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# Cellular Distribution of Glut-1 and Glut-5 in Benign and Malignant Human Prostate Tissue

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## ABSTRACT

Over-expression of hexose transporters (Gluts), specifically Glut-1, is a common event in human malignancies. In prostate cancer (CaP), however, expression of Gluts has been characterized poorly. In this study, expression and distribution of Glut-1 and Glut-5 proteins were characterized using immunohistochemistry in 76 specimens of benign prostate, 10 specimens of high-grade intraepithelial neoplasia (HGPIN), and 28 specimens of CaP. In addition, mRNA expression of Glut-2, Glut-7, Glut-9, and Glut-11 was analyzed in a set of five specimens of benign prostate and CaP. In benign prostate, Glut-1 localized to the basal cells and to the basolateral membrane of secretory/luminal epithelial cells. Glut-5, however, localized to the apical membrane of secretory/luminal epithelial cells. In HGPIN, Glut-1 was immunohistochemically undetectable. Glut-5, however, localized to the apical membrane of the neoplastic epithelial cells. In CaP, Glut-1 and Glut-5, were immunohistochemically undetectable. However, over-expression of GLUT1 was observed in some specimens of highly proliferative intraductal CaP. Glut-7, Glut-9, and Glut-11 mRNAs were detected in benign prostate and CaP, however, only Glut-11 mRNA was consistently up-regulated in CaP compared to benign prostate. Low levels of expression of Glut-1 protein in the majority of CaP could explain, at least in part, the limited clinical applicability of positron emission tomography using 2-[18F]-fluoro-2-deoxy-D-glucose for imaging CaP. Moreover, expression of Glut-5 in HGPIN suggested that fructose could be utilized as potential metabolic substrate in HGPIN. Understanding the molecular mechanisms involved in regulation/dysregulation of Gluts in CaP could provide insight in the understanding of hexose metabolism in CaP. J. Cell. Biochem. 113: 553–562, 2012. © 2011 Wiley Periodicals, Inc.

**KEY WORDS:** GLUCOSE; GLUCOSE TRANSPORTER; GLUT-1; GLUT-5; PROSTATE; PROSTATE CANCER

Glucose transport in human cells is mediated mostly by the mammalian facilitative hexose transporter (Glut) family. Fourteen members of the Glut family have been described: Glut-1 to Glut-12, Glut-14, and a proton-coupled myoinositol transporter (HMIT) [Joost and Thorens, 2001]. These transporters show tissue-specific expression and multifunctional transport capacity. Among all of the isoforms, only Glut-1, Glut-2, Glut-3, Glut-4, and Glut-5 have been characterized functionally in detail [Mueckler et al., 1985; Fukumoto et al., 1988, 1989; Kayano et al., 1988, 1990; Catalona et al., 1991; Nualart et al., 1999; Watanabe et al., 1999]. Glut-6, Glut-7, Glut-8, Glut-9, Glut-10, Glut-11, and Glut-12 have been identified recently using homology searches of EST databases and their function is less well understood [Nualart et al., 2009]. Glut-14 is a duplication of Glut-3 and Glut-13 corresponds to HMIT. Glut-1 has been reported to be the most ubiquitous isoform and Glut-1 transports glucose and galactose, but not fructose [Mueckler et al., 1985]. Glut-2 and Glut-5 have been reported to be the only proteins to mediate fructose transport in human cells [Fukumoto et al., 1988;

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Watanabe et al., 1999] and Glut-5 appears to be a pure fructose transporter [Kayano et al., 1990; Concha et al., 1997]. Glut-7, Glut-9, and Glut-11 have been suggested to transport fructose due to their high sequence homology with Glut-5 (36–40% identity) [Augustin et al., 2004; Li et al., 2004; Scheepers et al., 2005]. However, the functional role of these proteins as fructose transporters in human cells remains to be elucidated.

Activation of Glut gene expression, specifically Glut-1, enhances metabolism of glucose and is a molecular feature of the malignant phenotype in a variety of cancers [Warburg, 1956; Birnbaum et al., 1987; Flier et al., 1987; Yamamoto et al., 1990; Brown and Wahl, 1993; Younes et al., 1995, 1996a, b, 1997; Haber et al., 1998; Garcia et al., 2001; Godoy et al., 2006, 2009]. Broad immunohistochemical analyses, however, have failed to detect Glut-1 in a high percentage of tumors, which suggests other hexose transporters may be over-expressed in human cancer [Younes et al., 1996b; Godoy et al., 2006]. In support of this hypothesis, our laboratory [Godoy et al., 2006] reported over-expression of Glut-2 and Glut-5 in human tumors and we demonstrated that human choroid plexus papilloma, breast cancer (ZR-75-1), and hepatoma (HepG2) cell lines transported fructose efficiently in vitro. Furthermore, a recent study [Liu et al., 2010] indicated that fructose led to increased cell proliferation of pancreatic cancer cells. Therefore, several lines of evidence suggest that fructose may have an important role in maintaining cancer cell metabolism.

Prostate cancer (CaP) is the most common non-skin cancer in American men; approximately 217,730 Americans were diagnosed with CaP and approximately 32,050 men died from CaP in 2010 [Jemal et al., 2010]. In CaP, expression and distribution of Glut has been characterized poorly; Effert et al. [2004] and Steward et al. [2008] independently analyzed expression of Glut-1 in 45 and 67 human clinical specimens of benign hyperplasia and CaP, respectively. However, in these studies, analysis of the expression of Glut-1 was performed only at the mRNA level. Moreover, Chandler et al. [2003] analyzed the expression of Glut-1 and Glut-12 proteins in three clinical specimens of CaP. In this analysis, the three specimens stained for Glut-12 and one specimen stained weakly for Glut-1. These analyses, while interesting, were inconclusive in defining the molecular mechanisms (transport system/s) involved in hexose uptake in benign human prostate cells and the possible regulation of the expression of these transporters during prostate carcinogenesis. In this study, we used immunohistochemistry to characterize the cellular distribution of Glut-1 and Glut-5 in clinical specimens of benign and malignant human prostate tissue.

## MATERIALS AND METHODS

### CLINICAL SAMPLES AND FIXATION

Human prostate tissue was collected under approved protocol guidelines on the use of human subjects from University of Concepción, Concepción, Chile. Briefly, a portion of benign prostate (n = 76), high-grade prostatic intraepithelial neoplasia (HGPIN, n = 10) or CaP tissue (n = 28) was removed from each surgical specimen, fixed for 24 h by immersion in 10% formalin, and embedded in paraffin for histologic analyses. Another portion of the tissue was fixed for 3 days by immersion in Bouin's solution (5% glacial acetic acid, 9% formaldehyde and 0.9% picric acid), and embedded in paraffin for histologic analyses. All tissues and the images obtained from these tissues for analysis were reviewed by one pathologist (Carolina Delgado) (Table I)

### REVERSE TRANSCRIPTION AND PCR

Total RNA from tissue sections was prepared using the RNAeasy mini-kit (Qiagen, Valencia, CA). Reverse transcription from mRNA was performed using the SuperScript™ III First-Strand kit (Invitrogen) [Godoy et al., 2007]. Approximately 1 μl of the reverse transcribed cDNA product was used as template in a reaction mix that contained 200 nM of each primer using Platinum PCR Supermix (Invitrogen). PCR products were separated using electrophoresis on 2% agarose gels and visualized with ethidium bromide. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control in the analytical gels. Primer sequences for the PCR reactions were: Glut-1, forward: 5'-TGAACCTGCTGGCCTTC-3', reverse: 5'-TGTGCTAAAGAAGCTGC-3'. Glut-2, forward: 5'-TGGTGGGT-GGCTTGGGGACA-3', reverse: 5'-CCCCTGAGAGCGGTTGGAGC-3'. Glut-5, forward: 5'-GAATTCATGGAAGACTT-3', reverse: 5'-GC-CATCTACGTTTGCAA-3'. Glut-7, forward: 5'-GCCTACAGTTTCAT-CATCTTTGC-3', reverse: 5'-ATGGTTTCTTCTTTCTCCTCTGG-3'. Glut-9, forward: 5'-CCTCCTTCCTCTACGGCTAC-3', reverse: 5'-AACGGCAGGGACCACAATCA-3'. Glut-11, forward: 5'-TTGG-TGGGACTTTTCAGTTTG-3', reverse: 5'-AGGGACCACATAAGCAG-GACT-3'. GAPDH, forward: 5'-GCTCGTCGTCGACAACGGCTC-3', reverse: 5'CAAACATGATCTGGGTCATCTTCTC-3'.

### GALLEGO'S METHOD

Formalin-fixed paraffin-embedded prostate tissue sections were stained using Hematoxylin-chromotrope (nuclear staining) followed by acetic-fuchsin solution [distilled water 10 ml, glacial acetic acid 1 drop, and Ziehl's fuchsin 10 drops] for 2 min at room temperature. After that, sections were washed in distilled water and differentiated

TABLE I. Clinical and histologic characteristics of the invasive human prostate cancer specimens.

| Number | Age | Gleason Grade | Lymph Node Invasion |
|---|---|---|---|
| PC1 | 70 | 2 + 3 = 5 | NA |
| PC2 | 68 | 4 + 4 = 8 | + |
| PC3 | 63 | 1 + 4 = 5 | − |
| PC4 | 70 | 1 + 2 = 3 | − |
| PC5 | 65 | 3 + 3 = 6 | − |
| PC6 | 70 | 4 + 4 = 8 | − |
| PC7 | 71 | 3 + 3 = 6 | − |
| PC8 | 70 | 4 + 2 = 6 | − |
| PC9 | 57 | 3 + 4 = 7 | + |
| PC10 | 71 | 5 + 4 = 9 | + |
| PC11 | 83 | 4 + 4 = 8 | − |
| PC12 | 75 | 3 + 4 = 7 | − |
| PC13 | 75 | 2 + 3 = 5 | − |
| PC14 | 75 | 4 + 5 = 9 | + |
| PC15 | 70 | 2 + 4 = 6 | + |
| PC16 | 73 | 4 + 4 = 8 | + |
| PC17 | 75 | 3 + 2 = 5 | + |
| PC18 | 70 | 3 + 3 = 6 | − |
| PC19 | 73 | 3 + 3 = 6 | − |
| PC20 | 67 | 3 + 3 = 6 | − |
| PC21 | 74 | 4 + 4 = 8 | + |
| PC22 | 60 | 3 + 3 = 6 | − |

PC: prostate cancer specimen, NA: not analyzed.

in acetic-formol solution [distilled water 10 ml, formaldehyde 2 drops, and glacial acetic acid 1 drop] for 5 min at room temperature. Then, sections were washed in distilled water, counterstained using picro-indigo solution, dehydrated, and mounted with coverslips. Nuclei stained in magenta red, epithelial cytoplasm in red–yellow, connective tissue stained in brilliant green, muscle in olive green, and keratinized epithelium and blood in grass green.

### IMMUNOHISTOCHEMISTRY

Immunostaining was performed according to standard procedures [Godoy et al., 2006, 2009]. Briefly, antigens were retrieved using microwave irradiation and citrate buffer pH 6.0 for 15 min. Endogenous peroxidase activity was inhibited with 3.0% $H_2O_2$ in methanol and nonspecific binding of antibodies was blocked with 3% bovine serum albumin (BSA, EMD Chemicals, Gibbstown, NJ) for 30 min at room temperature. Tissue sections were incubated overnight with the following primary antibodies: anti-Glut-1 (1:1,000, Alpha Diagnostic, San Antonio, TX), anti-Glut-2 (1:500, Alpha Diagnostic), anti-Glut-5 (1:2,500, Alpha Diagnostic), anti-high molecular weight (HMW)-cytokeratin (1:50, monoclonal, Dako), anti-p-63 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), and alpha-methylacyl-coenzyme-A racemase (AMACR) (1/200, Dako, Carpinteria, CA). All antibodies were diluted in 100 mM Tris–HCl buffer [pH 7.8] that contained 8.4 mM sodium phosphate, 3.5 mM potassium phosphate, 120 mM NaCl, and 1% BSA. After washing three times for 10 min in Tris–HCl buffer, tissue sections were incubated with HRP-conjugated anti-mouse or rabbit IgG (1/100, Dako) secondary antibody for 2 h at room temperature. Peroxidase activity was developed using Tris–HCl buffer containing 3,3-diaminobenzidine tetrahydrochloride (1 μg/ml, Sigma-Aldrich) and $H_2O_2$ (1 μl/ml, VWR International, West Chester, PA). Immunostaining in the absence of primary antibody provided negative controls.

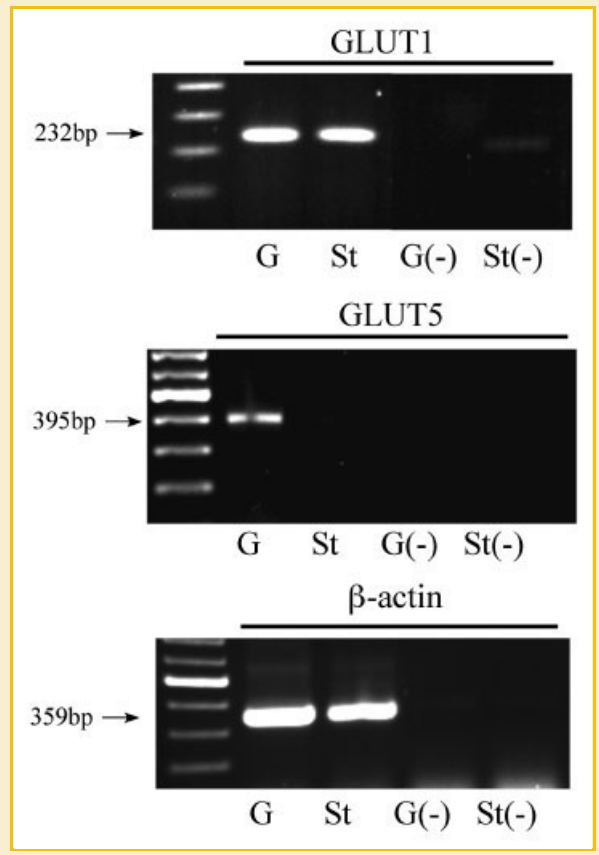


Fig. 1. Expression of GLUT1 and GLUT5 mRNA in benign prostate tissue. GLUT1 and GLUT5 mRNA expression was analyzed in areas of benign prostate tissue enriched in epithelial glands (G) or stroma (St) using RT-PCR analysis. As a negative control, PCR analyses were performed when reverse transcription step was omitted (G(−), St(−)). β-Actin was used as loading control.

## RESULTS

### ANALYSIS OF THE EXPRESSION OF Glut-1 AND Glut-5 mRNA IN BENIGN HUMAN PROSTATE TISSUE

A first attempt to analyze cellular distribution of Glut-1 and Glut-5 in human benign prostate tissue was performed using conventional RT-PCR. A pathologist dissected out regions of benign prostate tissue that were enriched for either stroma or glandular epithelium. A first screening found mRNA expression of both Glut-1 and Glut-5 in human benign prostate tissue (Fig. 1). Glut-1 mRNA was detected either in regions that were enriched in glandular epithelium or in stroma. However, Glut-5 mRNA was detected only in portions of prostate tissue that were enriched in glandular epithelium. These results suggested a differential cellular distribution of Glut-1 and Glut-5 isoforms in human benign prostate tissue.

### ANALYSIS OF EXPRESSION OF Glut-1 PROTEIN IN BENIGN PROSTATE, HGPIN, AND CAP

In order to confirm expression and differential cellular distribution observed for Glut-1 and Glut-5 mRNA at the protein level, human clinical specimens of benign, HGPIN, and CaP tissues were obtained from radical prostatectomy and analyzed using immunohistochemistry. Prostate tissue was dissected out by a pathologist and designated as benign (non-involved) or tumor tissue. The patients ages ranged from 57 to 83 years. Gleason grade in these patients varied between 1 + 2 and 5 + 4 (Table I). Gallego's staining confirmed tissue architecture of the glandular epithelium and stromal compartment in both benign and malignant human prostate tissue (Fig. 2). As expected, benign prostate tissue showed a higher proportion of surrounding stroma versus glandular epithelium compared to malignant prostate tissue (Fig. 2A,B,F,G). Confirmation of the presence of benign or malignant tissue was achieved analyzing the expression of the basal cell markers, HMW-cytokeratin and p-63, and the tumor epithelial cell marker, AMACR (Fig. 2). As expected, HMW-cytokeratin and p-63 were highly expressed at the basal cell level compartment in benign prostate (Fig. 2C,D). However, no immunostaining for these antigens was detected in CaP (Fig. 2H,I). On the contrary, AMACR was not detected in benign prostate (Fig. 2E), but was highly expressed in the cancer epithelial cells from CaP (Fig. 2J). These results confirmed the presence of benign and malignant prostate tissue in the corresponding clinical specimens.

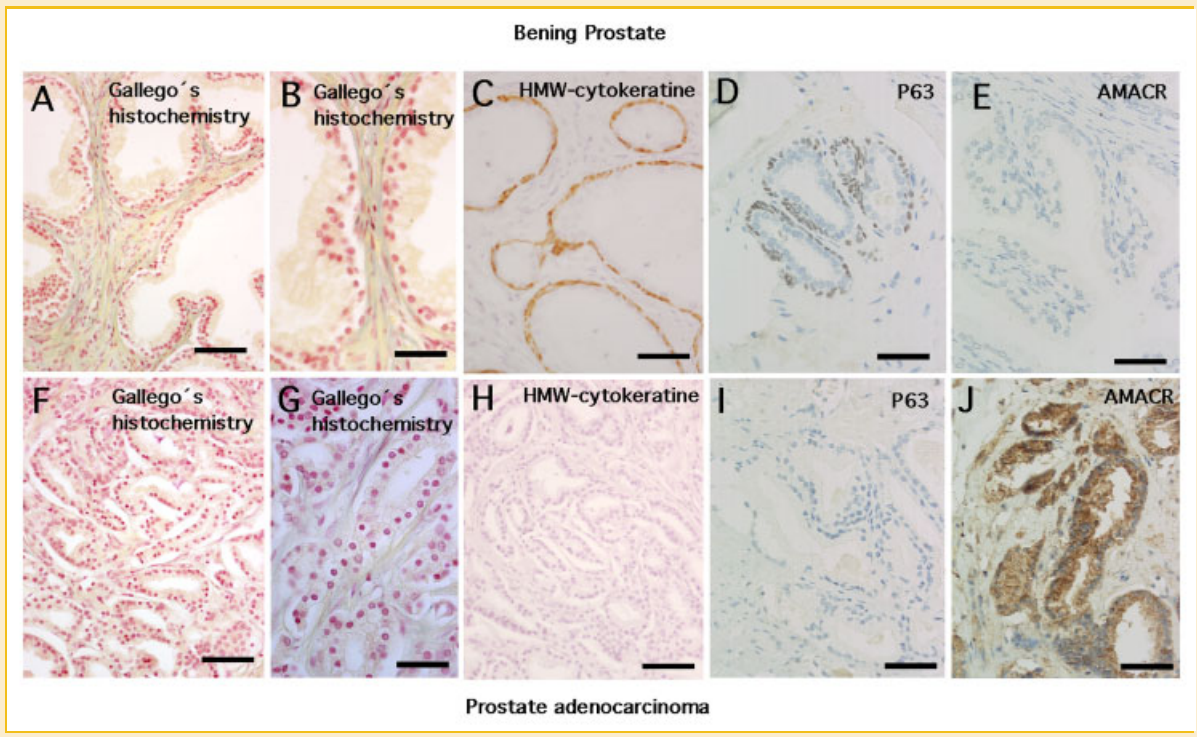


Fig. 2. Analysis of the expression of markers of benign prostate and CaP. Tissue architecture of benign (A,B) and malignant (F,G) human prostate tissue was analyzed using Gallego's histochemistry. Presence of human benign prostate tissue was confirmed using HMW-cytokeratin (C) and p63 (D) markers. Benign prostate tissue did not stain for AMACR (E). Whereas CaP tissue did (J). Malignant prostate tissue stained neither for HMW-cytokeratin (H) nor p63 (I) markers. Black bars in A,C,D,F,H,I 50 μm; in B,G 20 μm; E,J 30 μm.

Expression of Glut-1 in clinical specimens of benign prostate, HGPIN and CaP was analyzed in tissue sections previously fixed in either 10% formalin or Bouin's solution. The Glut-1 immunostaining was not found in benign prostate or CaP when the clinical specimens were fixed in 10% formalin (Fig. 3A,G,H). However, Glut-1 immunostaining was observed in erythrocytes (Fig. 3A,G,H, arrow), which represent an internal positive control for Glut-1 expression. In Bouin's-fixed clinical specimens of benign prostate, Glut-1 immunostaining was detected at the periphery of the benign glands, which suggests Glut-1 was expressed in basal cells. Under this experimental condition, very low levels of Glut-1 immunostaining were observed in the secretory/luminal epithelial cells and Glut-1 immunostaining was undetectable in stromal cells. To confirm expression of Glut-1 at the secretory/luminal epithelial cell level, thick section (40 μm) were analyzed using conventional immunohistochemistry and immunofluorescence (Fig. 3C,D). Glut-1 was expressed in basal cells and in secretory/luminal epithelial cells, in which Glut-1 preferentially localized to the basolateral portion of the plasma membrane (Fig. 3D, arrows). In HGPIN and CaP, Glut-1 was undetectable in both types of human prostate clinical specimens fixed in 10% formalin (Fig. 3E–G) or Bouin's fixative (not shown). Intense Glut-1 immunostaining was observed in a subset of intraductal CaP specimens (Fig. 4). In these samples, Glut-1 localized to the plasma membrane of cancer epithelial cells and was over-expressed in cancer epithelial cells located in the center of the cribriform/papillary growth of these tumors (Fig. 4). Contrary to prior observations in other human tissues [Godoy et al., 2006, 2009], the results indicated malignant transformation of prostate tissue was associated with decreased expression of GLUT1, which suggests glucose might not play an important role in maintaining prostate cancer cell metabolism.

## ANALYSIS OF THE EXPRESSION OF Glut-5 PROTEIN IN BENIGN PROSTATE, HGPIN AND CAP

The immunostaining analysis demonstrated Glut-5 expression in benign prostate using clinical specimens fixed in either 10% formalin (Fig. 5A) or Bouin's solution (Fig. 5B). In both experimental conditions, Glut-5 immunostaining was localized to the apical portion of the plasma membrane of secretory/luminal epithelial cells. No Glut-5 immunostaining was observed in the basolateral portion of the plasma membrane of secretory/luminal epithelial cells, nor in the basal cell or stromal compartment. These observations were confirmed using conventional immunohistochemistry (Fig. 5C, arrow) and immunofluorescence (Fig. 5D, arrow) applied to thick sections (40 μm) of benign prostate fixed in Bouin's solution. In HGPIN, Glut-5 immunostaining was observed in 10% formalin-fixed specimens (Fig. 5E,F). Glut-5 immunostaining was less intense than in benign prostate and was highly polarized to the apical portion of the plasma membrane of the neoplastic epithelial cells (Fig. 5E,F). In CaP, Glut-5 immunostaining was not detected in formalin-fixed or in Bouin's-fixed clinical specimens. Taken together, these results demonstrated that Glut-5

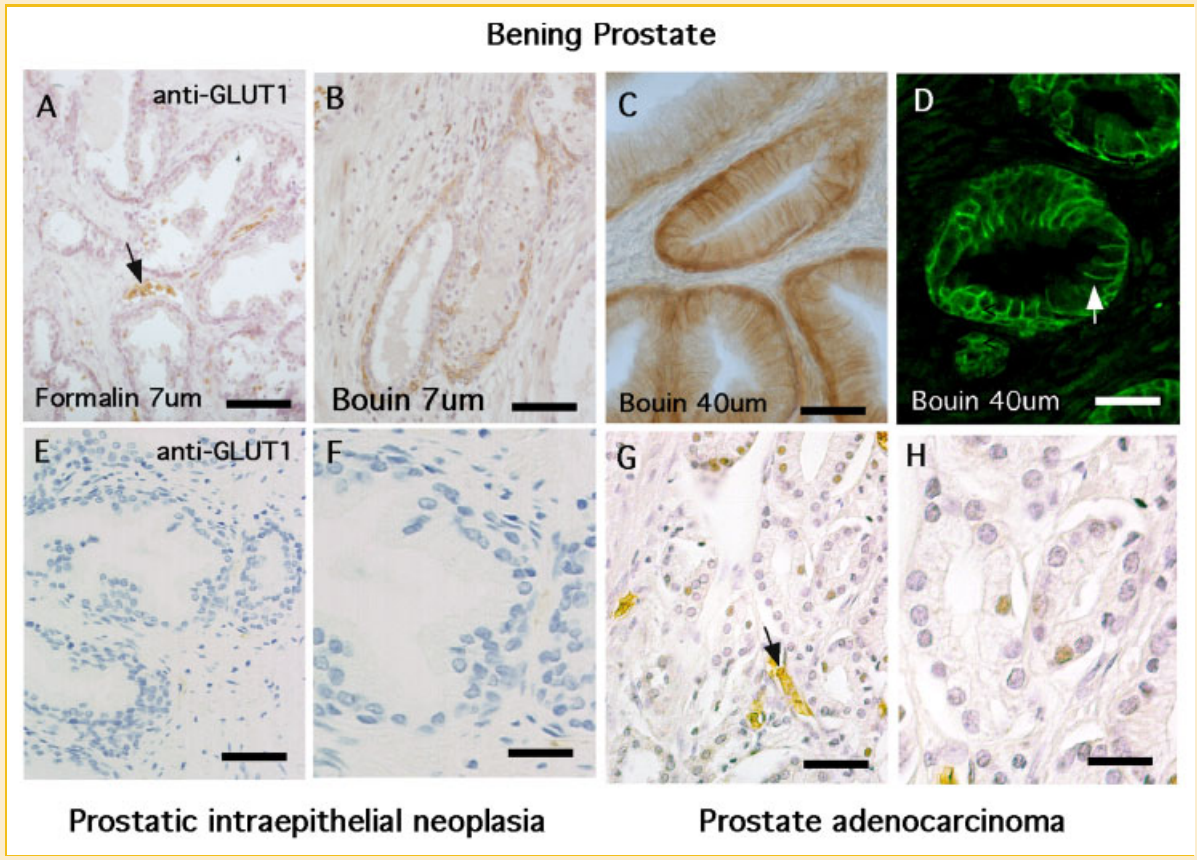


Fig. 3. Analysis of expression of GLUT1 in benign prostate, HGPIN and CaP. Glut-1 expression was analyzed in 10% formalin-fixed (A) or Bouin's-fixed (B) benign prostate tissue specimens. (C,D) Glut-1 immunostaining in benign prostate tissue was confirmed using thick sections (40 μm) and peroxidase immunostaining (C) or immunofluorescence (D). (E–H) Glut-1 immunostaining in HGPIN (E,F) and CaP (G,H) tissue specimens. (F) and (H) represent higher magnifications of (E) and (G), respectively. Black bars in A,B 50 μm; in C,D,G 30 μm; F,H 20 μm.

was expressed in benign prostate and HGPIN, but not in CaP. The levels of Glut-5 immunostaining intensity inversely correlate with malignant transformation of prostate tissue. Expression of Glut-5 in HGPIN suggests fructose might be a metabolic substrate in HGPIN.

### ANALYSIS OF THE EXPRESSION OF Glut-2, Glut-7, Glut-9, AND Glut-11 IN BENIGN HUMAN PROSTATE AND CAP

A lack of expression of Glut-5 protein in CaP could suggest a lower consumption of fructose by CaP cells. Subsequently, preliminary RT-PCR and immunocytochemistry analyses for expression of mRNA of Glut-2, Glut-7, Glut-9, and Glut-11, the alternative fructose transporters, were performed in 5 matched clinical specimens of non-involved benign (NT) and CaP (T) tissues (Fig. 6). These results indicated that Glut-2 mRNA was not present in any clinical specimen analyzed (Fig. 6A), which was confirmed using immunohistochemistry (Fig. 6B). Glut-7 mRNA showed lower expression in CaP compared to benign tissue (specimens 2–5). Glut-9 mRNA expression was variable; one specimen showed increased expression in CaP compared to benign prostate tissue (specimens 1), while the majority of the specimens showed the opposite result (specimens 3–5). Glut-11, however, showed increased mRNA expression in CaP compared to benign prostate tissue in all of the specimens analyzed, which suggests Glut-11 represents a candidate to mediate fructose transport in CaP cells.

## DISCUSSION

Analysis of Glut expression in different tumors has revealed an important role for Glut-1 during neoplastic transformation and tumor progression [Warburg, 1956; Birnbaum et al., 1987; Flier et al., 1987; Yamamoto et al., 1990; Brown and Wahl, 1993; Wahl et al., 1993; Younes et al., 1995, 1996a, b, 1997; Haber et al., 1998; Garcia et al., 2001; Godoy et al., 2006, 2009]. This cellular property has been utilized extensively in positron emission tomography (PET), a widely accepted tool for staging and evaluating the benefit of treatment for several human tumors, especially breast tumors, using 2-[18F]-fluoro-2-deoxy-D-glucose (FDG) [Minn and Soini, 1989; Tse et al., 1992; Nieweg et al., 1993; Wahl et al., 1993]. In CaP, however, FDG-PET imaging has shown limited clinical applicability [Bender et al., 1997; Salminen et al., 2002], probably due to: (1) low metabolic activity of this type of tumor [Singh et al., 1999] or (2) utilization of hexoses other than glucose, such as fructose, to maintain CaP cell metabolism [Godoy et al., 2006; Levi et al., 2007].

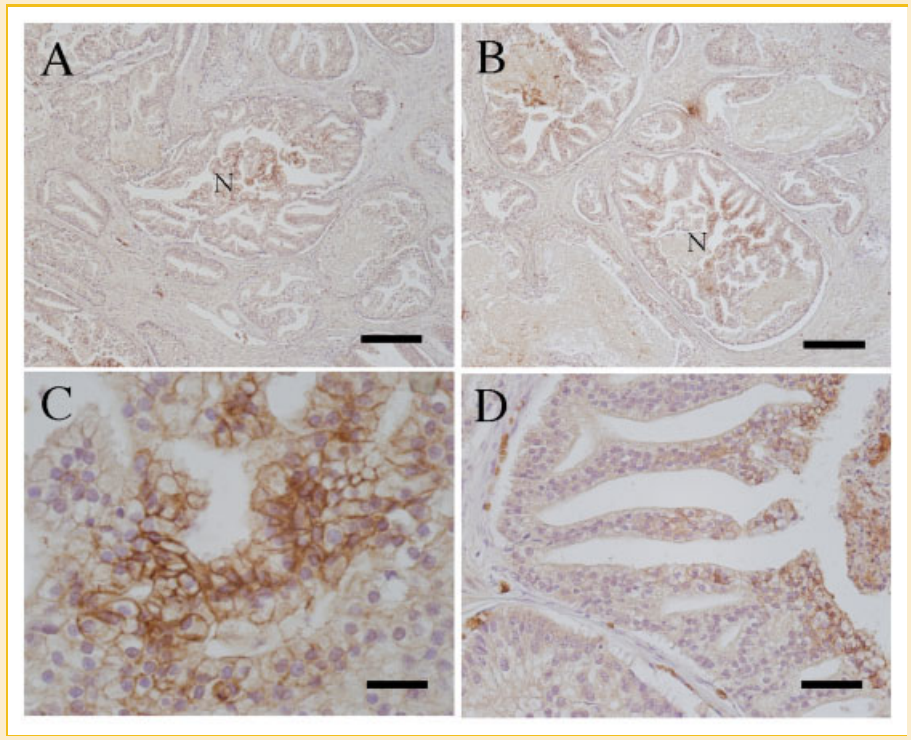


Fig. 4. Expression of Glut-1 in intraductal CaP. (A,B) Low magnification images of two cases of intraductal CaP showing a cribiform growth pattern. Necrosis was observed in the center of the tumor masses of proliferating cancer epithelial cells (N). Higher magnifications indicated GLUT1 immunostaining was observed in the center of these tumor masses (D) and specifically localized to the plasma membrane of cancer epithelial cells (C). Black bars in A,B 50 μm; C,D 20 μm.

However, to date, none of these possibilities has been examined in detail. Previous studies [Chandler et al., 2003; Effert et al., 2004; Stewart et al., 2008] are limited and inconclusive in terms of defining the molecular mechanisms (transport systems) involved in hexose uptake in CaP cells. Our data indicated a very low level of expression of Glut-1 in clinical specimens of CaP, which could explain, at least in part, the lack of effectiveness of FDG-PET for imaging CaP. Our data support the study by Chandler et al. [2003], which indicated that out of three clinical specimens of CaP analyzed, only one showed weak expression of Glut-1. Our results, however, contrast with the analyzes reported by Effert et al. [2004] and Steward et al. [2008], which indicated that expression of Glut-1 was higher in CaP than benign prostatic hyperplasia. In these studies, however, expression of Glut-1 was analyzed at the mRNA level and no attempt was made to analyze expression of Glut-1 protein. These inconsistencies could be explained by the fact that the presence of mRNA does not always correlate with expression of the corresponding protein, which highlights the importance of our immunostaining analysis.

Cellular distribution of Glut has been characterized with some detail in cell lines [Mitsumoto and Klip, 1992; Verhey et al., 1993; Cornford et al., 1994]. Our group was among the first to analyze subcellular localization of Glut-1 in benign and malignant human breast tissue [Godoy et al., 2009]. In this study, preferential distribution of Glut-1 to canaliculi-like structures of the plasma membrane was observed in breast cancer epithelial cells but not in benign epithelium. These observations suggest trafficking and distribution of Glut-1 was highly regulated in breast cancer cells. In benign prostate tissue, trafficking/distribution of Glut-1 and Glut-5 also was highly regulated; Glut-1 preferentially located to basolateral portion of the plasma membrane of secretory/luminal epithelial cells, whereas Glut-5 located to the apical portion of the plasma membrane of secretory/luminal epithelial cells. However, no special distribution of Glut-1 or Glut-5 was observed in basal cells or in any other cell type in benign prostate tissue. Location of Glut-1 at the basal cell level and to the basolateral membrane of secretory/luminal epithelial cells suggest these cells have the ability to incorporate glucose from the prostate capillary network present in the stroma immediately adjacent to benign glands and ducts [Bigler et al., 1993]. However, expression of Glut-5 at the apical membrane of secretory/luminal epithelial cells indicates that these cells might either efflux fructose to the prostate fluid or incorporate fructose from it. Prostate fluid represents approximately 25–30% of the seminal fluid, which contains one of the highest concentrations of fructose in the human body. It is generally accepted that fructose in semen is contributed by the seminal vesicles [Said et al., 2009]. However, other accessory glands, like prostate, may contribute fructose. Our results suggest the hypothesis that fructose also is secreted through the prostate epithelium. One possibility could be that fructose is synthesized from glucose in secretory/luminal epithelial cells and transported to the prostate fluid via Glut-5. Another possibility could be that fructose is incorporated directly from blood flow and transported through endothelial cells, basal cells and/or secretory/luminal epithelial cells using different transport mechanisms. More studies are necessary to test these two possibilities.

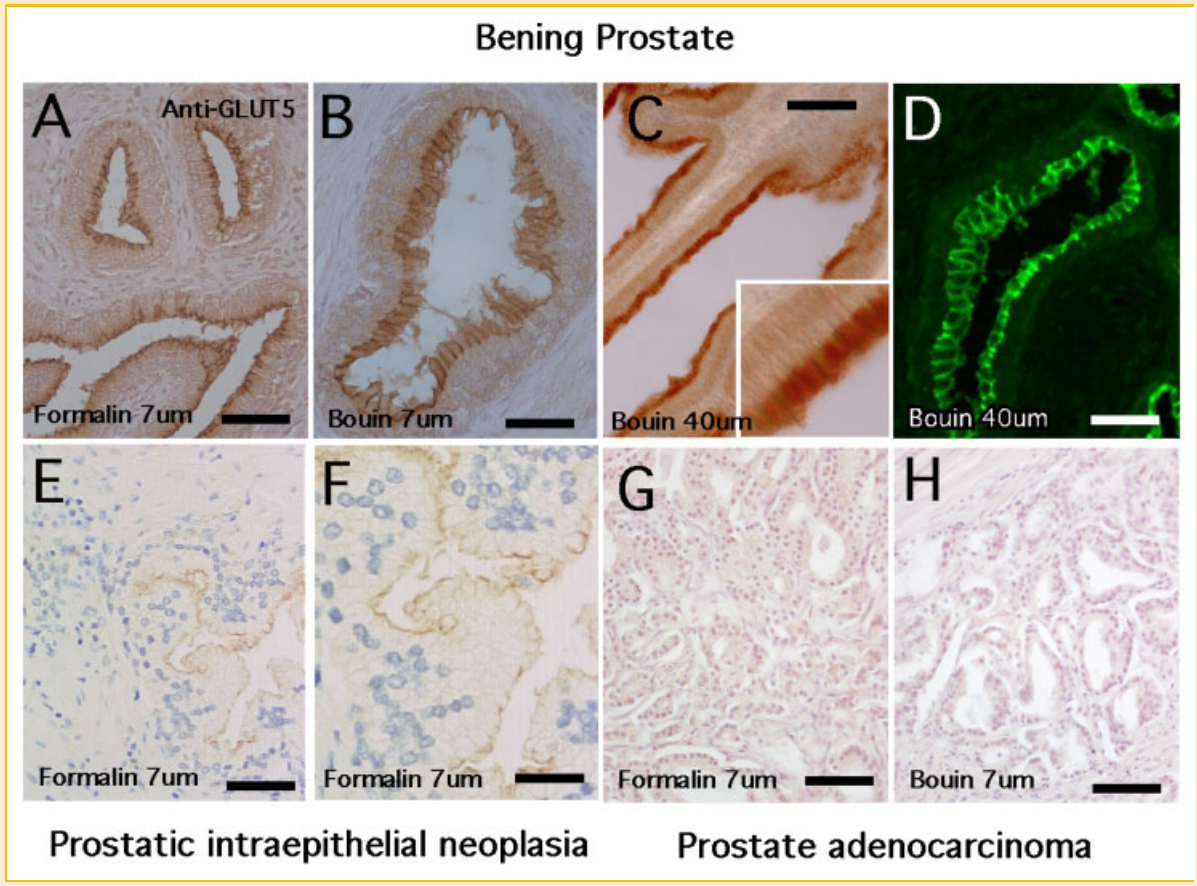


Fig. 5. Analysis of expression of GLUT5 in benign prostate, HGPIN and CaP. Glut-5 expression was analyzed in 10% formalin-fixed (A) or Bouin's-fixed (B) benign prostate tissue specimens. (C,D) Glut-5 Immunostaining in benign prostate tissue was confirmed using thick sections (40 μm) and peroxidase immunostaining (C) or immunofluorescence (D). (E,F) Glut-5 immunostaining in 10% formalin-fixed (E,F) HGPIN specimens. Image (F) represents a higher magnification of image (E). Glut-5 immunostaining in 10% formalin-fixed (G) or Bouin's-fixed (H) CaP tissue specimens. Black bars in A,B,E,G 30 μm; C,D,F,H 15 μm.

The identification of membrane antigens/biomarkers in malignant tissue sections using immunohistochemical techniques has become important for the diagnosis, prognosis, classification, and treatment evaluation in several types of cancers. Traditionally, 10% neutral buffered-formalin has been the preservative of choice for most specimens; however, Bouin's solution may be preferred instead of formalin in two situations. The first case is for small biopsies, because the yellow tinge imparted to the tissue facilitates visualization during embedding, without an additional step of dipping the biopsies in ink. The second is when excellent nuclear detail and glycogen preservation properties are essential for histopathological diagnosis [Ananthanarayanan et al., 2005]. Our study demonstrated plasma membrane antigens expressed at high levels were detected equally using either Bouin's- or formalin-fixed human prostate tissues (for example, Glut-5 in benign prostate). However, when low levels of expression of Glut-1 protein were observed (for example in benign prostate), Bouin's fixative was more efficient for detecting expression of Glut-1. These data suggest that Bouin's fixative could be the preferred choice when diagnosis is hampered by a low level of expression of a specific antigen.

Broad immunohistochemical analyses [Younes et al., 1996b; Godoy et al., 2006] have failed to detect Glut-1 expression in a high percentage of tumors, which suggests other members of the Glut family may be over-expressed in human cancer. Our group [Godoy et al., 2006] had demonstrated previously that, while Glut-1 was the most widely expressed Glut isoform in human cancers, Glut-2 and Glut-5 isoforms also were over-expressed in some tumors compared to their benign counterpart. In the present study, both Glut-1 and Glut-5 were expressed at lower levels in CaP compared to benign prostate. Lower expression of Glut-1 in CaP suggests low glucose metabolism in this type of tumor and correlates with the limited clinical applicability shown by FDG-PET imaging in CaP [Bender et al., 1997; Salminen et al., 2002]. Also, lower expression of Glut-5 in CaP suggests a lower consumption of fructose by prostate cancer cells compared to benign epithelial cells. However, this hypothesis needs to be confirmed since PET imaging using fructose derivatives as radiotracers to evaluate fructose metabolism in prostate cancer cells has not been considered yet [Levi et al., 2007]. Moreover, other Glut isoforms, such as Glut-2, Glut-7, Glut-9, and Glut-11, also have been suggested to transport fructose due to their high sequence homology with Glut-5 [Augustin et al., 2004;

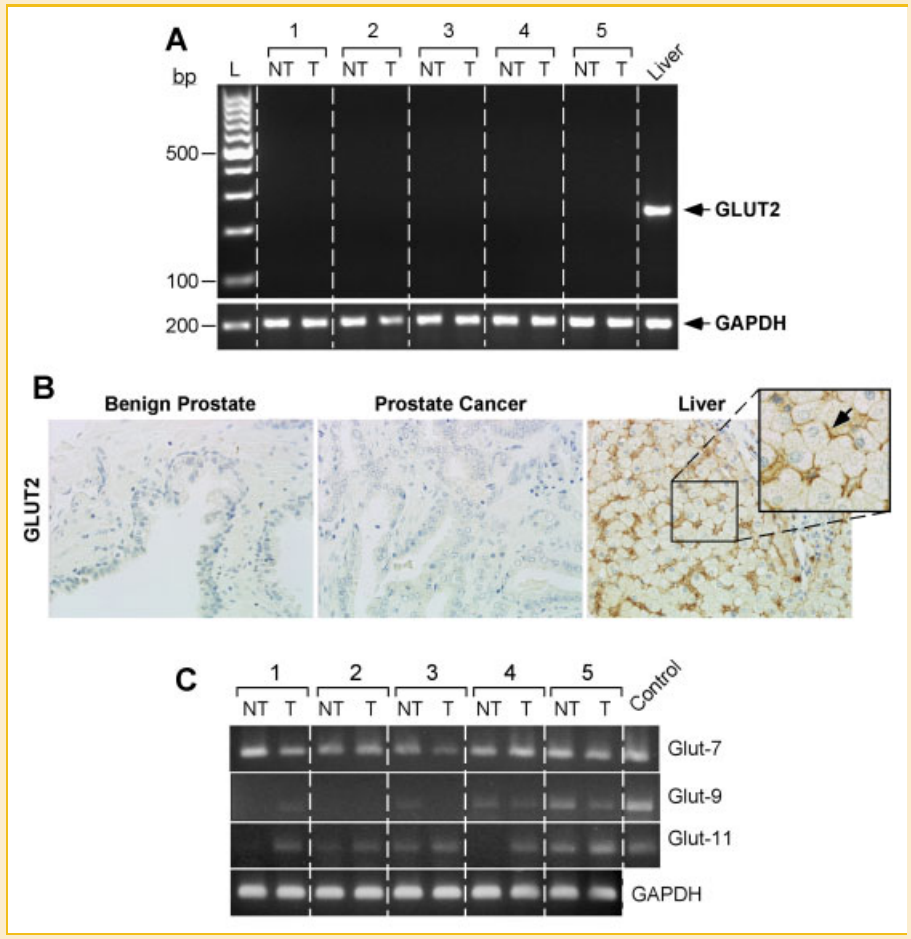


Fig. 6. Analysis of expression of Glut-2, Glut-7, Glut-9, and Glut-11 in benign prostate and CaP. (A) RT-PCR analysis of Glut-2 in 5 different specimens of human prostate tissue. (B) Immunostaining analysis of Glut-2 in benign and malignant human prostate tissue. (C) RT-PCR analysis of Glut-7, Glut-9, and Glut-11 in 5 different specimens of human prostate tissue. NT: non-tumor (non-involve) tissue; T: Tumor; Controls: Glut-2 (Liver), Glut-7 (small intestine), Glut-9 (kidney), and Glut-11 (kidney).

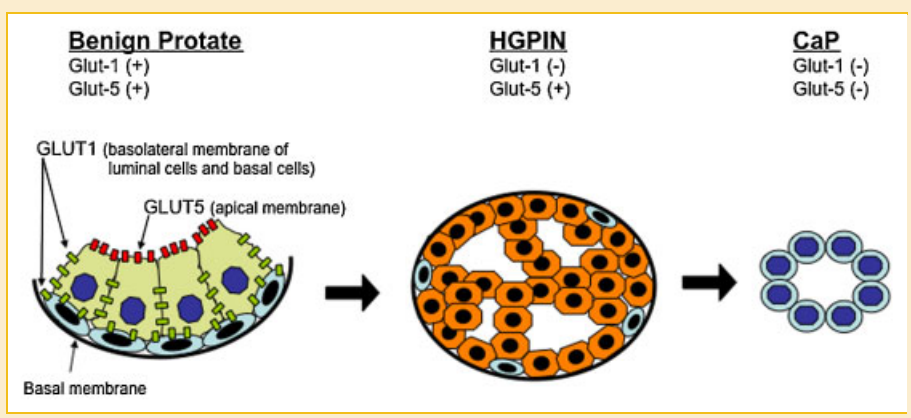


Fig. 7. Schematic representation of the expression of Glut-1 and Glut-5 in benign prostate, HGPIN and CaP. In benign prostate, Glut-1 and Glut-5 showed differential subcellular distribution; Glut-1 localized to the basal cells and to the basolateral portion of the plasma membrane of secretory/luminal epithelial cells. Glut-5, localized to the apical portion of the plasma membrane of secretory/luminal epithelial cells. In HGPIN, Glut-1 was immunohistochemically undetectable. Glut-5, however, localized to the apical portion of the plasma membrane of the hyper-proliferative neoplastic epithelial cells. In CaP, Glut-1 and Glut-5, were immunohistochemically undetectable.

Li et al., 2004; Scheepers et al., 2005]. However, antibodies that recognize Glut-7, Glut-9, and Glut-11, and suitable for immunohistochemistry, are not available commercially. Our preliminary analysis using RT-PCR in a subset of clinical samples indicated that out of these isoforms, only Glut-11 mRNA was consistently expressed at higher levels in malignant human prostate tissue compared to benign human prostate tissue, which suggests Glut-11 may represent a candidate to mediate fructose transport in CaP. A recent study indicated that pancreatic cancer cells metabolize fructose to increase proliferation [Liu et al., 2010]. These findings have a major significance for cancer patients, especially considering that fructose is one of the most highly consumed sugars in the American diet [Gross et al., 2004] and that refined fructose has been implicated in several diseases with rapidly increasing incidence, including obesity, diabetes and fatty liver [Havel, 2005; Montonen et al., 2007]. Further analyses are necessary to validate the role of fructose in the development and progression of CaP and the possible role of fructose transporters as potential biomarkers for CaP.

In the present study, a subset of intraductal CaP samples showed over-expression of Glut-1 compared to benign prostate. In all these cases, Glut-1 localized predominantly to the center of the intraductal neoplastic growth. Considerable hypoxia must occur to the center of these highly proliferative masses of cells and over-expression of Glut-1 might be a consequence of this process [Stewart et al., 2008]. Recently, we demonstrated a similar pattern of expression of Glut-1 in a subset of the highly proliferative breast cancers type SBRII [Godoy et al., 2009]. Glut-1 demonstrated a preferential localization to the portions of the cellular membrane that faced neighboring cells and formed canaliculi-like structures we named ''nutritional channels'' [Godoy et al., 2009]. These nutritional channels may represent morpho-functional adaptations of the hyper-proliferative breast cancer cells to facilitate nutrient supply, in general, and to increase glucose uptake, specifically, to complement tumor neo-vascularization. In case of intraductal CaP, because of the decrease in blood supply as the cells proliferate to the center of the gland and get far from the vasculature that surround the glandular compartment, over-expression and preferential localization of Glut-1 to specific cellular adaptations of the plasma membrane might be a potential mechanism to assure a more efficient nutrient supply to the cancer epithelial cells during their intra-glandular growth. However, more studies are necessary to define whether or not this particular type of tumor develops these morpho-functional adaptations.

In summary, our study is among the first to characterize the expression and cellular distribution of the glucose transporter Glut-1 and the fructose transporter Glut-5 proteins in benign and malignant human prostate tissue. Low levels of expression of Glut-1 protein in the majority of clinically relevant CaP could explain the failure of FDG-PET imaging for evaluation of the metabolic status of CaP (Fig. 7). Expression of Glut-5 in HGPIN suggested that fructose could be utilized as potential substrate to maintain metabolic requirements, and probably survival, of the neoplastic epithelial cells in HGPIN (Fig. 7). In addition, over-expression of Glut-11, at the mRNA level, in CaP compared to benign prostate suggests fructose might have an important role in accelerating CaP growth/survival. Understanding the molecular mechanisms involved in the down-regulation of hexose transporters in malignant human prostate tissue could provide insight to better understand CaP cell metabolism.

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## REFERENCES

Ananthanarayanan V, Pins MR, Meyer RE, Gann PH. 2005. Immunohistochemical assays in prostatic biopsies processed in Bouin's fixative. J Clin Pathol 58:322–324.

Augustin R, Carayannopoulos MO, Dowd LO, Phay JE, Moley JF, Moley KH. 2004. Identification and characterization of human glucose transporter-like protein-9 (GLUT9): alternative splicing alters trafficking. J Biol Chem 279: 16229–16236.

Bender H, Schomburg A, Albers P, Ruhlmann J, Biersack HJ. 1997. Possible role of FDG-PET in the evaluation of urologic malignancies. Anticancer Res 17:1655–1660.

Bigler SA, Deering RE, Brawer MK. 1993. Comparison of microscopic vascularity in benign and malignant prostate tissue. Hum Pathol 24:220–226.

Birnbaum MJ, Haspel HC, Rosen OM. 1987. Transformation of rat fibroblasts by FSV rapidly increases glucose transporter gene transcription. Science 235: 1495–1498.

Brown RS, Wahl RL. 1993. Overexpression of Glut-1 glucose transporter in human breast cancer. An immunohistochemical study. Cancer 72:2979–2985.

Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJ, Petros JA, Andriole GL. 1991. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med 324:1156–1161.

Chandler JD, Williams ED, Slavin JL, Best JD, Rogers S. 2003. Expression and localization of GLUT1 and GLUT12 in prostate carcinoma. Cancer 97:2035–2042.

Concha II, Velasquez FV, Martinez JM, Angulo C, Droppelmann A, Reyes AM, Slebe JC, Vera JC, Golde DW. 1997. Human erythrocytes express GLUT5 and transport fructose. Blood 89:4190–4195.

Cornford EM, Hyman S, Swartz BE. 1994. The human brain GLUT1 glucose transporter: ultrastructural localization to the blood-brain barrier endothelia. J Cereb Blood Flow Metab 14:106–112.

Effert P, Beniers AJ, Tamimi Y, Handt S, Jakse G. 2004. Expression of glucose transporter 1 (Glut-1) in cell lines and clinical specimens from human prostate adenocarcinoma. Anticancer Res 24:3057–3063.

Flier JS, Mueckler MM, Usher P, Lodish HF. 1987. Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. Science 235:1492–1495.

Fukumoto H, Kayano T, Buse JB, Edwards Y, Pilch PF, Bell GI, Seino S. 1989. Cloning and characterization of the major insulin-responsive glucose transporter expressed in human skeletal muscle and other insulin-responsive tissues. J Biol Chem 264:7776–7779.

Fukumoto H, Seino S, Imura H, Seino Y, Eddy RL, Fukushima Y, Byers MG, Shows TB, Bell GI. 1988. Sequence, tissue distribution, and chromosomal localization of mRNA encoding a human glucose transporter-like protein. Proc Natl Acad Sci USA 85:5434–5438.

Garcia MA, Carrasco M, Godoy A, Reinicke K, Montecinos VP, Aguayo LG, Tapia JC, Vera JC, Nualart F. 2001. Elevated expression of glucose transporter-1 in hypothalamic ependymal cells not involved in the formation of the brain-cerebrospinal fluid barrier. J Cell Biochem 80: 491–503.

Godoy A, Ormazabal V, Moraga-Cid G, Zuniga FA, Sotomayor P, Barra V, Vasquez O, Montecinos V, Mardones L, Guzman C, Villagran M, Aguayo LG, Onate SA, Reyes AM, Carcamo JG, Rivas CI, Vera JC. 2007. Mechanistic insights and functional determinants of the transport cycle of the ascorbic acid transporter SVCT2. Activation by sodium and absolute dependence on bivalent cations. J Biol Chem 282:615–624.

Godoy A, Salazar K, Figueroa C, Smith GJ, de Los Angeles Garcia M, Nualart FJ. 2009. Nutritional channels in breast cancer. J Cell Mol Med 13:3973–3984.

Godoy A, Ulloa V, Rodriguez F, Reinicke K, Yanez AJ, Garcia Mde L, Medina RA, Carrasco M, Barberis S, Castro T, Martinez F, Koch X, Vera JC, Poblete MT, Figueroa CD, Peruzzo B, Perez F, Nualart F. 2006. Differential subcellular distribution of glucose transporters GLUT 1-6 and GLUT9 in human cancer: ultrastructural localization of GLUT1 and GLUT5 in breast tumor tissues. J Cell Physiol 207:614–627.

Gross LS, Li L, Ford ES, Liu S. 2004. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. Am J Clin Nutr 79:774–779.

Haber RS, Rathan A, Weiser KR, Pritsker A, Itzkowitz SH, Bodian C, Slater G, Weiss A, Burstein DE. 1998. GLUT1 glucose transporter expression in colorectal carcinoma: a marker for poor prognosis. Cancer 83:34–40.

Havel PJ. 2005. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutr Rev 63:133–157.

Jemal A, Siegel R, Xu J, Ward E. 2010. Cancer statistics, 2010. CA: Cancer J Clin 60:277–300.

Joost HG, Thorens B. 2001. The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). Mol Membr Biol 18:247–256.

Kayano T, Burant CF, Fukumoto H, Gould GW, Fan YS, Eddy RL, Byers MG, Shows TB, Seino S, Bell GI. 1990. Human facilitative glucose transporters. Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle, and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). J Biol Chem 265:13276–13282.

Kayano T, Fukumoto H, Eddy RL, Fan YS, Byers MG, Shows TB, Bell GI. 1988. Evidence for a family of human glucose transporter-like proteins. Sequence and gene localization of a protein expressed in fetal skeletal muscle and other tissues. J Biol Chem 263:15245–15248.

Levi J, Cheng Z, Gheysens O, Patel M, Chan CT, Wang Y, Namavari M, Gambhir SS. 2007. Fluorescent fructose derivatives for imaging breast cancer cells. Bioconjug Chem 18:628–634.

Li Q, Manolescu A, Ritzel M, Yao S, Slugoski M, Young JD, Chen XZ, Cheeseman CI. 2004. Cloning and functional characterization of the human GLUT7 isoform SLC2A7 from the small intestine. Am J Physiol Gastrointest Liver Physiol 287:G236–G242.

Liu H, Huang D, McArthur DL, Boros LG, Nissen N, Heaney AP. 2010. Fructose induces transketolase flux to promote pancreatic cancer growth. Cancer Res 70:6368–6376.

Minn H, Soini I. 1989. [18F]fluorodeoxyglucose scintigraphy in diagnosis and follow up of treatment in advanced breast cancer. Eur J Nucl Med 15: 61–66.

Mitsumoto Y, Klip A. 1992. Development regulation of the subcellular distribution and glycosylation of GLUT1 and GLUT4 glucose transporters during myogenesis of L6 muscle cells. J Biol Chem 267:4957–4962.

Montonen J, Jarvinen R, Knekt P, Heliovaara M, Reunanen A. 2007. Consumption of sweetened beverages and intakes of fructose and glucose predict type 2 diabetes occurrence. J Nutr 137:1447–1454.

Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, Allard WJ, Lienhard GE, Lodish HF. 1985. Sequence and structure of a human glucose transporter. Science 229:941–945.

Nieweg OE, Kim EE, Wong WH, Broussard WF, Singletary SE, Hortobagyi GN, Tilbury RS. 1993. Positron emission tomography with fluorine-18-deoxy-glucose in the detection and staging of breast cancer. Cancer 71:3920–3925.

Nualart F, Godoy A, Reinicke K. 1999. Expression of the hexose transporters GLUT1 and GLUT2 during the early development of the human brain. Brain Res 824:97–104.

Nualart F, Los Angeles, Garcia M, Medina RA, Owen GI. 2009. Glucose transporters in sex steroid hormone related cancer. Curr Vasc Pharmacol.

Said L, Galeraud-Denis I, Carreau S, Saad A. 2009. Relationship between semen quality and seminal plasma components: alpha-glucosidase, fructose and citrate in infertile men compared with a normospermic population of Tunisian men. Andrologia 41:150–156.

Salminen E, Hogg A, Binns D, Frydenberg M, Hicks R. 2002. Investigations with FDG-PET scanning in prostate cancer show limited value for clinical practice. Acta Oncol 41:425–429.

Scheepers A, Schmidt S, Manolescu A, Cheeseman CI, Bell A, Zahn C, Joost HG, Schurmann A. 2005. Characterization of the human SLC2A11 (GLUT11) gene: alternative promoter usage, function, expression, and subcellular distribution of three isoforms, and lack of mouse orthologue. Mol Membr Biol 22:339–351.

Singh G, Lakkis CL, Laucirica R, Epner DE. 1999. Regulation of prostate cancer cell division by glucose. J Cell Physiol 180:431–438.

Stewart GD, Gray K, Pennington CJ, Edwards DR, Riddick AC, Ross JA, Habib FK. 2008. Analysis of hypoxia-associated gene expression in prostate cancer: lysyl oxidase and glucose transporter-1 expression correlate with Gleason score. Oncol Rep 20:1561–1567.

Tse NY, Hoh CK, Hawkins RA, Zinner MJ, Dahlbom M, Choi Y, Maddahi J, Brunicardi FC, Phelps ME, Glaspy JA. 1992. The application of positron emission tomographic imaging with fluorodeoxyglucose to the evaluation of breast disease. Ann Surg 216:27–34.

Verhey KJ, Hausdorff SF, Birnbaum MJ. 1993. Identification of the carboxy terminus as important for the isoform-specific subcellular targeting of glucose transporter proteins. J Cell Biol 123:137–147.

Wahl RL, Zasadny K, Helvie M, Hutchins GD, Weber B, Cody R. 1993. Metabolic monitoring of breast cancer chemohormonotherapy using positron emission tomography: initial evaluation. J Clin Oncol 11: 2101–2111.

Warburg O. 1956. On the origin of cancer cells. Science 123:309–314.

Watanabe T, Nagamatsu S, Matsushima S, Kondo K, Motobu H, Hirosawa K, Mabuchi K, Kirino T, Uchimura H. 1999. Developmental expression of GLUT2 in the rat retina. Cell Tissue Res 298:217–223.

Yamamoto T, Seino Y, Fukumoto H, Koh G, Yano H, Inagaki N, Yamada Y, Inoue K, Manabe T, Imura H. 1990. Over-expression of facilitative glucose transporter genes in human cancer. Biochem Biophys Res Commun 170: 223–230.

Younes M, Brown RW, Mody DR, Fernandez L, Laucirica R. 1995. GLUT1 expression in human breast carcinoma: correlation with known prognostic markers. Anticancer Res 15:2895–2898.

Younes M, Brown RW, Stephenson M, Gondo M, Cagle PT. 1997. Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. Cancer 80:1046–1051.

Younes M, Lechago LV, Lechago J. 1996a. Overexpression of the human erythrocyte glucose transporter occurs as a late event in human colorectal carcinogenesis and is associated with an increased incidence of lymph node metastases. Clin Cancer Res 2:1151–1154.

Younes M, Lechago LV, Somoano JR, Mosharaf M, Lechago J. 1996b. Wide expression of the human erythrocyte glucose transporter Glut1 in human cancers. Cancer Res 56:1164–1167.