Journal of Bioenergetics and Biomembranes, Vol. 29, No. 4, 1997

Oncogenes in Tumor Metabolism, Tumorigenesis, and Apoptosis

Chi V. Dang,[1-4] Brian C. Lewis,[2] Christine Dolde,[2] Gerard Dang,[1] and Hyunsuk Shim[1]

Received May 27, 1997; accepted July 15, 1997

The ability of cancer cells to overproduce lactic acid aerobically was recognized by Warburg about seven decades ago, although its molecular basis has been elusive. Increases in glucose transport and hexokinase activity in cancer cells appear to account for the increased flux of glucose through the cancer cells. Herein we review current findings indicating that the c-Myc oncogenic transcription factor and hypoxia-inducible factor 1 (HIF-1) are able to bind the lactate dehydrogenase A promoter *cis* acting elements, which resemble the core carbohydrate response element (ChoRE), CACGTG. These and other observations suggest that the normal cell responds physiologically to changes in oxygen tension or the availability of glucose by altering glycolysis through the ChoRE, which hypothetically binds c-Myc, HIF-1, or related factors. The neoplastic cell is hypothesized to augment glycolysis by activation of ChoRE/HIF-1 sites through direct interaction with c-Myc or through activation of HIF-1 or HIF-1-like activity. We hypothesize that oncogene products either stimulate HIF-1 and related factors or, in the case of c-Myc, directly activate hypoxia/glucose responsive elements in glycolytic enzyme genes to increase the ability of cancer cells to undergo aerobic glycolysis.

KEY WORDS: c-myc, oncogene; transcription; hypoxia; HIF-1; tumor metabolism; glycolysis; tumorigenesis; apoptosis.

The most striking and common feature of tumor cells is the production of large amounts of lactic acid which is due to enhanced glycolysis despite the presence of oxygen (Bodansky, 1975; Bustamante *et al.*, 1981; Goldman *et al.*, 1964; Pedersen, 1978; Racker and Spector, 1981) with an accompanying increased rate of glucose transport (Templeton and Weinberg, 1991). This phenomenon was recognized about seven decades ago (Warburg, 1930, 1956), although its molecular basis and connection to cancer genetics have remained poorly understood. Based on recent observations on hypoxia-inducible genes and c-Myc target genes reviewed herein, we propose a unifying hypothesis which links genetic alterations in cancer to altered tumor metabolism.

GLYCOLYTIC ENZYME GENES AND THE CACGTG MOTIF

Understanding of the regulation of glycolytic enzyme gene expression is beginning to emerge with the characterization of glycolytic enzyme gene promoters. Glucose is a major regulator of gene transcription in many life forms. Glucose is able to stimulate transcription of genes encoding glycolytic and lipogenic enzymes in adipocytes and hepatocytes through the carbohydrate response element (ChoRE) (Foufelle *et al.*, 1992; Jacoby *et al.*, 1989; Lefrancois-Martinez *et al.*, 1994; Thompson and Towle, 1991). Transcription of metabolic enzymes in response to carbohydrate

[1] Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.
[2] Program in Human Genetics and Molecular Biology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.
[3] Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.
[4] The Johns Hopkins Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

0145-479X/97/0800-0345$12.50/0

occurs through ChoRE, a CACGTG motif, although the transcription factors binding this site remain poorly understood. Carbohydrate feeding induces the liver-type pyruvate kinase (Liu *et al.*, 1993) and rat S_{14} genes (Shih and Towle, 1994; Shih *et al.*, 1995) at the transcriptional level to the increased glucose metabolism (Jump *et al.*, 1990; Vaulont *et al.*, 1986). The promoter regions of pyruvate kinase and S_{14} bear ChoRE motifs, and mutations in these motifs result in defective glucose stimulation (Liu *et al.*, 1993; Shih *et al.*, 1995). Thus, the CACGTG motif is important for the regulation of many glycolytic enzyme gene expression.

Freitas *et al.* (1996) have reported that enzyme activities of lactate dehydrogenase, glucose-6-phosphate dehydrogenase, dihydrofolate reductase, purine nucleoside phosphorylase, and acid phosphatase are significantly elevated in hypoxic tumor cells. These elevated enzyme activities give tumor cells the ability to thrive in hypoxia and remain resistant to most therapies, in particular radiotherapy. Although the transcriptional regulation of glycolytic enzyme genes remains to be elucidated, sequence analysis indicates that the ChoRE motif, CACGTG, also known as the Myc E-box, is found frequently within the promoter and intronic sequences glycolytic enzyme genes (Fig. 1).

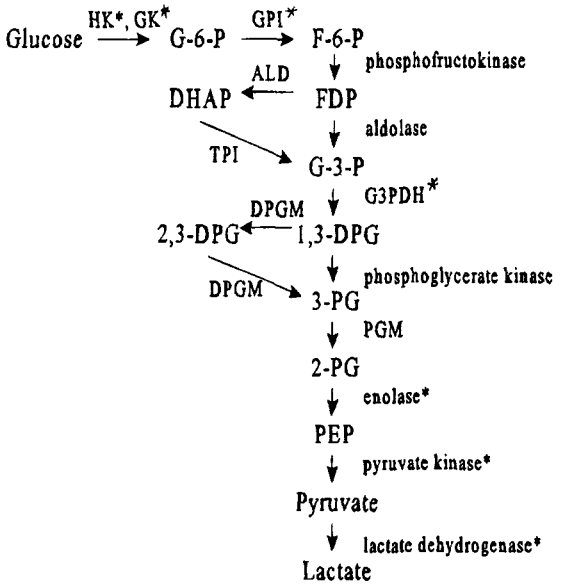

Fig. 1. The glycolytic pathway with enzymes (*) which have ChoRE in their promoter region indicated. HK, hexokinase; GPI, glucosephosphate isomerase; DHAP, dihydroxyacetone phosphate; TPI, trisephosphate isomerase; DPG, diphosphoglycerate; DPGM, diphosphoglycerate mutase; PEP, phosphoenolpyruvate; FDP, fructose diphosphate; PGM, phosphoglycerate mutase; G3PDH, glyceraldehyde-3-P dehydrogenase; GK, glucokinase.

Hexokinase II

One of the rate-limiting glycolytic enzymes is hexokinase type II which has ChoRE in its 5′ and intronic sequences. Hexokinase II is highly overexpressed in many cancer cells and its amplification correlates with the elevated glycolysis in tumor cells (Rempel *et al.*, 1996).

Pyruvate Kinase

L-type pyruvate kinase gene responds at the transcriptional level to the increased glucose metabolism (Vaulont *et al.*, 1986). The promoter region of L-type pyruvate kinase gene (Liu *et al.*, 1993) bears two consensus ChoRE motifs or E-boxes, and mutations in either motif result in defective glucose stimulation.

Enolase

Another glycolytic enzyme enolase was shown to be the c-*myc* promoter binding protein, MBP-1 (Chaudhary *et al.*, 1996), and enolase itself contains ChoRE in its promoter.

TRANSCRIPTION FACTORS WHICH MAY REGULATE GLYCOLYSIS THROUGH CACGTG

It is notable and intriguing that the carbohydrate response element (ChoRE) has a core consensus CACGTG which is identical to the c-Myc/Max binding site or E-box (Towle, 1995). Moreover, the consensus HIF-1 binding site also overlaps with the c-Myc E-box. Based on the structure of the basic region of ARNT/HIF-1, it is predictable that these transcription factors would also bind the Myc E-box, which is shared with other factors such as USF, TFE-3, and TFE-B (Dang *et al.*, 1992). In fact, ARNT and HIF-1 have been shown to interact with the CACGTG binding site (Antonsson *et al.*, 1995; Semenza *et al.*, 1996; Sogawa *et al.*, 1995; Swanson *et al.*, 1995).

c-Myc and Family Members

c-myc is an early serum response gene whose deregulated expression is the molecular signature of

Burkitt lymphomas and is frequently found in various commonly occurring solid tumors (for reviews see Cole, 1986; Dang and Lee, 1995). The c-Myc protein participates in the regulation of cell proliferation, differentiation, and apoptosis induced by serum deprivation (Eilers *et al.*, 1989, 1991; Evan *et al.*, 1992; Harrington *et al.*, 1994; Hermeking and Eick, 1994; Packham and Cleveland, 1995; Wagner *et al.*, 1994; White, 1996). c-Myc is a basic-helix-loop-helix/leucine zipper (bHLH/Z) transcription factor that heterodimerizes with another protein termed Max via the HLH/Z domain to bind a DNA consensus core sequence, CACGTG or E-box (Blackwood *et al.*, 1992; Dang and Lee, 1995; Evan and Littlewood, 1993; Meichle *et al.*, 1992; Prendergast and Ziff, 1992). Although c-Myc provides the transregulatory function for the heterodimer to activate through E-boxes or suppress gene transcription through initiator elements, its targets have not been comprehensively characterized and are only beginning to emerge from various studies (Ayer *et al.*, 1995; Bello-Fernandez *et al.*, 1993; Gaubatz *et al.*, 1995; Grandori *et al.*, 1996; Gu *et al.*, 1993; Jones *et al.*, 1996; Kretzner *et al.*, 1992; Lee *et al.*, 1996; Li *et al.*, 1994; Miltenberger *et al.*, 1995; Philipp *et al.*, 1994; Schuldiner *et al.*, 1996).

HIF-1

Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric basic-helix-loop-helix-PAS protein composed of HIF-1 alpha and HIF-1 beta/ARNT subunits whose expression is induced under conditions of reduced oxygen tension (Wang *et al.*, 1995; Wang and Semenza, 1995). Previous studies have demonstrated the ability of HIF-1 to stimulate the expression of vascular endothelial growth factor (VEGF), hemeoxygenase, and several glycolytic enzymes (Forsythe *et al.*, 1996; Lee *et al.*, 1997; Semenza *et al.*, 1994, 1996). Mice lacking HIF-1 beta show abnormal angiogenesis and responses to oxygen deprivation, underscoring the importance of HIF-1 as a critical hypoxia response factor (Maltepe *et al.*, 1997). To date, a role for HIF-1 in cellular transformation or apoptosis has not been demonstrated.

USF

USF (upstream stimulatory factor, also known as MLTF1 or UEF) was identified as the component of a HeLa cell nuclear extract which bound to, and activated transcription from, E-box sequence upstream of the adenovirus major late promoter. The gene encoding the 43kDa polypeptide, known as *USF,* was found to have sequences encoding a C-terminal bHLHZip motif. It was also found to possess an N-terminal transcriptional activation domain (TAD). USF forms homodimers, but are not known to dimerize with any other b-HLH/bHLHZip protein. The cellular function of USF is unclear, but some studies suggest that it possesses antiproliferative properties and that it may even antagonize Myc-induced transformation. USF is implicated in the transcription of many genes which possess E-boxes; however, it is difficult to assess the direct involvement of USF in the regulation of a particular gene (Gregor *et al.*, 1990). USF is abundant and ubiquitously expressed.

TFE-3 and TFE-B

TFE3 is a ubiquitous activator of the IgH enhancer that binds the canonical E-box as well as regulatory elements important in lymphoid-specific, muscle-specific, and some ubiquitously expressed genes (Beckmann *et al.*, 1990). A closely related protein, TFEB, is also known to bind E-box.

REGULATION OF LDH-A GENE EXPRESSION BY HELIX-LOOP-HELIX TRANSCRIPTION FACTORS

Alteration of LDH-A in Cancer

The *LDH-A* gene is an epidermal growth factor, cAMP and phorbol ester-inducible delayed early serum response gene whose role in neoplasia remains unestablished despite its widespread use as a prognostic tumor marker (Chung *et al.*, 1995; Huang and Jungmann, 1995; Matrisian *et al.*, 1985; Short *et al.*, 1994). Lactate dehydrogenase is a tetrameric enzyme with five isoforms which are composed of combinations of two subunits, LDH-A and LDH-B. The LDH-A subunit converts pyruvate to lactate under nonequilibrium anaerobic conditions in normal cells. The other isoenzyme, LDH-B, kinetically favors the conversion of lactate to pyruvate and is found at high levels in aerobic tissues such as the heart. Hereditary LDH-A subunit deficiency causes early postimplantation embryonic lethality in homozygotic mice (Merkle *et al.*, 1992). Human LDH-A deficiency presents clinically as an

exertional myopathy, which is associated with a severe inability of excercised muscles to produce lactic acid (Kanno *et al.*, 1988). In addition to its role in intermediary metabolism, the LDH-A isozyme may be functionally involved in the transcriptional modulation of gene expression and/or DNA replication, since its tyrosine-phosphorylated form localizes to the cell nucleus, and has been found to be a single-stranded DNA binding protein with DNA helix-destabilizing activity (Cooper *et al.*, 1983; Grosse *et al.*, 1986; Sharief *et al.*, 1986; Williams *et al.*, 1985; Zhong and Howard, 1990). In addition, isozymes of enolase, phosphoglycerate mutase, and lactate dehydrogenase were found to be tyrosine phosphorylated in cells transformed by Rous sarcoma virus (Cooper *et al.*, 1983); although the functional significance of phosphorylation is unclear.

Increased serum LDH is an independent prognostic indicator in Burkitt's lymphomas, in which deregulated c-Myc expression is a hallmark of the disease (Cowan *et al.*, 1989; Csako *et al.*, 1982; Endrizzi *et al.*, 1982; Fasola *et al.*, 1984; Jagannath *et al.*, 1985; Mintzer *et al.*, 1984; Pan *et al.*, 1991; Schneider *et al.*, 1980; Swan *et al.*, 1989). In the clinical setting, serum LDH is the most significant independent prognostic factor of survival after recurrence of breast cancer (Johansen *et al.*, 1995). The elevated LDH level yields 88% sensitivity and 85% specificity for detecting ovarian malignancy, while the significant shift to LDH-4 (3 subunits of LDH-A and 1 subunit of LDH-B) or LDH-5 (4 subunits of LDH-A) of the LDH isozyme pattern yields 84% sensitivity and 77.5% specificity, respectively. From the combined analysis of total LDH levels and isozyme patterns, the true positive of ovarian malignancy detection could reach 100% (Bose and Mukherjea, 1994; Chow *et al.*, 1991). In addition, LDH isozyme assay of nipple discharge is reported as a useful technique for diagnosis of breast cancer (Kawamoto, 1994); higher LDH-A levels predict poor prognosis. Similarly, serum LDH level is one of the diagnostic markers for cervical cancer (Patel *et al.*, 1993) and germ-cell tumors of the testis (Klein, 1993).

LDH-A and c-Myc

Shim *et al.* (1997) confirmed LDH-A as a direct target of c-Myc based on the following findings. They identified lactate dehydrogenase subunit A gene as one of the putative c-Myc up-regulated genes using representational difference analysis (RDA) (Hubank and Schatz, 1994; Lisitsyn *et al.*, 1993) in nonadherent Rat1a fibroblasts that only require ectopic c-Myc expression to be transformed (Barrett *et al.*, 1995; Small *et al.*, 1987; Stone *et al.*, 1987). The RNase protection assay shows elevated LDH-A expression in nonadherent Rat1a-Myc cells versus Rat1a cells. Nuclear run-on assays demonstrated an increased transcriptional rate of *LDH-A* in Rat1a-Myc as compared to Rat1a cells. To determine whether the *LDH-A* gene might be transcriptionally activated by Myc, they used a previously characterized Rat1a cell line expressing a Myc-estrogen-receptor (Myc-ER) fusion protein that was activated by an addition of hydroxytamoxifen (HOTM) to the growth medium (Eilers *et al.*, 1989; Grandori *et al.*, 1996). Activation of Myc-ER by HOTM in confluent cells causes induction of *LDH-A*, which is not inhibited by the protein synthesis inhibitor CHX. These observations suggest that induction of *LDH-A* expression by Myc is direct and does not require new protein synthesis.

The rat *LDH-A* promoter contains two consensus Myc/Max binding sites or E-boxes, CACGTG (located at -78 to -83 and -175 to -180 from the transcriptional start site), that are conserved in both mouse and human, suggesting that c-Myc may be able to regulate the transcription of *LDH-A* through these E-boxes (Fukasawa and Li, 1987; Short *et al.*, 1994; Takano and Li, 1990). Transient transfection experiments with a c-Myc expression vector demonstrated an E-box-dependent transactivation of the *LDH-A* promoter-luciferase reporter gene. Mutation of either or both E-boxes abrogated Myc-dependent transactivation. The expression vector producing a c-Myc mutant lacking the helix-loop-helix domain was unable to activate the *LDH-A* promoter, suggesting that dimerization with Max was required for transactivation. An expression vector for the nononcogenic basic-helix-loop-helix-leucine-zipper transcription factor USF (Gregor *et al.*, 1990), which also bound to CACGTG, was also able to stimulate the LDH-A promoter although only half as efficiently as the c-Myc expression vector. *In vitro* electrophoretic mobility shift DNA-protein binding assays demonstrated the ability of recombinant Myc/Max proteins to bind the *LDH-A* promoter E-boxes. These observations suggest that *LDH-A* is a direct Myc-responsive target gene.

LDH-A and HIF-1

LDH-A is a well-characterized hypoxia-inducible gene among many other glycolytic enzyme genes

(Semenza *et al.*, 1994). The *LDH-A* promoter was found to contain a HIF-1 site that appears to cooperate with a cAMP response element (CRE) to induce expression under hypoxic conditions (Firth *et al.*, 1995). Another element, CACGTG, which is a Myc binding site (located at -78 to -83 from the transcriptional start site), was also found to be critically important for response to hypoxia. The identity of this factor was not known in this study (Firth *et al.*, 1995). In a subsequent study by Semenza *et al.* (1996), *LDH-A*, among other glycolytic enzyme gene promoters, such as aldolase A and enolase 1, was found to contain essential binding sites for HIF-1. The E-box element located at -78 to -83 from the transcriptional start site of the *LDH-A* promoter was found to bind HIF-1 and an as yet unidentified factor. The *LDH-A* promoter provides a model to examine the interplay between c-Myc, HIF-1, and related factors in the regulation of *LDH-A* gene expression.

These observations taken together suggest the following model of induction of glycolytic enzymes in cancer cells. Given that induction of glycolysis is critical for the growth of cancer cells under adverse pathophysiological conditions of limited supply of oxygen and nutrients, it stands to reason that pathways capable of activating the genetic program encoding the glycolytic machinery would be altered (Fig. 2). As such, activation of the c-*myc* oncogene results in an oncogenic transcription factor capable of activating glycolytic enzyme gene expression through the ChoRE or HIF-1 sites. Further studies are necessary to test these specific hypotheses. Activation of other oncogenes, such as *ras* or *src* and *bcr-abl* tyrosine kinases, may in part increase c-Myc activity although their major effects may be through the activation of the HIF-1 pathway. Intriguingly, loss of the tumor suppressor VHL alters the metabolism or utilization of mRNAs encoding vascular endothelial cell growth factor (VEGF) (Gnarra *et al.*, 1996; Siemeister *et al.*, 1996; Wizigmann-Voos *et al.*, 1995), which contains HIF-1 sites, and GLUT-1 which is also hypoxia responsive. Therefore, it should be immediately testable as to whether other oncogenic events activate pathways that have overlapping or identical endpoints of activating glycolysis through HIF-1/ChoRE/Myc E-boxes.

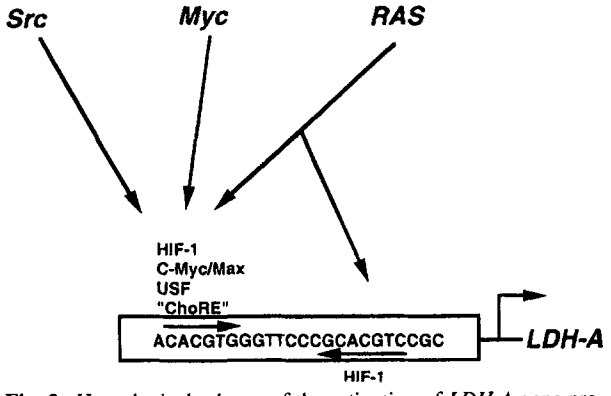

Fig. 2. Hypothetical scheme of the activation of *LDH-A* gene promoter by products of various oncogenes. c-Myc is shown to activate the promoter directly, whereas Src and Ras are shown to hypothetically activate HIF-1 or HIF-1-like factors, augmenting the transcription of *LDH-A* or genes encoding other glycolytic enzymes.

ROLE OF LDH-A IN TRANSFORMATION AND APOPTOSIS

LDH-A and Anchorage-Independent Growth

Shim *et al.* (1997) determined whether elevated LDH-A expression is necessary for c-Myc-mediated anchorage-independent growth by constructing Rat1a-Myc cells and c-Myc transformed lymphoblastoid cells expressing antisense *LDH-A*. Reduction of LDH-A activity in Rat1a-Myc cells by antisense expression, which did not alter ectopically expressed Myc protein levels, dramatically decreased soft agar clonogenicity of Rat1a-Myc cells in soft agar. Reduction of *LDH-A* expression also inhibited soft agar colony formation of human lymphoid cells transformed by c-Myc. EBV-immortalized human lymphoblastoid CB33 cells can be transformed by c-Myc *in vitro*, resulting in the ability to form soft agar colonies (Lombardi *et al.*, 1987). These results indicate that LDH-A is necessary for c-Myc-mediated transformation of Rat1a and Burkitt lymphoma cells, although *LDH-A* overexpression alone is insufficient to induce the extent of growth in soft agar characteristic of c-Myc-transformed Rat1a cells.

Reduced expression of LDH-A is able to block Myc-mediated soft agar colony formation, suggesting that elevated LDH-A expression in human cancers may be necessary for their neoplastic phenotype. These observations are instructive when the growth properties of Myc-transformed Rat1a or lymphoblastoid cells expressing antisense *LDH-A* in soft agar are compared with the same cells in the normal growing conditions. The growth rates of these Myc-transformed cells are virtually the same when they were grown without suspension in agar, suggesting that *LDH-A* overexpression is required for soft agar colony formation but not for

cell viability. The biochemical basis for this difference remains to be established; however, it is conceivable that a threshold level of glycolysis and production of lactate is necessary for soft agar colony formation. They speculate that the anaerobic conditions within an expanding soft agar colony may select against cells with low LDH-A levels, which are inefficient in anaerobic glycolysis (Hemlinger *et al.*, 1997; Sutherland *et al.*, 1986). In fact, the growth of rat fibroblasts under hypoxic conditions is heavily dependent on LDH-A levels. This contention is further supported by the early embryonic lethality of homozygous LDH-A deficient mice, which is probably due to the postimplantation anaerobic conditions (Ellington, 1987) that exist before formation of the chorioallantoic placenta (Merkle *et al.*, 1992).

LDH-A and Apoptosis

Since LDH-A is intimately linked to glucose metabolism and its expression is enforced by c-Myc, we determined whether glucose deprivation would alter the phenotype of c-Myc-transformed cells (Shim, Chun and Dang, unpublished observation). Glucose deprivation of nontransformed Rat1a cells causes a reduction in BrdU incorporation, an enrichment in G_1 phase cells, and a reduction in S-phase and G_2/M cells. Only 67% of the Rat1a-Myc cells with S-phase DNA content incorporated BrdU, indicating that the other 33% of cells in S-phase was either arrested or died. The nontransformed Rat1a cells displayed minimal apoptotic cell death with glucose deprivation determined by the terminal deoxynucleotidyl transferase (TdT) end-labeling flow cytometric assay. In contrast, 19% of the c-Myc-transformed cells had undergone apoptosis and were TdT positive after 20 h of glucose deprivation. The apoptotic cells have DNA content spreading from the G_1 into the S-phase pool as well as some in the G_2 pool. Coexpression of Bcl-2 completely blocked glucose deprivation-induced apoptosis of c-Myc-transformed cells. These observations uncover a novel glucose-dependent apoptotic pathway that is activated by c-Myc overexpression and is inhibited by coexpression of Bcl-2.

Intriguingly, overexpression of *LDH-A* increases the population of Rat1a cells in S-phase in the presence of glucose. Withdrawal of glucose, however, was associated with the dramatic reduction in BrdU-positive cells in S-phase. Ectopic *LDH-A*-expressing Rat1a cells, similar to c-Myc-overexpressing cells, displayed significant apoptotic cell death with glucose deprivation. Unlike Rat1a-Myc cells, Rat1a-LDH-A cell growth was arrested with serum withdrawal and did not display increased apoptotic cell death. These observations indicate that induction of apoptosis by glucose deprivation and serum deprivation are distinctly different pathways and that LDH-A links c-Myc to glucose-dependent apoptosis.

These results indicate that overexpression of *LDH-A* in rat fibroblasts is sufficient to sensitize cells to glucose deprivation-induced apoptosis. This observation supports the hypothesis that LDH-A is a downstream target of c-Myc which mediates this unique apoptotic phenotype. The connection between *LDH-A* overexpression, glucose deprivation, and the common pathway leading to apoptotic cell death remains to be elucidated. We speculate that constitutive generation of NAD^+ and lactate by LDH-A and the decrease in the regeneration of NADH by inhibition of glycolysis contribute to oxidative stress on the cells, which then triggers the final death pathway (Hockenbery *et al.*, 1993).

LDH-A AND WARBURG EFFECT

Seven decades ago, Warburg studied glycolysis in a variety of human and animal tumors and found that there was a trend toward an increased rate of glycolysis in tumor cells, resulting in the excessive production of lactic acid from glucose (Warburg, 1930, 1956). This phenomenon known as the Warburg effect was a subject of intense investigation, controversy, and intrigue, yet the molecular basis of the Warburg effect has remained unclear (Racker and Spector, 1981).

When stably transfected Rat1a cells that constitutively express rat *LDH-A* cDNA were subjected to a soft agar anchorage-independent growth assay, the Rat1a-LDH-A cells were unable to proliferate as strongly as Rat1a-Myc cells in suspension, indicating that increased *LDH-A* expression is insufficient to induce full transformation (Shim *et al.*, 1997). The Rat1a-LDH-A cells, however, do display more colonies with larger sizes (>100 mm) than control Rat1a cells. In contrast, we observed that both c-Myc-transformed and ectopic *LDH-A*-expressing Rat1a cells produce more lactate than the control stably transfected Rat1a cells (Table I). These observations suggest that the Warburg effect induced in fibroblasts by c-Myc is largely due to the deregulated expression of *LDH-A*.

Table I. Molar Ratios of Lactate Production to Glucose Consumption in Rat1a Cell Lines[a]

Rat1a	Rat1a-Myc	Rat1a-LDH-A
1.28	1.44	1.68
1.28	1.48	1.62
1.28	1.44	1.58
1.06	1.48	1.56
1.23 ± 0.11	1.46 ± 0.02	1.61 ± 0.05

[a] Values represent the moles of lactate produced per mole of glucose consumed for various cell lines after 24 h incubation with fresh medium. Values from four separate experiments and the means with standard deviations are shown for each cell line.

The findings of Shim *et al.* (1997) are intriguing when the Warburg effect and previous links between elevated LDH-A levels and human cancers are taken into consideration (Bodansky, 1975; Bredin *et al.*, 1975; Carda-Abella *et al.*, 1982; Csako *et al.*, 1982; Goldman *et al.*, 1964; Li *et al.*, 1988; Nevin and Mulholland, 1988; Nishikawa *et al.*, 1991; Schneider *et al.*, 1980; Tanaka *et al.*, 1984; Vergnon *et al.*, 1984; Woollams *et al.*, 1976). In particular, an elevated LDH-A level is an independent predictor of poor clinical outcome in Burkitt lymphoma, in which activation of the *c-myc* gene by chromosomal translocations is a *sine qua non* (Csako *et al.*, 1982; Dalla-Favera *et al.*, 1982; Magrath *et al.*, 1980; Schneider *et al.*, 1980). Our results indicate that c-Myc is able to activate the expression of *LDH-A*, increase lactate production, and perhaps account for the elevation of LDH-A levels in various forms of commonly occurring human cancers. An elevation of lactate production in a transgenic mouse model that overexpresses c-Myc in the liver, without development of liver tumor, further supports the induction of the Warburg effect by c-Myc (Valera *et al.*, 1995). Moreover, we observe that ectopic *LDH-A* expression is sufficient to induce the Warburg effect in fibroblasts without conferring the full transformed phenotype of anchorage-independent growth.

THE CELL CYCLE AND ENERGY METABOLISM

The observation that glucose is required for nontransformed cells to progress through the G_1-S boundary has been previously observed with 3T3 fibroblasts (Holley and Kiernan, 1974; Greiner *et al.*, 1994). Low glucose fluxes and repressed levels of glycolytic enzymes have been associated with the lengthening of G_1 (Beck and von Meyenburg, 1968). Furthermore, *Saccharomyces cerevisiae* growth was arrested prior to the START point when deprived of glucose (Gillies *et al.*, 1981). Although the oxygenated yeast cells were arrested by glucose deprivation, the starved yeast cells were able to synthesize ATP, suggesting that the signal for cell cycle progression is created during the catabolism of glucose and not strictly by the energy supply. Intriguingly, Aon *et al.* (1995) have shown that pyruvate kinase is one of the cell division cycle (cdc) proteins, cdc 19, demonstrating the direct connection of glycolysis and the cell cycle. Depletion of ATP *per se* through mitochondrial uncoupling also arrests mammalian cells in G_1, and also in G_2 with extensive ATP depletion (Sweet and Singh, 1995). This observation, along with our observation of a residual G_2-M population of nontransformed Rat1a cells after glucose deprivation, suggests that a glucose-dependent restriction of passage through G_2-M may also exist. These studies suggest that there exists an evolutionarily conserved glucose-dependent cell cycle checkpoint that appears to overlap with START in yeast and with the restriction point in mammalian cells (Pardee, 1974).

CLINICAL IMPLICATIONS

The glucose antimetabolite 2-deoxyglucose differentially induced apoptosis in c-Myc-transformed rat fibroblasts and lymphoblastoid cells (Shim, Chun, and Dang, unpublished observation). Moreover, 2-deoxyglucose induced apoptosis on two of three Burkitt lymphoma cell lines, the Ramos and DW6, but not the ST486; although all three cell lines overexpress c-Myc and have elevated LDH-A levels. Since the Ramos and the ST486 cell lines both contain mutant p53 (Gaidano *et al.*, 1991), it appears that the glucose deprivation-induced apoptosis is independent of wild-type p53 activity. In contrast, wild type p53 is required for c-Myc-induced apoptosis with serum deprivation (Evan *et al.*, 1992; Hermeking and Eick, 1994; Wagner *et al.*, 1994). The high Bcl-2 protein level in the ST486 Burkitt cell line and its low levels in the Ramos and DW6 cell lines suggest that Bcl-2 is a critical determinant of Myc-dependent glucose deprivation-induced apoptosis. Studies performed almost four decades ago indicate that infusions of 2-deoxyglucose into cancer patients were well tolerated (Landau *et al.*, 1958). In leukemic patients, the white cell count fell during the 24-hour period following a single 2-deoxyglucose

infusion and glycolysis was lowered in the leukemic cells. With the available modern molecular probes, cancer cells may be characterized with regard to their molecular characteristics including Bcl-2/Bcl-X_L status, and it is conceivable that this antimetabolite may be effective in activating apoptosis in Bcl-2/Bcl-X_L-negative neoplasms with high LDH-A or c-Myc levels.

ACKNOWLEDGMENTS

This work was supported in part by NIH grants (CA57341 to C.V.D.) and the Wilbur-Rogers Foundation. H.S. is a fellow of the Lymphoma Research Foundation of America and was supported in part by NIH grant T32HL07525. B.C.L. is a Howard Hughes predoctoral fellow. C.V.D. is a Scholar of the Leukemia Society of America.

REFERENCES

Antonsson, C., Arulampalam, V., Whitelaw, M. L., Pettersson, S., and Poellinger, L. (1995). *J. Biol. Chem.* **270**, 13968–13972.

Aon, M. A., Monaco, M. E., and Cortassa, S. (1995). *Exp. Cell. Res.* **217**, 42–51.

Ayer, D. E., Lawrence, Q. A., and Eisenman, R. N. (1995). *Cell* **80**, 767–776.

Barrett, J. F., Lewis, B. C., Hoang, A. T., Alvarez, J. R. J., and Dang, C. V. (1995). *J. Biol. Chem.* **270**, 15923–15925.

Beck, C., and von Meyenburg, H. K. (1968). *J. Bacteriol.* **96**, 479–486.

Beckmann, H., Su, L. K., and Kadesch, T. (1990). *Genes Dev.* **4**, 167–179.

Bello-Fernandez, C., Packham, G., and Cleveland, J. L. (1993). *Proc. Natl. Acad. Sci. USA* **90**, 7804–7808.

Blackwood, E. M., Kretzner, L., and Eisenman, R. N. (1992). *Curr. Opin. Genet. Dev.* **2**, 227–235.

Bodansky, O. (1975). *Biochemistry of Human Cancer,* Academic Press, New York.

Bose, C. K., and Mukherjea, M. (1994). *Cancer Lett.* **77**, 39–43.

Bredin, H. C., Daly, J. J., and Prout, G., Jr. (1975). *J. Urol.* **113**, 487–490.

Bustamante, E., Morris, H. P., and Pedersen, P. L. (1981). *J. Biol. Chem.* **256**, 8699–8704.

Carda-Abella, P., Perez-Cuadrado, S., Lara-Baruque, S., Gil-Grande, L., and Nunez-Puertas, A. (1982). *Cancer* **49**, 80–83.

Chaudhary, D., Gaurcello, V., Jones, D. E., and Miller, D. M. (1996). *Proc. Am. Assoc. Cancer Res.* **37**, 578.

Chow, S. N., Wu, C. C., Hwang, S. C., Tsai, Y. L., and H., C. C. (1991). *Proc. Nat. Sci. Council, Rep. China.* **15**, 101–4.

Chung, K. C., Huang, D., Chen, Y., Short, S., Short, M. L., Zhang, Z., and Jungmann, R. A. (1995). *Mol. Cell. Biol.* **15**, 6139–6149.

Cole, M. D. (1986). *Annu. Rev. Genet.* **20**, 361–384.

Cooper, J. A., Reiss, N. A., Schwartz, R. J., and Hunter, T. (1983). *Nature* **302**, 218–223.

Cowan, R. A., Harris, M., Jones, M., and Crowther, D. (1989). *Br. J. Cancer* **60**, 904–10.

Csako, G., Magrath, I. T., and Elin, R. J. (1982). *Am. J. Clin. Pathol.* **78**, 712–717.

Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C., and Croce, C. M. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 7824–7827.

Dang, C. V., and Lee, L. A. (1995). *c-Myc Function in Neoplasia,* R. G. Landes, Austin, Texas.

Dang, C. V., Dolde, C., Gillison, M., and Kato, G. J. (1992). *Proc. Natl. Acad. Sci. USA* **89**, 599–602.

Eilers, M., Picard, D., Yamamoto, K. R., and Bishop, J.M. (1989). *Nature* **340**, 66–8.

Eilers, M., Schirm, S., and Bishop, J. M. (1991). *EMBO J.* **10**, 133–41.

Ellington, S. K. L. (1987). *Development* **100**, 431–439.

Endrizzi, L., Fiorentino, M. V., Salvagno, L., Segati, R., Pappagallo, G. L., and Fosser, V. (1982). *Eur. J. Cancer Clin. Oncol.* **18**, 945–9.

Evan, G. I., and Littlewood, T. D. (1993). *Curr. Opin. Genet. Dev.* **3**, 44–49.

Evan, G. I., Wyllie, A. H., Gilbert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C. M., Penn, L. Z., and Hancock, D. C. (1992). *Cell* **69**, 119–128.

Fasola, G., Fanin, R., Gherlinzoni, F., Galieni, P., Taruscio, D., Frezza, G., Mazza, P., Pileri, S., and Baccarani, M. (1984). *Acta Haematol.* **72**, 231–8.

Firth, J. D., Ebert, B. L., Pugh, C. W., and Ratcliffe, P. J. (1994). *Proc. Natl. Acad. Sci. USA* **91**, 6496–6500.

Firth, J. D., Ebert, B. L., and Ratcliffe, P. J. (1995). *J. Biol. Chem.* **270**, 21021–21027.

Forsythe, J. A., Jiang, B. H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D., and Semenza, G. L. (1996). *Mol. Cell. Biol.* **16**, 4604–13.

Foufelle, F., Gouhot, B., Pegorier, J. P., Perdereau, D., Girard, J., and Ferre, P. (1992). *J. Biol. Chem.* **267**, 20543–6.

Freitas, I., Bono, B., Bertone, V., Griffini, P., Baronzio, G. F., Bonandrini, L., and Gerzeli, G. (1996). *Anticancer Res.* **16**, 1491–502.

Fukasawa, K. M., and Li, S.-L. (1987). *Genetics* **116**, 99–105.

Gaidano, G., Ballerini, P., Gong, J. Z., Inghirami, G., Neri, A., Newcomb, E. W., Magrath, I. T., Knowles, D. M., and Dalla-Favera, R. (1991). *Proc. Natl. Acad. Sci. USA* **88**, 5413–5417.

Gaubatz, S., Imho, F. A., Dosch, R., Werne, R. O., Mitchell, P., Buettne, R. R., and Eilers, M. (1995). *EMBO J.* **14**, 1508–1519.

Gillies, R. J., Ugurbil, K., den Hollander, J. A., and Shulman, R. G. (1981). *Proc. Natl. Acad. Sci. USA* **78**, 2125–2129.

Gnarra, J. R., Zhou, S., Merrill, M. J., Wagner, J. R., Krumm, A., Papavassiliou, E., Oldfield, E. H., Klausner, R. D., and Linehan, W. M. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 10589–94.

Goldman, R. D., Kaplan, N. O., and Hall, T. C. (1964). *Cancer Res.* **24**, 389–399.

Grandori, C., Mac, J., Siebelt, F., Ayer, D. E., and Eisenman, R. N. (1996). *EMBO J.* **15**, 4344–4357.

Gregor, P. D., Sawadogo, M., and Roeder, R. G. (1990). *Genes Dev.* **4**, 1730–1740.

Greiner, E. F., Guppy, M., and Brand, K. (1994). *J. Biol. Chem.* **269**, 31484–31490.

Grosse, F., Nasheuer, H. P., Scholtissek, S., and Schomburg, U. (1986). *Eur. J. Biochem.* **160**, 459–47.

Gu, W., Cechova, K., Tassi, V., and Dalla-Favera, R. (1993). *Proc. Natl. Acad. Sci. USA* **90**, 2935–2939.

Harrington, E. A., Fanidi, A., and Evan, G. I. (1994). *Curr. Opin. Genet. Dev.* **4**, 120–129.

Hemlinger, G., Yuan, F., Dellian M., and Jain, R. K. (1997). *Nature Med.* **3**, 177–182.

Hermeking, H., and Eick, D. (1994). *Science* **265**, 2091–2093.

Hockenbery, D. M., Oltvai, Z. N., Yin, X. M., Milliman, C. L., and Korsmeyer, S. J. (1993). *Cell* **75**, 241–251.

Holley, R. W., and Kiernan, J. A. (1974). *Proc. Natl. Acad. Sci. USA* **71**, 2942–2945.
Huang, D., and Jungmann, R. A. (1995). *Mol. Cell. Endocrinol.* **108**, 87–94.
Hubank, M., and Schatz, D. G. (1994). *Nucleic Acids Res.* **22**, 5640–5648.
Jacoby, D. B., Zilz, N. D., and Towle, H. C. (1989). *J. Biol. Chem.* **264**, 17623–6.
Jagannath, S., Velasquez, W. S., Tucker, S. L., Manning, J. T., McLaughlin, P., and Fuller, L. M. (1985). *J. Clin. Oncol.* **3**, 39–47.
Johansen, J. S., Cintin, C., Jorgensen, M., Kamby, C., and Price, P. A. (1995). *Eur. J. Cancer* **31A**, 1437–42.
Jones, R. J., Branda, J., Johnston, K. A., Polymenis, M., Gadd, M., Rustgi, A., Callanan, L., and Schmidt, E. V. (1996). *Mol. Cell. Biol.* **16**, 4754–4764.
Jump, D. B., Bell, A., and Santiago, V. (1990). *J. Biol. Chem.* **265**, 3474–8.
Kanno, T., Sudo, K., Maekawa, M., Nishimura, Y., Ukita, M., and Fukutake, K. (1988). *Clin. Chim. Acta* **173**, 89–98.
Kawamoto, M. (1994). *Cancer* **73**, 1836–41.
Klein, E. A. (1993). *Urol. Clin. North Am.* **20**, 67–73.
Kretzner, L., Blackwood, E. M., and Eisenman, R. N. (1992). *Nature* **359**, 426–429.
Landau, B. R., Laszlo, J., Stengle, J., and Burk, D. (1958). *J. Natl. Cancer Inst.* **21**, 485–494.
Lee, L. A., Dolde, C., Barrett, J., Wu, C. S., and Dang, C. V. (1996). *J. Clin. Invest.* **97**, 1687–1695.
Lee, P. J., Jiang, B. H., Chin, B. Y., Iyer, N. V., Alam, J., Semenza, G. L., and Choi, A. M. (1997). *J. Biol. Chem.* **272**, 5375–81.
Lefrancois-Martinez, A. M., Diaz-Guerra, M. J., Vallet, V., Kahn, A., and Antoine, B. (1994). *FASEB J* **8**, 89–96.
Li, L. H., Nerlov, C., Prendergast, G., MacGregor, D., and Ziff, E. B. (1994). *EMBO J.* **13**, 4070–4079.
Li, S. S., Pan, Y. E., Sharief, F. S., Evans, M. J., Lin, M. F., Clinton, G. M., and Holbrook, J. J. (1988). *Cancer Invest.* **6**, 93–101.
Lisitsyn, N., Lisitsyn, N., and Wigler, M. (1993). *Science* **259**, 946–951.
Liu, Z., Thompson, K. S., and Towle, H. C. (1993). *J. Biol. Chem.* **268**, 12787–95.
Lombardi, L., Newcomb, E. W., and Dalla-Favera, R. (1987). *Cell* **49**, 161–170.
Magrath, I., Lee, Y. J., Anderson, T., Henle, W., Ziegler, J., Simon, R., and Schein, P. (1980). *Cancer* **45**, 1507–1515.
Maltepe, E., Schmidt, J. V., Baunoch, D., Bradfield, C. A., and Simon, M. C. (1997). *Nature* **386**, 403–7.
Matrisian, L. M., Rautmann, G., Magun, B. E., and Breathnach, R. (1985). *Nucleic Acids Res.* **13**, 711–726.
Meichle, A., Philipp, A., and Eilers, M. (1992). *Biochim. Biophys. Acta* **1114**, 129–146.
Merkle, S., Favor, J., Graw, J., Hornhardt, S., and Pretsch, W. (1992). *Genetics* **131**, 413–421.
Miltenberger, R. J., Sukow, K. A., and Farnham, P. J. (1995). *Mol. Cell. Biol.* **15**, 2527–2535.
Mintzer, D. M., Andreeff, M., Filippa, D. A., Jhanwar, S. C., Chaganti, R. S., and Koziner, B. (1984). *Blood* **64**, 415–21.
Nevin, G. B., and Mulholland, P. (1988). *Tumour Biol.* **9**, 195–199.
Nishikawa, A., Tanaka, T., Takeuchi, T., Fujihiro, S., and Mori, H. (1991). *Br. J. Cancer* **63**, 819–821.
Packham, G., and Cleveland, J. L. (1995). *Biochim. Biophys. Acta* **1242**, 11–28.
Pan, L., Beverley, P. C., and Isaacson, P.G. (1991). *Clin. Exp. Immunol.* **86**, 240–5.
Pardee, A. B. (1974). *Proc. Natl. Acad. Sci. USA* **71**, 1286–1290.
Pardee, A. B., Dubrow, R., Hamlin, J. L., and Kletzien, R. F. (1978). *Annu. Rev. Biochem.* **47**, 715–750.
Patel, P. S., Rawal, G. N., and Balar, D. B. (1993). *Gynecol. Oncol.* **50**, 294–9.
Pedersen, P. L. (1978). *Prog. Exp. Tumor Res.* **22**, 190–274.
Philipp, A., Schneider, A., Vasrik, I., Finke, K., Xiong, Y., Beach, D., Alitalo, K., and Eilers, M. (1994). *Mol. Cell. Biol.* **14**, 4032–4043.
Prendergast, G. C., and Ziff, E. B. (1992). *Trends Genet.* **8**, 91–6.
Racker, E., and Spector, M. (1981). *Science* **213**, 303–307 (retracted by Racker, E. In: *Science* **213**, 1313).
Rempel, A., Mathupala, S. P., Griffin, C. A., Hawkins, A. L., and Pedersen, P. L. (1996). *Cancer Res.* **56**, 2468–71.
Schneider, R. J., Seibert, K., Passe, S., Little, C., Gee, T., Lee, B. D., Mike, V., and Young, C. W. (1980). *Cancer* **46**, 139–143.
Schuldiner, O., Eden, A., Ben-Yosef, T., Yanuka, O., Simchen, G., and Benvenisty, N. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 7143–7148.
Semenza, G. L., Roth, P. H., Fang, H. M., and Wang, G. L. (1994). *J. Biol. Chem.* **269**, 23757–23763.
Semenza, G. L., Jiang, B. H., Leung, S. W., Passantino, R., Concorde, J. P., Maire, P., and Giallongo, A. (1996). *J. Biol. Chem.* **271**, 32529–32537.
Sharief, F. S., Wilson, S. H., and Li, S. S. (1986). *Biochem. J.* **233**, 913–916.
Shih, H., and Towle, H. C. (1994). *J. Biol. Chem.* **269**, 9380–9387.
Shih, H. M., Liu, Z., and Towle, H. C. (1995). *J. Biol. Chem.* **270**, 21991–21997.
Shim, H., Dolde, C., Lewis, B. C., Wu, C., Dang, G., Jungmann, R. A., Dalla-Favera, R., and Dang, C. V. (1997). *Proc. Natl. Acad. Sci. USA* **94**, 6658–6663.
Short, M. L., Huang, D., Milkowski, D. M., Short, S., Kunstman, K., Soong, C. J., Chung, K .C., and Jungmann, R. A. (1994). *Biochem. J.* **304**, 391–398.
Siemeister, G., Weindel, K., Mohrs, K., Barleon, B., Martiny-Baron, G., and Marme, D. (1996). *Cancer Res.* **56**, 2299–301.
Small, M. B., Hay, N., Schwab, M., and Bishop, J. M. (1987). *Mol. Cell. Biol.* **7**, 1638–1645.
Sogawa, K., Nakano, R., Kobayashi, A., Kikuchi, Y., Ohe, N., Matsushita, N., and Fujii-Kuriyama, Y. (1995). *Proc. Natl. Acad. Sci. USA* **92**, 1936–40.
Stone, J., de Lange, T., Ramsay, G., Jakobovits, E., Bishop, J. M., Varmus, H., and Lee, W. (1987). *Mol. Cell. Biol.* **7**, 1697–709.
Sutherland, R. M., Sordat, B., Bmat, J., Gabbert, H., Bourat, B., and Mueller-Klieser, W. (1986). *Cancer Res.* **46**, 5320–5329.
Swan, F., Jr., Velasquez, W. S., Tucker, S., Redman, J. R., Rodriguez, M. A., McLaughlin, P., Hagemeister, F. B., and Cabanillas, F. (1989). *J. Clin. Oncol.* **7**, 1518–27.
Swanson, H. I., Chan, W. K., and Bradfield, C. A. (1995). *J. Biol. Chem.* **270**, 26292–302.
Sweet, S., and Singh, G. (1995). *Cancer Res.* **55**, 5164–5167.
Takano, T., and Li, S. S.-L. (1990). *J. Genet. Mol. Biol.* **1**, 34–44.
Tanaka, T., Fujii, M., Nishikawa, A., Bunai, Y., Obayashi, F., Sugie, S., Shima, H., Yoshimi, N., Kuniyashu, T., Kato, K., *et al.* (1984). *Cancer Detect. Prevent.* **7**, 65–71.
Tavtigian, S. V., Zabludoff, S. D., and Wold, B. J. (1994). *Mol. Biol. Cell.* **5**, 375–388.
Templeton, D. J., and Weinberg, R. A. (1991). Principles of cancer biology. In *American Cancer Society Textbook of Clinical Oncology* (Holleb, A. I., Fink, D. J., and Murphy, G. P., eds.) American Cancer Society, Atlanta, pp. 678–690.
Thompson, K. S., and Towle, H. C. (1991). *J. Biol. Chem.* **266**, 8679–82.
Towle, H. C. (1995). *J. Biol. Chem.* **270**, 23235–23238.
Valera, A., Pujol, A., Gregori, X., Riu, E., Visa, J., and Bosch, F. (1995). *FASEB J.* **9**, 1067–1078.
Vaulont, S., Munnich, A., Decaux, J. F., and Kahn, A. (1986). *J. Biol. Chem.* **261**, 7621–5.
Vergnon, J. M., Guidollet, J., Gateau, O., Ripoll, J. P., Collet, P., Louisot, P., and Brune, J. (1984). *Cancer* **54**, 507–511.
Wagner, A. J., Kokontis, J. M., and Hay, N. (1994). *Genes Dev.* **8**, 2817–2830.

Wang, G. L., and Semenza, G. L. (1995). *J. Biol. Chem.* **270**, 1230–1237.
Wang, G. L., Jiang, B. H., Rue, E. A., and Semenza, G. L. (1995). *Proc. Natl. Acad. Sci. USA* **92**, 5510–5514.
Warburg, O. (1930). *The Metabolism of Tumours,* Constable, London.
Warburg, O. (1956). *Science* **123**, 309–314.
White, E. (1996). *Genes Dev* **10**, 1–15.
Williams, K. R., Reddigari, S., and Patel, G. L. (1985). *Proc. Natl. Acad. Sci. USA* **82**, 5260–5264.
Wizigmann-Voos, S., Breier, G., Risau, W., and Plate, K. H. (1995). *Cancer Res* **55**, 1358–64.
Woollams, R., Barratt, P. J., Orwell, R. L., and Piper, D. W. (1976). *Digestion* **14**, 20–28.
Zhong, X. H., and Howard, B. D. (1990). *Mol. Cell. Biol.* **10**, 770–776.