Comparison of two sialosyl-Tn binding monoclonal antibodies (MLS102 and B72.3) in detecting pancreatic cancer

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Abstract
An immunohistochemical study has been carried out to compare and contrast the cellular distribution of two different sialosyl-Tn antigen binding monoclonal antibodies, MLS102 and B72.3, in the pancreas. MLS102 but not B72.3 monoclonal antibody binding increases with the content of the sialosyl-Tn epitopes. It was found that all 13 pancreatic cancer specimens bound both MLS102 and B72.3 monoclonal antibodies. Their cellular distribution in the cancer was virtually identical. Fifteen of 20 (75%) patients with chronic pancreatitis and five of 10 (50%) normal subjects were B72.3 positive, but MLS102 was completely negative in the latter group. Both monoclonal antibodies bound fetal pancreas diffusely. Thus, when pancreatic ductal cells have undergone malignant transformation, like the fetal pancreas, they express cell surface and secreted glycoconjugates with increased sialosyl-Tn epitopes suggesting enhanced 2-6 sialosyltransferase activity. This study shows that MLS102 is an extremely sensitive and specific tumour marker in the pancreas and that it is better than B72.3 in distinguishing pancreatic cancer from normal and chronic pancreatitis.

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Aberrant glycosylation of structural and secretory glycoconjugates are well documented in epithelial cancer cells. The end results include expression of a variety of tumour associated antigens which are recognised by different monoclonal antibodies. Some of these antigens represent blood group epitopes. Sialosyl-Tn (STn) blood group antigen, identified by monoclonal antibodies B72.3 and TKH2, is widely expressed in pancreatic cancer and has a sensitivity of between 74% and 100%.* B72.3 was developed against cell membrane enriched breast cancer metastasis and TKH2 against ovine submaxillary mucin which is known to possess a high concentration of sialosyl-Tn epitopes. Another STn binding monoclonal antibody, MLS102, has recently been developed against a colon cancer cell line, LS180, and has been found to bind strongly to mucin that bears a high concentration of STn epitopes. Its affinity for ovine submaxillary mucin is 130 fold higher than for rectal mucin glycoprotein, which is known to possess significantly fewer STn epitopes in its oligosaccharide side chains. In this study, we have compared and contrasted the cellular distribution of the MLS102 binding sites with B72.3 in the pancreas.

Methods
Pancreatic needle biopsy specimens were obtained from patients with pancreatic cancer (n=13), five well differentiated, five moderately differentiated, and three poorly differentiated adenocarcinoma); chronic pancreatitis (n=20); and normal subjects (n=10). Pancreas from a fetus of 15 weeks' gestation was obtained with consent for this study. These specimens were fixed in formalin and embedded in paraffin before they were sectioned for immunohistochemistry.

Sections were dewaxed, rehydrated, and then incubated in 3% H2O2 to eradicat endogenous peroxidase activity. This was followed by washing the sections repeatedly (×3) in Tris buffered saline (TBS, pH 7.6). They were then incubated in monoclonal antibody MLS102 (5 µg/ml, gift of Drs S Fukui and A Kurosaka, Kyoto, Japan) or B72.3 (1:160, gift of Dr J Schlam, National Cancer Institute, USA) at room temperature for one hour. Unbound antibody was removed by repeated washings (×3) with TBS and the bound mouse monoclonal antibodies were subsequently identified by peroxidase-tagged rabbit anti-mouse antibody (Sigma, USA). The latter was finally visualised by incubation with 3,3'diaminobenzidine/H2O2 containing solution. The slides were then viewed under ×10 magnification and they were scored as negative if the overall staining was <5%, patchy if it was >5% but <30%, and positive if it was >30%. Negative controls consisted of substituting normal mouse serum for MLS102 or B72.3 monoclonal antibody, which resulted in negative staining. Desialylation by using 0-5 mU/ml of Vibrio cholerae neuraminidase (Sigma, USA) at 37°C for 16 hours abolished both the MLS102 and B72.3 binding activity.

Results (Table)

Ductal tissues
The cell surface and the intracytoplasmic regions of all (n=13) the pancreatic cancer specimens were uniformly stained by both the MLS102
(Fig 1 (A) and (B) and B72.3 (Fig 2 (A) and (B)) monoclonal antibodies. The cellular distribution of these two STn binding monoclonal antibodies was virtually identical. Strong mucin staining (Figs 1 and 2) was similarly observed in all the 13 pancreatic cancers studied. MLS102 was negative in the normal controls and those with benign disease but B72.3 bound 75% (15 of 20) of the chronic pancreatitis specimens. Thirteen of 15 B72.3 positive chronic pancreatitis specimens showed diffused B72.3 staining in the ductal cell surface and in the intracytoplasmic regions (Fig 3). There was also weak B72.3 positive staining in some of the intraductal mucins (Fig 3, arrowed). Half (five of 10) of the normal ductal tissues were also B72.3 positive but mucins were positive in only one of these (Fig 4). The fetal pancreatic ductal tissue was positively stained by both of these monoclonal antibodies.

**ACINAR TISSUES**

MLS102 did not bind the normal or chronic pancreatic acinar tissues which were positively stained by B72.3 in 50% (five of 10) of the normal control subjects and 75% (15 of 20) of the patients with chronic pancreatitis. Diffuse staining was found in the surface and the intracytoplasmic regions of five normal subjects positive for B72.3 (Fig 4). The same pattern was observed in 13 of the 15 patients with chronic pancreatitis who were positive for B72.3 (Fig 3). The remaining two showed only patchy B72.3 positivity. Both the MLS102 and B72.3 monoclonal antibodies bound fetal pancreatic acinar tissue.

**Discussion**

MLS102 and B72.3 are both highly sensitive in detecting pancreatic cancer cells but the former is better because it is more specific. MLS102, but not B72.3, distinguishes extremely well between pancreatic cancer and normal subjects and patients with chronic pancreatitis in this study.

B72.3 has been characterised to bind STn antigen previously and there is very little difference between this antibody and TKH2 (another STn binding monoclonal antibody) in distinguishing pancreatic cancer from normal and benign controls. They have been shown to bind pancreatic ductal tissues in 33% and 44% respectively in previous studies. MLS102 is a recently developed anti-STn monoclonal antibody that has been characterised to bind more strongly to antigen that bears clusters of STn epitopes. Kuroskaka *et al* showed that a 3-6 fold increase in the disaccharides (N-acetylneuraminic acid 2-6 N-acetylgalactosamine, the STn epitope) led to a 130 fold enhancement of MLS102 binding activity. B72.3 has never been shown to possess similar characteristics before. We believe this may account for the apparent difference in their specificity.

Both monoclonal antibodies bind the fetal pancreas indicating that STn antigen is a commonly present fetal antigen. The absence of MLS102 positivity in the normal adult pancreas and its reappearance after the organ has under-
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Figure 3: The pancreatic ductal and acinar tissues of this chronic pancreatitis specimen are positively stained (brown) by B72.3 monoclonal antibody. There is weak B72.3 positive mucin (arrowed) present in some of the intraductal musins. (Magnification: ×25.)

Figure 4: This normal pancreas shows strong and diffuse B7.2.3 positivity (brown staining) in the ductal as well as the acinar tissues. (Magnification: ×10.)

gone malignant transformation suggests that there are increased STn epitopes, which probably form clusters, in the cell surface and secreted glycoconjugates in fetal and malignant pancreas suggesting the presence of enhanced 2-6 sialylltransferase activity.

A number of blood tests have been produced to help diagnosis of pancreatic cancer. In particular, the CA19-9 assay which detects a mucin antigen with the sialylated Lewis epitope has been shown to be the best serological marker and has a sensitivity for diagnosis of pancreatic cancer of 68-93%. However, false negative results are expected in patients who are Lewis+ and Lewis−, a situation which occurs in about 5% of whites. Specificity of CA19-9 radioimmunoassay is usually good, most studies reporting values of 80-93%, but raised CA19-9 values have been reported in up to 28% of patients with acute or chronic pancreatitis or benign obstructive jaundice. This specificity is therefore too low to allow its use as a screening test. B7.2.3 assay has been shown to detect 34-74% of pancreatic cancers. Sandwich enzyme linked immuno-sorbert assay (ELISA) is being developed by using the MLS102 monoclonal antibody. Its superior specificity compared with B7.2.3 as shown in this immunohistochemical study may suggest that a more sensitive and specific serological assay by incorporating the MLS102 monoclonal antibody may be achieved.

The study of changes in glycosylation of tissue and serum glycoconjugates in cancer have led to the discovery of tumour markers and provided valuable insights into tumour biology. Changes in cell surface or circulating glycoconjugates, or both, may have profound effects on the response of cells to growth factors and probably affect cell-cell interaction. Increased sialylation of cells is believed to be a very important characteristic of cells with high metastatic potential. STn expression has recently been found to be an independent predictor of poor prognosis in colon cancer indicating that colon cancer cells bearing this epitope have more aggressive biological behaviour. Like others we have observed a high frequency of STn expression in pancreatic cancer cells in this study. This suggests the overall poor prognosis of pancreatic cancer may be related to the presence of the STn epitope or simply because of the increased sialylation as previously postulated.

MLS102 is a promising monoclonal antibody that is directed against a STn blood group epitope which is present abundantly in excocrine pancreatic cancers. Its use in immunohistochemistry in distinguishing malignant from normal or pancreatic controls has been outstanding when compared with a similar STn binding monoclonal antibody B7.2.3. Its application in serology and immunocytography to help clinical diagnosis and tumour localisation remains to be defined by further studies.
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Notes