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OVER-EXPRESSION OF FACILITATIVE GLUCOSE TRANSPORTER GENES IN HUMAN CANCER

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SUMMARY The expression of five facilitative glucose transporter genes, GLUT1 (erythrocyte type), GLUT2 (liver type), GLUT3 (brain type), GLUT4 (muscle/fat type), and GLUT5 (small intestine type), was examined in human cancer tissues of the digestive system by RNA blotting analysis. The amounts of the GLUT1, GLUT2, and GLUT3 transcripts were elevated in most cancer tissues studied, although the expression of the GLUT2 gene is primarily restricted to the liver. On the other hand, mRNA levels of GLUT4 and GLUT5 were below sensitivity in all cancer tissues examined. These results suggest that over-expression of GLUT1 and GLUT3 might be closely related with tissue development and that the acceleration of glucose uptake by transformed cells may result, at least in part, from the increase in the expression of these two glucose transporters.

It is well known that the proliferation of transformed cells is accompanied by an acceleration of glucose uptake and its metabolism (1, 2). However, the molecular mechanism responsible for increased glucose uptake by transformed cells remains unclear.

Recent studies have demonstrated that facilitative glucose transport across the plasma membrane is mediated by a family of structually-related proteins (3-7). These human facilitative glucose transporters have a distinct tissue distribution and

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contribute to the disposal of glucose under varying conditions: mRNAs encoding GLUT1 and GLUT3 are present at various levels in most adult tissues and in term placenta (5,8). GLUT2 is the predominant glucose transporter expressed in liver (4). GLUT4 is abundant in insulin responsive tissues such as skeletal muscle and subcutaneous fat (6), and the highest levels of GLUT5 mRNA are observed in upper jejunum (7).

It is important to study how the expression of these glucose transporter genes is regulated in transformed cells. We have examined, therefore, the mRNA levels of five glucose transporter genes in various human cancer tissues.

Materials and Methods

Tissues and RNA extraction

Total RNA was isolated from human cancerous and normal tissues by the guanidium thiocyanate/cesium chloride method (9). These tissues were surgically removed from human esophagus, stomach, colon, liver, and pancreas.

RNA blotting analysis

Twenty µg of total RNA was denatured with glyoxal and, after electrophoresis through a 1.0% agarose gel, transferred to a nylon filter. The filter was hybridized with cDNA probes labeled by nick translation. The hybridization conditions have been described previously (5). The intensity of the autoradiographic spots was quantified by densitometric scanning.

Probes

The probes used were: GLUT1 cDNA, the 1396-bp EcoRI-HindIII insert from phGT2-1 (10); GLUT2 cDNA, the 1395-bp EcoRI insert from λhHTL-14 (4); GLUT3 cDNA, the 2190-bp EcoRI insert from phMGT-31 (5); GLUT4 cDNA, the 1730-bp EcoRI insert from phJHT-3 (6); GLUT5 cDNA, the 1890-bp EcoRI insert from λhJHT-5 (7); and TGF-β cDNA, the 1007-bp PstI-BamHI insert from phTGF-B-2.

Results and Discussion

RNA blotting analysis revealed a single GLUT1 trancript of 2.8 kb in normal esophagus, stomach, colon, and all cancer tissues examined, while GLUT1 mRNA was below the sensitivity of our assay in normal liver and pancreas. The GLUT1 gene was expressed at higher levels in all hepatoma (2/2) and pancreatic cancer (5/5) examined. The levels of GLUT1 mRNA also were elevated in 1/1 esophageal cancer, 1/2 gastric cancer, and 2/3

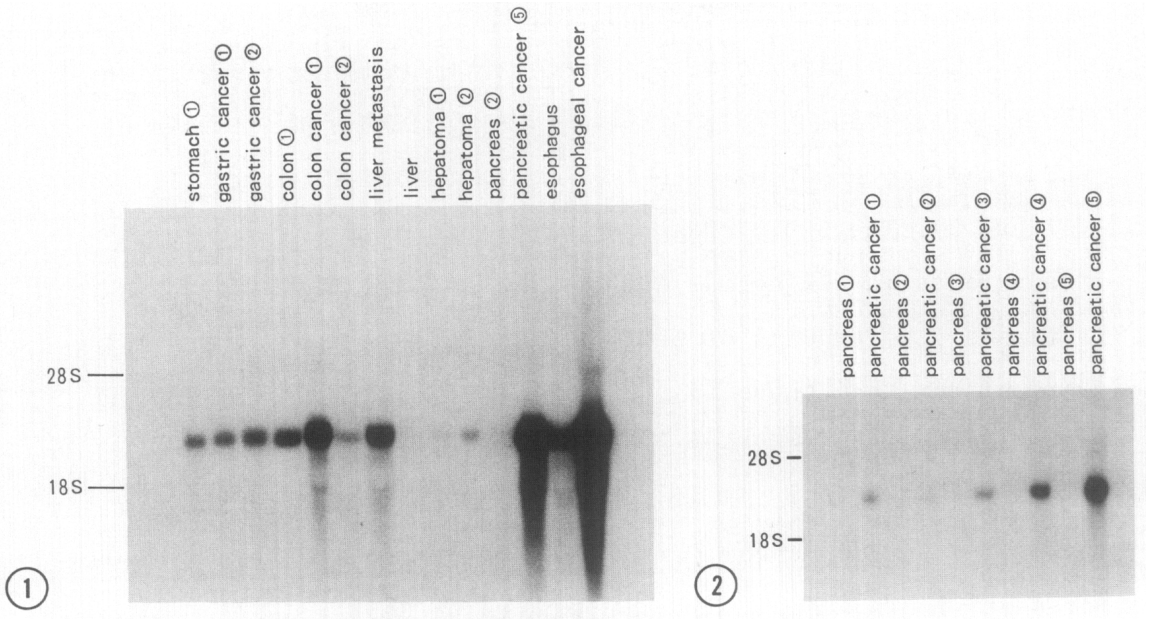


Figure 1. Expression of GLUT1 mRNA in human cancer of digestive system. Positions of 28S and 18S ribosomal RNA are indicated. Liver metastasis indicates liver metastasis of colon cancer.

Figure 2. Expression of GLUT1 mRNA in human pancreatic cancer. Positions of 28S and 18S ribosomal RNA are indicated.

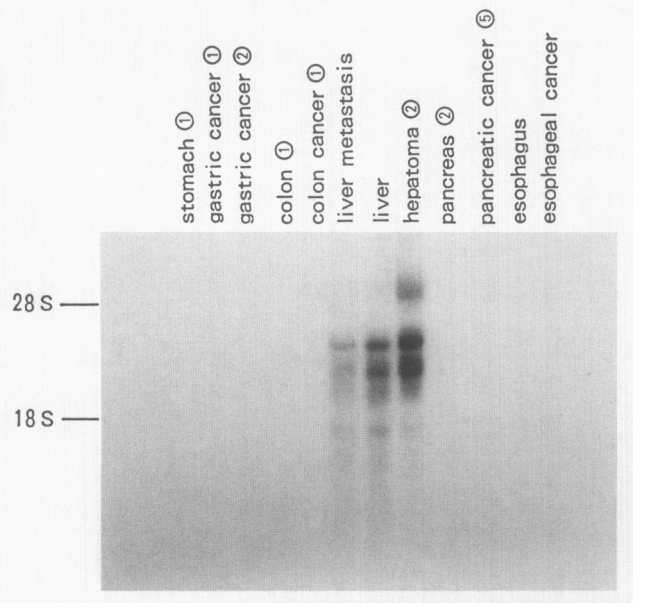
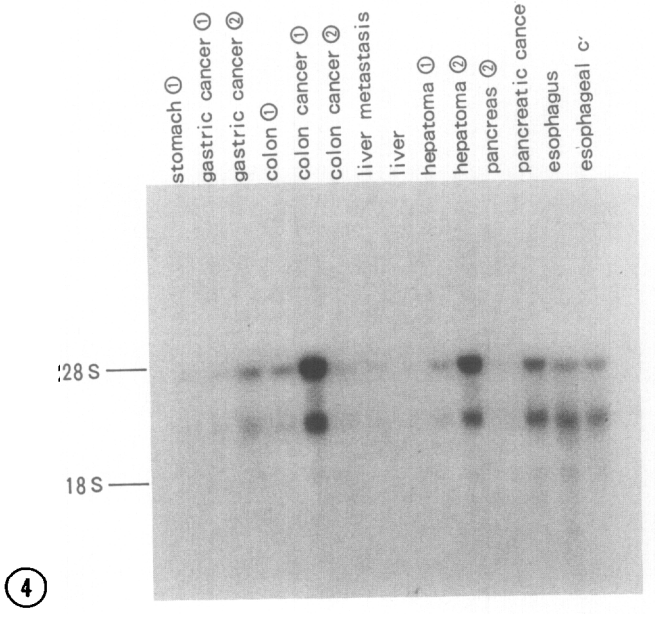


Figure 3. Expression of GLUT2 mRNA in human cancer of digestive system. Positions of 28S and 18S ribosomal RNA are indicated. Liver metastasis indicates liver metastasis of colon cancer.

colon cancer (Figure 1 and 2). The GLUT2 cDNA probe hybridized to three transcripts of 5.4, 3.4, and 2.8 kb only in normal liver, liver metastasis of colon cancer, and hepatoma. The levels of GLUT2 mRNA in hepatoma were higher than those in normal liver (Figure 3). The GLUT3 cDNA probe hybridized to 4.1 and 2.7 kb transcripts in all normal and cancer tissues except normal pancreas. The amounts of GLUT3 transcripts in all cancer tissues were higher than those in the respective normal tissues except esophagus, where the levels were almost the same (Figure 4). Like GLUT1, higher levels of GLUT3 mRNA were observed in all pancreatic cancer (5/5) than in normal pancreas (Figure 5). Our present findings show that the amounts of GLUT1, GLUT2, and GLUT3 transcripts are elevated in most cancer tissues studied, although the expression of the GLUT2 gene is restricted primarily to the



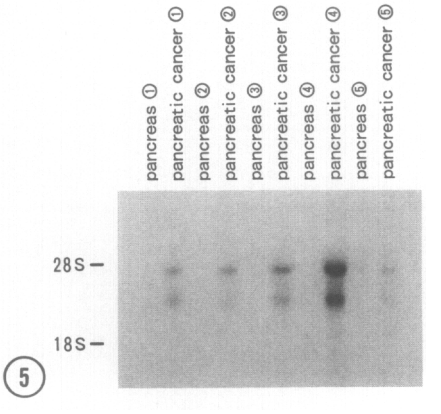


Figure 4. Expression of GLUT3 mRNA in human cancer of digestive system. Positions of 28S and 18S ribosomal RNA are indicated. Liver metastasis indicates liver metastasis of colon cancer.

Figure 5. Expression of GLUT3 mRNA in human pancreatic cancer. Positions of 28S and 18S ribosomal RNA are indicated.

liver. Werner et al. (11) have shown that GLUT1 mRNA levels in rat heart and liver are increased at the embryonic stage, but that they are markedly decreased at the postnatal stage. Although the GLUT3 cDNA is isolated from a human fetal skeletal muscle cDNA library, very low levels of GLUT3 mRNA are present in human adult skeletal muscle (5). Since GLUT1 and GLUT3 have been demonstrated to be closely related with tissue development, the over-expression of these genes in the present study might result from the rapid growth of the cancer tissues. It seems likely that GLUT4 and GLUT5 do not play an important role in cell proliferation, because mRNAs encoding these glucose transporters expressed in a tissue specific manner in the respective tissues and were not detectable in any cancer tissues examined.

Recent study has demonstrated that the transforming growth factor-β (TGF-β) stimulates glucose uptake by co-activation of the epidermal growth factor (EGF) receptor kinase system in vitro

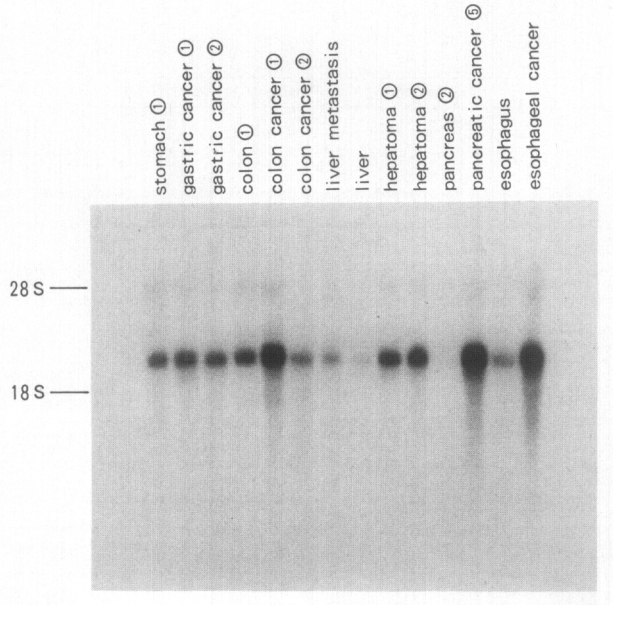


Figure 6. Expression of TGF-β mRNA in human cancer of digestive system. Positions of 28S and 18S ribosomal RNA are indicated. Liver metastasis indicates liver metastasis of colon cancer.

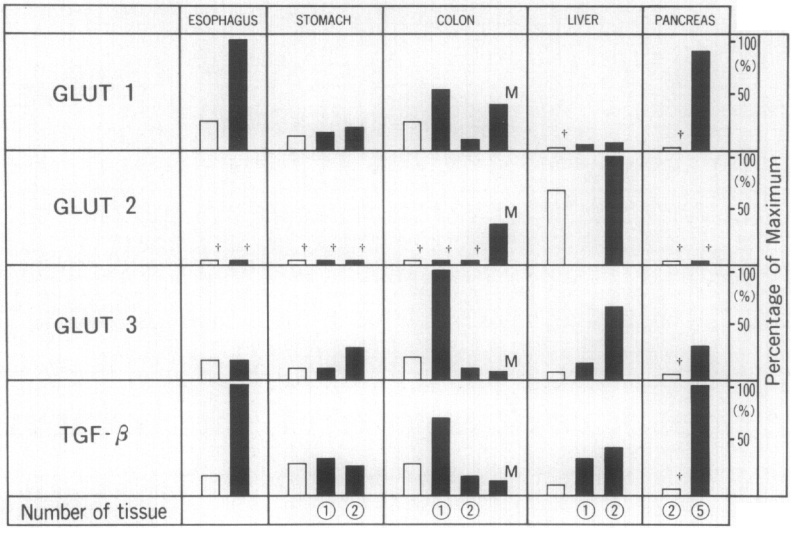


Figure 7. Comparison of gene expression of glucose transporters and TGF-β in human cancer of digestive system. The abundance of each mRNA is expressed relative to the amount present in tissue having the highest level of RNA.
Open column: normal region
Closed column: cancerous region
M: liver metastasis of colon cancer
†: no expression detected

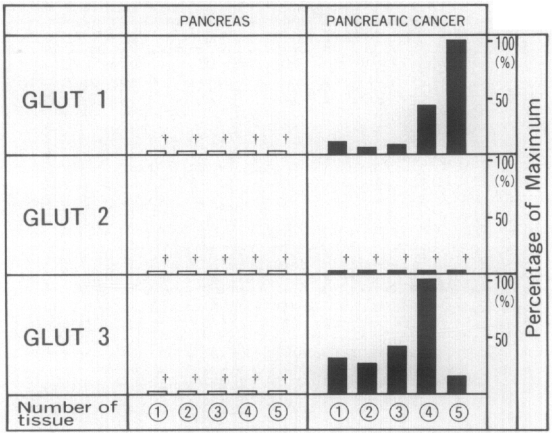


Figure 8. Comparison of gene expression of glucose transporters in human pancreatic cancer. The abundance of each mRNA is expressed relative to the amount present in tissue having the highest level of RNA.
Open column: normal region
Closed column: cancerous region
†: no expression detected

(12). Therefore, we have also examined the expression of the TGF-β gene in cancer tissues. The change of TGF-β mRNA levels in cancer tissues (Figure 6) was similar to that of GLUT1 and GLUT3, suggesting that the increase in the expression of GLUT1 and/or GLUT3 genes may be a consequence of the activated TGF-β gene product. A comparison of the gene expression of glucose transporters and TGF-β is summarized in Figure 7 and 8. We also compared the expression of several oncogenes (myc, Ha-ras, Ki-ras, N-ras, and src) with that of the glucose transporter genes, but found no significant correlation.

Although the precise mechanism still remains to be determined, the present results suggest that the induction of glucose transporter gene expression, such as GLUT1 and GLUT3, which are closely related with tissue development, may contribute to the increased glucose uptake by transformed cells.

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