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Contract nr: POSDRU/88/1.5/S/52826

Beneficiar
Universitatea de Medicină şi Farmacie din Craiova

UNIVERSITY OF MEDICINE AND
PHARMACY OF CRAIOVA

PhD Thesis
-Abstract-

Angiogenesis in gastric and pancreatic neuroendocrine tumours

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Craiova, 2012

CONTENTS

Introduction

The term “carcinoid” (“karzinoide”) was used for the first time by Oberndorfer in 1907 to define a particular type of tumours with an intermediary malignant potential. Actually the term carcinoid is as a synomin for neuroendocrine tumours (NETs).

NETs are malignancies that can arise from neuroendocrine cells throughout the body. These tumors produce peptides that cause characteristic hormonal syndromes.

Neuroendocrine tumours of the stomach arise from corpus enterochromaffin-like cells, that produce histamine that regulates acid secretion. The majority of gastric carcinoids are accompanied by hypergastrinaemia and achlorhydria. The diagnosis of gastric carcinoids has increased with the widespread use endoscopy techniques.

Originating from the diffuse neuroendocrine cell system, pancreatic neuroendocrine tumours (PNETs) are rare pancreatic tumours. Based on clinical manifestations PNETs are classified into functional and non-functional tumors. Functional tumors are associated with a clinical syndrome caused by the ectopic secretion of a hormone and includes: gastrinoma, insulinoma, glucagonoma, VIPoma, somatostatinoma, GRFoma, ACTHoma, GHRHomas, *(Ehehalt et al., 2009, Metz et al., 2008)*. Non-functional PNETs are tumors with endocrine differentiation but without clinical manifestations caused by hormone hypersecretion *(O'Grady HL, Conlon KC, 2008)*.

For the development of solid malignancies angiogenesis is a crucial event, allowing the local growth and spreading of the tumour by supplying oxygen and nutrients. Since the 1970s when Folkman proposed this concept, many researchers have aimed to find correlations between tumour angiogenesis and cancer invasiveness or patients survival and to inhibit angiogenesis as a part of cancer therapy *(Folkman, 1971)*.

It has also been shown that VEGF-A is expressed by well-differentiated neuroendocrine tumour cells, while its expression is variable in poorly differentiated neuroendocrine tumours *(La Rosa et al., 2003; Couvelard et al., 2006)*, but only few studies have been performed on gastric or pancreatic carcinoids, which are highly vascular tumours. In addition, unlike the most carcinomas, in neuroendocrine tumours the hypervascular phenotype seems to be associated with a low metastatic potential and good prognosis.

Furthermore, there are only few data in the literature about the role of contrast-enhanced power Doppler endoscopic ultrasound (EUS) in PNET. According to recently published data, contrast-enhanced EUS is a feasible method for the diagnosis of PNET but there are limitations in terms of differentiation between chronic pancreatitis and PNET, due to the same vascular pattern

(Napoleon et al., 2010). European Neuroendocrine Tumour Society (ENETS) established the role of EUS in diagnosis of PNET, EUS-FNA being mandatory for the diagnosis of these patients *(Atiq et al., 2011)*. In addition, the immunostaining with chromogranine A and synaptophysine is always necessary *(Lloyd, 2003; Bussolati et al., 2001; Klöppel et al., 2009)*.

Continuous research to elucidate the molecular events of neuroendocrine tumours carcinogenesis and behaviour, as well as establishing new markers associated with disease progression and prognosis are essential.

Keywords: gastric neuroendocrine tumours, pancreatic neuroendocrine tumours, carcinoids, angiogenesis, VEGF, EGFR, microvascular density, imunohistochemestry, Real-Time PCR, endoscopic ultrasound (EUS), Doppler.

I. LITERATURE REVIEW

Chapter 1, entitled ***"Neuroendocrine tumours of the stomach"***, describes the epidemiology, classification, histopathology, staging and diagnosis of gastric neuroendocrine tumours.

Chapter 2, entitled ***"Neuroendocrine tumours of the pancreas"***, describes aspects of the epidemiology, classification, histopathology, staging and imagistical diagnosis of pancreatic neuroendocrine tumors.

Chapter 3, entitled ***"Angiogenesis in gastric and pancreatic neuroendocrine tumours"***, presents aspects of normal and tumoral angiogenesis. It also describes the role of pro-angiogenic factors involved in the angiogenesis of gastrointestinal neuroendocrine tumours.

II. PERSONAL CONTRIBUTIONS

Chapter 4. MVD and VEGF-A expression in gastric neuroendocrine tumours

In this chapter, we aimed to characterise the gastric carcinoids from the perspective of microvascular density and VEGF-A gene and protein levels in order to gain insights into the process of angiogenesis in these particular tumours.

Material and Methods

Patients and samples. Eighteen patients with gastric carcinoids investigated by upper gastro-intestinal endoscopy followed by endoscopic biopsy, at The Royal Liverpool Hospital, between 2006 and 2008 were included in this study. For gene expression analysis, the samples were collected in RNALater and stored at -80°C until RNA isolation. Forty-two patients were included in the control group.

For the immunohistochemical evaluation of VEGF-A and MVD, specimens collected by upper GI endoscopy from seven patients with gastric carcinoids patients (carcinoids or micro-carcinoids) and two control patients were included (H. pylori negative).

RNA isolation and purification. Total RNA from all the specimens was isolated using *TRI Reagent® (Sigma Aldrich, USA)*. Total RNA was then purified using *High Pure RNA Tissue Kit (Roche Applied Science, Mannheim, Germany)*. Total purified RNA was stored at -80 ℃ until reverse-transcription.

Two-Step RT-PCR. In the first step The Transcriptor First Strand cDNA Synthesis Kit *(Roche Applied Science, Mannheim, Germany)* was used to reverse transcribe the total RNA. Anchored-oligo(dT)18 primers were used for reverse transcription in order to achieve specificity of the

reaction. 100 ng of total RNA from each specimen was used for reverse transcription. All the reactions were carried out in 20µl volumes, in sterile, nuclease-free, thin walled PCR tubes.

In the second step of the two-step PCR, the cDNA is amplified and quantified using the LightCycler® 480 Probes Master (*Roche Applied Science, Mannheim, Germany*). Specific primers for human VEGF-A and endogenous control GAPDH were purchased from Eurogentec (*Eurogentec, Belgium*). Specific probes from Universal Probe Library (*Roche Applied Science, Mannheim, Germany*) were used with each primer set.

Immunohistochemistry study. Both NETs and normal specimens were fixed in 10% formaldehyde for 24 hours. After automatically processing and embedding in paraffin, 4µm thick seriated sections were cut and mounted on APES (3- aminopropyltriethoxysilane) coated slides. The neuroendocrine areas were identified by immunostaining with chromogranin A. Seriated sections were stained with VEGF-A and von Willebrand factor (vW) antibodies.

Results and discussions.

VEGF-A mRNA expression. VEGF-A mRNA relative levels (VEGF-A/GAPDH) did not vary biological or statistically significant between the carcinoids and the control group, both groups showing the same expression pattern.

VEGF-A mRNA levels were analysed according to several clinico-pathological parameters: age, gender, H. pylori histopathology and pathological characteristics of the tumours. No correlations were found between VEGF-A expression and gender, H. pylori histopathology or pathological characteristics of the gastric carcinoids. On the contrary, VEGF-A expression was higher in patients under 65 years old and lower in patients over 65 years old ($p<0.001$, Mann-Whitney test).

We analysed possible correlations between VEGF-A expression, gastrin, vitamin B12 and ferritin levels, and gastroscopic characteristics of carcinoid nodules (number and maximum size). A strong negative correlation at a statistically significant level was found between VEGF-A relative mRNA and ferritin levels ($r=-0.76$, $p=0.04$). Also, without being the aim of our study, we have found a strong negative correlation between gastrin and vitamin B12 levels, at the borderline statistical significance ($r=-0.83$, $p=0.06$).

Immunohistochemistry study. The mean number of vessels that were stained with vW factor antibody, per microscopic field was 8.46±0.87 in the chromogranin A positive areas and 5.9±0.4 in normal specimens (1.43-fold increase in MVD). This difference in MDV between carcinoid areas and normal specimens was only borderline statistically significant ($p=0.06$, Mann Whitney test)

We analysed VEGF-A immunoexpression and we have noticed a cytoplasmic pattern with homogenous distribution for VEGF-A immunostaining with anti- VEGF-A antibody, VG1 clone. The intensity of reaction was variable between tumours and normal specimens and in different areas of the same specimens With the exception of one normal gastric mucosa specimen, all the slides

showed a moderately or strongly positive immunostaining for VEGF-A. VEGF-A immunoexpression was significantly higher in gastric carcinoids compared with normal mucosa of the stomach (p=0.01, Mann-Whitney test).

VEGF-A immunoexpression was significantly higher in carcinoids (specifically in the chromogranin-A - positive areas) when compared with normal gastric mucosa. This increase in VEGF-A immunoexpression was due mostly to an increase in the quantity of protein produced by each cell (immunointensity), characterised by a cytoplasmic pattern with homogenous distribution, whereas the percentage of positive cells only increased slightly. It has already been shown that VEGF-A is synthesised by normal gastro-pancreatic endocrine cells and well differentiated NETs (*Vidal et al., 2000; La Rosa et al., 2003*). VEGF-A has been even proposed as an indirect marker of differentiation (*Poncet et al., 2009*) and the hypothesis that VEGF-A, by maintaining vascular integrity also indirectly stimulates hormone secretion by NET cells was also proposed (*Terris et al., 1998)*.

High VEGF-A expression levels in neuroendocrine tumours have also been shown by other authors, but the reported results are controversially. While some authors have shown that a strong VEGF-A expression was correlated with increased angiogenesis and poor prognosis in GEP-NETs patients (*Zhang et al., 2007*), in a recent study no correlation has been established between VEGF-A tissue levels quantified by ELISA and clinicopathological parameters *(Kuiper et al., 2011)*.

In our study, MVD was higher in carcinoids than in normal gastric mucosa, and this difference was borderline statistically significant. Furthermore, VEGF-A immunoexpression levels and MVD were not correlated in our study. The lack of correlation between VEGF and VEGF receptors immunoexpression and MVD has also been found by other authors *(La Rosa el al., 2003)*. This finding is in accordance with the hypothesis that in digestive neuroendocrine tumours VEGF-A expression is not associated with progression, invasiveness and metastasising *(La Rosa et al., 2003; Kuiper et al., 2011)*. In contrast with the situation found in the majority of cancers, in GEP-NETs a high MVD is associated with good prognosis and low metastasizing. This hypothesis is supported by the lack of association in our study between VEGF-A mRNA expression and number and size of the nodules, as well as with other histopathologically characteristics.

We have also observed a strong negative correlation between ferritin and VEGF-A mRNA levels. Iron depletion increases the hypoxia-inducible factor-1 alpha (HIF-1α) production, which is a well known stimulator of VEGF-A secretion (*Dongiovanni et al., 2008*). Furthermore, an alteration of H:L chains ratio alters VEGF-A expression *(Harned et al., 2010)*. A serum iron and

ferritin deficiency has been observed in pre-menopausal women *(Ali et al., 2003)*. The majority of patients in our study were women. Moreover the VEGF-A mRNA expression was significantly lower in older than in younger patients. We can therefore reinforce the importance of ferritin levels in regulation of VEGF-A expression and thus in modulation of angiogenesis

In summary, we can propose the hypothesis that in gastric carcinoids, VEGF-A is involved in maintaining of endothelial architecture and functions and cannot be used as a marker associated with new blood vessels formation, thus with tumour invasiveness. Therefore, an anti-angiogenic therapy in these types of tumours would be inappropriate, but these does not exclude the utility of a clinical trial.

Conclusions.

VEGF-A protein is highly expressed by carcinoid cells (chromogranin-A positive cells), whereas no biological or statistical significant difference was observed for VEGF-A mRNA between carcinoid and control groups.

No correlation exists between VEGF-A and MVD. Thus, VEGF-A is mainly involved in maintaining endothelial architecture and functions and its use as a marker associated with new blood vessels formation is not feasible.

Gastrin does not promote VEGF-A gene transcription, but a post-transcriptional expression regulation could be possible.

Low ferritin levels, a marker of iron deficiency into the cells, is correlated with VEGF-A over-expression, being involved in modulation of angiogenesis in gastric carcinoids. Thus, ferritin levels may be indirectly be a serum marker for angiogenesis.

Chapter 5. VEGF and EGFR expression in pancreatic ductal adenocarcinomas and neuroendocrine tumours.

Material and Methods.

Patients and samples. Pancreatic tissue samples collected from thirty-five patients who had undergone endoscopic ultrasonography (EUS) and elastographic examination followed by fine-needle aspiration (FNA) of the focal pancreatic masses at the Research Centre in Gastroenterology and Hepatology of Craiova, Romania, between 2009-2011, were included in this study. The samples were collected in RNAlater solution (*Ambion, Inc., Austin, Texas, US*) and stored at -80°C.

RNA isolation, concentration, purity and integrity assessment. SV Total RNA Isolation System (Promega) was used for the isolation and purification of total RNA from tissue samples. The RNA concentration and purity were measured spectrophotometrically (Eppendorf Biophotometer) and the quality of RNA was assessed by denaturing agarose gel electrophoresis.

Reverse-Transcription. The reverse-transcription was performed using *High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA)*. The reverse-transcription reactions were carried out in 20µl volume; the input amount of total RNA was 100 ng diluted to a volume of 10µl in Nuclease Free Water.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). The cDNA was diluted 1:10 in Nuclease Free Water prior to use in PCR reaction. At least one no template control reaction (NTC) was performed in each run as negative control. 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 (VEGF - Hs00900054_m1, EGFR - Hs01076092_m1 and GAPDH - Hs99999905_m1). The amplifications were carried out in 20 µl volume, in triplicate, on a Rotor-Gene 6200 HRM (*Corbett Life Science, Sydney, Australia*). The cycling parameters were: 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.

Results and discussions.

VEGF expression was detected in all chronic pancreatitis and adenocarcinoma samples and in only 62.5% of pancreatic neuroendocrine tumours. EGFR expression was detected in only 40% of the cases of chronic pancreatitis, 76.9% of adenocarcinomas and in 50% of PNETs. Both VEGF and EGFR mRNA levels were significantly higher in pancreatic ductal adenocarcinoma when compared with normal tissue. VEGF expression inversely correlated with pancreatic ductal adenocarcinoma size, while EGFR expression was related to local invasiveness of adenocarcinomas.

Several immunohistochemistry studies based on surgical samples have shown an over-expression of VEGF in both pancreatic adenocarcinomas and PNETs, but the reported findings concerning the association between its expression and clinico-pathological features and prognosis are still controversial. For pancreatic adenocarcinoma patients it was found that VEGF expression

was associated with poor prognosis *(Zhang and Yuan, 2002)* whereas in PNETs there are no correlations between VEGF expression and tumour growth or spread *(Takahashi et al., 2007)*. Also, VEGF tissue levels measured by ELISA were not related to clinicopathological features and poor prognosis in PNETs patients *(Kuiper et al., 2011)*. Many studies have shown that VEGF expression is crucial for the development of pancreatic ductal adenocarcinoma, but the majority of the studies did not found a correlation between VEGF expression and tumour stage or patients' survival *(Ai et al., 2008; Chung et al., 2006)* . In other studies, high VEGF serum levels were correlated with tumor size, lymph node metastases and distant metastases in patients with pancreatic adenocarcinoma (Karayiannakis et al., 2003; Talar-Wojnarowska et al., 2010). In our study VEGF mRNA levels were not associated with tumour invasiveness, lymph nodes invasion or liver metastases neither in PNETs nor in pancreatic adenocarcinomas. On the other hand, VEGF mRNA relative expression in adenocarcinomas was higher in small tumours (tumours with the maximum diameter lower than 3 cm) compared with large tumours (tumours with the maximum diameter higher than 3 cm), suggesting that new blood vessels formation is a key process in the early stages of tumour development and becomes less evident in advanced stages, probably due to necrosis or to the strong desmoplastic reaction that accompanies advanced pancreatic adenocarcinoma. Thus, the defective angiogenesis in some of the advanced pancreatic adenocarcinoma cases might be responsible for the lack of response to chemotherapy and/or antiangiogenic therapy, which does not reach the tumour cells due to decreased vessel formation and intense desmoplastic reaction at the level of tumour stroma. Although a correlation with contrast-enhancement patterns during CT or EUS was not an objective of the current study, it has been already proven that most advanced pancreatic adenocarcinoma cases were hypovascular as compared to sorrounding normal pancreatic tissue *(Săftoiu et al., 2010)*.

EGFR gene is over-expressed in pancreatic ductal adenocarcinomas when compared with normal pancreatic cells *(Muslimov 2008)*. Over-expression of EGFR was associated with tumour stage (*Zhang et al., 2002)* and it was suggested that EGFR plays a crucial role in the progression of pancreatic adenocarcinoma, especially in the invasion and in the acquisition of aggressive clinical behavior *(Ueda et al., 2004)*. In contrast, other authors have shown no significant correlation between expression of EGFR and tumour size or lymph nodes status *(Bloomston et al., 2006)*. A recent meta-analysis of previous studies based on surgical samples reported that EGFR expression is a poor prognosis factor for survival in patients with pancreatic cancer *(Luo et al., 2011)*. The immunoexpression of EGFR in PNETs has been also correlated with the grade of malignancy *(Bergmann et al., 2009)*.

EGFR seems to play an important role in pancreatic fibrosis in both chronic pancreatitis and pancreatic adenocarcinoma, characterized by stromal expansion and excessive deposition of extracellular matrix (ECM) that replaces pancreatic tissue. This eventually leads to dysregulation of ECM turnover, production of cytokines and restricted blood flow *(Blaine et al., 2009)*. The

restriction of blood flow may be a stimulus for VEGF over-production by tumour cells, supported by the association between VEGF and EGFR expression shown in our study.

In summary, we have shown that VEGF is over-expressed in chronic pancreatitis, pancreatic adenocarcinomas and PNETs when compared with normal tissue, whereas EGFR was over-expressed only in adenocarcinomas and less than 25% of PNETs. Furthermore, EGFR expression was related to adenocarcinoma invasiveness, whereas VEGF was inversely correlated with tumour size. In conclusion, EGFR expression in EUS-guided FNA samples may be used as a diagnostic marker associated with invasiveness in pancreatic adenocarcinoma, although this small feasibility study has to be extended on a larger group of patients. Also, evaluation of EGFR or VEGF expression in EUS-guided FNA samples might be important to assess angiogenesis in pancreatic adenocarcinoma or PNETs, in order to choose the best therapeutic regimen.

Conclusions.

By using a two-step qPCR, we have generated a cDNA library that can be used for further analysis of other pro- or anti-angiogenic molecules involved in pancreatic pathology.

VEGF-A expressed in all chronic pancreatitis and adenocarcinoma samples and its expression in PDAC was significantly greater compared with normal tissue. On the contrary, no difference was noticed in VEGF-A expression in PNETs.

EGFR expressed in various degrees in the studied pathology. A significant over-expression of EGFR was noted in PDAC compared with normal tissue.

EGFR expression was related to adenocarcinoma invasiveness, whereas VEGF-A was inversely correlated with tumour size.

EGFR expression may be used as a diagnostic marker associated with prognosis in pancreatic adenocarcinoma patients.

Protein expression assays for the same patients are necessary to elucidate if there is a post-transcriptional regulation of the analysed transcripts.

Chapter 7. Power Doppler Endoscopic Ultrasound for the Assessment of Pancreatic Neuroendocrine Tumours

Materials and methods.

All consecutive patients with PNET assessed by power Doppler EUS in the Research Centre of Gastroenterology and Hepatology Craiova, Romania, in the past 51 months were included in the study. Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) was conducted in all the cases where a focal pancreatic mass was suspected. The samples collected from at least 3 passes were used for both cytology and micro-histology.

Power Doppler EUS procedures were performed using a linear EUS system - Pentax EG 3830 UTK coupled with the corresponding ultrasound system - Hitachi EUB 8500 or Hitachi Preirus. The following parameters were fixed in power Doppler mode.

After an initial examination in grey scale mode, all the EUS examinations were followed by power Doppler mode examinations, before and after contrast-enhancement. As a contrast agent, 2.4 ml of *SonoVue (Bracco SpA, Milan, Italy*) were used.

Each recorded EUS movie was further subjected to post-processing using a computer-enhanced dynamic analysis using a public domain Java-based image processing tool *(Image J, NIH, Bethesda, Maryland, USA)* with a special vascularity plug-in developed by the IT Department of the University of Medicine and Pharmacy, Craiova. The plug-in was used to calculate the percent of colour pixels of each frame of the movie. Thus, the EUS vascularity index (EUS-VI) was calculated before and after contrast-enhancement, as a percent of colour pixels in every frame of the movies, with an average calculated over a 10 second movie

For cytological exams, Giemsa and Papanicolau stainings were used in the first step. Then, an immunoassay with synaptophysine and/or chromogranine A was performed in all patients to demonstrate the neuroendocrine origin, based on cell blocks obtained through EUS-guided fine needle aspiration. The staining for the proliferation marker Ki-67 was realized only in patients who underwent surgery, based on histopathological exam of resection pieces.

Results and discussions.

Based on the analysis of all consecutive malignant focal pancreatic masses diagnosed in the study period, a total number of 131 consecutive patients were included: 14 patients with pancreatic neuroendocrine tumours and 117 patients with pancreatic adenocarcinoma. The sensitivity of the pre-contrast EUS-VI for the diagnosis of PNET was 71.43%, similar with EUS-FNA. After contrast enhancement, the EUS-VI is also higher in PNET (27.07%) as compared to pancreatic adenocarcinoma where it was significantly lower 9.82% ($p<0.001$). However, the sensitivity of EUS-VI after contrast enhancement for the diagnosis of PNET was 100%, higher than pre-contrast EUS-VI, with an acceptable specificity (79.49%) and better accuracy (81.68%)

In detection of PNET,s EUS seems to be the better imaging diagnostic tool, having an accuracy of 93%, even if it is compared to multidetector CT *(Ardengh et al., 2004; Khashab et al., 2011)*.

Although is well known that PNETs are hypervascular tumors, the quantification of vascularization using power Doppler parameters is not yet sufficiently studied. Our study comes to offer a feasibility study for establishing the role of EUS-VI in the assessment and diagnosis of PNETs. This parameter is already studied in pancreatic adenocarcinoma where it offers a good sensitivity, specificity and accuracy of 75.8%, 95.2%, and 83.3% respectively, while it can be used also in combination with real time elastography *(Saftoiu et al., 2010)*. Evaluation of vascularity through EUS-VI might thus be a good tool for differentiation between PNET and pancreatic adenocarcinoma after microbubble contrast enhancement *(SonoVue)*.

In pancreatic cancer, the sensitivity of EUS-FNA for the diagnosis of malignancy is around 85%, while specificity is 98%, according to a recently published meta-analysis *(Hewitt et al., 2012)*. In the setting of patients with PNET, we obtained a lower sensitivity of EUS-FNA (71.43%) for the diagnosis of PNET, but a better accuracy (96.95%). In the literature, the data are also discordant, EUS-FNA accuracy in PNETs varying between 46 and 90.1%, probably due to the small number of patients and lack of standardization of EUS-FNA sampling and evaluation techniques *(Atiq et al., 2011; Voss et al., 2000; Ardengh et al., 2004)*. The lower accuracy in some studies may be due to sampling error or hemorrhagic samples in these hypervascular tumors, as well as lack of routine performance of immunocytochemistry *(Rafique et al., 2007)*.

Conclusions

Power Doppler EUS represents a useful method in the initial assessment of PNET. Using evaluation of vascularity through EUS-VI, the differentiation between PNET and pancreatic cancer could be possible, especially in the subgroup of patients where EUS-FNA is falsely negative.

This does not preclude the use of EUS-FNA with immunocytochemistry, which has an acceptable sensitivity for the diagnosis of PNET and a very high specificity and accuracy.

Final conclusions

VEGF-A protein is highly expressed by carcinoid cells (chromogranin-A positive cells), whereas no biological or statistical significant difference was observed for VEGF-A mRNA between carcinoid and control groups.

No correlation exists between VEGF-A and MVD. Thus, VEGF-A is mainly involved in maintaining endothelial architecture and functions and its use as a marker associated with new blood vessels formation is not feasible.

Gastrin does not promote VEGF-A gene transcription, but a post-transcriptional expression regulation could be possible.

By using a two-step qPCR, we have generated a cDNA library that can be used for further analysis of other pro-or anti-angiogenic molecules involved in pancreatic pathology.

VEGF-A expressed in all chronic pancreatitis and adenocarcinoma samples and its expression in PDAC was significantly greater compared with normal tissue. On the contrary, no difference was noticed in VEGF-A expression in PNETs.

EGFR expressed in various degrees in the studied pathology. A significant over-expression of EGFR was noted in PDAC compared with normal tissue.

EGFR expression was related to adenocarcinoma invasiveness, whereas VEGF-A was inversely correlated with tumour size.

EGFR expression may be used as a diagnostic marker associated with prognosis in pancreatic adenocarcinoma patients.

ABL1 is over-expressed in pancreatic cancer compared with chronic pancreatitis.

Power Doppler EUS represents a useful method in the initial assessment of PNET. Using evaluation of vascularity through EUS-VI, the differentiation between PNET and pancreatic cancer could be possible, especially in the subgroup of patients where EUS-FNA is falsely negative.

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