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Is mitochondrial DNA copy number a good prognostic marker in resectable pancreatic cancer?

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ABSTRACT

Background: The aim of this prospective study was to investigate mitochondrial DNA (mtDNA) copy number in a group of resectable pancreatic cancer (PC) tumor tissues and adjacent normal pancreatic tissues, and to explore the correlation between the mtDNA content in tissues and the clinicopathological parameters and the overall survival.
Methods: Relative mtDNA copy number was measured by the quantitative PCR-based assay. The tumors specimens ($n = 43$) originated from the patients with pathologically confirmed pancreatic ductal adenocarcinoma who did not receive any neoadjuvant systemic therapy. The adjacent normal pancreatic tissue samples ($n = 31$) were obtained from surgical margins.
Results: mtDNA copy number was significantly lower in PC tissue ($P < 0.001$) compared to adjacent normal pancreatic tissue. Jonckheere-Terpstra trend testing indicated a statistically significant decrease in median mtDNA copy number across the differentiation (adjacent normal pancreatic tissue, low-grade, intermediate-grade, high-grade cancer), $P < 0.001$. However, the survival analyses failed to show a significant difference in survival between patients with high and low mtDNA copy number.
Conclusions: To the best of our knowledge, we provided the first evidence that mitochondrial DNA copy number was significantly lower in pancreatic cancer tissue ($P < 0.001$) compared to adjacent normal pancreatic tissue. Also, we demonstrated that mitochondrial copy number was not a significant marker for predicting prognosis in resectable pancreatic cancer.

Introduction

A mitochondrion is a semi-autonomous, self-reproducing organelle in the cytoplasm of eukaryotic cells. The copy numbers of mitochondrial DNA (mtDNA) per mitochondrion range from 1 to 15 and the average is almost 5. Each human cell contains approximately 100 mitochondria, giving a total number of mtDNA molecules per human cell of approximately 500 [1]. In comparison to nuclear genomic DNA, mtDNA reveals high mutation rates caused by constant exposure to mutagenic oxygen radicals and lack of the protective mechanisms of DNA repair. These characteristics of mtDNA suggest their potential importance in the pathophysiology of diverse medical conditions [2,3]. Several types of mtDNA alterations have been identified in human cancers. Among them, point mutations and copy number alterations are the two most common mtDNA alterations in cancers. Qualitative aberrations of mtDNA,

Abbreviations: AUC, area under the curve; EMT, epithelial-mesenchymal transition; G1, well-differentiated cancer (low grade); G2, moderately differentiated cancer (intermediate grade); G3, poorly differentiated (high grade); IQR, interquartile range; mtCN, mitochondrial DNA copy number; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; N0, no regional lymph node metastasis; N1, regional lymph node metastasis; Nx, undetermined lymph node involvement; PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; R0, tumor pathologically marked as R0, no cancer cells seen microscopically at the resection margin; R1, tumor pathologically marked as R1, cancer cells present microscopically at the resection margin; ROC, receiver operating characteristic; T2, tumor limited to the pancreas, with more than 2 cm in greatest dimension; T3, tumor that extend beyond the pancreas but without involvement of the celiac axis or the superior mesenteric artery; T4, tumor that involve the celiac axis or the superior mesenteric artery.

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such as mutations, have been found in hematological malignancies [4,5] and in solid tumors, such as hepatocellular carcinoma, breast, gastric, prostate and colorectal cancer [6]. Quantitative aberrations of mtDNA have also been observed in various tumors [2]. While increased mtDNA content has been found in lung adenocarcinoma and papillary thyroid cancer [2,7], reduced mtDNA content in bladder, breast, esophageal, renal and liver cancers has been reported [2,8,9]. Because of these aberrations involved in the carcinogenesis, mtDNA may have promising clinical applications for cancers, especially as a biomarker and prognostic factor. In the literature, there are studies investigated mtDNA copy number in peripheral blood leukocytes or in the tumor tissue. Regarding cancer biomarker, increased peripheral blood mtDNA copy number levels were associated with increased risk of renal cell carcinoma, breast, gastric and colorectal cancer, melanoma, and gliomas [10–14]. Low peripheral blood mtDNA copy number levels were associated with increased risk of esophageal adenocarcinoma, bladder cancer and non-melanoma skin cancers [15,16]. Peripheral blood mtDNA copy number has also been investigated in pancreatic cancer patients [17]. In that study higher mtDNA copy number was significantly associated with increased pancreatic cancer risk. Regarding the prognostic factor, low mitochondrial DNA copy number in tumor tissue was correlated with poorer prognosis in breast cancer patients [18]. Similarly, increased tumor mtDNA copy number levels were associated with better survival in adrenocortical carcinoma, chromophobe renal cell carcinoma and low-grade glioma [2]. The opposite trend, of poor survival in patients with high tumor mtDNA, was reported in clear-cell renal cell carcinoma, cervical cancer and melanoma [2,19].

In the present study, we developed a simple and accurate multiplex real-time PCR method for synchronized quantification of nuclear DNA (nDNA) and mtDNA in adjacent normal and cancerous pancreatic tissue samples from patients with resectable pancreatic ductal adenocarcinoma. To our knowledge, this is the first qPCR-based study to investigate the mitochondrial DNA copy number (mtCN) in pancreatic cancer (PC) tissue, the correlation between mtCN and the pathological parameters and the overall survival.

Materials and methods

Study population and data collection

Samples and matched clinical information were obtained from the Clinical Department of Gastroenterological Surgery and Transplantation Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw, Poland. Fresh frozen samples of 43 cases of pancreatic ductal adenocarcinoma and 31 cases of adjacent normal pancreatic tissue that were diagnosed and treated from December 2013 to June 2016 were prospectively retrieved. All operations in patients included in the study were performed due to a pancreatic tumor diagnosed in imaging studies. The histological type, stage, and grade of the tumors were classified according to WHO criteria. In the cancer group, all patients have resectable tumors that were pathologically confirmed as pancreatic ductal adenocarcinoma. Further, all PC patients did not receive any neoadjuvant systemic therapy and were treated with gemcitabine after the operation. Among the non-cancer group, the adjacent normal pancreatic tissue samples were obtained from the surgical margin. Within the study population, there were 7 paired samples of tumor and adjacent normal pancreatic tissue. The study was approved by the Medical Ethics Committee of the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw, Poland and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The patients gave their informed consent prior to their inclusion in the study. Outcome status and dates of recurrence, metastasis or death were obtained from the clinical and hospital records and by patient/family contact until January 2017.

DNA isolation

Total genomic DNA was isolated from pancreatic cancer tissue and adjacent normal tissue with Genomic Mini kit (A&A Biotechnology). DNA purification was performed according to the manufacturer's instructions. The concentration of extracted DNA was obtained by measuring the absorbance at 260 nm using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). Until DNA isolation tissues were stored at −80 °C.After isolation DNA was stored at −20 °C.

Measurements of mtDNA copy number

The relative mtDNA copy number in the 1) tumor tissue and 2) adjacent normal tissue was measured by a quantitative PCR-based method (qPCR) as previously described [17,20,21]. For qPCR analysis primers were designed in Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). PCR efficiency was obtained from the standard curve. PCR efficiency E(%) was in the range 1,92-2,15. We used two pairs of primers in order to perform the relative quantification of mtDNA copy number: *ND1*: fw_TACGGGCTACTACAACCCTTC, rv_ATGGTAGATGTGGCGGGTTT, was used to amplify *ND1* (Mitochondrially Encoded NADH: Ubiquinone Oxidoreductase Core Subunit 1) representing mitochondrial genome, and *SLCO2B1:* fw_CCTGATGCCTAGGTTTCTTTTCTTG, rv_GGTCATCTGCCTACCCTAGAAC) (Solute Carrier Organic Anion Transporter Family Member 2B1) representing nuclear genome. Genes representing mitochondrial (*ND1*) and nuclear (*SCLO2B1*) genome were chosen randomly [22,23]. For qPCR, 10ng of DNA were loaded with 0,25 μM of forward and reverse primers, 12,5 μl of iTaq ™ universal SYBR® Green Supermix (Bio-rad) onto 96-well plate for LightCycler® 96 (Roche Diagnostics GmbH). The qPCR was carried out onto 96-well plate for LightCycler® 96 (Roche Diagnostics GmbH) at the PCR reaction conditions: hot start at 95 °C for 3 min followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The ratio of mitochondrial DNA to nuclear DNA was calculated [24,25] on the basis of the quantification cycle (Cq) values and the baseline settings automatically calculated by the qPCR instrument software. Data were analyzed in the GenEX software (Multid Analyses AB, Göteborg, Sweden).

Statistical analysis

Each sample from each patient was running in the 4 replicates. Statistical analysis was performed with GraphPad Prism 5.0 (GraphPad Software Inc. La Jolla, USA) and PQStat 1.6.6 (PQStat Software, Poland). The Shapiro–Wilk test was used as a test of normality. The student t-test and Fisher's exact test were used to compare cancer and control group regarding age and sex, respectively. Mann Whitney tests, (***) $P < 0.001$, were used to assess the differences between relative mtCN in 1) pancreatic cancer tissue and adjacent normal tissue; 2) tissues from tumors localized in the head of the pancreas and from tumors in other localization; 3) tissues from tumors with negative lymph node status (N0) and from tumors with positive lymph node status (N1). Kruskal-Wallis tests were used to assess the differences between relative mtCN in 1) adjacent normal tissue and tissues from well-differentiated tumors (G1), moderately differentiated tumors (G2) and from poorly differentiated tumors (G3); 2) adjacent normal tissue and tissues from tumors with positive (R1) and negative (R0) surgical margin. Dunn's Multiple Comparison test (*) $P < 0.05$, (**) $P < 0.01$ was used

as a post test. The results shown on the charts were presented as a median with (±IQR). A ROC analysis was performed to determine an optimal threshold of mtDNA copy number for pancreatic cancer. Based on a ROC-generated mtCN cut-off point, cancer patients were ranked with either a high or low mtCN status. Subsequently, we have carried out a survival analysis with Kaplan-Meier method, the results were compared with the logrank (Mantel-Cox) Test. $P < 0.05$ was considered significant.

Results

Results were obtained from 74 samples (43 from a pancreatic cancer tissue, 31 from adjacent normal tissue). The mean age was 64.10 (ranging from 37 to 84) for the normal pancreatic tissue group and 63.44 (ranging from 40 to 84) for the cancer group. Within the cancer group, there were 44.19% females and 55.81% males, and within the control group, there were 48.39% females and 51.61% males. Table 1 summarises these basic characteristics of the study population. There were no significant differences between the two groups regarding age, ethnicity, and sex. Within the cancer group, available baseline characteristics included tumor size, lymph node involvement, differentiation grade, sublocation, and surgical margin status; these data are listed in Table 2.

Determination of relative mtDNA copy number *ND1/SCLO2B1* ratio in the pancreatic cancer tissue and adjacent pancreatic tissue.

The median mtDNA copy number (mtCN) and interquartile ranges (IQR) in the 43 pancreatic cancer specimens and in the 31 adjacent normal pancreatic tissue specimens were respectively 123 (IQR 75) and 195 (IQR 462). mtCN was significantly lower in pancreatic cancer tissue ($P < 0.001$) compared to adjacent normal pancreatic tissue. Data were shown in Fig. 1 A. Within the cancer group, most tumors have the relative mtCN ranging from 50 to 200. Data were shown in Fig. 1 B. Within the study population, there were 7 paired samples of tumor and adjacent normal pancreatic tissue. Similarly, in this subgroup, mtCN was lower in pancreatic cancer tissue compared to adjacent normal pancreatic tissue ($P > 0.05$). Data were shown in Figure 4 in the Supplement 1.

Determination of relation between relative mtDNA copy number and the clinicopathological characteristics of pancreatic cancer group.

mtDNA copy number (mtCN) was significantly lower in pancreatic cancer tissue ($P < 0.001$) compared to adjacent normal pancreatic tissue. Furthermore, we observed that mtCN decreased as the PC grading increased. Jonckheere-Terpstra trend testing indicated a statistically significant decrease in median mtCN across the differentiation (adjacent normal pancreatic tissue, low grade, intermediate grade, high grade), $P < 0.001$. Farther, G2 tumors had a significantly lower mtCN than control adjacent normal pancreatic tissue, $P < 0.01$. Data were shown in Fig. 2 A. Regarding lymph node involvement, there was no significant difference in mtCN between specimens from tumors pathologically marked as N1 (tumors with regional lymph node metastasis) and specimens from tumors marked as N0 (no regional lymph node metastasis). Data were shown in Fig. 2B. Concerning relation between relative mtDNA copy number and a resection margin, both specimens from tumors marked as R0 (no cancer cells seen microscopically at the resection margin) and from tumors marked as R1 (cancer cells present microscopically at the tumor resection margin) had statistically significantly less mtCN compared to surrounding tissue, which is not surprising due to the fact that the samples from all tumors had statistically significantly less mtCN compared to the surrounding tissue. Our results showed no significant differences in the mtCN values for tumors marked as R0 and tumors marked as R1. Data were shown in Fig. 2C. With regard to the PC localization, there were no differences in relative mtCN between tumors localized in the head of the pancreas and tumors in other localization. Data were shown in Fig. 2D.

The impact of relative mtDNA copy number on the overall survival of the patients with resectable pancreatic cancer.

We then evaluated the overall survival of the patients with resectable pancreatic cancer to assess the prognostic value of mtCN. A receiver operating characteristic (ROC) curve analysis was performed to determine an optimal threshold of mtDNA copy number for cancer. The area under the ROC curve (AUC) of mtCN for cancer was 0.742. The cut-off point for mtCN for cancer was 231, with a sensitivity of 90.7% and a specificity of 46.9%. Data were shown in Fig. 3A. Based on a ROC-generated mtCN cut-off point (231), cancer patients were ranked with either a high (>231) or low (≤231) mtCN

Table 1
Distribution of selected characteristics of pancreatic cancer cases and controls. For categorical variables, Fisher's exact test was used to examine the differences. For continuous variables, Student's t-test was used to examine the differences. ns – $P > 0.05$.

Characteristics	Cancer group (n = 43) N (%)	Controls (n = 31) N (%)	P-value
Ethnicity			
White	43 (100.0)	31 (100.0)	ns
Sex			
Male	24 (55.81)	16 (51.61)	
Female	19 (44.19)	15 (48.39)	ns
Age (mean; years)	63.44	64.10	ns

Table 2
Baseline characteristics of cancer group included pancreatic cancer staging (the extent of the tumor and lymph node involvement according to TNM classification: T2 refers to the tumors limited to the pancreas, with more than 2 cm in greatest dimension; T3 refers to the tumors that extend beyond the pancreas but without involvement of the celiac axis or the superior mesenteric artery; T4 refers to the tumors that involve the celiac axis or the superior mesenteric artery – borderline tumors; the N0 refers to the specimens from the pancreatic cancers without regional lymph node metastases, while N1 refers to the specimens from pancreatic cancers which have spread to nearby lymph nodes), differentiation grade, sublocation of the tumor and surgical margin status (the R0 corresponds to the specimens from the pancreatic cancers tumors that were pathologically marked as R0 (no cancer cells seen microscopically at the resection margin), while R1 corresponds to the specimens from the pancreatic cancers tumors that were pathologically marked as R1 (cancer cells present microscopically at the resection margin).

Characteristics	Cancer group (n = 43) N (%)
The extent of the primary tumor	
T2	2 (4.65)
T3	38 (88.37)
T4 (borderline)	3 (6.98)
Lymph node involvement	
N0	5 (11.63)
N1	37 (86.05)
Nx	1 (2.33)
Differentiation grade	
Poor	4 (9.30)
Moderate	29 (67.44)
Well	9 (20.93)
Undetermined	1 (2.33)
Sublocation	
Head	36 (83.72)
Head and body	3 (6.98)
Body and tail	2 (4.65)
Other	2 (4.65)
Surgical margin status	
R0	15 (34.88)
R1	27 (62.79)
Undetermined	1 (2.33)

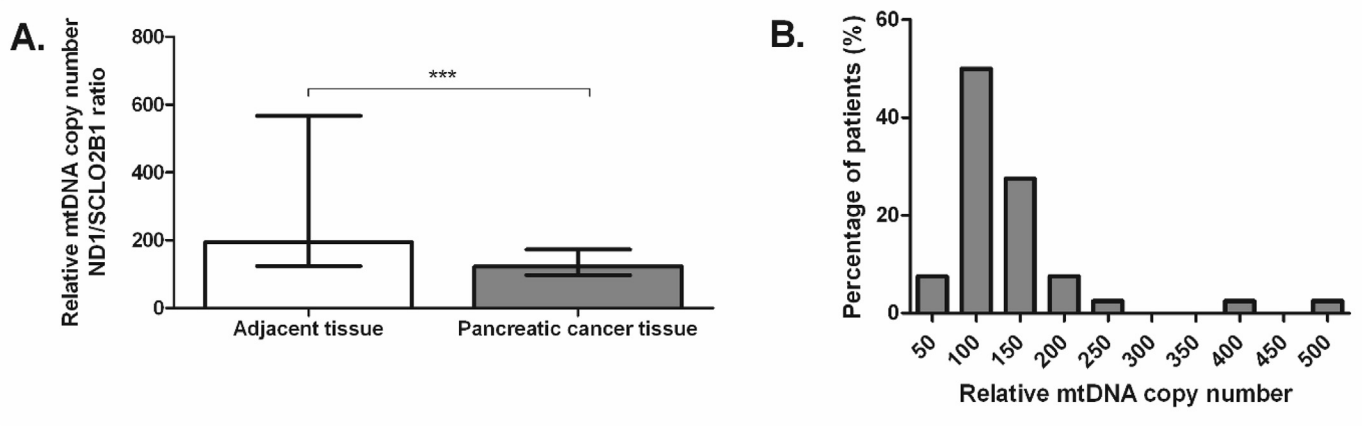

Fig. 1. (A) Relative mtDNA copy number in the adjacent normal pancreatic tissue and pancreatic cancer tissue. Relative mtDNA copy number was measured as a *ND1/SCLO2B1* ratio, with qPCR methods. Genes represent mitochondrial (*ND1*) and nuclear (*SCLO2B1*) genome were chosen randomly. The graph shows the statistically significant difference between adjacent pancreatic tissue vs. pancreatic cancer tissue (Mann Whitney test). Results shown in the graphs were obtained as a median (±IQR), (***) $P < 0.001$. (B) The frequency of relative mtDNA copy number (*ND1/SCLO2B1*) in pancreatic tumor tissue.

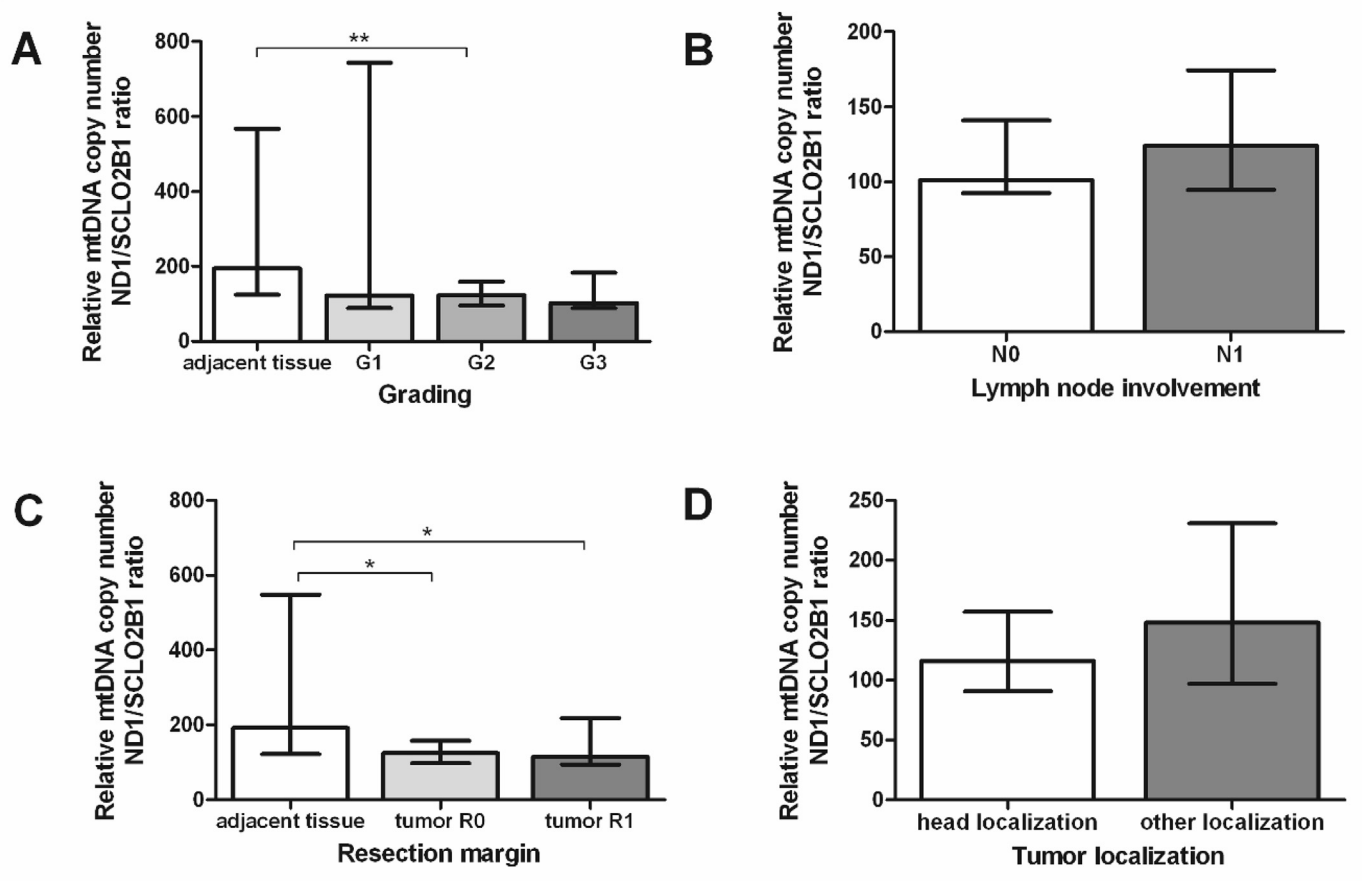

Fig. 2. (A) Relative mtDNA copy number measured as a *ND1/SCLO2B1* ratio in the adjacent normal pancreatic tissue and in pancreatic ductal adenocarcinoma pathologically marked as G1, G2 and G3 according to the tumor differentiation. The G1 refers to well-differentiated cancer (low grade); G2 - moderately differentiated cancer (intermediate grade); G3 - poorly differentiated (high grade). The graph shows the statistical significant difference between adjacent tissue and G2 tumors (Kruskal-Wallis test, post test Dunn's Multiple Comparison Test), (**), $P < 0.01$. Results shown in the graphs were obtained as a median (±IQR). (B) The impact of the relative mtDNA copy number on the lymph node involvement. The graph shows the non-significant difference between N0 and N1 cancers obtained with Mann Whitney test, $P > 0.05$. The N0 refers to the specimens from the pancreatic cancers without regional lymph node metastases, while N1 refers to the specimens from pancreatic cancers which have spread to nearby lymph nodes. Results shown in the graphs were obtained as a median (±IQR). (C) Relative mtDNA copy number level in the adjacent normal pancreatic tissue and in the tumors that were pathologically marked as R0 and R1 according to the resection margin status (Kruskal-Wallis test, post test Dunn's Multiple Comparison Test) *, $P < 0.05$. The graph shows the statistical significant difference between the adjacent tissue vs. tumor R0 and between adjacent tissue vs. tumor R1. The R0 corresponds to the specimens from the pancreatic cancers tumors that were pathologically marked as R0 (no cancer cells seen microscopically at the resection margin), while R1 corresponds to the specimens from the pancreatic cancers tumors that were pathologically marked as R1 (cancer cells present microscopically at the resection margin). Results shown in the graphs were obtained as a median (±IQR). (D) The impact of pancreatic cancer tumor localization on the relative mtDNA copy number. The graph shows no difference in mtCN between the tumors localized in the head of the pancreas vs. tumors with other localizations. Results were obtained with Mann Whitney test, $P > 0.05$. Other localizations refer to: the tumors in the body of the pancreas, in the tail of the pancreas and diffuse tumors.

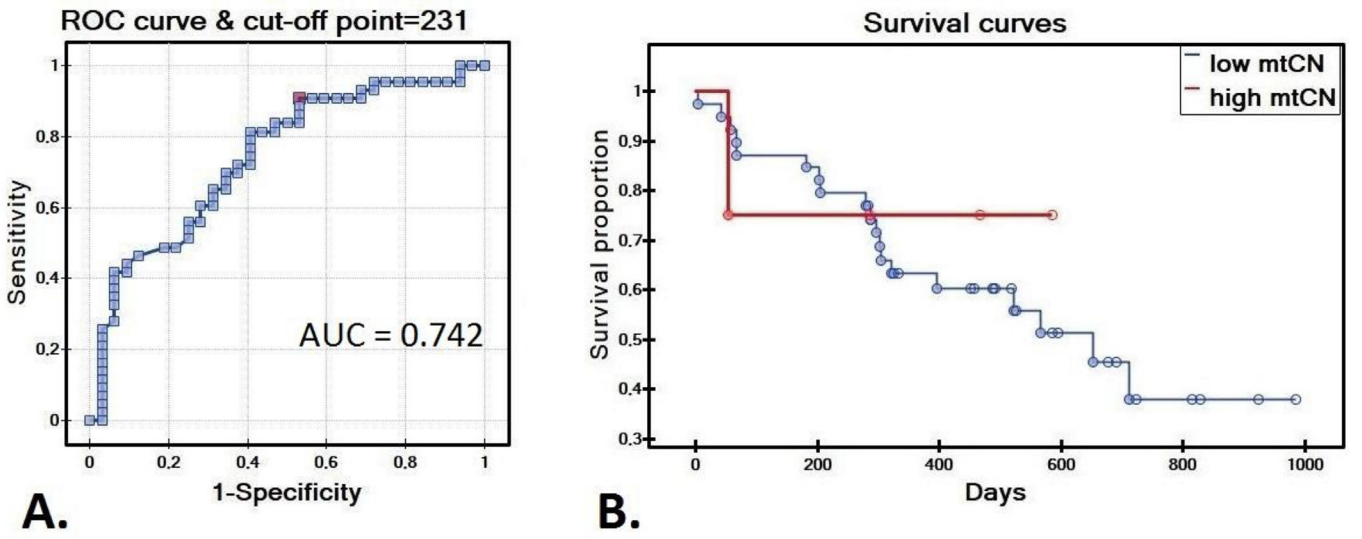

Fig. 3. (A) Receiver operating characteristic (ROC) curve was used to determine an optimal threshold of mtDNA copy number for pancreatic cancer. A P value < 0 .05 was considered statistically significant. AUC – area under the curve. (B) Relative mtDNA copy number measured as *ND1/SCLO2B1* ratio and survival. Kaplan–Meier curves present the overall survival of patients with resectable pancreatic ductal adenocarcinoma with higher (red curve) and lower (blue curve) tumor tissue mtDNA copy number, until 2.7 years of follow-up. Results were compared with the logrank (Mantel-Cox) Test. There were no significant differences in survival between the patients with higher and lower mtDNA copy number ($P > 0.05$).

status. Then we have carried out a survival analysis with the Kaplan-Meier method and compared the results with the logrank (Mantel-Cox) test. Obtained results failed to prove that mitochondrial DNA copy number is a good prognostic marker in resectable pancreatic ductal adenocarcinoma. Data were shown in Fig. 3B.

Discussion

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer with a dismal overall prognosis mainly unchanged over the past decades. Most patients have an unresectable disease with distant metastases at the time of diagnosis. The mean survival in this group is 3–6 months. Only a small number of patients (between 15 and 20%) can be treated completely, which includes radical surgery. The mean survival in this group is 11–24 months. What is more, pancreatic cancer incidence rates continue to rise. Therefore, it is crucial to know the factors affecting the prognosis for the patients to facilitate decision-making on potential individual therapy strategies [26–28].

The association between mtDNA copy number variation and cancer is still subject of debate, with different tumors yielding different results [2,29]. Here we report a comprehensive evaluation of the mitochondrial DNA copy number in pancreatic ductal adenocarcinoma tissue and adjacent normal pancreatic tissue and demonstrate decreased mtDNA content in PC tumor tissue compared to adjacent normal pancreatic tissue. A critical reduction in mtDNA content may compromise mitochondrial functioning with downstream effects. Subsequent changes in cellular processes such as aerobic respiration, calcium homeostasis or the intrinsic apoptotic pathway could, in turn, impact tumorigenic properties. Previous findings have pointed towards a link between low mtDNA content and cancer aggressiveness but the exact association remains uncertain [9]. Pancreatic cancer is known for its extremally aggressive biology and hypoxic microenvironment [30]. It is plausible that a hypoxic environment can reduce tumor mtDNA content as suggested previously [31].

To the best of our knowledge, we provided the first qPCR-based study investigating the mitochondrial DNA copy number (mtCN) in pancreatic cancer (PC) tissue derived from a resected tumor. In the literature, there is the study exploring the mitochondrial genome in pancreatic cancer cell lines and xenografts [32]. Jones et al. performed a Southern blot hybridization to determine the relative amounts of mtDNA in two sets of paired pancreatic cancer xenografts and tissue from the normal duodenum. The results from this study showed an increased intracellular mass of mtDNA in pancreatic cancer cells, which does not correspond to our results. However, Jones et al. comparison included only two pairs of samples. Here, it is worth to notice, that Reznik et al. (who indicated mtCN in over 1000 pairs of tumor/adjacent-normal samples from 15 cancer types) reported that in case of decreased mtDNA content tumors, despite a tendency towards mtDNA depletion, all tumor types contained at least one sample with higher mtDNA content than adjacent normal tissue [2].

The relation between mtDNA content and clinicopathological findings has been reported before for several types of cancer, but not for pancreatic ductal adenocarcinoma. Here, we report that mtDNA copy number decreased as the PC grading increased. Jonckheere-Terpstra testing indicated a statistically significant decrease trend in median mtCN across the differentiation (adjacent normal pancreatic tissue, low grade, intermediate grade, high grade). These observations are consistent with results of a study conducted by Yu et al., who demonstrated that lower mtDNA contents were significantly correlated with a higher histological grade in breast cancer patients [18]. Similarly, Zhang et al. demonstrated that mtDNA copy number was significantly negatively associated with tumor grade in glioma patients [33]. Regarding the lymph node involvement, our results showed no significant differences in mtCN between samples from tumors pathologically marked as N1 and specimens from tumors marked as N0. These results correspond to observations of studies carried out by Chang et al. (colorectal cancer) [34], Lee et al. (gastric cancer) [35] and by Yu et al. (breast cancer) [18]. Regarding margin status, both samples from tumors marked as R0 and from tumors marked as R1 had statistically significantly less mtCN compared to surrounding tissue, which is not surprising due to the fact that the samples from all tumors had statistically significantly less mtCN

compared to the surrounding tissue. Our results showed no significant differences in the mtCN values for tumors marked as R0 and tumors marked as R1. These results are consistent with observations of studies carried out by Chang et al. (colorectal cancer) [34] and Yamada et al. (hepatocellular carcinoma) [36]. Further, our results showed that there were no differences in relative mtDNA copy number between tumors localized in the head of the pancreas and tumors in other localization. This finding also corresponds to observations of studies carried out by Chang et al. (colorectal cancer) [34] and Zhang et al. (gastric cancer) [37].

To address a possible relation between mtDNA content and aggressive behavior *in vivo*, we analyzed primary resectable pancreatic tumor mtDNA content and prognosis in homogenous group of 43 pancreatic cancer patients. This article was the first to analyze the prognostic value of mitochondrial DNA copy number in pancreatic cancer. Our results failed to show an association between the tissue mtCN and overall survival. Nevertheless, they do correspond to conclusions of several studies on such cancers as colon and gastric cancer [35,38] as well as hepatocellular carcinoma [36].

A number of related open questions remain to be resolved, including what mechanisms determine the mtDNA depletion in pancreatic cancer, what are the consequences for cancer biology of mtDNA depletion at various levels and which agents affecting tissue mtCN could indicate the patients who may benefit from the evaluation of tissue mtCN as a potential important prognostic factor in resectable pancreatic cancer.

To conclude, we provided the first qPCR-based study investigating the mitochondrial DNA copy number in pancreatic cancer tissue and the impact of mtCN on overall survival in PC patients. We demonstrate significantly lower mtDNA content in pancreatic cancer tissue compared to adjacent normal tissue. Also, our results failed to prove that mitochondrial DNA copy number is a good prognostic marker in resectable pancreatic ductal adenocarcinoma. Larger cohorts of uniformly treated patients are necessary to validate these results and to further unravel the clinical relevance of mtDNA content determination in cancer.

Author contributions

1. Julia Tuchalska-Czuroń: project conceptualization, data curation, funding acquisition, investigation, resources, writing – original draft.

2. Jacek Lenart: project conceptualization, investigation, methodology, resources, writing – review, and editing.

3. Justyna Augustyniak: project conceptualization, formal analysis, methodology, resources, writing – review, and editing.

4. Marek Durlik: project conceptualization, funding acquisition, resources, supervision, writing – review, and editing.

Conflict of interest disclosure

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2018.11.009.

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