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EXPRESSION OF YPEL1 IN PANCREATIC CANCER CELL LINES AND TISSUES

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Pancreatic cancer is one of the most aggressive tumors of the digestive tract. The locally advanced stage of the disease at the time of diagnosis and early recurrence after curative resection makes pancreatic cancer the fourth leading cause of cancer-related deaths in the Western world [1,2]. Thus, the prognosis of pancreatic cancer is extremely poor with a 5-year-survival rate of less than 5% when all stages are combined [2]. Absence of early symptoms leading to late diagnosis, the highly aggressive nature of the tumor with rapid metastasis to and invasion of lymph nodes, liver, intra- and extrapancreatic nerve structures are the main characteristics of this malignancy. Increased trans-differentiation from an epithelial cellular structure to a more mesenchymal phenotype is also an important molecular aspect. This particularly involves an increased migratory and invasive potential which is a "prerequisite" for invasion and distant metastasis. Perineural invasion (PNI) is a prominent and general feature of pancreatic cancer, known as an important prognostic factor since it causes retropancreatic tumor extension and therefore precludes curative resection [3]. Furthermore perineural invasion has a strong impact on local recurrence after curative tumor resection.

YPEL1 is a previously classified human protein which belongs to YPEL family. YPEL1 demonstrates a striking degree of conservation between species but has no known biochemical function nor can any function be inferred due to a lack of homology with any other characterized genes [4]. It appears to be a ubiquitous intracellular protein with a potential to bind zinc and a capacity to interact with itself [5]. Recently we have identified YPEL1 as potentially involved in perineural invasion of pancreatic cancer [6]. Since it was previously reported that YPEL1 is related to mesenchymal to epithelial transformation of cells [4], and since pancreatic cancer is characterized by a loss of an epithelial phenotype, in the current study we analyzed the expression of this gene in bulk pancreatic cancer tissues and cell lines.

Materials and methods. *Cell Culture*
Pancreatic cancer cells were routinely grown in RPMI medium supplemented with 10% fetal calf serum (FCS), 100 units/mL penicillin, and 100 µg/mL streptomycin (complete medium). Cells were maintained at 37°C in a humid chamber with 5% CO2 and 95% air atmosphere.

Tissue Sampling. Pancreatic tissue specimens were obtained from patients who underwent pancreatic resection at the surgical clinics of the University Heidelberg and the Technische Universität München or through an organ donor program from previously healthy individuals. The Human Subjects Committee of the University of Heidelberg and the Technische Universität München, Germany, approved all studies. Written informed consent was obtained from all patients.

Real-time quantitative polymerase chain reaction (QRT-PCR). All reagents and equipment for mRNA/cDNA preparation were supplied by Roche Applied Science (Mannheim, Germany) [7]. Quantitative RT-PCR (QT-PCR) was carried out using the Light-Cycler FastStart DNA SYBR Green kit. The number of specific transcripts was normalized to the housekeeping gene cyclophilin B (cpb) and presented as copies/10,000 copies cpb. All primers were obtained from Search-LC (Heidelberg, Germany).

Results were expressed as mean ± standard error of the mean (SEM). For statistical analyses the non-parametric Mann-Whitney U test was used unless indicated otherwise. Significance was defined as $p<0.05$. Survival analysis was carried out using the Kaplan-Meier method for estimation of event rates and the log-rank test for survival comparison between patient groups.

Results and discussion. Recently we have developed an ex-vivo perineural invasion model for pancreatic cancer and described the consensus transcriptome signature of perineural invasion [6]. One of the genes which were found to be deregulated in perineural invasive cells was YPEL1. In this study we sought to analyze endogenous expression of YPEL1 in 9 cultured pancreatic cancer cell lines. Quantitative real time PCR demonstrated moderate to low expression of this gene in all cell lines (Figure 1). Highest levels of YPEL1 expression was observed in Panc-1 cells (88.0±11.0 copies/10,000 copies cpb) whereas lowest expression was detected in Colo357 pancreatic cancer cells (4.3±0.3 copies/10,000 copies cpb). We also performed hypoxic treatment of Capan2 cells; however these experiments did not reveal induction or repression of YPEL1 expression by hypoxia in these cancer cells (Figure 1).

Next we analyzed the expression of YPEL1 mRNA in pancreatic bulk tissues. 19 normal pancreas (organ donor), 19 chronic pancreatitis and 31 pancreatic ductal adenocarcinoma tissue samples were used for this experiment. Quantitative PCR revealed significantly downregulated expression of YPEL1 in human pancreatic cancer tissues compared to normal pancreatic tissues (Figure 2, $p<0.05$). Previous studies have demonstrated a role of YPEL1 in

the regulation of mesenchymal to epithelial-like transition [4]. It is well known that pancreatic cancer cells lose their epithelial morphology and gain more mesenchymal characteristics during malignant transformation [8]. It is difficult to say whether reduced expression of YPEL1 in pancreatic cancer (Figure 2) has a key regulatory role in this process; however, downregulation of YPEL1 appears to be due to the loss of epithelial morphology of these tumors. We studied the survival of pancreatic cancer patients depending on YPEL1 tissue expression. The median YPEL1 value in pancreatic cancer patients was taken as a cut-off to compare patients with high/low YPEL1 mRNA levels using the Kaplan-Meier method. Interestingly higher YPEL1 mRNA levels indicated a tendency for a worsened prognosis for patients (Figure 3). Thus, the median survival in the high YPEL1 group was 13 months versus 16 months in the YPEL1 low group. However this difference did not reach statistically significance (p=0.17).

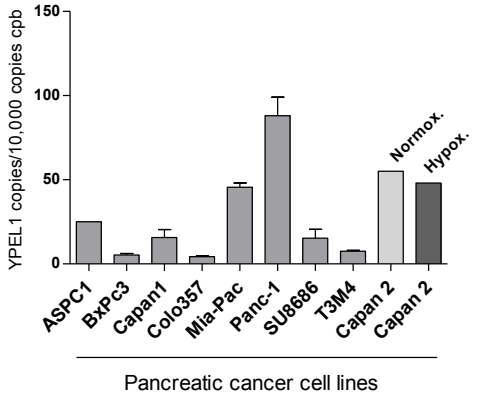

Fig. 1. Expression of YPEL1 in pancreatic cancer cells

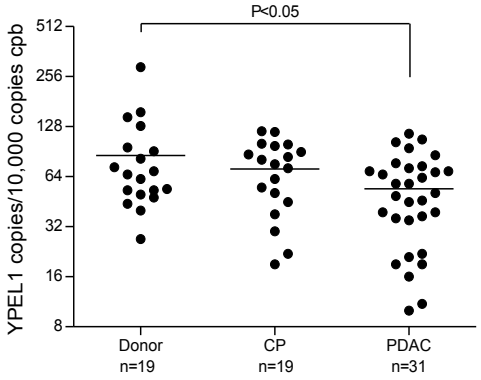

Fig. 2. Expression of YPEL1 in human pancreatic tissues

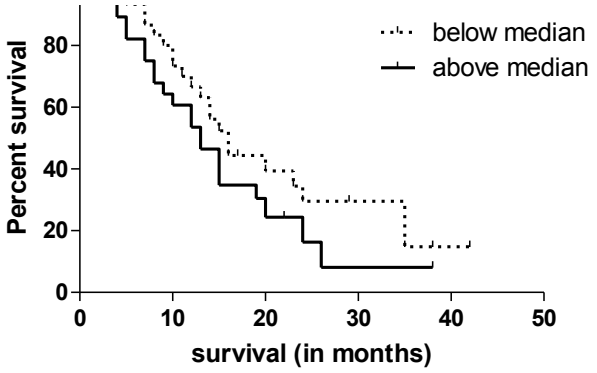

Fig. 3. Survival of pancreatic cancer patients depending on YPEL1 tissue expression

YPEL1 is localized at centrosomal part of nuclei [9] and is conserved between humans, mice and birds. Over-expression of this protein in fibroblasts alters vimentin and actin cytoskeletal components [4], thus changing the morphogenetic behavior towards an epithelial-like phenotype. One of the important events of normal and malignant morphogenesis is the regulation of a cellular phenotype between a mesenchymal, motile cell type, and an epithelial, sessile cell type. Therefore YPEL1 might be an important factor during the development and malignant transformation of tissues. Further studies are required to better assess the role of human YPEL1 in pancreatic cancer pathogenesis.

REFERENCES

1. Jemal A., et al. Cancer statistics, 2009. CA Cancer J Clin, 2009. 59(4): p. 225-49.
2. Kleeff J. et al. Pancreatic cancer: from bench to 5-year survival. Pancreas 2006; 33(2): 111-8.
3. Esposito I. et al. Most pancreatic cancer resections are R1 resections. Ann Surg Oncol. 2008; 15(6): 1651-60.
4. Farlie P. et al. Ypel1: a novel nuclear protein that induces an epithelial-like morphology in fibroblasts. Genes Cells 2001; 6(7): 619-29.
5. Roxstrom-Lindquist K., Faye I. The Drosophila gene Yippee reveals a novel family of putative zinc binding proteins highly conserved among eukaryotes. Insect Mol Biol. 2001; 10(1): 77-86.
6. Abiatari I. et al. Consensus transcriptome signature of perineural invasion in pancreatic carcinoma. Mol Cancer Ther. 2009; 8(6): 1494-504.
7. Abiatari I. et al. Moesin dependent cytoskeleton remodeling is associated with an anaplastic phenotype of pancreatic. Cancer. J Cell Mol Med. 2009.
8. Arumugam T. et al. Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. Cancer Res. 2009; 69(14): 5820-8.

9. Hosono K. et al. Identification and characterization of a novel gene family YPEL in a wide spectrum of eukaryotic species. Gene 2004; 340(1): 31-43.

SUMMARY

EXPRESSION OF YPEL1 IN PANCREATIC CANCER CELL LINES AND TISSUES

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YPEL1 is a nuclear protein that is suggested to be involved in mesenchymal to epithelial-like transition during tissue development. Recently we have identified YPEL1 as a gene whose expression is deregulation in perineural invasive pancreatic cancer cells. In this study we assessed the expression of YPEL1 in normal and diseased pancreatic tissues and pancreatic cancer cell lines. Quantitative real time polymerase chain reaction was used to analyze the expression of YPEL1 mRNA in nine cultured pancreatic cancer cell lines and pancreatic bulk tissues of the normal pancreas (n=19), chronic pancreatitis (n=19) and pancreatic adenocarcinoma tissues (n=31). Quantitative real time polymerase chain reaction analysis revealed a significant down-regulation of YPEL1 mRNA expression in pancreatic adenocarcinoma tissues compared to normal tissues (54.1±5.2 vs. 85.8±14.1 copies/10,000 copies cpb) and low expression of this gene indicated a tendency for better survival of pancreatic cancer patients (16 vs. 13 months; p=0.17). Expression of YPEL1 mRNA was present in all tested pancreatic cancer cell lines with comparably low to moderate expression levels of 4.3 – 88.0 copies/10,000 copies cpb. Reduced expression of YPEL1 in pancreatic cancer might be related to perineural invasion. and prognosis. YPEL1 might be an important factor during the development and malignant transformation of tissues. Further studies are required to better assess the role of human YPEL1 in pancreatic cancer pathogenesis.

Key words: pancreatic cancer, Yippee-like 1, YPEL.

РЕЗЮМЕ

ЭКСПРЕССИЯ ГЕНА YPEL1 В КЛЕТКИ И ТКАНИ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ ПРИ РАКЕ

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Предполагается, что ген YPEL1 вовлечен в процесс мезенхимально-эпителиальной трансформации клеток в течение развития тканей. Авторами выявлен ген YPEL1, экспрессия которого была дерегулирована в периневрально-инвазивных опухолевых клетках поджелудочной железы. В данном исследовании была проанализирована экспрессия YPEL1 в нормальных и патологических тканях поджелудочной железы, а также в клеточных линиях рака поджелудочной железы. Методом полимеразной цепной реакции в реальном времени исследовали экспрессию гена YPEL1 в клеточных линиях рака поджелудочной железы, в ткани поджелудочной железы доноров, в ткани поджелудочной железы при хроническом панкреатите, а также в ткани протоковой аденокарциномы поджелудочной железы. Выявлено значительное понижение YPEL1 в ткани протоковой аденокарциномы поджелудочной железы по сравнению с тканью поджелудочной железы доноров (54,06 5,22; 85,84 14,07 копий/10.000 копий циклофилина Б, соответственно). При низких уровнях экспрессии гена была выявлена тенденция к увеличению продолжительности жизни больных с протоковой аденокарциномой поджелудочной железы (16 против 13-и месяцев; p=0.17). Экспрессия YPEL1, от сравнительно низкого до умеренного уровней, была выявлена во всех исследованных клеточных линиях рака поджелудочной железы (4,3 - 88,0 копий/10.000 копий циклофилин Б). Понижение экспрессии YPEL1 при раке поджелудочной железы возможно связано с периневрально инвазией. YPEL1 можно рассматривать как значимый фактор злокачественного перерождения ткани поджелудочной железы.

Необходимы дальнейшие исследования для оценки роли человеческого YPEL1 в патогенезе рака поджелудочной железы.