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RESEARCH HIGHLIGHT

Anti-cancer effects of vitamin C revisited

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Vitamin C was first suggested to have cancer-fighting properties in the 1930s and has been the subject of controversy ever since. Despite repeated reports of selective cancer cell toxicity induced by high-dose vitamin C treatment *in vitro* and in mouse models, the mechanism of action has remained elusive.

Yun *et al*. [1] have recently shed light on what was until now the elusive mechanism by which vitamin C (*aka* ascorbate) induces toxicity in selected oncogene-driven cancers. They reported that in cells with mutations of *KRAS* or *BRAF*, death is not caused by vitamin C itself, but rather by its oxidized form dehydroascorbate (DHA). Whereas vitamin C enters cells through sodium cotransporters, DHA competes with glucose for uptake through glucose transporters (particularly GLUT1 and GLUT4) and then is reduced back to vitamin C in cells [2] (Figure 1). It was previously observed that while melanoma cell lines take up DHA at much higher rates than vitamin C, normal melanocytes do not, demonstrating that transformation-driven upregulation of GLUTs leads to increased uptake of DHA [3]. More recently, using Magnetic Resonance Spectroscopy Imaging, it was demonstrated that hyperpolarized ^{13}C-labeled DHA is rapidly taken up by cancer cells and converted to vitamin C, illustrating the tumors' reducing state [4]. Yun *et al*. [1] now show that the reduction of DHA back to vitamin C is at the crux of the vitamin C-induced cell death observed in these cancer cells.

Mutations in *KRAS* or *BRAF* are found in approximately half of the cases of colorectal cancer (CRC) and their expression correlates with an increase in GLUT1 expression, glucose uptake and reliance on glycolysis. Yun *et al*. [1] observed that vitamin C is oxidized to DHA in tissue culture media and that *KRAS* or *BRAF* mutated CRC cell lines take up more DHA compared to their wild-type counterparts. More importantly, they found that DHA induces death in the mutant lines, but not in wild-type counterparts overexpressing GLUT1, suggesting that additional oncogenic reprogramming is necessary for DHA-induced toxicity. The authors then profiled metabolic changes after treatment with vitamin C. In cells with *KRAS* or *BRAF* mutations, they found an accumulation of the glycolytic intermediates upstream of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) whereas those downstream of GAPDH were depleted. This indicated a decrease in GAPDH activity and a concomitant diversion of glucose into the oxidative phase of the pentose phosphate pathway (PPP), a metabolic shift that, upon oxidative stress, helps restore NADPH levels and cellular reducing potential (Figure 1). Indeed, Yun and co-workers found that the intracellular reduction of DHA back to vitamin C depleted the cellular stores of reduced glutathione (GSH, the major antioxidant in cells), leading to an increase in reactive oxygen species (ROS). Furthermore, they found that, upon exposure to DHA, GAPDH itself was oxidized (on Cys^{152}), and consequently inhibited. Inhibition of GAPDH caused energy stress in the highly glycolytic mutant cell lines, leading to cell death. In mice harboring tumor xenografts with either *KRAS* or *BRAF* mutations, treatment with high doses of vitamin C reduced tumor size. Treatment also reduced the number and size of polyps in an *Apc*-driven transgenic mouse model of intestinal cancer, but again, only in tumors expressing mutant *Kras*. Moreover, in addition to showing the direct inhibition of GAPDH by oxidation, the authors demonstrated that its activity is further hindered by DHA-induced NAD^+ depletion, since GAPDH activity relies on the availability of NAD^+ as a co-substrate. The major NAD^+ consumer, the DNA repairing enzyme poly ADP-ribose polymerase (PARP) was then investigated. It was found that the increase in ROS after high-dose vitamin C treatment also induced DNA damage and PARP activation in *KRAS* or *BRAF* transformed cells. Providing the cells with a PARP inhibitor or an NAD^+ precursor partially rescued their viability. Thus, ROS cause the inhibition of GAPDH activity in cells on two fronts: first, via inducing its direct oxidation and second, by causing NAD^+ depletion (Figure 1). Yun *et al*. [1] thus demonstrated an intricate mechanism by which oncogenic reprogramming, which causes glycolysis addiction, induces a metabolic vulnerability which can be exploited with high doses of DHA that elevate intracellular ROS as it is converted back to vitamin C.

Despite numerous clinical studies, the anti-cancer property of vitamin C has remained controversial. Potential translation of the mechanism presented by Yun *et al*. [1] to therapeutic application raises concerns regarding toxicities of high-dose vitamin C treatment. Though the authors do not report side effects in their mice (treated daily with 4-8 g/kg body weight IP), the upper dose equates to over half a kilogram for the average person. High-dose

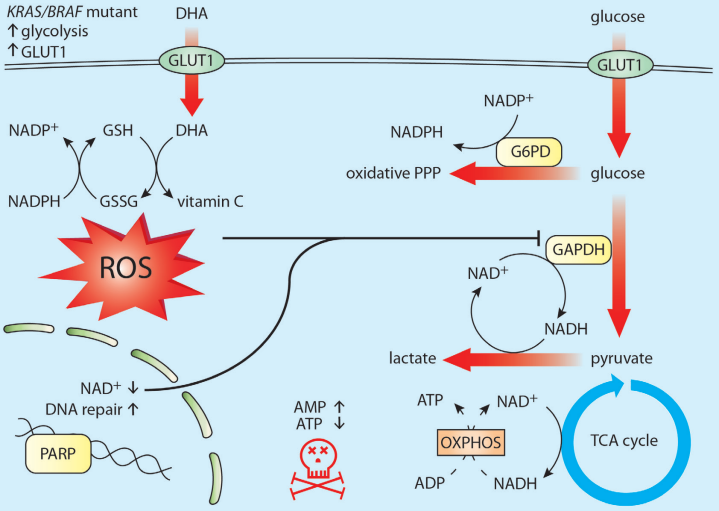

Figure 1 Mechanistic overview of proposed vitamin C toxicity in CRCs driven by *KRAS* and *BRAF* mutations. *KRAS* and *BRAF* mutations induce metabolic reprogramming by upregulating GLUT1, glucose uptake, and glycolytic flux. Upon vitamin C treatment and its extracellular oxidation, DHA (the oxidized form of vitamin C) is taken up through GLUT1 and is reduced back to vitamin C in the cells, depleting GSH and NADPH. Consequently, an increase in ROS leads to GAPDH oxidation, and with it, to a decrease in glycolytic flux. In parallel, ROS-mediated oxidative DNA damage induces PARP activation and subsequently, NAD^+ levels fall and cause additional inhibition of GAPDH and glycolysis, resulting in energy crisis and cell death.

oral supplementation of vitamin C is associated with increased kidney stone incidence, and clinical studies demonstrated significant renal, cardiac, and metabolic toxicity upon vitamin C administration. Still, overall reports of toxicity are variable, poorly graded, and therefore inconclusive [5, 6]. Affinity studies of DHA for GLUT1 may help establish a lower effective dose, though unwanted side effects in tissues that highly express GLUT1 need to be considered. The brain obtains vitamin C by uptake of DHA through GLUT1 at the blood-brain barrier and its subsequent reduction [7]. Erythrocytes express high levels of GLUT1 and are crucial for ascorbate recycling, keeping DHA levels low [8]. Importantly, erythrocytes rely solely on glycolysis for energy production. Thus, high DHA levels may induce brain toxicity and haemolysis via mechanisms similar to those described by Yun *et al*. [1].

Though vitamin C oxidizes rapidly in tissue culture media, it acts mainly as an antioxidant *in vivo*. It remains unclear how and where circulating vitamin C is oxidized *in vivo*, an issue not addressed by Yun *et al*. [1]. Oxidation of vitamin C to DHA by tumor stroma has been suggested [9], complicating the ability to predict tumor responsiveness to the treatment. As such, without being able to control the extent of vitamin C oxidation to DHA, effectiveness and toxicity of vitamin C treatment cannot be predicted.

Finally, the authors report that, following DHA uptake, NAD^+ depletion by PARP activation contributes to the inhibition of glycolysis (and potentially to the stimulation of oxidative PPP flux). The observation that PARP inhibition partly rescues vitamin C-treated cells may suggest that the toxic effect of DHA uptake is not caused by ROS alone, since restoring NAD^+ levels and glycolysis with the PARP inhibitor may actually decrease PPP flux and NADPH production, and aggravate the redox stress. It remains to be demonstrated that PARP inhibition indeed restores NAD^+ levels in vitamin C-treated cells, and how this affects the balance between energy and redox metabolism. This also raises the question whether PARP activity in BRCA1/2-deficient tumors produces a similar metabolic phenotype via NAD^+ depletion and whether the use of a PARP inhibitor (e.g., olaparib) to treat these tumors [10] might restore NAD^+ levels and counterbalance GAPDH inhibition by other oxidative agents.

In summary, Yun *et al*. [1] show that, in glycolysis-addicted *KRAS* and *BRAF* driven cancer cells, high-dose vitamin C treatment induces cell death via the uptake and reduction of its oxidized form DHA back to vitamin C. DHA reduction, through scavenging GSH, induces oxidative stress, leading to GAPDH inactivation, inhibition of glycolysis and the subsequent energy crisis and cell death. This study further elucidates the mechanism by which ROS can induce cell death, and neatly shows how vitamin C, an antioxidant, can work as a double edged sword. However, further work is necessary to determine whether there is a therapeutic potential for vitamin C in cancer patients.

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