Aberrant Glycolytic Metabolism of Cancer Cells: A Remarkable Coordination of Genetic, Transcriptional, Post-translational, and Mutational Events That Lead to a Critical Role for Type II Hexokinase

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For more than two-thirds of this century we have known that one of the most common and profound phenotypes of cancer cells is their propensity to utilize and catabolize glucose at high rates. This common biochemical signature of many cancers, particularly those that are poorly differentiated and proliferate rapidly, has remained until recently a "metabolic enigma." However, with many advances in the biological sciences having been applied to this problem, cancer cells have begun to reveal their molecular strategies in maintaining an aberrant metabolic behavior. Specifically, studies performed over the past two decades in our laboratory demonstrate that hexokinase, particularly the Type II isoform, plays a critical role in initiating and maintaining the high glucose catabolic rates of rapidly growing tumors. This enzyme converts the incoming glucose to glucose-6-phosphate, the initial phosphorylated intermediate of the glycolytic pathway and an important precursor of many cellular "building blocks." At the genetic level the tumor cell adapts metabolically by first increasing the gene copy number of Type II hexokinase. The enzyme's gene promoter, in turn, shows a wide promiscuity toward the signal transduction cascades active within tumor cells. It is activated by glucose, insulin, low oxygen "hypoxic" conditions, and phorbol esters, all of which enhance the rate of transcription. Also, the tumor cell uses the tumor suppressor p53, which is usually modified by mutations to debilitate cell cycle controls, to further activate hexokinase gene transcription. This results in both enhanced levels of the enzyme, which binds to mitochondrial porins thus gaining preferential access to mitochondrially generated ATP, and in a decreased susceptibility to product inhibition and proteolytic degradation. Significantly, these multiple strategies all work together to enable tumor cells to develop a metabolic strategy compatible with rapid proliferation and prolonged survival.

KEY WORDS: Cancer; tumor metabolism; hexokinase; Type II hexokinase; gene amplification; gene regulation; tumor suppressor genes; p53; hypoxic conditions.

INTRODUCTION

Many tumor cells have a very distinctive metabolism, characterized by a high rate of aerobic glycolysis.

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Significantly, the glycolytic capacity of a given tumor is characteristic of its state of differentiation, where slow growing tumors in their formative stages with a more differentiated phenotype show glycolytic rates on par with those seen in parental cells, whereas highly de-differentiated rapidly growing tumors show a marked increase in glycolytic capacity.[1] The ability of cancer cells to maintain a high rate of glycolysis was initially identified by Warburg[2] when he noted

the absence of the Pasteur effect, i.e., the lack of inhibition of glycolysis when tumor cells are exposed to normal oxygen conditions. However, his hypothesis that glycolysis is the primary causative basis for malignancy has not borne out.[3–5] Rather, research carried out over the past 25 years has made it clear that cancer is fundamentally a genetic disease, and has indicated that specific "cancer genes," and the proteins they encode, hold, in part, the key to improved cancer detection, diagnosis, and treatment. Nevertheless, the fact remains that the ability to sustain an enhanced glycolytic rate represents one of the most consistent and profound biochemical phenotypes of many cancer cells. This high rate of glycolysis is important for rapidly growing tumors, as they may obtain as much as 50% of their energy from this process.[6] Moreover, glycolytic intermediates are important precursors for cell "building blocks" essential for cell growth and division. Therefore, a better definition of the molecular events giving rise to the high glycolytic phenotype of many cancers also holds, in part, the key to improved cancer detection, diagnosis, and treatment, and as we report here also has a genetic basis.

ENHANCED GLYCOLYSIS AND THE ROLE OF HEXOKINASE

Enhanced rates of glucose intake and turnover and alterations of cellular levels and kinetic properties of key glycolytic enzymes, notably hexokinase, phosphofructokinase-1, and pyruvate kinase, have been suggested to account, at least in part, for the enhanced glycolysis of many tumors.[1,5,7,8] For example, in comparison to normal cells, the activity of hexokinase is markedly elevated in rapidly growing tumors that exhibit the high glycolytic phenotype.[1,7,9] This elevated activity can be attributed to changes which can be traced back from the post-translational stages for this enzyme to altered levels of the gene itself within the tumor cell (see below).

In mammalian cells, four isoforms of hexokinase (HK I, II, III, and IV) exist, most of which show a tissue-specific distribution profile, with Type I being predominantly expressed in brain and erythrocytes, Type II in skeletal muscle and adipocytes, and Type IV, also known as glucokinase, in liver and pancreas. In many highly malignant rapidly growing tumor cells, the Type II hexokinase, and to a lesser extent the Type I, is highly expressed, regardless of the tumor's tissue of origin.[10–14]

EFFECTS OF POST-TRANSLATIONAL CHANGES OF HEXOKINASE ON TUMOR CELL GLYCOLYSIS

At the protein level, two mechanisms come into play to alter the glycolytic capacity of tumor cells. First is the overproduction of the enzyme, for example in liver derived tumors, where a striking alteration in the isoenzyme profile is seen upon tumorigenesis.[4,13,14] In rapidly growing hepatomas the ratio of hexokinase to glucokinase is markedly increased in relation with normal liver.[15] The second mechanism is the propensity of this overabundant enzyme to bind to porins on the outer mitochondrial membrane,[16] where the bound hexokinase gains preferential access to mitochondrially generated ATP.[9] Significantly, mitochondrial bound hexokinase accounts for as much as 70% of the total cellular hexokinase in hepatoma cells in contrast to the negligible hexokinase levels found on the mitochondria of normal liver cells.[17] When the glucokinase (in normal liver) to hexokinase (in hepatoma) transition has taken place, a striking kinetic effect results with the isoenzyme in tumors exhibiting an approximately 100-fold higher affinity for glucose. In addition, the binding of hexokinase to the outer mitochondrial membrane results in reduced sensitivity of hexokinase to feedback inhibition by the product glucose-6-phosphate,[7] an important regulatory property of normal cells. Thus, it is clear from these studies that upon tumorigenesis, normal cells alter their hexokinase profile to generate a form that has higher affinity, and better access, to glucose and ATP, and lower feedback inhibition from the product, facilitating enhanced glycolysis.

Cloning and sequencing of the Type II hexokinase isoform that is expressed in two rapidly growing tumor cell lines, the Novikoff ascites[12] and AS-30D hepatoma,[18] has shown, at least at the primary sequence level, that the expressed Type II isoform is essentially identical to the Type II hexokinase expressed in skeletal muscle. However, it is quite possible that tumor-specific post-translational modifications do exist due to altered signal transduction pathways which may alter the kinetic properties of the Type II isoform expressed in tumor cells.

EFFECTS OF TRANSCRIPTIONAL REGULATION OF HEXOKINASE ON TUMOR CELL GLYCOLYSIS

It has been known for many years that hexokinase is highly expressed in rapidly growing tumor cells.

However, the relationship between the gene product and the activity of the gene itself has not been known until recently. At the transcriptional level, a 10-fold enhancement of the rate of transcription was shown for hexokinase from tumors using an *in vitro* transcription assay.[19] Also, the increased levels of hexokinase isoforms has been correlated, at least in part, to enhanced levels of the mRNA using Northern blot analysis.[13,14,20] Following the cloning and sequence analysis of the promoter for Type II hexokinase from the hepatoma AS-30D,[14] (Fig. 1) it became possible to analyze the effect of various signal transduction pathways on the regulation of Type II hexokinase gene expression. For example, reporter gene analysis of the 4.3-kbp cloned proximal promoter region indicated that it is up-regulated by glucose, insulin, glucagon, and by pathways for both protein kinase A and protein kinase C. The activation of the promoter by both insulin and glucagon, which are normally opposing hormones, reveals its promiscuous nature, which for survival purposes may help tumors maintain an enhanced glucose catabolic rate regardless of the host's nutritional status.

Interestingly, the response element for glucose [CACGTG] on the tumor Type II hexokinase promoter was found to overlap a recently discovered element [CACGTGCT] that responds to hypoxia.[21] Analysis of the Type II hexokinase promoter under hypoxic conditions also indicated up regulation,[22] adding another interesting facet to physiological observations made for enhanced glycolysis of tumor cells. It is not yet known whether these two elements act synergistically in the presence of both glucose and hypoxia.

Significantly, when the same promoter construct was transfected into the normal cells (hepatocytes) basal level expression was observed, although up regulation by glucose, insulin, glucagon, and protein kinase A and C were absent. These results indicate that (1) in normal cells, the gene is silenced, most probably by mechanisms involving methylation and histone modification, and (2) a different level or set of transcription factors or cascades are involved in normal (hepatocytes) and hepatoma cells for the control of hexokinase gene expression, and therefore the glycolytic rate. Furthermore, cloning and sequence analysis of the promoter region from hepatocytes have indicated a similarity of 99% at the nucleotide level for the promoter when compared with the promoter from the tumor cells.[23] Therefore, altered DNA sequences within the respective promoters cannot be involved in the changes observed during reporter gene expression studies.

Another finding of considerable interest is the activation of the promoter by glucose.[24] This contrasts with previous suggestions that an intermediate, perhaps glucose-6-phosphate, is the mediator for the glucose response. However, detailed studies using glucose analogs and inhibitors of hexokinase implicate glucose itself rather than one or more glycolytic intermediates in mediating the glucose response.[24]

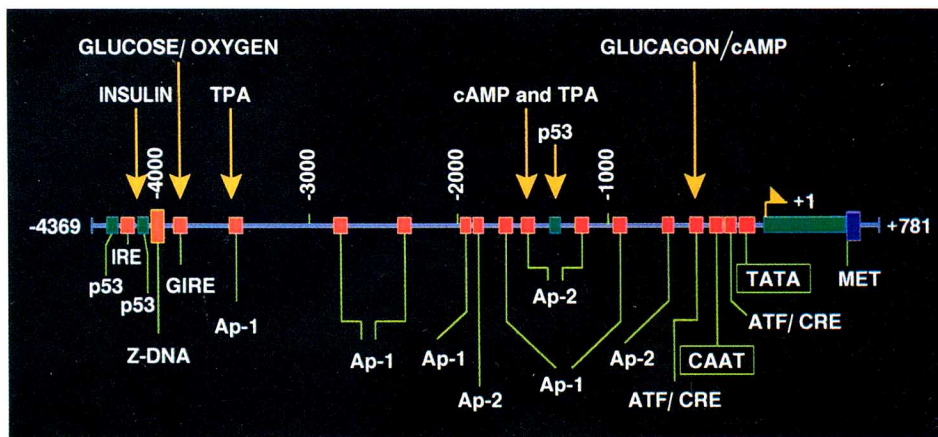Recent studies have shown another interesting observation for the Type II hexokinase promoter within

Fig. 1. The positions of the response elements for PKA, PKC, insulin, glucose, hypoxia, cAMP, and p53 on the 4.3-kbp Type II hexokinase promoter. +1, transcription start site; Met, translation start site; the glucose, insulin, hypoxia, and p53 response elements are located within the distal 4-kbp region of the promoter. [From Ref. 14].

tumor cells, where functional p53 elements were identified within the same promoter region that harbors the glucose and hypoxia responsive elements[25] (Fig. 2). This correlated with the presence of a p53 protein with an enhanced half-life expressed in the same tumor cells. Co-expression of this protein with the Type II hexokinase promoter during reporter gene analysis resulted in enhanced transcription. The proximity of the p53 elements to the hypoxia and glucose response elements, as well as the recent observation that tumors within hypoxic regions promote p53 mutations at a high rate, implicate an important relationship between hexokinase expression, the expression of mutated p53,

hypoxia, enhanced glucose catabolism, and cell-cycle progression or proliferative capacity of highly glycolytic, rapidly growing tumors.

EFFECTS OF ALTERED TYPE II HEXOKINASE GENE STRUCTURE AND ENHANCED GLYCOLYSIS

The previous studies showed how highly elevated glycolytic rates are initiated by enhanced Type II hexokinase gene transcription which in turn results in enhanced translation. Another facet to this cascade of
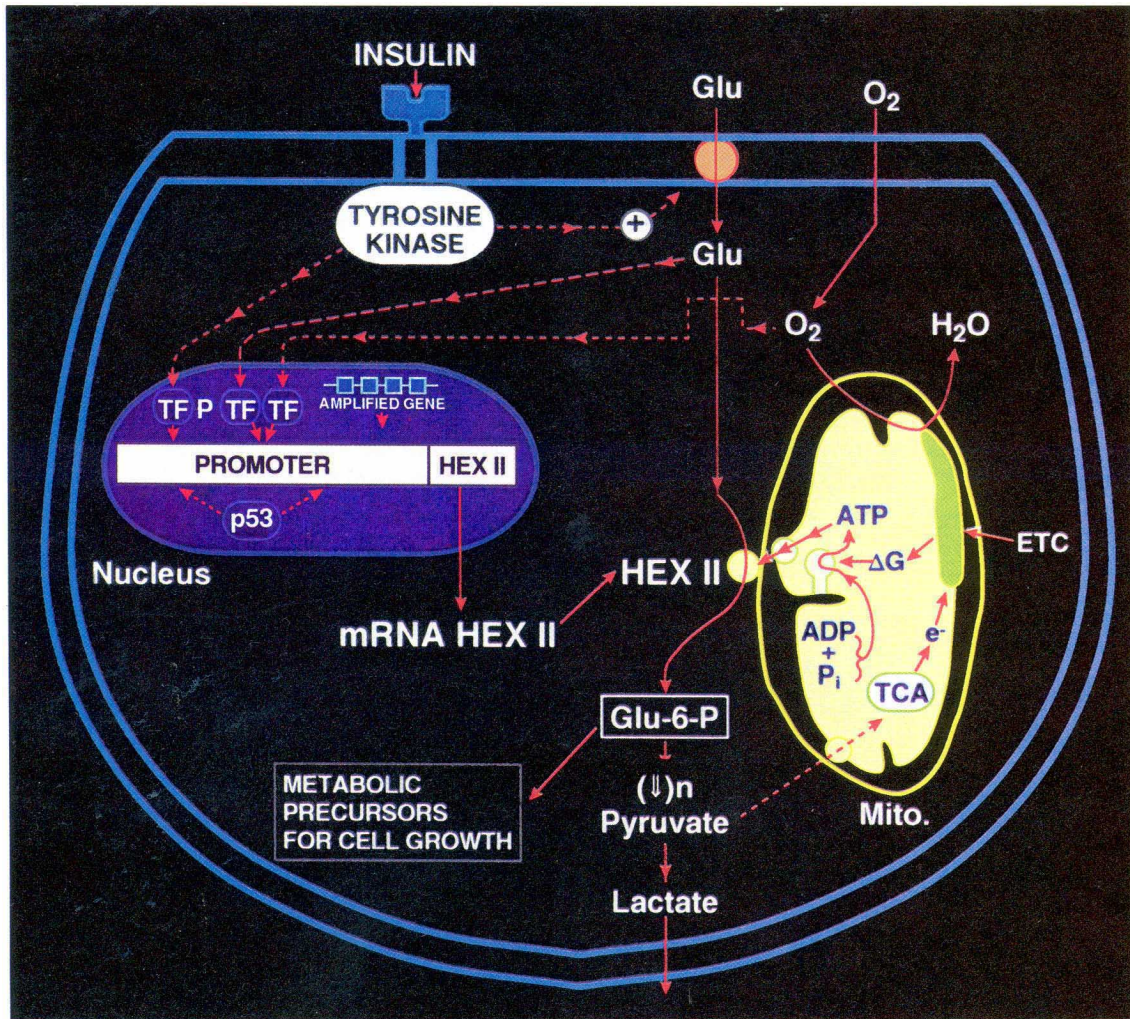

Fig. 2. Current view of the genetic, biochemical, and bioenergetic events responsible for the propensity of cancers to catabolize glucose at high rates to support cell growth and division. Upon tumorigenesis, the Type II hexokinase gene is amplified, activated, and induced by multiple signal transduction cascades to overexpress its message. The resulting overexpressed protein binds to porins on the outer mitochondrial membrane, where phosphorylation of incoming glucose is facilitated by ATP generated via oxidative phosphorylation.

events was seen when the tumor cells were analyzed for DNA amplification. Thus, when the hepatoma and hepatocyte DNA were analyzed for altered Type II hexokinase gene copy number, an approximately 5-fold enhancement was seen for the gene in tumor cells. These studies indicated that the entire gene was amplified, where the amplification was localized to a single chromosome within the nucleus, suggesting that the amplification occurs on only one chromosome. The ~ 5-fold amplification of the gene should result in a similar-fold contribution to the enhanced hexokinase message,[26] and may contribute significantly to the highly glycolytic phenotype.

CONCLUSIONS

All studies described above indicate a strategy used by highly malignant tumors to survive as well as thrive within the host using a remarkable set of coordinated molecular mechanisms. These mechanisms, which are very similar to those utilized by some highly successful parasites, indicate a sophisticated strategy devised by tumors to survive even the most inhospitable microenvironments within the host. It is quite possible that the strategies outlined for the Type II hexokinase gene product apply, at least in part, to other genes involved in the glycolytic pathway of highly glycolytic tumor cells, e.g., recent studies by Dang and coworkers[27] on lactate dehydrogenase. Further studies in progress involving a detailed analysis of specific transcription factors that influence Type II hexokinase gene regulation will reveal another level of modulation of this key enzyme critical for the high glycolytic phenotype of numerous cancers.

ACKNOWLEDGMENT

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