Editorial Commentary



# New role for GCH1 in cancer

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GTP cyclohydrolase I (GTPCH1; also known as GCH1, EC 3.5.4.16) is the rate-limiting enzyme of the *de novo* tetrahydrobiopterin (known as BH4) synthesis pathway. BH4 is an essential cofactor for several enzymes including the aromatic amino acid hydroxylases (tyrosine-, tryptophan-, and phenylalanine-hydroxylases) which produce dopamine, serotonin and noradrenaline. BH4 is also required for nitric oxide synthases (NOS) to make nitric oxide (NO) and by alkylglycerol monooxygenase in lipid metabolism (1). Furthermore, in addition to its role as an obligate cofactor for these enzymes, BH4 also exerts cofactor-independent roles such as superoxide scavenging, mitochondrial respiration, iron metabolism, chaperone functions, and protection from iron-dependent cell death (ferroptosis). BH4 is, therefore, a very important metabolite not only for the nervous system in terms of neurotransmitter synthesis but also in the cardiovascular, immune and hepatic systems. Thus, its levels are tightly controlled by three main pathways—*de novo*, salvage, and recycling pathways. GCH1 is the rate-limiting enzyme of the *de novo* pathway involved in the first enzymatic step converting GTP to dihydroneopterin triphosphate which can then be metabolized further to BH4 or, as an alternative, to the closely related, though less reactive and studied metabolite, neopterin (2). Patients with *GCH1* deficiencies exhibit strong reductions in BH4 levels which can lead to severe neurodevelopmental and cognitive deficits (including dopa-responsive dystonia, and Parkinson's disease), as well as malignant hyperphenylalaninemia (HPA) and cardiac dysfunctions due in large part to high circulating levels of phenylalanine, resulting from a deficiency of phenylalanine hydroxylase activity in the liver (which converts phenylalanine to tyrosine). GCH1 itself is also tightly regulated at the transcriptional level. Moreover, there exists a negative feedback mechanism whereby the GCH1 regulated feedback protein (GRFP) binds to GCH1 forming an inhibitory GCH1-GFRP complex in the presence of BH4—this binding of GCH1-GRFP is BH4-dependent so when enough BH4 is synthesized any surplus will be used to reduce GCH1 activity via GFRP binding. DAHP (2,4-diamino-6-hydroxypyrimidine) is an indirect inhibitor of GCH1—it engages this negative feedback inhibitory system through direct allosteric binding of GFRP (3).

## The BH4 pathway in cancer

Most of the research on the BH4 pathway in cancer has focused on the end product, BH4 and how it is used to either promote proliferation, scavenge reactive oxygen species (ROS) or as an obligate cofactor for endothelial NOS in the production of NO. NO promotes angiogenesis which supports tumor growth. Endothelial NOS requires BH4 to produce NO and increasing GCH1/BH4 leads to increased tumor angiogenesis via NO; conversely reducing GCH1 levels exerted the opposing effects (4). This was also seen in highly vascularized hepatocellular carcinoma where DAHP treatment lead to reduced BH4 and NO levels and a corresponding reduction in angiogenesis and tumor growth (5). Recently, a novel function of BH4 was attributed to protect against ferroptosis (6,7). Certain cancers have increased iron metabolism compared to

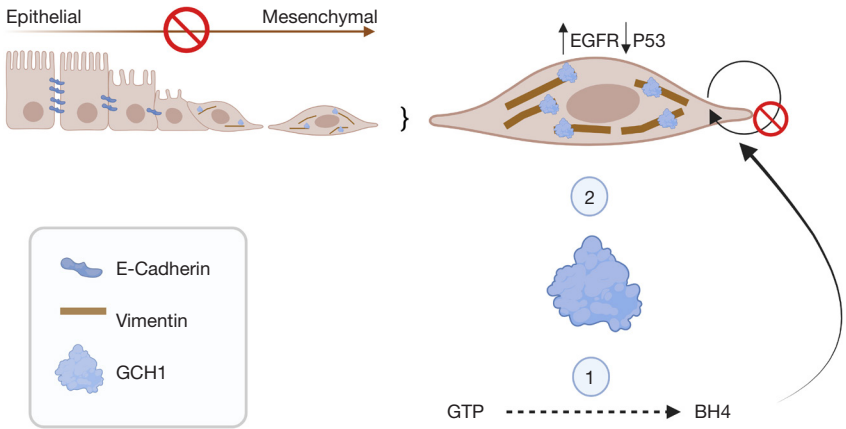


**Figure 1** GCH1 induces EMT independent of its BH4-synthesizing activity. In addition to its canonical enzymatic role in synthesizing BH4 which promotes cancer in by itself ①, GCH1 has now been shown to bind and stabilize Vimentin, and in doing so promote epithelial-mesenchymal transitioning ②. By understanding these distinct functions of GCH1, it opens novel specific, or combinatorial, therapeutic breast cancer treatment options which can target mechanistic routes 1 or 2, or both. GCH1, GTP cyclohydrolase I; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition.

healthy cells and so are prone to high iron-dependent ferroptosis.

BH4 was also shown to promote proliferation and tumor growth *in vivo* of multiple myeloma where it also increased the resistance of these cancer cells to proteasomal inhibition treatment (8). Recently we have reported that in lung cancer, activated KRAS drives BH4 production and that deletion of *Gch1* specifically in lung epithelial cells harboring mutated *Kras* mutation reduced tumor burden and extended survival (9). Moreover, tumor spheroids treated with an inhibitor against sepiapterin reductase (SPR; EC 1.1.1.153, another enzyme of the *de novo* and salvage BH4 biosynthesis pathway) also decreased lung tumor spheroid formation *in vitro*, further suggesting that in mutated *Kras*-driven lung cancer BH4 exerts tumorigenic activity. In a triple-negative breast cancer (TNBC) cell line, MDA-MB-231, researchers knocked down SPR which reduced BH4, increased ROS and lead to a dramatic decrease in the proliferation of breast cancer cells (10). SPR has also been shown to drive neuroblastoma proliferation and indeed an inhibitor against SPR called sulfasalazine was shown to exert anti-tumor effects (11,12). Indeed, *SPR* expression is significantly correlated to unfavorable neuroblastoma characteristics such as oncogenic MYC amplification and increased aggressiveness (12). SPR was also demonstrated to drive hepatocellular carcinoma proliferation *in vitro* (13).

Some of these studies link the effects of SPR enzymatic inhibition to increased ROS levels exerting anti-proliferative and pro-apoptotic effects on the cancer cell lines, while others point to non-enzymatic functions of SPR in promoting cancer cell apoptosis (11,13). Non-enzymatic roles in cancer have also recently been attributed to GCH1. A recent study has shown that GCH1 induces IDO1 expression in TNBC tumors. IDO1 catabolizes tryptophan into downstream metabolites called kynurenines, thus limiting the amount of tryptophan in the tumor microenvironment for infiltrating T cells which creates an immunosuppressive environment (14). Moreover, kynurenines have been shown to generate Treg cells from T cells, thus further potentiating local immune suppression in which the tumor thrives.

## GCH1 drives epithelial-mesenchymal transitioning

A new study has further strengthened the non-enzymatic functions of GCH1 in promoting breast cancer. A report in *Cancer Research* has uncovered an unexpected role for GCH1 in driving breast cancer and promoting metastasis [via epithelial-mesenchymal transition (EMT)] independent of its enzymatic activity (and of BH4) (*Figure 1*) (15). EMT is the process whereby an epithelial cell in contact with the basement membrane undergoes structural and biochemical alterations resulting in a transition to a mesenchymal-type cell which enhances its motility and migratory abilities. It is

a normal physiological process during embryogenesis and organ development but has also been coopted by cancers to metastasize and spread. The authors show GCH1 expression varies in multiple cancer cells lines—with estrogen-positive ($ER^+$) cells lines having the highest expression while certain TNBC cells lines having undetectable *GCH1* levels. Overexpression of GCH1 in a TNBC cell line increased BH4 levels, cell proliferation and transwell invasion *in vitro*. When these cells were then transplanted into mice, the resulting tumors also demonstrated increased growth as well as a switch from E-Cadherin (CDH1) expression to Vimentin (Vim) expression, indicative of EMT induction (15). CDH1 is an adherens junction protein which maintains cell adhesion and epithelial structural integrity while VIM is a cytoskeletal protein which modulates cell mechanics, cell adhesion and gene expression profiles to promote a more migratory phenotype. Importantly, the authors could reduce the increased BH4 levels and hyperproliferative effects of GCH1 overexpression by DAHP treatment but not the EMT induction (*Figure 1*). Moreover, GCH1 overexpression could transform non-tumor human mammary epithelial cells, again by inducing EMT-related gene expression profiles (*VIM* and *SLUG*) and downregulating *CDH1*. Mechanistically, the study shows that GCH1 interacts with VIM, mediated by the chaperone HSP90. This GCH1-VIM interaction seems to stabilize VIM, increasing its half-life and thus prolonging its function in promoting cell motility and EMT in TNBC and human epidermal growth factor receptor 2-positive ($HER2^+$) breast cancer cell lines (15). Additionally, they show that increased GCH1 also leads to increased epidermal growth factor receptor (EGFR) levels. Conversely, when *GCH1* was genetically ablated, proliferation and tumor growth were significantly reduced *in vitro* and *in vivo*. VIM and EGFR levels were also reduced, neither of which were restored with exogenously added BH4, further suggesting that these effects may be independent of GCH1 enzymatic function and of BH4. The authors also demonstrate that GCH1 suppresses the P53 pathway in estrogen receptor-positive ($ER^+$) breast cancer cells, a protective pathway which puts a brake on cancer cell proliferation, and that in the absence of GCH1, P53 is upregulated, and EGFR is downregulated, both of which lead to cancer inhibition *in vitro* and *in vivo*. The authors followed up with bioinformatical analysis from a large cohort of breast cancer patients in which *GCH1* showed significantly higher expression in breast cancer tissue compared to normal breast tissue, and that its levels correlated with worse relapse-free survival and overall survival especially in $ER^+$ breast cancer, replicating a previous study (16).

## GCH1—a potential target for breast cancer

This new data further advances our understanding of the role that members of the BH4 biosynthetic pathway play in cancer, and importantly their cancer-promoting functions can also be independent of their enzymatic activities and of BH4 itself. SPR has been demonstrated to promote hepatocellular carcinoma progression in an enzymatic-independent fashion through interactions with FoxO3a/Bim signaling (13). And now GCH1 promotes breast cancer tumorigenesis via interaction and stabilization of the EMT-inducing factor VIM (15). Indeed, in the nervous system GCH1 has been proposed to stabilize tyrosine hydroxylase via direct interaction (17). However, as BH4 itself exhibits stabilizing chaperone activity for phenylalanine hydroxylase, the contribution of the end-product BH4 is difficult to entirely rule out and further studies will be needed to unravel the emerging complexity of this pathway in cancer. As mentioned before, DAHP does not directly inhibit GCH1 enzymatic activity *per se* but rather acts on GFRP to allosterically promote an inhibitory GCH1-GFRP complex which would block its enzymatic function while possibly still allowing certain protein-protein interactions, with VIM for example. Both GCH1 and GFRP interact as pentamers establishing a large protein complex. Both N-terminal and C-terminal residues of GCH1 have been implicated in GFRP interaction (18). Mapping these distinct functions of GCH1 (enzymatic activity and protein interactions with VIM) will be important to functionally separate its roles in cancer and to ultimately design function-specific blockers of GCH1.

GCH1 thus far has been difficult to pharmacologically target, unlike the more distal enzyme SPR in which a growing number of small molecules are being developed in the treatment of chronic pain among other pathological conditions (19). As mentioned earlier, BH4 is a very physiologically important metabolite. Patients deficient in SPR do exhibit milder symptoms than those presenting with GCH1 deficiencies, especially in the periphery. For example, HPA is associated with reduced GCH1 levels but not so much with reduced SPR levels. The reason for this may be due to the fact than unlike GCH1, SPR activity can be compensated for by other reductases such as carbonyl reductases and aldose reductases, which are quite active in the liver (19). Additionally, neopterin, an often-thought

 

chemically-inert, inflammatory-diagnostic byproduct of GCH1 activity, has recently been implicated in enhancing learning and memory (20). Therefore, targeting the enzymatic function of GCH1 in the body will have many unwanted side effects. However, this recent study offers important insights into the possibility of targeting the non-enzymatic roles of GCH1 to abrogate EMT in breast cancer while sparing its BH4-synthesizing activity. Additionally, as cancer-homing delivery of drugs is advancing, GCH1 is an attractive target to block both the BH4-dependent pro-tumorigenic functions of the metabolite as well as the GCH1-depedent (BH4-indepedent) roles in EMT. As the multifaceted functions of BH4 itself are expanding, so are the proteins associated with its biosynthesis such as SPR and GCH1.

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