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Investigation of the resistance of immortalized primary pancreatic carcinoma cell lines as a predictor of the effectiveness of adjuvant therapy

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Pancreatic cancer is a highly lethal disease that challenges current treatment strategies in clinical work. Among these, chemotherapy remains a reliable strategy. Commercialized cell lines are not suitable for oncological research because they lose some of the characteristics of cancer. In our study, we hypothesized that immortalized primary pancreatic cancer cells can not only retain their initial traits but also retain their high proliferative capacities.

To verify our hypothesis, primary pancreatic cancer cell lines (MaPac107, PaCaDD159, and PaCaDD165 were immortalized. We then compared the differences between primary (Pri-) and immortalized (Im-) cells via their characteristics of morphology and growth, drug resistance, redox-regulatory situation, and bioinformatics analysis. No obvious morphological differences were observed between primary and immortalized cells. In addition to PaCaDD159, Im-MaPac107 and Im-PaCaDD165 grew faster than their counterparts in 2D and 3D models after seven days of observation. Furthermore, both Pri-PaCaDD159 and Im-PaCaDD159 failed to form spheroids. After the first screening of candidate chemotherapeutic drugs using MTT assays on primary cells, gemcitabine and oxaliplatin were used to compare the differences in drug resistance between primary and immortalized cells. The sigmoidal fitting of the dose-response curves of Pri-MaPac107, Im-MaPac107, Pri-PaCaDD165, and Im-PaCaDD165 basically matched our expectations after treatment with these two chemotherapeutic drugs for 48 and 72 h, except for Pri-PaCaDD159 and Im-PaCaDD159. Subsequently, we further evaluated the redox-regulatory situation of primary and immortalized cells expressing Grx1-roGFP3+ at three different concentrations based on the serum peak concentration and IC_{50} values of gemcitabine and oxaliplatin. Fewer differences were observed between Pri-PaCaDD165 and Im-PaCaDD165, Pri-PaCaDD159, and Im-PaCaDD159 in the 2D model as compared with those of Pri-MaPac107 and Im-MaPac107. Moreover, fewer differences were observed between Pri-PaCaDD165 and Im-PaCaDD165, Pri-PaCaDD159, and Im-PaCaDD159 in the 2D model after treatment with oxaliplatin for 48 h as compared with gemcitabine. Interestingly, the difference between Pri-PaCaDD165 and Im-PaCaDD165 in the 2D model after treatment with oxaliplatin for 48 h was different from that in the 3D model. To explain these findings, we obtained RNA sequencing data from all cell lines (to compare primary and immortalized cells). After analysis of differentially expressed genes and pathways from KEGG database, we found that some pathways in the KEGG database may explain the differences of proliferation of primary and immortalized pancreatic cancer cells, which contained PI3K-Akt, NF-kappa B, EGFR tyrosine kinase inhibitor resistance, Hippo, MAPK, Hedgehog, JAK-STAT, Ras, TGF-beta, Toll-like receptor and Chemokine pathway. Then, we found that some differentially expressed genes may elucidate the drug resistance of gemcitabine and oxaliplatin. For MaPac107, SLC28A3, ABCG2, HCP5, and KDR were found to relate to gemcitabine; ABCG2, PTGS2, PARD3B, and CXCL10 were found to relate to oxaliplatin. TNF and CXCL10 were correlated with gemcitabine and oxaliplatin respectively in PaCaDD159. Two de-regulated genes related to gemcitabine and oxaliplatin were expressed by PaCaDD165: IGF2, and DKK1. Besides, some pathways in the KEGG database may also affect the drug resistance, including NF-kappa B, EGFR tyrosine kinase inhibitor resistance, PI3K-Akt, AMPK, TGF-beta, Necroptosis, Nucleotide excision repair, Hippo, JAK-STAT, MAPK and FoxO pathway. As for the evaluation of redox-regulatory situation of primary and immortalized cells expressing Grx1-roGFP3+, cluster analysis correlation and 3D PCA map found that primary and immortalized MaPac107 were less correlated with each other. Furthermore, some pathways like JAK-STAT, PI3K/Akt, NF- kappa B, Lysosome, FoxO, AMPK, and MAPK in the KEGG database may be involved in ROS production or ROS-induced apoptosis of pancreatic cancer cells. Importantly, aforementioned genes

and pathways are reported by published papers and the adjusted p-value of these pathways are less than 0.05.

In conclusion, the immortalization skills used in our study cannot conserve the characteristics of primary pancreatic cancer cell lines but also overcome the shortcomings to some extent, which is an effective tool for prioritizing cancer research.