Immunohistochemical expression of glucose transporter Glut1 and cyclin D1 in breast carcinomas with negatine lymph nodes

P. Ravazoula[1], A. Batistatou[1], C. Aletra[1], J. Ladopoulos[2], G. Kourounis[2], B. Tzigounis[2]

Department of Pathology[1] and Obstetrics and Gynaecology[2], University Hospital of Patras, Rion (Greece)

Summary

All malignant cells appear to have increased needs for glucose *in vitro* as well as *in vivo*. The enhanced glucose uptake is mediated through transporters (Gluts), whose action and expression are regulated by oncogenes and growth factors. Cyclin D1 is a nuclear protein that plays an important role in regulating the cell cycle by promoting entry of cells from the G1 to S phase. Increased expression of Glut1 glucose transporter and cyclin D1 have been reported in several neoplasms. In the present study we examined the expression of Glut1 and cyclin D1 in breast carcinomas with negative lymph nodes. We studied 78 infiltrating ductal carcinomas (25 grade 1, 36 grade 2, and 17 grade 3) with negative lymph nodes. Glut1 was expressed in 28% of grade 1, 63.8% of grade 2 and 58.7% of grade 3 carcinomas. Nuclear expression of cyclin D1 was detected in 32% of grade 1, 44.4% of grade 2, and 41.2% of grade 3 carcinomas. From our study it appears that in breast carcinomas with negative lymph nodes Glut1 expression is better correlated to the grade of the neoplasm than cyclin D1.

Key words: Glucose transporter; Cyclin D1; Breast cancer.

Introduction

Malignant cells exhibit increased needs for glycolysis and glucose uptake [1]. This process is believed to be mediated through transporters (Gluts), whose action and expression are regulated via oncogenes and growth factors [2, 3]. The glucose transporters belong to a family of transmembrane proteins with six members Gluts1-5 and Glut7 [4, 5]. These transporters have different distributions and functions in animals and humans. In rats Glut1 mRNA and protein have been detected in the brain, kidneys and breast. In humans they have been found in endothelial cells of the blood-brain barrier, liver, placenta, and embryonic membranes [6-8]. Glut1 has been detected in the cytoplasmic membrane of endothelial cells from brain vessels with certain restricted permeability, while it was not detected in blood vessels from tissues which permit the passage of molecules of protein size [9]. However Glut1 immunoexpression is absent in most types of normal epithelial cells [10]. Studies in humans have shown increased expression of Glut1 mRNA in carcinomas of the oesophagus, colon and pancreas, while increased expression of Glut1 protein has been shown in neoplasms of the kidneys, breast, thyroid and in non-small cell lung carcinoma [11-15]. In addition it has been reported that Glut1 expression can be utilized as a sensitive marker of malignancy in body cavity effusions [16].

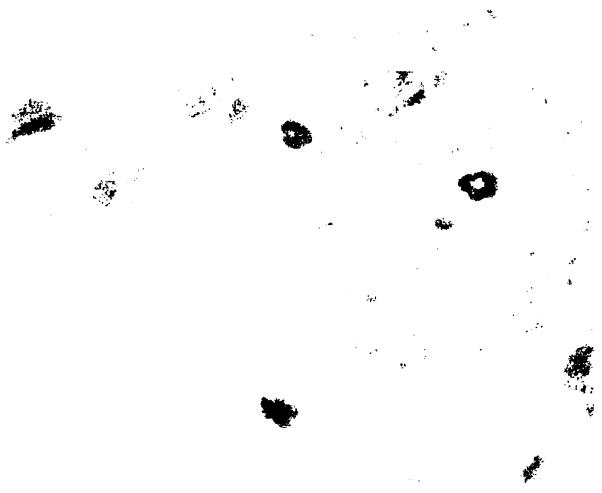
Recently it has been shown that the growth and progression of malignant neoplasms is regulated by molecules that are involved in the regulation of the cell cycle. A class of such molecules are the cyclins and cyclin-dependent kinases [17-20]. Cyclin D1 is involved in the regulation of cell cycles by promoting entry of cells from the G1 to S phase [21]. There are three types of cyclin D, namely cyclin D1, D2 and D3. Increased expression of cyclin D1 has been detected in cells that have remained in the G1 phase and not exited to the G0 phase, an observation that suggests that this cyclin is involved in the regulation of this exact point of the cell cycle [22]. Cyclin D1 is coded by the gene CCND-1, which is located in chromosome 11q13 [23]. Several studies have shown that gene amplification and increased expression of cyclin D1 are tumor promoting [24, 25]. Gene amplification of cyclin D1 has been detected in breast cancer cell lines and in breast cancers in humans [26-28]. The aim of the present study was the detection of Glut1 and cyclin D1 in breast carcinomas with negative lymph nodes.

Materials and Methods

Seventy-eight infiltrating ductal carcinomas with negative lymph nodes were examined. Among these, 25 were grade 1, 36 grade 2, and 17 grade 3. The tissues were fixed in buffered formalin and embedded in paraffin. In tissue sections of 5 μm the immunohistochemical method streptavidine-biotin was applied. Microwave pretreatement was employed to umask the antigen sites. The polyclonal antibody for Glut1 (Chemicon, USA, dilution 1:400) and the monoclonal antibody for cyclin D1 (Novocastra, UK, dilution 1:10) were used. Sections were incubated in RT for 12 hours. DAB was used as a chromogene. The sections were counterstained with hematoxylin. A quantitative method was used for the evaluation of immunohistochemical results: Negative (–) if none, + if < 10% and ++ if > 10% of tumor cells were positive.

Revised manuscript accepted for publication February 15, 2003

'ig. 1A



ig. 1B

Figure 1. — Positive membranous immunostaining for Glut1 in **A.** Ductal carcinoma in situ (x400). **B.** Infiltrating ductal carcinoma (x400).

Fig. 2A

Fig. 2B

Figure 2. — Positive nuclear immunostaining for cyclin D1 in **A.** Ductal carcinoma in situ (x200). **B.** Infiltrating ductal carcinoma (x200).

Results-Discussion

Glut1 was expressed in the cytoplasmic membrane of 55.5% of malignant neoplasms (40/78) overall. Specifically, Glut1 was detected in 7/40 (28%) grade 1, 23 (63.8%) grade 2 and 10 (58.7%) grade 3 carcinomas (Figure 1). In 17/40 of the neoplasms that were positive for Glut1 the percentage of positive tumor cells was < 10% and in 23/40 was > 10% (Table 1). Protein Glut1 was expressed not only in the invasive ductal carcinoma cells, but also in the in situ component of the neoplasms. In addition it was expressed in the endothelial cells of the vessels from all tumors examined, even those with negative immunostaining of neoplastic cells.

Positive nuclear staining for cyclin D1 was detected in 31/78 (40%) of breast carcinomas, eight (32%) grade 1, 16 (44.4%) grade 2, and seven (41.2%) grade 3 (Figure 2). In 21/31 of the neoplasms that were positive for Glut1 the percentage of positive tumor cells was < 10% and in 10/31 was > 10% (Table 1).

Table 1. — *Immunohistochemical expression of Glut1 and cyclin D1 in breast carcinomas.*

% of positive cells	Glut1	Cyclin D1
0	38	47
< 10%	17	21
> 10%	23	10
# of cases	78	78

Our results indicate that Glut1 expression correlates positively with the grade of invasive ductal carcinoma in patients with negative lymph nodes. This finding is in accordance with reports correlating overexpression of Glut1 with poorer prognosis in breast and lung carcinomas [14, 15]. The molecular changes that lead to increased Glut1 expression are not currently understood [1]. However, it can be postulated Glut1 overexpression could be part of a molecular cascade leading to tumor progression. Regarding the cases in our study of breast carcinoma that did not overexpress Glut1, it is possible that the glucose transport of neoplastic cells is mediated by transporters other that Glut1. The detected Glut1 protein in the endothelium of blood vessels in all tumors examined suggests that regulation of Glut1 expression in endothelial cells is different than that in tumor cells.

The role of cyclin D1 has not been fully elucidated despite intense investigation. Our results indicate that cyclin D1 is quite frequently overexpressed in breast carcinomas (~40%). It has been postulated that cyclin D1 amplification and/or overexpression may be involved in early stages of breast carcinogenesis, even as early as in hyperplasias and ductal carcinoma in situ [26-28]. Our results are consistent with this hypothesis. Cyclin D1 overexpression was not significantly different in the three grades of infiltrating ductal carcinoma, indicating that if

cyclin D1 is overexpressed, then this deregulation happens early in breast carcinogenesis, even in grade 1 tumors with negative lymph nodes.

References

[1] Flier J.S., Mueckler M.M., Usher P., Lodish H.F.: "Elevated levels of glucose transport and transporter messanger RNA are induced by ras or src oncogenes". *Science,* 1987, *235,* 1492.

[2] Hiraki Y., Rosen O.M., Birnbaum M.J.: "Growth factors rapidly induce expression of the glucose transporter gene". *J. Biol. Chem.*, 1988, *263,* 13655.

[3] Merrall N.W., Plevin R., Gould G.W.: "Growth factors, mitogens, oncogenes and the regulation of glucose transport". *Cell. Sign.*, 1993, *5*, 667.

[4] Mueckler M.: "Facilitative glucose transporters". *Eur. J. Biochem.* 1994, *219,* 713.

[5] Pessin J.E., Bell G.I.: "Mammalian facilitating glucose transporter family:structure and molecular regulation". *Annu. Rev. Physiol.,* 1992, *54*, 911.

[6] Maher F., Vannucci S.J., Simpson I.A.: "Glucose transporter proteins in brain". *FASEB J.,* 1994, *8* (13), 1003.

[7] Camps S., Vilaro S., Testar X., Palacin M., Zorzano A.: "High and polarized expression of GLUT1 glucose trasporters in epithelial cells from mammary gland: acute downregulation of GLUT1 carriers by weaning". *Endocrinology,* 1994, *134,* 924.

[8] Jansson T., Wennergren M., Illsley N.P.: "Glucose trasporter protein expression in human placenta throughout gestation and in intrauterine growth retardation". *J. Clin. Endocrinol. Metab.,* 1993, *77,* 1554.

[9] Guerin C., Laterra J., Drewes L.R., Brem H., Goldstein G.: "Vascular expression of glucose trasporter in experimental brain neoplasms". *Am. J. Pathol.,* 1992, *140,* 417.

[10] Younes M., Lechago L.V., Somoano J.R., Morcharaf M., Lechago J.: "Wide expression of the human erythrocyte glucose transporter Glut1 in benign and malignant human tissues". *Cancer Res.,* 1996, *56,* 1164.

[11] Mellanen P., Minn H., Grenman R., Harkonen P.: "Expression of glucose transporters in head-and-neck tumors". *Int. J. Cancer,* 1994, *56,* 622.

[12] Haber R.S., Weiser K.R., Pritzker A., Reder I., Burstein D.E.: "Glut1 glucose transporter expression in benign and malignant thyroid nodules". *Thyroid.,* 1997, *7,* 363.

[13] Nagase Y., Takata K., Moriyama N., Aso Y., Murakami T., Hirano H.: "Immunohistochemical localization of glucose transporters in human renal carcinoma". *J. Urol.,* 1995, *153*, 798.

[14] Younes M., Brown R.W., Stephenson M., Gondo M., Cagle P.: "Overexpression of Glut1 and Glut3 in Stage I nonsmall cell lung carcinoma is associated with poor survival". *Cancer,* 1997, *80,* 1046.

[15] Younes M., Brown R.W., Mody D.R., Fernandez L., Laucirica R.: "Glut1 expression in human breast carcinoma: correlation with known prognostic markers". *Anticancer Res.,* 1995, *15*, 2895.

[16] Burstein D.E., Reder I., Weiser K., Tong T., Protsker A., Haber R.S.: "Glut1 glucose transporter: A highly sensitive marker of malignancy in body cavity effusions". *Mod. Pathol.,* 1998, *11,* 392.

[17] Sherr C.J.: "G1 phase progression: cyclin on cue". *Cell.,* 1994, *79*, 551.

[18] Morgan D.O.: "Principles of cdk regulation". *Nature,* 1995, *371,* 131.

[19] Hengst L., Dulic V., Slingerland J.M., Lees E., Reed S.I.: "A cell-cycle regulated inhibitor of cyclin-dependent kinases". *PNAS,* 1994, *91*, 5291.

[20] Reed S.I., Bailly E., Dulic V., Hengst L., Resnitzky D., Slingerland J.: "G1 control in mammalian cells". *J. Cell. Sci.,* 1994, *18,* 69.

[21] Murray A.W.: "Creative blocks: cell-cycle checkpoints and feedback controls". *Nature,* 1992, *359,* 599.

[22] Zwijsen R.M.L., Klompmarker R., Wientjens E., Kristel P., van der Burg B., Michalidis R.: "Cyclin D1 triggers autonomous growth of breast cancer cells by governing cell cycle exit". *Mol. Cel. Biol.,* 1996, *16,* 2554.

[23] Xiong Y., Menninger J., Beach D., Ward D.C.: "Molecular cloning and chromosomal mapping of CCND genes encoding human D-type cyclins". *Genomics,* 1992, *13,* 574.

[24] Lin B., Brynes R.K., Gelb A.B., McCourty A., Amin M., Medeiros J.: "Cyclin D1 expression in renal cell carcinomas and oncocytomas: An immunohistochemical study". *Mod. Pathol.,* 1988, *11*, 1075.

[25] Bartkova J., Lukas J., Strauss M., Bartek J.: "Cyclin D1 oncoprotein abberantly accumulates in malignancies of diverse histogenesis". *Oncogene,* 1995, *10*, 775.

[26] Bartkova J., Lukas J., Muller J., Lutzhoft D., Strauss M., Bartek J.: "Cyclin D1 protein expression and function in human breast cancer". *Int. J. Cancer,* 1994, *57,* 353.

[27] Zhang S.Y., Caamano J., Cooper F., Guo X., Klein-Szanto A.: "Immunohistochemistry of cyclin D1 in human breast cancer". *Am. J. Clin. Pathol.,* 1994, *102,* 695.

[28] Zhu X.L., Hartwick W., Rohan T., Kandel R.: "Cyclin D1 gene amplification and protein expression in benign breast disease and breast carcinoma". *Mod. Pathol.,* 1998, *11,* 1082.

Address reprint requests to:
J. LADOPOULOS, M.D.
Patr. Ioakim, 44
Athens 10675 (Greece)