Title page

Title:

Estrogen Receptor Expression in Pancreatic Adenocarcinoma: Time to Reconsider Evidence

Running title:

Estrogen Receptors and Pancreatic Cancer

Authors:

Panagis M. Lykoudis MD MSc[1]

John Contis MD PhD FACS[1]

Affiliation:

[1]2nd Department of Surgery, Aretaieio University Hospital, Athens School of Medicine, National & Kapodistrian University of Athens, Athens, Greece

Disclosure:

The authors of this study received no relevant funding and have no conflict of interest to disclose.

Corresponding author:

Dr Panagis M Lykoudis, MD MSc DIC MRCS FHEA

2nd Department of Surgery

Aretaieio University Hospital,

76, Vas. Sofias ave.

11528

Athens

Greece

Phone: +30 6948087674

Email: p.lykoudis@ucl.ac.uk

Abstract

Pancreatic adenocarcinoma remains a chemotherapy resistant and refractory malignancy with high mortality, unaffected by recent progress in anticancer treatment. Expression of estrogen receptors was detected almost 50 years ago, in both benign and malignant pancreatic cells. However, early preclinical studies in pancreatic cancer led to contradictory findings, and most clinical studies failed to demonstrate an effect with tamoxifen treatment. The identification of a second form of estrogen receptor seems to provide some explanation for these discrepancies. Predominantly expressed in malignant cells, and structurally different to what was considered the only estrogen receptor, Estrogen Receptor β was recognized as a negative prognostic factor as well as a possible therapeutic target in pancreatic ductal adenocarcinoma. Therefore, findings of research prior to the identification of Estrogen Receptor β should be reconsidered and further studies should be designed to re-assess the expression and effect of this specific Estrogen Receptor type in pancreatic cancer.

Keywords

Pancreatic cancer, adenocarcinoma, estrogen receptor, ER-beta

Main text

Introduction

Over the past 30 years, cancer treatment has achieved astonishing milestones. A steady decrease in cancer mortality has been documented in all surveillance programs, and mortality of malignancies such as lung cancer in men and breast cancer in women has decreased from peak by almost 50%.[1] In this auspicious context, pancreatic cancer is the unfortunate exception. According to predictions, in 2020, 47000 deaths are expected in the United states[2] and almost 46000 in Europe.[1] These numbers correspond to an increase of about 0.1% per year over the past decade, leading to a 5-year survival of 9%, the lowest among estimations of common cancers.[2] Interestingly enough, this is despite the relatively smaller increase in incidence of pancreatic cancer, as opposed to other malignancies, which reveals a gap in the effectiveness of respective treatments. In other words, despite ground-breaking discoveries in cancer molecular biology and treatment, effective treatment for pancreatic cancer, and especially inoperable stages, is still lacking. Researchers have tried to identify why pancreatic cancer remains one of the most chemotherapy resistant and refractory types of malignancies. Dysregulation of pivotal pathways[3] and immune evasion[4] seem to contribute to this aggressive behavior, that leads to failure of most modern anticancer approaches. In situations like this, when mainstream methods do not yield the desired results, thinking outside the box might help. With regards to pancreatic cancer, that might involve looking into pathways and treatments that were previously abandoned but under the light of recent evidence, might merit reconsideration.

Almost 50 years ago, Sandberg et al noticed that estrogen receptors existed in pancreas, which till then was not considered a target of the relevant pathways.[5] The sophisticated economy of multicellular organisms guaranteed that this could not be random, therefore authors wondered what role estrogens might play in pancreatic cells' physiology. Gradually, research revealed that estrogens are involved in the endocrine function of beta-cells[6] but also in the tumorigenesis of several types of pancreatic neoplasms.[7] This observation, along with the epidemiological observations regarding deviating incidences in females, pregnancy and oral contraceptive administration[8], as well as the histological observation of presence of ovarian-type stroma in pancreatic lesions[9], quickly created optimism that a novel therapeutic approach in pancreatic cancer might emerge.[10-12] However, initial enthusiasm was succeeded by skepticism as early studies did not demonstrate a significant impact with tamoxifen treatment in unresectable pancreatic ductal adenocarcinoma.[13,14] Consequently, research interest declined until Mosselman et al identified another isoform of the estrogen receptor (ER), leading to the differentiation of ER-alpha and ER-beta.[15] It soon became apparent that various cell types differentially express the two aforementioned isoforms, immunohistochemical methods mainly assess the presence of ER-alpha and established antagonists might not be equally effective in tissues that predominantly express ER-beta.[16] This finding reignited interest in the correlation between ER and pancreatic cancer, with recent studies confirming the higher expression of ER-beta and its prognostic as well as potentially therapeutic role.[17,18]

This study aimed to review the historical evolution of research, to identify the evidence around the critical turning point, and to highlight current and future perspectives regarding ER and pancreatic cancer.

Materials and Methods

A systematic literature search was carried out, including three independent databases (Pubmed, Library of Congress and LISTA). Two blocks of search terms were created, the first one including the terms “oestrogen” and “estrogen” and the second one including the terms “pancreas” and pancreatic”. Specific blocks for cancer and receptors were not used, as the anticipated large variability in relevant terms would make search too complex and thus not reproducible. Therefore, four total combinations were sought in tile and in abstracts, without any restriction regarding year of publication. Search was limited to articles written in English language. Duplicates were removed, and abstracts and full texts were accessed to assess for relevance, focusing on pancreatic adenocarcinoma. Finally, references of maintained articles were reviewed to identify any further articles, missed during the aforementioned process.

Results

Initial search yielded 859 articles and eventually 42 articles were identified as relevant to the topic and are discussed below. Figure 1 depicts the flow of the search process.

Early years

In 1973, Sandberg et al described the presence of a cytosol protein that binds estriol and estradiol-17β, in three different species, including humans.[5] Five years later, the same research group published two more studies. The first one aimed to assess if this binding protein was also expressed in cells of other non-target organs and found that it was only present in pancreas, suggesting some type of specialized action.[19] The second study focused on the expression of the estrogen-binding protein in the exocrine cells of the pancreas and prostate.[20] High expression of the protein as well as high affinity for estrogens was confirmed and authors reiterated the need for further research to elucidate the role of estrogens in pancreatic cell biology. The first studies correlating pancreatic malignancies and ER were published at the same time. Molteni et al confirmed ER binding activity in rat pancreatic cancer model[21] suggesting that estrogens might be involved not only in normal pancreatic tissue metabolism but also in pancreatic malignancies development. Moreover, Greenway et al, reported high affinity binding, both in the cytosol as well as in the nucleus of pancreatic cancer cells, deriving from 6 patients with pancreatic adenocarcinoma.[7] In the same study, authors examined pancreatic tissue from 5 fetuses and ER activity was high too. Therefore, authors supported that estrogens might be associated with cell proliferation, in fetal life and malignancy, and were the first to suggest that ER might serve as a target for anticancer treatment. Pousette et al went on to further describe ER macromolecule, confirmed the binding properties of the purified protein and following the example of estramustine in prostate cancer, suggested that estrogen-based cytotoxic agents could benefit pancreas cancer patients as

well.[22] The same team were the first to design a clinical study to assess the efficacy of tamoxifen, and reported their preliminary results in 1983[10], a decade after the first relevant study. In 14 patients that were treated with the estrogen antagonist for unresectable pancreatic adenocarcinoma, median survival was 8.5 months. Although this was not a comparative study, at that time, reported median survival of patients at the same stage was 2.5 months.

For the following five years, until 1989, research mainly revolved around further characterization of molecular properties and, binding and nuclear presence of ER[11,23-26], while studies in the '90s focused more on the potential therapeutic role of ER. Based on the epidemiological observation that males developed pancreatic cancer more frequently than females, and pre-menopause females had a higher risk post oophorectomy, some researchers suggested that estrogens might have a protective role against pancreatic cancer and consequently estrogens could be admitted with a preventive or curative intent.[27,28] Another research group tested various hormonal manipulations such as castration, ovariectomy, and administration of estradiol, tamoxifen and testosterone in Fischer rats, and found that estrogens attenuated nodules of precancerous pancreatic acinar cells.[29,30] Further research in Lewis rats demonstrated a protective role of estrogens against precancerous and cancerous lesions of the pancreas, while tamoxifen did not demonstrate a benefit.[31] Contrary to this concept, Benz et al documented an inhibitory activity of tamoxifen, danazol and especially of progestins in MiaPaCa cell lines.[32]

In four studies that examined pancreatic cancer tissue, only one specimen out of 80 was found positive for ER expression.[33-36] In terms of clinical studies regarding treatment with tamoxifen, two groups reported improved median

survival in patients with unresectable pancreatic ductal adenocarcinoma. The first study demonstrated a median survival of 7 months in 10 patients[37] and the second one 7 months in 24 patients[38], with a median survival of historic control groups of 2-3 months. Another cohort study did not detect any response in 14 patients.[39] Bakkevold et al randomized 176 patients with unresectable pancreatic ductal adenocarcinoma, into one group of 92 patients that received tamoxifen and 84 that received placebo and detected no statistically significant difference in median survival (115 vs 122 days).[13] They did however detect a subgroup of patients, consisting of females with N1M0 disease, with a median survival of 191 days in the tamoxifen group versus 45 days in the placebo group (P = 0.011) but argued this was incidental rather than treatment related. This was the first suggestion in literature that a subgroup of patients might exist, with specific biological characteristics that might benefit from ER targeted treatment. Taylor et al, randomized 44 patients of the same disease and stage as the aforementioned study, and presented a median survival of 75 days in the 22 patients that received tamoxifen versus 131 days in remaining patients of the placebo group (P = 0.2).[14]

In order to shed some light in this discrepancy, Kuramoto et al examined 7 different pancreatic cancer cell lines. They found that tissue plasminogen activator correlated with the functional state of ER.[40] Moreover, testing various ER modulators, they found that medroxy-progesterone acetate had a significant antitumor effect in 3 cell lines. Finally, Singh et al determined the quantity of ER messenger RNA in pancreatic cancer tissue, and they found that even in those positive for ER, the actual quantity is very low.[41] Therefore, authors argued that this might be the reason why immunohistochemistry studies might fail to detect

the presence of ER, and thus relevant treatment might only be effective in the subgroup of patients with significant amounts of ER. These two studies suggested that both ER expression assessment as well as therapeutic targeting need to be more precise and focused on specific subgroups of patients.

ER-α versus ER-β

The field was meant to change in 1996 when Mosselman et al described another estrogen receptor, with extensive similarities to the already known and investigated receptor, now named ER-β as opposed to the novel ER-β, but also some differences that could expect different biological function.[15] The authors suggested that Northern blot technique was not accurate in terms of ER-β detection, which could be the reason why previous studies failed to detect it. Moreover, small structural differences might mean that ER-β has a lower affinity for previously examined ligands and targets different gene targets. Overall, they concluded that cloning of ER-β mandated re-evaluating findings of previous studies. Two studies from the same research group demonstrated that higher ER-β expression, in both its unphosphorylated and phosphorylated forms, is associated with adverse prognosis following resection of pancreatic ductal adenocarcinoma. The first study was conducted on specimens obtained from 84 patients and ER-β positive patients had a median survival of 13.5 months compared to 23.5 months for those ER-β negative ($P = 0.029$), which was also confirmed in multifactorial analysis (hazard ratio, 1.938, $P = 0.047$).[17] The second study examined the phosphorylated form of the receptor in 175 patients, with similar findings associating higher expression with lower median survival (29 versus 15.1 months, $P = 0.016$), again confirmed in multifactorial analysis (hazard ratio, 1.9, $P = 0.013$).[42] Kondoh et al finally demonstrated that 15-

deoxy-Δ12,14-prostaglandin-J2 inhibits MAP kinase signaling, thus reduces ER-β phosphorylation and consequently downregulates the expression of human telomerase reverse transcriptase, with a final apoptotic effect.[43]

The importance of structural differences between ER-α and ER-β was seconded by Barkhem et al who examined the interaction of known ligands and the two receptors.[16] Tamoxifen for instance had a partial agonist/antagonist effect on ER-α but a pure antagonist effect on ER-β. They thus suggested that ER modulation effect should be studied through receptor-specific ligands. The hypothesis that the two receptors are not equally expressed in all types of cells was confirmed by Iwao et al that compared the differential mRNA expression of ER-α and ER-β in pancreatic and breast cancer.[44] They found that ER-α mRNA was higher in ER-positive breast cancer, ER-β mRNA was higher in ER-negative breast cancer, ER-α/ER-β mRNA ratio was significantly lower in pancreatic cancer. Konduri et al assessed this ratio in 8 pancreatic adenocarcinoma cell lines and found that ER-β expression was higher than that of ER-α in 7 out of 8 cell lines.[45] This ratio correlated with estradiol and genistein effect and the addition of the latter two ligands was attenuating the cytotoxic effect of gemcitabine. Authors thus concluded that only selective ER-β antagonists could possibly demonstrate an antitumor effect. Towards this direction, Martin-Santamaria et al, synthesized 4 new compounds that can be used as scaffolds to create ER ligands with tailored affinity and agonist/antagonist action.[46] Two of these actually demonstrated antitumor effect in *in vitro* studies of Panc-1 and L36PL cell lines. The authors also explained that their findings could lead to the development of agonists

exclusively for ER-β, with subsequent use in further research and targeted anticancer therapy.

Conclusions

The detection of estrogen receptors in pancreatic cancer cells raised hopes that a novel therapeutic target could emerge. Despite early encouraging results, subsequent studies did not demonstrate a consistent expression pattern and clinical trials with tamoxifen did not demonstrate a statistically significant benefit. Further studies revealed a second type of receptor, namely ER-β, with different properties compared to the ER-α, which was till then though to be the only one. Its prognostic as well as potential therapeutic role have been supported by *in vitro* and clinical studies recently. There are certainly aspects regarding expression assessment, function and therapeutic implementations that have not been elucidated yet. It is thus suggested that further studies should be designed to examine the role of ER-β specifically in order not to miss a possible opportunity to gain ground against a stubborn, lethal disease.

References

1. Carioli G, Bertuccio P, Boffetta P, et al. European cancer mortality predictions for the year 2020 with a focus on prostate cancer. *Ann Oncol.* 2020;31:650-658.

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin.* 2020;70:7-30.

3. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science.* 2008;321:1801-1806.

4. Maitra A. Pancreatic cancer hidden in plain sight. *Nature.* 2020;581:34-35.

5. Sandberg AA, Kirdani RY, Varkarakis MJ, et al. Estrogen receptor protein of pancreas. *Steroids.* 1973;22:259-271.

6. Tiano J, Mauvais-Jarvis F. Selective estrogen receptor modulation in pancreatic beta-cells and the prevention of type 2 diabetes. *Islets.* 2012;4:173-176.

7. Greenway B, Iqbal MJ, Johnson PJ, et al. Oestrogen receptor proteins in malignant and fetal pancreas. *Br Med J (Clin Res Ed).* 1981;283:751-753.

8. Fernandez E, La Vecchia C, D'Avanzo B, et al. Menstrual and reproductive factors and pancreatic cancer risk in women. *Int J Cancer.* 1995;62:11-14.

9. Fukushima N, Fukayama M. Mucinous cystic neoplasms of the pancreas: pathology and molecular genetics. *J Hepatobiliary Pancreat Surg.* 2007;14:238-242.

10. Theve NO, Pousette A, Carlstrom K. Adenocarcinoma of the pancreas--a hormone sensitive tumor? A preliminary report on Nolvadex treatment. *Clin Oncol.* 1983;9:193-197.

11. Pettersen A, Lea O, Bakkevold K, et al. Progress in pancreatic oestrogen receptors. *Eur J Surg Oncol.* 1986;12:321-324.

12. Andren-Sandberg A, Johansson J. Influence of sex hormones on pancreatic cancer. *Int J Pancreatol.* 1990;7:167-176.

13. Bakkevold KE, Pettersen A, Arnesjo B, et al. Tamoxifen therapy in unresectable adenocarcinoma of the pancreas and the papilla of Vater. *Br J Surg.* 1990;77:725-730.

14. Taylor OM, Benson EA, McMahon MJ. Clinical trial of tamoxifen in patients with irresectable pancreatic adenocarcinoma. The Yorkshire Gastrointestinal Tumour Group. *Br J Surg.* 1993;80:384-386.

15. Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Lett.* 1996;392:49-53.

16. Barkhem T, Carlsson B, Nilsson Y, et al. Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists. *Mol Pharmacol.* 1998;54:105-112.

17. Seeliger H, Pozios I, Assmann G, et al. Expression of estrogen receptor beta correlates with adverse prognosis in resected pancreatic adenocarcinoma. *BMC Cancer.* 2018;18:1049.

18. Younes M, Ly CJ, Singh K, et al. Expression of estrogen receptor beta isoforms in pancreatic adenocarcinoma. *Oncotarget.* 2018;9:37715-37720.

19. Rosenthal HE, Sandberg AA. Estrogen binding proteins in rat pancreas. *J Steroid Biochem.* 1978;9:1133-1139.

20. Sandberg AA, Rosenthal HE. Steroid receptors in exocrine glands: the pancreas and prostate. *J Steroid Biochem.* 1979;11:293-299.

21. Molteni A, Bahu RM, Battifora HA, et al. Estradiol receptor assays in normal and neoplastic tissues. A possible diagnostic acid for tumor differentiation. *Ann Clin Lab Sci.* 1979;9:103-108.

22. Pousette A, Carlstrom K, Skoldefors H, et al. Purification and partial characterization of a 17 beta-estradiol-binding macromolecule in the human pancreas. *Cancer Res.* 1982;42:633-637.

23. Grossman A, Richardson SB, Altszuler N, et al. Evidence that the [3H]estradiol-binding protein in pancreas is localized in exocrine cells. *Endocrinology.* 1985;116:2221-2226.

24. Pousette A, Fernstad R, Theve NO, et al. Further characterization of the estrogen binding macromolecule in human pancreas. *J Steroid Biochem.* 1985;23:115-119.

25. Andren-Sandberg A. Estrogens and pancreatic cancer: some recent aspects. *Scand J Gastroenterol.* 1986;21:129-133.

26. Pousette A, Fernstad R, Skoldefors H, et al. Analysis of an estrogen binding macromolecule in human pancreas by radioimmunoassay. *J Steroid Biochem.* 1987;26:439-442.

27. Bourhis J, Lacaine F, Augusti M, et al. Protective effect of oestrogen in pancreatic cancer. *Lancet.* 1987;2:977.

28. Devesa SS, Silverman DT. Protective effect of oestrogen in pancreatic cancer. *Lancet.* 1988;2:905-906.

29. Lhoste EF, Roebuck BD, Brinck-Johnsen T, et al. Effect of castration and hormone replacement on azaserine-induced pancreatic carcinogenesis in male and female Fischer rats. *Carcinogenesis.* 1987;8:699-703.

30. Sumi C, Longnecker DS, Roebuck BD, et al. Inhibitory effects of estrogen and castration on the early stage of pancreatic carcinogenesis in Fischer rats treated with azaserine. *Cancer Res.* 1989;49:2332-2336.

31. Longnecker DS, Sumi C. Effects of sex steroid hormones on pancreatic cancer in the rat. *Int J Pancreatol.* 1990;7:159-165.

32. Benz C, Hollander C, Miller B. Endocrine-responsive pancreatic carcinoma: steroid binding and cytotoxicity studies in human tumor cell lines. *Cancer Res.* 1986;46:2276-2281.

33. Targarona EM, Pons MD, Gonzalez G, et al. Is exocrine pancreatic cancer a hormone-dependent tumor? A study of the existence of sex hormone receptors in normal and neoplastic pancreas. *Hepatogastroenterology.* 1991;38:165-169.

34. Taylor OM, Teasdale J, Cowen PN, et al. Classical oestrogen receptor is not detectable in pancreatic adenocarcinoma. *Br J Cancer.* 1992;66:503-506.

35. Mao C, Desaty D, Howard JM. Cytosolic estrogen and progesterone receptors in exocrine pancreatic adenocarcinoma. *Int J Pancreatol.* 1994;15:155-156.

36. Ollayos CW, Riordan GP, Rushin JM. Estrogen receptor detection in paraffin sections of adenocarcinoma of the colon, pancreas, and lung. *Arch Pathol Lab Med.* 1994;118:630-632.

37. Tonnesen K, Kamp-Jensen M. Antiestrogen therapy in pancreatic carcinoma: a preliminary report. *Eur J Surg Oncol.* 1986;12:69-70.

38. Wong A, Chan A, Arthur K. Tamoxifen therapy in unresectable adenocarcinoma of the pancreas. *Cancer Treat Rep.* 1987;71:749-750.

39. Crowson MC, Dorrell A, Rolfe EB, et al. A phase II study to evaluate tamoxifen in pancreatic adenocarcinoma. *Eur J Surg Oncol.* 1986;12:335-336.

40. Kuramoto M, Yamashita J, Ogawa M. Tissue-type plasminogen activator predicts endocrine responsiveness of human pancreatic carcinoma cells. *Cancer.* 1995;75:1263-1272.

41. Singh S, Baker PR, Poulsom R, et al. Expression of oestrogen receptor and oestrogen-inducible genes in pancreatic cancer. *Br J Surg.* 1997;84:1085-1089.

42. Pozios I, Knosel T, Zhao Y, et al. Expression of phosphorylated estrogen receptor beta is an independent negative prognostic factor for pancreatic ductal adenocarcinoma. *J Cancer Res Clin Oncol.* 2018;144:1887-1897.

43. Kondoh K, Tsuji N, Asanuma K, et al. Inhibition of estrogen receptor beta-mediated human telomerase reverse transcriptase gene transcription via the suppression of

mitogen-activated protein kinase signaling plays an important role in 15-deoxy-Delta(12,14)-prostaglandin J(2)-induced apoptosis in cancer cells. *Exp Cell Res.* 2007;313:3486-3496.

44. Iwao K, Miyoshi Y, Ooka M, et al. Quantitative analysis of estrogen receptor-alpha and -beta messenger RNA expression in human pancreatic cancers by real-time polymerase chain reaction. *Cancer Lett.* 2001;170:91-97.

45. Konduri S, Schwarz RE. Estrogen receptor beta/alpha ratio predicts response of pancreatic cancer cells to estrogens and phytoestrogens. *J Surg Res.* 2007;140:55-66.

46. Martin-Santamaria S, Rodriguez JJ, de Pascual-Teresa S, et al. New scaffolds for the design of selective estrogen receptor modulators. *Org Biomol Chem.* 2008;6:3486-3496.

Figure legends

Figure 1. Flow-chart of literature search process