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Transferrin receptor is a marker of malignant phenotype in human pancreatic cancer and in neuroendocrine carcinoma of the pancreas

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Abstract

Transferrin receptor (TFRC) is a membrane-bound protein expressed in larger amounts in proliferating, e.g., malignant, cells than in quiescent cells. The specific expression of TFRC can represent a diagnostic tool or a therapeutic target in solid tumours expressing this antigen. Whether TFRC is expressed in human pancreatic tumours is unknown. The aim of this study was the investigation of the expression of TFRC and transferrin in human pancreatic cancer and in neuroendocrine tumours of the pancreas. Fifty one specimens of human pancreatic cancer and 14 samples of pancreatic neuroendocrine tumours were obtained after surgery. The expression of TFRC, transferrin and cytokeratin was studied by standard immunohistochemistry. Flow cytometry was used for the investigation of TFRC expression in nine cell lines of ductal pancreatic cancer *in vitro*. In contrast to normal tissue, 93% of pancreatic tumour cells showed positive (82%) or heterogeneous (11%) expression of TFRC. It was strongly expressed by malignant epithelial cells; normal stromal and endothelial cells were not stained by anti-TFRC antibodies. Primary tumours and metastases showed a similar frequency of TFRC expression. Three neuroendocrine carcinomas showed positive expression of TFRC by malignant tumour cells. The expression of TFRC was negative in benign neuroendocrine tumours of the pancreas. The cell lines of pancreatic cancer were characterised by a low expression of TFRC *in vitro*. In contrast to normal pancreatic tissue and benign neuroendocrine tumours of the pancreas, pancreatic cancer and neuroendocrine carcinoma are therefore characterised frequently by high expression of TFRC. Hence, TFRC represents a marker of malignant transformation in the pancreas that could be applied as potential diagnostic and therapeutic target.

Keywords: Pancreas; Pancreatic tumours; Neuroendocrine; Tumours; Transferrin; Transferrin receptor; Immunocytochemistry; Flow cytometry

1. Introduction

Ductal adenocarcinoma of the pancreas is still the fifth most common cause of cancer deaths in Europe and the USA [1]. The therapeutic results for pancreatic carcinoma are disappointing and the mean survival is very low. More than 80% of patients with pancreatic carcinoma come to clinical treatment in stage III or IV of tumour growth. Surgery is so far the only possibility for curing this malignant tumour. The mean survival time after diagnosis of pancreatic carcinoma does not exceed 6 months. Adjuvant therapeutic modalities such as chemo- and radiochemotherapy can increase the recurrence-free time, but do not improve the mean survival rates [1–3]. Despite attempted curative operations, only a minority of patients with ductal adenocarcinoma survive 5 years [4].

The development of specific molecular tracers for the diagnosis and treatment of this lethal cancer has a major goal. Advances in oncological research have led to the identification of many tumour-associated mutations. Some of them, such as p53, p16INK [5], kiras [6] and SMAD4 [1,7] have been already evaluated

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as diagnostic factors in pancreatic carcinoma. However, the majority of these markers are characterised by limited specificity or sensitivity and can be used only in combination with conventional diagnostic tools [1].

Iron is an essential element for cell proliferation and cell metabolism. The high proliferative rates in malignant cells are characterised by an increased requirement for iron. For this purpose, cells have developed a specific mechanism for iron uptake from the plasma. The initial step in cellular iron uptake is the binding of transferrin–iron complexes to the transferrin receptor (TFRC) on the plasma membrane. After binding, this complex may be internalised. Because of its pivotal role in iron uptake, the TFRC is expressed in larger amounts in proliferating, e.g., malignant, cells than in quiescent cells [8]. Among normal tissues, the liver, epidermis, intestinal epithelium, brain endothelial cells as well as some populations of haematopoietic cells express constitutively the TFRC [8].

The TFRC is also expressed by most carcinomas, sarcomas and some lymphomas and leukaemias [9–12]. Due to its increased expression by many malignant cells, the receptor has been suggested as a promising target in anti-cancer therapy [13].

Although the expression of TFRC was already well characterised in many malignant tumours, there have been no substantial reports about the role of this molecule in pancreatic tumours. A single investigation mentions two cases of pancreatic cancer that expressed TFRC [8]. In the present study, the expression of TFRC in human pancreatic carcinoma and in neuroendocrine tumours of the pancreas was investigated in a large number of tissue samples. This study demonstrated that TFRC is not expressed in normal pancreatic tissue and in most benign neuroendocrine tumours of the pancreas. TFRC was frequently upregulated in primary pancreatic cancer and its metastases, and in neuroendocrine carcinoma.

2. Materials and methods

2.1. Patients and tissue samples

Patients admitted to the study were undergoing surgery for pancreatic tumours at the Department of Surgery of the University of Heidelberg. All gave their informed consent to the protocol, which was approved by the ethics committee at the University of Heidelberg. Fifty one samples of ductal pancreatic carcinoma (12 metastases and 39 primary tumours) and 12 samples of neuroendocrine tumours (five insulinomas, one gastrinoma, one glucagonoma, one carcinoid and four neuroendocrine carcinomas) were collected. Eight samples of non-malignant pancreas were obtained from the patients operated on for pancreatic tumours, these samples were obtained 5–10 cm away from the tumour. Each sample was snap-frozen and stored in liquid nitrogen. Clinical information on the operated patients was documented prospectively for further statistical analysis.

2.2. Immunohistochemical staining

Sections were cut at 5 μm, air-dried and fixed in acetone. The slides were stored at −20 °C until further use. The sections were stained by indirect three-step immunohistochemistry using the LSAB Kit (Dako, Hamburg, Germany) and counterstained with Mayer's acid haemalum (Fluka, Steinheim, Germany). Monoclonal anti-cytokeratin (clone AE1/AE3; Dako) and anti-TFRC (clone Ber-T9; Dako) antibodies were used. Transferrin was stained by polyclonal antibodies (Dako). Positive expression of TFRC was recorded if more than 80% of cells were stained positively. Heterogeneous expression was recorded if 25–80% of cells were stained. Staining of <5% of cells was defined as negative expression.

2.3. Flow cytometry

The expression of TFRC *in vitro* was investigated in nine cell lines of ductal pancreatic cancer: AsPC1, Capan1, MiaPaca1, KCl MOH1, Panc1, FAMPAC, Patscl52, PaTu8902 and PaTu8988t. The cells were allowed to grow for 2–3 days before investigation in Iskove medium (CCPro, Neustadt, Germany) supported by 10% of fetal calf serum (CCPro). Cells were harvested 2–3 days after seeding. They were non-confluent and proliferating actively at this time. A suspension of single cells was obtained by filtration through a 40-μm nylon mesh. Cells were incubated with fluorescein isothiocyanate (FITC)-labelled anti-TRFC antibodies (clone DF1513; Serotec, Düsseldorf, Germany). FITC-labelled mouse isotypic antibodies (BD Pharmingen, Heidelberg, Germany) were used as a negative control. Flow cytometry was performed on a Becton–Dickinson (Mannheim, Germany) flow cytometer.

3. Results

3.1. Transferrin receptor/transferrin

No samples of normal pancreatic tissue were stained by anti-TRFC antibodies. The expression of TFRC was also negative in most neuroendocrine tumours of the pancreas (all insulinomas, gastrinoma, glucagonoma and one neuroendocrine carcinoma) (Table 1). Three of the four neuroendocrine carcinomas were characterised

Table 1
Expression of transferrin receptor by tumour cells in neuroendocrine tumours of the pancreas

Tumour	*n*	Positive	Negative
Insulinoma	5	0	5
Gastrinoma	1	0	1
Glucagonoma	1	0	1
Carcinoid	1	0	1
Neuroendocrine carcinoma	4	3	1

by positive expression of TFRC that was strongly related to malignant tumour cells (Table 1; Fig. 1).

In contrast to normal tissue, 93% of pancreatic tumour cells showed positive (82%) or heterogeneous (11%) expression of TFRC. It was strongly expressed by malignant epithelial cells; these cells were identical to cells positively stained by anti-cytokeratin antibodies (Fig. 2). Primary tumours and metastases showed a similar frequency of positive/heterogeneous/ negative expression of TFRC, as shown in Table 2. Three primary tumours and two metastases demonstrated negative expression of TFRC (Table 2). Statistical analysis showed no significant difference between primary tumours and metastases ($P > 0.05$, χ^2 test). Normal stromal and endothelial cells were not stained by anti-TFRC antibodies.

The immunohistochemical analysis of transferrin showed diffuse homogeneous staining in tissues investigated (Fig. 2).

3.2. Expression of transferrin receptor by cell lines

The expression of TFRC by the nine cell lines *in vitro* varied, but in general only a low amount was found, in contrast to the situation *in vivo*. The percentages of TFRC-expressing cells were: 3% (AsPC1), 2% (Capan1), 13% (MiaPaca1), 19% (KCl MOH1), 5% (Panc1), 7% (FAMPAC), 3% (Patscl-52), 8% (PaTu8902) and 11% (PaTu8988t).

4. Discussion

The expression of TFRC in human pancreatic tumours had not, to the best of our knowledge, been analysed systematically before. There is only one report on the expression of transferrin receptor in normal pancreatic tissue and in two pancreatic cancer samples only [8]. We have investigated for the first

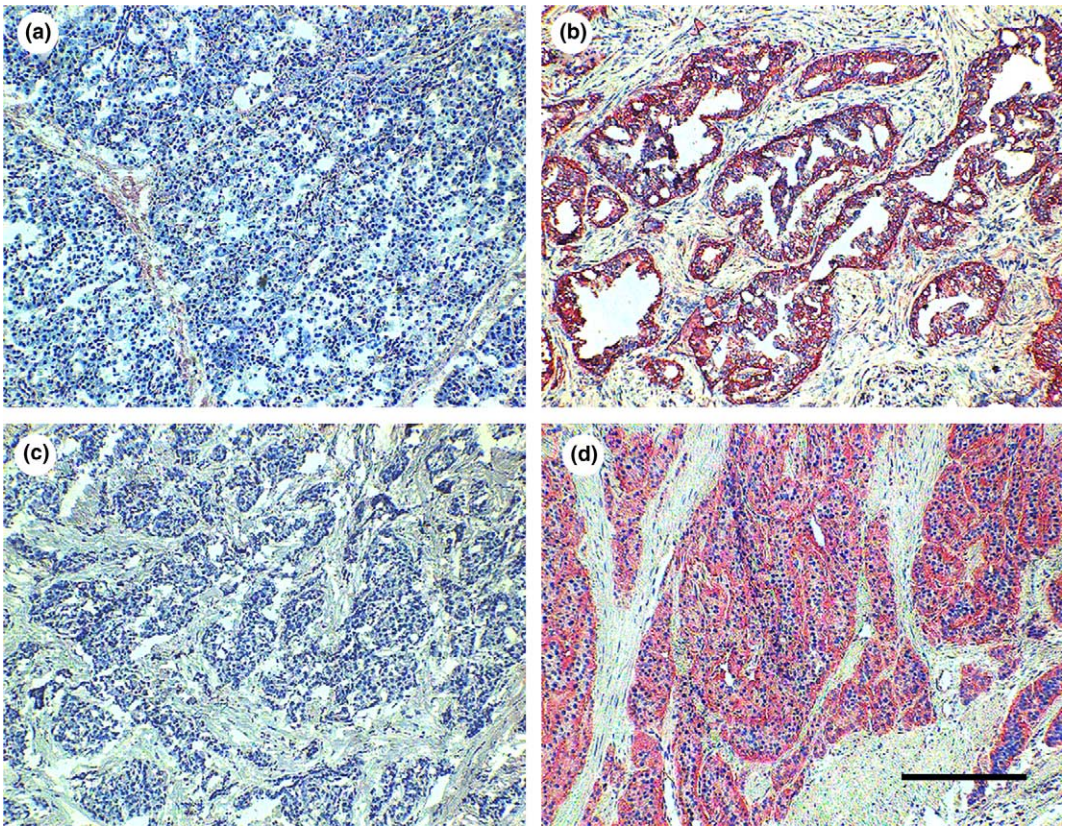

Fig. 1. Immunohistochemical staining of transferrin receptor (TFRC) in normal pancreas (a), pancreatic cancer (b), insulinoma (c) and neuroendocrine carcinoma (d). Normal pancreas and benign neuroendocrine tumours (insulinomas) are not stained by anti-TFRC antibodies. Malignant cells in pancreatic cancer and neuroendocrine carcinoma show a strongly positive staining of the receptor. Bar = 250 μm.

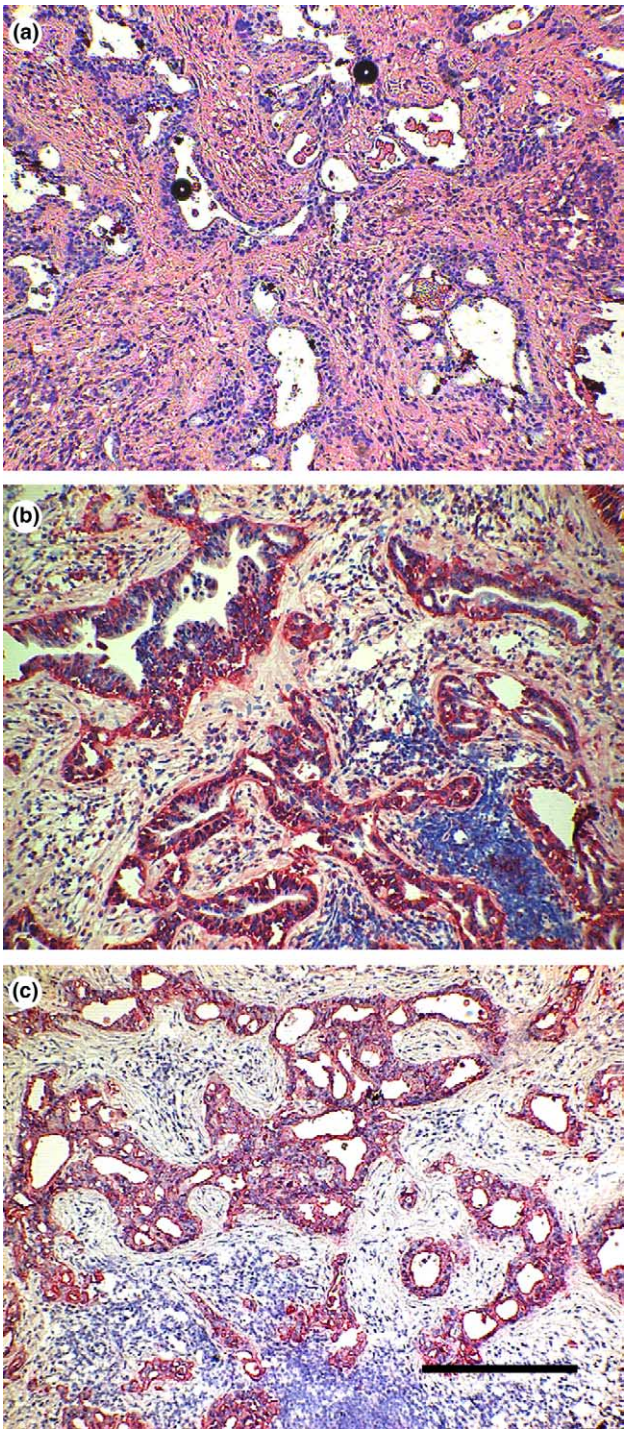

Fig. 2. Immunohistochemical staining of transferrin (a), transferrin receptor (TFRC) (b) and cytokeratin (c) in pancreatic cancer. Transferrin shows diffuse staining, whereas malignant epithelial cells are exclusively stained by anti-TFRC and anti-cytokeratin antibodies. (a–c) show the same tumour. Bar = 250 μm.

time the expression of TFRC in a significant number of pancreatic cancers and in neuroendocrine tumours of the pancreas. We demonstrate that the TFRC was expressed exclusively by malignant cells in 90% of pancreatic cancers. Primary tumours and metastases showed a similar frequency of positive expression of TFRC. Tumour cells in three of the four neuroendocrine carcinomas also expressed the receptor. Normal pancreatic tissue and benign neuroendocrine tumours were characterised by a loss of TFRC expression. The earlier study found positive staining for TFRC in the islets of Langerhans with one of the four different monoclonal antibodies [8]. We found no positive reaction in pancreatic exocrine and endocrine tissue with the antibody used here.

Table 2
Expression of transferrin receptor by tumour cells in human pancreatic cancer

Expression	Primary tumours	Metastases	All tumours
Positive	32 (82%)	9 (75%)	41 (80%)
Heterogeneous	4 (11%)	1 (8%)	5 (10%)
Negative	3 (7%)	2 (17%)	5 (10%)
Total	39	12	51

That TFRC was expressed exclusively by malignant pancreatic tumours is very interesting. It provides evidence that the receptor is a specific marker of a malignant phenotype in pancreatic tissue. We believe that this finding may have potential for diagnostic and therapy. The selective expression of TFRC by malignant cells has already been shown to improve significantly the tumour-targeted delivery of immunocytokines [14], cytotoxic drugs [15,16] and genes [17] in cancer models [17]. Another aspect of TFRC expression might be the targeting of tumour cells with monoclonal antibodies against the receptor. These antibodies were useful in selectively inhibiting tumour growth in a lymphoid tumour model [13].

We found that the expression of TFRC by proliferating malignant cells *in vitro* was very low under standard conditions, in obvious contrast to the findings *in vivo*. This difference is easily explained by the fact that the abundant supply of iron under cell-culture conditions does not necessitate upregulation of TFRC. Thus, we present evidence that TFRC is not a direct marker of proliferation, but that its expression on malignant cells is induced by the local microenvironment competing for iron under limited supply. This finding accords well with other studies on the influence of tumour hypoxia on TFRC. Tacchini and colleagues [18] showed that expression of the TFRC is strongly dependent on tumour hypoxia. Bianchi and colleagues [19] demonstrated that the hypoxia-inducible factor-1 activates the transcription of TFRC in hepatoma cells.

In conclusion, the present study demonstrates a high expression of TFRC in pancreatic cancer and in neuroendocrine carcinoma of the pancreas. We believe that this observation may have implications for diagnosis and the delivery of cytotoxic agents, and for the direct targeting of malignant cells.

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