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ORIGINAL PAPER

Overexpression of Glucocorticoid Receptor in Human Pancreatic Cancer and in Xenografts. An Immunohistochemical Study

Sándor Békási · Attila Zalatnai

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Abstract Glucocorticoid receptor overexpression has been reported in a variety of human solid tumors, but much less in known about its presence in pancreatic cancer. Only one report is available in the literature, back to 1994, since that no peculiar attention has been paid to this issue. Immunohistochemical analysis of paraffin-embedded tissue sections was performed in human normal pancreata and well differentiated pancreatic adenocarcinomas (monoclonal primary antibody, ABCAM, Cambridge, UK). As positive control invasive ductal adenocarcinoma of the breast was used. In the normal non-tumorous pancreas a strong positivity was detected in all acinar cells, typically in the cytoplasm. Nuclear staining was not visible. The distribution of the positive reaction was homogenous. The ductal pancreatic carcinoma cells also displayed a strong positivity. The location of the immune reaction was mainly cytoplasmic but in some tumors a strong nuclear reaction was also noticed. In some slides acini remained also positive in the close vicinity of the tumor. Although the positivity of the ductal tumor cells was a constant finding in our samples, surprisingly, the liver metastasis was completely negative. Strong glucocorticoid receptor expression was also found in xenografted human pancreatic cancer showing a diffuse, mainly cytoplasmic positivity. Our studies have shown that the human pancreatic carcinomas do overexpress a strong glucocorticoid receptor positivity, but its significance is not clear. However, this finding might have a clinical relevance.

S. Békási · A. Zalatnai (✉)
First Department of Pathology and Experimental Cancer Research, Faculty of Medicine, Semmelweis University,
Üllői út 26,
1085 Budapest, Hungary
e-mail: zalatnai@korb1.sote.hu

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Abbreviations

GR	glucocorticoid receptor
GRE	glucocorticoid response element
CBG	corticosteroid-binding globulin
TFIID	transcription factor IID

Introduction

A number of the basic physiological cell functions, such as growth, defense and apoptosis [1–3], are regulated by glucocorticoid hormones through glucocorticoid receptors (GRs).

As a specific signal, the ligand-activated receptor acts directly on the transcription of several genes by binding to the adequate recognition element (glucocorticoid response element, GRE) in the promoter region of the target gene or indirectly on interaction with other proteins.

In the circulation majority of the glucocorticoids is bound to a specific carrier molecule (corticosteroid-binding globulin, CBG) and only a trace circulating as free hormones has the ability to reach the target cells.

The glucocorticoid hormone after getting through the plasma membrane interacts with the constitutively expressed glucocorticoid receptor complex located mainly in the cytoplasm of the cell. The high-affinity ligand-receptor binding results in conformational changes and dissociation of chaperone proteins, phosphorylation, homodimer formation [4] and translocation into the nucleus through the nucleopore. The response intensity of the cell depends on the fluctuating intracellular concentration of the glucocorticoid receptors.

The indirect effect of glucocorticoids is based on protein-protein interactions: with other transcriptional regulators, such as activating protein-1 (AP-1), c-jun/c-myc, nuclear factor κB (NF-κB), transcription factor IID (TFIID) complex, STAT5, and numerous newly described co-repressor and co-activator proteins constituting a huge network of interacting signaling pathways [5–7].

The presence of glucocorticoid receptors was analyzed in several different types of human solid tumors, such as malignant melanoma [8], leiomyosarcoma [9, 10], hepatocellular carcinoma [11, 12], gastric carcinoma [13, 14], colon cancer [15], prostate carcinoma [16, 17], small [18] and non-small cell lung cancer [19], breast cancer [20], meningioma [21, 22], ovarian carcinoma [23], thyroid cancer [24] and B cell type malignant lymphoma [25] by biochemical or immunohistochemical techniques, but its level was found to be highly variable, from negative cases to 100 % positivity in these tumors. In this respect, pancreatic carcinoma has surprisingly rarely been investigated.

Some of these studies investigated whether the GR expression correlated with clinicopathological characteristics or disease progression but the results are controversial. For example, in ovarian cancer no prognostic impact of the amount of glucocorticoid receptor was seen [23], in colonic cancer GR expression correlated with the expression of cell cycle-related molecules [15]. Also a difference in non-small cell lung cancer GR expression was associated with the outcome of the disease [19]. Patients with a higher GR amount had a significantly longer progression-free and overall survival.

In pancreatic cancer samples GR expression correlated inversely with the degree of tumor differentiation: the poorly differentiated neoplasms showed lower glucocorticoid receptor expression [26]. In addition, growth suppression of a unique human pancreatic cancer cell line (HPAC) expressing glucocorticoid receptor was also found after dexamethasone treatment [26].

In numerous other tumor cell lines [17–19, 27–29] and animal experiments [30, 31] a tumor growth inhibition was found after glucocorticoid treatment suggesting an anti-proliferative effect of these hormones. In a small cell lung cancer cell line an apoptotic effect was also seen connected to GR overexpression [18]. Other lung cancer cell lines were also analyzed where beside the presence of glucocorticoid receptor the growth inhibitory effect of dexamethasone was also seen [27].

In contrast, GR expression in Kaposi's sarcoma was associated with cytokines promoting growth, and glucocorticoid hormones had a direct stimulatory effect on tumor cell proliferation [32].

Although the above mentioned effects of glucocorticoid hormones suggested some therapeutical benefits, apoptosis inhibition of dexamethasone was also observed during chemotherapy in a breast cancer xenograft system [33]. The study concluded that a pretreatment with glucocorticoids decreases the tumor response to Paclitaxel and the rate of chemotherapy-induced apoptosis. Similarly, an induced resistance to chemotherapy was seen in pancreatic surgical resections, pancreatic cancer xenografts and cell lines [34].

The aim of the present study was to investigate the presence of GR expression in human pancreatic cancer and in xenografted tumors.

Materials and Methods

Normal pancreata (n=3), resected pancreatic cancer cases (n=14) and xenografted tumors (n=10) have been selected from the biopsy archives of the department. The tumors were well/moderately differentiated ductal adenocarcinomas with no acinar and endocrine areas. The surgically removed fresh pancreatic cancer tissues were minced and subcutaneously transplanted into the back region of artificially immunosuppressed CBA mice.

Immunohistochemistry

Paraffin-embedded tissue sections subjected to immunostaining were deparaffinized, rehydrated and then rinsed in tap water for 5 min. Antigen retrieval was completed in 10 mM citrate buffer at pH6.0 by microwaving the slides. Endogenous peroxidase activity was quenched by incubation in methanol containing 0.3% hydrogen peroxide at room temperature for 30 min. Slides were then washed in phosphate buffered saline (PBS) for 5 min and blocked with normal horse serum for 20 min. To localize the glucocorticoid receptors, slides were incubated with specific mouse monoclonal primary antibody (ABCAM, Cambridge, UK) diluted in PBS for 60 min at room temperature. Thereafter, tissue sections were washed with PBS for 5 min and then incubated with an anti-mouse HRPO-labeled rabbit IgG reagent ImmPRESS (Vector Laboratories Inc., Burlingame, CA) for 30 min. After washing in PBS, each section was subjected to DAB-chromogen/substrate reagent and counterstained with hemotoxylin and eosin. As a positive control invasive ductal breast cancer was used.

Results

The pathologic findings are summarized in Table 1. In the positive control, the breast cancer cells displayed a strong positivity mainly in the cytoplasm but in some areas a slight nuclear reaction was also found (Fig. 1a). In the tumorous stroma some lymphoid nodules were seen and lymphocytes

Table 1 Immunohistochemical expression of glucocorticoid receptor in human pancreatic cancer samples

	Parenchyma		Stroma		
	Cytoplasm	Nucleus	Fibroblasts	Vessels	Lymphocytes
Control, breast cancer	90	10	–	–	80
Normal pancreas			10	–	80
acini	100	–	10	–	80
small ducts	±	–			
larger ducts	10	–			
Pancreatic cancer	80	20	10	±	70
Pancreatic cancer xenografts	80	20	–	–	N.A.

(proportion of positive cells)

± some areas exhibit a weak, inconstant positivity

N.A. not available

also expressed glucocorticoid receptor, but the stromal cells and vessels were negative.

In the normal, non-tumorous pancreata a strong positivity was detected in all acinar cells typically in the cytoplasm. Nuclear staining was not visible. The small/intermediate ducts expressed a very weak and inconstant positivity (Fig. 1b) except for one area where the small ducts increased in number forming a tubular complex.

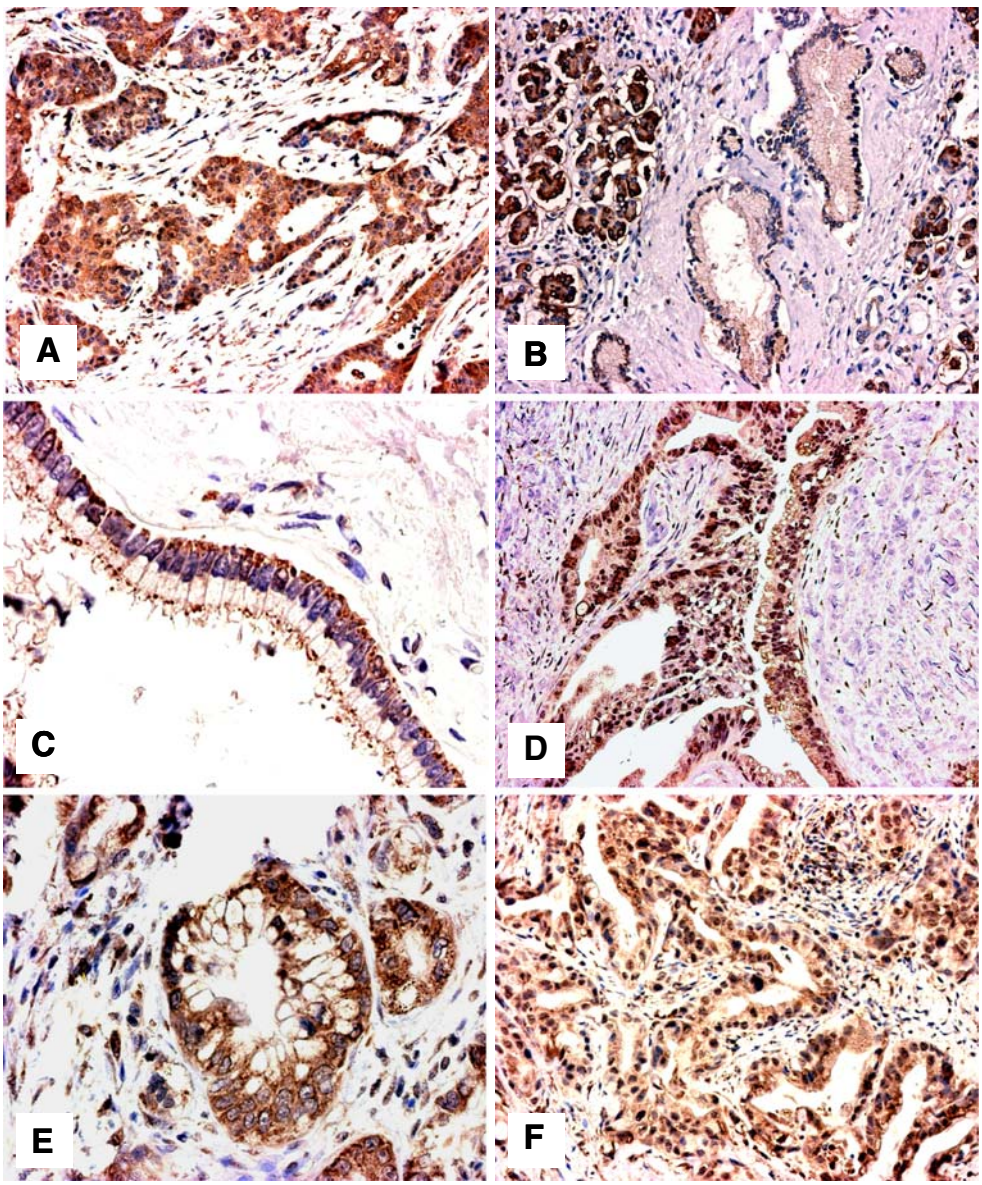

Fig. 1 **a** Positive control—breast cancer. (× 100). **b** Normal pancreas. On the left the acini show strong positivity mainly in the cytoplasm, but the intermediate ducts express a very weak reaction. The stromal mesenchymal elements do not stain while the lymphocytes show moderate positivity. (× 100). **c** Normal pancreas. In the large ducts the subnuclear region shows positive reaction. (× 200). **d**–**e** Pancreatic cancer. Strong positive reaction is seen in most of the tumorous glands (especially in the cytoplasm), but in some tumor cells nuclear reaction was also noticed. (**d** × 200, **e** × 400). **f** Pancreatic cancer xenograft. (× 200)

Some large ducts displayed positive reaction in the subnuclear region (Fig. 1c). In the stromal elements apart from the lymphocytes the myofibroblastic cells displayed a slight positive reaction, similarly to the Langerhans islets.

The ductal pancreatic carcinoma cells also expressed a strong positivity. The location of the immune reaction was mainly cytoplasmic but in some tumors a nuclear reaction was also noticed (Fig. 1d–e). When the tumor was positive for glucocorticoid receptor, over 80% of the tumor cells exhibited positivity, and this feature did not change with differentiation. The staining properties of the stroma was identical but stronger than that of the non-tumorous pancreata. The endothelial cells were mainly negative. Although the positivity of the tumor cells was a constant finding in our samples, surprisingly the liver metastases were completely negative.

The strong glucocorticoid receptor expression has been retained in xenografted human pancreatic cancer (Fig.1f) showing a diffuse, mainly cytoplasmic positivity.

Discussion

In this study it was shown that the glucocorticoid receptor was overexpressed in the tumor cells of the human ductal pancreatic cancer.

The role of the glucocorticoids in malignant tumors is controversial. They are well established inducers of apoptosis in lymphoblastic leukemia cells which has been exploited in the therapy of malignant lymphoproliferative disorders. However, in solid tumors, they behave differently. Dexamethasone has led to downregulation of the proapoptotic element of death receptors and mitochondrial apoptotic pathway in cervical or lung cancer [35]. It was also found a significant apoptosis inhibition in response to various cytostatics and irradiation in experiments with hepatocellular and colorectal carcinoma [36]. Dexamethasone has also promoted the growth of cisplatin or 5-FU-treated breast, cervical cancer, melanoma or neuroblastoma cell lines [37]. In 140 of 157 analyzed tumors (89%) glucocorticoid induced resistance for chemotherapy *in vitro* or *in vivo* as evidenced by inhibition of apoptosis, promotion of viability and cell cycle progression. These effects, however, proved to be reversible upon removal the steroid [38, 39], but raised concern about the application of steroids in non-hematological malignancies.

Although many different non-endocrine tumors contain measurable level or immunohistochemically detectable GR, its significance is largely unknown. Most of the papers provide descriptive results, but their applicability is vague. One cannot generalize about the presence or absence of GRs in malignant neoplasms because their level is highly variable from tumor to tumor.

For example, 41% of gastric cancer [14], 48% of colonic cancer [15], 51% of non-small cell lung cancer [19], 66% of malignant fibrous histiocytomas [9] contained these receptors but on the other side in human hepatocellular carcinomas the amount of GRs were right between 1,9–66,8% [11]. There are some tumors where the receptor content is extremely low or lacking, for example, the prostatic cancer, where 70–85% of these tumors exhibit very low expression [16, 17], or the small cell lung cancer [18]. There are some data available that the tumor and the surrounding tissue contain different amount of receptors, as it was presented in human or rat liver cell cancer [11, 12], or in gastric cancer [13]. Most of these publications do not provide data about the correlation with the survival, just a few papers mention it, but no clear cut conclusions have been drawn. In gastric cancer the glucocorticoid receptors have been associated with worse prognosis [14] but in non-small cell lung cancer patients a survival benefit was noted in positive cases [19]. Yemelyanov et al. claimed that the GR functions as a tumor suppressor inhibiting multiple signaling pathways and transcriptional factors involved in proliferation and transformation [17]. In rat liver cancer however no differences were found in genomic DNA-patterns of GR gene [12].

As for pancreatic cancer the available data are very limited. *In vivo* experiments have shown that dexamethasone inhibited the drug-induced apoptosis and enhanced tumor growth in all pancreatic carcinoma cell lines, and prevented the cytotoxic effects of gemcitabine and cisplatin in 90% of freshly isolated cell lines. Similarly in xenograft model dexamethasone has completely abolished the cytotoxic effect of chemotherapy. In these experiments the glucocorticoids upregulated the antiapoptotic genes [34].

The hormonal responsiveness of the pancreas has long been investigated, but treatment with steroids in experimental models similarly yielded controversial results. In acinar cell tumors (AR42J) dexamethasone resulted in transcriptional stimulation of amylase gene expression [40], and increased the amylase content and number of secretory granules [41]. Cortivazol, a potent synthetic glucocorticoid has doubled the GR mRNA levels in H2T hamster pancreatic adenocarcinomas, inhibited the tumor weight, DNA, RNA and protein content [42]. In addition, dexamethasone was able to induce differentiation in pancreatic AR42J cells [43]. Other pancreatic tumors, however, respond differently which was pointed out by Benz et al [44]: *in vitro* in Colo-357 tumor 30% increase of proliferation was observed, in AR42J tumor the proliferation rate has decreased by 50% but the RWP-2 tumor proved to be completely resistant.

Dexamethasone exerted no antiproliferative effect *in vitro* but it reduced the invasiveness of pancreatic carcinoma cells, and *in vivo* significant reduction of recurrent

tumor volume and the number of liver and spleen metastases were observed. Therefore, this steroid results in a profound influence on the malignant phenotype but it is not clear whether this effect is due to direct action or inhibition of proinflammatory singals [45].

In the oncological practice glucocorticoids are frequently coadministered with cytostatics to reduce the hyperemesis and the acute toxicity on the nontumorous tissues. However, concerns are raised about this combination because experimental data may show that it could be a harmful combination [34]. Steroid treatments are frequently applied for differential diagnosis of autoimmune pancreatitis and pancreatic cancer. Autoimmune pancreatitis, a mass forming tumor-like pancreatic lesion could be differentiated by a two-week steroid treatment due to the fact that a marked improvement in pancreatic duct narrowing is observed in the former one, but the malignant process remained unchanged [46, 47].

Although the overexpression of the glucocorticoid receptor in our pancreatic cancer cases was well demonstrated, there are many open questions. We do not know if the presence of immunohistochemical positivity means functionally active receptors, or just an aberrant expression. Some studies suggested that these receptors would interfere with cytostatic treatment [37, 38] resulting in a decreased efficacy of chemotherapy. Similarly, the dynamics of the receptor expression during pancreatic carcinogenesis is obscure, but it would be important, because the ductal system in our study displayed a faint and inconsistent positivity, however, the ductal adenocarcinomas were strongly positive. So far, systemic investigations on the possible association between the glucocorticoid receptor and its clinical impact are also still lacking. In the treatment of pancreatic cancer steroids are not approved, but the presence of these receptors should have a biological significance. The applicability of this finding is still far from clear, however, it is worth further investigating.

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