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Gene Section
Review

PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3)

Laura Novellasdemunt, Àurea Navarro-Sabaté, Anna Manzano, Ana Rodríguez-García, Ramon Bartrons

Departament de Ciencies Fisiologiques, Campus de Ciencies de la Salut, Universitat de Barcelona, Feixa Llarga s/n, E-08907, L'Hospitalet de Llobregat, Barcelona, Spain (LN, ÀNS, AM, ARG, RB)

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Identity

Other names: IPFK2, PFK2
HGNC (Hugo): PFKFB3
Location: 10p15.1
Note: The human PFKFB3 gene is located on the short arm of chromosome 10 at position 10p15.1 (Manzano et al., 1998) (Figure 1).
Location in the mouse: chromosome 2, 8,73 cM, 11471430-11502101 bp, complement strand (MGI).
For a comparison of the gene of Homo sapiens, mouse, rat, cattle, chimpanzee and domestic dog see: (MGI). Also for all species known gene tree, see: Treefam database.

Figure 1

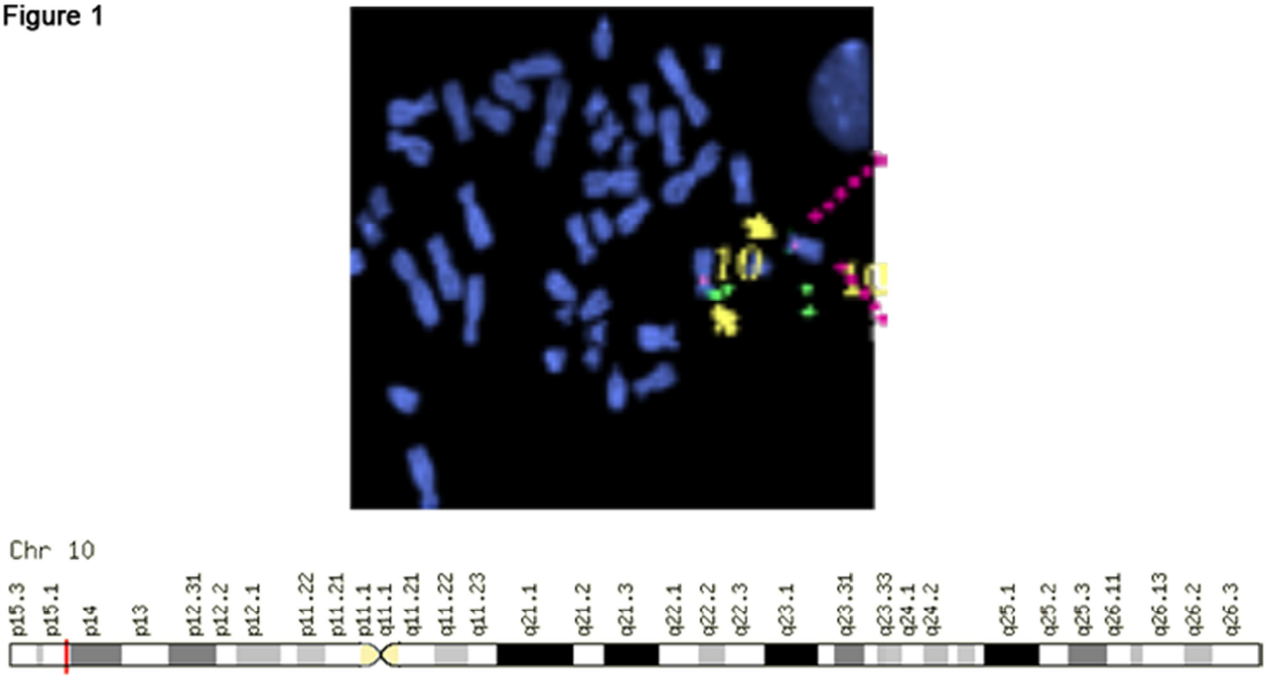

Figure 1. Localization of human PFKFB3 gene by in situ hybridization.

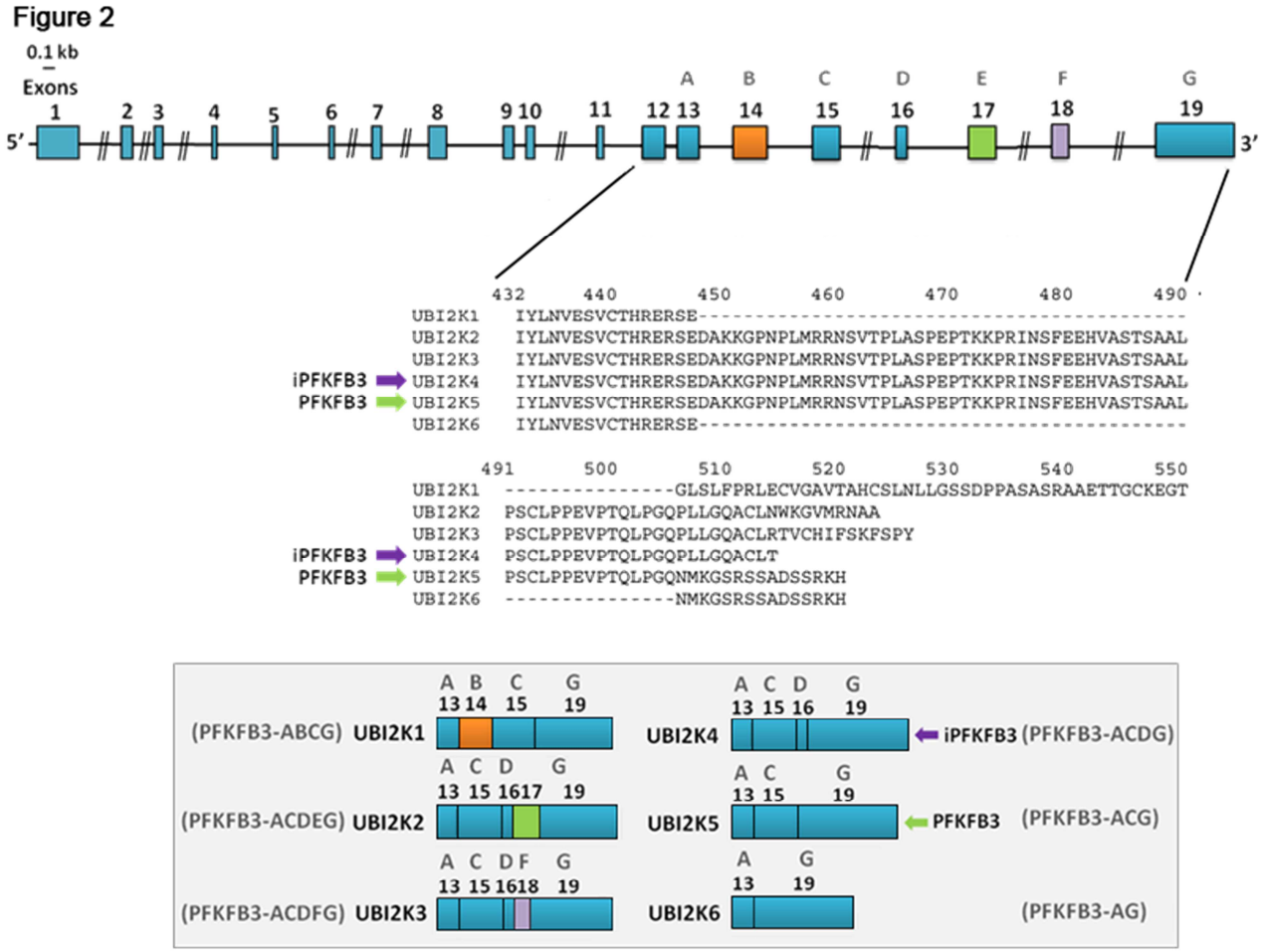

Figure 2. Splicing pattern of the human PFKFB3 gene. Schematic organization of the human PFKFB3 gene generated by alternative splicing and the protein sequence derived. The sequences of UBI2K4 and UBI2K5 isoforms corresponds to iPFKFB3 and uPFKFB3, respectively.

DNA/RNA

Description

The human PFKFB3 is composed of 19 exons spanning genomic region about 90,6 Kb (GenBank).

Alternative splicing variants have been reported. The main variants corresponding to mRNAs of 4453 bp and 4224 bp for the variant 1 u-PFK2 (NM_004566.3) and variant 2 i-PFK2 (NM_001145443.1), respectively (Figure 2).

Transcription

The human PFKFB3 coding sequence consists of 1503 bp for i-PFK2 and 1563 bp for u-PFK2 from the start codon to the stop codon.

Multiple alternatively spliced transcript variants have been found for this gene. (Entrez Gene) (OTTMUSG00000011314).

Pseudogene

No pseudogene of PFKFB3 known.

Protein

Description

PFKFB3 protein consists of two homodimeric subunits of 520 amino acids, with a molecular weight of 59609 Da for each subunit.

The monomer structure is divided into two functional domains within the same polypeptidic chain (El-Maghrabi et al., 1982; Okar et al., 2001; Pilkis et al., 1995).

The finding of mutations that affect kinase activity of the enzyme (Kurland et al., 1995; Li et al., 1992b; Rider et al., 1994), and the localization of ATPγS in the structure, lead to the identification of the N-terminal domain as the kinase domain, which catalyses the synthesis of Fru-2,6-P_2, using fructose-6-phosphate and adenosine-5-triphosphate (ATP) as substrates (Figure 3).

On the other hand, structural and sequence homology with yeast phosphoglycerate mutase (Winn et al., 1981) and rat acid phosphatase (Schneider et al., 1993), along with the location of

mutations that affect Fru-2,6-Pase activity (Li et al., 1992a; Li et al., 1992c; Lin et al., 1992; Tauler et al., 1990) suggested the C-terminal domain to contain the bisphosphatase activity of the enzyme.
This domain is responsible for the hydrolytic degradation of Fru-2,6-P_2 into fructose-6-P and inorganic phosphate (Pi).
These two mutually opposing catalytic activities are controlled by different mechanisms such that either activity is predominant in a given physiological condition. Ultimately, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2) activities control Fru-2,6-P_2 synthesis and degradation, regulating the rate of glucose metabolism.
Different PFK-2/FBPase-2 isoenzymes have been described, sharing high sequence similarity (85%). PFKFB3 isoenzyme has some structural differences with the other isoforms.
The conformations of the substrate loops in the kinase domain are different from those of other isoforms (Hasemann et al., 1996; Lee et al., 2003), giving a structural explanation for the higher kinase activity. Moreover, the N-terminus binds to the bisphosphatase domain to produce a conformational change in the active pocket to enhance inhibitory binding of product (Kim et al., 2006). Residues 4-15 of the kinase domain form a β-hairpin structure and the rest is used as an arm connecting the hairpin to the bisphosphatase domain. Additionally, the contacting area of the bisphosphatase domain is functionally very sensitive due to the residues critical for binding of both product and substrate are located very close (Kim et al., 2006). The low bisphosphatase activity of PFKFB3, which is lower than that of other isoforms by an order of magnitude, is due to the presence of a serine at residue 302 instead of an arginine as conserved in the other isoforms. This residue is said to interact with the 2-phosphate and further stabilizes the transitions state (Cavalier et al., 2012; Kim et al., 2006).

Expression

The PFKFB3 gene product is present in proliferating tissues (Duran et al., 2008a; Duran et al., 2009; Goren et al., 2000; Manzano et al., 1998; Sakai et al., 1996) and transformed cells (Calvo et al., 2006; Chesney et al., 1999; Hamilton et al., 1997; Minchenko et al., 2002; Novellasdemunt et al., 2012; Obach et al., 2004; Riera et al., 2002) and various tumors (Atsumi et al., 2002; Fleischer et al., 2011; Kessler et al., 2008). The high kinase/ bisphosphatase activity ratio of this isoenzyme can explain the high Fru-2,6-P_2 found in the cells where it is present, which in turn sustains high glycolytic rates and it is crucial in supporting growing and proliferant cell metabolism.

Figure 3

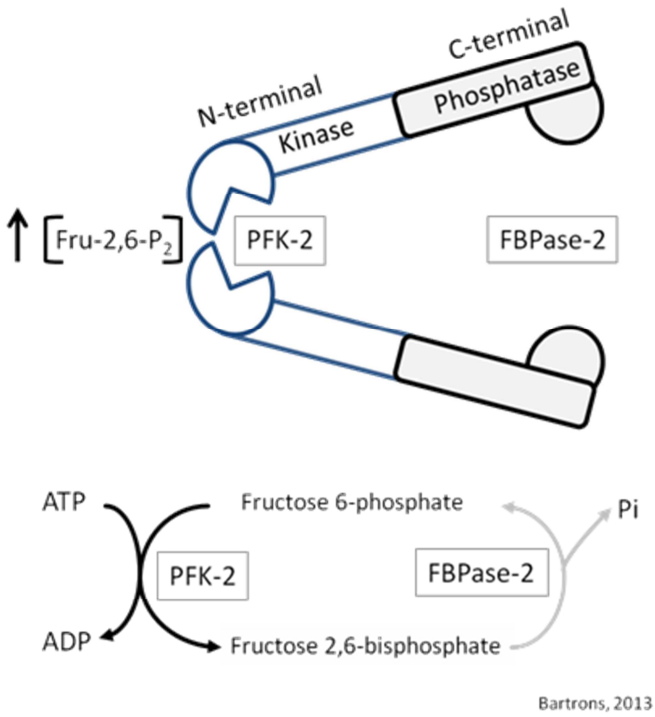

Figure 3. Scheme of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2) activity. PFKFB3 protein consists of two homodimeric subunits of 59,6 KDa. The monomer structure contains two functional domains; kinase and bisphosphatase, located at N-terminal and C-terminal regions, respectively. PFKFB3 has the highest Kinase/Bisphosphatase activity ratio (K/B=700) which promotes high concentrations of Fru-2,6-P_2.

PFKFB3 gene has been found to be expressed in different cell systems. During C2C12 myogenic cell differentiation, the 6-phosphofructo-2-kinase isoenzyme, product of the PFKFB3 gene, is downregulated, being the PFKFB3 isoenzyme degraded through the ubiquitin-proteasome proteolytic pathway (Riera et al., 2003). In neurons, PFKFB3 is constantly subjected to proteasome degradation after ubiquitylation by the E3 ubiquitin ligase APC/C-Cdh1. The activity of this complex is determinant in controlling the protein levels of PFKFB3 and hence the rate of glucose consumption through glycolysis. The function of APC/C is strictly dependent on the presence of Cdh1, which is very abundant in these cells. In contrast, astrocytes express very low Cdh1 levels and therefore APC/C activity is negligible. Accordingly, PFKFB3 protein levels are high and glycolysis is active in astrocytes owing to the low levels of Cdh1. In fact, overexpression of Cdh1 in astrocytes destabilizes PFKFB3 and concomitantly decreases the rate of glycolysis (Fernandez-Fernandez et al., 2012). The later finding that the activity of the PFKFB3 isoenzyme is regulated in cancer cells by APC/C-Cdh1 (Almeida et al., 2010) led to the identification of the role of this ubiquitin ligase in the metabolic regulation of the cell cycle and therefore of cell proliferation (Moncada et al., 2012).

The regulatory component of this complex, Cdh1, has been shown to be downregulated during malignant progression and tumor formation. A decrease in the activity of APC/C-Cdh1 in mid-to-late G1 phase, that has been described as the nutrient-sensitive restriction point and is responsible for the transition from G1 to S, leads to the accumulation of PFKFB3 and enhances the glycolytic flux in malignant cells. PFKFB3 is also a substrate at the onset of S-phase for the ubiquitin ligase SCF (Skp1/cullin/F-box)-β-TrCP (β-transducin repeat-containing protein), so that the activity of PFKFB3 is short-lasting, coinciding with a peak in glycolysis in mid-to-late G1, demonstrating that proliferation and the induction of aerobic glycolysis are both essential components of neoplastic transformation (Moncada et al., 2012).

PFKFB3 has also been found in testis. In adult testes both PFKFB3 and PFKFB4 isoenzymes are present. PFKFB3 is located across the seminiferous epithelium, whereas expression of PFKFB4 is restricted to the spermatogenic cells, being the only one present in mature spermatozoa (Gomez et al., 2005). This differential distribution supports the idea that the cell-specific isoenzymes are able to adapt their kinetic and regulatory enzymatic properties to the metabolic demand of a particular tissue or cell status. Thus, in parallel with spermatogenesis and spermiogenesis, PFKFB isoenzyme expression switches from PFKFB3, which is required during the proliferative phase, to the testis isoform PFKFB4, which is germ cell specific (Gomez et al., 2012; Gomez et al., 2009).

In mouse hypothalami, PFKFB3 mRNA levels are increased by 10-fold in response to re-feeding. In the hypothalamus, re-feeding also decreases the phosphorylation of AMP-activated protein kinase (AMPK) (Thr172) and the mRNA levels of agouti-related protein (AgRP), and increases the mRNA levels of cocaine-amphetamine-related transcript (CART).

In addition, knockdown of PFKFB3 in N-43/5 neurons causes a decrease in rates of glycolysis, which is accompanied by increased AMPK phosphorylation, increased AgRP mRNA levels and decreased CART mRNA levels. In contrast, overexpression of PFKFB3 in N-43/5 neurons causes an increase in glycolysis, which was accompanied by decreased AMPK phosphorylation and decreased AgRP mRNA levels and increased CART mRNA levels. Together, these results suggest that PFKFB3 responds to re-feeding, which in turn stimulates hypothalamic glycolysis and decreases hypothalamic AMPK phosphorylation and alters neuropeptide expression in a pattern that is associated with suppression of food intake (Li et al., 2012).

Recent results have shown that the tumour suppressor PTEN promotes APC/C-Cdh1 activity (Song et al., 2011) and that cells from mice overexpressing PTEN exhibit reduced glucose and glutamine uptake and are resistant to oncogenic transformation (Garcia-Cao et al., 2012). Studies on the possible connection between APC/C-Cdh1 and the metabolic effects of other tumour suppressors such as p53 (Feng and Levine, 2010) or known proto-oncogenes such as c-Myc (Morrish et al., 2008) and Akt (Matheny and Adamo, 2009) are also likely to be highly relevant.

Localisation

PFKFB3 protein is predominantly cytoplasmic but it has been found also into the nucleus. PFKFB3 overexpression produces a marked increase in cell proliferation.

Figure 4

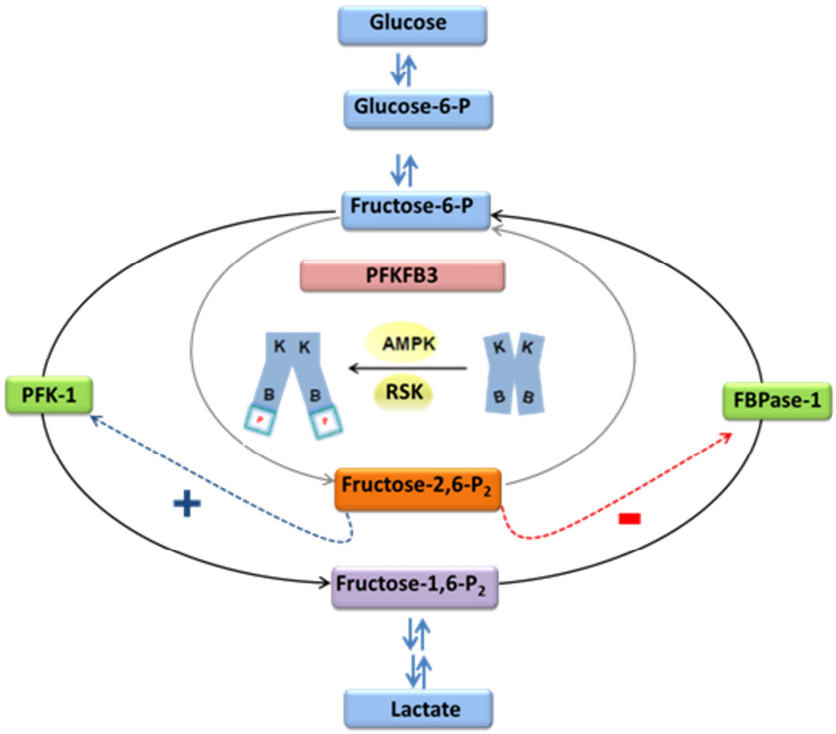

Figure 5

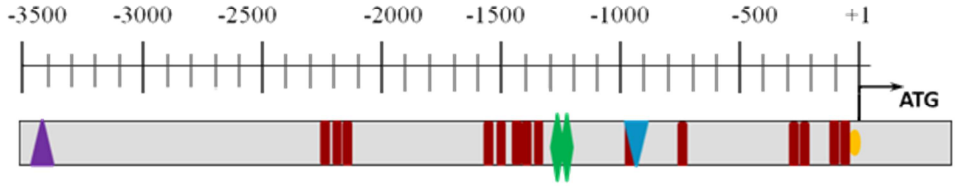

Table I

Gen	Localization		Ratio Kinase: Bisphosphatase	Isoenzymes	Regulation by kinases
	Human	Rat			
PFKFB1	Xq27-q28	Xq22-q31	2,5 (rat liver) 0,4 (rat muscle)	lPFK-2 (liver) mPFK-2 (muscle) fPFK-2 (foetal)	PKA
PFKFB2	1q31	13q24-q25	1,8 (bovine heart)	hPFK-2 (heart)	PKA, PKC, PKB, AMPK
PFKFB3	10p14-p15		710 (human placenta) 3,1 (bovine brain)	uPFK-2 (brain, placenta) iPFK-2 (inducible)	AMPK, PKC, PKA, RSK, MK2
PFKFB4	3p22-p21		0,9 (human testis) 4,9 (rat testis)	tPFK-2 (testis)	

Figure 4. PFKFB3 activities and function in the glycolytic pathway.
Figure 5. Analysis of the 5' PFKFB3 human gene promoter region. Putative regulatory elements in the PFKFB3 gene promoter.
Table I. Properties of PFKFB genes.

Function

These effects on proliferation were completely abrogated by mutating either the active site or nuclear localization residues of PFKFB3, demonstrating a requirement for nuclear delivery of Fru-2,6-P_2 which produced an increase in the expression of several key cell cycle proteins, including cyclin-dependent kinase Cdk1, Cdc25C, and cyclin D3 and decreased the expression of the cell cycle inhibitor p27, a universal inhibitor of Cdk-1 and the cell cycle, indicating that Fru-2,6-P_2 may couple the activation of glucose metabolism with cell proliferation (Yalcin et al., 2009a).

Synthesis and degradation of fructose-2,6-bisphosphate:

- Kinase catalytic activity: ATP + D-fructose-6-phosphate = ADP + beta-D-fructose-2,6-bisphosphate

- Phosphatase catalytic activity: Beta-D-fructose-2,6-bisphosphate + H_2O = D-fructose-6-phosphate + phosphate

The rate of glycolytic flux is controlled at different levels and by different mechanisms: substrate availability, enzyme concentrations, allosteric effectors and covalent modification on regulatory enzymes. One of the critically modulated steps is that catalysed by 6-phosphofructo-1-kinase (PFK-1), with fructose-2,6-bisphosphate (Fru-2,6-P2) being its most powerful allosteric activator (Okar and Lange, 1999; Rider et al., 2004; Van Schaftingen, 1987).

Fru-2,6-P2 relieves ATP inhibition and acts synergistically with AMP, and in addition it inhibits fructose 1,6-bisphosphatase (Van Schaftingen, 1987). These properties confer to this metabolite a key role in the control of fructose-6-P/fructose-1,6-P2 substrate cycle and hence critically regulate carbohydrate metabolism (Figure 4).

PFKFB genes

In mammals, there are four PFKFB genes (PFKFB1, PFKFB2, PFKFB3 and PFKFB4) which code for the different PFK-2/FBPase-2 isoenzymes, characterized by their cellular expression patterns. These isoforms share highly conserved core catalytic domains (85%) but differ greatly in their kinetic properties and responses to regulatory signals (Okar et al., 2001). These differences are mostly due to highly divergent N- and C-terminal regulatory domains; however, a few but significant sequence differences in the catalytic domains that constitute the secondary residue shells surrounding the active sites also contribute to the kinetic differences (Cavalier et al., 2012).

These isoforms show differences in their distribution and kinetic properties in response to allosteric effectors, hormonal, and growth factor signals (Okar et al., 2001). The expression of these genes is dependent on tissue and on development stage (Goren et al., 2000). Importantly, tissue- and cell-specific isoenzymes are not totally exclusive and several cells express more than one isoenzyme (Calvo et al., 2006; Minchenko et al., 2005a; Minchenko et al., 2005b; Telang et al., 2006).

This pattern of expression suggests that each isoenzyme plays a key role under different physiological conditions or in response to different stimuli.

Although the PFKFB isoenzymes have the same enzymatic activities and share the same substrates, indicating functional redundancy, their biological function and regulation is different in the specific cells (Table I).

PFKFB1 is mainly expressed in liver and skeletal muscle, PFKFB2 in heart tissue, PFKFB3 is expressed ubiquitously in several tissue and proliferating cells, and PFKFB4 was originally found in testis (Okar et al., 2001).

PFKFB3 has a uniquely large 6-phosphofructo-2-kinase to fructose-2,6-bisphosphatase activity ratio compared to other isoforms (Sakakibara et al., 1997).

This isoform, which has a native activity ratio of roughly 700-fold kinase-to-phosphatase activity, dramatically increases upon phosphorylation of Ser461 by protein kinase A (PKA), AMP-dependent protein kinase (AMPK) or other kinases.

The low bisphosphatase activity of PFKFB3, which is lower than that of other isoforms by an order of magnitude, is solely due to the presence of a serine at residue 302 instead of an arginine as conserved in the other isoforms (Cavalier et al., 2012; Kim et al., 2006).

PFKFB3 gene was cloned from a fetal brain library (Manzano et al., 1998; Ventura et al., 1995), human placenta (Sakai et al., 1996) and breast cancer cells (Hamilton et al., 1997).

PFKFB3 is expressed ubiquitously and it is present in proliferating tissues, transformed cells and in tumours (Almeida et al., 2010; Atsumi et al., 2002; Bando et al., 2005; Calvo et al., 2006; Chesney et al., 1999; Duran et al., 2008a; Duran et al., 2009; Kessler et al., 2008; Novellasdemunt et al., 2012; Riera et al., 2002; Yalcin et al., 2009b). An inducible PFK-2/FBPase-2 (iPFK-2) with proto-oncogenic features was cloned from cancer cell lines (Chesney et al., 1999).

The iPFK-2 represents a splice product of the PFKFB3 gene, as does the ubiquitous PFK-2/FBPase-2 (uPFK-2) (Navarro-Sabate et al., 2001). In human brain, have been demonstrated the occurrence of six alternatively spliced PFKFB3 transcripts, designated UBI2K1-6 splice isoforms of ubiquitous 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (Kessler and Eschrich, 2001).

Regulation:

PFKFB3 gene is regulated by different mechanisms. Induction of its expression has been reported in response to different stimuli, amongst these are hypoxia (Bartrons and Caro, 2007; Minchenko et al., 2002; Obach et al., 2004) and progestins (Hamilton et al., 1997; Novellasdemunt et al., 2012), through HIF (Hypoxia Inducible Factor) and PR (progesterone

receptor) interactions within their binding to the consensus HRE (Hypoxia response element) and PRE (progesterone response element) sites located at PFKFB3 promoter, respectively. Growth factors such as insulin (Riera et al., 2002) and pro-inflammatory molecules (Chesney et al., 1999) such as IL-6 (Ando et al., 2010), LPS and adenosine (Ruiz-Garcia et al., 2011) or in response to stress stimuli (NaCl, H_2O_2, UV radiation or anisomycin) through SRF (Serum Response Factor) and its binding to SRE (Serum response element) site (Novellasdemunt et al., 2013) (Figure 5). The proinflammatory cytokine interleukin (Il-6) enhances glycolysis through activation of PFKFB3 as a consequence of the STAT3 activation (Ando et al., 2010). PFKFB3 is also a target gene of PPARγ. Additionally, PFKFB3 is involved in the antidiabetic effect of PPARγ activation, at least, by suppressing excessive fatty acid oxidation-related reactive oxygen species (ROS) production and inflammatory responses in adipose tissue/adipocytes (Huo et al., 2010).

The product of the PFKFB3 gene, the bifunctional enzyme 6-phosphofructo-2-kinase/ fructose-2,6-bisphosphatase, is also controlled by different mechanisms. In addition to the gene expression regulation, the C-terminal domain can be phosphorylated at Ser461 by different protein kinases, such as AMP-activated protein kinase (AMPK) (Bando et al., 2005; Marsin et al., 2002), RSK (Novellasdemunt et al., 2012) and MK2 (Novellasdemunt et al., 2013) (Figure 6).

Ser461 can also be phosphorylated by PKC and PKA making it responsive to multiple external signals (Okamura and Sakakibara, 1998). Phosphorylated PFKFB3 kinetics shows an increase in Vmax of the kinase activity and a decreased Km for Fru-6-P (Marsin et al., 2002; Novellasdemunt et al., 2012). Furthermore, the PFKFB3 isoenzyme was found to be regulated through the PI3K (phosphoinositide 3-kinase)/Akt/mTOR (mammalian target of rapamycin) pathway, turning it into a target of growth factors signalling (Duran et al., 2009; Garcia-Cao et al., 2012) (Figure 7).

Furthermore, the mRNAs of all PFKFB3 isoforms contain multiple copies of the AUUUA instability motif in its 30 untranslated region (30UTR) (Chesney et al., 1999). AU-rich elements (AREs) target them RNAs of proto-oncogenes and pro-inflammatory cytokines for rapid degradation and regulate the efficiency of their translation into proteins (Chen and Shyu, 1995).

Also, PFKFB3 isoenzyme is regulated by modulation of its protein stability. Thus, it is degraded through the ubiquitin/proteasome proteolytic pathway (Riera et al., 2003). PFKFB3 but not the other isoenzymes, contains a recognition signal composed of a K-E-N box (KENXXXN), where K is lysine, E is glutamate and N is asparagine, that is recognized by the anaphase-promoting complex/cyclosome (APC/Cdh1), an E3 ubiquitin ligase complex that plays an essential role in G1 phase and mitosis through the degradation of several cell cycle proteins and PFKFB3 (Almeida et al., 2010).

Homology

It appears that the use of Fru-2,6-P_2 as a regulatory metabolite is a specifically eukaryotic phenomenon. The most plausible hypothesis for the origin of the PFK-2/FBPase-2 would be the fusion of two ancestral genes coding for a kinase functional unit and a phosphohydrolase/mutase unit, respectively. From protein sequence alignments, it is clear that the bisphosphatase activity located in the C-terminal domain of the PFK-2/FBPase-2 is homologous to the phosphoglyceratemutases (PGMs) and the acid phosphatase family (Jedrzejas, 2000; Okar et al., 2001). Alignments of the bisphosphatase domain with PGM and acid phosphatase can be accessed at InterPro.

The N-terminal PFK-2 domain is sequentially and structurally homologous to several nucleotide binding proteins, primarily that of adenylate kinase of E. coli.

Mutations

Note

No PFKFB mutations have been collected in the COSMIC database. A lot of SNPs have been described, but they are not affecting to coding sequences. 2319 NCBI SNPs in PFKFB3 are shown (GeneCard).

Germinal

No germinal mutations of PFKFB3 have been described.

Somatic

Loss of heterozygosity (LOH) of PFKFB3 has been found in 55% of glioblastomas. The allelic deletion of PFKFB3 resulted in a decrease of PFKFB3 mRNA level accompanied by a lower PFKFB3 protein level. The expression of growth-inhibiting splice variant UBI2K4 was effectively reduced in glioblastomas with PFKFB3 LOH and a positive correlation with overall PFKFB3 expression was observed. The PFKFB3 LOH as well as the resulting low UBI2K4 expression level was associated with a poor prognosis of glioblastoma patients. It was concluded that LOH on 10p14-p15 in glioblastomas targets PFKFB3 and in particular splice variant UBI2K4, a putative tumor suppressor protein in glioblastomas (Fleischer et al., 2011).

Figure 6

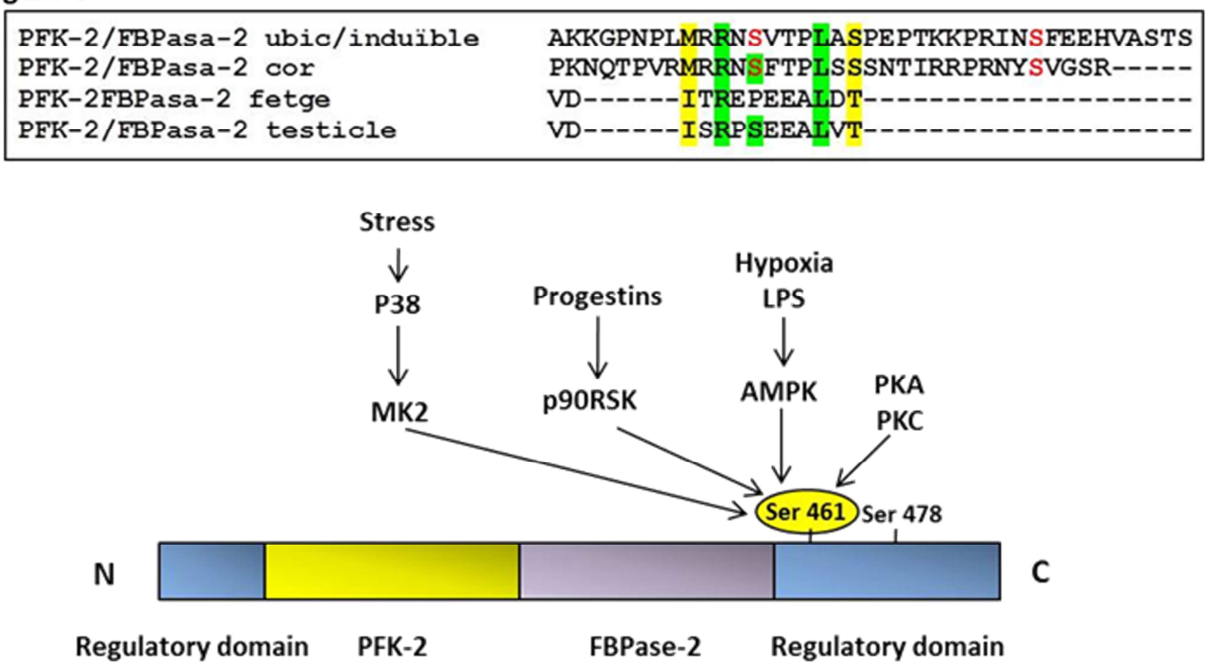

Figure 7

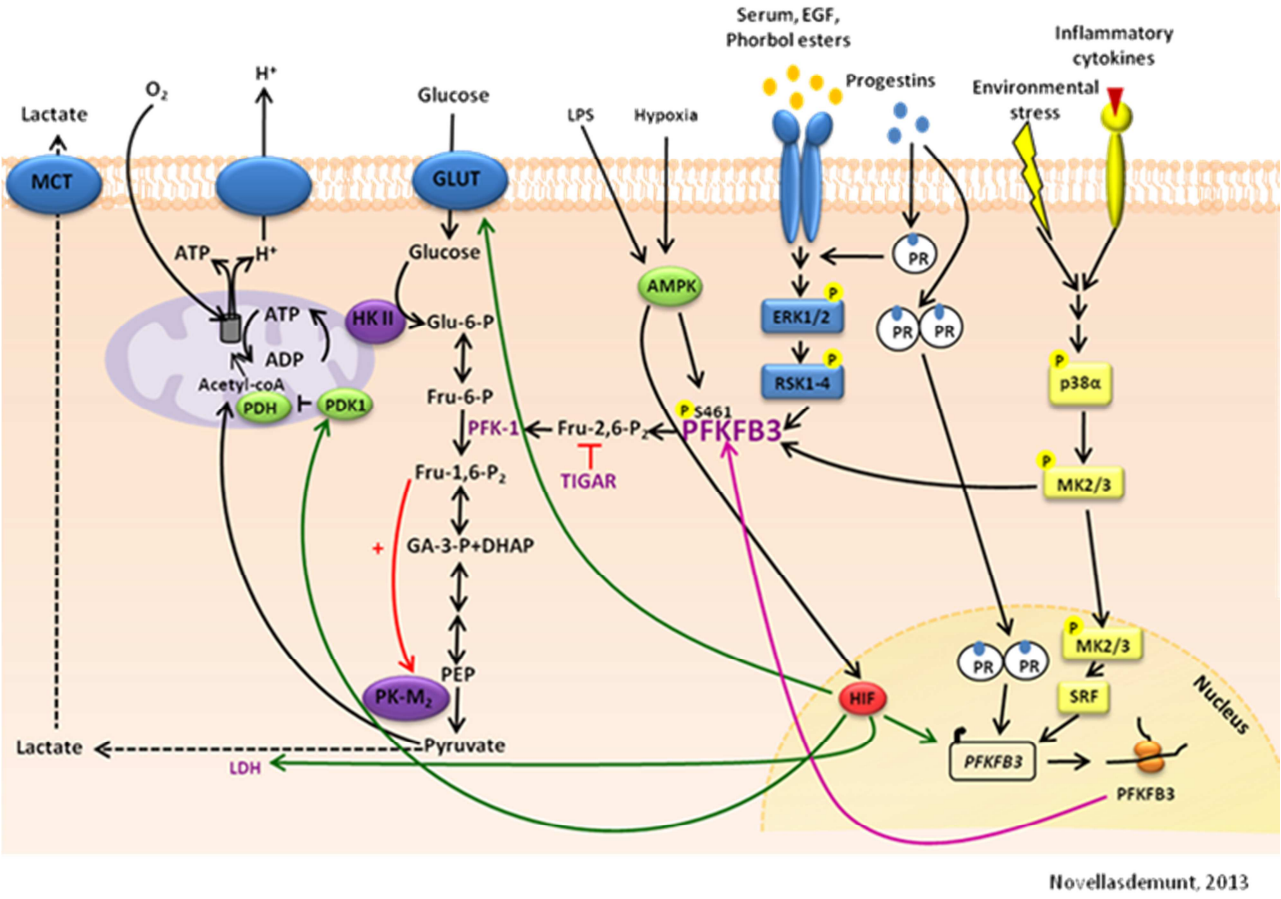

Figure 6. PFKFB3 phosphorylation sites. The residues Ser461 and Ser478 correspond to PFKFB3 isoenzyme and are equivalent to Ser466 and Ser483 of the PFKFB2 isoenzyme. The colours represent the phosphorylation residues (red), residues fully conserved in all or at least one of the isoforms (green), similar residues (yellow) and different residues (black).

Figure 7. Differential physiological factors regulate PFKFB3 gene expression and its isoenzyme activity through phosphorylation of Ser461.

Implicated in

Various cancers

Prognosis

At least 6 alternatively spliced variants of PFKFB3 mRNA are known in humans (UBI2K1-6) (Figure 2). Thus, each might have a different contribution to prognosis of tumors. UBI2K5 and UBI2K6 are the predominant splice variants found in rapidly proliferating high-grade astrocytomas while the expression of UBI2K3 and UBI2K4 is mainly restricted to low-grade astrocytomas and non-neoplastic brain tissue (Zscharnack, 2009).

Oncogenesis

Enhanced glycolysis is important for oncogenesis and for the survival and proliferation of cancer cells in the tumor microenvironment. Neoplastic cells metabolize abundant glucose relative to normal cells in order to satisfy the increased energetic and anabolic needs of the transformed state. Moreover, most of tumours are subjected to hypoxic conditions due to the abnormal vasculature that supply them with oxygen and nutrients. A key step in controlling glycolytic rate is Fru-2,6-P_2 levels. This is controlled, in tumoral cells, mainly by PFKFB3 which, in turn, is activated by mitogenic, inflammatory and hypoxic stimuli, and is found to be constitutively expressed by several human leukemias and solid tumor cells (Bartrons and Caro, 2007; Chesney, 2006).

Thyroid and ovarian cancer

Note

Increments in the expression of PFKFB3 mRNA and protein were reported in ovarian and thyroid carcinomas using in situ hybridization and immunohistochemistry analysis (Atsumi et al., 2002).

Breast cancer

Note

PFKFB3 was firstly identified as a progesterone responding gene (PRG1) in human breast cancer cell lines (Hamilton et al., 1997). Afterwards, overexpression of PFKFB3 in T47D and MCF7 cells has been probed and the regulatory mechanism of the protein and gene regulation have been described (Novellasdemunt et al., 2012; Obach et al., 2004).

PFKFB3 has also been identified, using loss-of-function screen, as a gene required for cell growth and proliferation in CD44+CD24- human breast cancer cells (Marotta et al., 2011).

Lung cancer

Note

Heterozygotic genomic deletion of the inducible PFKFB3 gene in ras-transformed mouse lung fibroblasts suppresses Fru-2,6-P_2 production, glycolytic flux to lactate, and growth as soft agar colonies or tumors in athymic mice (Atsumi et al., 2002; Telang et al., 2006).

Gastric and pancreas cancer

Note

The expression of PFKFB3 mRNA was observed in normal human gastric tissue and was increased in malignant gastric tumors. PFKFB3 gene is also expressed in gastric (MKN45, NUGC3) and pancreatic cancer cells (Panc1) which strongly respond to hypoxia via a HIF-1alpha dependent mechanism (Bobarykina et al., 2006).

Colorectal cancer

Note

PFKFB3 gene expression is controlled by insulin in the human colon adenocarcinoma HT29 cell line (Riera et al., 2002). Overexpression of PFKFB3 isoenzyme and particularly the phosphorylated form (phospho-PFKFB3 (Ser461)) has also been described in the human colon malignant tumors (Bando et al., 2005).

Glioblastoma

Note

PFKFB3 protein levels were markedly elevated in high-grade astrocytomas relative to low-grade astrocytomas and corresponding non-neoplastic brain tissue, whereas no significant increase of PFKFB3 mRNA was observed in high-grade astrocytomas when compared with control tissue. In the group of glioblastomas the PFKFB3 protein inversely correlates with EGFR expression. The findings demonstrate that PFKFB3 up-regulation is a hallmark of high-grade astrocytomas offering an explanation for high glycolytic flux and lactate production in these tumors (Kessler et al., 2008).

The study of the different PFKFB isoenzymes showed that the splice variant UBI2K4 impeded the tumour cell growth and might serve as a tumour suppressor in astrocytic tumours (Zscharnack, 2009). In this sense, loss of heterozygocity on 10p14-p15 was detected in 55% of glioblastomas and the allelic deletion of PFKFB3 splice variant UBI2K4 was associated with a poor prognosis (Fleischer et al., 2011).

Neuronal diseases

Note

Strong evidence of linkage to late-onset Alzheimer disease (LOAD) has been observed on chromosome 10, which implicates a wide region and at least one disease-susceptibility locus. Although significant associations with several biological candidate genes, such as PFKFB3, on chromosome 10 have been reported, these findings have not been consistently replicated, and they remain controversial (Grupe et al., 2006).

Increased expression of HIF- 1α, as well as its target genes, VEGF, and PFKFB3 in both major depressive and bipolar disorder patients have been found in a

depressive state compared to healthy control subjects. The data suggest that altered expression of HIF-1 and its target genes mRNA in peripheral blood cells are associated - mainly in a state-dependent manner - with mood disorders. In addition, altered expression of HIF-1 and its target genes may be associated with the pathophysiology of depression (Shibata et al., 2013).

Diabetes and obesity

Note

Expression of PFKFB3 gene has been demonstrated in adipocytes (Atsumi et al., 2005). Single nucleotide polymorphism rs1064891, located in the 3' UTR of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) gene, was nominally associated with obesity in combined analysis of human cohorts (Jiao et al., 2008). In addition, it has been found that disruption of PFKFB3 gene ameliorates diet-induced adiposity but exacerbates systemic insulin resistance and adipose tissue inflammatory response (Huo et al., 2010).

In contrast, overexpression of ubiquitous 6-phosphofructo-2-kinase in the liver of transgenic mice results in weight gain (Duran et al., 2008b).

Pfkfb3 gene expression is increased in streptozotocin-induced diabetic mouse liver. During streptozotocin-induced diabetes, phosphorylation of both p38 mitogen-activated protein kinase and Akt was detected, together with the overexpression of the proliferative markers cyclin D and E2F.

These findings indicate that PFKFB3 induction is coupled to enhanced hepatocyte proliferation in streptozotocin-induced diabetic mouse liver. Expression decreased when hepatocytes were treated with either rapamycin or LY 294002, suggesting that PFKFB3 regulation is phosphoinositide 3-kinase-Akt-mammalian target of rapamycin dependent (Duran et al., 2009).

Inflamatories diseases (arthritis and coeliac disease)

Note

Rheumatoid arthritis (RA) and coeliac disease (CD) are two autoimmune disorders which have similarities in their pathogenesis. In a meta-analysis of Dutch and UK data sets, shared association with six loci, one of them being PFKFB3 (Coenen et al., 2009). The proinflammatory cytokine interleukin (IL)-6 enhanced glycolysis in mouse embryonic fibroblasts and human cell lines through STAT3 activation which enhanced the expression of the glycolytic PFKFB3 isoenzyme (Ando et al., 2010). Furthermore, cooperation of adenosine with macrophage Toll-4 receptor agonists leads to increased glycolytic flux through the enhanced expression of PFKFB3 gene, showing that, in macrophages, endogenously generated adenosine cooperates with bacterial components to increase PFKFB3 isoenzyme activity, resulting in greater Fru-2,6-P_2 accumulation. This process enhances the glycolytic flux and favours ATP generation helping to develop and maintain the long term defensive and reparative functions of the macrophages (Ruiz-Garcia et al., 2011).

Osteoporosis

Note

PFKFB3 has been also associated with osteoclast differentiation by the immune system response or to various stimuli (Xiao et al., 2012).

Targeting PFKFB3 for cancer therapy

Note

PFKFB3 plays an important role in cancer cell metabolism and it has been considered as a potential target for cancer therapy. Different authors reported that PFKFB3 isoforms suppression inhibited anchorage-independent growth and induced cell-cycle delay, proposing that PFKFB3 protein product may serve as an essential downstream metabolic mediator of oncogenic ras (Calvo et al., 2006; Telang et al., 2006). The JAK2V617F oncogene requires expression of PFKFB3 for cell growth and increased metabolic activity. JAK2V617F as well as active STAT5 increases the expression of PFKFB3, which is required for JAK2V617F-dependent lactate production, oxidative metabolic activity and glucose uptake. Targeted Knockdown of PFKFB3 limited cell growth under normoxic and hypoxic conditions and blocked in vivo tumor formation in mice (Reddy et al., 2012).

Clinical development of PFKFB3 inhibitors as chemotherapeutic agents has been published (Clem et al., 2008, Seo et al., 2011).

For example, a cell-permeable dipyridinyl-propenone (3PO) compound that selectively blocks PFK-2 (6-phosphofructo-2-kinase) activity has been found (Telang et al., 2006). 3PO decreases Fru-2,6-P_2 and lactate production, arresting proliferation of transformed cells and suppressing tumor growth in several xenograft mouse models (Telang et al., 2006, Clem et al., 2008).

In addition, other small molecule inhibitors (N4A, YN1) have been identified. When tested on cultured cancer cells both, N4A and YN1, inhibited PFKFB3, decreasing Fru-2,6-P_2 concentration and glycolysis and, ultimately, led to cell death (Seo et al., 2011).

KO and transgenic animals

Note

Models of KO and transgenic animals have been developed showing that after disruption of the mouse PFKFB3 gene, no homozygous PFKFB3 (-/-) embryos were detected after 8 days of embryogenesis, indicating that the loss of PFKFB3 activity via targeted disruption of the gene leads to loss of embryonic cell proliferation, differentiation, and/or implantation.

Serial analysis of gene expression has indicated that the mouse PFKFB3 mRNA becomes detectable during the blastocyst stage of embryogenesis.

In contrast, normal phenotype is observed in PFKFB3 (+/-) mice despite the 50% reduction in PFKFB3 expression and activity (Chesney et al., 2005).
Using PFKFB3-disrupted (PFKFB3+/-) mice, it has been shown that the animals are protected against diet-induced insulin resistance and adipose tissue inflammatory response (Huo et al., 2010).
A transgenic mouse model that overexpressed pfkfb3 and which was subjected to diet-induced obesity revealed production of high Fru-2,6-P_2 levels and changes in hepatic gene expression profiles of key gluconeogenic and lipogenic enzymes, as well as an accumulation of lipids in periportal cells, and weight gain (Duran et al., 2008b).

References

Winn SI, Watson HC, Harkins RN, Fothergill LA.. Structure and activity of phosphoglycerate mutase. Philos Trans R Soc Lond B Biol Sci. 1981 Jun 26;293(1063):121-30. (REVIEW)

El-Maghrabi MR, Claus TH, Pilkis J, Fox E, Pilkis SJ.. Regulation of rat liver fructose 2,6-bisphosphatase. J Biol Chem. 1982 Jul 10;257(13):7603-7.

Van Schaftingen E.. Fructose 2,6-bisphosphate. Adv Enzymol Relat Areas Mol Biol. 1987;59:315-95.

Tauler A, Lin K, Pilkis SJ.. Hepatic 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Use of site-directed mutagenesis to evaluate the roles of His-258 and His-392 in catalysis. J Biol Chem. 1990 Sep 15;265(26):15617-22.

Li L, Lin K, Correia JJ, Pilkis SJ.. Lysine 356 is a critical residue for binding the C-6 phospho group of fructose 2,6-bisphosphate to the fructose-2,6-bisphosphatase domain of rat liver 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. J Biol Chem. 1992a Aug 15;267(23):16669-75.

Li L, Lin K, Kurland IJ, Correia JJ, Pilkis SJ.. Site-directed mutagenesis in rat liver 6-phosphofructo-2-kinase. Mutation at the fructose 6-phosphate binding site affects phosphate activation. J Biol Chem. 1992b Mar 5;267(7):4386-93.

Li L, Lin K, Pilkis J, Correia JJ, Pilkis SJ.. Hepatic 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. The role of surface loop basic residues in substrate binding to the fructose-2,6-bisphosphatase domain. J Biol Chem. 1992c Oct 25;267(30):21588-94.

Lin K, Li L, Correia JJ, Pilkis SJ.. Glu327 is part of a catalytic triad in rat liver fructose-2,6-bisphosphatase. J Biol Chem. 1992 Apr 5;267(10):6556-62.

Schneider G, Lindqvist Y, Vihko P.. Three-dimensional structure of rat acid phosphatase. EMBO J. 1993 Jul;12(7):2609-15.

Rider MH, Crepin KM, De Cloedt M, Bertrand L, Hue L.. Site-directed mutagenesis of rat muscle 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: role of Asp-130 in the 2-kinase domain. Biochem J. 1994 May 15;300 (Pt 1):111-5.

Chen CY, Shyu AB.. AU-rich elements: characterization and importance in mRNA degradation. Trends Biochem Sci. 1995 Nov;20(11):465-70. (REVIEW)

Kurland I, Chapman B, Lee YH, Pilkis S.. Evolutionary reengineering of the phosphofructokinase active site: ARG-104 does not stabilize the transition state in 6-phosphofructo-2-kinase. Biochem Biophys Res Commun. 1995 Aug 15;213(2):663-72.

Pilkis SJ, Claus TH, Kurland IJ, Lange AJ.. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase: a metabolic signaling enzyme. Annu Rev Biochem. 1995;64:799-835. (REVIEW)

Ventura F, Ambrosio S, Bartrons R, el-Maghrabi MR, Lange AJ, Pilkis SJ.. Cloning and expression of a catalytic core bovine brain 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Biochem Biophys Res Commun. 1995 Apr 26;209(3):1140-8.

Hasemann CA, Istvan ES, Uyeda K, Deisenhofer J.. The crystal structure of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase reveals distinct domain homologies. Structure. 1996 Sep 15;4(9):1017-29.

Sakai A, Kato M, Fukasawa M, Ishiguro M, Furuya E, Sakakibara R.. Cloning of cDNA encoding for a novel isozyme of fructose 6-phosphate, 2-kinase/fructose 2,6-bisphosphatase from human placenta. J Biochem. 1996 Mar;119(3):506-11.

Hamilton JA, Callaghan MJ, Sutherland RL, Watts CK.. Identification of PRG1, a novel progestin-responsive gene with sequence homology to 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Mol Endocrinol. 1997 Apr;11(4):490-502.

Sakakibara R, Uemura M, Hirata T, Okamura N, Kato M.. Human placental fructose-6-phosphate,2-kinase/fructose-2,6-bisphosphatase: its isozymic form, expression and characterization. Biosci Biotechnol Biochem. 1997 Nov;61(11):1949-52.

Manzano A, Rosa JL, Ventura F, Pérez JX, Nadal M, Estivill X, Ambrosio S, Gil J, Bartrons R.. Molecular cloning, expression, and chromosomal localization of a ubiquitously expressed human 6-phosphofructo-2-kinase/ fructose-2, 6-bisphosphatase gene (PFKFB3). Cytogenet Cell Genet. 1998;83(3-4):214-7.

Okamura N, Sakakibara R.. A common phosphorylation site for cyclic AMP-dependent protein kinase and protein kinase C in human placental 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Biosci Biotechnol Biochem. 1998 Oct;62(10):2039-42.

Chesney J, Mitchell R, Benigni F, Bacher M, Spiegel L, Al-Abed Y, Han JH, Metz C, Bucala R.. An inducible gene product for 6-phosphofructo-2-kinase with an AU-rich instability element: role in tumor cell glycolysis and the Warburg effect. Proc Natl Acad Sci U S A. 1999 Mar 16;96(6):3047-52.

Okar DA, Lange AJ.. Fructose-2,6-bisphosphate and control of carbohydrate metabolism in eukaryotes. Biofactors. 1999;10(1):1-14. (REVIEW)

Goren N, Manzano A, Riera L, Ambrosio S, Ventura F, Bartrons R.. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase expression in rat brain during development. Brain Res Mol Brain Res. 2000 Jan 10;75(1):138-42.

Jedrzejas MJ.. Structure, function, and evolution of phosphoglycerate mutases: comparison with fructose-2,6-bisphosphatase, acid phosphatase, and alkaline

phosphatase. Prog Biophys Mol Biol. 2000;73(2-4):263-87. (REVIEW)

Kessler R, Eschrich K.. Splice isoforms of ubiquitous 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase in human brain. Brain Res Mol Brain Res. 2001 Mar 5;87(2):190-5.

Navarro-Sabate A, Manzano A, Riera L, Rosa JL, Ventura F, Bartrons R.. The human ubiquitous 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene (PFKFB3): promoter characterization and genomic structure. Gene. 2001 Feb 7;264(1):131-8.

Okar DA, Manzano A, Navarro-Sabat A, Riera L, Bartrons R, Lange AJ.. PFK-2/FBPase-2: maker and breaker of the essential biofactor fructose-2,6-bisphosphate. Trends Biochem Sci. 2001 Jan;26(1):30-5. (REVIEW)

Atsumi T, Chesney J, Metz C, Leng L, Donnelly S, Makita Z, Mitchell R, Bucala R.. High expression of inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (iPFK-2; PFKFB3) in human cancers. Cancer Res. 2002 Oct 15;62(20):5881-7.

Marsin AS, Bouzin C, Bertrand L, Hue L.. The stimulation of glycolysis by hypoxia in activated monocytes is mediated by AMP-activated protein kinase and inducible 6-phosphofructo-2-kinase. J Biol Chem. 2002 Aug 23;277(34):30778-83. Epub 2002 Jun 13.

Minchenko A, Leshchinsky I, Opentanova I, Sang N, Srinivas V, Armstead V, Caro J.. Hypoxia-inducible factor-1-mediated expression of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) gene. Its possible role in the Warburg effect. J Biol Chem. 2002 Feb 22;277(8):6183-7. Epub 2001 Dec 14.

Riera L, Manzano A, Navarro-Sabate A, Perales JC, Bartrons R.. Insulin induces PFKFB3 gene expression in HT29 human colon adenocarcinoma cells. Biochim Biophys Acta. 2002 Apr 3;1589(2):89-92.

Lee YH, Li Y, Uyeda K, Hasemann CA.. Tissue-specific structure/function differentiation of the liver isoform of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. J Biol Chem. 2003 Jan 3;278(1):523-30. Epub 2002 Oct 11.

Riera L, Obach M, Navarro-Sabate A, Duran J, Perales JC, Vinals F, Rosa JL, Ventura F, Bartrons R.. Regulation of ubiquitous 6-phosphofructo-2-kinase by the ubiquitin-proteasome proteolytic pathway during myogenic C2C12 cell differentiation. FEBS Lett. 2003 Aug 28;550(1-3):23-9.

Obach M, Navarro-Sabate A, Caro J, Kong X, Duran J, G—mez M, Perales JC, Ventura F, Rosa JL, Bartrons R.. 6-Phosphofructo-2-kinase (pfkfb3) gene promoter contains hypoxia-inducible factor-1 binding sites necessary for transactivation in response to hypoxia. J Biol Chem. 2004 Dec 17;279(51):53562-70. Epub 2004 Oct 5.

Rider MH, Bertrand L, Vertommen D, Michels PA, Rousseau GG, Hue L.. 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: head-to-head with a bifunctional enzyme that controls glycolysis. Biochem J. 2004 Aug 1;381(Pt 3):561-79. (REVIEW)

Atsumi T, Nishio T, Niwa H, Takeuchi J, Bando H, Shimizu C, Yoshioka N, Bucala R, Koike T.. Expression of inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase/PFKFB3 isoforms in adipocytes and their potential role in glycolytic regulation. Diabetes. 2005 Dec;54(12):3349-57.

Bando H, Atsumi T, Nishio T, Niwa H, Mishima S, Shimizu C, Yoshioka N, Bucala R, Koike T.. Phosphorylation of the 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase/PFKFB3 family of glycolytic regulators in human cancer. Clin Cancer Res. 2005 Aug 15;11(16):5784-92.

Chesney J, Telang S, Yalcin A, Clem A, Wallis N, Bucala R.. Targeted disruption of inducible 6-phosphofructo-2-kinase results in embryonic lethality. Biochem Biophys Res Commun. 2005 May 27;331(1):139-46.

Gomez M, Manzano A, Navarro-Sabate A, Duran J, Obach M, Perales JC, Bartrons R.. Specific expression of pfkfb4 gene in spermatogonia germ cells and analysis of its 5'-flanking region. FEBS Lett. 2005 Jan 17;579(2):357-62.

Minchenko OH, Ogura T, Opentanova IL, Minchenko DO, Ochiai A, Caro J, Komisarenko SV, Esumi H.. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene family overexpression in human lung tumor. Ukr Biokhim Zh. 2005a;77(6):46-50.

Minchenko OH, Opentanova IL, Ogura T, Minchenko DO, Komisarenko SV, Caro J, Esumi H.. Expression and hypoxia-responsiveness of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 in mammary gland malignant cell lines. Acta Biochim Pol. 2005b;52(4):881-8. Epub 2005 Jul 11.

Bobarykina AY, Minchenko DO, Opentanova IL, Moenner M, Caro J, Esumi H, Minchenko OH.. Hypoxic regulation of PFKFB-3 and PFKFB-4 gene expression in gastric and pancreatic cancer cell lines and expression of PFKFB genes in gastric cancers. Acta Biochim Pol. 2006;53(4):789-99. Epub 2006 Dec 4.

Calvo MN, Bartrons R, Castano E, Perales JC, Navarro-Sabate A, Manzano A.. PFKFB3 gene silencing decreases glycolysis, induces cell-cycle delay and inhibits anchorage-independent growth in HeLa cells. FEBS Lett. 2006 May 29;580(13):3308-14. Epub 2006 May 8.

Chesney J.. 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase and tumor cell glycolysis. Curr Opin Clin Nutr Metab Care. 2006 Sep;9(5):535-9. (REVIEW)

Grupe A, Li Y, Rowland C, Nowotny P, Hinrichs AL, Smemo S, Kauwe JS, Maxwell TJ, Cherny S, Doil L, Tacey K, van Luchene R, Myers A, Wavrant-De Vrieze F, Kaleem M, Hollingworth P, Jehu L, Foy C, Archer N, Hamilton G, Holmans P, Morris CM, Catanese J, Sninsky J, White TJ, Powell J, Hardy J, O'Donovan M, Lovestone S, Jones L, Morris JC, Thal L, Owen M, Williams J, Goate A.. A scan of chromosome 10 identifies a novel locus showing strong association with late-onset Alzheimer disease. Am J Hum Genet. 2006 Jan;78(1):78-88. Epub 2005 Nov 15.

Kim SG, Manes NP, El-Maghrabi MR, Lee YH.. Crystal structure of the hypoxia-inducible form of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3): a possible new target for cancer therapy. J Biol Chem. 2006 Feb 3;281(5):2939-44. Epub 2005 Nov 29.

Telang S, Yalcin A, Clem AL, Bucala R, Lane AN, Eaton JW, Chesney J.. Ras transformation requires metabolic control by 6-phosphofructo-2-kinase. Oncogene. 2006 Nov 23;25(55):7225-34. Epub 2006 May 22.

Bartrons R, Caro J.. Hypoxia, glucose metabolism and the Warburg's effect. J Bioenerg Biomembr. 2007 Jun;39(3):223-9. (REVIEW)

Clem B, Telang S, Clem A, Yalcin A, Meier J, Simmons A, Rasku MA, Arumugam S, Dean WL, Eaton J, Lane A, Trent JO, Chesney J.. Small-molecule inhibition of 6-phosphofructo-2-kinase activity suppresses glycolytic flux and tumor growth. Mol Cancer Ther. 2008 Jan;7(1):110-20. doi: 10.1158/1535-7163.MCT-07-0482.

Duran J, Gomez M, Navarro-Sabate A, Riera-Sans L, Obach M, Manzano A, Perales JC, Bartrons R.. Characterization of a new liver- and kidney-specific pfkfb3 isozyme that is downregulated by cell proliferation and dedifferentiation. Biochem Biophys Res Commun. 2008a

Mar 21;367(4):748-54. doi: 10.1016/j.bbrc.2008.01.005. Epub 2008 Jan 10.

Duran J, Navarro-Sabate A, Pujol A, Perales JC, Manzano A, Obach M, Gomez M, Bartrons R.. Overexpression of ubiquitous 6-phosphofructo-2-kinase in the liver of transgenic mice results in weight gain. Biochem Biophys Res Commun. 2008b Jan 11;365(2):291-7. Epub 2007 Nov 6.

Jiao H, Kaaman M, Dungner E, Kere J, Arner P, Dahlman I.. Association analysis of positional obesity candidate genes

based on integrated data from transcriptomics and linkage analysis. Int J Obes (Lond). 2008 May;32(5):816-25. doi: 10.1038/sj.ijo.0803789. Epub 2008 Jan 8.

Kessler R, Bleichert F, Warnke JP, Eschrich K.. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3) is up-regulated in high-grade astrocytomas. J Neurooncol. 2008 Feb;86(3):257-64. Epub 2007 Sep 6.

Morrish F, Neretti N, Sedivy JM, Hockenbery DM.. The oncogene c-Myc coordinates regulation of metabolic networks to enable rapid cell cycle entry. Cell Cycle. 2008 Apr 15;7(8):1054-66. Epub 2008 Feb 8.

Coenen MJ, Trynka G, Heskamp S, Franke B, van Diemen CC, Smolonska J, van Leeuwen M, Brouwer E, Boezen MH, Postma DS, Platteel M, Zanen P, Lammers JW, Groen HJ, Mali WP, Mulder CJ, Tack GJ, Verbeek WH, Wolters VM, Houwen RH, Mearin ML, van Heel DA, Radstake TR, van Riel PL, Wijmenga C, Barrera P, Zhernakova A.. Common and different genetic background for rheumatoid arthritis and coeliac disease. Hum Mol Genet. 2009 Nov 1;18(21):4195-203. doi: 10.1093/hmg/ddp365. Epub 2009 Jul 31.

Duran J, Obach M, Navarro-Sabate A, Manzano A, Gomez M, Rosa JL, Ventura F, Perales JC, Bartrons R.. Pfkfb3 is transcriptionally upregulated in diabetic mouse liver through proliferative signals. FEBS J. 2009 Aug;276(16):4555-68. doi: 10.1111/j.1742-4658.2009.07161.x. Epub 2009 Jul 18.

Gomez M, Navarro-SabatŽ A, Manzano A, Duran J, Obach M, Bartrons R.. Switches in 6-phosphofructo-2-kinase isoenzyme expression during rat sperm maturation. Biochem Biophys Res Commun. 2009 Sep 18;387(2):330-5. doi: 10.1016/j.bbrc.2009.07.021. Epub 2009 Jul 10.

Matheny RW Jr, Adamo ML.. Current perspectives on Akt Akt-ivation and Akt-ions. Exp Biol Med (Maywood). 2009 Nov;234(11):1264-70. doi: 10.3181/0904-MR-138. Epub 2009 Jul 13. (REVIEW)

Yalcin A, Clem BF, Simmons A, Lane A, Nelson K, Clem AL, Brock E, Siow D, Wattenberg B, Telang S, Chesney J.. Nuclear targeting of 6-phosphofructo-2-kinase (PFKFB3) increases proliferation via cyclin-dependent kinases. J Biol Chem. 2009a Sep 4;284(36):24223-32. doi: 10.1074/jbc.M109.016816. Epub 2009 May 27.

Yalcin A, Telang S, Clem B, Chesney J.. Regulation of glucose metabolism by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases in cancer. Exp Mol Pathol. 2009b Jun;86(3):174-9. doi: 10.1016/j.yexmp.2009.01.003. Epub 2009 Jan 14. (REVIEW)

Zscharnack K, Kessler R, Bleichert F, Warnke JP, Eschrich K.. The PFKFB3 splice variant UBI2K4 is downregulated in high-grade astrocytomas and impedes the growth of U87 glioblastoma cells. Neuropathol Appl Neurobiol. 2009 Dec;35(6):566-78. doi: 10.1111/j.1365-2990.2009.01027.x. Epub 2009 Apr 15.

Almeida A, Bolanos JP, Moncada S.. E3 ubiquitin ligase APC/C-Cdh1 accounts for the Warburg effect by linking glycolysis to cell proliferation. Proc Natl Acad Sci U S A. 2010 Jan 12;107(2):738-41. doi: 10.1073/pnas.0913668107. Epub 2009 Dec 22.

Ando M, Uehara I, Kogure K, Asano Y, Nakajima W, Abe Y, Kawauchi K, Tanaka N.. Interleukin 6 enhances glycolysis through expression of the glycolytic enzymes hexokinase 2 and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3. J Nippon Med Sch. 2010 Apr;77(2):97-105.

Feng Z, Levine AJ.. The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein. Trends Cell Biol. 2010 Jul;20(7):427-34. doi: 10.1016/j.tcb.2010.03.004. (REVIEW)

Huo Y, Guo X, Li H, Wang H, Zhang W, Wang Y, Zhou H, Gao Z, Telang S, Chesney J, Chen YE, Ye J, Chapkin RS, Wu C.. Disruption of inducible 6-phosphofructo-2-kinase ameliorates diet-induced adiposity but exacerbates systemic insulin resistance and adipose tissue inflammatory response. J Biol Chem. 2010 Feb 5;285(6):3713-21. doi: 10.1074/jbc.M109.058446. Epub 2009 Nov 30.

Fleischer M, Kessler R, Klammer A, Warnke JP, Eschrich K.. LOH on 10p14-p15 targets the PFKFB3 gene locus in human glioblastomas. Genes Chromosomes Cancer. 2011 Dec;50(12):1010-20. doi: 10.1002/gcc.20914. Epub 2011 Aug 30.

Marotta LL, Almendro V, Marusyk A, Shipitsin M, Schemme J, Walker SR, Bloushtain-Qimron N, Kim JJ, Choudhury SA, Maruyama R, Wu Z, Gonen M, Mulvey LA, Bessarabova MO, Huh SJ, Silver SJ, Kim SY, Park SY, Lee HE, Anderson KS, Richardson AL, Nikolskaya T, Nikolsky Y, Liu XS, Root DE, Hahn WC, Frank DA, Polyak K.. The JAK2/STAT3 signaling pathway is required for growth of $CD44^{+}CD24^{-}$ stem cell-like breast cancer cells in human tumors. J Clin Invest. 2011 Jul;121(7):2723-35. doi: 10.1172/JCI44745.

Ruiz-Garcia A, Monsalve E, Novellasdemunt L, Navarro-Sabate A, Manzano A, Rivero S, Castrillo A, Casado M, Laborda J, Bartrons R, D'az-Guerra MJ.. Cooperation of adenosine with macrophage Toll-4 receptor agonists leads to increased glycolytic flux through the enhanced expression of PFKFB3 gene. J Biol Chem. 2011 Jun 3;286(22):19247-58. doi: 10.1074/jbc.M110.190298. Epub 2011 Apr 4.

Seo M, Kim JD, Neau D, Sehgal I, Lee YH.. Structure-based development of small molecule PFKFB3 inhibitors: a framework for potential cancer therapeutic agents targeting the Warburg effect. PLoS One. 2011;6(9):e24179. doi: 10.1371/journal.pone.0024179. Epub 2011 Sep 21.

Song MS, Carracedo A, Salmena L, Song SJ, Egia A, Malumbres M, Pandolfi PP.. Nuclear PTEN regulates the APC-CDH1 tumor-suppressive complex in a phosphatase-independent manner. Cell. 2011 Jan 21;144(2):187-99. doi: 10.1016/j.cell.2010.12.020.

Cavalier MC, Kim SG, Neau D, Lee YH.. Molecular basis of the fructose-2,6-bisphosphatase reaction of PFKFB3: transition state and the C-terminal function. Proteins. 2012 Apr;80(4):1143-53. doi: 10.1002/prot.24015. Epub 2012 Jan 31.

Fernandez-Fernandez S, Almeida A, Bolanos JP.. Antioxidant and bioenergetic coupling between neurons and astrocytes. Biochem J. 2012 Apr 1;443(1):3-11. doi: 10.1042/BJ20111943.

Garcia-Cao I, Song MS, Hobbs RM, Laurent G, Giorgi C, de Boer VC, Anastasiou D, Ito K, Sasaki AT, Rameh L, Carracedo A, Vander Heiden MG, Cantley LC, Pinton P, Haigis MC, Pandolfi PP.. Systemic elevation of PTEN induces a tumor-suppressive metabolic state. Cell. 2012 Mar 30;149(1):49-62. doi: 10.1016/j.cell.2012.02.030. Epub 2012 Mar 6.

Gomez M, Manzano A, Figueras A, Vinals F, Ventura F, Rosa JL, Bartrons R, Navarro-Sabate A.. Sertoli-secreted FGF-2 induces PFKFB4 isozyme expression in mouse spermatogenic cells by activation of the MEK/ERK/CREB pathway. Am J Physiol Endocrinol Metab. 2012 Sep 15;303(6):E695-707. doi: 10.1152/ajpendo.00381.2011. Epub 2012 Jul 17.

Moncada S, Higgs EA, Colombo SL.. Fulfilling the metabolic requirements for cell proliferation. Biochem J. 2012 Aug 15;446(1):1-7. doi: 10.1042/BJ20120427. (REVIEW)

Novellasdemunt L, Obach M, Millan-Arino L, Manzano A, Ventura F, Rosa JL, Jordan A, Navarro-Sabate A, Bartrons R.. Progestins activate 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) in breast cancer cells. Biochem J. 2012 Mar 1;442(2):345-56. doi: 10.1042/BJ20111418.

Reddy MM, Fernandes MS, Deshpande A, Weisberg E, Inguilizian HV, Abdel-Wahab O, Kung AL, Levine RL, Griffin JD, Sattler M.. The JAK2V617F oncogene requires expression of inducible phosphofructokinase/fructose-bisphosphatase 3 for cell growth and increased metabolic activity. Leukemia. 2012 Mar;26(3):481-9. doi: 10.1038/leu.2011.225. Epub 2011 Aug 23.

Xiao H, Shan L, Zhu H, Xue F.. Detection of significant

pathways in osteoporosis based on graph clustering. Mol Med Rep. 2012 Dec;6(6):1325-32. doi: 10.3892/mmr.2012.1082. Epub 2012 Sep 13.

Li H, Guo X, Xu H, Woo SL, Halim V, Morgan C, Wu C.. A role for inducible 6-phosphofructo-2-kinase in the control of neuronal glycolysis. J Nutr Biochem. 2013 Jun;24(6):1153-8. doi: 10.1016/j.jnutbio.2012.08.016. Epub 2012 Dec 14.

Novellasdemunt L, Bultot L, Manzano A, Ventura F, Rosa JL, Vertommen D, Rider MH, Navarro-Sabate A, Bartrons R.. PFKFB3 activation in cancer cells by the p38/MK2 pathway in response to stress stimuli. Biochem J. 2013 Jun 15;452(3):531-43. doi: 10.1042/BJ20121886.

Shibata T, Yamagata H, Uchida S, Otsuki K, Hobara T, Higuchi F, Abe N, Watanabe Y.. The alteration of hypoxia inducible factor-1 (HIF-1) and its target genes in mood disorder patients. Prog Neuropsychopharmacol Biol Psychiatry. 2013 Jun 3;43:222-9. doi: 10.1016/j.pnpbp.2013.01.003. Epub 2013 Jan 16.

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