

Pyruvate Kinase Type M2: A Key Regulator Within the Tumour Metabolome and a Tool for Metabolic Profiling of Tumours

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Abstract. Normal proliferating cells and tumour cells in particular express the pyruvate kinase isoenzyme type M2 (M2-PK, PKM2). The quaternary structure of M2-PK determines whether the glucose carbons are degraded to pyruvate and lactate with production of energy (tetrameric form) or channelled into synthetic processes, debranching from glycolytic intermediates such as nucleic acid, amino acid and phospholipid synthesis. The tetramer:dimer ratio of M2-

PK is regulated by metabolic intermediates, such as fructose 1,6-P2 and direct interaction with different oncoproteins, such as pp60^{v-src} kinase, HPV-16 E7 and A-Raf. The metabolic function of the interaction between M2-PK and the HERC1 oncoprotein remains unknown. Thus, M2-PK is a meeting point for different oncogenes and metabolism. In tumour cells, the dimeric form of M2-PK is predominant and has therefore been termed Tumour M2-PK. Tumour M2-PK is released from tumours into the blood and from gastrointestinal tumours also into the stool of tumour patients. The quantification of Tumour M2-PK in EDTA plasma and stool is a tool for early detection of tumours and therapy control.

1 Introduction

The first oncogene discovered was the src oncogene. The discovery goes back to the year 1910 and Peyton Rous who found that cell-free cell extracts of sarcomas from Plymouth Rock hens transmit the disease when injected in other chickens (Rous 1910). Nearly contemporary to Peyton Rous, Otto Warburg began his investigations into the metabolism of tumour cells and in 1924 described for the first time that tumour cells produce high levels of lactate even in the presence of oxygen (Warburg et al. 1924). Both findings initiated two new fields of extensive and fundamental investigation. The basic observations of Peyton Rous resulted in the discovery of tumour viruses, oncogenes and proto-oncogenes. The transforming principle of the Rous sarcoma virus was isolated in 1977 and termed pp60^{v-src} (Brugge and Erikson 1977). Three years later, it was demonstrated that pp60^{v-src} is a protein tyrosine kinase, which was the first identified member of this class of enzymes (Hunter and Sefton 1980). In the field of metabolic research, it turned out that the glycolytic phenotype of tumour cells is the result of multiple mechanisms, which include activation of oncogenes as well as stabilization of transcription factors (Shim et al. 1997; Gatenby and Gillies 2004) and correlates with an upregulation of most glycolytic enzymes as well as changes in the isoenzyme composition of certain glycolytic enzymes (see Sect. 2). The tumour-specific metabolic phenotype is summarized as the tumour metabolome (<http://www.metabolic-database.com>). One of the glycolytic enzymes which was found to be consistently altered during tumorigenesis is pyruvate kinase. Tumour cells are characterized by the expression of the pyruvate kinase isoenzyme type M2 (M2-PK,

PKM2), which was found to be a target of the pp60^{v-src} kinase (Presek et al. 1980, 1988) as well as other oncoproteins. Thus, M2-PK is a meeting point for different oncogenes and metabolism.

2 The Pyruvate Kinase Isoenzymes

In glycolysis, first two moles of ATP have to be invested in the hexokinase and 6-phosphofructo 1-kinase reaction before the ATP is regained in the phosphoglycerate kinase reaction. Net ATP production occurs in the last step within the glycolytic sequence, the dephosphorylation of phosphoenolpyruvate (PEP) to pyruvate catalyzed by pyruvate kinase (Fig. 1). In contrast to mitochondrial respiration, energy regeneration by pyruvate kinase is independent of oxygen and allows survival of the cells in the absence of oxygen, which is of special importance in tumour cells, often growing in areas with varying oxygen supply.

There are four pyruvate kinase isoenzymes in mammals which differ widely in their occurrence according to the type of tissue, kinetic characteristics and regulation mechanisms. The pyruvate kinase isoenzyme type L (L-PK) has the lowest affinity to its substrate PEP and is expressed in tissues with gluconeogenesis, such as liver, kidney and intestine (Brinck et al. 1994; Steinberg et al. 1999). L-PK is allosterically activated by fructose 1,6-P₂ as well as ATP and phosphorylated by a cAMP-dependent protein kinase under the control of glucagon. The phosphorylation of L-PK leads to a reduction of the PEP affinity and an inactivation of the enzyme under physiological conditions. Furthermore, L-PK expression is regulated by nutrition. Whereas a carbohydrate-rich diet enhances protein synthesis of L-PK, hunger reduces L-PK expression.

The pyruvate kinase isoenzyme type M1 (M1-PK) has the highest affinity to its substrate PEP and is not allosterically regulated, phosphorylated or influenced by diet. M1-PK is expressed in skeletal muscle and brain, both organs which are strongly dependent upon a high rate of energy regeneration (Yamada and Noguchi 1999). The pyruvate kinase isoenzyme type R (R-PK) is found in erythrocytes and is very similar to L-PK in respect to its kinetic characteristics and regulation mechanisms (Noguchi et al. 1987). The pyruvate kinase isoenzyme type M2 (M2-

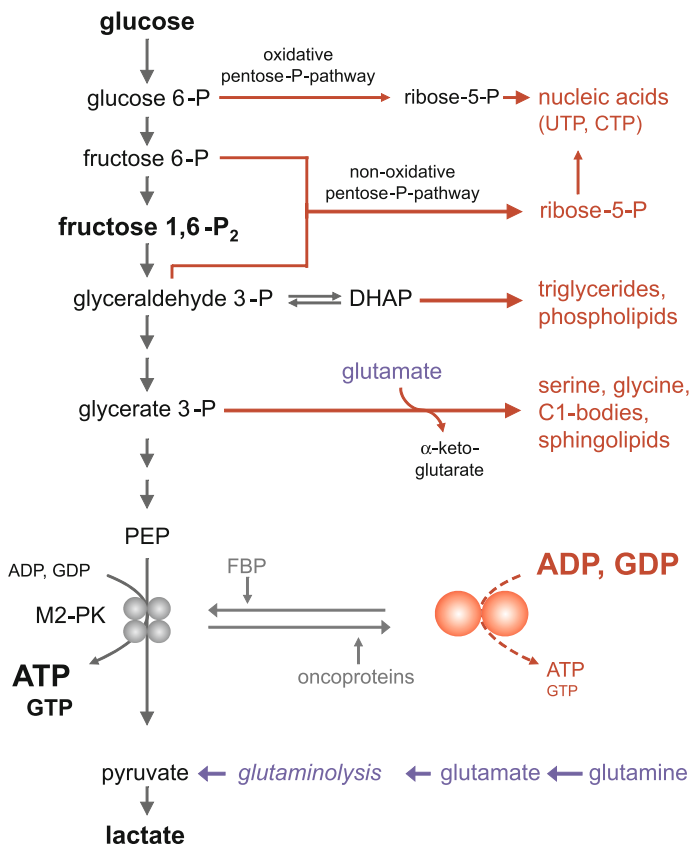


Fig. 1. Glycolysis with debranching synthetic processes. A large level of the highly active tetrameric form correlates with high levels of ATP and GTP, high ATP:ADP and GTP:GDP ratios as well as a high (ATP+GTP):(UTP+CTP) ratio. In contrast, high levels of the nearly inactive dimeric form of M2-PK correlate with low ATP and GTP levels, low ATP:ADP and GTP:GDP ratios as well as a low (ATP+GTP):(UTP+CTP) ratio

PK, PKM2) is expressed in some differentiated tissues, such as lung, fat tissue, retina as well as in all cells with a high rate of nucleic acid synthesis, which are all proliferating cells including normal proliferat-

M2-PK

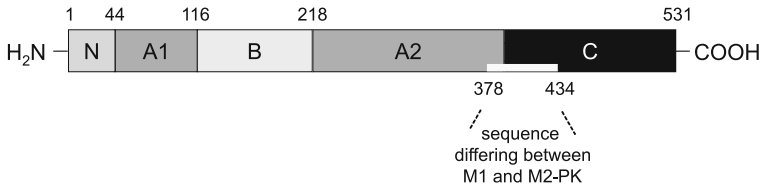


Fig. 2. Molecular structure of the M2-PK protein

ing cells, embryonic cells, stem cells and especially tumour cells. When embryonic cells differentiate, the M2-PK isoenzyme is progressively replaced by the respective tissue-specific pyruvate kinase isoenzymes. Conversely, during tumorigenesis the tissue-specific isoenzymes disappear and M2-PK is expressed (Reinacher and Eigenbrodt 1981; Staal and Rijksen 1991; Steinberg et al. 1999). The kinetic characteristics of the M2-PK isoenzyme depend on the quaternary structure of the enzyme (see Sect. 3).

The R and L isoenzymes of pyruvate kinase are encoded by the same gene and are expressed under the control of different tissue specific promoters (Noguchi et al. 1987). In the same way, the M1 and M2-PK isoenzymes are encoded by one gene but result from alternative splicing of exons 9 and 10. The human M1 and M2-PK isoenzyme differ in 23 amino acids within a stretch of 56 amino acids (Fig. 2) (Noguchi et al. 1986; Yamada and Noguchi 1999; Dombrackas et al. 2005).

3 Bifunctional Role of the Pyruvate Kinase Isoenzyme Type M2 Within the Tumour Metabolome

The human M2-PK isoenzyme consists of 531 amino acids and can be subdivided into the N-terminal domain from aa 1–43, the A-domain which is composed of aa 44–116 as well as 219–389, the B-domain from aa 117–218 and the C-domain from aa 390–531 (Fig. 2) (Dombrackas et al. 2005). The A-domain is responsible for the intermolecular subunit contact to compose a dimeric form. The interfaces of the

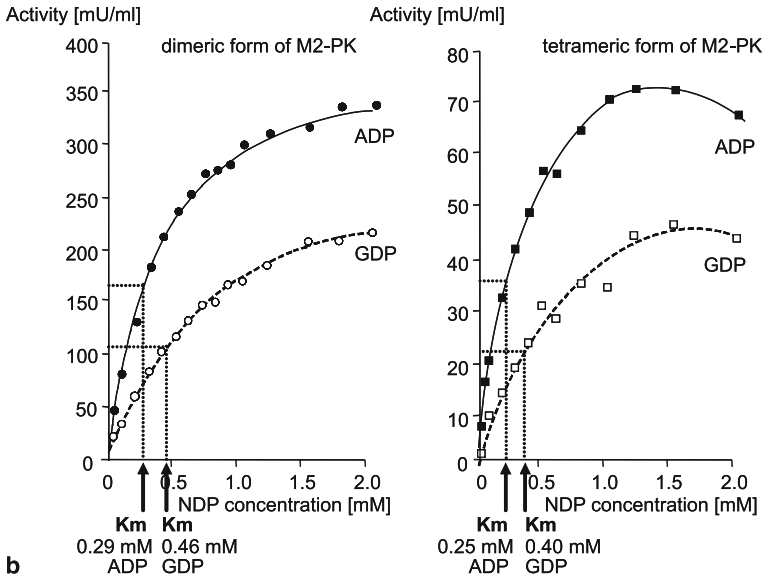
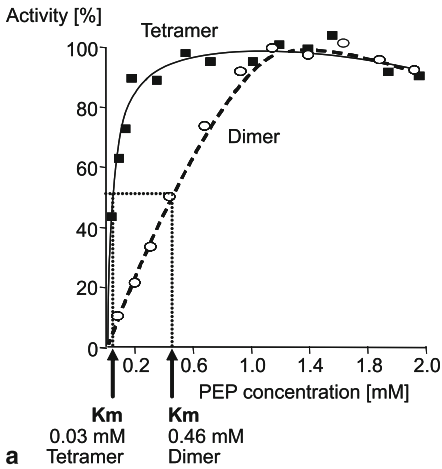
C-domain of two dimeric forms then associate to a tetrameric form. The C-domain contains 44 amino acids of the 56 amino acid stretch, which differs between the M1 and M2-PK subunits and is responsible for the different kinetic characteristics and regulation mechanisms found for M1 and M2-PK.

The upregulation of M2-PK is controlled by ras and the transcription factors SP1 and SP3 (Discher et al. 1998; Mazurek et al. 2001b). The M-gene furthermore has two HIF-binding sites (Kress et al. 1998; Stubbs et al. 2003; Brahimi-Horn and Pouyssegur 2007).

Whereas the other pyruvate kinase isoenzymes are characterized by a tetrameric quaternary structure, M2-PK may occur in a tetrameric form but also in a dimeric form. The tetrameric form of M2-PK has a high affinity to its substrate PEP and is highly active at physiological PEP concentrations. The dimeric form is characterized by a low affinity to PEP and is nearly inactive under physiological conditions (Fig. 3a).

Furthermore, the tetrameric form of M2-PK is associated with other glycolytic enzymes, such as hexokinase, glyceraldehyde 3-P dehydrogenase, phosphoglycerate kinase, enolase and lactate dehydrogenase, other enzymes such as nucleotide diphosphate kinase and adenylate kinase, components of the protein kinase cascade such as RAF, MEK and ERK as well as RNA in a cytosolic glycolytic enzyme complex (Hentze 1994; Nagy and Rigby 1995; Zwerschke et al. 1999; Mazurek et al. 2001a,b). The glycolytic enzyme complex can be isolated by isoelectric focusing using a buffer with low salt concentration. Proteins associated within the glycolytic enzyme complex focus at a common isoelectric point which is different than the isoelectric point of the purified proteins (Fig. 4). Migration of proteins in or out of the glycolytic enzyme complex are reflected by shifts in their isoelectric points. Accordingly, the dimeric form of M2-PK focuses outside the glycolytic enzyme complex at a more alkaline pH value. The close spatial proximity of the highly active tetrameric form of M2-PK to the other glycolytic enzymes of the

Fig. 3a,b. Affinity of the tetrameric and dimeric form of M2-PK (a) to their glycolytic substrate PEP and (b) to their dinucleotide substrates ADP and GDP. The tetrameric and dimeric form of M2-PK were isolated by gel permeation from MCF-7 cells



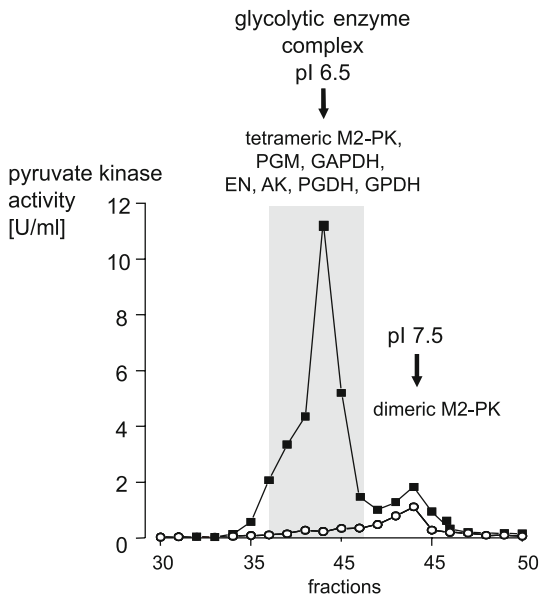


Fig. 4. Glycolytic enzyme complex in NRK cells. Migration of M2-PK into the glycolytic enzyme complex in ras-expressing NRK cells. Result of isoelectric focusing. \circ , parental NRK cells; \blacksquare , ras expressing NRK cells. (Mazurek et al. 2001a)

complex allows an effective conversion of glucose to pyruvate and lactate and correlates with the high rate of aerobic glycolysis described first by Otto Warburg (Warburg et al. 1924). The tumour-derived lactate lowers the pH value of the tumour environment and has been found to alter the phenotype and functional activity of dendritic cells in multicellular spheroid models (Gottfried et al. 2006).

Regarding the dinucleotide substrates, M2-PK has the highest affinity to ADP, but may also use GDP with lower affinity as a phosphate acceptor. In contrast to the PEP affinity, which is high in the case of the tetrameric form and low in the case of the dimeric form, the affinities to ADP and GDP do not differ between the tetrameric and dimeric form (Fig. 3b). The affinities to the dinucleotides UDP, CDP and TDP are low

(Mazurek et al. 1998). Accordingly, a high amount of the tetrameric form of M2-PK correlates with high ATP and GTP levels and a high ATP:ADP and GTP:GDP ratio (Fig. 1) (Zwerschke et al. 1999; Mazurek et al. 2001a,b).

The tetrameric form of M2-PK predominates in differentiated cells expressing M2-PK such as the lung. In tumour cells, M2-PK was found to be mainly in the inactive dimeric form, which at first glance appears to be inconsistent with the increased conversion of glucose to lactate described for a wide variety of tumours (Eigenbrodt and Glossmann 1980; Eigenbrodt et al. 1992). However, energy regeneration is not the only metabolic function of glycolysis in proliferating and especially tumour cells. Glycolytic intermediates are important precursors for the synthesis of cell building blocks, which are required in large amounts by proliferating cells (Fig. 1) (Eigenbrodt and Glossmann 1980; Pressek et al. 1988; Eigenbrodt et al. 1992, 1998; Zwerschke et al. 1999; Mazurek et al. 2001b; Miccheli et al. 2006). Glycerate 3-P is the precursor for the synthesis of serine and glycine, C₁ units, cysteine and sphingosine. Dihydroxyacetone-P provides the backbone for phospholipids. Ribose 5-P, the sugar component of nucleotides, can be synthesized from glucose 6-P via the oxidative pentose P pathway or from fructose 6-P and glyceraldehyde 3-P via the nonoxidative pentose-P pathway, whereby studies with C¹⁴ marked glucose revealed that in tumour cells 85% of the ribose 5-P is synthesized by thiamine-dependent transketolase via the nonoxidative pentose-phosphate pathway (Boros et al. 1998). Therefore, cell proliferation is only possible if enough energy-rich phosphometabolites are available. This regulation mechanism has been termed the metabolic budget system (Eigenbrodt et al. 1992). A high level of the nearly inactive dimeric form of M2-PK as found in tumour cells induces an increase in all glycolytic phosphometabolites above the pyruvate kinase reaction and favours channelling of glucose carbons into synthetic processes. Because of its low activity, the dimeric form of M2-PK correlates with low ATP and GTP levels and low ATP:ADP and GTP:GDP ratios. On the other hand, a high level of the dimeric form correlates with high rates of nucleic acid synthesis, which is especially reflected by an increase in the UTP and CTP concentrations. Thus, cell proliferation and a high amount of the dimeric form of M2-PK was found to correlate with a low ratio between purines

(ATP+GTP) and pyrimidines (UTP+CTP), whereas a high amount of the tetrameric form of M2-PK is accompanied by a high (ATP+GTP):(UTP+CTP) ratio (Fig. 1) (Ryll and Wagner 1992; Zwerschke et al. 1999; Mazurek et al. 2001a, 2001b). When M2-PK is mainly in the inactive dimeric form and not available for glycolytic ATP production, energy can be provided by the degradation of the amino acid glutamine to glutamate, aspartate, CO₂, pyruvate, citrate and lactate, a pathway termed glutaminolysis (Lobo et al. 2000; Mazurek et al. 2001a; Rossignol et al. 2004). Glutaminolysis and the truncated citric acid cycle have the metabolic advantage that the amount of acetyl CoA infiltrated into the citric acid cycle is low and that the acetyl CoA is saved for fatty acid and cholesterol de novo synthesis. Fatty acids can be used for phospholipid synthesis or can be released. Fatty acids and glutamate are immunosuppressive and may be capable of protecting tumor cells from immune attacks (Mazurek et al. 2002).

The tetramer:dimer ratio of M2-PK is not a stationary value in tumour cells and may oscillate depending on the concentration of key metabolites as well as oncoproteins. A key regulator of the tetramer:dimer ratio of M2-PK and the metabolic budget system is the glycolytic intermediate fructose 1,6-P2 (Eigenbrodt and Glossmann 1980; Eigenbrodt et al. 1992; Ashizawa et al. 1991; Mazurek and Eigenbrodt 2003). High fructose 1,6-P2 levels induce the reassociation of the inactive dimeric form of M2-PK to the highly active tetrameric form. Consequently, glucose is converted to pyruvate and lactate with the production of energy until fructose 1,6-P2 levels drop below a critical value to allow the dissociation to the dimeric form. Another activator of M2-PK is the amino acid L-serine, which allosterically increases the affinity of M2-PK to its substrate PEP and reduces the amount of fructose 1,6-P2 necessary for tetramerization. Serine is synthesized from the glycolytic intermediate glycerate 3-P and the glutaminolytic intermediate glutamate, thereby linking both pathways (Fig. 1). Serine is an essential precursor for phospholipid and sphingolipid synthesis as well as for glycine and activated methyl groups, which are necessary substrates in purine and pyrimidine synthesis. However, if the synthesis of activated methyl groups from serine exceeds a certain rate, tetrahydrofolate is irreversibly converted to N5-methyl-tetrahydrofolate, a methyl trap, and is consequently no longer available for nucleic acid de novo synthesis.

Therefore, the activation of M2-PK by serine is an effective regulatory feedback mechanism to prevent serine over-production and the methyl trap. An inhibition of M2-PK is induced by the glutaminolytic intermediate L-alanine as well as, L-cysteine, L-methionine, L-phenylalanine, L-valine, L-leucine, L-isoleucine and saturated and mono-unsaturated fatty acids. Furthermore, M2-PK is a target of the thyroid hormone 3,3',5-triiodo-L-thyronine (T₃), which binds to the monomeric form of M2-PK and prevents its association to the tetrameric form (reviewed in Eigenbrodt et al. 1992).

4 Interaction of M2-PK with Different Oncoproteins

4.1 Interaction Between M2-PK and pp60^{v-src}

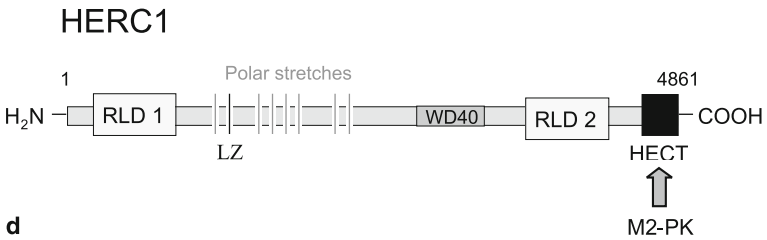
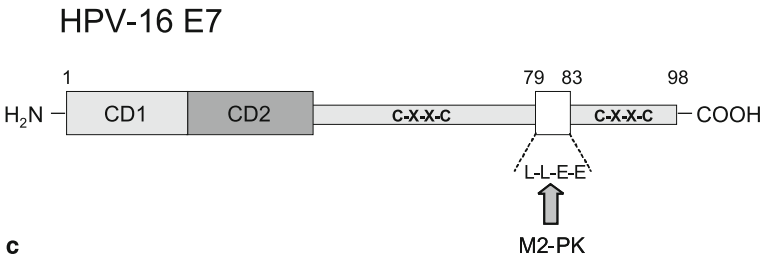
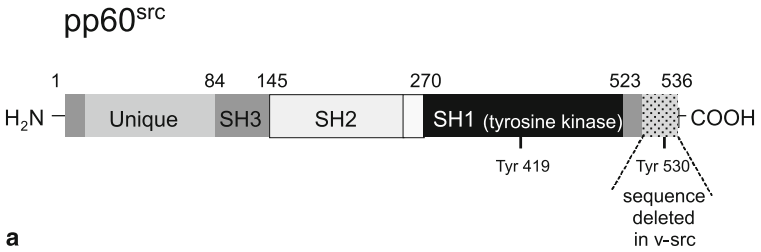
pp60^{v-src} is a 60 kDa nonreceptor tyrosine kinase. Expression of v-src in avian and mammalian cells leads to transformation. The normal cellular homologue of v-src is the proto-oncoprotein c-src. All src kinases contain a poorly conserved unique domain at the N-terminus, three conserved Src homology domains (SH3, SH2 and SH1, whereby SH1 harbours the tyrosine kinase domain) and a C-terminal regulatory domain (Fig. 5a) (Roskoski 2004; Prakash et al. 2007). Autophosphorylation of tyrosine 419 (human c-src) within the SH1 tyrosine kinase domain is necessary for optimal activity and leads to a stabilization of the active form. Inactivation is induced by binding of phosphorylated Tyr 530 (within human C-terminal regulatory domain) to its own SH2 domain. In contrast to c-src, within the C-terminal regulating domain, the viral counterpart lacks 19 amino acids, which include the negative regulating phosphorylation site, resulting in a high level of kinase activity and a high transforming potential. V-src has been shown to phosphorylate lactate dehydrogenase, enolase and the pyruvate kinase isoenzyme type M2 both in vitro and in vivo. Whereas in vitro phosphorylation activities of pp60^{v-src} and pp60^{c-src} were found to be qualitatively similar, in vivo the phosphorylation activities of pp60^{c-src} were only weak (Presek et al. 1980, 1988; Cooper et al. 1983; Eigenbrodt et al. 1983; Coussens et al. 1985). In chicken embryo cells, transfection with the temperature-sensitive mutant NY 68 of the Schmidt-Ruppin strain of Rous sarcoma virus induced tyrosine phosphorylation and dimerization

Fig. 5a–d. Molecular structure of oncoproteins interacting with M2-PK. **a** Human src protein: *SH*, src homology domain. **b** A-Raf protein: the conserved regions CR1 and CR2 represent the regulatory N-terminus of the enzyme. The CR3 domain harbours the catalytic activity. M2-PK binds to the very C-terminus, which is not conserved between the different Raf isoforms. **c** HPV 16-E7: deletion of aa 79–83 resulted in decreased affinity to M2-PK. *CD*, conserved domain; *CXXC*, putative zinc finger motifs. **d** HERC 1: *RLD*, RCC1 like domain; *LZ*, leucine zipper; *HECT*, Homologous to E6-AP-CARboxyl-terminus

of M2-PK within 3 h after the shift to the permissive temperature, with a maximal peak after 12 h. Similar results have been obtained with NIH 3T3 cells transfected with RSV ts LA90. The dimerization of M2-PK was accompanied by an increase in fructose 1,6-P₂, P-ribose-PP and 1,2 diacylglycerol (Presek et al. 1980, 1988; Eigenbrodt et al. 1998).

4.2 Interaction Between M2-PK and A-Raf

M2-PK can also be phosphorylated in serine. In tumour cells, serine phosphorylation of M2-PK was shown to be cAMP-independent and inducible by EGF (Oude Weernink et al. 1991; Moule and McGivan 1991; Eigenbrodt et al. 1998). However, a corresponding serine kinase remained long undiscovered. The yeast two-hybrid technique revealed that M2-PK specifically interacts with the A-Raf isoenzyme. The interaction between A-Raf and M2-PK takes place within the C-terminal domain of A-Raf (Fig. 5b). The interacting region, although part of the conserved domain 3, is not conserved between the different Raf isoforms, which may explain why the two other Raf isoforms B-Raf and c-Raf were not found to interact with M2-PK within the yeast two hybrid test (Le Mellay et al. 2002). Deletion of the N-terminal regulatory domain leads to constitutive active Raf forms. A fusion between the kinase domain of A-Raf and the retroviral gag-protein (gag-A-Raf) is able to transform NIH 3T3 cells. Co-transfection of NIH 3T3 cells with a kinase dead mutant of M2-PK (M2-PK K366M) reduced colony formation of stably A-Raf-expressing NIH 3T3 cells, whereas co-transfection of NIH 3T3 cells with gag-A-Raf and wild type M2-PK led to a doubling of focus formation, which points to a cooperative effect of A-Raf



d

and M2-PK in cell transformation (Le Mellay et al. 2002). The effect of A-Raf on the quaternary structure of M2-PK seems to depend on the basic metabolism of the individual cell line. In primary mouse fibroblasts, which are characterized by glutamine production and serine degradation, A-Raf wild type expression induced a dimerization and inactivation of M2-PK, which resulted in a reduction of the glycolytic flux rate. In immortalized NIH 3T3 fibroblasts characterized by glutamine degradation and serine production, gag-A-Raf transformation increased the highly active tetrameric form of M2-PK and favoured degradation of glucose to lactate under the regeneration of energy. High serine levels activate M2-PK. Thus the activation and tetramerization of M2-PK found in gag-A-Raf transformed NIH 3T3 cells may be a secondary metabolic effect induced by high serine levels (Mazurek et al. 2007).

4.3 Interaction Between M2-PK and Protein Kinase C Delta

Protein kinase C delta (PKC δ) was shown to play a role in apoptosis, metastasis and tumour suppression (Kiley et al. 1999; Perletti et al. 1999; Zhong et al. 2002). It remains unknown whether PKC δ is an oncogenic or tumour suppressive protein. Two-dimensional isoelectric focusing electrophoresis in combination with MALDI mass spectroscopy identified M2-PK as a new substrate of PKC δ (Siwko and Mochly-Rosen 2007). Immunoprecipitation experiments suggest that PKC δ binds to M2-PK and rapidly releases the enzyme after phosphorylation. In vitro incubation of M2-PK with purified PKC δ neither influenced the activity nor the tetramer:dimer ratio of M2-PK. However, an in vivo effect of PKC δ on M2-PK has not yet been investigated and therefore cannot be ruled out at this point. In PKC $\delta^{-/-}$ mice, an approximately twofold decrease in M2-PK levels was observed in comparison to the PKC $\delta^{+/+}$ mice suggesting that phosphorylation of M2-PK by PKC δ may regulate stability or degradation of M2-PK (Mayr et al. 2004).

4.4 Interaction Between M2-PK and HPV-16 E7

The E7 oncoprotein of the human papillomavirus type 16 (HPV-16 E7) cooperates with the HPV-16 E6 oncoprotein to immortalize human keratinocytes (Münger and Howley 2002). Thus, HPV-16 belongs to the

high-risk types of human papillomavirus and is linked to malignant human cervix cancer (zur Hausen 2002). HPV-16 E7 consists of 98 amino acids and contains two conserved domains CD1 and CD2 at the N-terminus and two zinc finger motifs (C-X-X-C) at the carboxy terminus (Fig. 5c). The conserved domain 2 (CD2) within the N-terminus mediates binding of E7 to proteins of the retinoblastoma gene family, thereby contributing to the deregulation of the cell cycle (Münger and Howley 2002). The carboxy terminus of HPV-16 E7 acts as an interaction domain for M2-PK. Deletion of the amino acids 79–83 (Leu, Leu, Glu, Glu) within the HPV-16 E7 protein leads to a reduced affinity of HPV-16 E7 to M2-PK and reduces the transforming potential of E7, suggesting that binding of M2-PK may play a role in cell transformation (Zwerschke et al. 1999). Thus, the transforming activity of E7 is sensitive to mutations in both the N-terminus as well as the C-Terminus (Jewers et al. 1992). NIH 3T3 cells which are already immortal are transformed by E7 alone, whereas transformation of primary normal rat kidney cells (NRK) require expression of a second oncoprotein ras (Zwerschke et al. 1999; Mazurek et al. 2001a,b). The parental NRK cells were characterized by low glycolytic enzyme activities and a low glycolytic flux rate. The stable expression of ras induced an increase in most of the glycolytic enzymes, including 6-phosphofructo 1 kinase (PFK) and M2-PK as well as an increase in the glycolytic flux rate. The increase in PFK activity correlated with an increase of fructose 1,6-P2 levels, which resulted in a tetramerization and migration of M2-PK into the glycolytic enzyme complex in close proximity to adenylate kinase (AK) (Fig. 4). The close association between the highly active M2-PK and AK led to a decrease in ATP and an increase in AMP levels. High AMP levels inhibit cell proliferation by inhibiting P-ribose PP synthetase, a key enzyme in purine and pyrimidine synthesis (Mazurek et al. 1997). Accordingly, in ras-expressing cells, cell proliferation was inhibited. However, the expression of ras dramatically boosts tumour metabolism, thereby preparing the metabolome of the cells for transformation. In E7-transformed cells, binding of E7 to M2-PK induced a dimerization and migration of M2-PK out of the glycolytic enzyme complex which favoured the channelling of glucose carbons into synthetic processes. Accordingly, UTP and CTP levels increased, whereas ATP and GTP levels decreased in E7 transformed cells.

4.5 Interaction Between M2-PK and HERC1

HERC 1, also termed oncH according to its identification in a nude mouse tumorigenicity assay and p532 according to its molecular weight, is one of four proteins within the human HERC protein family (Rosa et al. 1996). HERC proteins contain a HECT (homologous to E6 AP carboxyl terminus) domain in their carboxyl-terminus and one or more RCC1-like domains (RLDs) elsewhere in their amino acid sequence. RCC1 (regulator of chromosome condensation 1-protein) is a guanine nucleotide exchange factor (GEF) for RAN, a small GTP-binding protein which is predominantly located in the nucleus and involved in the nuclear transport of proteins with nuclear localization signals. HERC 1, which was shown to be consistently over-expressed in several tumour cell lines, contains two RLD domains (Fig. 5d). RLD1 is a GEF for ARF-1, Rab3a and Rab5, which are all three GTPases involved in cellular membrane trafficking (Rosa et al. 1996). RLD2, for which as yet no GEF activity has been shown, specifically binds to ARF-1 in the Golgi apparatus as well as to clathrin and Hsp70 (Rosa and Barbacid 1997). The yeast two hybrid technique, in vitro pull-down experiments, as well as in vivo pull-down experiments in Sf9 insect cells infected with baculovirus encoding full-length M2-PK and the His-tagged HECT domain of HERC1 (last 366 aa of HERC1), revealed that M2-PK specifically binds to the HECT domain of the HERC1 protein (Garcia-Gonzalo et al. 2003). The M2-PK sequence involved in HERC1 binding contains the critical residues for fructose 1,6-P2 binding as well as for the intersubunit contact. HECT domains confer E3 ubiquitin protein ligase activity and are involved in protein degradation. However, all results so far appear to indicate that the interaction of M2-PK with HERC1 influences neither M2-PK activity nor the tetramer:dimer ratio of M2-PK, nor does it induce ubiquitination and increased degradation of M2-PK (Garcia-Gonzalo et al. 2003). Therefore, the physiological function of the interaction between M2-PK and HERC1 is still not known. Since M2-PK also phosphorylates GDP, it is conceivable that M2-PK may function as a local GTP producer (nano machine) for the RLDs as well as for the GTPases ARF-1 and Rab5.

5 Role of M2-PK in the Nucleus

M2-PK contains an inducible nuclear translocation signal (NLS) in its C-domain, which, in contrast to classical NLS, is not rich in arginine and lysine (Hoshino et al. 2007). The role of M2-PK within the nucleus is complex since pro-proliferative as well as pro-apoptotic stimuli have been described. In BB13 cells, an interleukin 3-dependent haematopoietic cell line, which ectopically expresses the EGF receptor, IL-3 stimulation induced a translocation of M2-PK into the nucleus within 30 min. The IL-3 stimulated nuclear translocation of M2-PK was dependent on JAK2. In the same cell system, the over-expression of a construct of the M2-PK protein fused with the NLS from SV40-T antigen enhanced EGF-stimulated cell proliferation in the absence of IL-3 (Hoshino et al. 2007). The mechanism by which nuclear M2-PK enhances cell proliferation is yet not clear. In Morris hepatoma 7777 tumour cells, nuclear M2-PK was found to participate in the phosphorylation of histone 1 by direct phosphate transfer from PEP to histone 1 (Ignacak and Stachurska 2003). On the other hand, nuclear translocation of M2-PK induced by the somatostatin analogue TT 232, H₂O₂ or UV light has recently been linked to the induction of a caspase-independent programmed cell death (Stetak et al. 2007).

6 Tumour M2-PK: A Biomarker for Metabolic Profiling of Tumours

Measurements of v-max activities in different cell lines allowed the classification of proliferating cell lines in the following three groups: nontumour normal proliferating cells with PK-v-max activities between 30 and 950 mU/mg protein, tumour cell lines with PK-v-max activities between 900 and 1300 mU/mg protein and metastatic tumour cell lines with PK-v-max activities between 1590 and 1630 mU/mg protein (Board et al. 1990). V-max activities of enzymes are measured at saturated substrate concentrations. In the case of M2-PK v-max, activities were measured at saturated PEP concentrations, which means that both the tetrameric and dimeric form are highly active. In contrast, at physiological PEP concentrations, only the tetrameric form of M2-PK

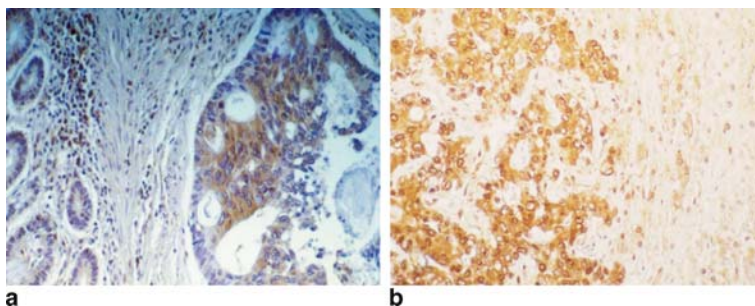


Fig. 6a,b. Immunohistochemistry staining of (a) rectum carcinoma and (b) metastases of the rectum carcinoma in the liver with the monoclonal anti M2-PK antibody clone DF4. (Hardt et al. 2004a; Mazurek 2008)

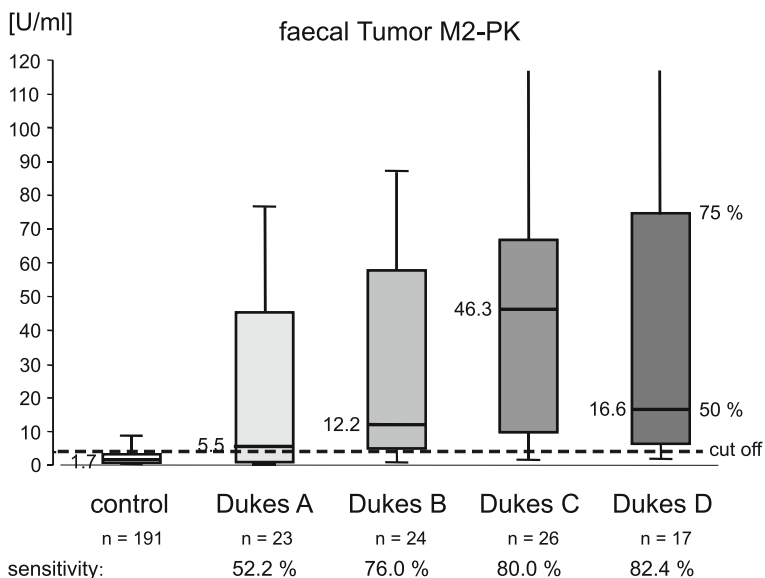


Fig. 7. Correlation between faecal tumour M2-PK and TNM staging. (Hardt et al. 2004b)

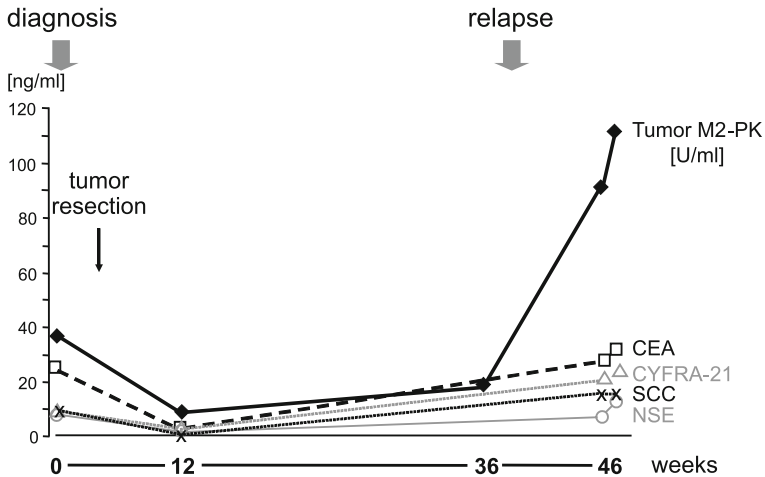


Fig. 8. Follow-up study of a patient with a squamous carcinoma ($T_2N_1M_0$) of the lung. After surgical resection, a local relapse surrounding the arteria pulmonalis and metastases in infracranial and paratracheal lymph nodes occurred between weeks 36 and 46. (Schneider et al. 2002)

is highly active, whereas the dimeric form is nearly inactive (Fig. 3a). Immunohistological staining of various tumours with monoclonal antibodies which specifically recognize the dimeric form of M2-PK allows the visualization of the pyruvate kinase isoenzyme shift in tumour cells. This technique shows that the distribution of M2-PK in primary tumours can be heterogeneous, whereas their metastases are always stained very homogeneously (Fig. 6).

Furthermore, the dimeric form of M2-PK (tumour M2-PK) is released from tumour cells into the blood and from gastrointestinal tumours also into the stool of tumour patients, most likely by tumour necrosis and cell turnover, providing the possibility of diagnostic application. Thus, the amount of tumour M2-PK was found to increase in the EDTA plasma of patients with renal cell carcinoma, melanoma, lung, breast, cervical, ovarian, oesopharyngeal, gastric, pancreatic and colorectal cancer as well as in stool samples of patients with gastric and colorectal cancer and to correlate with tumour stages (Fig. 7) (Wech-

sel et al. 1999; Lüftner et al. 2000; Schneider et al. 2002; Hardt et al. 2004b; Kaura et al. 2004; Ahmed et al. 2007; Koss et al. 2008; Kumar et al. 2007).

Therefore, tumour M2-PK is an organ-unspecific biomarker which reflects the metabolic activity and proliferation capacity of tumours. An important field of application of the plasma test are follow-up studies to monitor failure, relapse or success during therapy (Fig. 8).

Interestingly, in different human gastric carcinoma cell lines, cisplatin resistance was found to correlate with low M2-PK protein levels and activity (Yoo et al. 2004). Low PK activities promote synthetic processes debranching from glycolysis, such as the oxidative pentose P-shuttle, an important source for NADPH production within cells. NADPH is necessary for reduction of glutathione (GSSG) and activation of the thioredoxin system, both of which have been shown to be involved in cisplatin resistance.

7 Conclusions

The expression of the pyruvate kinase isoenzyme type M2, which can switch between a highly active tetrameric form and a nearly inactive dimeric form, is an important metabolic sensor to adapt tumour metabolism to different metabolic conditions, such as nutrient supply. The quantification of the dimeric form of M2-PK in plasma and stool is a tool for early detection of tumours and therapy control.

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