ABL1 mRNA LEVELS IN PANCREATIC CANCER AND CHRONIC PANCREATITIS

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Summary
Pancreatic cancer is one of the most aggressive malignant tumours, being the fourth cancer-related death cause in Western countries. In this study we evaluated the expression profiles of ABL1 mRNA in pancreatic cancer and chronic pancreatitis. 31 patients with pancreatic focal masses biopsied by EUS-guided FNA were included in this study. A two-step qRT-PCR was performed for evaluation of ABL1 expression. Total RNA was successfully isolated from all the pancreatic tissue samples. ABL1 expressed in 70% of chronic pancreatitis and in 76.19% of pancreatic cancers, and its expression was significantly higher in pancreatic cancer compared with chronic pancreatitis. In conclusion, despite of the relatively small number of patients and consequent limitations in statistical analysis, this study has demonstrated that ABL1 is over-expressed in pancreatic cancer compared with chronic pancreatitis.

Key words: pancreatic cancer, chronic pancreatitis, EUS-FNA, ABL1, qRT-PCR.

Introduction
Pancreatic cancer is one of the most aggressive malignant tumours, being the fourth cancer-related death cause. The five-year survival rate is less than 5% (Jemal et al., 2010). The main reason for this is represented by the failure to achieve significant improvements in survival with conventional therapies, such as chemotherapy, radiotherapy, anti-hormonal therapy or monoclonal antibodies.

Various studies have focused on identifying molecular markers for pancreatic carcinogenesis, and possible correlation with clinico-pathological parameters and prognosis. Over 200 genes are involved in the pathogenesis of pancreatic cancer. In pancreatic ductal adenocarcinoma (PDAC), the most common pancreatic cancer, a series of changes regarding the pattern of tumour progression starting from intraductal epithelium to the invasive pancreatic cancer (Garcea et al., 2005).

Pancreatic neuroendocrine tumours (PNETs) usually occur sporadically, but they can also belong to a number of inherited syndromes and are associated with mutations in oncogenes and tumor suppressor genes (Gu et al., 2012).

Chronic pancreatitis is a pancreatic inflammation, with symptoms that can mimic pancreatic cancer, and differentiating the two diseases is difficult in the absence of well-established biomarkers.

The ABL1 proto-oncogene encodes a cytoplasmic and nuclear protein tyrosine kinase, involved in processes of cell differentiation, cell division, cell adhesion, and stress response. When DNA damage occurs and ABL1 is activated, two responses may occur: cell cycle arrest and apoptosis, but the underlying mechanisms are unclear.

Selective tyrosine kinase inhibitors have been developed, among them being STI571 (Gleevec, imatinib mesylate, Novartis Pharmaceuticals) which has...
selectivity for ABL1, PDGF receptor and c-kit receptor tyrosine kinases. Gleevec inhibits pancreatic cancer cell growth in a tyrosine kinase receptor independent manner (Junsheng et al., 2003).

The aim of our study was to evaluate the gene expression profiles of ABL1 mRNA in pancreatic cancer and chronic pancreatitis and to determine the correlations between the expression levels and clinico-pathological parameters of the tumors.

Material and methods

Patients and samples. We included in this study 31 patients who had undergone endoscopic ultrasonography (EUS) followed by fine-needle aspiration (FNA) of the focal pancreatic masses at the Research Centre of Gastroenterology and Hepatology of Craiova, between 2009-2011. The samples were collected in RNALater solution (Ambion Inc., Austin, Texas, US), kept at 4°C for 12-24 hours, and further stored at -80°C until RNA isolation. All the samples were examined at the Department of Pathology, University of Medicine and Pharmacy of Craiova. The study was approved by the Ethical Committee of the University of Medicine and Pharmacy of Craiova, Romania and informed consent for EUS-guided FNA, followed by molecular studies was obtained from each of the patients.

RNA isolation. Total RNA was isolated from the tissue using the SV Total RNA Isolation System (Promega, Madison, WI, USA). The RNA concentration and purity were measured spectrophotometrically (Eppendorf Biophotometer, Eppendorf, AG, Hamburg, Germany) and the integrity was assessed using the Agilent 2010 Bioanalyzer (Agilent Technologies Inc., US).

Quantitative RT-PCR. For the evaluation of mRNA levels, a two step quantitative Real-Time PCR was performed. In the first step 100ng total RNA was reverse transcribed into complementary DNA (cDNA). The reverse-transcription was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, US). The reverse-transcription reactions were carried out in 20µl volume and prior to use in PCR reaction, the cDNA was diluted 1:10 in Nuclease Free Water. Quantitative Real-Time PCR was performed using TaqMan® Gene Expression Master Mix (Applied Biosystems, Foster City, CA, US) with specific primers and TaqMan® probes for target genes and for endogenous control gene (ABL1 - Hs 00245443_m1 and GAPDH - Hs99999905_m1). The amplifications were carried out in 20 µl volume, in triplicate, on a ViiA™ 7 System (Applied Biosystems, Foster City, CA, US). The cycling parameters were as follows: 50°C for 2-minutes, 95°C for 10 minutes, followed by 50 cycles of PCR at 95°C for 15 seconds and 60°C for 1 minute. Three no template control reactions (NTC) were performed as negative control.

The expression of the target gene was normalised to the GAPDH endogenous control gene and the results are shown as relative mRNA expression.

Statistics. Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to determine the distribution of the values. F test was performed to compare variances between groups and Unpaired t test with Welch's correction was used for statistical analysis of ABL1 mRNA expression between groups of samples. For assessing differences in genes expression according to clinico-pathological parameters, Mann-Whitney tests were performed. Two-tailed p values <0.05 were considered statistically significant. The data were analysed using GraphPad Prism 5 and GraphPad InStat software (GraphPad Software, Inc, CA, US).

Results

Pancreatic tissue was collected by EUS-guided FNA from 31 patients with focal pancreatic masses, at the Research Centre in Gastroenterology and Hepatology
of Craiova. Based on EUS, elastography and cytopathological examination, 21 patients were diagnosed with pancreatic cancer and 10 with chronic pancreatitis (CP).

Total RNA was successfully isolated from all the pancreatic tissue samples. At least 200 ng total RNA was isolated from each sample, and the 260/280 and 260/230 ratios were within the recommended ranges for using in reverse-transcription reaction. The RNA Integrity Numbers (RINs) were generally lower than 5. To investigate ABL1 mRNA expression, relative mRNA levels (ABL1/GAPDH) were assessed in all the samples and compared between pancreatic cancer and chronic pancreatitis.

ABL1 expressed in 70% of chronic pancreatitis and in 76.19% of pancreatic cancers. ABL1 expression was significantly higher in pancreatic cancer compared with chronic pancreatitis ($p=0.018$, Unpaired t test with Welch’s correction) (Figure 1).

ABL1 mRNA levels in pancreatic cancer were correlated with several clinico-pathological characteristics: age, gender, tumor location, tumor size, local invasiveness, lymph nodes metastasis and liver metastasis (Table 1). ABL1 expression was higher in tumors smaller than 3 cm in size compared with tumors greater than 3 cm.

**Discussions**

ABL1 is the product of the cellular homologue of the transforming gene of Abelson murine leukaemia virus. ABL1, namely the BCR-ABL1 tyrosine-kinase, has been involved in the pathogenesis of chronic myeloid leukemia (Ernst and Hochhaus, 2012), but the data regarding ABL1 in solid tumours are scarce.

![Figure 1](image_url). Comparative ABL1 relative expression in chronic pancreatitis and pancreatic cancer. $n=31$. Unpaired t test with Welch correction
In this study we evaluated the mRNA expression of ABL1 in chronic pancreatitis and malignant tumours of the pancreas (adenocarcinomas and PNETs), in EUS-guided FNA samples. Total RNA was successfully isolated from all the samples, with acceptable concentrations and purities. The quality of RNA, assessed by RIN calculation, revealed a partially degraded RNA, without effects on the evaluation of gene by qRT-PCR, since it has been shown that PCR efficiency does not vary with RIN when small amplicons are generated (Fleige and Pfaffl., 2006). Our results suggest that ABL1 is over-expressed in pancreatic cancer compared with chronic pancreatitis.

ABL1 protein is localised in nucleus and cytoplasm. According to its localisation, ABL1 has distinct roles: nuclear ABL1 induces arrest of the cell cycle in G1-phase and apoptosis, whereas cytoplasmic ABL1 activates mitogenic and anti-apoptotic pathways (Vigneri and Wang, 2001). The effects of ABL1 occur upon multiple protein-protein and protein-DNA interactions and its tyrosine kinase domain. Nuclear ABL1 tyrosine-kinase promotes apoptosis as a response to the stress produced by DNA (Kharbanda et al., 1995; Van Etten, 1999;

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<table>
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<th>Patients characteristics</th>
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<tr>
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Table 1. Correlation between ABL1 relative expression and clinico-pathological parameters. Relative expression mean±SEM. Mann-Whitney test
Shaul Y, 2000). The over-expression shown in our study in pancreatic cancer compared with chronic pancreatitis, can be explained by DNA damage that triggers ABL1 expression in malignant cells.

ABL1 has been found constitutively activated in breast cancer cell lines (Srinivasan and Plattner, 2006) and anaplastic thyroid cancers cells (Podtcheko et al., 2003).

Furthermore, ABL1 is activated after platelet-derived-growth factor receptor (PDGFR) stimulation and modulates the chemotactic response, thus playing an important role in cancer invasion (Plattner et al., 2003). The over-expression of ABL1 in pancreatic cancer found in our study may be a marker of invasiveness of small pancreatic tumours, in which the expression was higher than in greater tumours.

Conclusions
In conclusion, despite of the relatively small number of patients and consequent limitations in statistical analysis, this study has demonstrated that ABL1 is over-expressed in pancreatic cancer compared with chronic pancreatitis. Further studies, on higher number of patients, are required to determine the feasibility of using ABL1 as a biomarker to differentiate between chronic pancreatitis and pancreatic cancer.

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