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Review

Hypoxia inducible factor-1 and facilitative glucose transporters GLUT1 and GLUT3: Putative molecular components of the oxygen and glucose sensing apparatus in articular chondrocytes

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Summary. Articular cartilage is an avascular connective tissue in which the availability of oxygen and glucose is significantly lower than synovial fluid and plasma. Glucose is an important metabolic fuel and structural precursor that plays a key role in the synthesis of extracellular matrix macromolecules in articular cartilage. However, glucose concentrations in cartilage can fluctuate depending on age, physical activity and endocrine status. Chondrocytes are glycolytic cells and must be able to sense the quantities of oxygen and glucose available to them in the extracellular matrix and respond appropriately by adjusting cellular metabolism. Consequently chondrocytes must have the capacity to survive in an extracellular matrix with limited nutrients and low oxygen tensions. The molecular mechanisms responsible for allowing chondrocytes to adapt to these harsh environmental conditions are poorly understood. In this article we present a novel "dual" model of oxygen and glucose sensing in chondrocytes based on recent experimental data. This model incorporates the hypoxia-inducible factor alpha (HIF-1α) as an oxygen sensor and the hypoxia responsive facilitative glucose transporters, GLUT1 and GLUT3 as putative components of the glucose sensing apparatus in chondrocytes. Recent studies have shown that GLUT1 and GLUT3 are both expressed in chondrocytes and their HIF-1α-mediated transcription may be dually stimulated in response to hypoxia and low glucose conditions which in turn promote anaerobic glycolysis in favor of oxidative phosphorylation. This working model provides, for the first time, a unifying hypothesis to explain how chondrocytes might sense and respond to low oxygen tensions and alterations in extracellular glucose.

Key words: Articular cartilage, Chondrocyte, Oxygen-regulated transcription factor, Hypoxia-inducible factor alpha, Glucose sensing, Glucose transporter, GLUT1, GLUT3

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Introduction

The physiological maintenance of oxygen and nutrient homeostasis in mammalian cells is essential for numerous subservient cellular functions including cell division, proliferation, differentiation, excitability and secretion (Rolland et al., 2001). Oxygen and nutrient maintenance is also critical to cell fate, senescence (Nemoto et al., 2004) and apoptosis (Martens et al., 2005). Cells can generally "sense" the levels of available oxygen for oxidative phosphorylation but only a limited number of specialized cell types can switch to anaerobic glycolysis when deprived of oxygen for extended periods of time. Cells can also adapt to changes in the extracellular concentrations of nutrients by regulating their transport across the plasma membrane and, subsequently, their compartmentalization and metabolism. Such adaptation normally involves transcriptional control of cell surface and intracellular sensors, transcription factors and transporter genes (Zhang et al., 1999; Heilig et al., 2003; Semenza 2004). Alterations in the expression of genes involved in anaerobic glycolysis, oxidative phosphorylation, oxygen delivery and angiogenesis are predominantly mediated by oxygen-related transcription factors such as the

hypoxia inducible factor 1 (HIF-1).

It is generally accepted that the quantities of available oxygen and glucose can fluctuate considerably in connective tissues such as articular cartilage, growth plate and intervertebral disc (Mobasheri et al., 2002b; Rajpurohit et al., 2002; Pfander et al., 2003). Therefore, the cells that make up these tissues must be able to sense the amount of oxygen and glucose available and respond appropriately to shortages or surpluses by altering metabolic rate. Articular cartilage chondrocytes which are the main focus of this article consume less oxygen in comparison with most other cell types (Lee and Urban, 2002). Consequently anaerobic glycolysis forms the principal source of cellular ATP in cartilage. Recent studies have clearly demonstrated the importance of oxygen-related transcription factors such as HIF-1 and its target genes in the maintenance of anaerobic glycolysis (Pfander et al., 2003) and cellular glucose levels (Mobasheri et al., 2002a; Richardson et al., 2003) in response to hypoxia and nutrient stress (Rajpurohit et al., 2002). In this article we review the recent literature on HIF-1 in cartilage and its gene targets in chondrocytes (particularly the hypoxia responsive GLUT1 and GLUT3 glucose transporters) and put forward a new hypothesis for oxygen and glucose sensing in articular cartilage.

Regulation of oxygen homeostasis by hypoxia-inducible factor 1 (HIF-1)

Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric basic-helix-loop-helix-PAS domain transcription factor which consists of several subunits: HIF-1 alpha (HIF-1α), HIF-1 beta (HIF-1ß), HIF-2 alpha (HIF-2α) and HIF-3 alpha (HIF-3α) (Semenza 1998, 1999). The role of HIF-1α and HIF-1ß in the cellular response to hypoxia is well established, but less is known about HIF-2α and HIF-3α with respect to organ distribution and transcriptional regulation by hypoxia. The expression and transcriptional activity of HIF-1α itself increases when oxygen availability becomes a limiting factor and several dozen target genes are acutely transactivated by HIF-1α. The most important genes in the context of this review are those involved in glucose transport, glycolysis and tissue vascularization (Fig. 1). HIF-1α activates mRNAs encoding erythropoietin and the following glycolytic enzymes: aldolase, phosphoglycerate kinase, pyruvate kinase, enolase, lactate dehydrogenase, and phosphofructokinase (Semenza et al., 1994, 1996). Other HIF-1 target genes include those encoding vascular endothelial growth factor (VEGF), and the GLUT1 and GLUT3 facilitative glucose transporters (Vannucci et al., 1996, 1998; Ouiddir et al., 1999; Semenza 1999, 2001). Appropriate regulation of many of the above genes is essential for embryonic development and survival since HIF-1α is required for a variety of responses to chronic hypoxia (Iyer et al., 1998; Kotch et al. 1999). Improper regulation of these gene targets has been implicated in carcinogenesis and various lines of evidence indicate that genetic alterations in tumor suppressor genes and oncogenes induce HIF-1 activity which in turn contributes to tumor progression. Furthermore, the GLUT1 glucose transporter gene is an early target of HIF-1 in hypoxic conditions in a variety of tumors (Airley et al., 2003; Hoskin et al., 2003; Lund et al., 2004).

Regulation of hypoxia and stress-responsive genes by HIF-1α in chondrocytes

Studies of embryonic and epiphyseal chondrocytes have shown that HIF-1α is essential for chondrocyte growth arrest and survival *in vivo (*Schipani et al., 2001; Pfander et al., 2003). Recent work on nucleus pulposus cells in the intervertebral disc has also suggested that HIF-1α is important for the maintenance of anaerobic glycolysis and the response to hypoxia and nutrient stress (Rajpurohit et al., 2002; Pfander et al., 2003). HIF-1α is known to upregulate stress-responsive genes and one such gene is the vascular endothelial growth factor (VEGF). Studies of mouse epiphyseal chondrocytes have shown that soluble isoforms of VEGF, VEGF(120) and VEGF(164), are abundantly expressed splice variants in cells exposed to low oxygen levels (Cramer et al., 2004). Thus the biological effects of VEGF in low-oxygen conditions are HIF-1α dependent since functional inactivation of HIF-1α abolishes the hypoxic increase of VEGF expression in chondrocytes (Cramer et al., 2004). HIF-1α may also be involved in the poorly understood process of mechanotransduction; elegant recent studies have shown that mechanical overload increases HIF-1α expression and immunoreactivity in cartilage which, in turn, induces VEGF expression in chondrocytes (Pufe et al., 2004). VEGF is an important agent for angiogenesis and vascularization but it also

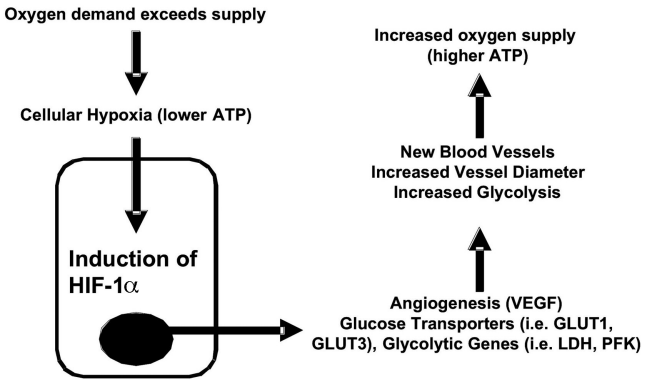

Fig. 1. Regulation of genes involved in angiogenesis (vascular endothelial growth factor, VEGF), glucose transport (facilitative glucose transporters GLUT1 and GLUT3) and glycolysis (lactate dehydrogenase, LDH; phosphofructokinase, PFK) by HIF-1α in response to hypoxia or reduced intracellular ATP levels (i.e. low intracellular glucose).

participates in cytokine mediated inflammatory processes (Pufe et al., 2001, 2004). Therefore HIF-1α regulated target genes are expressed in chondrocytes and are involved in diverse stress response processes.

Glucose sensors: lessons from other organisms

Studies in bacteria, yeasts and plants suggest that glucose acts as a "signalling nutrient" and a hormone (Moriya and Johnston, 2004). Glucose is a major source of carbon and energy for many bacteria, yeasts and plants. Studies in *Saccharomyces cerevisiae* suggest that glucose transporters in the yeast cell membrane are key components of the glucose sensing pathway. Since extracellular glucose needs to be determined by yeast cells it would make sense to place the sensors on the cell surface. The rate of glucose utilization in yeast is dictated by the abundance of glucose transporters in the plasma membrane. Molecular analysis of sugar transporters in *Saccharomyces cerevisiae* has revealed the existence of a multigene family of sugar carriers (Bisson et al., 1993) and many of the genes encoding sugar carriers are putative glucose sensors due to their plasma membrane localization. Indeed the yeast genome encodes at least eighteen hexose (Hxt) transporters (Ozcan and Johnston, 1999). Yeast cells can detect extracellular medium glucose and respond to generate subcellular signals that affect gene expression to regulate the cellular response to alterations in the concentration of extracellular glucose. These alterations occur at the mRNA level by activating or repressing genes that encode proteins involved in glucose transport and metabolism (Johnston, 1999). Discussion of yeast glucose transporters and the G-protein coupled receptor mediated sensing of extracellular glucose in yeast is beyond the scope of this review and has been covered elsewhere (Ozcan and Johnston 1999; Forsberg and Ljungdahl, 2001). However, a brief overview of the mammalian glucose transporter gene families is important for following the arguments and hypotheses that we intend to present in this article and is introduced in the following section.

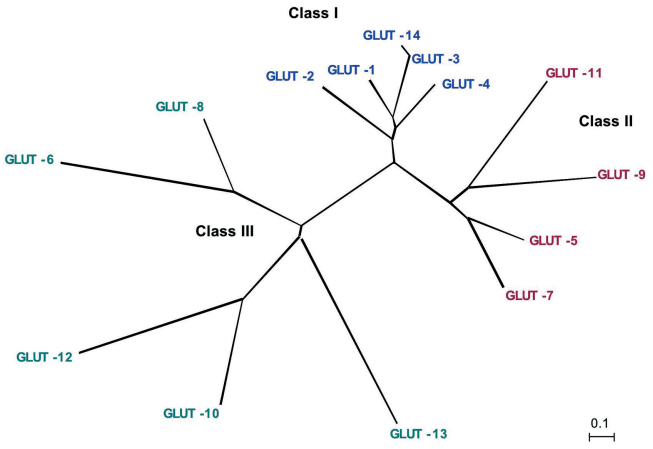

Fig. 2. Unrooted radial phylogram drawn from multiple sequence alignments of fourteen members of the human GLUT/SLC2A family. The tree was constructed using neighbour-joining analysis of a distance matrix generated with PHYLIP software. The three classes of GLUT proteins are colour-coded as: blue, class I; red, class II; green, class III. The scale bar represents 0.1 substitutions per amino acid position. HMIT; H^+-coupled myo-inositol transporter. Modified from Wood and Trayhurn, 2003 with kind permission and assistance of the authors.

The GLUT/SLC2A and SGLT/SLC5A sugar transporter families

Monosaccharide transport across the plasma membranes of mammalian cells is mediated by members of the GLUT/SLC2A family of facilitative sugar transporters and the SGLT/SLC5A family of Na^+-dependent sugar transporters (Wood and Trayhurn 2003). Fourteen members of the GLUT/SLC2A family have been identified in the human genome in addition to several non-expressed pseudogenes (Joost and Thorens, 2001; Wu and Freeze, 2002; Wood and Trayhurn, 2003) (see Fig. 2 and Table 1). Five of the mammalian facilitated glucose carriers (GLUTs 1-5) have been well characterized in terms of function and regulation but very little is known about the remaining 9 glucose transporters (GLUTs 6-14) (Joost and Thorens 2001) and much remains to be learned about their expression, tissue distribution and transport functions (Uldry and Thorens 2004). Of these 14 glucose transporter genes, at least nine isoforms are known to be expressed in chondrocytes (Richardson et al., 2003) (Fig. 3) including the hypoxia responsive GLUT1 and GLUT3 isoforms, the GLUT5 fructose transporter and the recently described GLUT9 isoform[1] (Mobasheri et al., 2002a, 2005; http://www.ncbi.nih.gov/IEB/Research/Acembly/av.cgi?db=35&c=Gene&1=SLC2A9).

Functional roles of glucose transporters 1-5

GLUT1, GLUT3 and GLUT4 are high-affinity transporters whereas GLUT2 is a low-affinity transporter isoform; GLUT-5 is a fructose carrier (Thorens, 1996). High-affinity transporters are found in many metabolically active tissues, but their expression is higher in cells with a high glycolytic activity (i.e. hepatocytes, absorptive intestine epithelial cells, and proximal tubule cells of the kidney nephron) (Tal et al., 1990; Thorens et al., 1990). Studies using human tissue microarrays in our laboratories have shown that GLUT1 protein is expressed in many human tissues (Fig. 4) including articular cartilage. GLUT1 is also abundantly expressed in embryonic cartilage and the intervertebral disc (Fig. 5). GLUT1 is abundantly expressed in the brain (Flier et al., 1987), erythrocytes (Mueckler et al., 1985) and the liver but is present in significantly lower quantities in cardiac and skeletal muscle which also express other glucose transporters including GLUT3

(Shepherd et al., 1992; Guillet-Deniau et al., 1994; Hocquette and Abe 2000) and GLUT4 (James et al., 1988; Charron et al., 1989). GLUT2 is expressed in tissues involved in bulk glucose transport, such as the pancreas, intestine, kidney and liver (Thorens 1996), as well as the brain where it is involved in maintaining glucose homeostasis, and in cells where glucose-sensing is essential (i.e. pancreatic ß cells and hypothalamic neurons) (Waeber et al., 1995). In response to variations in metabolic conditions, the expression of the GLUT1-5 transporters is regulated by glucose and different hormones (Thorens 1996).

Dual sensing of oxygen and glucose by HIF-1α, GLUT1 and GLUT3 in the brain

Cells of the central nervous system are dependent on glucose and oxygen for energy metabolism. A number of studies have investigated the effects of hypoxia, glucose deprivation, and hypoxia plus glucose deprivation on the transcription and translation of glucose transporters in neurons (Choeiri et al., 2002) and astroglia (Morgello et al., 1995; Yu et al., 1995). Cerebral hypoxia-ischemia produces major alterations in energy metabolism and glucose utilization in brain. Studies of glucose transport and metabolism in hypoxia-ischemia in the rat brain have also revealed new and valuable information about the specific roles of the GLUT1 and GLUT3 isoforms in regulating glucose uptake in low oxygen conditions. Cerebral hypoxia increases the expression of GLUT1 and GLUT3 proteins via HIF-1α to increase glucose transport and glycolytic rate. These proteins could therefore function as glucose sensing receptors in nervous tissues exposed to hypoxic conditions. The

Table 1. The human GLUT family of facilitative sugar/polyol transporters (gene name SLC2A) (modified from Wood and Trayhurn, 2003).

ISOFORM	PREVIOUS NAME	CLASS	MAIN TISSUE LOCALIZATION	INSULIN SENSITIVE	FUNCTIONAL CHARACTERISTICS (TRANSPORT)	PRESENT IN SKELETAL MUSCLE	PRESENT IN WHITE ADIPOSE TISSUE	PRESENT IN CARTILAGE
GLUT1	-	I	erythrocytes, brain, cartilage, ubiquitous	no	glucose	yes	yes	yes
GLUT2	-	I	liver, pancreas, intestine, kidney	no fructose	glucose (low affinity);	no	no	no
GLUT3	-	I	brain and many other tissues including cartilage	no	glucose (high affinity)	no	yes (mRNA)	yes
GLUT4	-	I	heart, muscle, white and brown adipose tissue, brain	yes	glucose (high affinity)	yes	yes	no (only in embryonic chondro-blasts and growth plate)
GLUT5	-	II	intestine, testis, kidney	no	fructose; glucose (very low affinity)	yes	yes	yes (mRNA)
GLUT6	GLUT 9	III	brain, spleen, leukocytes	no	glucose	no	n.d.	yes
GLUT7		II	intestine	n.d.	fructose; glucose	n.d.	n.d.	n.d.
GLUT8	GLUT X1	III	testes, brain and other tissues	no; (yes in the blastocyst)	glucose	yes (mRNA)	yes (mRNA)	yes
GLUT9	GLUT X	II	liver, kidney and cartilage	n.d.	n.d.	no	n.d.	yes
GLUT10		III	liver, pancreas	no	glucose	yes (mRNA)	yes (mRNA)	yes (mRNA)
GLUT11*	GLUT 10	II	heart, muscle	no	glucose (low affinity); fructose (long form)	yes (mRNA)	no	yes (mRNA)
GLUT12	GLUT 8	III	heart, prostate, muscle, small intestine, white adipose tissue	yes	glucose	yes	yes	yes
HMIT	-	III	brain	n.d.	H+/myo-inositol	no (mRNA)	yes (mRNA)	n.d.
GLUT-14	-	I	testis	n.d.	n.d.	no	no	n.d.

*: GLUT11 occurs in two splice variants: a short form (low affinity glucose transport) and a long form (which may be a fructose transporter). The presence of each transporter in skeletal muscle and white adipose tissue are also shown since these are the major sites of insulin-stimulated glucose uptake. n.d.: not determined.

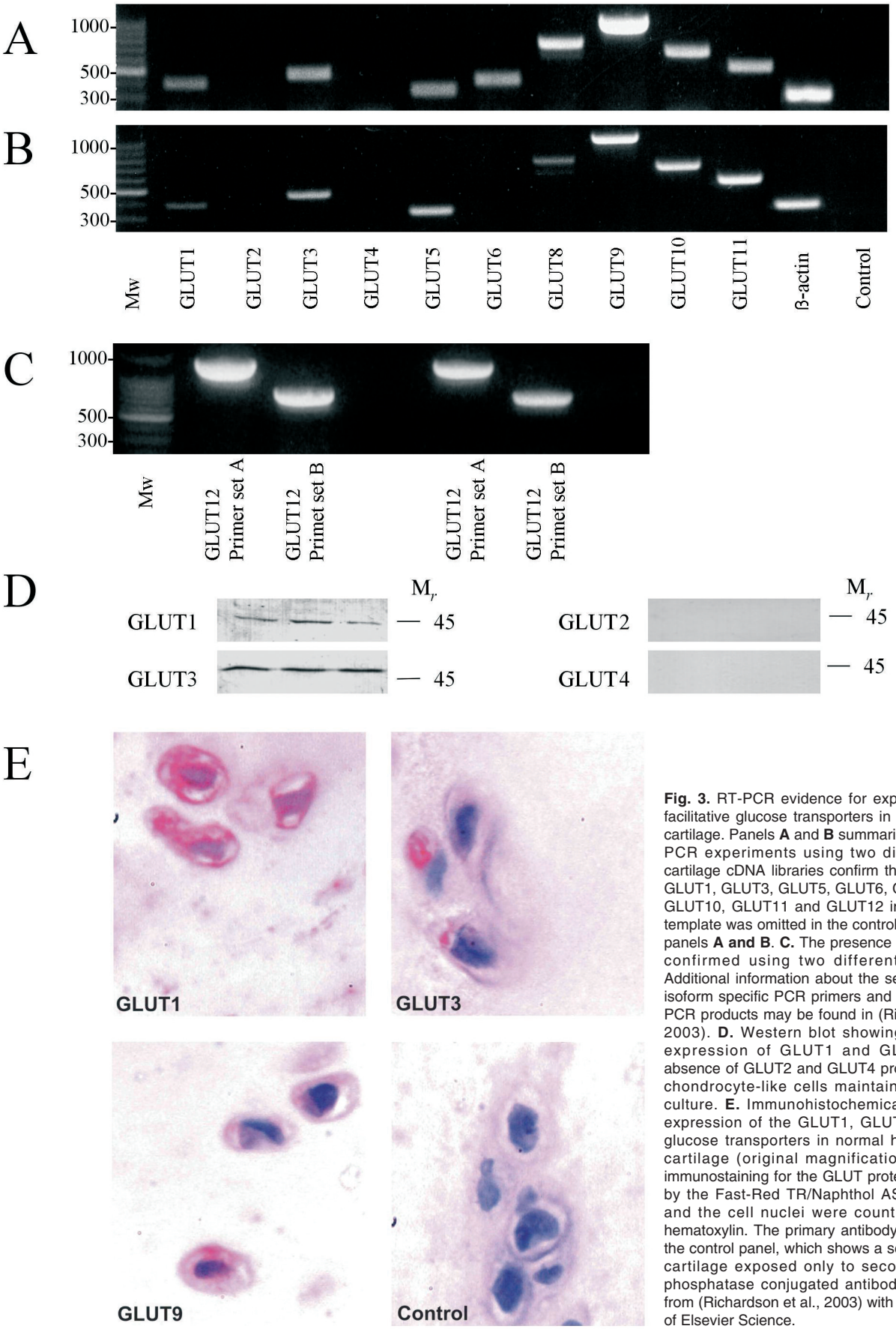

Fig. 3. RT-PCR evidence for expression of nine facilitative glucose transporters in human articular cartilage. Panels **A** and **B** summarize the results of PCR experiments using two different human cartilage cDNA libraries confirm the expression of GLUT1, GLUT3, GLUT5, GLUT6, GLUT8, GLUT9, GLUT10, GLUT11 and GLUT12 in cartilage. The template was omitted in the control lanes shown in panels **A and B**. **C.** The presence of GLUT12 was confirmed using two different primer sets. Additional information about the sequences of the isoform specific PCR primers and the sizes of the PCR products may be found in (Richardson et al., 2003). **D.** Western blot showing evidence for expression of GLUT1 and GLUT3 and the absence of GLUT2 and GLUT4 proteins in human chondrocyte-like cells maintained in alginate culture. **E.** Immunohistochemical evidence for expression of the GLUT1, GLUT3 and GLUT9 glucose transporters in normal human articular cartilage (original magnification x 400). The immunostaining for the GLUT proteins is indicated by the Fast-Red TR/Naphthol AS-MX substrate and the cell nuclei were counterstained with hematoxylin. The primary antibody was omitted in the control panel, which shows a section of human cartilage exposed only to secondary alkaline phosphatase conjugated antibody. Reproduced from (Richardson et al., 2003) with kind permission of Elsevier Science.

GLUT1 isoform mediates the transport of glucose across the blood-brain barrier whereas both GLUT1 and GLUT3 mediate glucose uptake into neurons and glia (Vannucci et al., 1996). Hypoxia-ischemia in the rat brain causes upregulation of GLUT1 and GLUT3 glucose transporter gene expression (Vannucci et al., 1998). Cells treated with the hypoxia mimetic cobalt chloride (a chemical agent that stimulates the expression of a set of hypoxia-responsive genes by preventing the oxygen dependent degradation of HIF-1α under normoxic conditions) also upregulate GLUT1 and GLUT3 (Badr et al., 1999). Glucose deprivation alone produces minimal effects on GLUT mRNA levels in the brain but hypoxia and glucose deprivation synergize to markedly increase GLUT gene expression (Bruckner et al., 1999). Among the various hypoxia-responsive genes, GLUT1 was the first gene whose rate of transcription was shown to be dually regulated by hypoxia (Zhang et al., 1999). It is now well appreciated that brain GLUT1 and GLUT3 gene and protein expression is acutely regulated by the HIF-1α.

Physiological significance of glucose transporter diversity in chondrocytes

Fully developed adult chondrocytes express multiple

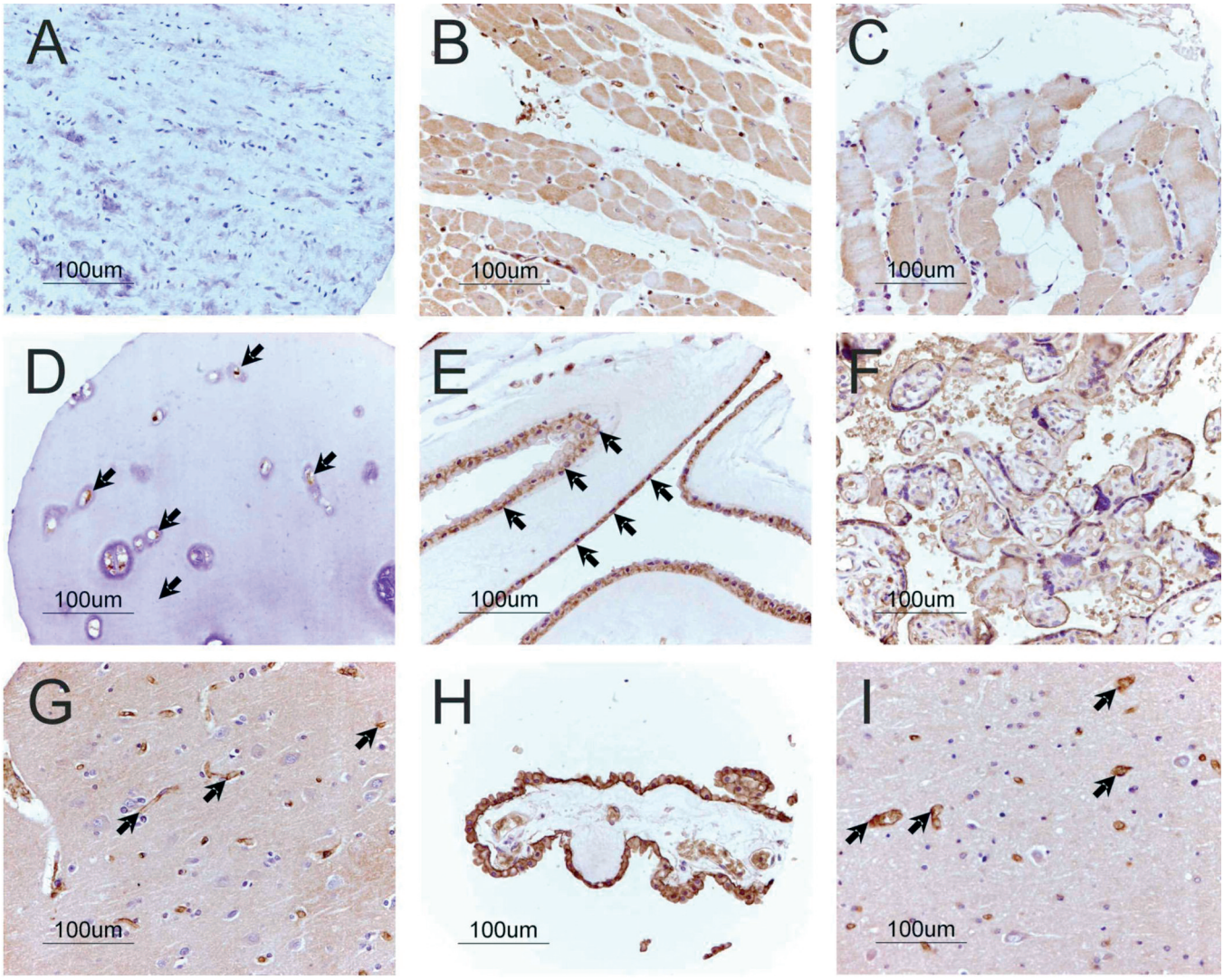

Fig. 4. Differential distribution of the GLUT1 glucose transporter in human tissues. GLUT1 is not detectable in aortic smooth muscle **(A)** but is moderately expressed in cardiac muscle **(B)** and skeletal muscle **(C)**. GLUT1 is present in articular chondrocytes (Richardson et al., 2003) **(D)**, amniotic membranes **(E)** (Wolf and Desoye, 1993), placenta **(F)** (Arnott et al., 1994) and in microvessels (Takakura et al., 1991; Virgintino et al., 1997) in various regions of the central nervous system including cerebral cortex **(G)**, choroid plexus **(H)** and hippocampus **(I)**. Expression in the choroid plexus epithelium was predominantly basolateral (Kurosaki et al., 1995). Arrows indicate sites of GLUT1 expression. Bars:10 μm.

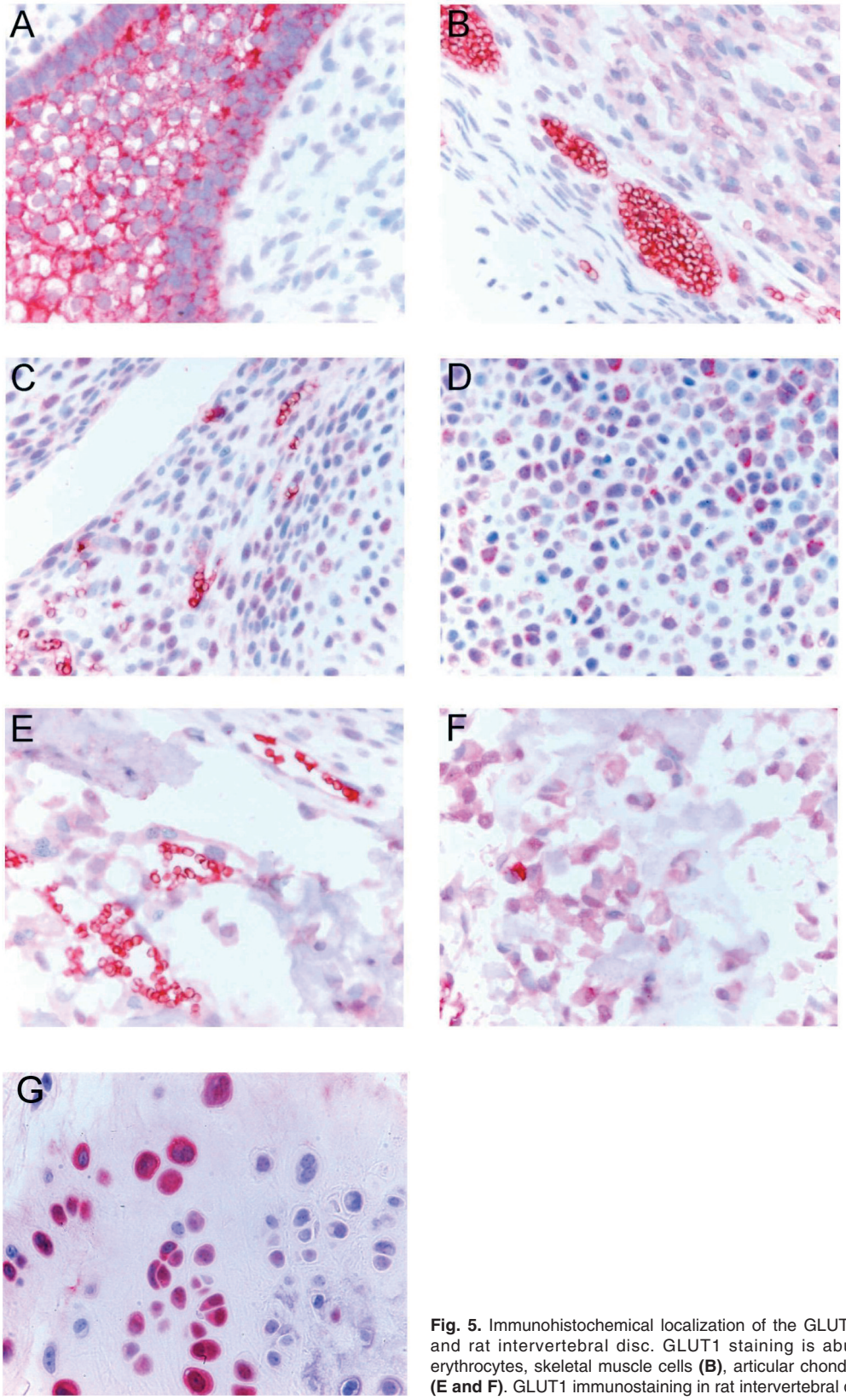

Fig. 5. Immunohistochemical localization of the GLUT1 transporter in embryonic ovine limbs and rat intervertebral disc. GLUT1 staining is abundant in laminar keratinocytes **(A),** erythrocytes, skeletal muscle cells **(B)**, articular chondrocytes **(C and D)** and in bone marrow **(E and F)**. GLUT1 immunostaining in rat intervertebral disc is shown in panel **G**.

isoforms of the GLUT/SLC2A family of glucose transporters including GLUT1, GLUT3, GLUT5, GLUT6, GLUT8, GLUT9, GLUT10, GLUT11 and GLUT12 (Shikhman et al., 2001, 2004; Mobasheri et al., 2002a,b; Richardson et al., 2003) (Fig. 3). Several hypotheses have been put forward to explain the reasons for such GLUT isoform diversity in chondrocytes. We have previously suggested that the transmembrane uptake of glucose, fructose and other related hexose sugars is highly specialized in chondrocytes and may reflect a cartilage-specific requirement for 'fast' (i.e. GLUT3) and baseline (GLUT1) glucose transporters that operate more efficiently at low substrate concentrations under physiological conditions (Mobasheri et al., 2002b; Richardson et al., 2003). We now suggest that the presence of GLUT1 in chondrocytes may be linked to the acute requirement of these cells for glycolytic energy metabolism under the low oxygen tension conditions that are prevalent in avascular load-bearing articular cartilage and intervertebral disc (Schipani et al., 2001; Rajpurohit et al., 2002; Pfander et al., 2003). GLUT1 has also been shown to be a cytokine inducible glucose transporter in cartilage since it is induced by catabolic, pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1ß) (Shikhman et al., 2001, 2004; Richardson et al., 2003; Phillips et al., 2005).

Importance of GLUT1 and GLUT3 in embryonic development

High levels of GLUT1 and GLUT3 are present in a wide range of embryonic and fetal tissues, but expression of these transporters greatly decreases after birth. Abundant levels of the GLUT1 and GLUT3 proteins are present in pre-implantation mouse embryos, since glucose is the main substrate consumed (Pantaleon and Kaye, 1998; Pantaleon et al., 2001). GLUT1 is primarily responsible for glucose supply to the dividing and differentiating cells during the early period of organ formation (i.e. brain, heart, skeletal muscle and kidney) (Santalucia et al., 1992; Matsumoto et al., 1995). In this phase of development high affinity glucose transporters are required because embryonic mammalian cells may be exposed to hypoxia and may have to rely upon anaerobic glycolysis (Matsumoto et al., 1995). Provision of glucose to growing musculoskeletal tissues is particularly important during fetal development, when

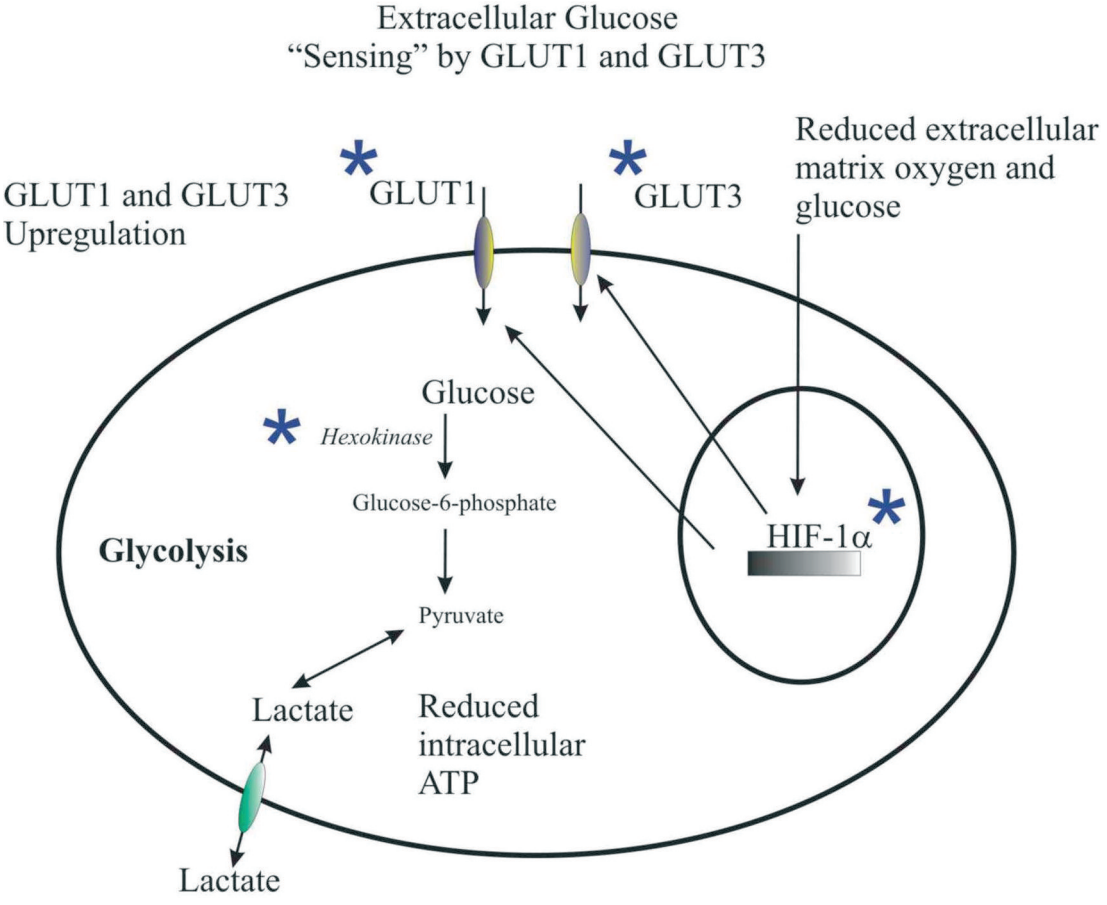

Fig. 6. Model of oxygen and glucose sensing by chondrocytes in which GLUT1, GLUT3 act as sensors for extracellular glucose and HIF-1α as an oxygen sensor and intracellular regulator of GLUT1/GLUT3 transcription. Triggers for GLUT1 and GLUT3 upregulation may include reduced extracellular matrix oxygen and glucose or reduced intracellular ATP. *: Putative glucose sensors.

cells are rapidly dividing and differentiating (Santalucia et al., 1992; Vannucci, 1994; Matsumoto et al., 1995; Pantaleon and Kaye, 1998) and involves the GLUT1 isoform (Mobasheri et al., 2005) (see Fig. 5). Developmental studies have shown that the post-natal expression of GLUT1 and GLUT3 is greatly decreased in many cell types. Studies in our laboratory have shown that GLUT1 and GLUT3 are persistently expressed at mRNA and protein levels in mature chondrocytes from a variety of species including human (Mobasheri et al., 2002a,b), equine (Phillips et al., 2005) and ovine (Mobasheri et al., 2005).

HIF-1α and GLUT1/GLUT3 as oxygen and glucose sensors in chondrocytes

Recent studies from our laboratories and several others have provided new information that will enable us to gradually construct a new phenotypic signature for articular chondrocytes based on the "metabolic" characteristics and nutritional requirements of these cells. Chondrocytes have been shown to express a number of "metabolic" phenotypic markers which include HIF-1α (Schipani et al., 2001; Rajpurohit et al., 2002; Pfander et al., 2003), GLUT1 and GLUT3 (Mobasheri et al., 2002a; Richardson et al., 2003). We propose that the metabolic adjustment of chondrocytes to a low oxygen, low glucose environment in the extracellular matrix of articular cartilage involves HIF-1α as an "oxygen sensor" capable of activating hypoxia responsive target genes involved in extracellular "glucose sensing" and uptake (i.e. GLUT1 and GLUT3). The model proposed is shown in Figure 6. Studies on glucose sensors in the small intestine suggest that a G-protein-coupled receptor linked to a cyclic AMP-Protein Kinase A pathway involved in sensing the lumenal glucose concentration by modulating the expression of apical glucose transporters (Dyer et al., 2003). G-protein coupled receptors might be involved as signaling molecules in the chondrocyte glucose sensing mechanism but these proteins remain to be identified and implicated in the glucose sensing process. It is also possible that other HIF-1α gene targets concerned with anaerobic glycolysis (i.e. lactate dehydrogenase (LDH) and phosphofructokinase (PFK) (Semenza et al., 1994, 1996, 1997) could also be involved in the glucose sensing pathway.

Conclusions and implications for cartilage metabolism and the pathogenesis of arthritis

In the last five years we have witnessed a considerable proliferation of literature on cartilage metabolism. A number of research groups including ours have used multidisciplinary approaches to identify the molecules responsible for glucose uptake in articular and growth plate chondrocytes (Wang et al., 1999; Ohara et al., 2001; Shikhman et al., 2001, 2004; Ishizeki et al., 2002; Macheda et al., 2002; Richardson et al., 2003; Mobasheri et al., 2005). Other groups have discovered that HIF-1α is expressed in chondrocytes and have begun to define its role in metabolism under low oxygen tensions and its link to VEGF activity (Schipani et al., 2001; Pfander et al., 2003; Cramer et al., 2004; Pufe et al., 2004). It is clear from this short review that more experimental work is required to identify key components of the oxygen and glucose sensing apparatus in chondrocytes. This review article has attempted to link these studies in an effort to stimulate interest and debate and consolidate research efforts in this important area of connective tissue biology. To conclude, we would like to leave the reader with several important issues that have remained unresolved and should be the focus of future expanded studies in this area:

1. Could other oxygen-regulated transcription factors be present in chondrocytes in addition to HIF-1α?
2. Is the HIF-1α target erythropoietin involved in oxygen delivery and sensing in chondrocytes as it has been shown to be in other systems?
3. Does the enzyme hexokinase participate in "intracellular glucose sensing" and metabolic regulation in chondrocytes?
4. Are G-protein-coupled receptors involved in the chondrocyte glucose sensing apparatus? If so, are they linked to Protein Kinase A or Protein Kinase C signaling pathways?
5. Could any other GLUT isoforms be involved in glucose sensing in the extracellular matrix of cartilage?
6. Are these oxygen and glucose sensing mechanisms affected in degenerative joint disorders such as osteoarthritis and osteochondritis dissecans and could they be targets of future therapies?

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