New Pancreatic Cancer Biomarkers eIF1, eIF2D, eIF3C and eIF6 Play a Major Role in Translational Control in Ductal Adenocarcinoma

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Abstract. *Background/Aim:* Pancreatic cancer is one of the deadliest forms of cancer and ranks among the leading causes of cancer-related death worldwide. The most common histological type is ductal adenocarcinoma (PDAC), accounting for approximately 95% of cases. Deregulation of protein synthesis has been found to be closely related to cancer. The rate-limiting step of translation is initiation, which is regulated by a broad range of eukaryotic translation initiation factors (eIFs). *Patients and Methods:* Human PDAC samples were biochemically analyzed for the expression of various eIF subunits on the protein level (immunohistochemistry, immunoblot analyses) in 174 cases of PDAC in comparison with non-neoplastic pancreatic tissue (n=10). *Results:* Our investigation revealed a significant down-regulation of four specific eIF subunits, namely eIF1, eIF2D, eIF3C and eIF6. Concomitantly, the protein (immunoblot) levels of eIF1, eIF2D, eIF3C and eIF6 were reduced in PDAC samples as compared with non-neoplastic pancreatic tissue. *Conclusion:* Members of the eIF family are of relevance in pancreatic tumor biology and may play a major role in translational control in PDAC.

Consequently, they might be useful as potential new biomarkers and therapeutic targets in PDAC.

Pancreatic cancer, including ductal adenocarcinoma (PDAC), its most common manifestation, is still one of the deadliest forms of cancer. It usually affects the elderly, with a 5-year survival rate of 5% that has not improved over the past 20 years (1). PDAC, originates from the exocrine portion of the gland and accounts for approximately 80% of cases (1). Compared to other hepatobiliary cancers, only little is known about the risk factors and molecular mechanisms underlying PDAC. Several studies have shown that alcohol and nicotine abuse are the main risk factors, although genetic studies have identified a large number of mutations that affect signaling pathways (2). These genetic variations include *K-RAS*, the tumor suppressors *p53* and *p16*, as well as changes in *TGF-β* and *WNT/NOTCH* signaling (1-4).

However, the cellular alterations promoting carcinogenesis still need to be investigated for their possible function as new diagnostic markers, prognostic tools and novel therapeutic targets.

Translation, the process of protein synthesis crucially needed for cellular homeostasis, is subdivided into four major steps: initiation, elongation, termination and ribosome recycling. Initiation constitutes the rate-limiting step and is concerted by an abundance of eukaryotic translation initiation factors (eIFs), acting in different ways to generate the fully functional ribosomal apparatus with its associated interaction partners, such as the formyl-methionine-tRNA and the consecutively translated messenger RNA (mRNA) (5). Briefly, translation initiation starts with the formation of the 43S-preinitiation complex, consisting of the 40S ribosomal subunit, the ternary

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complex (which is composed of the initiating methionyl-tRNA bound to eIF2-GTP), eIF1, eIF1A and eIF3. This assembly consecutively scans the mRNA for the first start codon (AUG) in the 5' untranslated region (UTR), leading to codon-anticodon interaction assisted by eIF1 and eIF1A. The scanning process is further assisted by the heterotrimeric eIF4F complex (eIF4A, eIF4E and eIF4G). Binding to the matching start codon leads to eIF2-GTP hydrolysis and dissociation of the respective eIF2-GDP. Ribosomal subunit joining is mediated through eIF5B, forming the fully functional ribosome (5).

Dysfunction in translational control is an important downstream endpoint of oncogenic pathways, such as the PI3K/AKT/mTOR and RAS/RAF/MAPK pathways, which support cellular transformation and tumor development (6). In normal cells, these pathways operate as sensors of nutrient availability, stress or energy, adapting ribosome production and gene expression to the cellular conditions. eIFs have recently been discovered to be involved in tumor development in a variety of malignancies (6, 7), and have therefore been suggested as potential therapeutic targets with some substances currently under preclinical and early clinical investigation (7-10). Dysregulation of translation initiation and its interaction with the PI3K/AKT/mTOR signaling pathway are just starting to be understood in pancreatic cancer. Previously described alterations include up-regulation of eIF3A, eIF4E and eIF5A, as well as down-regulation of eIF3F (11-16). However, the molecular mechanism regulating eIFs in tumor biology is still not fully understood.

To address this issue, we investigated the involvement of the PI3K/AKT/mTOR signaling cascade and various eIF subunits, focusing on PDAC. For this purpose, we used protein analyses, immunohistochemistry and immunoblotting to characterize the expression pattern of the mTOR signaling members and various eIF subunits.

Materials and Methods

Ethics statement. The collection and use of human derived pancreas specimens were approved by the Ethics Committee of the Medical University of Graz, Graz, Austria, according to the ethical guidelines of the 1975 Declaration of Helsinki (EK 28-294 ex 15/16). All samples and medical data used in this study were irreversibly anonymized, and their clinical and pathological data are listed in Tables I and II.

Tissue microarray (TMA). A total of 174 formalin-fixed, paraffin-embedded patient samples were retrospectively collected from the Institute of Pathology, Technical University Munich, Germany. Hematoxylin-eosin stained (H/E) slides were reviewed by experienced, board certified pathologists (J.H. and I.B.), who confirmed the diagnosis and identified the areas of tumor and non-neoplastic tissue for each tissue microarray core. Tissue cylinders of 1.2 mm in diameter were punched out from the chosen tumor areas and embedded as TMA in a fresh paraffin block according to a specific pattern. Tissue sections were cut (4 µm) and placed on adhesive-coated glass slides followed by immunohistochemical analysis.

Table I. Clinical and pathological characteristics of 174 patients with pancreatic cancer. For each biochemically assessed PDAC patient, the clinical and pathological characteristics, including sex, age, TNM, stage and the presence of positive lymph nodes, are listed.

	Total (%)	Male (%)	Female (%)
n	174 (100)	98 (56.3)	76 (43.7)
Mean age	64.14	62.44	66.33
pT			
1/2	35 (20.1)	15 (15.3)	20 (26.3)
3/4	139 (79.9)	83 (84.7)	56 (73.7)
pN			
0	42 (24.1)	22 (22.4)	20 (26.3)
1	132 (75.9)	76 (77.6)	56 (73.7)
pM			
0	168 (96.6)	93 (94.9)	75 (98.7)
1	6 (3.4)	5 (5.1)	1 (1.3)
Grading			
1	7 (4)	4 (4.1)	3 (3.9)
2	92 (52.9)	51 (52)	41 (54)
3	75 (43.1)	43 (43.9)	32 (42.1)

Immunohistochemistry. IHC was performed on a Ventana Immunostainer XT (Ventana Medical Systems, Tucson, AZ, USA) by heat-induced epitope retrieval (HIER) in cell conditioning solution for 30 min and an ultra-VIEW universal DAB Detection Kit (Ventana Medical Systems, Tucson, AZ, USA). The primary antibodies were incubated for 30 minutes using different dilutions (Table II). Each core was semi-quantitatively scored, and an intensity score was defined as follows: 0=no staining, 1=weak staining, 2=moderate staining and 3=strong staining. Additionally, the percentage of stained tumor cells was determined (0-100%) and assigned to 5 groups, 0=0%, 1=1-49%, 3=50-79% and 4= 80-100%. The intensity score (0-3) was then multiplied by the percentage score (0-4), and the values obtained were subdivided as follows: 0=no staining, 1-4=weak staining, 5-8=moderate staining, 9-12=strong staining, and displayed in stacked plots.

Human pancreatic cancer patient samples. Human pancreas cancer samples (n=56) and non-neoplastic tissue (NNT) (n=56) were provided by the Department of Pathology, Medical University of Magdeburg, Germany. Informed consent was obtained from all patients. All samples were acquired during surgery, immediately snap frozen in liquid nitrogen, and stored at -80°C until protein or RNA extraction.

Protein extraction and western blot analysis. To prepare protein lysates, frozen tissue samples were homogenized with a MagNA Lyser homogenizer (Roche Diagnostics, Risch-Rotkreuz, Switzerland) and lysed in Nonidet-P40-based lysis buffer (0.05 M Tris-HCl, 0.15 M NaCl, 0.5% NP-40, 0.1 mM pefabloc, 1 mM DTT, complete mini EDTA-free, PhosSTOP (Roche Austria GmbH, Vienna, Austria). Protein concentrations were determined using Bradford protein assay (Biorad Protein Assay Dye Reagent; BioRad Laboratories GmbH, Munich, Germany). Thirty ng of total protein lysate was loaded onto sodium dodecyl sulphate (SDS)-polyacrylamide gels (30% acrylamide/bisacrylamide solution; ROTH, Karlsruhe, Germany), subjected to electrophoresis in mini-vertical electrophoresis units

Table II. *Antibodies for immunohistochemistry.*

Primary antibody	Company	Dilution	Second antibody
eIF1	Sigma Aldrich (HPA043003) (Bavaria, Germany)	1:50	Rabbit
eIF2 α (D7D3) XP	Cell Signaling (#5324) (Hessen, Germany)	1:2000	Rabbit
eIF3A	Cell Signaling (#2538) (Hessen, Germany)	1:50	Rabbit
eIF6	Gene Tex (GTX63642) (Irvine, CA, USA)	1:75	Rabbit

Table III. *Primary antibodies for immunoblotting.*

Primary antibody	Company	Dilution	Second antibody
GAPDH	Sigma (A2103) (Bavaria, Germany)	1:1000	Rabbit
eIF1	Sigma (HPA043003) (Bavaria, Germany)	1:500	Rabbit
eIF2 α (D7D3) XP	Cell Signaling (#5324) (Hessen, Germany)	1:1000	Rabbit
eIF3C	Cell Signaling (#2068) (Hessen, Germany)	1:1000	Rabbit
eIF6	GeneTex (GTX63642) (Irvine, CA, USA)	1:1000	Rabbit

(Hoefer Inc, Richmond, VA, USA), and blotted onto PVDF membranes (Immobilin-P Transfer Membrane; Millipore, MA, USA) using a Semi Dry Blotting Unit (SCIE-PLAS; Cambridge, England). The membranes were blocked in 5% non-fat dried milk (AppliChem, Darmstadt, Germany) in 0.1% TBS tween (TBST) for 1 h at room temperature. The primary antibodies (Table III) were diluted in 0.1% TBS tween (TBST) with 5% BSA and incubated at 4°C overnight. The membranes were washed with 0.1% TBST, followed by incubation with a horseradish peroxidase conjugated secondary antibody (anti-mouse 1:3000 and anti-rabbit 1:5000; GE Healthcare Life Sciences, Buckinghamshire, UK). Proteins were visualized using the chemiluminescence Amersham ECL Western Blotting Detection Reagent (GE Healthcare Life Sciences, Buckinghamshire, UK) and the Image Quant LAS 500 (GE Healthcare, Little Chalfont, UK) detection system. Signals were normalized to the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) loading control (mAb dilution 1:1000, Cell Signaling, Frankfurt, Germany). Densitometric analysis was performed using ImageJ software.

Statistical analysis. The Cancer Genome Atlas (TCGA) public dataset, including 125 PDCA subjects, was analyzed to identify the association between gene expressions stratified by the median survival. Kaplan–Meier curves were generated using the survival R package. The log rank test was applied to test for an association of survival and gene expression. All results were expressed as mean±standard deviation (SD). Differences between groups were assessed using Students *t*-test or Mann-Whitney *U*-test based on data distribution. $p < 0.05$ was considered as statistically significant. Statistical analysis and graph generation were performed using GraphPad PRISM version 5.0 (GraphPad software Inc., La Jolla, CA, USA).

Results

eIF1, eIF2D, eIF3C and eIF6 are down-regulated in PDAC patients. To address the relevance of eIF1, eIF2D, eIF3C and eIF6 in PDAC, we investigated their expression by

immunohistochemistry (IHC) on tissue microarrays (TMA) in comparison with non-neoplastic tissue (NNT) (Figure 1). In PDCA, 174 patient samples were analyzed, and 10 NNT pancreatic tissues served as controls. Clinical data of patients analyzed by IHC are listed in Table I. Staining was mainly observed in the cytosol. Down-regulation of eIF1 was demonstrated in well and poorly differentiated samples ($p < 0.0001$) (Figure 1A, B and I). eIF2D was found to be down-regulated at the same significance level ($p < 0.0001$), while significant differences were present between well (G1/2) and poorly (G3) differentiated samples ($p = 0.0119$) (Figure 1C, D, J and 2B). Furthermore, expression of eIF3C was mitigated in tumor samples, independent of differentiation ($p < 0.0001$) (Figure 1E, F, K and 2C). Finally, eIF6 was also down-regulated in PDAC (Figure 2D) as compared with NNT, ($p = 0.0083$) (Figure 1G, H, L, and 2D). Analysis of staining intensity in PDAC G1/G2 and G3 tumors revealed significant differences in eIF1, eIF2D, eIF3C and eIF6 expression within these groups. eIF1 expression displayed a significant decrease in G1/G2 ($p < 0.0001$) and high grade G3 tumors ($p < 0.0001$) as compared with NNT (Figure 2A). eIF2D staining was also significantly decreased in G1/G2 ($p < 0.0001$) and high grade G3 tumors ($p < 0.0001$) as compared with NNT (Figure 2B). When comparing G1/2 ($p < 0.05$) versus G3 ($p < 0.05$), significant changes were observed (Figure 2B) among the different grades. For eIF3C, we noted a significant decrease in the expression for G1/G2 ($p < 0.0001$) and high grade G3 tumors ($p < 0.0001$) as compared with NNT (Figure 2C). Analysis of staining intensity in PDAC G1/2 and G3 tumors revealed significant differences in eIF6 expression within these groups. eIF6 expression displayed a significant decrease in G1/G2

Figure 1. *eIF1*, *eIF2D*, *eIF3C* and *eIF6* expression in PDAC in comparison with NNT. (A) Representative pictures of immunohistochemical staining of *eIF1* in non-neoplastic tissues (NNT). (B) Representative pictures of immunohistochemical staining of *eIF1* in PDAC. (I) Densitometric analyses of immunohistochemical staining of *eIF1* in PDAC in comparison with NNT. (C) Representative pictures of immunohistochemical staining of *eIF2D* of NNT. (D) Representative pictures of immunohistochemical staining of *eIF2D* in PDAC. (J) Densitometric analyses of immunohistochemical staining of *eIF2D* in PDAC in comparison with NNT. (E) Representative pictures of immunohistochemical staining of *eIF3C* in NNT. (F) Representative pictures of immunohistochemical staining of *eIF3C* in PDAC. (K) Densitometric analyses of immunohistochemical staining of *eIF3C* in PDAC in comparison with NNT. (G) Representative pictures of immunohistochemical staining of *eIF6* in NNT. (H) Representative pictures of immunohistochemical staining of *eIF6* in PDAC. (L) Densitometric analyses of immunohistochemical staining of *eIF6* PDAC compared to NNT. Scale bars: 400 μ m.

tumors ($p < 0.05$) and high grade G3 tumors ($p < 0.01$) as compared with NNT (Figure 2D).

Low eIF2D, eIF3C and eIF6 expression correlates with better overall survival of PDAC patients. Based on the

results shown above, we investigated the correlation between the expression of *eIF1*, *eIF2D*, *eIF3C* and *eIF6* and patients' overall survival. An *in silico* analysis, including 125 patients of The Cancer Genome Atlas (TCGA) database, was performed. To determine the statistical

Figure 2. *eIF1*, *eIF2D*, *eIF3C* and *eIF6* expression in sub-grouped PDAC samples (well and poorly differentiated) compared to NNT. (A) Expression analyses of immunohistochemical staining of *eIF1* in sub-grouped well and poorly differentiated PDAC samples compared to non-neoplastic tissues (NNT). (B) Expression analyses of immunohistochemical staining of *eIF2D* in sub-grouped well and poorly differentiated PDAC samples compared to non-neoplastic tissues (NNT). (C) Expression analyses of immunohistochemical staining of *eIF3C* in sub-grouped well and poorly differentiated PDAC samples compared to NNT. (D) Expression analyses of immunohistochemical staining of *eIF6* in sub-grouped well and poorly differentiated PDAC samples compared to NNT. Bars represent mean $\pm$ SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. Statistical analysis: Mann-Whitney U-test.

significance, the log rank test was carried out. Kaplan-Meier curves were generated to assess a potential association of *eIF1*, *eIF2D*, *eIF3C* and *eIF6* expression with overall survival in PDAC patients. Lower expression of *eIF2D* ($p=0.002$), *eIF3C* ($p=0.026$) and *eIF6* ($p=0.132$) in PDAC was associated with better overall survival as compared with high expression of *eIF2D*, *eIF3C* and *eIF6* (Figure 3B, C and D).

Additionally, the influence of *eIF1* on overall survival of PDAC patients was investigated (Figure 3A). Lower expression of *eIF1* in PDAC indicated a significantly poorer overall survival as compared with high *eIF1* ($p=0.048$) expression. These findings suggest that *eIF1*, *eIF2D*, *eIF3C* and *eIF6* might have a different function and might serve as novel prognostic markers for the overall survival of PDAC patients.

eIF1, *eIF2D*, *eIF3C* and *eIF6* are markers of PDAC with good prognosis. To examine whether *eIF1*, *eIF2D*, *eIF3C* and *eIF6* protein levels are down-regulated in fresh frozen pancreatic tumor tissue compared to NNT, we prepared protein lysates of 56 PDAC tissue samples and 56 NNT. The lysates were analyzed by immunoblotting. *eIF1* demonstrated down-regulation in tumor samples ($p<0.001$) when compared with NNT (Figure 4A and B). A significant down-regulation of *eIF2α* was also detected ($p<0.0001$) when compared with NNT (Figure 4A and C). Furthermore, expression of *eIF3C* was lower in tumor samples ($p<0.001$) as compared with NNT (Figure 5A and B). Finally, *eIF6* showed significant differences ($p<0.001$) between NNT and tumor samples on the protein level (Figure 5A and C). Densitometric evaluation (*eIF1*; *eIF2D*; *eIF3C* and *eIF6*/GAPDH) of all samples analyzed by immunoblotting is

Figure 3. *eIF1*, *eIF2D*, *eIF3C* and *eIF6* are clinically relevant candidates in PDAC. (A) Kaplan–Meier curves reflect the effect of *eIF1* expression on overall survival for PDAC. Cases are divided into low or high expression according to whether expression is below or above median, and survival was compared using the log-rank test. (B) Kaplan–Meier curves reflecting the effect of *eIF2D* expression on overall survival for PDAC. Cases are divided into low or high expression according to whether expression was below or above median, and survival was compared using the log-rank test. (C) Kaplan–Meier curves reflecting the effect of *eIF3C* expression on overall survival for PDAC. Cases are divided into low or high expression according to whether expression was below or above median, and survival was compared using the log-rank test. (D) Kaplan–Meier curves reflecting the effect of *eIF6* expression on overall survival for PDAC. Cases were divided into low or high expression according to whether expression was below or above median, and survival was compared using the log-rank test.

shown in Figures 4B, C and 5B and C. These data suggest that *eIF1*, *eIF2D*, *eIF3C* and *eIF6* may play an important role during tumorigenesis of PDAC.

Discussion

Accounting for 95% of pancreatic cancers, PDAC is still one of the deadliest forms of cancer. It usually affects the elderly, with an overall 5-year survival rate of 8% that has not improved during the past 20 years (1). Biomarkers to monitor disease progression and novel therapeutic alternative targets for these tumors are strongly required.

eIFs are involved in the translation of growth factors, in proteins influencing cell cycle, growth, as well as in apoptosis

and malignant transformation. A broad range of cancer types, including pancreatic cancer, have been candidates for targeting the PI3K/AKT/mTOR pathway due to its common deregulation via amplification, mutation or loss of key regulators.

eIFs are overexpressed in various malignancies, such as squamous cell carcinoma of the head and neck, lung, thyroid, breast and colorectal cancer (17-19). However, data on *eIFs* in PDAC is still limited.

We presented evidence that compared with normal pancreatic tissue, *eIF1*, *eIF2D*, *eIF3C* and *eIF6* are down-regulated in PDAC, and are predictors for overall survival in PDAC patients.

The *in silico* analysis of The Cancer Genome Atlas (TCGA) database revealed a significant influence of *eIF2D*,

Figure 4. *eIF* expression in PDAC samples ($n=56$) versus non-neoplastic pancreatic tissue ($n=56$) on protein level. (A) Graph shows immunoblot analysis for PDAC patients as compared with NNT. Equal amounts of protein were resolved on SDS PAGE and immunoblotted with *eIF1*, *eIF2D* and GAPDH (loading control) antibodies. (B) Densitometric analyses of immunoblot of *eIF1* in PDAC compared with NNT. (C) Densitometric analyses of immunoblot of *eIF2D* in PDAC compared with NNT. Bars represent mean $\pm$ SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Statistical analysis: Two-way ANOVA with Bonferroni post-test.

eIF3C and *eIF6* on overall survival of PDAC patients. The TCGA analysis revealed a significantly longer overall survival in patients with low *eIF2D*, *eIF3C* and *eIF6* expression. Moreover, IHC staining of PDAC compared to NNT for *eIF1*, *eIF2D*, *eIF3C* and *eIF6*, revealed their down-regulation in the cytoplasm of pancreatic tumor cells, whereas staining intensity and density were higher in NNT. This decrease in cytoplasmic *eIF1*, *eIF2D*, *eIF3C* and *eIF6* in human FFPE tissue specimens has not yet been reported. However, there was no change in the expression patterns of *eIF1*, *eIF2D*, *eIF3C* and *eIF6* in the nucleus when comparing PDAC tissues and the non-neoplastic tissues. Our study is the first to provide evidence that *eIF1*, *eIF2D*, *eIF3C* and *eIF6* are down-regulated in PDAC compared to non-neoplastic pancreatic tissue, and that they are predictors for overall survival in PDAC.

eIF2D is an important target of regulation, and its activity is often rate-limiting for protein synthesis (20, 21). Initiation of mRNA translation requires *eIF2*-bound GTP and the initiator methionyl-tRNA, leading to the formation of a ternary complex associated with the 40S ribosomal subunit and the initiation factors *eIF1*, *eIF1A* and *eIF3*, resulting in the formation of the 43S pre-initiation complex (20). In stressed cells, increased *eIF2D* switches *eIF2* from a

substrate to a complete inhibitor of *eIF2B*, resulting in a global inhibition of protein synthesis (22). Previously, transient *eIF2D* expression was increased in healthy cells, whereas constitutive overexpression supported tumor initiation and progression (7). Our study also displayed increased expression of *eIF1*, *eIF2D*, *eIF3C* and *eIF6* in healthy cells compared to PDAC. *eIF6* affects the maturation of 60S ribosomal subunits (23) as well as formation of the 80S ribosome as anti-association factor (24). It can be found in the nucleolus and in the cytoplasm of a cell. *eIF6* was overexpressed in various cancer types, for example metastatic colorectal cancer (17) or non-small cell lung cancer (19). *eIF6* expression was indicated to limit cell growth and transformation (25). Regulation of *eIF6* is controlled neither transcriptionally *via* c-Myc nor post-transcriptionally *via* PI3K/AKT/mTOR signaling (24). The specific molecular mechanisms underlying the role of *eIF6* in these processes remains unclear (26-31). Thus, reduced expression of *eIF6* and or inhibition of its activity may be used as a biomarker in PDAC. Furthermore, we identified *eIF1*, *eIF2D*, *eIF3C* and *eIF6* as promising novel targets for PDAC therapies as they were down-regulated on the protein-level and, except for *eIF2*, *eIF3C* and *eIF6*, also affected patients' overall survival.

Figure 5. *eIF* expression in PDAC samples ($n=56$) versus non-neoplastic pancreatic tissue ($n=56$) on protein level. (A) Graph shows immunoblot analysis for PDAC patients compared to NNT. Equal amounts of protein were resolved on SDS PAGE and immunoblotted with *eIF3C*, *eIF6* and GAPDH (loading control) antibodies. (B) Densitometric analyses of immunoblot of *eIF3C* in PDAC compared with NNT. (C) Densitometric analyses of immunoblot of *eIF6* in PDAC compared with NNT. Bars represent mean $\pm$ SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Statistical analysis: Two-way ANOVA with Bonferroni post-test.

Although our study provides promising results, further analyses are required to elucidate the role of *eIFs* in pancreatic carcinogenesis in more detail. Future experiments should focus on tumor cell type-specific expressions of *eIFs* under consideration of tumor heterogeneity.

Conclusion

In conclusion, we suggest that *eIF1*, *eIF2D*, *eIF3C* and *eIF6* might serve as prognostic biomarkers for overall survival in PDAC patients, and that their regulation could serve as a potential new therapeutic approach in PDAC. Still, there is a need for future investigations of *eIF1*, *eIF2D*, *eIF3C* and *eIF6* expression in PDAC to determine whether *eIF1*, *eIF2D*, *eIF3C* and *eIF6* drive pancreatic carcinogenesis.

Conflicts of Interest

All Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

NGS, PP and JH were responsible for the concept and design of this study. NGS, PP, and MGK performed the experiments and

procedures. NGS and PP wrote the article. WW and BG participated in building up the study patient cohort. NGS, PP, MGK, WW, BG and JH commented on the manuscript and interpreted data during the course of the project. JH acquired funding for conducting the entire study. All Authors read and approved the final manuscript.

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