# The Search for the Achilles Heel of Cancer

## Introduction

Numerous studies have demonstrated that the altered glucose metabolism in tumor cells and the increased proliferative and invasive potentials of cancer cells are frequently associated with specific mutations in oncogenes and/or tumor suppressor genes. The combination of these mutations and the hypoxic conditions in many tumors is widely held to dictate the overall metabolic state of individual tumor cells and their aggressiveness. Although the precise mechanisms underlying the unholy coupling of aerobic glycolysis with tumorigenesis have not been fully resolved, a major breakthrough in our understanding of the molecular link between the glycolytic and tumorigenic phenotypes has been made. More specifically, it has been demonstrated that the production of fructose-2-6-bisphosphate (F-2-6-BP), a well-established tumor survival factor, is differentially up-regulated in the cytoplasm and nucleus of cancer cells to coordinately enhance aerobic glycolysis and cell cycling, respectively (1). The potential exploitation of this finding for selective intervention with cancer will be discussed here in the context of our current understanding of cancer biology.

## I: Hypoxia and anaerobic glycolysis

Glycolysis is a ten-step process that breaks-down glucose to pyruvate, takes place in the cytoplasm of virtually all eukaryotic cells, and requires the presence of NAD+, but not oxygen, as an oxidizing agent (Figure 1). Under aerobic conditions, NAD+ is regenerated from NADH by oxidative phosphorylation (OXPHOR), a five-step mitochondrial process in which a pair of electrons is transferred from electron donors, such as NADH and NADPH, to oxygen. The energy released during this process is efficiently utilized to generate ATP from ADP. In the absence of oxygen, NAD+ is regenerated in mammalian cells through the action of lactate dehydrogenase A, an enzyme that uses NADH as a cofactor to convert pyruvate to lactate. To sustain glycolysis under anaerobic conditions, known as anaerobic glycolysis, lactate is excreted from the cells as a waste product. Thus, in the absence of oxygen, the net cellular gain from glycolytic breakdown of one glucose molecule is two ATP molecules, which constitute less than 7% of the total ATP generated by complete oxidation of glucose to $CO_2$ and $H_2O$. For this reason, cells normally switch from slow aerobic to rapid anaerobic consumption of glucose, a phenomenon first noted by Louis Pasteur in 1857, and known as the Pasteur effect. The avid consumption of glucose by tumor cells in the presence of oxygen (the Warburg effect) is associated with enhanced glycolytic flux, increased glucose oxidation in the pentose phosphate pathway (PPP), and down-regulation of mitochondrial respiration. Although such a mode of glucose utilization compromises ATP yields, it confers tumor cells a significant growth advantage by: (I) attenuating generation of OXPHOR-derived reactive oxygen species (ROS), which are known to induce cellular senescence and/or apoptosis, and (II) providing a ready supply of NADPH and other essential reagents for *de novo* biosynthesis of macromolecules needed for cell proliferation and invasion (Figure 1).

Under the hypoxic conditions that prevail in many tumors, anaerobic glycolysis is often accompanied by significant acidosis of the cellular microenvironment due to increased release of lactate and slow removal of the glycolytic waste product, lactic acid. By contrast, lactic acid produced under anaerobic conditions in normal tissues, as in overworked skeletal muscles, is readily transported, via blood, to the liver for recycling. Thus, in the inhospitable environment of tumor cells, the conversion of pyruvate to lactate is a double-edged sword. On one hand, it facilitates anaerobic glycolysis by regenerating its essential oxidizing agent, NAD+, but on the other, it impairs the ability of the cells to sustain it, because of increased acid accumulation. It has been shown that tumor cells lacking functional p53 expression possess an increased tolerance to low intracellular and extracellular pH. This confers them not only the ability to sustain high rates of acid-producing glycolysis, but also a powerful growth advantage over adjacent cells that express functional p53 and are therefore highly vulnerable to acid-induced, p53-dependent apoptosis (2-3). Moreover, the lowering of the external pH by these cells may lead to increased degradation of the extracellular matrix, which is known to promote angiogenesis and metastasis.

## II: Glucose Metabolism in Cancer: Close Examination of the Warburg Effect

Under normal oxygen concentrations (normoxia), tumor tissues, but not adjacent normal tissues, exhibit a high rate of glucose consumption. This phenomenon, known as aerobic glycolysis or the Warburg effect, has been widely exploited for the diagnosis and staging of human solid cancers by FDG-PET, an imaging technique for detecting increased glucose uptake by positron emission tomography (PET) of the glucose analogue tracer, [2-18F]-2-deoxyglucose (FDG) (4).

Increased consumption of nutrients, particularly glucose and glutamine, is exhibited not only by cancer cells, but also by normal proliferating mammalian cells. Given the increased energetic and biosynthetic needs of dividing cells and their greater sensitivity to oxidative stress from OXPHOR-derived ROS, it is not surprising that upon stimulation of glucose uptake, both cancer and normal proliferating cells down-regulate OXPHOR along with up-regulating glucose processing via aerobic glycolysis and the pentose phosphate pathway (PPP). Furthermore, close examination of the energetic and biosynthetic needs of dividing cells reveals that although such a switch in glucose metabolism compromises ATP yields, it is essential for successful cell division, for the following reasons: (I) the need for NADPH in biosynthetic processes often exceeds that of ATP (e.g. fatty-acid biosynthesis requires twice as many NADPH than ATP molecules), (II) NADPH plays a key role in maintaining the cellular redox state by regenerating reduced glutathione, which is critical for cellular protection against ROS-associated DNA damage, (III) the PPP is the major cellular source for NADPH and ribose-5-phosphate, an essential precursor for nucleic-acid biosynthesis, (IV) satisfying increased cellular demands for ATP by OXPHOR would jeopardize the cell by severely impairing its antioxidant status, both through generation of ROS and depletion of NADPH, and (V) enhanced glycolysis combined with increased



**Figure 1**
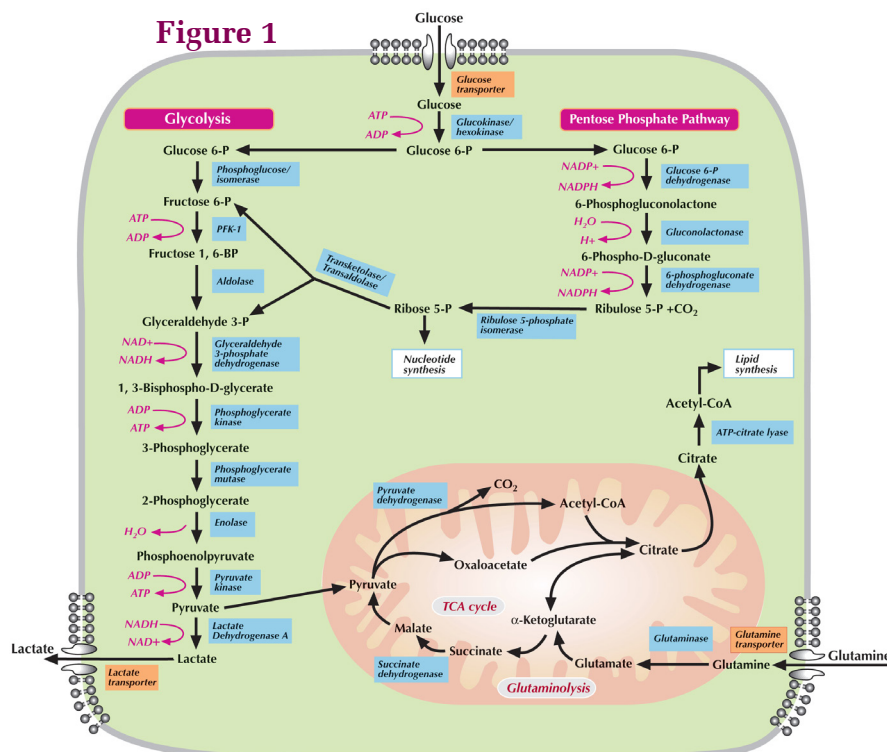
glutaminolysis can rapidly generate sufficient amounts of ATP as well as key building blocks for lipid and protein biosynthesis.

In normal dividing cells, upregulation of nutrient consumption is critically dependent on the presence of compatible growth factors, i.e. lineage-specific growth factors capable of activating growth-promoting (mitogenic) signaling pathways, such as the PI3K/AKT/mTOR and Ras/Raf-MAPK pathways. In cancer cells, these pathways are constitutively activated as a result of activation of oncogenes and/or inactivation of tumor suppressor genes. Taken together, these data clearly indicate that the cell-autonomous activation of nutrient consumption and not the Warburg effect *per se* is the key metabolic characteristic that distinguishes cancer cells from normal proliferating cells.

## III: Control of the Mammalian Cell Cycle during and after Tissue Neogenesis

For a newly divided postnatal mammalian cell to become competent for another entry into mitosis (M) it must first exit quiescence ($G_0$ state), and then proceed through the G1, S, and G2 cell-cycle phases, collectively known as interphase (Figure 2). The $G_0$/G1 transition is reversible and dependent upon the presence of compatible growth factors and nutrients in the extracellular milieu. The progression through interphase requires sequential activation, by cyclins, of at least two cyclin-dependent kinases (Cdks), Cdk4 or Cdk6, which appear to be redundant, and Cdk2. Activation of Cdk4/6 by cyclin D is required for overcoming the suppression of G1 progression by retinoblastoma proteins (pRB), whereas successive activation of Cdk2, first by cyclin E and then by cyclin A, is required for crossing the G1/S restriction point and advancing through the S and G2 phases, respectively. Crossing the G2/M checkpoint and proceeding through the M phase is governed by cyclin B/Cdk1 complex (Figure 2).

The entry to, progression through, and exit from cell-cycle phases require sequential accumulation and degradation of D-, E-, A-, and B-type cyclins. However, although the concentration of cyclin B in late G2 phase is sufficient to drive
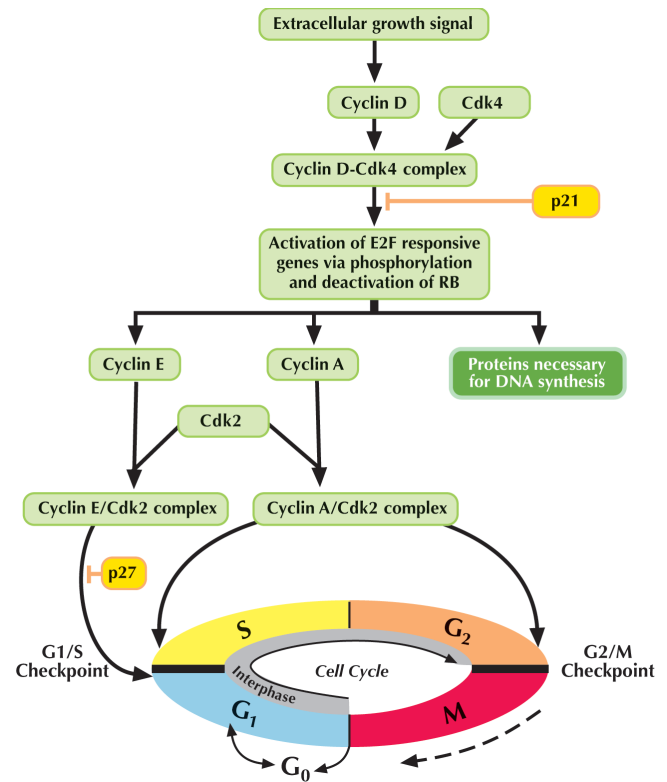


**Figure 2:** Schematic illustration of the mammalian cell cycle.

the formation of cyclin B/Cdk1, this complex is held inactive until its action is needed. To prevent premature mitotic entry, on one hand, and to relieve the cell from the otherwise unbearable load of substances prepared for cell replication, on the other, the activation of cyclin B/Cdk1 must be accurately timed. The forestalling of cyclin B/Cdk1 action is accomplished through phosphorylation of two Cdk1 residues (Thr14 and Tyr15) by specific protein kinases, Wee1 and Myt1, whose action renders cyclin B/Cdk1 complex inactive. Activation of this complex requires dephosphorylation of these residues by Cdc25 phosphatase (e.g. Cdc25B and Cdc25C) whose activation requires successive phosphorylation of several sites, first by Polo-like kinase 1(Plk1) which targets Cdc25 to the nucleus, and then by a nuclear kinase whose identity is still unknown. Since activated Cdc25 phosphatase is the final activator of cyclin B/Cdk1 complex and therefore also the ultimate effector of mitotic entry, the timing of its activation must be accurately coordinated. This necessitates reliable messenger(s) from the cytoplasm to induce Cdc25-activating phosphorylation in the nucleus shortly after the completion of biomass preparation for cell replication. Although the

exact mechanism underlying Cdc25 activation is still unresolved, evidence strongly suggests, as later discussed, that the conversion of fructose-6-phosphate (produced in the cytoplasm) to fructose-2-6-bisphosphate in the nucleus plays an important role in coordinating and triggering the timely entry of cells into mitosis.

It has been demonstrated that mouse embryos lacking all interphase Cdks (i.e. Cdk2, Cdk4, and Cdk6) undergo organogenesis and develop normally to mid-gestation at embryonic day 12.5 (E12.5), indicating that during embryonic tissue neogenesis, Cdk1 alone is sufficient to drive the cell cycle (5). However, these embryos were not normal and began to die at E13.5, with all dying by E15.5. This phenomenon was associated with substantial apoptosis at E13.5-E14.5, indicating that post mid-gestation, the control of tissue growth and remodeling is more stringent than during tissue neogenesis, requiring the activation of at least one interphase Cdk. *In vitro* proliferation of primary fibroblasts isolated from these embryos at E12.5 was partially compromised, yet they became immortal on continuous passage. Interestingly, although the absence of interphase Cdks in mouse embryos at E12.5 did not significantly affect the expression levels of cyclins and other cell-cycle regulators, the protein levels, but not mRNA levels, of cyclin dependent kinase inhibitor 1b (Cdkn1b) were significantly lower than in E12.5 wild-type mouse embryos (5).

Cdkn1b, also known as p27Kip1 or p27, is one of three Cip/Kip family members. The other two are p21Cip1 (p21) and p57Kip2 (p57). Although Cip/Kip proteins share the ability to inhibit all interphase cyclin/Cdk complexes, they differ from one another by the regulatory roles that they play in the cell cycle. The main function of p21, which is a transcriptional target of p53, is to mediate cell-cycle arrest, primarily during the G1 phase, in response to DNA damage. The main roles of p57 and p27 are to regulate $G_0$/G1 transition during and after tissue neogenesis, respectively. To forestall premature entry of growth factor-starved and/or nutrient-famished cells into the cell cycle, the protein levels of p27 and p57 are elevated in quiescent cells. Upon entry into G1 phase, the cellular level of these proteins sharply declines as a result of increased protein degradation, which is triggered by their phosphorylation. The rapid degradation of p27 has been shown to involve phosphorylation of its threonine residue at position 187 (1).

E2f1, E2f2, and E2f3, collectively referred to as E2F1, form heterodimeric complexes with Dimerization Partner (DP) proteins. E2F1/DP complexes, referred to as E2F transcription factors, are considered to be the ultimate effectors of the G1/S transition of the cell cycle. E2F1 and DP proteins have a conserved N-terminal DNA binding domain and a dimerization domain. When bound to DNA as free E2F1/DP heterodimers, E2F transcription factors stimulate the expression of all the proteins involved in cell cycle progression through interphase including: (I) cyclin E, whose complex with Cdk2 is critical for the completion of G1 phase and entry into the S phase, (II) Cyclin A, whose complex with Cdk2 is required for the completion of S and G2 phases, (III) proteins involved in DNA synthesis during the S phase, and (IV) E2F1, E2f7, and E2f8. Unlike other E2fs, E2f7 and E2f8 have two DNA binding sites rather than one. This renders them the ability not only to effectively compete with E2F1/DP heterodimers for their DNA binding sites, but also to keep their action in balance via a negative feedback-loop mechanism. Ectopic expression of E2f7 or E2f8 in mouse embryos resulted in cell-cycle arrest at G1, whereas their combined ablation in mouse embryos resulted in embryonic lethality by mid-gestation (6). *In vitro* proliferation of cells lacking both E2f7 and E2f8 was associated with substantial DNA damage and increased p53-dependent apoptosis, underscoring the importance of E2f7/E2f8 proteins in cell-cycle regulation. Interestingly, E2F1-null mouse embryos develop normally until E11.5 and then die (6), indicating that E2F1 expression is not required for tissue neogenesis, at least during embryonic organogenesis. In $G_0$ and early G1 phase, E2F1/DP transcriptional activity is repressed by the retinoblastoma tumor-suppressor proteins (pRB), which bind to E2F1 and block the activation of E2F1/DP target

genes. The mechanisms by which these proteins suppress E2F1/DP activity have not been fully resolved, but it has been shown that pRB can actively inhibit DNA transcription by recruiting histone deacetylases and lysine/arginine methyl transferases. Ablation of pRB repression in late G1 phase is dependent on mitogenic or oncogenic signaling and involves phosphorylation of a specific set of sites on pRB by cyclin D complexes with either Cdk4 or Cdk6. Thus far, *in vitro* studies have failed to detect significant differences between the activities of Cdk4 and Cdk6 complexes with either one of the three D-type cyclins (D-1, D-2, and D-3).

## IV: Coupling Aerobic Glycolysis and Cell Cycling by Fructose-2-6-Bisphophate

Note: Since the glycolytic process *per se* does not require the presence of oxygen, the adjective "aerobic" and "anaerobic" are somewhat misleading when used in conjunction with glycolysis. To underscore the metabolic significance of enhanced glycolysis under aerobic and anaerobic conditions, the terms anabolic glycolysis and catabolic glycolysis, respectively, will be used henceforth.

Studies have established that the switch from normal glucose metabolism to either anabolic glycolysis (the Warburg effect) or catabolic glycolysis (the Pasteur effect) requires significant elevation of the cytoplasmic level of fructose-2-6-bisphoshate (F-2-6-BP). At micromolar concentrations, F-2-6-BP is a powerful activator of 6-phosphofructose-1 kinase (PFK-1), the glycolytic enzyme that catalyzes the phosphorylation of fructose-6-phosphate (F6P) to fructose-1-6-bisphosphate (F-1-6-BP). This conversion constitutes the rate-limiting step and a key checkpoint of glycolysis. Unlike F-1-6-BP, F-2-6-BP is an extremely acid-labile molecule which readily decomposes to F6P and inorganic phosphate even under slightly acidic conditions, e.g., in the presence of 10µM HCL at 20°C (7). Although the 2' phosphoryl group of F-2-6-BP renders it instable, it confers F-2-6-BP the ability to enhance the phosphorylation of F6P by PFK-1 through a mechanism that involves: (I) formation of the ternary complex, F6P/PFK-1/F-2-6-BP, through binding of F6P and F-2-6-BP to distinct binding sites on PFK-1, (II) transfer of the 2' phosphoryl group of F-2-6-BP to a histidyl residue of PFK-1, and (III) substrate-level phosphorylation of F6P by PFK-1. The net result of these events is the conversion of F-2-6-BP to F-1-6-BP. The frequency at which this ternary complex is formed is influenced not only by the cytoplasmic concentrations of the complex's components, but also by those of ATP and citrate, which compete with F-2-6-BP for its binding site on PFK-1. As later discussed, several lines of evidence suggest that the binding of F-2-6-BP to PFK-1 can be competitively inhibited by vitamin C (ascorbate), which appears to serve as a global regulator of metabolic transformations by controlling the action of PFK-1 and adenylate cyclase, the enzyme that catalyzes the conversion of ATP to cyclic AMP (cAMP). Binding of either ATP or citrate to PFK-1 can forestall the glycolytic process at the PFK-1 checkpoint, an event that occurs when their cytoplasmic concentrations exceed their homeostatic set-point values. However, if these values were meant to prevail during the S phase of the cell cycle, cell growth would not be possible. To overcome the suppression of PFK-1 activity by either ATP or citrate, the cytoplasmic level of F-2-6-BP must be considerably elevated upon cell entry into the S phase. This requires increased expression and activation of PFKFB3, a 6-phosphofructose-2-kinase (PFK-2), which subsequent to its activation by AMP activated protein kinase (AMPK) can rapidly increase the cytoplasmic level of F-2-6-BP to a concentration range in which F-2-6-BP can override the suppression of its binding to PFK-1 by either ATP and/or citrate. AMPK, also known as the "energy gauge" of the cell, is a key regulator of the intracellular AMP/ATP setpoint ratio. When this ratio assumes values above its homeostatic set point, AMPK acts to suppress ATP consuming processes (e.g. *de novo* macromolecule biosyntheses) along with activating ATP-generating processes. An important pathway that leads to increased ATP production involves the activation of PFKFB3 through phosphorylation of its Ser461 residue by AMPK (8). PFKFB3 belongs to the PFKFB family of bifunctional enzymes which interconvert their substrates, F6P and F-2-6-BP. Members of this family are encoded by four independent genes (PFKFB1-4) and display

distinct tissue expression profiles. With the exception of PFKFB3, the kinase/phosphatase activity ratio of PFKFB isozymes is approximately 1:1. As such, they play an important role in the regulation of the low steady-state levels of F-2-6-BP in the tissues of their expression. In contrast, PFKFB3 is an inducible enzyme whose expression is increased in response to hypoxic challenge and during the S phase of the cell cycle. Its high kinase/phosphatase activity ratio (approx. 740:1) enables PFKFB3 to rapidly elevate the cellular level of F-2-6-BP, which is critical for both cell survival under hypoxia and cell proliferation under normoxia. The pivotal role of PFKFB3 in promoting both catabolic and anabolic glycolysis is underscored by the findings that cells that cannot express PFKFB3, such as neurons, die rapidly following inhibition of mitochondrial respiration, whereas PFKFB3 overexpressing cells, such as those found in virtually all human cancers, exhibit increased glucose consumption and uncontrolled cell proliferation. A novel PFKFB3 inhibitor, PFK158, has recently been shown to promote lipophagy and chemosensitivity in gynecologic cancers, suggesting an important clinical role for PFKFB3 (9).

PFKFB3 is the only metabolic enzyme known to contain multiple copies of the AUUUA sequence in the 3' untranslated region of its mRNA. This motif confers mRNA instability and enhanced translational activity and is found in mRNAs of several inflammatory cytokines and proto-oncogenes that are members of the early response gene family. Alternative splicing of the PFKFB3 mRNA generates 6 variants encoding distinct C-terminal domains. Two of these variants, designated PFKFB3-ACG and PFKBFB3-AG are the predominant isoforms expressed in human cancers (8). The C-terminal domain of PFKFB3-ACG (also known as splice variant 5) has been shown to localize the enzyme to the nucleus (1). Unexpectedly, over-expression of this variant in HeLa cells had no effect on glucose metabolism, but rather markedly increased their proliferation, an effect that was associated with increased expression of Cdk1, cyclin D-3, and Cdc25C, and decreased protein level of p27Kip1. Taken together, these findings indicate that F-2-6-BP may couple the activation of anabolic glycolysis with cell proliferation (1). If this is indeed the case, then F6P must be the messenger from the cytoplasm that informs the nucleus that biomass preparation for cell replication has been completed. Upon completion of this preparation, anabolic glycolysis is inhibited at the PFK-1 checkpoint by ATP and citrate, resulting in increased cytoplasmic concentration of F6P. Apparently, F6P can diffuse or otherwise reach the nucleus, where it is converted by PFKFB3-ACG to F-2-6-BP. Although the mechanism underlying the induction of mitotic entry by F-2-6-BP is unknown, it is reasonable to assume that it facilitates the activation through phosphorylation of the kinase that activates Cdc25. As previously discussed, activated Cdc25 is the ultimate effector of mitotic entry.

## V: Suppression of Cancer by Vitamin C

Vitamin C, also known as L-ascorbic acid (AA) or ascorbate is one of the most abundantly produced substances in plants and living organisms, indicating a major physiological significance for this molecule. In mammals, endogenously-derived vitamin C is produced in the liver from D-glucose via the glucuronic pathway, a four-step process that requires the action of four different enzymes. The gene encoding one of these enzymes, L-gulonolactone oxidase, is defective in humans, guinea pigs, and few other animal species, rendering them dependent on dietary sources for proper supply of vitamin C. Numerous studies have suggested that insufficient dietary intake levels of vitamin C may adversely affect health and normal life span in man, and could be one of the reasons for the relatively high incidence of cancer in humans. Although vitamin C has been known for more than 80 years, the optimal vitamin C requirements for humans are still unknown and remain a topic of controversy. The question whether vitamin C, like other vitamins, should be considered a required **micro**nutrient or an essential **macro**nutrient whose consumption is needed for optimal human health has not been settled yet. However, findings indicating a critical role for vitamin C in restraining excessive cellular consump-

tion of glucose strongly suggest that the recommended dietary allowance (RDA) for vitamin C may not be sufficient to prevent the initiation of disease processes that could eventually lead to cancer and possibly other human diseases. Glucose and vitamin C are six-carbon sugars whose over-consumption positively and negatively affects neoplasia, respectively. Addictive cellular consumption of glucose, the hallmark of neoplastic cells, promotes cell proliferation and increases the risk of cancer, whereas levels of vitamin C at or near saturation in plasma and tissues protect multi-cellular organisms from ROS damage and uncontrolled cell proliferation. In humans, low levels of vitamin C were found in patients with lung cancer, bronchial asthma, cystic fibrosis, chronic obstructive pulmonary disease, and in smokers (10-14). Unlike glucose, vitamin C cannot be processed in any metabolic pathway and its urinary excretion does not necessarily indicate any disorder or its degree of saturation in bodily tissues, particularly in lungs and respiratory tract, where the level of vitamin C may rapidly decline as a result of oxidation by exogenously-derived oxidants.

Vitamin C is a reducing sugar with an extremely low toxicity capable of donating electrons to electron acceptors such as oxidized metal ions (e.g. $Fe^{3+}$ and $Cu^{2+}$) and ROS. Donation of a pair of electrons by vitamin C converts it to dehydroascorbic acid (DAA). The intracellular ratio between the reduced and oxidized forms of vitamin C depends on the cellular redox status, which in turn is influenced by the rate of mitochondrial production of ROS. As depicted in Figure 3, the intracellular levels of vitamin C and D-AA are controlled through the expression of two types of transporters: sodium-ascorbate cotransporters (SVCT1 and SVCT2), which exclusively import AA, and hexose transporters (GLUT1 and GLUT3), which transfer D-AA across the plasma membrane. The physiological significance of SVCT transporters is underscored by the finding that mice lacking functional SVCT2 gene die shortly after birth (15).

Vitamin C is the major water-soluble antioxidant in cells and biologic fluids, and an essential cofactor for hydroxylases, i.e. enzymes that
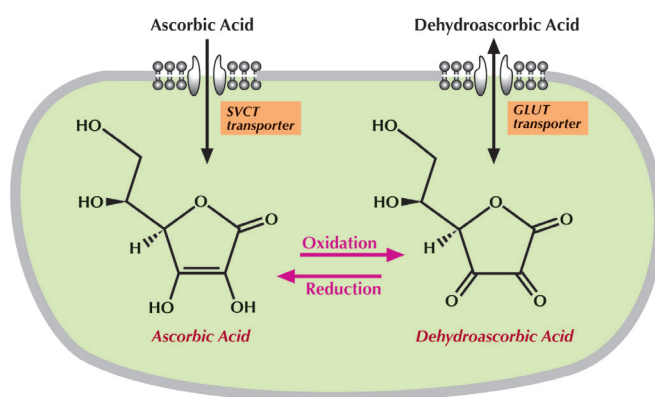
catalyze hydroxylation reactions. In these reactions, vitamin C plays a critical role in maintaining the prostatic metal ions of hydroxylases in their reduced form (e.g. $Fe^{2+}$ and $Cu^{+}$). Two hydroxylation reactions with significant relevance to cancer prevention are: (I) hydroxylation of prolyl and lysyl residues in procollagen which facilitates formation of a highly stable collagen, which in turn stabilizes the extracellular matrix and prevents tumor growth and metastasis. In contrast, reduced hydroxylation of these residues destabilizes the triple helix of collagen and results in increased collagen degradation, and (II) prolyl hydroxylation of hypoxia-inducible factor-1 alpha (HIF-1α) which promotes its association with Von Hippel-Lindau (VHL), a tumor suppressor protein responsible for targeting hydroxylated HIF-1α for ubiquitination and proteosomal degradation. HIF-1α is one of two subunits of the heterodimeric transcriptional factor, HIF-1, whose target genes encode glucose transporters, glycolytic enzymes, and other proteins involved in anabolic and catabolic glycolysis, including PFKFB3. Although counterintuitive, hypoxia increases, rather than decreases the intracellular level of ROS, whose increased intracellular level leads to significant oxidation of vitamin C, resulting in decreased prolyl hydroxylation of HIF-1α and activation of HIF-1. It level of F-2-6-BP by inhibiting PFKFB3 expression, vitamin C promotes glucose processing via the PPP, which, as previously discussed, is essential for maintaining the cellular redox state and genomic stability.

It has been proposed that vitamin C is a global regulator of the intracellular level of cAMP, an important second messenger in many cellu-

lar responses to hormonal signaling (16). The data obtained in studies on the potential usage of high doses of vitamin C in treating patients with Charcot-Marie-Tooth disease strongly suggest that vitamin C regulates cAMP action through competitive inhibition of ATP binding to adenylate cyclase (AC). This proposition is supported by structural analysis showing that the 3-D structures of the ribosyl moiety of ATP and the vitamin C molecule are almost identical (16). If such is the case, then a similar role for vitamin C in regulating F-2-6-BP action is suggested. Given that ATP and vitamin C compete for the same binding site on AC, and ATP and F-2-6-BP compete for the same binding site on PFK-1, then it is reasonable to assume that vitamin C and F-2-6-BP compete for the same site on PFK-1. This notion is vastly supported by data indicating that while F-2-6-BP is a powerful activator of glycolysis, vitamin C is a master regulator of glucose metabolism.

It is noteworthy that vitamin C is potentially more effective in preventing the initiation and progression of disease processes when not used in combination with supplemental vitamin A and/or vitamin E (17). Adding vitamin A and/or E negatively affected the ability of vitamin C to effectively inhibit PMP22 overexpression, which is the underlying cause for Charcot-Marie-Tooth Disease, a syndrome characterized by progressive weakness and atrophy of distal limb muscles. Here, we propose that vitamin C is the Achilles Heel of cancer whose beneficial effects in preventing the initiation and progression of cancer and other human diseases should be further explored.

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