

Invited Review

Glucose transporter expression in developing fetal lungs and lung neoplasms

T. Ito¹, Y. Noguchi², N. Udaka¹, H. Kitamura¹ and S. Satoh³

Departments of ¹Pathology, ²Surgery and ³Medicine, Yokohama City University School of Medicine, Yokohama, Japan

Summary. Glucose uptake and metabolism are essential for proliferation and survival of cells, and are supposed to be enhanced in actively proliferating cell systems such as embryonic and cancer tissues. Glucose uptake is usually carried out through glucose transporters.

In the developing fetal lung, metabolism of glucose is thought to be an important process in cell proliferation, differentiation and maturation. Active glucose uptake could result in accumulation of glycogen in epithelial cells, and utilization of glycogen could be a critical phenomenon for lung epithelial development. In hamsters, although facilitative glucose transporter isoform 1 (GLUT1) and isoform 4 (GLUT4) are not detected in adult lungs, expression of them is detected with immunohistochemical and Western blot analyses in the developing fetal lungs.

In human lung carcinomas, GLUT1 expression is seen in most cases of lung carcinoma, and is seen especially frequently in squamous cell carcinoma. GLUT1 expression in adenocarcinoma of the lung is correlated with reduced cell differentiation, larger tumor size and positive lymph node metastasis. A few cases of lung carcinoma show positive staining for GLUT3 and GLUT4.

Thus, expression of some facilitative glucose transporter isoforms is detected in developing fetal epithelium and in lung carcinomas. Overexpression of them could enhance uptake of glucose into these cells, and the increased influx of glucose could be involved in active cell proliferation, which is a common character of the developing lung epithelium and carcinoma.

Key words: Glucose transporter, Lung, Fetal development, Lung carcinoma, Immunohistochemistry

1. Introduction

Glucose transporters are integral membrane proteins that transport glucose and its analogues across the

cellular membrane. Two types of glucose transporters, Na⁺-dependent active glucose transporter and facilitative glucose transporter have been reported to exist in mammalian cells. In the lumen of absorptive cells of the small intestine and renal tubule, there is an energy-dependent Na⁺/glucose cotransporter, and this transporter family utilizes the movement of Na⁺ to drive the uptake of glucose (Hediger et al., 1987; Coady et al., 1990). Unlike the normally distributed Na⁺-dependent glucose transporter, all mammalian cells contain one or more members of the facilitative glucose transporter gene family (reviewed in Gould and Bell, 1990; Mueckler, 1994; Olson and Pessin, 1996). These facilitative glucose transporters regulate the movement of glucose between the extracellular and intracellular spaces with passive diffusion solely down the glucose concentration gradient.

The facilitative glucose transporters share significant sequence similarity, and seven members of this gene family have been reported. These proteins are named GLUT (for glucose transporters) 1-7, based on the chronological order of their identification and isolation. They are expressed in a tissue- and cell-specific manner, and exhibit different kinetic and regulatory properties. GLUT1 is widely expressed in adult and fetal tissues and provides many cells with their basal glucose requirement (Mueckler et al., 1985). In adult tissues, GLUT1 is abundant in erythrocytes (Kasahara and Hinkle, 1977) and endothelial cells (Pardridge et al., 1990). In fetal tissues, GLUT1 is expressed at high levels (Devaskar et al., 1991; Hogan et al., 1991; Santalucia et al., 1992; Postic et al., 1994), and GLUT1 is upregulated in a wide spectrum of human malignant neoplasms (Yamamoto et al., 1990; Younes et al., 1996). GLUT2 is a high-K_m isoform expressed in hepatocytes, pancreatic β cells, and absorptive cells of intestinal and renal tubular epithelia (Fukumoto et al., 1988; Thorens et al., 1988). GLUT2 has been proposed to play a role in blood glucose sensing (Orci et al., 1989; Thorens, 1992). GLUT3 is a low-K_m isoform, and is abundant in neuronal tissue (Kayano et al., 1988; Nagamatsu et al., 1992). GLUT4 is predominantly expressed in insulin-sensitive cells, which include skeletal and cardiac muscle cells as well as adipocytes (Birnbaum, 1989; Charron et al., 1989;

Offprint requests to: Takaaki Ito, MD, Department of Pathology, Yokohama City University School of Medicine, 3-9 Fuku-Ura, Kanazawa-ku, Yokohama 236-0004, Japan. Fax: +81-45-789-0588

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Fukumoto et al., 1989; James et al., 1989; Kaestner et al., 1989). GLUT5 principally transports fructose rather than glucose, and is primarily expressed in intestinal epithelium (Kayano et al., 1990; Burant et al., 1992). GLUT6 gene is a pseudogene (Kayano et al., 1990). GLUT7 is located in the endoplasmic reticulum of the liver and forms a complex with glucose-6-phosphatase (Waddell et al., 1992).

In the developing fetal lung, glycogen accumulates to significant levels in epithelial cells, which has been known since Claude Bernard (1859) first described glycogen in the fetal lung epithelium. The precise role of glycogen in fetal pulmonary tissues is, as yet, unknown. Sorokin (1965) suggested that it might serve as an energy source for cell proliferation and mitotic activity, and Brandstrup and Kretchmer (1965) proposed that lung glycogen might play a role in regulating blood glucose levels and carbohydrate metabolism in other organs. At least in type II alveolar cells, glycogen is thought to be utilized for synthesis of surfactant and for maturation of the alveolar epithelial cells (Kikkawa et al., 1971; Farrell and Bourbon, 1986; Rannels et al., 1991, 1997). The source of stored glycogen must be blood glucose, and GLUT should be involved in glycogenesis in fetal lung epithelium, as GLUT1 is

suggested to mediate the rate-limiting step in glucose uptake in fetal rat lung (Hart et al., 1998). In transgenic mice overexpressing GLUT1, it has been demonstrated that GLUT1 contributes to the accumulation of glycogen in muscle (Ren et al., 1993). Because GLUT1 was reported to be important for intrauterine growth of the lungs (Simmons et al., 1992, 1993), it was necessary to elucidate the distribution and modulation of the glucose transporter expression in the lungs during the fetal developmental period.

Since the early observations of Warburg (1931), it has become widely accepted that most tumor cells display increased rates of respiration, glucose uptake, and glucose metabolism as compared to healthy cells. The molecular mechanisms for the accelerated rate of glucose transport seen in tumor cells have not been completely clarified, but the elevated uptake of glucose is considered to be mediated by glucose transporters (Merrall et al., 1993). Younes et al. (1996) demonstrated immunohistochemically that GLUT1 was largely undetectable in normal epithelial tissues and benign epithelial tumors but was expressed in a significant proportion of a variety of human carcinomas. Apparent overexpression of certain types of GLUT isoforms has been reported in human cancers, including cancers of the

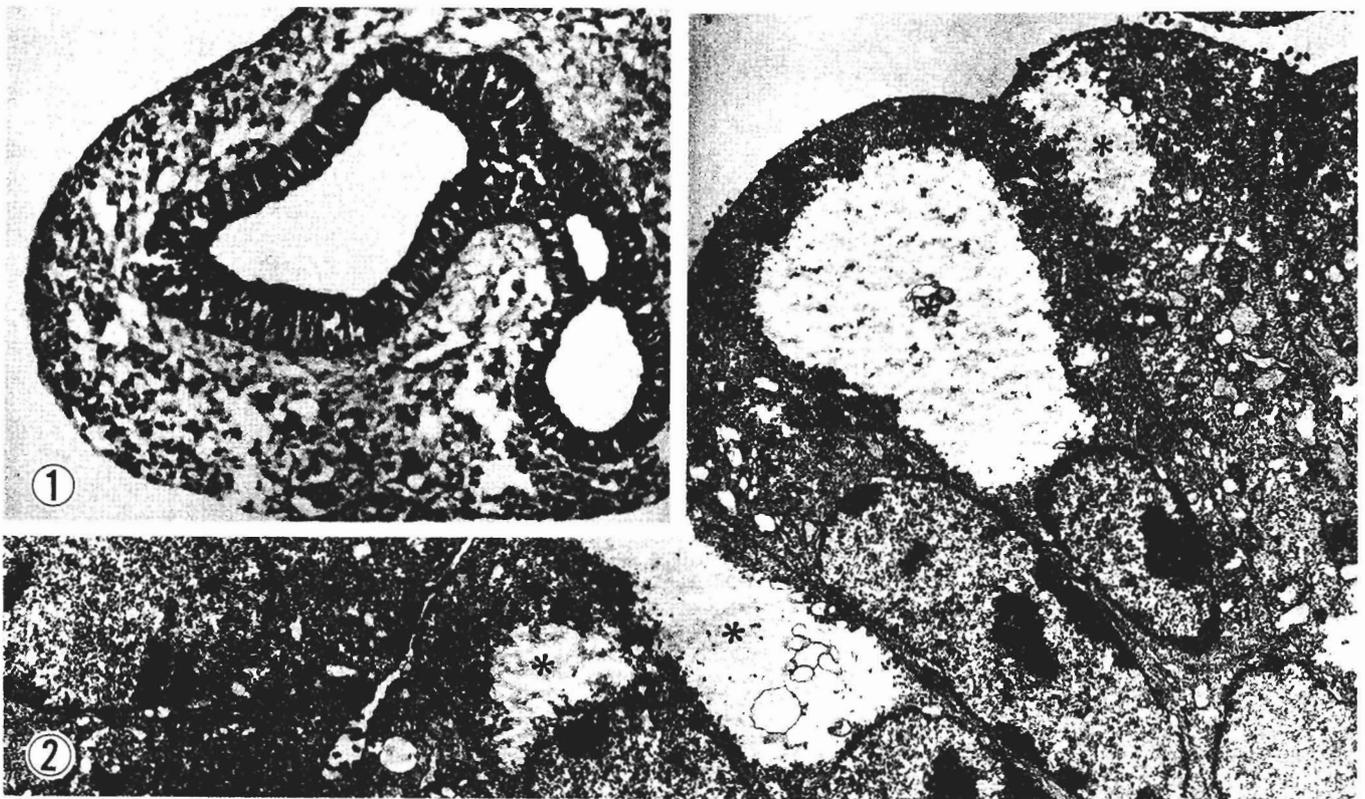


Fig. 1. Accumulation of glycogen in developing hamster lung epithelia on gestational day 12. Note the small amount of glycogen in the mesenchyme. Periodic acid-Schiff reaction and hematoxylin stain. x 175

Fig. 2. Electron micrograph of immature terminal tubule epithelium of a fetal hamster on gestational day 13. Asterisk: glycogen storage. x 4,500

esophagus (Yamamoto et al. 1990), stomach (Noguchi et al., 1999), colon, pancreas, liver (Yamamoto et al., 1990), breast (Brown and Wahl, 1993), head and neck (Mellanen et al., 1994), kidney (Nagase et al., 1995), bladder, ovary, testis, skin (Younes et al., 1996) and lung (Younes et al., 1996; Ogawa et al., 1997; Ito et al., 1998). In human lung cancers, expression of GLUT isoforms is related to histologic type, differentiation grade and tumor stage (Ito et al., 1998), and GLUT1 overexpression may be of prognostic value (Ogawa et al., 1997).

The study of lung development provides important information relevant to many areas of pulmonary physiology and pathology. Precise evaluation of histological and histochemical changes of lung epithelial cells is fundamental to our understanding of the phenotypic alterations in lung neoplasms as well as epithelial renewal in injured lungs. In this short review, we describe histology and immunostaining of GLUT isoforms in fetal lung tissues of hamster, and compare expression of GLUT isoforms in hamster fetal lung tissues with those in normal and neoplastic lung tissues of human.

2. Normal development and glycogen store in fetal hamster lungs

The mammalian lung develops from an endodermal tube which derives from an invagination of the primitive foregut. This embryonic stage is followed by progressive tubular morphogenesis, which accompanies cell proliferation, migration and cell differentiation.

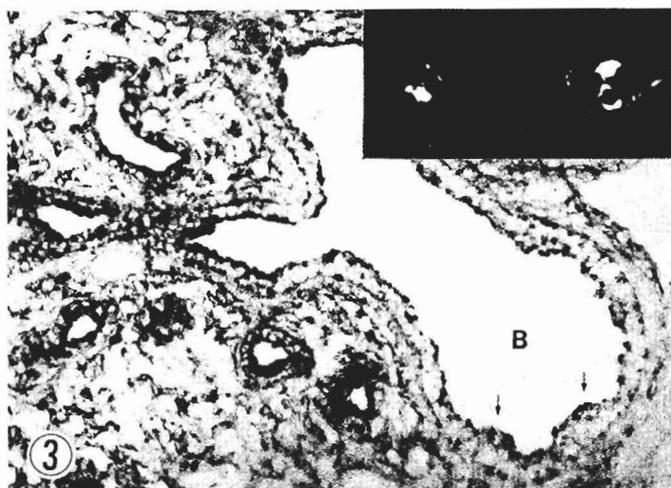


Fig. 3. In the developing fetal lung of hamster on gestational day 14, a small amount of glycogen is seen in the lobar bronchus (B) instead of a large amount of glycogen in developing alveolar epithelial cells. Clustered neuroendocrine cells (arrows). Periodic acid-Schiff reaction and hematoxylin stain. $\times 220$. **Inset:** A serial section of Fig. 3 with immunofluorescence staining for calcitonin gene-related peptide. The clustered neuroendocrine cells seen in Fig. 3 are positively stained. $\times 280$

Development of the respiratory system starts in the trachea and progresses as a wave distally to include the developing bronchi, bronchioles, and alveoli (Sorokin, 1965; Jeffery and Reid, 1977; Sturgess, 1985; Cutz, 1987). Early during normal development of Syrian golden hamsters, the undifferentiated epithelial cells contain abundant glycogen at all airway levels (Figs. 1, 2). Then, coincident with the appearance of the first neuroendocrine cells, the glycogen stores are depleted (Fig. 3). Thereafter, glycogen accumulates in pre-secretory cells until birth (Fig. 4), but by 2 days after birth the glycogen stores are again depleted. The initial depletion of glycogen followed by replenishment is observed at all levels of the conducting airways; changes in the trachea precede those in the bronchi and bronchioles by 1 and 2 days, respectively (McDowell et al., 1985, 1990; Sarikas et al., 1985; Ito et al., 1990). The pattern of glycogenolysis in the developing alveoli is different from that in the conducting airway epithelium: the glycogen breakdown in the alveoli is monophasic, and occurs before birth.

The biphasic pattern of glycogenolysis observed in the fetal conducting airway epithelia must have biological relevance, although its significance is still obscure. Glycogenolysis in the developing fetal lungs seems to involve epithelial cell differentiation and maturation. In the developing airways of hamsters, the first depletion of glycogen coincides with the appearance of neuroendocrine cells at all airway levels (trachea, bronchus and bronchiole). Pulmonary neuroendocrine cells are the first type of cells to differentiate in the fetal airway epithelium of various animal species, followed by ciliated, secretory, and basal cells (McDowell et al., 1985; Sarikas et al., 1985; Plopper et al., 1986; Cutz, 1987). In the second glycogen depletion in the developing airway epithelia during the perinatal period,

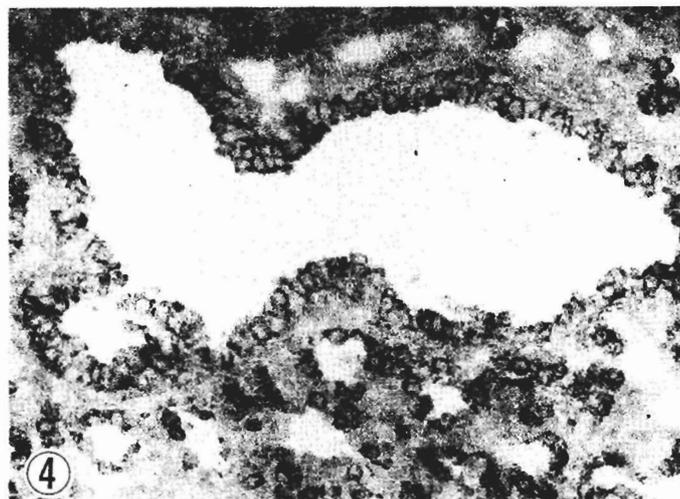


Fig. 4. In the developing bronchioles on gestational day 15, glycogen storage is seen. Periodic acid-Schiff reaction and hematoxylin stain. $\times 220$

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marked changes occur in the presecretory cells (Otani et al., 1986; Ito and Kanisawa, 1990). In the developing alveolar epithelial cells, the glycogen breakdown is considered to be an important event in the maturation of type 2 alveolar cells, as a lack of glycogenolysis is associated with decreased phosphatidylcholine synthesis and a reduced number of lamellar bodies in fetal type 2 alveolar cells (Rannels et al., 1991), and as glucose, originated from glycogen, is a substrate for synthesis of fatty acid and glycerol which are incorporated into surfactant phosphatidylcholine (Farrell and Bourbon,

1986).

3. Expression of glucose transporter in fetal lungs

Glucose is one of the most important metabolic fuels for the fetus, and glucose transporter may be critical in the control of fetal growth, as glucose transporters regulate cell glucose utilization.

Expression of glucose transporters in normal adult lung tissues has been studied in humans and animals using various methods. Western blot analyses have



Fig. 5. Immunofluorescence staining for facilitative GLUT1 in fetal hamster lung on gestational day 13. Positive staining is seen in the epithelium. x 360

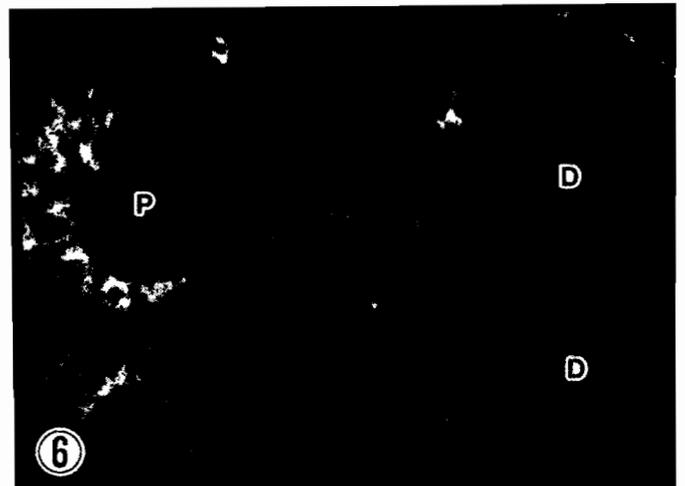


Fig. 6. Immunofluorescence staining for GLUT1 in a fetal hamster lung on gestational day 14. Proximal epithelium (P) shows stronger staining than distal epithelium (D). x 360

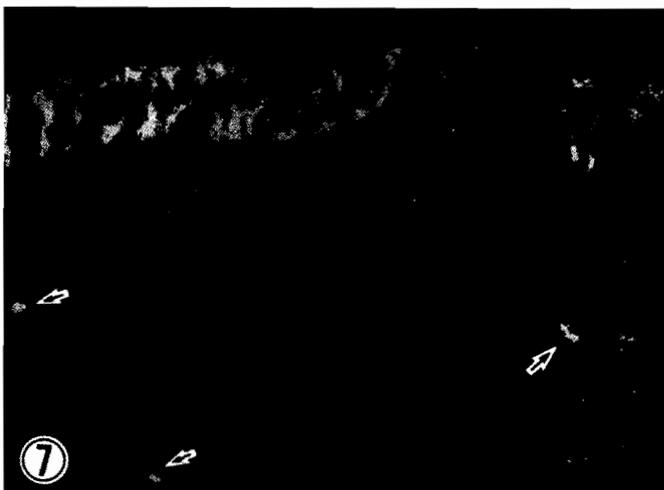


Fig. 7. Immunofluorescence staining for GLUT1 in a fetal hamster lung on gestational day 16 (the day of birth). Positive staining is demonstrated in the lobar bronchus, but no staining is seen in the alveolar region, except for red blood cells (arrows). x 480

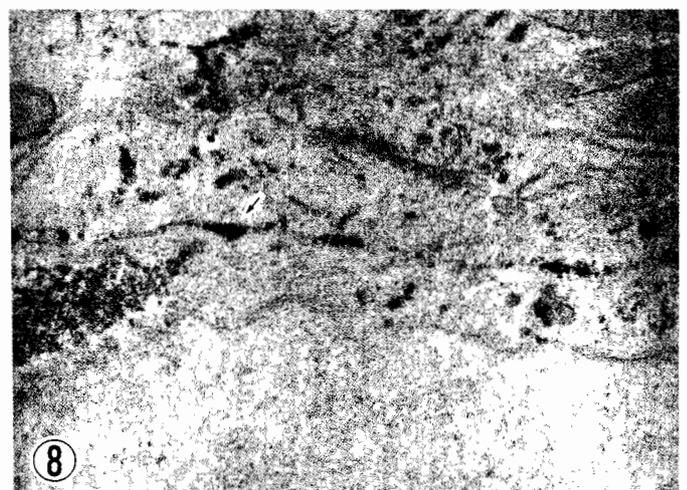


Fig. 8. Immuno-electron micrograph of a fetal hamster lung stained for GLUT1 on gestational day 13. Immunoreaction is localized mainly on the lateral cell membrane (arrow). x 22,000

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demonstrated expression of GLUT1 and GLUT2 in human whole lung tissues (Devaskar and deMello, 1996), of GLUT1 in fetal and adult mouse lung tissues (Manty et al., 1991) and of GLUT1 in fetal rat lung (Simmons et al., 1993; Hart et al., 1998), but not of GLUT3, GLUT4 or GLUT5 (Haber et al., 1993; Devaskar and deMello, 1996). Immunohistochemical studies have revealed positive immunoreaction for GLUT1 in red blood cells and Schwann cells in the lungs of adult humans (Devaskar and deMello, 1996) and fetal and adult mice (Manty et al., 1991), but no epithelial cells of human lung show positive staining (Devaskar and deMello, 1996; Younes et al., 1996; Ito et al., 1998). Positive immunostaining for GLUT2 has been reported in bronchial epithelial cells of humans (Ito et al., 1998) and rats (Devaskar and deMello, 1996). Devaskar and deMello (1996) suggested that pulmonary neuroendocrine cells may be positive for GLUT3, although they did not prove this by additional histochemical methods. In adult lung tissues, positive immunostaining for GLUT4 and GLUT5 has not been detected. Northern blot analyses have revealed that GLUT1 mRNA was abundant in fetal rat tissues (Werner et al., 1989; Hart et al., 1998) but GLUT3 mRNA is not detected (Haber et al., 1993).

We have studied expression of GLUT1 and GLUT4 in fetal and adult lung tissues of Syrian golden hamsters by immunostaining and Western blot analysis. Positive GLUT1 immunostaining is seen in the developing epithelial cells during the fetal period (Figs. 5-8), but during the later stage of gestation, the staining intensity is stronger in the airway epithelial cells than in the alveolar epithelial cells (Figs. 6, 7). Clustered neuroendocrine cells in the fetal period are not positively stained for GLUT1. Ultrastructurally, positive staining predominated on the cell membrane (Fig. 8). The

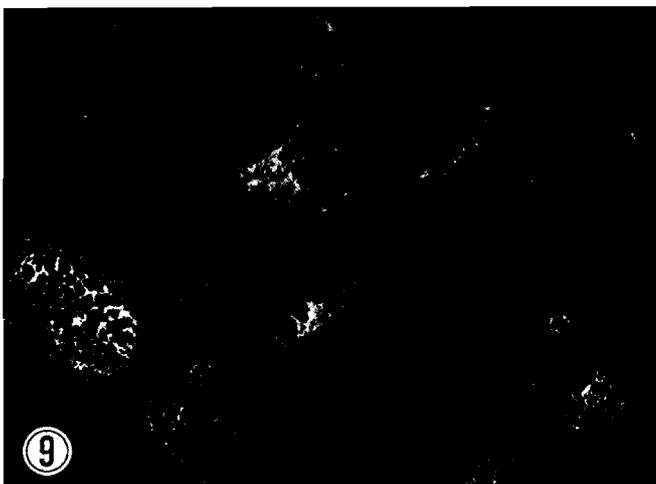


Fig. 9. Immunofluorescence staining for facilitative GLUT4 in a fetal hamster lung on gestational day 13. Immature epithelial cells are positively stained. x 240

staining pattern does not coincide with the pattern of fluctuation of glycogen storage in the epithelial cells. Positive GLUT4 immunostaining is detected in the developing airway epithelial cells on gestational days 12-14 (Fig. 9), but the immunoreactivity is unclear on gestational days 15 and 16 (the day of birth). The adult lung does not show positive reaction for GLUT4. The results of Western blot analysis agree with those from this immunohistochemical study: GLUT1 is positive throughout in the fetal period, and GLUT4 expression is seen in the fetal lungs up to gestational day 15 (Fig. 10). Thus, in the hamster lung, GLUT1 and GLUT4 are expressed much more in the fetal lung than in the adult lung, and these GLUT isoforms may play important roles in cell proliferation and differentiation, and in maturation of the lung during the fetal developmental period. Moreover, the expression of GLUT4 observed early in the development of fetal lung is rather unique, since expression of GLUT4 is restricted to insulin-sensitive organs, and usually does not occur in epithelial cells. The significance of the expression of GLUT4 in the immature lung epithelium could be related to the regulation of lung development by hormonal factors, especially insulin and insulin-like growth factors. Regenerating alveolar epithelial cells in adult human lungs are also positively immunostained for GLUT4 (Ito et al., 1998), which suggests that reappearance of GLUT4 in the adult lung may be related to alveolar cell proliferation and maturation.

Glucose uptake into mammalian cells is mediated by glucose transporters in cells, and once inside the cell, glucose is converted to glucose-6-phosphate by a hexokinase. Four hexokinase isoforms have been characterized, and they show different tissue distributions (Katzen and Schimke, 1965). Hexokinase I is ubiquitous, hexokinase II is found primarily in insulin-sensitive tissues and hexokinase IV isoform, also referred to as glucokinase, is specifically expressed in liver and pancreatic β -cells (Printz et al., 1993). Tissue-specific, coordinated expression of glucose transporters and hexokinases may play an important role in the regulation of glucose uptake and metabolism under

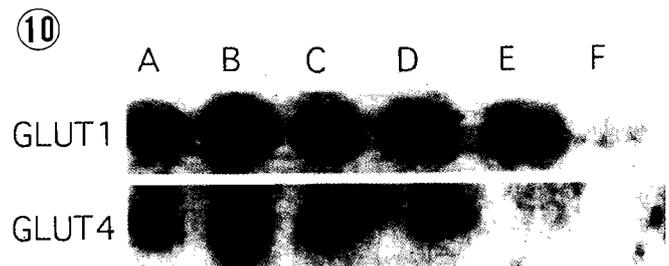


Fig. 10. Western blot analysis for GLUT1 and GLUT4 in fetal and adult whole lung tissues. Lane A: gestational day 12; lane B: day 13; lane C: day 14; lane D: day 15; lane E: day 16; lane F: adult.

various nutritional and hormonal conditions (Postic et al., 1994). We studied hexokinase I expression immunohistochemically in the developing hamster lung tissues. On gestational days 12 and 13, diffuse positive staining is seen in the developing lung epithelia (Fig. 11). But, during the perinatal period and thereafter, bronchial cells are stained more strongly than alveolar cells (Figs. 12, 13). Although the immunostaining pattern of hexokinase I is not the same as that of GLUT1 or GLUT4, changes in expression of hexokinase I during the fetal developmental period may be related to active glucose metabolism in the developing lung tissues.

4. Expression of glucose transporter in lung carcinomas

The rate of glucose uptake by glucose transporters may be regulated under conditions related to cell proliferation, differentiation, transformation, and nutrition (Merrall et al., 1993), and the state of glucose transporter expression can reflect the biological behavior of some cancer cells. We studied expression of GLUT isoforms immunohistochemically in human lung

carcinomas, and looked for differences in the expression pattern among various histological subtypes (Table 1). GLUT1 is expressed in most of lung carcinomas (Figs. 14, 15), and appears to be positive in all squamous cell carcinomas. No lung carcinomas show GLUT2 immunoreactivity, although the supranuclear region of the airway epithelial cells is positively stained. A few cases of lung carcinoma show positive immunostaining for GLUT3 and GLUT4 (Figs. 16, 17). Reverse transcriptase-polymerase chain reaction is a useful adjunct method to confirm the results obtained with glucose transporter immunohistochemistry (Fig. 18; Ogawa et al., 1997; Ito et al., 1998). Overexpression of glucose transporters is expected to enhance uptake of glucose into lung carcinoma cells, and the increased glucose influx could be involved in cell biological activities including cell proliferation. Overexpression of GLUT1 and GLUT3 is related to increased cell proliferation activity, which is evaluated using proliferating nuclear cell antigen immunohistochemistry (Ogawa et al., 1997). Changes in cell biological activity related to glucose transporter overexpression may reflect clinicopathological features of lung carcinomas. In

Table 1. Expression of facilitative glucose transporter isoforms in lung carcinomas.

HISTOLOGICAL SYBTYP	GLUT1	GLUT2	GLUT3	GLUT4	GLUT5
Squamous cell carcinoma	21/21	0/21	2/21	1/21	0/21
Adenocarcinoma	15/27	0/27	4/27	2/27	0/27
Small cell carcinoma	7/11	0/11	1/11	1/11	0/11
Large cell carcinoma	5/7	0/7	2/7	0/7	0/7
Total	48/66	0/66	9/66	4/66	0/66

Number of positive specimens/total.

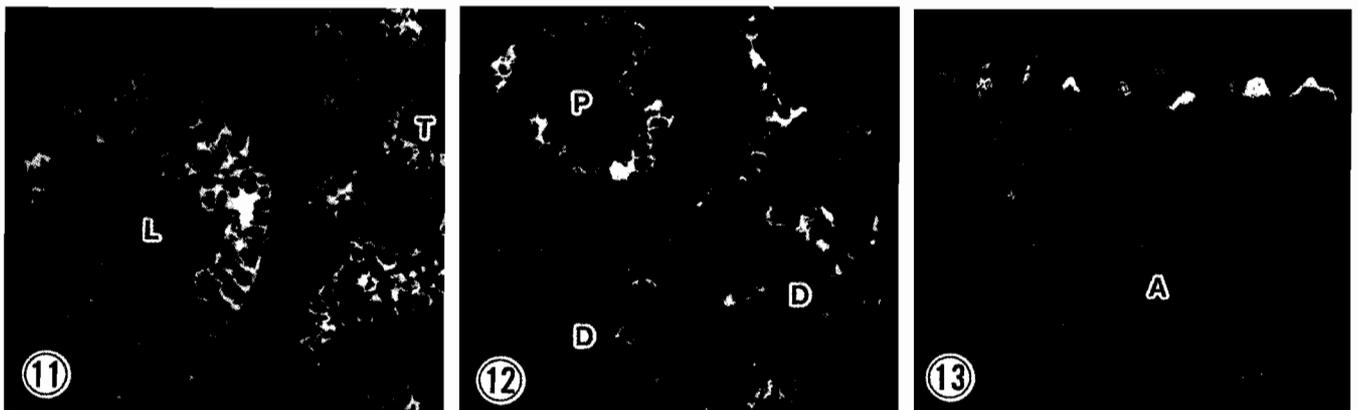


Fig. 11. Immunofluorescence staining for hexokinase I in a fetal hamster lung on gestational day 13. Positive staining is seen in the large airway (L) and terminal bud (T). x 210

Fig. 12. Immunofluorescence staining for hexokinase I in a fetal hamster lung on gestational day 14. The proximal part of the airway epithelium (P) shows stronger staining than the distal epithelium (D). x210.

Fig. 13. Immunofluorescence staining for hexokinase I in an adult hamster lung. Bronchial epithelium shows strong staining. No staining is seen in the alveolus (A). x 410

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Table 2. GLUT1 immunostaining of lung adenocarcinoma and clinicopathological data.

DIFFERENTIATION GRADE OF ADENOCARCINOMA	TUMOR SIZE				LYMPH NODE METASTASIS	
	t1	t2	t3	t4	n(-)	n(+)
Well	0/3	6/11	1/2	0/0	4/12	3/4
Modderate	0/3	2/2	2/2	1/1	1/3	4/5
Poor	0/0	3/3	0/0	0/0	2/2	1/1
Total	0/6	11/16	3/4	1/1	7/17	8/10

GLUT1 expression is less frequently positive in smaller tumor (t1 vs t2, $p < 0.5$), and GLUT1 expression is more common in cases with lymph node metastasis (n(-) vs n(+), $p < 0.5$).

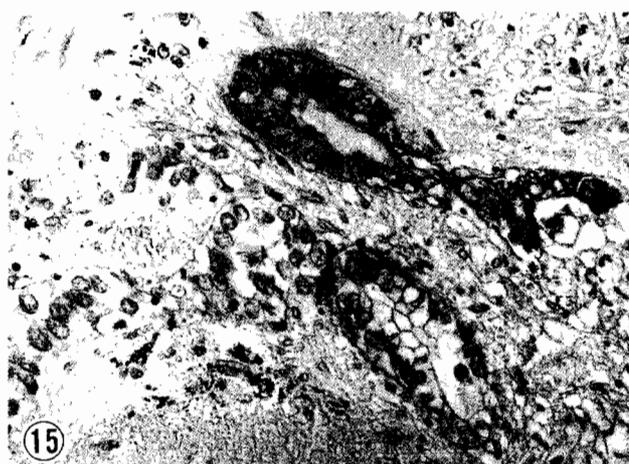
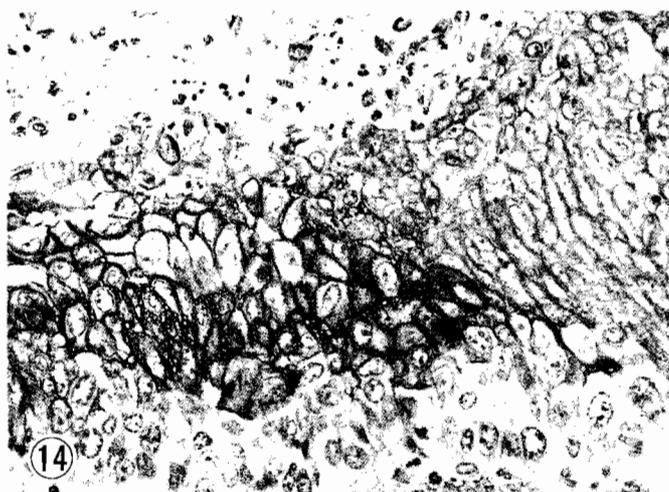


Fig. 14. Immunostaining for GLUT1 in squamous cell carcinoma of the lung. Tumor cells in suprabasal area are most prominently stained. Counterstained with hematoxylin. x 280

Fig. 15. Immunostaining for GLUT1 in adenocarcinoma of the lung. Counterstained with hematoxylin. x 280

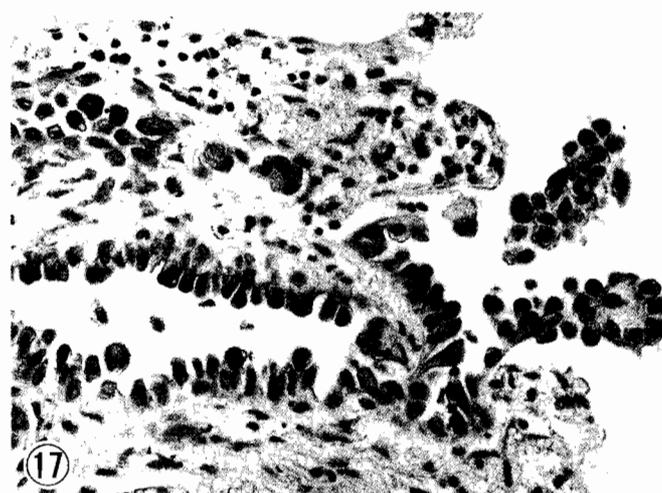
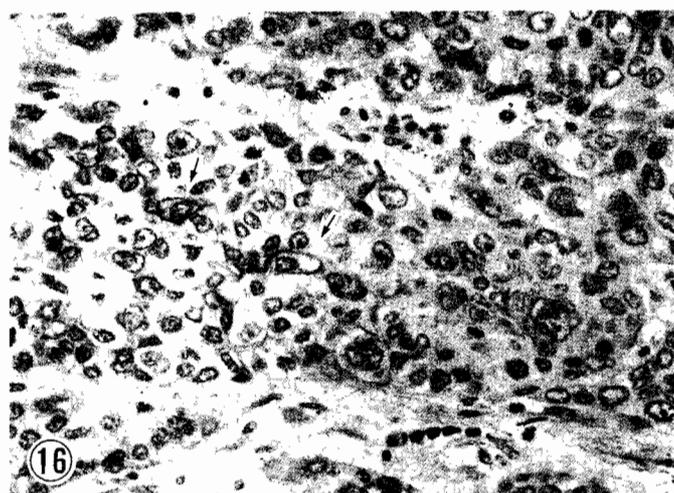


Fig. 16. Immunostaining for GLUT4 in squamous cell carcinoma of the lung. Only a few cells are positively stained (arrows). Counterstained with hematoxylin. x 280

Fig. 17. Immunostaining for GLUT4 in adenocarcinoma of the lung. Counterstained with hematoxylin. x 280



Fig. 18. Reverse transcriptase-polymerase chain reaction for GLUT1, GLUT3 and GLUT4. Lanes A and B: squamous cell carcinoma; lanes C and D: adenocarcinoma; lane E: small cell carcinoma; lane F: large cell carcinoma. High incidence of positive reaction for GLUT4 could be derived contamination of reactive alveolar epithelium.

adenocarcinoma, positive GLUT1 expression is correlated with reduced differentiation, larger tumor size and positive lymph node metastasis (Table 2; Ito et al., 1998).

5. Conclusion

In the developing fetal lung, active glucose metabolism is an important biochemical process in cell proliferation, differentiation and maturation. In comparison to normal adult lung tissues, fetal lung tissues show overexpression of GLUT1 and GLUT4, and increased glucose uptake resulting from overexpression and modulation of glucose transporter should facilitate rapid lung growth. The unique spatiotemporal GLUT4 expression pattern in early developing lungs is intriguing, and GLUT4 expression may be regulated by hormonal factors. The fetal expression pattern of glucose transporters seems to appear in the adult lung in pathological conditions such as epithelial regeneration after injury, and in neoplasms (Ito et al., 1998). In a sense, overexpression of glucose transporters in lung carcinomas may be considered as oncofetal phenotypic expression, and this expression could generally occur in proliferating lung cells with immature phenotypes. In cultured cells, GLUT1 expression is induced by transfection of *ras* and *src* oncogenes (Flier et al., 1987; White and Weber, 1990) and by treatment with growth factor treatment such as epidermal growth factor, fibroblast growth factor and platelet-derived growth factor (Hiraki et al., 1988; Mischoulon et al., 1992). The mechanisms regulating glucose transporter expression and the significance of glucose transporter in biological phenomena still remain to be discovered. Studies of normal fetal lung development may afford valuable clues toward the discovery of the regulatory mechanisms and significance of glucose transporter expression in lung

carcinomas, and vice versa.

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