Glycine Decarboxylase Cleaves a "Malignant" Metabolic Path to Promote Tumor Initiation

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Tumor-initiating cells (TICs) are thought to be critical for promoting tumorigenesis. In a recent *Cell* article, Zhang and colleagues found that non-small cell lung cancer TICs overexpress the metabolic enzyme glycine decarboxylase, which leads to increases in pyrimidine synthesis and is critical for proliferation and tumor initiation.

Tumors are frequently composed of a heterogeneous population of cancer cells, and accumulating evidence suggests that, in many tumor types, only a subpopulation of these cells, named cancer stem cells or tumor-initiating cells (TICs) (Nguyen et al., 2012), are responsible for tumor maintenance and progression. There is also evidence that TICs are more resistant to many conventional chemotherapies and radiotherapies and, as such, are suspected to be responsible for tumor recurrence after treatment (Singh and Settleman, 2010). Hence, understanding the vulnerabilities of TICs could enable more effective cancer therapies.

Within this context, Zhang et al., (2012) describe the isolation of TICs from non-small cell lung cancer (NSCLC) and characterization of some of the TICs' unique phenotypic features in a recent *Cell* article. Remarkably, NSCLC TICs have robust increases in glycolysis as well as in glycine/serine metabolism, most notably at the level of the glycine cleavage system enzyme, leading to increased pyrimidine synthesis and proliferation. This study supports a large body of work indicating that cancer cells differ from non-transformed cells in their programming for nutrient metabolism (Ferreira et al., 2012).

In a very deft series of experiments, Zhang et al., (2012) report that within the otherwise heterogeneous NSCLCs, there exists a subpopulation of $CD166^{+}$ cells that are extremely potent in their ability to induce tumor formation in immunocompromised NOD/SCID *Il2r*$\gamma^{-/-}$ mice and form tumor spheres in vitro. As such, they can be considered TICs. Yet another remarkable feature of the TICs is their metabolic profile. Concomitant with an upregulation of glycolytic genes, there is also a strong upregulation of genes involved in serine, glycine, and one-carbon metabolism. Intracellular metabolite levels from TICs accord well with the gene expression profiles, as there is a relative increase in glycolytic intermediates as well as intermediates associated with glycine/serine and nucleotide metabolism. Among all of the gene expression changes observed in TICs, the most striking one is a powerful increase in glycine decarboxylase (GLDC), a member of the protein complex that catabolizes glycine into carbon dioxide, ammonia, and 5,10-methylene-tetrahydrofolate.

Convincingly, Zhang et al., (2012) showed that GLDC overexpression promotes glycolysis, serine/glycine metabolism, and the accumulation of pyrimidine nucleotides. Consistent with the idea that the deviant serine/glycine metabolism of TICs facilitates their tumorigenic capacity, $CD166^{-}$ lung tumor cells, which are otherwise non-tumorigenic, were able to establish tumors at low frequency when made to overexpress GLDC. Furthermore, GLDC overexpression alone was able to transform NIH 3T3 cells in vitro and drive tumor formation in vivo, which required GLDC's enzymatic activity to be intact. Knockdown of this enzyme, on the other hand, was effective in diminishing many of these parameters, including tumorigenicity, in cells that express high levels of GLDC. Together, these results suggest that GLDC could be a new NSCLC oncogene—a finding that will be strengthened if recurrent genomic alterations that increase GLDC activity are found in primary tumors. The potential clinical relevance of GLDC overexpression in NSCLC was given further salience by the revelation that high expression of GLDC in primary NSCLC tumors is significantly associated with a higher risk of patient mortality.

Although originally identified in TICs derived from primary lung tumors, Zhang et al., (2012) also show that GLDC is expressed at high levels in many other tumor types and in about 25% of cancer cell lines tested. Knockdown of GLDC in these high expressing cell lines, such as A549 lung adenocarcinoma cells and CACO2 colon cancer cells, reduces their proliferation and tumorigenic potential. Importantly, to demonstrate that this is a newly acquired vulnerability of these cancer cell lines and not an activity that is generally required for proliferation, Zhang et al., (2012) showed that GLDC knockdown did not affect the proliferation of normal human lung fibroblasts. They also hint on the possibility of exploiting this new metabolic vulnerability for therapeutic purposes by showing that cells that overexpress this enzyme are more sensitive to the antifolate drug

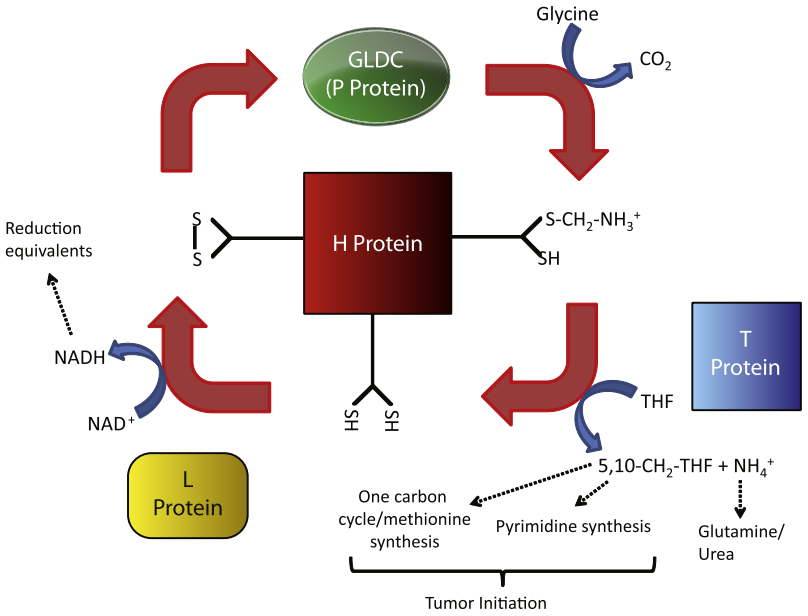

Figure 1. A Summary of the Protein Constituents of the Glycine Cleavage Complex and the Reactions that They Catalyze

The oxidative decarboxylation and deamination of glycine begins with glycine decarboxylase (GLDC; P protein), which removes carbon dioxide from glycine and transfers a methylamine group to the dithiolane ring of a lipoic acid molecule attached to H protein. The methylamine moiety is transferred to tetrahydrofolate (THF) by T protein, generating 5,10-methylene-tetrahydrofolate and reduced lipoate-H protein in the process. The reduced lipoate group of H protein is subsequently oxidized by L protein using NAD^+. This reaction regenerates the dithiolane ring of H protein's lipoate functional group, permitting it to participate in another catalytic cycle. Reducing equivalents in the form of NADH, one-carbon groups in the form of 5,10-methylene-THF and ammonium are important products of this reaction system. Based upon the work of Zhang et al., (2012), it is the enhanced supply of 5,10-methylene-THF available for use in the one-carbon cycle as well as for the synthesis of pyrimidines whereby upregulation of GLDC promotes tumor initiation.

methotrexane. It will be interesting to investigate if GLDC expression is a predictor of response to antifolate drugs in some cancers.

The work presented by Zhang et al., (2012) is a technical tour-de-force for its isolation of a rare subpopulation of TICs from primary NSCLC and for providing the scientific community with an explanation for what is genetically and metabolically unique about them. Although it certainly adds to our understanding about the metabolic differences between cancerous and non-cancerous cells, it also raised several fascinating questions.

The first—and most general—question concerns the nature of the recurring association between serine/glycine metabolism and tumorigenesis/cell proliferation. Other groups have also reported that many cancers have elevated levels of enzymes involved in the processing of serine/glycine (Possemato et al., 2011; Locasale et al., 2011; Vazquez et al., 2011; Vié et al., 2008), and overexpression of some of these enzymes is sufficient to enhance the potential for cellular transformation and/or increase the rate of cellular proliferation (Locasale et al., 2011; Vié et al., 2008). How does an increase in this pathway facilitate proliferation and tumorigenesis? Does it provide extra pyrimidines for DNA replication and prevent uracil accumulation? Are there epigenetic effects on proliferative gene expression, perhaps due to changes in one-carbon metabolism and cellular methylation capacity? Or are there other explanations? The metabolic fate of the amino acids serine and glycine is complexly intertwined with pathways associated with the TCA cycle, glycolysis, protein synthesis, generation of intermediates for one-carbon metabolism, phospholipid synthesis, nucleotide synthesis, and maintenance of cellular osmolarity. Carefully dissecting the contribution of changes in the flux of these subsidiary metabolic pathways as a consequence of a change in the rate in which a cell processes serine/glycine is an arduous task and will require more than a simple measurement of steady-state metabolite levels—an often deceptive marker of both the rate and directionality of flux (Fell, 1992; Snell and Fell, 1990). This later point is particularly important when evaluating the serine/glycine pathway, as many of the enzymes operate with only a slight displacement from equilibrium and flux determining steps are not intuitive.

The second question, as a corollary to the first, is how upregulated flux through the serine/glycine pathway is sustained in cancerous cells given all of the feedback mechanisms that are present in normal cells that prevent excess cycling through this pathway. Zhang et al., (2012) provide a tantalizing hint that, at least in the case of GLDC, oncogenes may drive the chronically high expression of serine/glycine metabolic enzymes, but how this occurs is unknown.

Finally, in regard to the biology of GLDC, it is curious that overexpression of only one component of the glycine cleavage complex is sufficient to massively perturb glycine dissimilation (Figure 1). The glycine cleavage complex is a multi-enzyme complex composed of four different subunits (P-[aka, GLDC] and H-, T-, and L-subunits) that are present in a ratio of 2P:27H:9T:1L and cooperate to channel substrates to reaction completion. Given this information, why are other subunits of the glycine cleavage complex not elevated in TICs? Is there a stoichiometric deficiency of GLDC in non-TICs that normally limits flux through the glycine cleavage complex? Is there a rearrangement of the complex ratios or modifications to the constituent subunits to handle an increase in GLDC-mediated product formation?

Overall, the paper presented by Zhang et al., (2012) makes a number of important contributions toward our understanding of the genetic and metabolic heterogeneity that is found in the cells within human tumors. It also suggests that inhibition of GLDC can be used in conjunction with existing antifolate chemotherapeutic regiments for the treatment of certain types of cancer. We shall watch with great interest as the story behind serine/glycine metabolism in cancer unfolds over the coming years.

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PI3King on *MYCN* to Improve Neuroblastoma Therapeutics

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MYCN is an oncogenic driver of childhood neuroblastoma, a frequently lethal pediatric tumor. In a recent paper in *Science Translational Medicine*, Chanthery and colleagues demonstrate that PI3K inhibition leads to the dual therapeutic benefits of enhanced MYCN degradation and loss of a paracrine angiogenic signal mediated by MYCN.

Despite decades of ever-improving outcomes across diverse pediatric cancers, neuroblastoma has remained a frustrating clinical entity. Most children are diagnosed with tumors that harbor genetic and biological features highly correlated with a poor treatment outcome. Current therapy for such high-risk patients includes dose-intensive chemotherapy, radiotherapy, and retinoids. Though there have been recent impressive translational successes for this tumor, such as immunotherapy using an antibody targeting cell-surface GD2 given with immunostimulatory cytokines (Yu et al., 2010), 3 year relapse free survival estimates for high-risk disease remain under 50%.

Further compounding the frustration is the fact that the genome of neuroblastoma is one of the most comprehensively characterized among pediatric cancers, but it has not yet led to more effective treatment. The recent discovery that the *ALK* receptor tyrosine kinase is constitutively activated in ~10% of neuroblastomas (Mossé et al., 2008) provides one such therapeutic opportunity, as *ALK* inhibitors have been in development due to the involvement of this kinase in a subset of non-small-cell lung cancers and anaplastic lymphomas. First generation *ALK* inhibitors such as crizotinib are already in Phase 2 trials for children with relapsed or refractory neuroblastoma and may make their way soon into upfront therapy for those patients with *ALK*-mutated tumors.

Contrast that with *MYCN*, the only other bona fide oncogene yet discovered in neuroblastoma that was initially identified almost 30 years ago (Brodeur et al., 1984). Despite this lead-time and a great deal of effort, no therapeutic has yet emerged to be able to target this clear oncogenic driver of the most aggressive subset of neuroblastomas. *MYCN*, which is a homolog of the *MYC* proto-oncogene, is somatically amplified in the tumor cells of ~20% of neuroblastoma patients (and in ~40% of those with a high-risk phenotype). *MYCN* amplification is independently correlated with advanced stage disease and poor outcome and therefore is used worldwide in risk classification algorithms. Moreover, genetically engineered mouse models with *MYCN* expression targeted to neural crest tissue develop tumors that resemble human neuroblastoma (Weiss et al., 1997). MYC proteins, including MYCN, serve pleiotropic roles in malignancy, such as altering metabolic programs, supporting angiogenesis, promoting self-renewal and "stemness," and driving proliferation while inhibiting differentiation.

ALK as a kinase is a pharmacologically tractable target, and a wealth of experience suggests that inhibition of activated kinases can lead to clinically impressive tumor responses. MYCN, in contrast, has long been seen as a problematic therapeutic target, as inactivating a highly abundant nuclear transcription factor that operates through a network of protein-protein interactions is pharmacologically daunting. Still, tumors are remarkably heterogeneous and cancer cells are remarkably adaptive. Resistance to targeted therapeutics can be efficiently selected for, especially when cells have