Evolution of GAPDH as a druggable target of tumor glycolysis?

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1. Introduction

Deregulation of energy metabolism, also known as the metabolic reprogramming, is a frequently witnessed biochemical phenotype of many solid malignancies, and has recently been recognized as one of the hallmarks of cancer [1]. This metabolic alteration in cancer is exemplified by rapid utilization of glucose, a functional property that is currently exploited in the clinical diagnosis of cancer by PET imaging [2]. Unlike the diagnostic application, successful therapeutic targeting of glucose metabolism or glycolysis in cancer still remains elusive. Plausibly, tumor heterogeneity, metabolic plasticity, and intratumoral variations in the metabolic phenotype and other factors may confound the outcome of antiglycolytic therapeutics. Recently, it has been shown that besides the expression level of specific glycolytic genes, assessment of the efficacy of the glycolytic phenotype of cancer may enable us to achieve selective targeting of cancer metabolism [3]. More importantly, the findings also demonstrated the specificity and efficacy of a natural compound, the Koningic acid (KA) in targeting the glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Overall, the current report on targeting GAPDH with KA is a significant step forward in the development of potential novel therapeutics, and may enable us to design effective interventional strategies to target cancer’s altered energy metabolism.

Cellular oxidation of glucose primarily involves glycolysis (i.e. the process of conversion of glucose into pyruvate) followed by the mitochondrial oxidation of pyruvate (an oxygen-dependent process referred as oxidative phosphorylation (OxPhos)). During the conditions of low-oxygen (hypoxia), OxPhos remains mitigated hence the glycolytic product, pyruvate gets oxidized into lactate (i.e. anaerobic glycolysis). The pioneering work of the German scientist, Otto Warburg documented that cancer cells metabolize glucose via glycolysis and produce lactate (referred as fermentation) even in the presence of sufficient oxygen (i.e. aerobic glycolysis), popularly referred as the Warburg effect [4]. Mathematically, the net amount of cellular energy (e.g. ATP) produced by glycolysis is far less than the complete oxidation of glucose by mitochondrial OxPhos. Though Warburg believed that dysfunctional mitochondria could be the causal factor for aerobic glycolysis, later reports have established that cancer cells exhibit glycolytic phenotype despite functionally active mitochondria [5]. Several elegant reviews have explicated that cancer’s propensity to aerobic glycolysis (despite the mitochondrial capacity) indicates that glycolytic phenotype is sufficient to meet the fundamental requirements that are essential for cancer cell’s proliferation and progression [6,7]. Furthermore, rapid oxidation of glucose by glycolysis facilitates increased rate of glucose uptake, the clinically witnessed metabolic signature of most, if not, all solid tumors. Therefore, tumor glycolysis is a significant and clinically relevant metabolic phenotype.

Comprehensibly, the effectiveness of antiglycolytic strategy also depends upon the magnitude of glycolytic phenotype in cancer. For example, the expression of monocarboxylate transporters (MCTs), which are crucial for the extrusion of lactate produced by fermentative glycolysis, directly correlates with the glycolytic phenotype. Accordingly, using a panel of cancer cell lines, Birsoy et al. [8] elegantly demonstrated that the sensitivity to a specific antiglycolytic agent strongly correlated with the level of specific MCT (i.e.) MCT1 (Figure 1). Nevertheless, the translational potential of any antiglycolytic strategy relies on additional factors besides the evaluation of the level of glycolytic capacity. First, the recognition of a critical yet sensitive molecular target of glycolysis. Next, the identification of a specific antagonist that can selectively and specifically inhibit the preferred molecular target of glycolysis. As demonstrated by Liberti et al. [3], besides the evaluation of glycolytic status, selection and validation of a potent and specific inhibitor of the glycolytic target (i.e.) GAPDH is critical to achieve the desired anticancer effects.

Conceivably, targeting any glycolytic enzyme (e.g. LDH) is likely to promote antiglycolytic and anticancer effects [9]. The distinctive feature of GAPDH is that its catalytic function is one of the critical rate-limiting steps of glycolysis. First, GAPDH-dependent hydrolysis of glyceraldehyde-3-phosphate is the first glycolytic step that produces the essential redox molecule NADH (refer [10]) which in turn is involved in several cellular processes. Further, data from computational modeling and metabolomics show that aerobic glycolysis in cancer flux through GAPDH [11], underscoring its critical role in the regulation of rate of tumor glycolysis. Accordingly, due to the metabolic adaptation to glycolysis cancer cells may be more
sensitive to antiglycolytic, anti-GAPDH approach than their healthy counterparts or non-glycolytic cells [11]. Furthermore, as a multifunctional protein, besides glycolysis, GAPDH is also involved in several cellular processes that are associated with cancer progression [12]. Hence, disruption of glycolysis by GAPDH inhibition could have multipronged effects in cancer cells. Several chemical and nucleic acid-based inhibitors have previously been shown to inhibit GAPDH in cancer cells underscoring the anticancer potential of GAPDH inhibition (Table 1). However, the cellular abundance as well as ubiquitous nature of GAPDH generated little enthusiasm largely due to the concerns of potential systemic toxicity. In fact, a literature review indicates that until recently, majority of the articles on cancer metabolic targets or targeting glucose or glycolytic pathway in cancer have carefully avoided including GAPDH as a potential target. For example, an elegant review highlights some of the key enzymes of glucose metabolism as potential therapeutic targets [13]. Understandably, GAPDH is excluded perhaps due to toxicity concerns or lack of substantial preclinical data. Thus, although the anticancer effect of targeting GAPDH has been known for few decades, the primary challenge of unwanted toxicity due to the ubiquitous nature of GAPDH downcast its translational potential and remained a major impediment to consider it as a therapeutic target.

Recent advances in our understanding of cancer-related roles of GAPDH (Figure 2) [14], along with the progress in drug delivery methods have alleviated the skepticism, leading to a renewed interest in targeting GAPDH in cancer. For instance, clinically relevant percutaneous ablative procedures

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**Table 1. GAPDH antagonists in preclinical investigations.**

| Antagonist          | Key reference(s) | Experimental evidence                                                                 |
|---------------------|------------------|----------------------------------------------------------------------------------------|
| Iodoacetate         | McKee et al. (1965) | *In vitro* evidence for partial inhibition of GAPDH by iodoacetate                     |
| Koningic acid       | Endo et al. (1985) | *In vitro* evidence documenting the inhibition of GAPDH                                   |
| 3-Bromopyruvate (3-BrPA) | Barnard et al. (1993) | First report indicating 3-BrPA binding with GAPDH                                         |
|                     | Geschwind et al. (2002) | First loco-regional therapy demonstrating anticancer efficacy of 3-BrPA                  |
|                     | Pereira de Silva et al. (2009) | First biochemical evidence in human HCC cells indicating that GAPDH could be the primary target of 3-BrPA |
|                     | Ganapathy-Kanniappan et al. (2009) | First autoradiographic evidence in human HCC cells demonstrating GAPDH as the primary target of 3-BrPA |
|                     | Ganapathy-Kanniappan et al. (2012) | First report demonstrating percutaneous ablation of human HCC by targeting GAPDH through 3-BrPA |
| Methylglyoxal       | Ray et al. (1997) | Demonstrates GAPDH of tumor cells as the principal target                                 |
| Saframycin A        | Lee et al. (2005) | *In vitro* evidence showing biochemical modification of GAPDH by methylglyoxal         |
|                     | Xing et al. (2004) | *In vitro* evidence demonstrating GAPDH as the target of Saframycin A – DNA adducts. Suggests GAPDH could be a chemotherapeutic target |
| Oligonucleotide     | Kim et al. (1999) | First report showing that GAPDH inhibition by antisense oligonucleotides affects proliferation, and induces apoptosis in cancer cells |
| siRNA               | Phadke et al. (2009) | Demonstrates that GAPDH-siRNA induces cell cycle arrest                                   |
| shRNA               | Ganapathy-Kanniappan et al. (2012) | First report demonstrating antitumorigenic effects of GAPDH silencing in human HCC both *in vitro* and *in vivo* |

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as well as intra-arterial therapies that are known to minimize or eliminate potential systemic toxicities have encouraged the molecular targeting of GAPDH [15]. Nonetheless, such approaches have the limitation of relevance only in the anatomic targeting rather than systemic therapy which is indispensable to treat metastases. Remarkably, the intraperitoneal administration of KA demonstrates profound effect on cancer cells implying minimal or negligible systemic toxicity [3]. The results indicate the selectivity and molecular specificity of KA in targeting of GAPDH, providing a window of opportunity to inhibit GAPDH in cancer.

2. Expert opinion

GAPDH is an indispensable enzymic protein for glucose metabolism, and is markedly upregulated in glycolytic cancer cells. Emerging reports show the anticancer potential of anti-GAPDH strategy, resulting in the revival of interest to consider GAPDH as a potential therapeutic target [12,16]. Accordingly, several natural and synthetic antagonists have been evaluated for their effectiveness in targeting GAPDH [8,17]. However, like any other molecular target, a potent antagonist or inhibitor of GAPDH must also fulfill the two cardinal principles (i.e.) selectivity and specificity which determine the safety and efficacy of a potential therapeutic. In this context, recent data indicate that KA may possess the efficacy as well as selectivity to inhibit GAPDH in cancer. Although the anti-GAPDH property of KA has been known for more than 30 years (Table 1), there has been a paucity in the documentation of its relevance in cancer therapy and the translational potential. The recent findings reinvigorate the opportunity to exploit the anti-GAPDH, anticancer potential of KA. Furthermore, such data also instigate hope and enthusiasm to consider a safe and efficacious targeting of GAPDH in cancer. Future studies on KA with reference to the mechanism of cellular uptake, transport, metabolism, stability, half-life, etc., may shed light to design advanced-delivery methods to treat cancer with KA. Also, it may enable us to develop analogous effective approaches to selectively target GAPDH in cancer. Taken together, the prediction of cancer’s glycolytic phenotype and its sensitivity to glycolytic/GAPDH inhibitor is a significant step toward the development of an effective interventional strategy to treat cancer.

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