Humangenetik 30, 181—185 (1975)

Hexokinase Isozymes in Human Neoplastic and Fetal Tissues: The Existence of Hexokinase II in Malignant Tumors and in Placenta

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Received June 30, 1975

Summary. The distribution of electrophoretic fractions of hexokinase (HK E.C. 2.7.1.1) in extracts of 53 human primary tumors, homologous normal tissues and fetal organs was investigated. It was found that the fraction HK II is present in every malignant tumor and in placenta tissue.

Zusammenfassung. Die Verteilung elektrophoretischer Fraktionen der Hexokinase (HK, E.C.: 2.7.1.1) in Extrakten 53 menschlicher Primärtumoren, homologen normalen Gewebes und fetaler Organe wurde untersucht. Die Fraktion HK II existiert in allen malignen Tumoren und im Placentagewebe.

Several authors reported a replacement of the isozyme hexokinase IV, *i.e.* glucokinase, by hexokinase II in poorly differentiated rat hepatomas making the distribution pattern similar to that in rat fetal liver (Farina *et al.*, 1968; Sato *et al.*, 1969). In human malignant hepatomas Balinsky *et al.* (1973) found a similar isozymic shift.

We extended these studies upon a variety of human tumors, searching for isozymic patterns characteristic of human cancer in general. We, furthermore, looked for similarities between isozymic patterns of neoplastic and fetal tissues.

Materials and Methods

Tissues. Extracts of the following primary human tumors and of homologous normal organ tissues were studied: 6 glioblastomas, 3 astrocytomas, 4 meningiomas, 4 melanoblastomas, 7 lung-, 11 kidney-, 8 stomach-, 4 rectal-, 2 colonic-, 1 sigmoid-, 1 caecum- and 2 skin carcinomas. Furthermore, organs of 6 fetuses between the second and seventh month of gestation period and corresponding placenta tissues were examined. The extraction method is described elsewhere (Kamel, 1974; Kamel and Schwarzfischer, 1975).

Electrophoretic Assay. We employed a variation of the method described by Grossbard and Schimke (1966). Horizontal starch gel electrophoresis was performed with the same buffer system, but hexokinase bands were visualized by a more sensitive method: Without NBT and PMS, 1.4 mM NADP and 70 i.u. of glucose-6-phosphate DH were sufficient for detecting

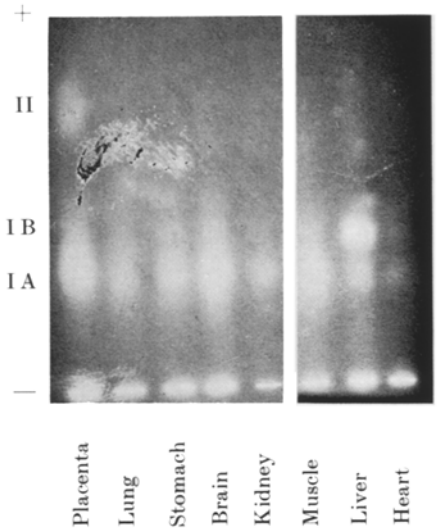

Fig. 1

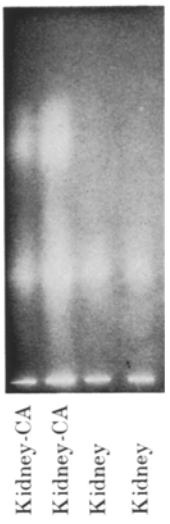

Fig. 2

Fig. 1. Electropherogram of hexokinase fractions in organs of a 3-months fetus. HK II exists in placenta extract

Fig. 2. Distribution of HK I and II in a kidney carcinoma and in corresponding normal kidney. A strong HK II fraction appears in the tumor

fluorescent NADPH-bands (356 nm) at the sites of hexokinase activity after 2—3 hrs of gel incubation at 37°C; other data agree with those reported by the authors.

Results

The separated electrophoretic fractions of hexokinase, which are designated HK I and HK II, consist of further bands, the sharpness of which very much depends on the charge of CONNOUGHT-starch used. HK I occurs in every tissue and represents the main fraction. Fig. 1 shows a division of this fraction into two HK-bands in the liver of a 3-months fetus. Here HK-IB is the more intensive band. In the other organs a strong HK-IA band occurs. An additional fraction HK II appears only in placenta.

The same fraction HK II is found to exist in every tumor, except in the rather benign meningiomas and astrocytomas of the first degree. Glioblastomas, melanoblastomas and kidney carcinomas show HK I and HK II, while in normal brain, chorioidea and kidney only HK I exists (Fig. 2). In lung, stomach, intestine and skin HK I and a faint HK II are encountered. This HK II fraction becomes more intensive in corresponding carcinomas.

Discussion

The regular appearance of a strong hexokinase II-component in addition to the main form HK I is correlated with the regular isozymic shift from pyruvate

kinase IV to PK I in all malignant human tumors, which we have studied (Kamel, 1974; Kamel and Schwarzfischer, 1975).

Like pyruvate kinase, hexokinase is a regulatory enzyme functioning at a delicate point in the glycolytic sequence; it is controlling the entrance of the first substrates of glycolysis into the cell. That such key enzymes own particular forms, which are required and synthesized in malignant cells, is now an evidence.

In the case of hexokinase, we do not encounter an isozymic shift towards a more active form, but an additional component, which leads to the same result: the elevation of total enzyme activity and thus the more effective sugar phosphorylation. A highly active hexokinase in cooperation with a highly active pyruvate kinase form is responsible for the elevated glycolytic capacity in malignant cells, which leads to the production of high levels of pyruvic acid required for mobilising the citric acid cycle and the respiratory pathways. These are known to function at maximal rates and without reserves in malignant cells. Excessive pyruvic acid is converted to lactate by lactate dehydrogenase, of which the form L.D.H.A is elevated in all tested human malignant tumors (Kamel, 1974). L.D.H.A is the more convenient isozyme for this task, as it is not inhibited by excess of pyruvic acid.

The model for cooperation of the isozymes of HK, PK and L.D.H. in human malignant cells could be roughly demonstrated as follows:

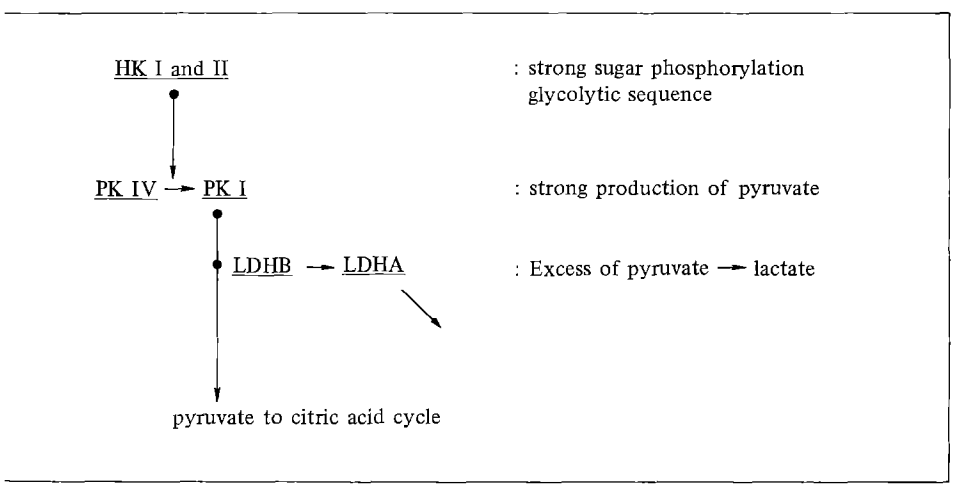

Diagram 1. Cooperation of glycolytic isozymes in human cancer cells

We do not postulate that these isozymic aberrations are the first cause of neoplastic transformation in general, as they are absent from benign tumors (meningiomas and astrocytomas of the first degree), but we think, that such aberrations are necessary as further events, if transformation of cell metabolism towards malignancy is to be accomplished.

The genetic basis of these correlated isozymic alterations remains unclear; we would like to suggest two possibilities:

1. If synthesis of the multiple molecular forms of HK, PK and LDH is controlled by the same mechanism (regulatory gene ?), then only one mutative event could lead to the aberrant synthesis of HK II, PK I and LDHA in human cancer cells.

2. If more than one mechanism is involved, then more than one mutation is necessary: to allow synthesis of the "malignancy isozymes" and, thus, to accomplish malignant transformation.

The hypothesis of derepression of fetal genes in human cancer cells has been supported by showing that the "malignancy-isozyme" PK I is predominant in fetal life between the 2nd and 7th month of gestation period (Kamel, 1974; Kamel and Schwarzfischer, 1975).

In contrast, the hexokinase fraction HK II, also typical for human cancer, shows a strong band only in placenta, while the other fetal organ tissues have HK-isozyme patterns very similar to those of normal adult organs.

Whether a strong HK II is synthesized in other fetal tissues before the 2nd month of gestation period remains to be investigated.

We would like to call HK II a "malignancy — and placental component" in analogy to the "Regan Isozyme" of alcaline phosphatase (Fishman *et al.*, 1968), looking at it as a further support for the hypothesis of derepression of early fetal genes in human cancer. Neither in placenta, nor in the other fetal organs, an isozymic shift from LDH B to LDH A occurs, as it does in malignant tumors (Kamel, 1974).

So the correlation between HK II, PK I and LDHA, typical for human cancer, is not realized in fetal organs and only partially realized in placenta:

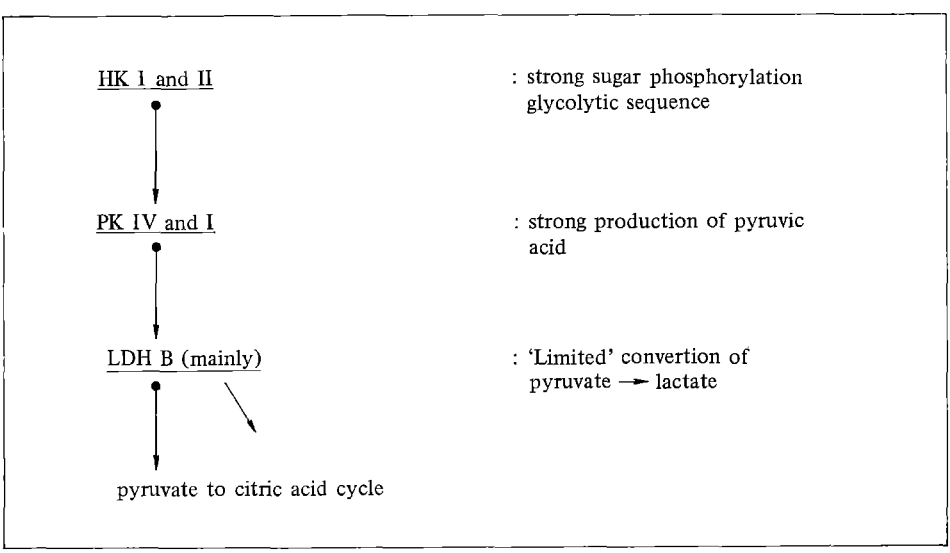

Diagram 2. Cooperation of glycolytic isozymes in human placenta tissue

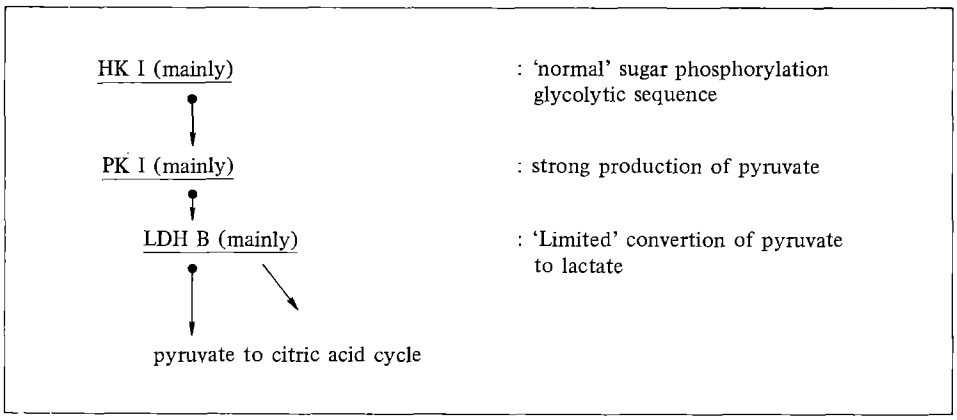

Diagram 3. Cooperation of glycolytic isozymes in other human fetal organ tissues

In conclusion we want to suggest:

1. The first mutative events starting the transformation procedure remain unclear.

2. As a further event, some other mutations are necessary to allow or amplify synthesis of particular glycolytic isozymes, of which HK II, PK I, and LDH A were discussed here. This is essential for elevating the glycolysis capacity and, thus, for finishing the malignant transformation procedure.

3. Not all activated genes in cancer are fetal ones, so malignant transformation is more, than just throwing the cell back to a fetal status.

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