Pathology – Research and Practice 212 (2016) 38–43

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original Article

Prognostic role of sex steroid receptors in pancreatic adenocarcinoma

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ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 25 October 2015
Accepted 9 November 2015

Keywords:
Sex steroid hormones
Pancreatic adenocarcinoma
Image analysis

ABSTRACT

From the available literature, it is unclear what proportion of pancreatic adenocarcinomas express estrogen receptors (ERα, ERβ), progesterone receptors (PR), and androgen receptors (AR), and if any of these markers have prognostic significance. We aimed to assess (1) the expression and (2) the correlation of the aforementioned markers with clinicopathological parameters and prognosis in patients with pancreatic adenocarcinoma. During a five-year period, 60 patients with pancreatic ductal adenocarcinoma underwent surgical resection at a single institution. Immunohistochemical stains of the studied markers were quantified by Image analysis system. ERα expression was positively associated with PR expression. Moreover, ERβ was inversely associated with the presence of metastases, whereas no significant associations implicated AR. As far as the prognostic significance of the studied receptors is concerned, higher ERα expression correlated with poorer survival at the univariate analysis, but the finding dissipated at the multivariate approach. No significant associations with overall survival were noted regarding the other receptors. The role of sex hormone receptors in the survival from pancreatic adenocarcinoma seems rather limited. Further prospective studies assessing those receptors should ideally be designed in order to confirm our results and possibly outline additional correlations between other steroid receptors and features of pancreatic adenocarcinoma.

Introduction

Pancreatic ductal adenocarcinoma is the fifth most common cause of death from cancer worldwide [1,2] and represents one of the most lethal malignancies. Approximately 80% of patients are diagnosed with locally advanced or metastatic disease, ruling out potentially curative resection [3]. For these patients, the disease generally progresses rapidly, with few patients surviving more than 1 year after diagnosis.

Imaging methods are not accurate enough to detect early lesions and bear difficulties in the differentiation of benign from malignant lesions [4]. For this reason, the search and identification of new biologic targets in patients with pancreatic ductal adenocarcinoma is auspicated and may contribute to improvement in their outcome.

Sex steroid hormones (estrogens, progesterone, and androgens) exert their effects through binding to their cognate receptors, estrogen receptors (ER), progesterone receptors (PR), and androgen receptors (AR), respectively. All steroid hormone receptors belong to a nuclear receptor superfamily of ligand-dependent transcription factors [5,6]. The specific functions of steroid hormone receptors are believed to be dependent on tissue- and cell-specific contexts.

There are two receptors for estrogens, ERα and ERβ. The two ERs, which are highly homologous, are derived from two separate genes [7]. These receptors are able to transduce extracellular signals into transcriptional response; nevertheless, their tissue distribution and possible functions are different [8,9]. ERα and ERβ have been detected in different tumor tissues and cell lines, such as breast, prostate, bladder, lung, colon and gastric neoplasms [10–15]. Hormonal therapy, which targets estrogen receptors, has

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http://dx.doi.org/10.1016/j.prp.2015.11.007
0344-0338/

played a significant role in breast cancer treatment [16,17]. Recent studies have shown that blocking estrogen receptor pathways may also provide new therapy options for patients with prostate and lung cancer [18–20]. This, in conjunction with the fact that there are few studies, with inconsistent results, evaluating the expression of both ERα and ERβ receptors using isoform-specific antibodies in human pancreatic cancer [21] prompted us to evaluate estrogen receptors status in this cancer.

Progesterone has two receptors, PRA and PRB. The two PR receptors are transcribed from the same gene, through alternative promoter usage [22]. Despite the fact that progesterone receptors expression has been extensively studied in mucinous cystic [23–26] and solid pseudopapillary [27–30] tumors of the pancreas, their expression in pancreatic adenocarcinoma has not been adequately examined [31–33]. The recent publication regarding the potential efficacy of the PR antagonist mifepristone for palliative therapy of patients with a variety of advanced cancer types, among them pancreatic adenocarcinoma [34], induced us to investigate the expression of PR in the latter tissue specimens.

So far, only one AR type has been reported [35,36]. Similarly to progesterone receptors, AR expression in pancreatic adenocarcinoma is vaguely studied, with conflicting results concerning its expression [33,37,38]. In light of several publications about the potential role of testosterone in pancreatic carcinogenesis [39], as well as the putative role of androgen receptor blocking agents as flutamide in combination with other current adjuvant therapies in patients with pancreatic cancer [40], we further focused on the expression of these receptors.

Sex hormone receptors play a critical role in the pathogenesis of various adenocarcinomas, such as breast, prostate, and colon. We hypothesized that there is a putative role of these receptors in the pathogenesis of pancreatic adenocarcinoma. From the available literature, it is unclear what proportion of pancreatic adenocarcinomas express estrogen receptors (ERα, ERβ), PR, and AR and if any of these markers have prognostic significance. At any case, expression of these markers is of interest because they are predictive of response to endocrine therapies. The objectives of our study were to: (1) evaluate the expression of ERα, ERβ, PR and AR in pancreatic adenocarcinoma and (2) assess the correlation of these factors with clinicopathological parameters and prognosis of pancreatic cancer patients utilizing long term clinical follow-up data.

Patient and tissue samples

This retrospective study comprised 60 consecutive patients who underwent whipple procedure ($n = 26$), whipple pylorus preserving procedure ($n = 18$) and distal pancreatectomy ($n = 16$) for adenocarcinoma of the head or body-tail of the pancreas by one single surgeon at the 5th Department of Surgery, "Eugenidio" Athens General Hospital during the period from December 1999 to July 2004. Any other pathology of the pancreas, treated during the same time period, as well as by-pass operations due to unresectable pancreatic adenocarcinomas, was excluded from the study.

All patients that were included in the study had a preoperative evaluation that was in concordance with intraoperative findings, with the exception of four patients (two with tumor of the head and two with tumor of the body-tail), in whom one single liver metastasis <1 cm was found during the exploration of the abdomen although the preoperative evaluation was negative for metastases. Those patients underwent whipple and distal pancreatectomy in combination with liver tumor excision.

The study was approved by the local ethics committee of Hippocratio Medical Hospital and is in accordance with the Helsinki Declaration.

Immunohistochemical staining

One formalin-fixed, paraffin-embedded block was selected for analysis in each patient's case on the basis of enclosing representative tumor tissue. Tissue blocks were sectioned into 3.5 μm and placed to 56 °C overnight. The next day they were placed in PT Link (device for deparaffinization, rehydration and epitope retrieval) with Tris/EDTA buffer, pH 9. After having been washed in water for 5 min, slides were incubated in 3% hydrogen peroxide for 10 min in a dark room, to block endogenous peroxidase activity and then were rinsed with water and TBST for 5 min. The sections were then incubated overnight at 4 °C (at least for 10 h) with monoclonal, rabbit, anti-human ERα clone EP1 (Dako, Denmark 1:40,), monoclonal, mouse, anti-human ERB1 clone PPG5/10 (Dako, Denmark 1:10), monoclonal, mouse, anti-human androgen receptor clone 441 (Dako, Denmark 1:60), and monoclonal, mouse, anti-human progesterone receptor clone 636 (Dako, Denmark 1:40). It should also be mentioned that according to antibody datasheets, monoclonal anti-human ERα, ERβ1 (both short and long form), progesterone receptor (reaction for both PR-A and PR-B forms, free and hormone-bound) and androgen receptor clones used in the study specifically immunostain positive cells in human tissues. Moreover, regarding estrogen receptors, no cross-reactivity was found between ERα and ERβ; the anti-ERβ1 antibody does not react with ERβ2.

The next day they were left for 30 min in room temperature and then rinsed with TBST three times followed by incubation with rabbit HRP-labelled polymer (Envision + System-HRP, Dako-Cytomation, Glostrup, Denmark) for 45 min. Slides were immersed in TBST for 5 min three times each and then chromogen DAB-Ni was placed for 5 min and rinsed afterwards with TBST three times. Light green was used as a counter stain which was placed for 40 s and was washed with abundant water afterwards for 5 min. Finally, tissue sections were dehydrated with graded alcohols, cleared with xylene and coversplipped with GLC mounting medium. From each paraffin block, a corresponding H&E-stained reference slide was available. All cases were stained simultaneously with appropriate specimens which served as positive controls. We used as a positive control breast cancerous tissue known to express ERα, ERβ and progesterone receptors in a very high percentage. Prostatic tissue from patients with benign prostatic hyperplasia served as androgen receptor-positive control tissue. For negative controls the primary antibody was omitted.

Image acquisition

Evaluation of immunohistochemical results was performed using an image analysis system known as Image Pro Plus Media Cybernetics, version 6, Rockville, USA. After thorough examination of each immunohistochemical slide at a low power magnification (using 4x objective lens) by two experienced pathologists (SS and AL), three to five representative pictures of the carcinomatous areas were acquired (SC30 Olympus Camera, Tokyo, Japan) and stored as jpeg files with consecutive numbers (Fig. 1). The details of the scanning technique and intensity quantification are available on the manufacturer's Web site (http://www.mediacy.com). For the purpose of immunohistochemical analysis, we selected the pancreatic neoplastic foci in each picture and each focus was quantified separately by the analysis system for staining intensity (0–3) and percentage of positively staining cells (0%-100%) with regard to nuclear positivity (Fig. 2). The H-score, which is a method of assessing intensity in combination with percentage of nuclear immunoreactivity applicable to steroid receptors [41], was obtained by the image analysis system automatically. The score is obtained by the formula: 3 × percentage of strongly staining nuclei + 2 × percentage of moderately staining nuclei + percentage of weakly staining nuclei, giving a range of 0 to 3.