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Perspective Article

Vitamin C in Cancer Therapeutics and Metastasis

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Abstract

There have been significant increased publications of preclinical studies and clinical trials of vitamin C (ascorbate) on cancer therapeutics in the past a few years. In this communication reflecting my personal opinions, I will highlight the main points of current research status; discuss impacts of vitamin C on immune cell function and inflammation at tumor microenvironment, and tumor metastasis; and propose stimulating questions and direction for future research in this area.

Adsorbate has long been used for cancer therapeutics

Unlike most animals, human can't make vitamin C due to the mutated Gulo gene; therefore, vitamin C is an essential micronutrient for us. In the 1970s, Linus Pauling suggested several biologically important roles of vitamin C in health and disease development. He and Ewan Cameron reported that there appeared to be an improved outcome of survival and quality of life when using a combination of mega dose oral with high dose intravenous (IV) vitamin C (ascorbate) for some late stage cancer patients. The mechanisms proposed mainly were due to enhance immunity and inhibitory effect on hyaluronidase [1]. However, the use of vitamin C in cancer treatments remained controversial due to the negative results of Mayo Clinic's two oral vitamin C clinical trials. With the discoveries of pharmacokinetics of ascorbate in human, its biological roles as biological cofactors, e.g. hydroxylases, the generation of H_2O_2 in rat at the tumor microenvironment, the advancements of cancer biology, the accumulated beneficial effects of ascorbate cancer patient case reports, the results of preclinical research and early phase clinical trial, further investigating the mechanisms and conducting larger well designed efficacy clinical trials of using ascorbate as cancer therapeutics agent in combination with standard care are needed and warrant [2]. The results showed that bone cancer cells (G292 cells) in vitro with 1 mM ascorbate decreased differentiation and maturation of osteoblastic, and increased cell apoptosis [3]. Several potential therapeutic mechanisms of IV ascorbate, including generating H_2O_2 at the extracellular tumor microenvironment and/or modulating epigenetic effect through cofactor by enhancing the activity of Ten-Eleven Translocation (TET) family enzymes, have

been summarized in several recently published papers [2,4].

Ascorbate, Immune and Inflammation at Tumor Microenvironment

Several review papers have summarized the potential mechanisms of ascorbate on immune cell functions, including through Hypoxia-Inducible Factors (HIF)s and TETs [5]. The antioxidant role of ascorbate can also be important at the tumor site modulating immune cell functions. The mixed results of the effect of ascorbate on immune cells have been reported, but their potential effects on cancer therapeutics are under researched. Ascorbate can enhance the proliferation and maturation of T cells [6]. Additionally, it can increase the proliferation of Natural Killer (NK) cells, but the effect on its immune function is unknown [5] The results of the effect of ascorbate on Tregs are conflicted or mixed[6].

Several studies reported the potential protective effects of IV ascorbate on sepsis by reducing the formation of Neutrophil Extracellular Traps (NETs)[7]. NETs were found in several cancer animal models' tumor microenvironments (such as, pancreatic carcinomas and Lewis lung carcinoma) and played potential roles in promoting tumor growth and/or metastasis. NETs also contributed to the immune-related adverse events from checkpoint blockade treatment in melanoma patients [8, 9]. A deficiency in vitamin C for neutrophils at the tumor microenvironment is highly possible. It points toward the fact that the IV ascorbate could potentially reduce the formation and enhance the clearance of NETs to control tumor cell proliferation, metastasis, and improve the efficacy of PD L-1 immunotherapy. However, further research is needed. In

addition, the number of infiltrating neutrophils before or during treatment has indicated the correlation with tumor progression and patient survival. The Neutrophil to Lymphocyte Ratio (NTLR) at the tumor microenvironment may predict the treatment responsiveness [10]. Whether IV ascorbate can reduce the NTLR is unknown, but it is likely and is important to investigate.

In the tumor microenvironment, chronic inflammation senescence cells, high ROS level tumor cells and reactive immune cells can stimulate releasing of interleukin-6 (IL-6) [11]. The epigenetic regulation can generate cytokines and induce tumor development and metastasis [12]. It is reported that IL-6 plays important roles in suppressing tumor immune response to anti-PD-L1 treatments in colorectal cancer, pancreatic cancer, and melanoma [13, 14]. TET2 can suppress the IL-6 production [15]. Inflammation marker of C-Reactive Protein (CRP) has been shown as potential predictive marker for nivolumab in lung cancer [16]. Ascorbate can enhance TET2 activity, especially in vitamin C deficient and/or TET2 mutation tumor cells and decrease CRP [2,17]. Ascorbate is expected to reduce IL-6. The investigation of the potential modulate effect of ascorbate on immunotherapy is clearly needed. The effect of vitamin C/TET2 on ADAR1 role in immunotherapy sensitivity needs to be investigated [18].

Ascorbate Inhibition of Metastasis

Regarding cancer cell metastasis, bone is one of the common metastasis sites for many types of cancer. The inflammatory cytokines, such as IL-6 is one of the major factors modulating cancer caused metastasis [11]. Matrix Metalloproteinases (MMPs) in tumor and stromal cells play important roles modulating tumor cell metastasis process and progression. Hyaluronidases that catalyze the degradation of hyaluronic acid (HA) have a family of five enzymes. Both preclinical and clinical investigations have shown that hyaluronidases are involved in tumor progression and metastasis [19, 20]. Some metastasis tumor cells secrete high amounts of hyaluronidases. High molecular weight hyaluronan polymers can prevent tumor cell growth and metastasis [19]. Research has shown that ascorbate can reduce IL-6,inhibit activity of the matrix metalloproteinases, and directly and/or indirectly reduce activity of the hyaluronidases [20-22]. Ascorbate inhibited metastasis can also result from ascorbate inhibition of HIF and enhanced TET 2 function [2]. In addition, a report of a pilot retrospective study showed encouraging result for need of further research on low dose, or maybe high dose as well of IV ascorbate to treat bone metastases and its related pain after radiotherapy [23]. The naked mole-rat is reported to have high amounts of high molecular weight hyaluronan polymers which contribute to its resistance to cancer cell growth and metastasis. Elephant and certain whale produce high amounts of vitamin C, for their tight and strong skin, which might be one of the reasons that they have low chance of dying from cancer.

Discussion and Future Directions

The leak of damaged DNA and/or mtDNA to cytosol caused by both endogenous and exogenous risk factors, such as aging induced ROS and mitochondrial dysfunction due to the lack of vitamin C, smoking, or reduced genome stability due to reduced TET activity and vitamin C deficiency, can activate the innate immunity cytosolic DNA-sensing cGAS-STING pathway and link inflammation (e.g. IL-6 generation), cell senescence and cancer [24]. We can imagine that there is an increased need for vitamin C to maintain "healthy" metabolism and respiratory chain functions, stem cell regeneration, in addition to the need for co-factors in all tissues during aging, especially brain and immune cells. Chronic vitamin C deficiency resulting accumulated high level of ROS and chronic inflammation in elderly can be one of the key risk factors that links aging and cancer development, and other aging diseases, such as neurodegeneration.

The overall mechanisms of IV ascorbate therapeutics and metastasis control can involve all these pathways: H2O2 generated at the extracellular tumor microenvironment with increased intracellular ROS, DHA and GSH metabolism driven ROS oxidative stress, TET 2 epigenetics function, inhibition MMPs and Hyaluronidases, reduction of IL 6 and improvement immune cells function [25]. The acute direct effect might mainly via H_2O_2/ROS synergize with standard treatment. However, it is very possible that additional long-term effects can also be via immune cells, TET2 epigenetics, and inhibition of MMPs and hyaluronidases. Highly proliferative cancer cells cause deficiency of vitamin C or no vitamin C in subset tumor cells, especially due to poor vasculature, perfusion and high ROS at tumor microenvironment. It is reasonable to anticipate that IV ascorbate alone with right frequency (e.g. daily, or twice/day) and lower IV pharmacological dose (plasma, 2-5 mM) may also control tumor growth and metastasis, especially for early stage cancer (in addition to high bulk dose, 2-3 times/week). This needs to be tested in vivo mice, before a human trial. Quercetin is another strong natural product inhibitor of hyaluronidases. Epigallocatechin gallate is also has inhibitory activity besides its epigenetic effect. More research is needed to investigate and develop combination natural products and micronutrient formula/product to target hyaluronidases and MMPs, modulate epigenetics and metabolism pathways, enhance immune cell function, and reduce inflammation cytokines (e.g. IL-6) to enhance the outcome of the standard treatment or immunotherapy and control the metastasis.

In summary, the limited, mixed, but encouraging data on the effect of ascorbate on immune cell functions and inflammation in the tumor microenvironment suggests the possibility and timely need to systematically further research the effect of ascorbate on immune cell functions. Furthermore, it would be important to explore the use of ascorbate in combination with cancer

immunotherapy, with the latter having no report or clinical trials yet. Developing ascorbate for cancer metastasis control is an important area to investigate. Hyaluronidase and HA/CD44 can be one of the important targets for tumor cell growth and metastasis control. Reducing or inhibiting of developing NETs using IV ascorbate is a potential promising area to control tumor metastasis and recurrence. Due to multi-targets and multi-modes of actions, using computation modeling, network analysis, and systems medicine approaches for developing ascorbate cancer therapeutics is needed.

Due to the deficiency of vitamin C in tumor cells and in cancer patients, the current used preclinical models or approaches for understanding the cancer biology and cancer drug development may need re-thinking in the context of vitamin C and its related physiological and pathological parameters. Clinical trials may need consider vitamin C as one of the biological variables for therapeutic outcomes. Its roles as a co-enzyme in epigenetic regulation of human development, aging and disease prevention/treatment; and its effects on stem cell regeneration and cancer stem cell death are just beginning to be understood. Finally, we should not forget that vitamin C is a powerful antioxidant. We should not forget its role in protecting normal cell, stem cell and tissue during cancer treatments and aging process. After all these years overlooked and under studied, the field is on the horizon.

Measurement of vitamin C, other micronutrients and metabolites at single tumor cell and in situ, tumor tissue level, besides in plasma are very critical for understanding the potential mechanisms of nutrients and metabolites in cancer development, response or resistance to treatment and metastasis. The imaging technology is essential for such a task. Developing "The Cancer Nutrient Atlas" at single cell and tissue level will be valuable resources for the public and research. Evaluate the adverse effect or interference of nutrients with cancer treatment is also much needed.

The following is a list of stimulating and remaining questions for IV ascorbate cancer therapeutics:

Mechanisms ascorbate cancer therapeutics and technology development

Despite the recent increased in research interests in preclinical studies and clinical trials, proposed several potential mechanisms, and the implications of possible beneficial therapeutic outcome from using IV ascorbate, our ability to determine the extent of developing standard protocol is hampered by our limited understanding of the molecular mechanisms of ascorbate cancer therapeutics in human and the lack of prognostic and predictive biomarkers for patient selection. Vitamin C at tumor tissue and plasma, TET2, 5hmc, SVCT, Glut1, transferrin and receptor, ADAR1, IL-6, HA/CD44, hyaluronidase and AQP (aquaporin) can be additional potential biomarkers to explore.

❖ **Stimulating questions**

➢ What are the molecular mechanisms of ascorbate cancer therapeutics?

➢ Why does ascorbate selectively kill tumor cell but not normal cell in vivo?

➢ Why does ascorbate therapy work for some patients, but not for others?

➢ What mechanisms ascorbate does control tumor cell metastasis?

➢ How to use ascorbate to enhance cancer immunotherapy?

➢ How does vitamin C deficiency affect cancer initiation, progression and development?

➢ How to use vitamin C for cancer prevention and tumor recurrence?

➢ Why do humans have mutated Gulo gene?

➢ How heterogenous levels of vitamin C contribute to cancer treatment outcome or resistance?

➢ What is possibility tumor cells develop acquired-resistance to ascorbate therapy?

➢ What are the risks long term of taking mega dose oral vitamin C in healthy person?

➢ What are the mechanisms by which vitamin C improves cognition, pain and cancer treatment quality of life?

Standardize clinical protocols

The field needs further systematic evaluation into the optimal clinical treatment protocol and to conduct preclinical in vivo research. The measurement of vitamin C in cancer patients should be evaluated. Imaging technology for ascorbate therapeutic application should be developed. A coordinated and standard protocol is needed to identify optimal application of ascorbate cancer therapy, such as, mono-therapy or adjuvant medicine, the route, dose, length, and duration. In addition to high doses IV ascorbate, a daily infusion of lower doses of IV ascorbate (e.g. 2-5 mM plasma concentration) may also need be considered to explore in preclinical study for certain types of cancer, and maybe for immunotherapy.

❖ **Stimulating questions**

➢ How to develop the optimal and most efficient ascorbate therapeutic protocol as mono-, or combination therapeutic agent?

➢ What are biomarkers for selection and how to stratify cancer patients to use ascorbate cancer therapy?

➢ What is the possibility that ascorbate therapy can be effective for all the cancer patients?

➢ How does circadian clock and age affect the outcome of ascorbate therapeutics?

➢ What is the technology need for developing ascorbate cancer therapeutics?

➢ How to develop effective oral ascorbate cancer therapeutics?

Acknowledgement

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