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Glucose transporter-1 expression and prognostic significance in pancreatic carcinogenesis

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Summary. The purposes of this study were to evaluate the prognostic significance of Glut-1 expression in patients with pancreatic ductal adenocarcinoma, and to analyse its expression in pancreatic intraepithelial neoplasias (PanIN) and non invasive intraductal papillary mucinous neoplasms (IPMN). Glut-1 expression was studied by immunohistochemistry in 60 pancreatic ductal adenocarcinomas and scored on a 4-point scale (1: <25%; 2: 25-50%; 3: 50-75%; 4: >75%). Relationships between Glut-1 score, histological grade and MIB-1 score were evaluated by the Spearman rank correlation test. Significant correlations were found between Glut-1 expression and histological grade ($P<0.001$) and MIB-1 score ($P<0.01$). Significant prognostic factors by univariate analysis were stage ($P<0.0001$), histological grade ($P<0.001$) and Glut-1 expression ($P<0.005$). Independent prognostic factors after multivariate analysis were stage ($P<0.001$) and Glut-1 expression ($P<0.05$), stratified as <50% and >50%. The correlation of Glut-1 score with histological grade and MIB-1 score indicated a higher glucose uptake in poorly differentiated and highly proliferative pancreatic cancer cells. Glut-1 immunohistochemical expression provides a useful prognostic factor in pancreatic ductal adenocarcinoma. Glut-1 expression was not found in PanINs 1 but in 27.8% and 43.8% of PanINs 2 and 3, and was not found in IPMNs with low- and moderate-grade dysplasia but in 60% of IPMNs with high-grade dysplasia, indicating Glut-1 involvement in a relatively early phase of pancreatic carcinogenesis.

Key words: Pancreatic adenocarcinoma, PanIN, Glut-1, Immunohistochemistry, Survival

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Introduction

Pancreatic cancer represents the fourth leading cause of cancer-related deaths in the United States and shows the lowest 5-year survival rate among the 10 most common cancers (Jemal et al., 2006). Late clinical presentation, intrinsic biological aggressiveness, and resistence to conventional chemotherapy and radiotherapy represent the predominant reasons for its poor prognosis. Clinicopathologic factors, such as tumor stage and grade (Lim et al., 2003), R0 resection (Wittekind et al., 2002), post-operative normalization of tumor markers (Sperti et al., 1993) and demonstration of disseminated tumor cells (Vogel et al., 2002) have shown prognostic significance, although differing survival rates among patients within the same stage groups have also been detected (Sperti et al., 2003).

Malignant cells have high constitutive glucose uptake and metabolism compared with normal cells. Glucose uptake is mediated by an expanding family of facilitative glucose transporters (GLUTs), the expression and activity of which have been found to be regulated by oncogenes and growth factors. Glut-1 protein is physiologically expressed in a few normal cells, such as erythrocytes, endothelial cells of the blood-brain and blood-nerve barriers, liver, placenta, and basal cells of benign squamous epithelium (Pessin and Bell, 1992; Cornford et al., 1994), but increases in Glut-1 expression have been shown in some preneoplastic lesions and in many malignant tumors, in which a negative prognostic significance has frequently been found (reviewed in Airley and Mobasheri, 2007).

In pancreatic cancer, immunohistochemical overexpression of Glut-1 and higher 18F-fluorodeoxyglucose (FDG) uptake, quantified by positron emission tomography and determined as standardized uptake value (SUV), have been found.

SUVs correlated with Glut-1 transcription or protein expression (Reske et al., 1997; Higashi et al., 1997). Thus, increased expression of Glut-1 molecules in human pancreatic tumors has been suggested to contribute to the higher rate of FDG uptake into tumor cells compared with normal pancreatic tissue (Higashi et al., 1997). SUV has been found to be a predictor of survival in patients with pancreatic cancer (Sperti et al., 2003; Nakata et al., 1997; Pedrazzoli et al., 2005). Literature data regarding the prognostic significance of immunohistochemical Glut-1 expression in pancreatic ductal adenocarcinoma are limited and non consistent, as a prognostic significance of Glut-1 expression has been found by one research group (Sun et al., 2007) but not by another one (Lyshchik et al., 2007). Therefore, in a highly selective series of patients with resected pancreatic ductal adenocarcinoma, we studied the correlations of Glut-1 immunohistochemical expression with clinicopathological and histopathological parameters, and evaluated its prognostic significance.

A progression model has recently been proposed for pancreatic ductal adenocarcinoma, ranging from normal epithelium to invasive carcinoma through a series of preneoplastic lesions, termed "pancreatic intraepithelial neoplasias" or PanINs (Hruban et al., 2000). PanIN-1A is a flat lesion composed of tall columnar cells with basally located nuclei and supranuclear mucin; PanIN-1B is similar to the preceding lesion, but with additional papillary or micropapillary architecture; PanIN-2 is a flat or papillary lesion with mild to moderate nuclear atypia; PanIN-3 shows severe atypias, i.e., papillary, micropapillary or cribriform architecture with budding and/or bridging, loss of nuclear polarity, pleomorphism, prominent nucleoli, frequent mitoses, and luminal necrosis (Hruban et al., 2000; Biankin et al., 2003). The premalignant significance of such lesions has recently been strengthened by studies showing the genetic aberrations of pancreatic adenocarcinoma also in PanINs. Mutations in HER-2/neu, K-ras and $p21^{WAF1/CIP1}$ occur early in the development of PanIN, followed by Id-1/Id-2, p53, cyclin D1 and $p16^{INK4A}$, with DPC4/Smad4 and BRCA2 abnormalities occurring in the late phases of PanIN progression (Biankin et al., 2003). Although Glut-1 overexpression has been found to play a role in the early carcinogenesis of some tumors, it has never been studied in pancreatic preneoplastic lesions. Intraductal papillary mucinous neoplasms (IPMN) of the pancreas represent another disease presenting proliferation of the epithelium of the pancreatic ducts, which usually forms papillae and leads to cystic dilations (Rickaert et al., 1991). IPMNs may show low-, moderate- or high-grade dysplasia and in about one third of the cases may be associated with invasive carcinoma of either tubular or colloid types (Adsay et al., 2000). Thus, in the present study, we also evaluated Glut-1 immunohistochemical expression in the above preneoplastic lesions, i.e., the PanINs found in non-neoplastic pancreatic tissue resected together with ductal adenocarcinoma and non invasive IPMN.

Materials and methods

Patients

The study was performed on a total of 60 consecutive patients (30 males, 30 females; age range 40-84 years; mean age 65.4 years) who underwent surgical pancreatic resection for pancreatic ductal adenocarcinoma and on 9 patients (5 males, 4 females; age range 50-74 years; mean age 67.3 years) who underwent surgical pancreatic resection for intraductal papillary mucinous neoplasms (IPMN). The completeness of tumor removal was assessed according to the TNM Residual Tumor Classification of the American Joint Committee of Cancer. None of the patients received pre-operative chemotherapy or radiation therapy. Informed consent was obtained from all patients, the study was approved by the local ethical committee, and conforms to the ethical standards of the World Medical Association Declaration of Helsinki. Tumors were classified according to the American Joint Committee of Cancer pTNM staging system (Greene et al., 2002), and grading was assessed using World Health Organization criteria (Kloppel et al., 2000). Survival was calculated from the date of surgical pancreatectomy to the date of the latest follow-up visit or death due to recurrent pancreatic cancer. Any patient who died from diseases other than pancreatic cancer was considered excluded.

Immunohistochemistry

Each surgical specimen was fixed in 10% buffered formalin, and multiple samples were collected from neoplastic and non-neoplastic pancreatic tissue. For diagnostic purposes, paraffin-embedded 5µm thick sections were stained with hematoxylin-eosin. All histological slides were reviewed by two pathologists who had no prior knowledge of the prognostic factors and/or clinical outcomes.

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue, using a standard avidin-biotin immunoperoxidase complex technique (Dako, Milan, Italy). Tissue sections (4 µm thick) were mounted on silanized slides, dewaxed in xylene, dehydrated in ethanol, boiled in 0.01 M citrate buffer (pH 6.0) for 15 min in a microwawe oven (95 °C), and incubated with 3% hydrogen peroxide for 5 min. After washing with phosphate-buffered saline (PBS), they were incubated in PBS containing 10% BSA for 5 min, followed by incubation for 45 min with mouse anti-Ki-67 monoclonal antibody (diluted 1:100; Dako, Milan), or rabbit anti-Glut-1 polyclonal antibody (diluted 1:200; A3536, Dako, Milan). Anti-Glut-1 antibody has been demonstrated to specifically localize Glut-1, without cross-reactivity with other glucose transporters. After washing, sections were incubated with biotinylated goat anti-mouse immunoglobulin (LSAB kit, Dako, Milan) and anti-rabbit

immunoglobulin (Biogenex, Space Import Export, Milan), respectively. Peroxidase-conjugated avidin (Dako, Milan) was used at a dilution of 1:500. Lastly, 0.02% diaminobenzidine and 1% hydrogen peroxide (Dako, Milan) in PBS were used as substrates in the color development reaction. Sections were then counterstained with hematoxylin. Immunohistochemical analysis of MUC1 (mouse monoclonal antibody, diluted 1:800; UCS Diagnostic, Rome) and MUC2 (mouse monoclonal antibody, diluted 1:400; UCS Diagnostic, Rome) was also performed on samples from resections for pancreatic ductal adenocarcinoma containing PanINs, in order to help their identification and grading.

Glut-1 expression was considered positive only when distinct plasma membrane staining was present. Red blood cells and the perineurium of nerves were considered as internal positive controls for Glut-1 staining. Positive controls for anti-MUC1 and -MUC2 stainings were mammary carcinomas and colonic tumours, respectively. Negative control sections were prepared with normal mouse or rabbit immunoglobulin instead of the specific primary antibody. In each case, Glut-1 immunostaining of neoplastic cells was blind-examined in two representative slides of neoplastic tissue by two independent, experienced pathologists. Ten fields at 40X were evaluated for each slide. The percentage of positive cells was calculated as the average of stained cancer cells in the 20 fields examined and was scored on a 4-point scale (1: <25%; 2: 25-50%; 3: 50-75%; 4: >75%).

PanINs were selected from outside the invasive cancer nest, in which the original structure of the pancreas was kept. The above immunohistochemical expressions in PanINs were not scored but were evaluated in terms of the presence or absence of immunostaining.

Computer-assisted image analysis was performed in order to determine the percentage of MIB-1 positive cells in neoplastic tissue samples. The image analysis system was composed of a Leica DM-R microscope (Leica Microsystems, Wetzlar, Germany) and a high-resolution digital camera (DC200, Leica Microsystems, Wetzlar) which transmits image data to a PC equipped with appropriate software for image acquisition and analysis (QWin, Leica Microsystems, Weitzlar). Two slides for each case were examined and, for each one, 10 fields were randomly chosen in order to best reflect the overall immunostaining of the tissue. The image of each field was acquired in full color (24-bit) at a final magnification of 40x, and processed to correct shading and enhance contrast. The area measured was outlined in order to restrict the analysis to the epithelial neoplastic component. Color thresholding was then applied to identify immunostained MIB-1 nuclei. A second thresholding operation was also applied to identify all hematoxylin-stained nuclei. The MIB-1 labeling index was obtained by dividing the area showing positive MIB-1 immunostaining by the total area showing positive staining.

Statistical analysis

Relationships between Glut-1 score, histological grade and MIB-1 proliferation index were evaluated by the Spearman rank correlation test. Univariate analysis of the prognostic significance of histological grade, staging and Glut-1 expression was performed by the log rank test. Univariate analysis of the prognostic significance of the MIB-1 proliferation index was performed by the Cox regression model. Variables associated with survival, with a P value of less than 0.05, were entered into the multivariate analysis with the Cox proportional hazards regression model, to identify independent prognostic factors in predicting survival. In the multivariate analysis, Glut-1 immunostaining was stratified into two groups, corresponding to low (scores 1-2: ≤50%) and high (scores 3-4: >50%) expression. $P<0.05$ was considered to be statistically significant. Statistical calculations were carried out on Prism 3.0.3 (GraphPad Software Inc., San Diego, CA, USA).

Results

Details of the clinicopathologic characteristics of the patients with pancreatic ductal adenocarcinoma are listed in Table 1. Forty-four patients (73%) underwent pancreaticoduodenectomy, 13 (22%) distal pancreatectomy, and 3 (5%) total pancreatectomy. In 54 cases (90%), tumor removal was complete (R0), according to the TNM Residual Tumor Classification; in 4 (6.7%) and 2 (3.3%) patients, microscopic (R1) and macroscopic (R2) residual tumors were present, respectively. Four patients (7%) were in stage I, 12 (20%) stage II, 34 (56%) stage III, and 10 (17%) stage IV. The median and mean (±SD) overall survivals for all 60 patients were 16 and 18.4 (±12.2) months, respectively. Forty patients (60%) died of cancer-related diseases, with median and mean (±SD) overall survivals of 13.5 and 15.5 (±7.9) months, respectively. The follow-up range of patients still alive was 7-69 months, with median and mean (±SD) follow-up periods of 20 and 24.2 (±16.8) months, respectively.

Of the 60 ductal adenocarcinomas, five (8%) were classified as well-differentiated (G1), 26 (44%) as moderately differentiated (G2) and 29 (48%) as poorly differentiated (G3). The MIB-1 index range of all patients was 4.8-49.7; the median and mean MIB-1 indexes (±SD) were 15.4 and 18.5 (±10.5), respectively. Plasma membrane immunoreactivity for Glut-1 was detected in all cases of pancreatic ductal adenocarcinoma, with a mean score of 2.3. In a linear regression analysis, histological grade correlated with MIB-1 index, with an R value of 0.383 ($P<0.01$). The mean values of Glut-1 expression in G1, G2 and G3 pancreatic adenocarcinomas were 2.8(±0.45), 2.81(±0.56), and 3.76(±0.43), respectively. Significant correlations were also found between Glut-1 expression and histological grade ($R=0.740$; $P<0.001$) and MIB-1 index ($R=0.402$; $P<0.01$) (Fig. 1; Table 2).

According to univariate analysis stage (P<0.001), histological grade (P<0.001) and Glut-1 score (P=0.003) were statistically significant prognostic factors (Table 3). Patients with Glut-1 scores of 1 and 2 had a median overall survival period of 22 and 29 months, respectively; those with Glut-1 scores of 3 and 4 had 16 and 12 months, respectively. Thus, for multivariate analysis, Glut-1 expression was stratified as low (scores 1-2: ≤50%) and high (scores 3-4: >50%). After multivariate analysis, stage (P<0.001) and Glut-1 expression (P<0.05) proved to be independent prognostic factors (Fig. 2; Table 4). The mean survival periods of the cases with low and high Glut-1 expression were 26 and 13 months, respectively (P<0.01).

The non-neoplastic pancreatic tissue sampled with the ductal adenocarcinomas showed ductal preneoplastic lesions of various grades in 58 out of 60 cases. PanINs 1A were detected in 41/60 cases, PanINs 1B in 37/60, and PanINs 2 and 3 in 18/60 and 16/60 cases, respectively. Anti-MUC1 positivity was not found in PanIN1, was focally found in 3/18 (16.7%) cases of PanIN2, while PanIN3 showed focal or diffuse MUC1 immunostaining in 9/16 (56.3%) cases. PanINs did not show MUC2 immunostaining (Fig. 3). PanIN 1 lesions did not show Glut-1 immunostaining, whereas PanINs 2 and 3 showed positivity in the ductal epithelium in 5/18 (27.8%) and 7/16 (43.8%) cases, respectively (Fig. 4). In positive PanIN 2 Glut-1 immunostaining was focal (Fig. 4B), in PanIN 3 it was focal (Fig. 4C) or diffuse (Fig. 4D). Correlations between Glut-1 immunostainings of the PanIN lesions and of the correspondent carcinoma have not been found.

IPMNs showed low-, moderate-, and high-grade dysplasia in 2/9 (22.2%), 2/9 (22.2%), and 5/9 (55.6%)

Table 1. Descriptive, clinicopathological and histopathological characteristics of sample (Total 60 cases).

Variable	No. Cases	Percent
Age at diagnosis (years)		
40-49	5	9
50-59	11	18
60-69	20	33
≥ 70	24	40
Gender		
Male	30	50
Female	30	50
Type of surgery		
Pancreaticoduodenectomy	44	73
Total pancreatectomy	3	5
Distal pancreatectomy	13	22
Completeness of tumor removal		
R0	54	90
R1	4	7
R2	2	3
UICC classification		
pT		
T1	0	0
T2	7	12
T3	47	78
T4	6	10
pN		
N0	16	27
N1	44	73
N2	0	0
pM		
M0	55	92
M1	5	8
pStage		
I	4	7
II	12	20
III	34	56
IV	10	17
Follow-up		
Alive	20	33
Dead	40	67
Histological grade		
G1	5	8
G2	26	44
G3	29	48
Glut-1 score		
1	14	23
2	23	38
3	16	27
4	7	12

Table 2. Correlation between histopathological characteristics.

Variables	Spearman's rho	Kendall's tau	*P*
Histological grade - Glut-1	0.7403	0.6792	<0.001
Histological grade - MIB-1	0.3825	0.3196	<0.01
Glut-1 - MIB-1	0.4015	0.2610	<0.01

Table 3. Univariate associations of clinicopathologic variables with overall survival.

Variables	Overall survival *P*
Stage	< 0.001 (Log-rank)
Histological grade	< 0.001 (Log-rank)
MIB-1 index	0.528 (Cox regression)
Glut-1 score	0.003 (Log-rank)

Table 4. Multivariate predictors of survival.

	Overall survival	
Variables	Hazard Ratio (95% CI)	*P*
Stage	2.15 (1.5-3.1)	<0.001
Histological grade	1.69 (0.7-4.1)	0.240
Glut-1 score*	2.81 (1.1-8.0)	0.034

*: Stratified as low (scores 1-2: <50%) and high (scores 3-4: >50%) expression.

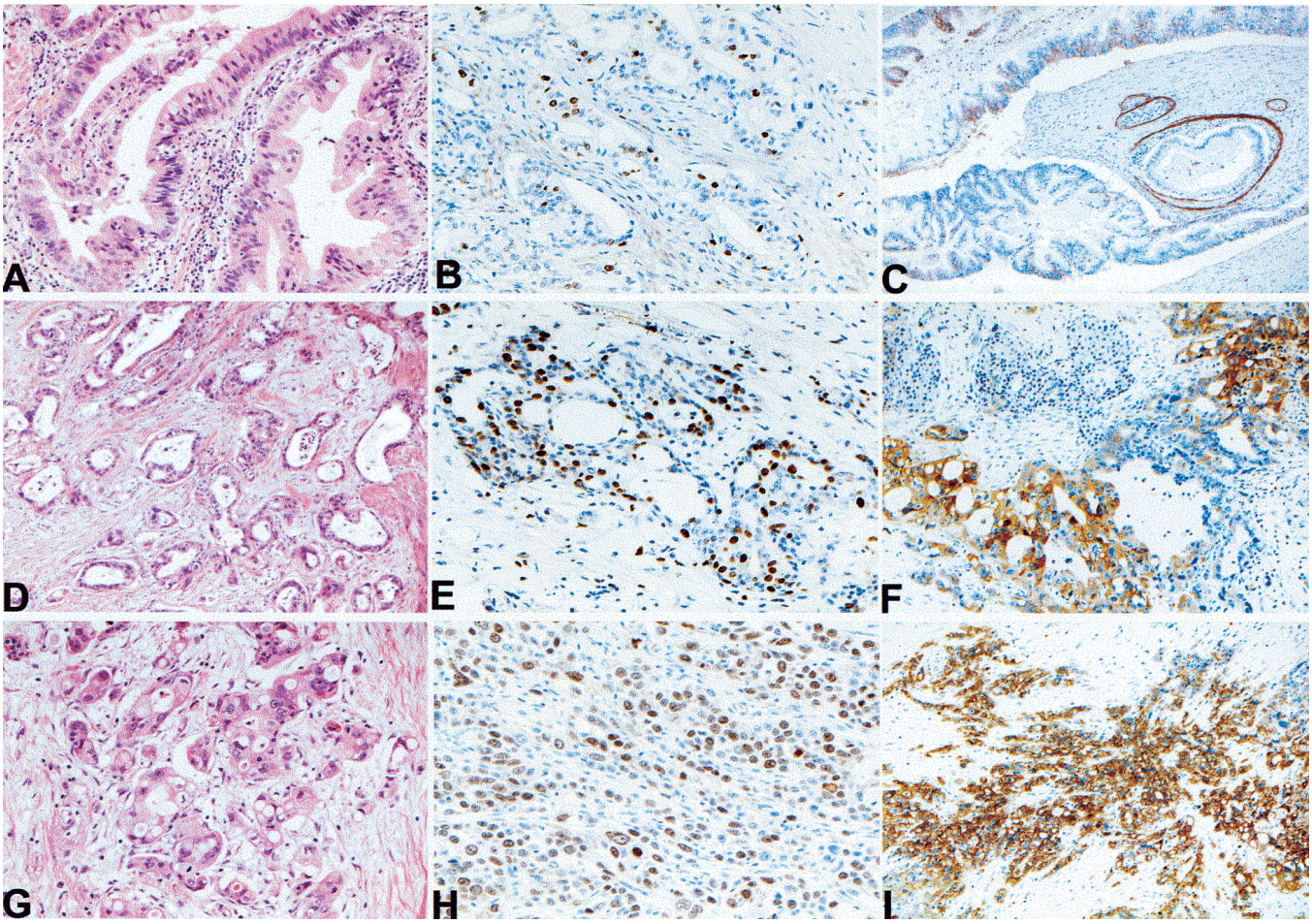

Fig. 1. G1 (**A**: H.E.; **B**: anti-Ki-67; **C**: anti-Glut-1), G2 (**D**: H.E.; **E**: anti-Ki-67; **F**: anti-Glut-1) and G3 (**G**: H.E.; **H**: anti-Ki-67; **I**: anti-Glut-1) pancreatic ductal adenocarcinomas, showing the correlation of Glut-1 expression with grading and MIB-1 staining. A, B, D, E, H, x 20; C, F, I, x 10; G, x 40.

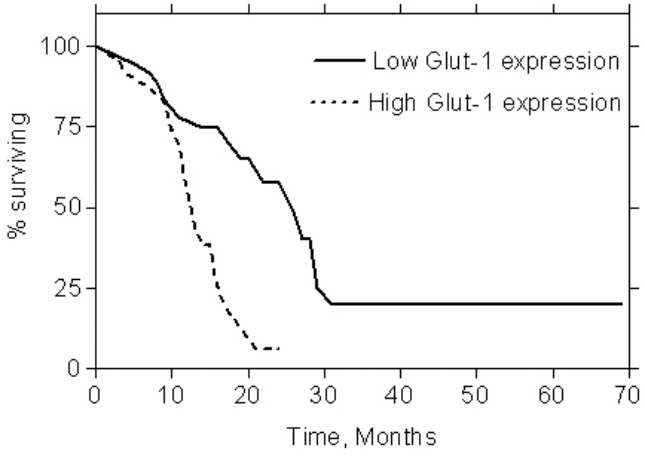

Fig. 2. Comparison of Glut-1 expression with overall survival. Data were condensed into 2 categories: low (Glut-1 scores = 1 and 2) and high (Glut-1 scores = 3 and 4) expression, and Kaplan-Meier survival curves for each group are shown. The difference of survival between patients with low or high Glut-1 expression was significant (P<0.01).

cases, respectively. IPMNs with low- and moderate-grade dysplasia did not show Glut-1 immunostaining, whereas 3/5 (60%) cases of IPMNs with high-grade dysplasia showed anti-Glut-1 positive reaction (Fig. 5). In IPMNs with high-grade dysplasia the mean percentage of Glut-1 immunostained cells was 21.4(±38%).

Discussion

Glut-1 protein overexpression represents a negative prognostic factor for bladder carcinoma, lung tumors, gastric cancer, breast carcinoma, cervix carcinoma, esophageal squamous cell carcinoma, oral squamous cell carcinoma, ovarian carcinoma, hypopharyngeal carcinoma, gallbladder carcinoma and colorectal carcinoma (Reviewed in Airley and Mobasheri, 2007). As it concerns the pancreatic cancer, Sun et al. (2007) revealed that high expression of Glut-1, i.e., >50% positive cells, was correlated with poor prognosis,

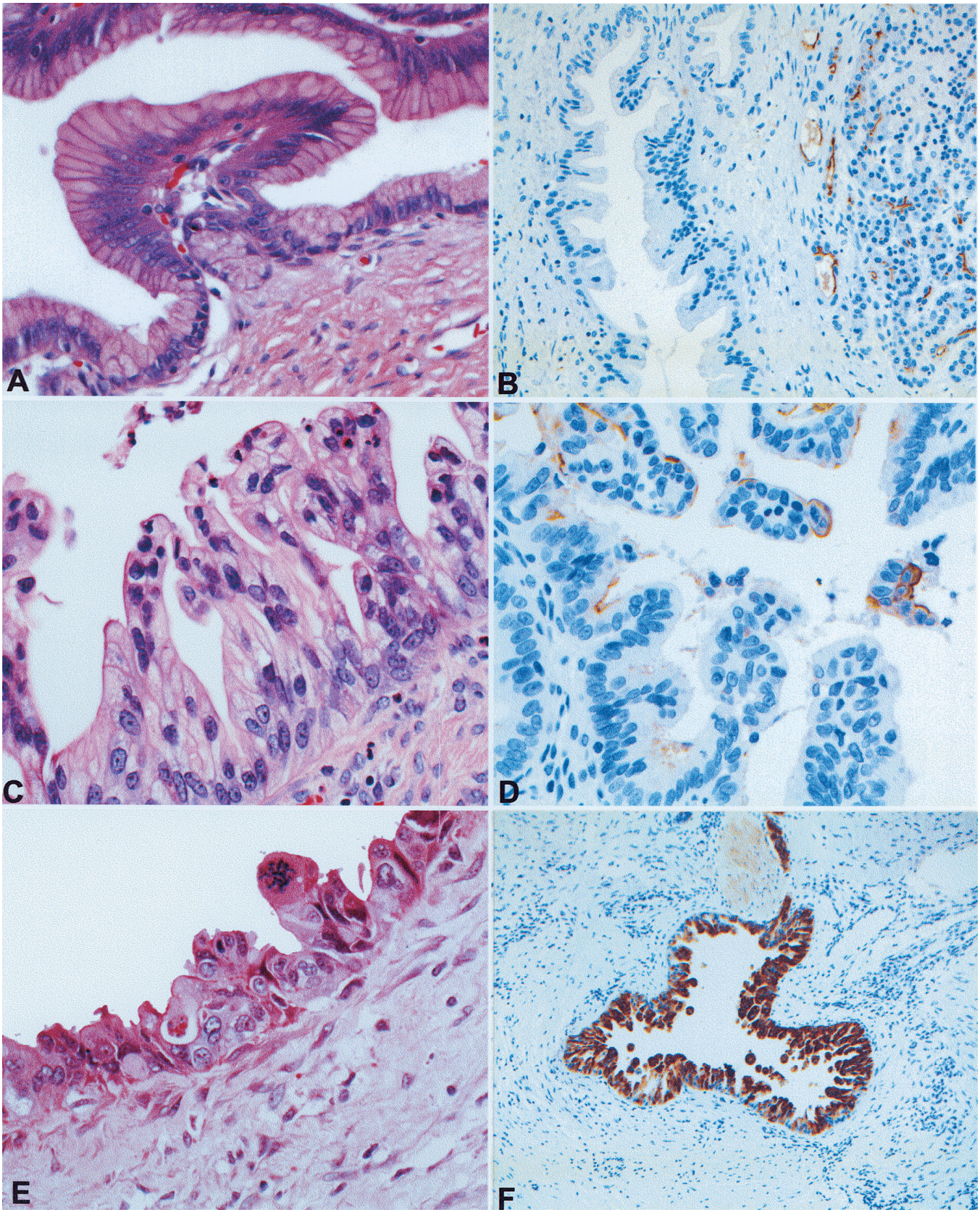

Fig. 3. PanIN1 (**A**: H.E.; **B**: anti-MUC1), PanIN2 (**C**: H.E.; **D**: anti-MUC1) and PanIN3 (**E**: H.E.; **F**: anti-MUC1), showing focal and diffuse MUC1 expression in PanIN2 and PanIN3, respectively. A-E, x 40; F, x 20.

although only in univariate analysis. On the contrary, Lyshchik et al. (2007) did not find a prognostic significance in the expression of Glut-1, scored on a 5-point scale. Our study shows that Glut-1 immunohistochemical expression provides a prognostic factor in pancreatic ductal adenocarcinoma, in agreement with Sun et al. (2007). Moreover, our study shows a statistically significant correlation with poor prognosis also in multivariate analysis. Different results in Lyshchik et al. (2007) may be ascribed to heterogeneity of histological types of pancreatic cancer, including tubular adenocarcinoma, intraductal papillary carcinoma and mucinous cystadenocarcinoma, and to the different scoring system. *In vitro and in vivo* studies show that forced overexpression of Glut-1 induces an increase in matrix metalloproteinase-2 and promotes pancreatic cell invasiveness (Ito et al., 2004). Our study provides a further explanation for the negative prognostic significance of this marker, as the correlation of Glut-1 scores with histological grade and MIB-1 index indicate higher glucose uptake in undifferentiated and highly proliferative pancreatic cancer cells. A

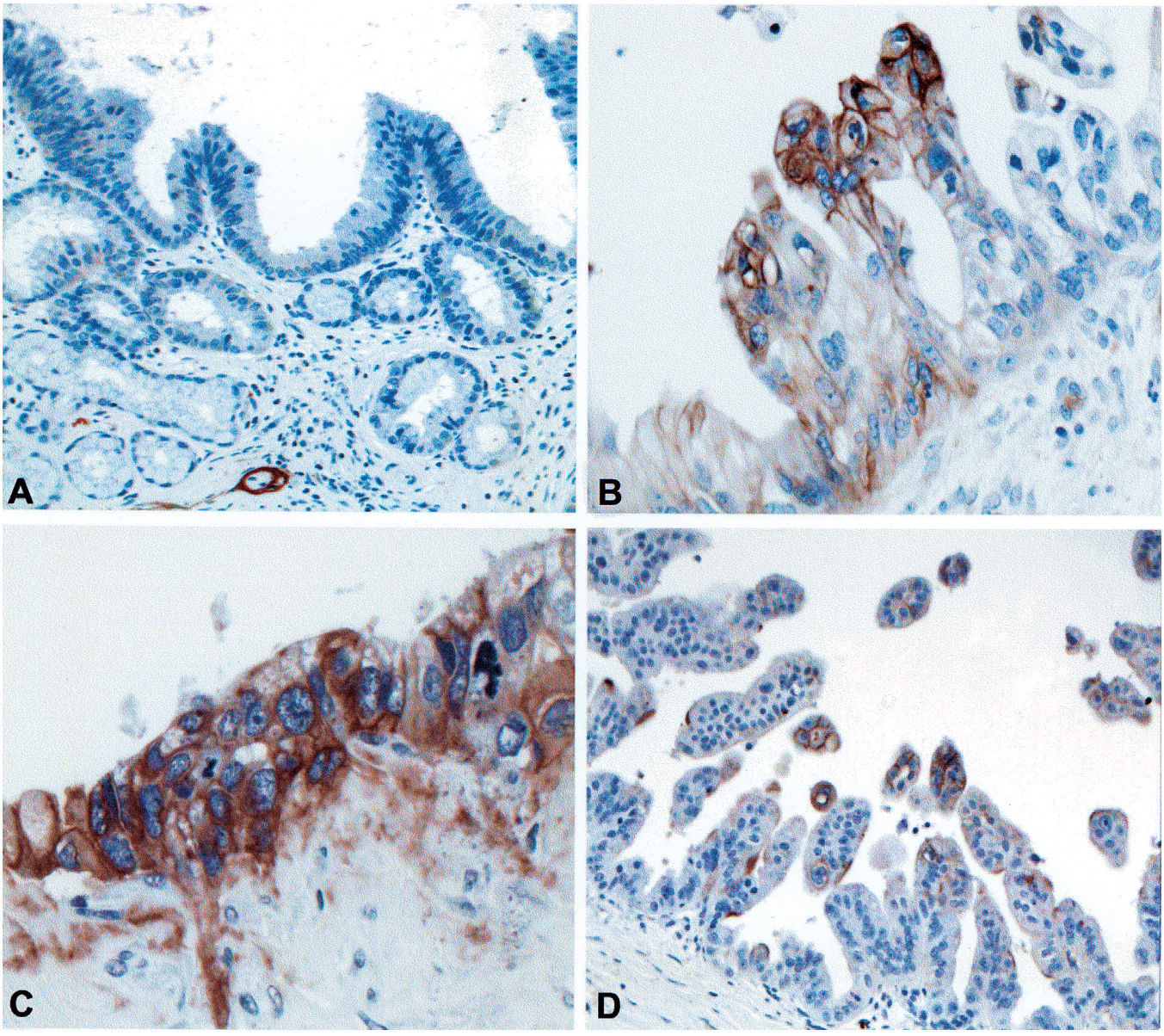

Fig. 4. PanIN1 **(A)**, PanIN2 **(B)** and PanIN3 **(C-D)**, showing increasing expression of Glut-1. Note the presence of different patterns of Glut-1 immunostaining, i.e., focal **(B-C)** and diffuse **(D)**. x 40.

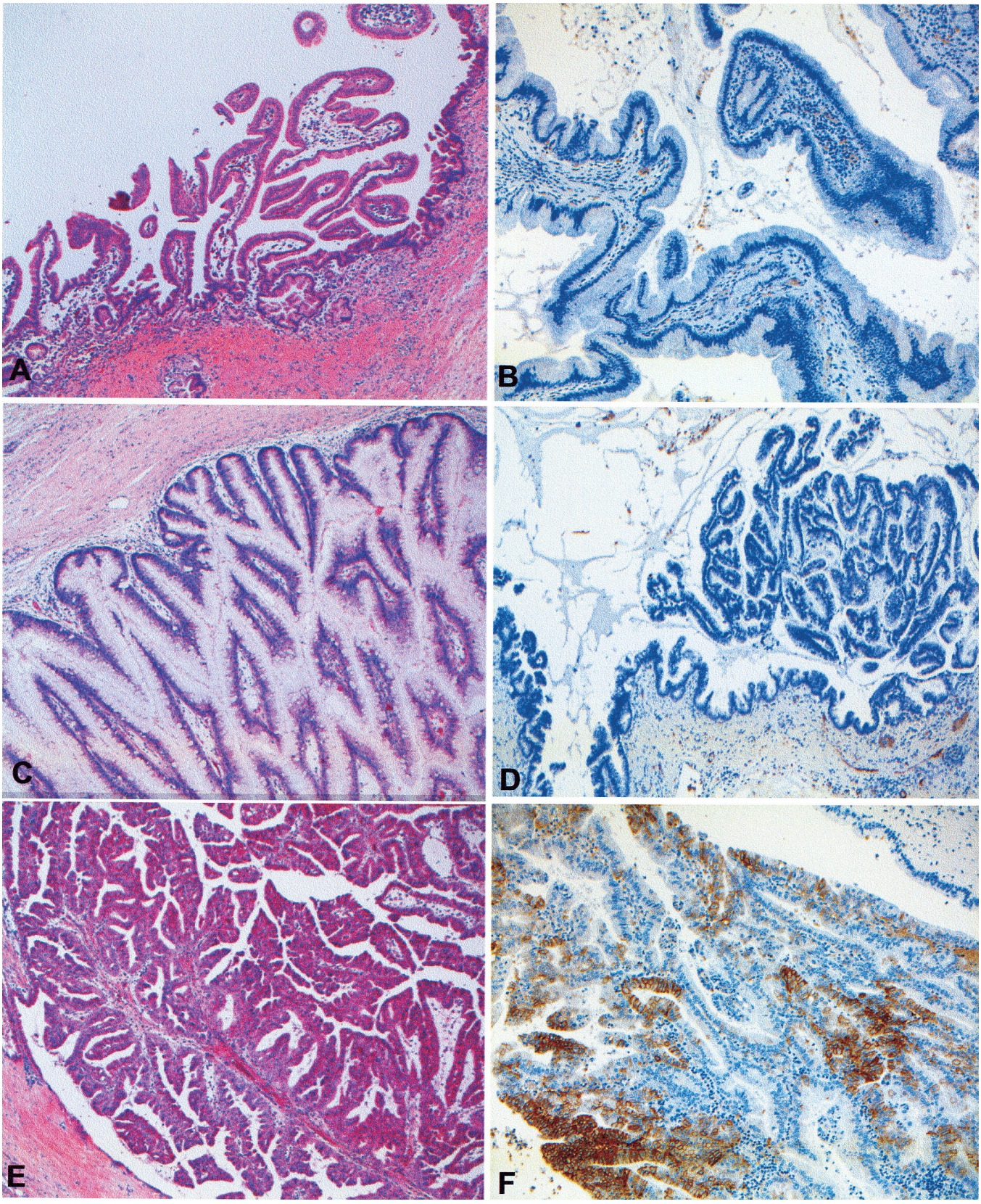

Fig. 5. Intraductal papillary mucinous neoplasms (IPMNs) with low- (**A**: H.E.; **B**: anti-Glut-1), moderate- (**C**: H.E.; **D**: anti-Glut-1) and high-grade (**E**: H.E.; **F**: anti-Glut-1) dysplasia, showing Glut-1 expression only in IPMN with high-grade dysplasia. x 10.

correlation between Glut-1 expression and grading has also been found in many other tumors, i.e., colorectal cancer (Younes et al., 1996), bladder carcinoma (Chang et al., 2000), ovarian tumors (Cantuaria et al., 2001; Kurokawa et al., 2004), lung adenocarcinoma (Minami et al., 2002), cervical cancer (Mendez et al., 2002) and bone and soft tissue sarcomas (Tateishi et al., 2006). However, it must also be taken into consideration that such correlations have not been confirmed in other papers (Mineta et al., 2002; Cooper et al., 2003; Rudlowski et al., 2004), and in our multivariate analysis Glut-1 expression was an independent prognostic factor, whereas histological grade was not, indicating that the biological significance of Glut-1 may not be reduced to correlation with histological grade. Correlation between Glut-1 expression and proliferative rate has been found in lung adenocarcinoma (Minami et al., 2002), breast cancer (Bos et al., 2002), epithelial tumors of the ovary (Kurokawa et al., 2004) and bone and soft tissue sarcomas (Tateishi et al., 2006). Our study confirms the role played by Glut-1 overexpression in cancer cell proliferation also in pancreatic cancer. At the moment, prognosis of pancreatic ductal adenocarcinoma is still poor, and chemotherapy and radiotherapy are relatively ineffective, so that novel therapeutic approaches targeting the disease at the molecular level have been investigated. For instance, gene therapies targeting oncogenes (K-ras, cancer-associated sm-like oncogene) and oncosuppressors (p16INK4a, p53) involved in pancreatic carcinogenesis have recently been proposed (Halloran et al., 2000). Our findings also indicate that Glut-1 represents a potential therapeutic target for strategies designed to inhibit the progression of pancreatic cancer. A significant reduction in proliferation has been demonstrated in cell lines from gastric cancers (Noguchi et al., 2000) and leukemias (Chan et al., 1999) after suppression of Glut-1 mRNA by transfection with cDNA for antisense Glut-1. High Glut-1 overexpression in pancreatic ductal adenocarcinoma and its negative prognostic significance suggest the possibility of developing and applying efficient gene therapy targeted at this transporter.

Glut-1 overexpression has been found in many malignancies, but many studies have also investigated it in hyperplastic/dysplastic lesions and benign neoplasms. Wang et al. (2000) reported Glut-1 immunostaining in endometrial atypical hyperplasia, associated with a high risk of adenocarcinoma, but not in simple and complex hyperplasia. In low-grade cervical intraepithelial neoplasia, Glut-1 immunostaining was detected in less than one-third of the epithelium, whereas it was present in over one-half of the epithelium in high-grade lesions (Mendez et al., 2002). Reisser et al. (1999) reported Glut-1 expression in the basal area in mild dysplastic epithelium, in basal and suprabasal areas in moderate dysplastic lesions, and in all levels of highly dysplastic lesions of the oro- and hypopharingeal mucosa. Glut-1 expression has also been detected in colorectal adenomas, with particular reference to the villous type having the greatest potential for malignant transformation, but not in hyperplastic polyps (Younes et al., 1996; Haber et al., 1998). Instead, Glut-1 expression was not found in low-grade and high-grade dysplasias in Barrett's metaplasia, but only in esophageal adenocarcinoma (Younes et al., 1997), and was not detected in gallbladder dysplasias, but only in 5% of adenomas and 52% of carcinomas (Kim et al., 2002), indicating Glut-1 involvement as a late event during these types of neoplastic progression. As regards benign neoplasms, Glut-1 protein overexpression has not been found in bladder transitional cell papillomas (Chang et al., 2000) or benign ovarian neoplasms, although moderate and strong immunostaining has been detected in borderline and malignant ovarian tumors, respectively (Kalir et al., 2002; Kurokawa et al., 2004; Rudlowski et al., 2004). Detection of Glut-1 in PanIN-2 and -3 and in IPMNs with high-grade dysplasia indicates its involvement in a relatively early phase of pancreatic carcinogenesis, and provides a possible interpretation of its mechanism of overexpression. Experimental studies have shown that hypoxia enhances the expression of Glut-1 (Ebert et al., 1995). Thus, Glut-1 overexpression in many tumors has been ascribed to local hypoxia due to insufficient blood supply. Glut-1 overexpression in pancreatic preneoplastic lesions (PanIN-2 and -3, and IPMN with high-grade dysplasia), which are characterized by many genetic aberrations, supports the hypothesis, also proposed for other tumors (Rudlowski et al., 2004), of a direct oncogene-triggered mechanism.

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