in our study, it provides us a new perspective to simultaneously perform the analysis of genetic alterations and clinical features via data mining.

\section*{Supporting information}

\begin{itemize}
\item \textbf{S1 Dataset.} Mutations. (TXT)
\item \textbf{S2 Dataset.} Putative copy-number alterations from GISTIC. (TXT)
\end{itemize}

\section*{Acknowledgments}

This research was supported by Guangdong Natural Science Foundation (project no. 2016A030310290) and The Specific Research Fund for TCM Science and Technology of Guangdong Provincial Hospital of Chinese Medicine (No. YN2015QN20). The funder had no further role in study design, data collection, analysis and interpretation of data, writing of the report or the decision to submit the paper for publication.

\section*{Author Contributions}

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References

1. Raimondi S, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: an overview. Nat Rev Gastroenterol Hepatol. 2009; 6(12):699–708. https://doi.org/10.1038/nrgastro.2009.177 PMID: 19806144

2. Bosetti C, Bertuccio P, Negri E, La Vecchia C, Zeeegers MP, Boffetta P. Pancreatic cancer: overview of descriptive epidemiology. Mol Carcinog. 2012; 51(1):3–13. https://doi.org/10.1002/mc.22162227

3. Siegel R, Ward E, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011; 61(4):212–236. https://doi.org/10.3322/caac.20121 PMID: 21685461

4. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015; 65(2):87–108. https://doi.org/10.3322/caac.21262 PMID: 25651787

5. Malvezzi M, Bertuccio P, Levi F, La Vecchia C, Negri E. European cancer mortality predictions for the year 2013. ANN ONCOL. 2013; 24(3):792–800. https://doi.org/10.1093/annonc/mdt010 PMID: 23402763

6. Shin SH, Kim SC, Hong SM, Kim YH, Song KB, Park KM, et al. Genetic alterations of K-ras, p53, c-erbB-2, and DPC4 in pancreatic ductal adenocarcinoma and their correlation with patient survival. PANCREAS. 2013; 42(2):216–222. https://doi.org/10.1097/MPA.0b013e31825b6ab0 PMID: 23944532

7. Ines C, Donia O, Rahma B, Ben AA, Sameh A, Khalfallah T, et al. Implication of K-ras and p53 in colorectal cancer carcinogenesis in Tunisian population cohort. Tumour Biol. 2014; 35(7):7163–7175. https://doi.org/10.1007/s13277-014-1874-4 PMID: 24763823

8. Conlin A, Smith G, Carey FA, Wolf CR, Steele RJ. The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. GUT. 2005; 54(9):1283–1286. https://doi.org/10.1136/gut.2005.066514 PMID: 15843421

9. Zhao H, Wang Q, Wang X, Zhu H, Zhang S, Wang W, et al. Correlation Between RAB27B and p53 Expression and Overall Survival in Pancreatic ductal adenocarcinoma. PANCREAS. 2016; 45(2):204–210. https://doi.org/10.1097/MPA.000000000000453 PMID: 26418905

10. Singh N, Gupta S, Pandey RM, Chauhan SS, Saraya A. High levels of cell-free circulating nucleic acids in pancreatic cancer are associated with vascular encasement, metastasis and poor survival. CANCER INVEST. 2015; 33(3):78–85. https://doi.org/10.3109/07357907.2014.1001894 PMID: 25647443

11. Qin R, Smyrk TC, Reed NR, Schmidt RL, Schneidendorfer T, Chari ST, et al. Combining clinicopathological predictors and molecular biomarkers in the oncogenic K-RAS/K67/HIF-1alpha pathway to predict survival in resectable pancreatic cancer. Br J Cancer. 2015; 112(3):514–522. https://doi.org/10.1038/bjc.2014.659 PMID: 25584484

12. Chen R, Dawson DW, Pan S, Ottenhof NA, de Wilde RF, Wolfgang CL, et al. Proteins associated with pancreatic cancer survival in patients with resectable pancreatic ductal adenocarcinoma. LAB INVEST. 2015; 95(1):43–55. https://doi.org/10.1038/labinvest.2014.128 PMID: 25347153

13. Ormanns S, Siveke JT, Heinemann V, Haas M, Sipos B, Schlitter AM, et al. pERK, pAKT and p53 tissue biomarkers in erlotinib-treated patients with advanced pancreatic cancer: a translational subgroup analysis from AIO-PK0104. BMC CANCER. 2014; 14:624. https://doi.org/10.1186/1471-2407-14-624 PMID: 25164437

14. Sheng W, Dong M, Zhou J, Li X, Liu Q, Dong Q, et al. The clinicopathological significance and relationship of Gli1, MDM2 and p53 expression in resectable pancreatic cancer. HISTOPATHOLOGY. 2014; 64(4):523–535. https://doi.org/10.1111/his.12273 PMID: 24289472

15. Yuan QY, Gu YP, Wang CJ, Zhang H, Wang XP. Identification of dysregulated pathways associated with pancreatic cancer by survival analysis. MOL MED REP. 2015; 11(1):277–282. https://doi.org/10.3892/mmr.2014.2693 PMID: 25333741

16. Castells A, Puig P, Mora J, Boadas J, Boix L, Urgell E, et al, Fernandez-Cruz L, Navarro S and Farre A. K-ras mutations in DNA extracted from the plasma of patients with pancreatic carcinoma: diagnostic
utility and prognostic significance. J CLIN ONCOL. 1999; 17(2):578–584. https://doi.org/10.1200/JCO.1999.17.2.578 PMID: 10080602

20. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. CANCER DISCOV. 2012; 2(5):401–404. https://doi.org/10.1158/2159-8290.CD-12-0095 PMID: 22588877

21. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. SCI SIGNAL. 2013; 6(269):1.

22. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. CANCER DISCOV. 2012; 2(5):401–404. https://doi.org/10.1158/2159-8290.CD-12-0095 PMID: 22588877

23. Fang B, Mehran RJ, Heymach JV, Swisher SG. Predictive biomarkers in precision medicine and drug development against lung cancer. Chin J Cancer. 2015; 34(7):295–309. https://doi.org/10.1186/s40880-015-0028-4 PMID: 26134262

24. Smit VT, Boot AJ, Smits AM, Fleuren GJ, Cornelisse CJ, Bos JL. KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. NUCLEIC ACIDS RES. 1988; 16(16):7773–7782. PMID: 3047672

25. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. NAT REV CANCER. 2003; 3(6):459–465. https://doi.org/10.1038/nrc1097 PMID: 12778136

26. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucchini M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. CELL. 1988; 53(4):549–554. PMID: 2453289

27. Shin SH, Kim SC, Hong SM, Kim YH, Song KB, Park KM, et al. Genetic alterations of K-ras, p53, c-erbB-2, and DPC4 in pancreatic ductal adenocarcinoma and their correlation with patient survival. PANCREAS. 2013; 42(2):216–222. https://doi.org/10.1097/MPA.0b013e31825b6ab0 PMID: 23344532

28. Biankin AV, Kench JG, Morey AL, Lee CS, Biankin SA, Head DR, et al. Overexpression of p21(WAF1/CIP1) is an early event in the development of pancreatic intraepithelial neoplasia. CANCER RES. 2001; 61(24):8830–8837. PMID: 11751405

29. Boschman CR, Stryker S, Reddy JK, Rao MS. Expression of p53 protein in precursor lesions and adenocarcinoma of human pancreas. AM J PATHOL. 1994; 145(6):1291–1295. PMID: 7992834

30. Kawesha A, Ghaneh P, Andreu-Sandberg A, Ograed D, Skar R, Dawiskiba S, et al. K-ras oncogene subtype mutations are associated with survival but not expression of p53, p16(INK4A), p21(WAF-1), cyclin D1, erbB-2 and erbB-3 in resected pancreatic ductal adenocarcinoma. INT J CANCER. 2000; 89(6):469–474. PMID: 11102889

31. Salek C, Minarikova P, Benesova L, Nosek V, Strnad R, Zavoral M, et al. Mutation status of K-ras, p53 and allelic losses at 9p and 18q are not prognostic markers in patients with pancreatic cancer. ANTI-CANCER RES. 2009; 29(5):1803–1810. PMID: 19443408

32. Talar-Wojnarowska R, Gasiorowska A, Smolarz B, Romanowicz-Makowska H, Strzelczyk J, Janiak A, et al. Comparative evaluation of p53 mutation in pancreatic adenocarcinoma and chronic pancreatitis. Hepatogastroenterology. 2006; 53(70):608–612. PMID: 16995472

33. Jeong J, Park YN, Park JS, Yoon DS, Chi HS, Kim BR. Clinical significance of p16 protein expression loss and aberrant p53 protein expression in pancreatic cancer. YONSEI MED J. 2005; 46(4):519–525. https://doi.org/10.3349/ymj.2005.46.4.519 PMID: 16127777

34. Dong M, Nio Y, Yamashita K, Toga T, Yue L, Harada T, p53 alteration is not an independent prognostic indicator, but affects the efficacy of adjuvant chemotherapy in human pancreatic cancer. J SURG ONCOL. 2003; 82(2):111–120. https://doi.org/10.1002/jso.10186 PMID: 12561067

35. Yeo CJ, Cameron JL. Prognostic factors in ductal pancreatic cancer. Langenbecks Arch Surg. 1998; 383(2):129–133. PMID: 9641885

36. Nakamori S, Yoshima K, Murakami Y, Ishikawa O, Ohigashi H, Imaoka S, et al. Association of p53 gene mutations with short survival in pancreatic adenocarcinoma. Jpn J Cancer Res. 1995; 86(2):174–181. PMID: 7730141