

2-Deoxy Glucose Reduces Angiogenic and Metastatic Potency of Tumor Cells

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Commentary

Recent reports from our group has revealed that 2-DG at sub-lethal doses are effective in reducing the angiogenic potency and migratory potential of tumor cells. Discussion on this topic forms the subject matter of the present commentary.

Tumor growth and metastasis has become a major cause of mortality and morbidity worldwide [1,2]. Several strategies to stop or rather slow down the growth of tumor have been under rigorous investigation for past few decades [3]. Blocking angiogenic process (The process of formation of new blood vessels from preexisting ones) has been one of them [4]. Angiogenesis is critical in the process of tumor growth and metastasis owing to the fact that (1) tumors are not viable beyond 2 mm diameter and induction on neo vessel formation is warranted for further tumor survival [5]. Further (2) the neo vessels, so formed are leaky in nature, which significantly contribute to the escape of tumor cells into the circulation leading to metastasis [6]. Considering the importance of metabolic shift in tumor cells from aerobic respiration to aerobic glycolysis, several studies have been undertaken to check if blocking glycolysis can contribute to retarded tumor growth [7]. Of the many reports available few of them have proved the cytotoxic nature of several glycolytic inhibitors [7]. The major glycolytic inhibitors reported to be anti tumor in nature include a) 3-Bromo pyruvate which has been suggested to act by inhibiting hexokinase [8,9]. b) Lonidamine, which act by inhibiting mitochondrially bound hexokinase [10] and 2 deoxy glucose (2-DG).

2-Deoxy glucose (2-DG) is a metabolic analogue of glucose where, hydroxyl group at the position of 2 is replaced by hydrogen. It can be converted to 2-Deoxy glucose 6 phosphate by hexokinase causing its accumulation in tumor cells [11,7]. It has been reported that 2-DG at higher concentration shows cytotoxic effect on tumor cells under both *in vitro* and *in vivo* conditions [12]. The tolerance of cells towards 2-DG has been studied extensively. In one study concentrations from 4-10 mM is reported to decrease cell survival in HeLa cells from 20-90% due to profound disruptions in thiol metabolism [13]. 4 mM concentration of 2-DG has also been reported to decrease survival of human primary fibroblast by regulating GAPDH levels [14]. Numerous studies have already revealed the radio and chemo sensitizing nature of 2-DG at concentrations above 5 mM due to metabolic instabilities caused by 2-DG [15]. The cytotoxic nature of the glycolytic inhibition has to be cautiously addressed since, using that as a therapeutic strategy would inflict serious harm to highly glycolytic organs such as brain etc. [16]. Further 2-DG at higher concentrations has also been reported to elicit unfolded protein response (UPR) [17]. In addition to the studies on the cytotoxic nature of 2-DG, there are some studies that reveal the tendency of cancer cells to develop resistance against 2-DG. p53 is reported to protect lung cancer cells against the 2-DG induced metabolic stress [18]. More studies are however required to address this issue of resistance since;

concentrations of 2-DG ranging beyond 1mM are being reported to be cytotoxic against a wide range of cell lines. 2-DG other than being reported to be cytotoxic in nature has been reported to be antiangiogenic in nature [19]. Merchan et al. has reported that 2-DG inhibits angiogenesis by inhibiting glycosylation, induction of ER stress and thereby compromising with the viability of endothelial cells [20]. Since this strategy may encounter a serious hazard when checked *in vivo*, a parallel strategy was thought of, to check if 2-DG at sub lethal concentration that do not induce ER stress could be used to alter the angiogenic potency of tumor cells. The result of the study published in Journal of Cellular Biochemistry showed that tumor cells when treated with sub lethal dose of 2-DG, that fails to elicit an UPR, makes the tumor cells significantly less angiogenic in nature. The angiogenic potency has been reported to be reduced due to a decrease in the production and secretion of VEGF as well as through the mechanism that regulate poly ADP ribosylation of VEGF and thereby making it biologically less active [21]. It is reported that transcriptional co-activator PGC-1 α stimulates angiogenesis by inducing VEGF expression through interacting with estrogen-related receptor- α (ERR- α), thereby preparing the tissue for oxidative metabolism [22]. The two important transcriptional factors which regulate the expression of VEGF in response to metabolic changes are, HIF and FOXO [23]. These two factors can be deacetylated by SIRT 1 [24], the expression of which was higher in response to 2-DG causing a reduction in VEGF expression [25]. Glycosylation is a major post translational modification which affect the biological activity of VEGF [33]. Since, angiogenesis is essential for tumor growth and metastasis [26] and vascular endothelial growth factor (VEGF) functions as a key mediator in this process [27], suppression of VEGF expression is crucial for restricting tumor invasion and metastasis. Mechanistically, it has been shown that proper recruitment of heterochromatic histone methyl transferase G9a is essential for silencing VEGF [28]. Since, apart from establishment of H3K9me2 [29], G9a also interacts with DNA methyl transferase and plays a role in the maintenance of DNA methylation [30], further studies on the pattern of H3K9me2 as well as DNA methylation at promoter of VEGF in tumour cells that underwent metastasis, may shed light on the a crucial epigenetic mechanisms that is involved. Even though the VEGF expression is inducible, it can be regulated transcriptionally, translationally and post translationally [31,32].

The biologically active form of VEGF165 is glycosylated at Asn 74 and exists as homodimers of 23 kDa units [33]. The binding of VEGF165 to endothelial cells are strength end by glycosaminoglycan (GAGs). Glycosylated VEGF 165 is the natural ligand of heparin, and it binds with high affinity than non-glycosylated one [34].

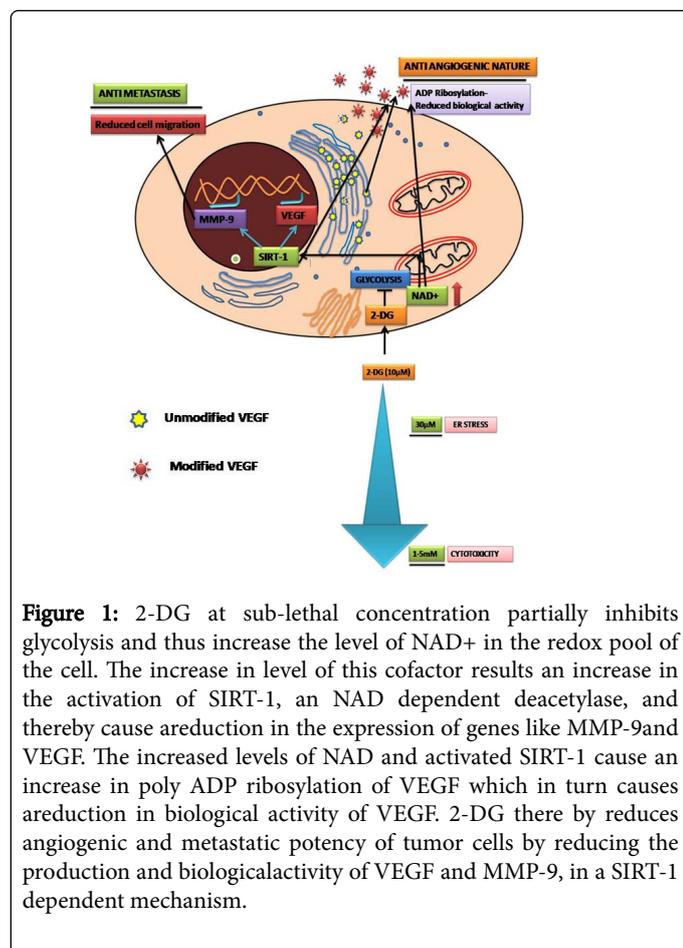


Figure 1: 2-DG at sub-lethal concentration partially inhibits glycolysis and thus increase the level of NAD⁺ in the redox pool of the cell. The increase in level of this cofactor results in an increase in the activation of SIRT-1, an NAD dependent deacetylase, and thereby cause areduction in the expression of genes like MMP-9 and VEGF. The increased levels of NAD and activated SIRT-1 cause an increase in poly ADP ribosylation of VEGF which in turn causes areduction in biological activity of VEGF. 2-DG there by reduces angiogenic and metastatic potency of tumor cells by reducing the production and biological activity of VEGF and MMP-9, in a SIRT-1 dependent mechanism.

ADP ribosylation (PAR induced) is another major post translational modification which affects the biological activity of VEGF. PAR modification is catalyzed by PARP (Poly ADP Ribose Polymerase) family of proteins; the activity of which depends on the metabolic status of the cell i.e. NAD⁺ concentration [35]. Metabolites like Lactate has been reported to inhibit poly-ADP ribosylation of VEGF, resulting in its enhanced angiogenic activity [36]. Treatment of tumor cells with 2-DG increased ADP ribosylation of VEGF and thereby reducing its biological activity [37]. This report shows that the 2-DG mediated reduction in the production and activity of VEGF depends on SIRT1 [38]. Here 2-DG is proposed to alter the redox potential of cells where by the ratio of NAD/NADH increase, leading to the activation of SIRT 1. The activated SIRT 1 thereafter has been reported to be responsible for the reduced production and biological activity of VEGF [39]. The extracellular matrix remodeling has an important role in cell migration, differentiation and thereby metastasis. MMP-9 is one of the important matrix remodeling enzymes, which plays an important role in cancer progression. A number of studies have reported that tumors subjected to hypoxia enhances its migratory property and metastasis [40]. It is evident that under conditions of hypoxia there is an increase in the rate of glycolysis and accumulation of lactate [41]. Among many other genes like VEGF and TNF α , one of the genes gets activated downstream of this phenomenon is MMP-9 [42]. The increase in the expression of MMP-9 is reported to be mediated by either CD147, AP1 or NF-kB [40-42], depending on the cells involved. Yet another report published by our group in Molecular and Cellular Biochemistry has addressed the production of MMP-9 (the major metallo protease

involved in tumor metastasis) by tumour cells in response to 2-DG. Data presented by Lincy et al. suggest that MMP-9 production get down regulated upon 2-DG treatment in a SIRT -1 dependant mechanism [41]. Selective anticancer therapeutics against new metabolic targets is made possible as a result of better understanding of tumor growth and its microenvironment. Anti-glycolytic drugs are promising candidates, as they exploit the hyper glycolytic nature of cancer cells. Toxicity and lack of specificity though pose a barrier to the antitumor efficacy of these compounds; 2-DG is currently being recruited under the combination therapy regime with irradiation in attenuated doses [40].

Altogether, these reports shows that 2-DG at low concentration partially inhibits glycolysis without inducing cytotoxicity or UPR responses. 2-DG also reduces cellular migration and angiogenic potency of tumor cells (Figure 1). Although 2-DG is under clinical trials, this information can significantly add onto the safety and efficiency of 2-DG being tested as an anticancer drug.

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