Distribution of Characteristic Mutations in Native Ductal Adenocarcinoma of the Pancreas and Pancreatic Cancer Cell Lines

Wagenhäuser MU*, Rückert F*, Niedergethmann M2, Grützmann R, and Saeger HD

Abstract

Introduction: Although oncological treatment of pancreatic adenocarcinoma (PDAC) has improved within the last few years, PDAC continues to have a very low 5-year survival rate. Basic research is needed to improve and develop new therapeutic strategies. Pancreatic tumour cell lines (CLs) are a prerequisite for such basic research. However, the isolation of CLs is challenging. Outgrowth of cells is only achieved in a small proportion of tumour samples, although the reason for this poor growth remains unknown.

Material and methods: To assess whether mutations play a role during the isolation process, we compared the genetic mutations of KRAS, p53, p16INK4A and SMAD4/DPC4 in CLs and native pancreatic tumour tissue (NT). We conducted a comprehensive literature search to assess the distribution of mutations in NT and CLs. Statistical analyses was performed using a chi-squared test with a two-tailed non-directional Fisher-Irwin test (Fisher’s exact test). The difference in the mutational rate between CLs and NT was considered statistically significant if p < 0.05.

Results: The mutations rates of KRAS and SMAD4/DPC4 in CLs and in NT were comparable. However, we observed a higher mutation rate of p53 in CLs. The same holds true for mutations of p16INK4A which were observed more frequently in CLs. Both p53 and p16INK4A are tumour suppressor genes that have a role in cell cycle regulation. Therefore, mutations in these genes might promote invivo tumour cell growth. Further experimental studies could provide evidence to support or refute this hypothesis.

Keywords
Pancreatic cancer; Pancreatic carcinoma; Pancreatic neoplasms; Cell line

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive carcinoma, and its incidence nearly equals its mortality [1]. Although remarkable achievements in the treatment of this tumour have been made within the few last years, especially concerning surgical management and perioperative care, PDAC continues to have a very poor prognosis, with an overall 5-year survival rate of less than 5% [2-4]. At the time of diagnosis, up to 80-90% of PDAC patients suffer from advanced disease and are therefore not eligible for surgical treatment [5,6]. To improve therapy, it is necessary to better understand the tumour pathophysiology. For this purpose, primary pancreatic carcinoma cell lines (CLs) are a prerequisite. The isolation of such cell lines in PDAC is an arduous process and has a success rate of only 3-8% [7]. The reason for this infrequent outgrowth of cells is unclear. However, our own experimental data suggest that intracellular mutations might play a role in successful out growth [8]. Numerous studies have observed a relatively unique molecular fingerprint in PDAC that comprising frequent alterations of four genes: KRAS, p53, p16INK4A and SMAD4 [9].

KRAS is an oncogene encoding a 21 kDa membrane protein. KRAS exhibits mutations in a high percentage of PDACs and appears to correlate with a poor prognosis [10]. Physiologically, KRAS exhibits pleiotropic effects on different cellular functions, including proliferation and migration. Mutations prevent the hydrolysis of GTP and lead to the activation of several intracellular pathways [11].

The transcription factor p53 is a tumour suppressor that plays an important role in the regulation of apoptosis and cell cycle arrest. Mutations have been reported in PDAC, although this mutation can also rarely be observed in chronic pancreatitis [11,12]. Mutated p53 accumulates in the cell and can be detected by immune histochemistry. Although p53 mutations play a major role in many tumour entities, its prognostic value in PDAC remains uncertain [13].

SMAD4/DPC4 is another important oncogene involved in TGF-β signalling [14]. SMAD4/DPC4 is located on chromosome 18q21.1 [15]. The inactivation of SMAD4/DPC4 by mutation leads to the inhibition of the TGF-β signalling pathway [14,16]. As TGF-β is an inhibitor of cellular proliferation in most known cell types, it gets obvious that mutations in the oncogene SMAD4/DPC4 may lead to unregulated cell proliferation [17].

The p16INK4A protein regulates the cell cycle by inhibition of phosphorylation of the retinoblastoma protein [18]. Low expression levels of p16INK4A are correlated with advanced disease, a high risk of early metastases and poor prognosis [19].

The aim of the present review was to analyse the frequency and distribution of mutations in the above-mentioned genes in NT and CLs. In doing so, we attempted to assess the importance of these genes in the process of cell line isolation.

Materials and Methods

A literature search was performed to analyse mutations in CLs described within the last 25 years. The keywords for this search included the name of the cell line in conjunction with the terms “KRAS”, “p53”, “SMAD4/DPC4” and “p16INK4A”. We only included peer-reviewed studies that were published after 1990. The distribution of these mutations in NT was also assessed by means of a literature search. For this purpose, we used the following keywords: “pancreatic cancer”, “KRAS”, “p53”, “SMAD4/DPC4”, “p16INK4A” and “mutation rate”. We included peer-reviewed studies that considered the
mutational status in fine-needle aspirations and surgical specimens. The patient cohort of the studies had to include at least 20 patients. We included studies that were published between 1990 and 2011.

The results are reported as frequency counts and percentages for the mutational status of each individual gene in CLs and NT. For the statistical analyses, we used a chi-squared test with a two-tailed non-directional Fisher- Irwin test (Fisher’s exact test). The statistical analyses were performed using SPSS 9.0 for Windows. The difference in the mutational rate between CLs and NT was considered statistically significant if \( p < 0.05 \).

**Results**

As a first step, we analysed the mutational status of the CLs. The search resulted in the identification of 71 published CLs. 60 of the cell lines were derived from explant cultures, 6 were derived from xenografts, 2 were derived from pleural effusions, and 3 were derived from malign as cited. Table 1 presents an overview of all 71 CLs. We were able to find data on the KRAS mutational status in 47 of the 71 CLs. 36(76.6%) of those cell lines exhibited mutations of the KRAS oncogene, and 11 (23.4%) CLs expressed wild type KRAS. 45 of the CLs (63.3%) were analysed with respect to the mutational status of p53. Mutations of p53 were observed in 33 of those CLs (73.3%). 12CLs expressed wild type p53 (26.6%). All of those cell lines were derived from explant cultures. No data on the mutational status of 26 of the CLs were available. Data on the mutation status of p16\(^{INKA} \) could only be obtained in 17 (23.9%) of the CLs. Mutations of p16\(^{INKA} \) were observed in 10 of those cell lines (58.8%). 7(41.1%) cell lines expressed wild type p16\(^{INKA} \). Again, all of those CLs derived from explant cultures. Mutations of p16\(^{INKA} \) were not investigated in 54 CLs, so their mutational status remains unclear. Data on the mutational status of SMAD4/DPC4 were published for 28 (39.4%) of the CLs. 22/28 of all CLs (78.6%) exhibited a mutation in that gene. So all of those cell lines were derived from explant cultures. The literature search did not identify data on the mutational status of any gene in 43 of the investigated CLs.

As a second step, we searched and analysed publications concerning the mutational status of NTs. We identified 6 studies that fulfilled the predetermined criteria. 4 studies [44-47] assessed the mutation status of KRAS. Altogether, 273 patient samples were analysed. Of those, 191 (70%) exhibited mutations in KRAS (Table 2). Concerning p53, we also identified 4 studies [44,45,48,49] that investigated the mutational status of 192 NT samples. Positive results were observed in 77 of the samples (40%) (Table 2). The literature search identified only one study that fulfilled the predetermined search criteria [44]. In that study, the p16\(^{INKA} \) mutational status was investigated in 81 samples. Positive results could be identified in 12.5% of all investigated samples (10/81). The same study analysed the mutational status of SMAD4/DPC4 and identified mutations in that gene in 63 patients (77.6%).

Table 3 presents an overview of the prevalence of mutations of these four genes in the CLs and NT investigated in this study.

In total, CLs exhibited a higher mutation rate of p53 and p16\(^{INKA} \) compared with NT. Our results reached statistical significance for both genes (\( p < 0.05 \)). The mutation rate of KRAS and SMAD4/ DPC4 was also higher in CLs than in NT. These results did not reach statistical significance (\( p > 0.05 \)).

![Table 1: Mutations in pancreatic cancer cell lines (CLs).](http://dx.doi.org/10.4172/2324-9293.1000104)

**Table 1: Mutations in pancreatic cancer cell lines (CLs).** The table lists all analysed cell lines and provides an overview of the mutation rates of the following genes: KRAS, p53, p16\(^{INKA} \) and SMAD4/DPC4. % indicates the ratio of positively tested cell lines compared to all analysed cell lines; *=mutated gene; wt=wildtype; - = no data available.

<table>
<thead>
<tr>
<th>Name of the cell line</th>
<th>Origin</th>
<th>KRAS</th>
<th>p53</th>
<th>p16(^{INKA} )</th>
<th>SMAD4/ DPC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA Panc-3</td>
<td>explant culture</td>
<td>+</td>
<td>+</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>CFPAC-1 [22]</td>
<td>explant culture</td>
<td>+</td>
<td>+</td>
<td>wt</td>
<td>+</td>
</tr>
<tr>
<td>KP-1N, KP-2, KP-3 [23]</td>
<td>xenograft</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PC-1, PC-2 [25,26]</td>
<td>xenograft</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PA-TU-898BS, PA-TU-898BT [27]</td>
<td>explant culture</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hu-P-T3, Hu-P-T4 [28]</td>
<td>ascites</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PA-TU-8902 [29]</td>
<td>explant culture</td>
<td>+</td>
<td>42.9%</td>
<td>71.4%</td>
<td>-</td>
</tr>
<tr>
<td>no name available</td>
<td>12 exocrine 2 endocrine [30]</td>
<td>explant culture</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IMIM-PC-1, IMIM-PC-2, SK-PC-1, SK-PC-3 [31]</td>
<td>explant culture</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MZ-PC-1 [32]</td>
<td>pleural effusion</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A818 [33]</td>
<td>explant culture</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KMP-1, KMP-2, KMP-3, KMP-4, KMP-5, KMP-6 [34]</td>
<td>explant culture</td>
<td>+</td>
<td>100%</td>
<td>50%</td>
<td>83.3%</td>
</tr>
<tr>
<td>UK Pan-1 [35]</td>
<td>explant culture</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>SNU-213, SNU-341, SNU-494 [36]</td>
<td>explant culture</td>
<td>+</td>
<td>75%</td>
<td>50%</td>
<td>wt</td>
</tr>
<tr>
<td>FAMPAC [37]</td>
<td>explant culture</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>SUIT-1 [38]</td>
<td>Ascites</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p 34 [39]</td>
<td>pleural effusion</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PP 78, PP 109, PP 116, PP 117, PP 115 [40]</td>
<td>explant culture</td>
<td>+</td>
<td>100%</td>
<td>100%</td>
<td>+</td>
</tr>
<tr>
<td>PaCa 5061 [41]</td>
<td>explant culture</td>
<td>+</td>
<td>wt</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>a 99 [42]</td>
<td>xenograft</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PaCaDD-3, PaCaDD-60, PaCaDD-119, PaCaDD-135, PaCaDD-137 [8]</td>
<td>explant culture</td>
<td>+</td>
<td>80%</td>
<td>60%</td>
<td>-</td>
</tr>
<tr>
<td>Capan-1 [43]</td>
<td>explant culture</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Colo 357 [43]</td>
<td>explant culture</td>
<td>+</td>
<td>wt</td>
<td>wt</td>
<td>+</td>
</tr>
<tr>
<td>MiPaCa-2 [43]</td>
<td>explant culture</td>
<td>+</td>
<td>+</td>
<td>wt</td>
<td>+</td>
</tr>
<tr>
<td>BxPC-3-1 [43]</td>
<td>explant culture</td>
<td>wt</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A5Pc-1 [43]</td>
<td>explant culture</td>
<td>+</td>
<td>+</td>
<td>wt</td>
<td>-</td>
</tr>
<tr>
<td><strong>Results:</strong></td>
<td></td>
<td>36/47</td>
<td>76.6%</td>
<td>33/45</td>
<td>73.3%</td>
</tr>
</tbody>
</table>
The isolation of primary CLs is a tedious process. Only approximately 10% of the samples isolated eventually resulted in the establishment of cell lines [7]. To date, it is unclear why isolation is successful in some samples but unsuccessful in others. There seems to be no correlation with clinical-pathological data [8]. To provide insight into the question of whether the prevalence of mutations in PDAC is responsible for the successful isolation process, we analysed the frequency of genetic mutations of KRAS, p16\textsuperscript{INKA} and SMAD4/DPC4 in CLs and NT. The literature search identified 6 studies that analysed the distribution of these genes in NT. Those studies analysed patient KRAS mutation status (n=273), p53 mutation status (n=192) and p16\textsuperscript{INKA} and SMAD4/DPC4 mutation status (n=81). We also reviewed 71 CLs that were established within the last 25 years. The majority of those cultures were derived from explant cultures. Approximately 76.6% of the samples exhibited positive KRAS mutation status, 73.3% exhibited mutations in p53, and 58.6% and 78.6% exhibited mutations in p16\textsuperscript{INKA} and SMAD4/DPC4, respectively. Our analysis therefore did not reveal any differences in the mutational status of the KRAS oncogene and SMAD4/DPC4 in CLs and NT samples.

Interestingly, we observed a different distribution of mutations of tumour suppressor genes in NT compared with CLs. Generally, CLs exhibited high rates of mutated tumour suppressor genes. Although the p53 mutation rates in NTs exhibited high variation, ranging from 23.5% up to 70.4%, we observed that 73.3% of the 45 investigated CLs exhibited mutations in p53. p53 is a crucial regulator of the cell cycle in multicellular organisms [50] and possesses anticaner functions [51]. The most important function of p53 is the induction of apoptosis after structural genomic damage. p53 further initiates growth arrest by halting the cell cycle [52]. These cellular functions could explain why cells possessing mutations of p53 might be selected during the process of cell line isolation. The isolation process imposes a high load of apoptotic stimuli on the cell. Among such stimuli are the mechanical stress of cell culture medium replacement and the lack of many essential growth factors and the intermittent non-physiological conditions. In this regard, the distribution of p53 we observed in the literature is very interesting. The data presented in this review suggest that cells carrying mutations within the p53 gene might indeed be selected during the isolation process. p16\textsuperscript{INKA} also exhibited different distribution of mutation rates in NT and CLs. In CLs, 58.8% of samples exhibited mutations, whereas only 12.3% of NT samples exhibited mutations in p16\textsuperscript{INKA}. p16\textsuperscript{INKA} is a tumour suppressor gene that acts on the retinoblastoma protein (pRB). pRB induces G1 arrest by repressing transcriptional activation of E2F target genes [18,53]. It has been previously established that p16 alterations are very common in PDAC but not in cultured cell lines [54]. These findings also suggest that cell lines carrying mutations in p16\textsuperscript{INKA} are preferentially selected during the isolation process. In this regard, it is very interesting that different alternatively spliced variants of p16\textsuperscript{INKA} have been reported. One of the transcripts contains an alternate open reading frame (ARF) that specifies a protein that is structurally unrelated to the products of the other variants. This ARF product functions as a stabiliser of the tumour suppressor protein p53, as it can interact with and sequester MDM2, a protein responsible for the degradation of p53 [55]. This relationship might be a link to the previously mentioned importance of p53 in cell line isolation. Although ARF possesses a p53 regulatory function, p16\textsuperscript{INKA} exhibits an important role in cell cycle G1 progression. Therefore, ARF and p16\textsuperscript{INKA} share a common functionality in cell cycle control. In conclusion, the presented data suggest that mutations in genes that have a tumour suppressor function might play a role in the isolation of CLs. However, this thesis should be tested in an experimental setting. Isolation of CLs might be achieved through their anti-apoptotic function and influence cell cycle regulation.

References


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