Chapter **47**

Tumor M2-PK: A Marker of the Tumor Metabolome

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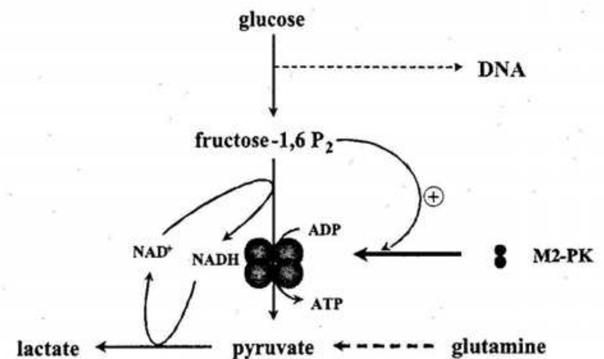
Analogous to *genome* and *proteome*, the expression *metabolome* was coined to summarize the metabolic network of the cells. The tumor metabolome is generally characterized by a high glycolytic capacity, high phosphometabolite levels, a high channeling of glucose carbons to synthetic processes (such as nucleic acid, phospholipid, and amino acid synthesis), a low (ATP+GTP) : (CTP+UTP) ratio, and a high glutaminolytic capacity (Figure 1) (1,2).

GENERAL ROLE OF TUMOR M2-PK IN THE TUMOR METABOLOME

The key regulator of the metabolic alterations found in tumor cells is the glycolytic isoenzyme pyruvate kinase type M2 that is generally expressed in all proliferating cells and overexpressed in all tumor cells investigated to date (1,2) (*http://www.pubmed.gov*; see nucleotides and tumor pyruvate kinase type M2).

During carcinogenesis a shift in the pyruvate kinase isoenzyme equipment always takes place, such that the tissue-specific isoenzymes disappear (such as M1-PK in muscle and brain, L-PK in liver and kidney, and R-PK in red blood cells), and M2-PK is expressed (1,2).

M2-PK can exist in a highly active tetrameric form with a high affinity to its substrate, phosphoenolpyruvate (PEP), and in a dimeric form with a low PEP affinity. The dimeric form of M2-PK is virtually inactive at cellular PEP concentration. When M2-PK is dimeric, glucose carbons are channeled to synthetic processes, such as nucleic acid, phospholipid, and amino acid synthesis (Figure 1). Furthermore, there is increased conversion of the amino acid glutamine to lactate, which has been termed *glutaminolysis* in analogy to *glycolysis,* to ensure the energy production of the tumor cells. This is due to the lack of pyruvate caused by the inhibition of pyruvate kinase in tumor cells. High levels of the glycolytic phosphometabolite fructose-1,6-P_2 (FBP) and of the amino acid serine induce reassociation from the dimeric to the tetrameric form (Figure 1). Pyruvate kinase catalyzes the transfer of phosphate from PEP to adenosine diphosphate (ADP) or guanosine diphosphate (GDP) under the production of pyruvate and adenosine triphosphate (ATP) or guanosine triphosphate



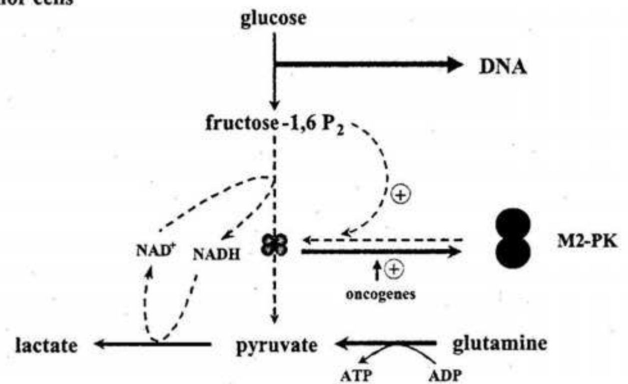


Figure 1 Metabolic scheme for the regulatory role of pyruvate kinase type M2. **Color representation of figure appears on Color Plate 9.**

(GTP), respectively. Thus, within the glycolytic sequence, pyruvate kinase is responsible for net energy production. Consequently, pyruvate kinase controls the ATP:ADP and GTP:GDP ratio and also the adenosine monophosphate (AMP) levels (together with adenylate kinase and 6-phosphofructo-1-kinase) (2). An increase in AMP levels inhibits DNA synthesis and cell proliferation by decreasing nicotinamide adenine dinucleotide (NAD), uridine triphosphate (UTP), and cytidine triphosphate (CTP) synthesis. By this mechanism M2-PK adapts energy-consuming cell proliferation and nutrient supply (1, 2).

In tumor cells a high amount of the less active, dimeric form of M2-PK is always found. Therefore, the dimeric form of M2-PK has been termed Tumor M2-PK (Tu M2-PK) (1).

The ability of Tumor M2-PK to reassociate to the highly active tetrameric form at high FBP and serine concentrations shifts the tumor metabolome from anabolic processes, in which the glucose carbons are channeled to synthetic processes, to energy-producing (catabolic) processes (2). In contrast to mitochondrial respiration, energy production by pyruvate kinase is independent of oxygen supply. Therefore the oscillation of M2-PK between the highly active tetrameric and the less active dimeric form allows tumor cells to proliferate despite the poor vascularization and strong variations in glucose and oxygen supply that are generally found in solid tumors (1). Presumably due to its central role in regulating catabolism and anabolism, M2-PK is directly targeted by certain oncoproteins, such as $pp60^{v\text{-}src}$ kinase and the E7 oncoprotein of the human papilloma virus type 16 (HPV-16). The $pp60^{v\text{-}src}$ kinase phosphorylates M2-PK in tyrosine; the HPV-16 E7 oncoprotein directly binds to M2-PK. Both interactions lead to a dimerization of M2-PK and higher FBP and serine levels are necessary to reassociate M2-PK to the tetrameric form (1,2).

QUANTIFICATION OF TUMOR M2-PK

In order to study the central role of pyruvate kinase in cell metabolism, monoclonal antibodies against the different isoenzymes of pyruvate kinase, as well as to the tetrameric and dimeric forms of M2-PK, have been produced. Immunohistological studies of various tumors have revealed a heterogeneous distribution of Tumor M2-PK in the primary tumors, whereas their metastases are characterized by a homogeneously large amount of M2-PK.

An enzyme-linked immunosorbent assay (ELISA) for the determination of Tumor M2-PK in ethylenediaminetetraacetic acid (EDTA)-plasma is available from ScheBo Biotech AG, Giessen, Germany.

In accordance with results from immunohistological staining, the amount of Tumor M2-PK in EDTA-plasma samples from patients with renal cell carcinoma, pancreatic, lung, breast, and colon cancer directly reflected the metastatic state of the tumors (3–9). Therefore, Tumor M2-PK is a strong marker of metastasis, but not an organ-specific marker.

The recommended cut-off value for Tumor M2-PK of 15 U/mL corresponds to a specificity of 90% in a control group with nonmalignant diseases. Investigations in patients with a variety of diseases other than tumors revealed an increase of Tumor M2-PK only in severe inflammation and polytrauma (3,10). This Tumor M2-PK mainly derives from activated granulocytes, which elevate PK levels more than 20-fold after polytrauma (10).

Tumor M2-PK plasma concentration reflects tumor size and growth in various tumor cells and is well correlated with tumor staging (3–9). Increasing Tumor M2-PK plasma concentrations were observed with progressive tumor stages in lung cancer patients (Figure 2). Lung cancer patients in disease stages III or IV had significantly higher Tumor M2-PK concentrations than those in stage I.

Figure 2 shows the high correlation between Tumor M2-PK levels and the staging of different tumors. In lung tumors the sensitivity of Tumor M2-PK is calculated to be 28% in stage I, increasing progressively to 73% in stage IV. A similar correlation between Tumor M2-PK and the Robson score is found for kidney tumors, increasing from 25% in stage I to 71% in stage IV, as well as for gastrointestinal tumors (5–7,9).

However, the sensitivity for nonmetastatic tumors can be strongly improved by the combination of Tumor M2-PK with appropriate "traditional" organ-specific markers. These include carcinoembryonic antigen (CEA) in colorectal cancers; CA19-9 in pancreatic cancer; and CA-72-4 in esophageal and gastric cancers; alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) in testicular cancer; and CYFRA 21-1 or neuron-specific enolase (NSE) in lung cancer (2,5,7,9). In patients suffering from silicosis, the use of X-rays for the detection of lung cancer is of limited diagnostic value. Efficiency in pre-invasive diagnosis of suspicious lesions will be improved by using tumor markers in the differential diagnosis between malignant and benign diseases.

Overall, the greatest advantage of Tumor M2-PK as an organ-unspecific marker is that it detects metastatic occurrence and aggressively growing tumors with a sensitivity of 60–70 % by a simple blood measurement.

STUDIES MONITORING THE EFFICACY OF TUMOR THERAPY USING TUMOR M2-PK

Several chemotherapeutic drugs, such as cisplatin, cyclophosphamide, and vinblastine, or modalities such as radiation that target DNA, depress NAD levels and glycolysis (1). These effects take place since poly-(ADP-ribose) polymerase (PARP), the enzyme that is responsible for DNA repair, consumes NAD. The decreased NAD level inhibits glyceraldehyde 3-phosphate dehydrogenase and lactate dehydrogenase, and increases FBP. In normal proliferating cells, these FBP levels are high enough to reassociate the dimeric form of M2-PK to the tetrameric form, which is the opposite of the situation in tumor cells. Therefore, in normal cells, sufficient nucleotide triphosphates

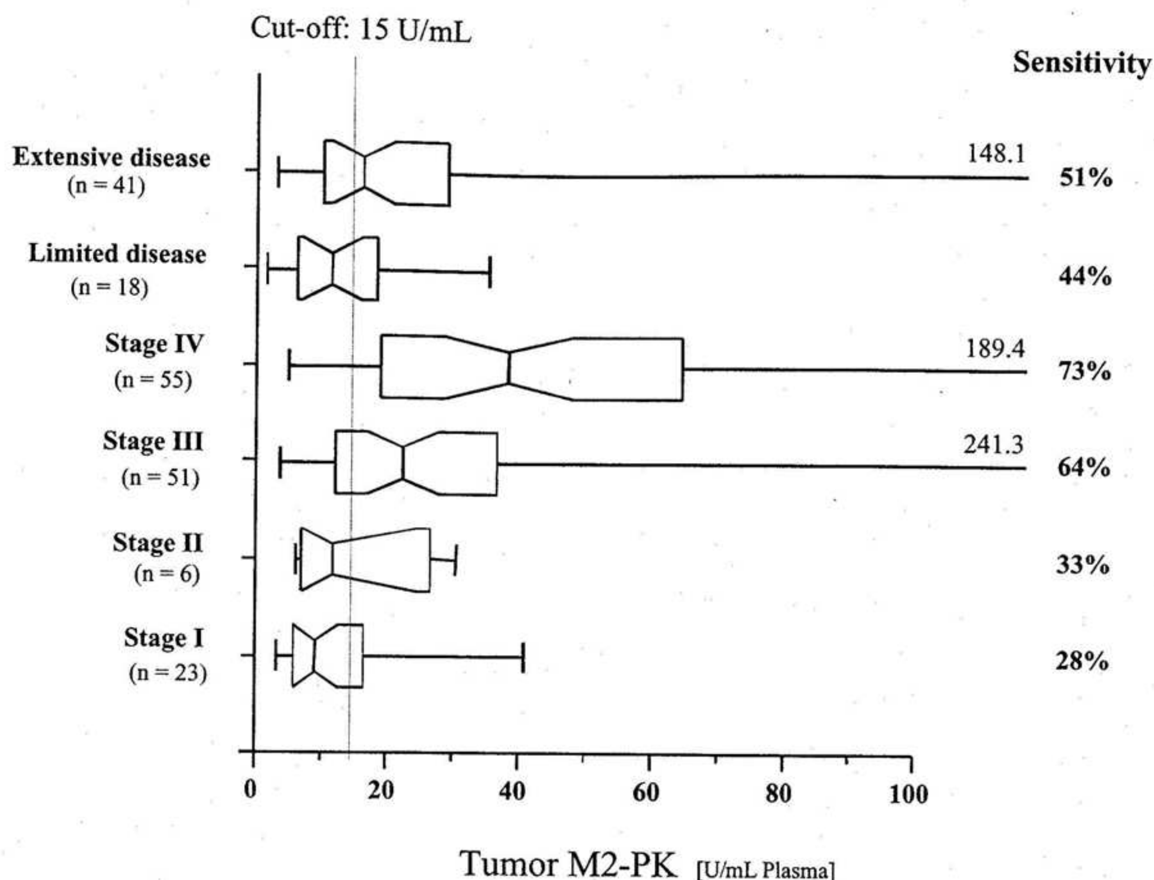


Figure 2 Correlation between Tumor M2-PK plasma concentrations and staging in lung cancer. The whiskers indicate the 25th and 75th percentiles. The center of the notches indicates the median. Lack of overlap indicates significant differences between the groups. Limited disease is compared with extensive disease for small-cell lung cancers. Stages I–IV are presented for all non-small-cell carcinomas. The sensitivity was calculated using the manufacturer's cut-off. **Color representation of figure appears on Color Plate 10.**

(NTPs) are available for DNA repair and the cells survive (Figure 1).

In tumor cells, the increase of FBP levels is not high enough for the tetramerization of M2-PK. Consequently tumor cells are unable to maintain sufficiently high NTP levels for DNA repair and the cells undergo apoptosis (1). The amount of Tumor M2-PK has been demonstrated to increase accordingly in necrotic areas in renal cell carcinomas.

Thus, the measurement of Tumor M2-PK in EDTA plasma samples from tumor patients allows direct monitoring of the energy status of the tumor cells during tumor therapy. Indeed, studies with breast cancer, metastatic renal cancer, and lung cancer patients revealed that the levels of Tumor M2-PK directly reflected the success or failure of the tumor therapy protocol (Figures 3A,B) (3,8,9).

Therefore, the measurement of Tumor M2-PK levels in EDTA plasma samples during tumor therapy promises to be an efficient tool to enable clinicians to apply an individualized, enzyme-guided tumor therapy for each patient (e.g., choice of therapy, dose, and duration).

FUTURE ASPECTS

Tumor M2-PK levels will be compared with glucose uptake by positron emission tomography (PET) in future studies in order to characterize the metabolic status of the tumor and to further individualize tumor therapy based on the individual tumor metabolome (1).

Furthermore, using long-term measurements of Tumor M2-PK levels in EDTA-plasma samples from healthy controls and from high-risk groups (such as smokers or workers with prior exposure to carcinogenic agents), Tumor M2-PK will be tested as a "generalized" marker for the early detection of malignant tumors, both alone and in combination with other markers.

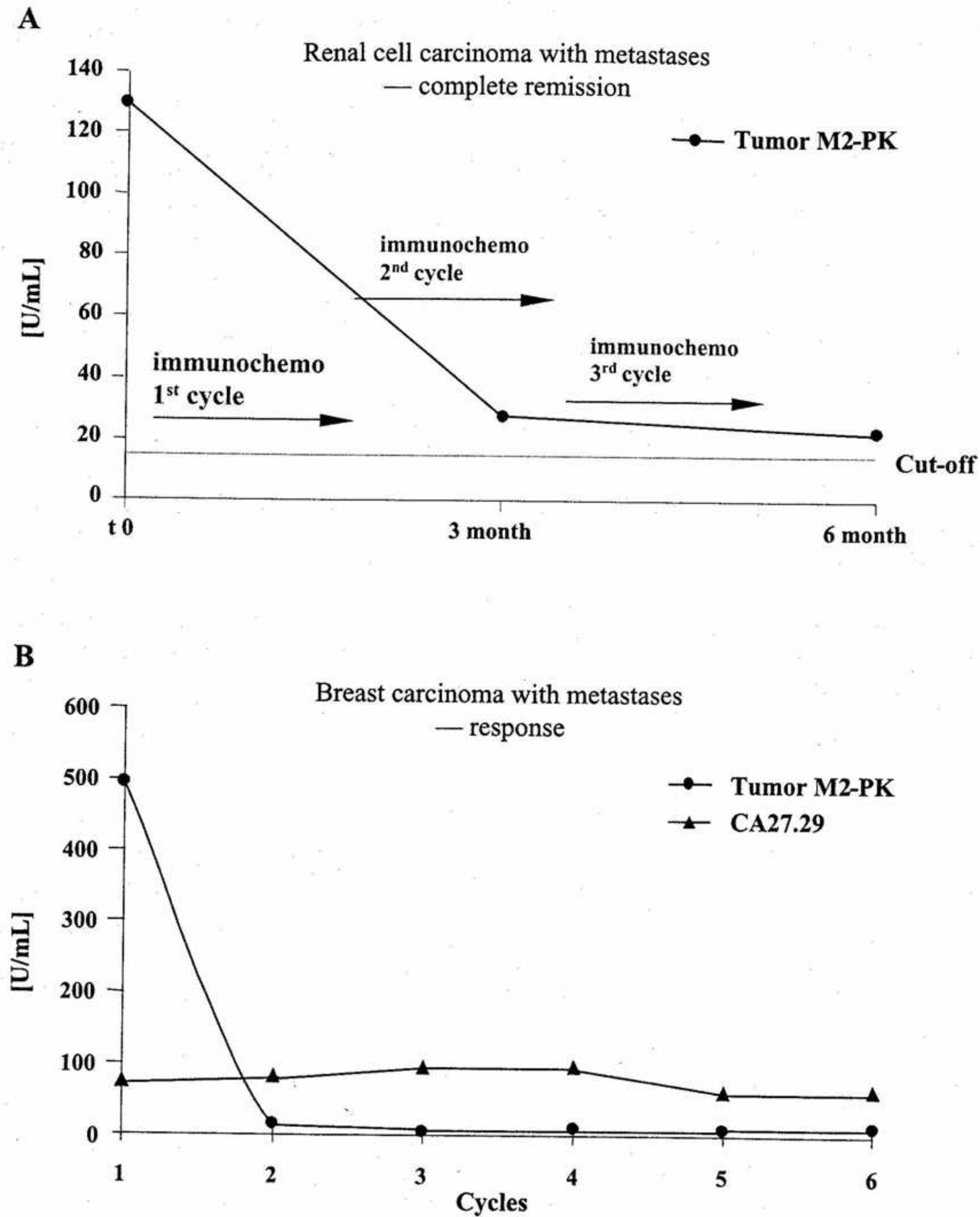


Figure 3 (A) Metastasized renal cell carcinoma: follow up with Tumor M2-PK. Tumor M2-PK levels in a patient with metastatic renal cell cancer (Robson IV) under immunochemotherapy, with complete remission after eight months. Radiological monitoring correlated well with Tumor M2-PK levels. (B) Longitudinal levels of Tumor M2-PK and CA27.29 levels in relation to response. Patient with partial response: elevated baseline level with prompt normalization after the start of chemotherapy. **Color representation of figure appears on Color Plate 11.**

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