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COMMENTARIES AND HISTORICAL REFLECTIONS OPEN ACCESS

Licensing instead of fueling: Glutamine synthetase promotes mitotic progression via a non-metabolic mechanism

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Abstract – A recent report published in *Nature Metabolism* identified that glutamine synthetase (GS), the only enzyme in mammals to produce glutamine from glutamate, can directly control cancer cell mitosis by governing the APC/C complex via a metabolism-independent mechanism. It reported that GS can directly interact with the nuclear pore protein NUP88 to abolish its binging with CDC20, therefore licensing the activation of APC/C^{CDC20} to permit proper metaphase to anaphase transition of mitosis. These findings illustrated a dual-function mode of action of GS in cancer cells, in which GS's metabolic and non-metabolic functions coordinate with the concentration change of glutamine in the tumor microenvironment (TME) to ensure cell survival or proliferation, respectively. These findings revealed the multi-faceted roles of glutamine synthetase in tumor development and underscored the potential to target non-canonical functions of glutamine synthetase for cancer treatment.

Key words: Glutamine synthetase, Mitosis, APC/C.

Main

Uncontrolled cell proliferation is the most striking hallmark of cancer [1]. To sustain the ceaseless cell division, cancer cells reprogram metabolic pathways to provide sufficient nutrients and energy for mitotic progression [2]. It is well documented that metabolic reprogramming of cancer cells is orchestrated by dysregulated metabolic enzymes, the products of which can fuel and boost cell cycle progression by meeting the cellular needs for biosynthesis and bioenergenesis. In addition to the canonical functions in metabolism, it has been increasingly appreciated that metabolic enzymes can also conduct non-canonical or nonmetabolic functions that are referred to as "moonlighting" functions during specific physiological and pathological processes [3]. It was recently found that phosphoglycerate kinase 1 (PGK1) can bind to the cell division cycle 7 (CDC7) and convert local adenosine diphosphate (ADP) to adenosine triphosphate (ATP), thereby abrogating the inhibitory effect of ADP on CDC7-apoptosis signal-regulating kinase 1 (ASK1) and promoting DNA replication [4]. In addition, pyruvate kinase M2 isoform (PKM2), another key enzyme of glycolysis, can directly regulate mitosis and cytokinesis by interacting with the spindle checkpoint protein budding uninhibited by benzimidazoles 3 homolog (Bub3) and myosin light chain 2 (MLC2), respectively [5, 6]. These findings revealed that metabolic enzymes can govern mitosis by directly affecting essential protein complexes in cell cycle regulation.

The anaphase-promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase, is a critical regulator of cell mitosis by triggering the transition from metaphase to anaphase [7]. The APC/C polyubiquitinates and degrades securin and synthesis phase (S) and mitosis phase (M) cyclins. APC/C-mediated cohesion degradation releases separase, a protease, to cleave cohesion, which is the protein complex that binds sister chromatids together. Consequently, sister chromatids become free to move to opposite poles for anaphase [8]. The regulation of APC/C has been intensively studied. However, whether APC/C is regulated by metabolic enzymes is unknown.

In a recent study published in *Nature Metabolism*, we reported an unexpected finding that glutamine synthetase (GS), the only enzyme that synthesizes glutamine *de novo* from glutamate and ammonia, can promote cancer cell proliferation by licensing mitotic progression via a nucleoporin 88 (NUP88)- cell division cycle 20 (CDC20)-dependent regulation on the APC/C complex. Importantly, this novel function of GS in cancer does not rely on its catalytic activities [9] (Video 1).

Using various research models, we found that GS depletion, but not impairment of its enzymatic activity, results in the repressed proliferation of cancer cells when extracellular glutamine is sufficient. To validate this non-metabolic function

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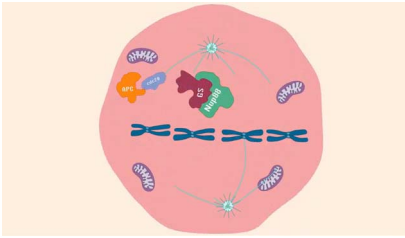

Video 1. GS interacts with NUP88 to license APC/C-mediated mitotic progression. https://vcm.edpsciences.org/10.1051/vcm/2022009#V1.

of GS, we employed a wild-type (WT) GS or a catalytically inactive GS-R324C mutant in our study. We found that under glutamine deprivation conditions, WT GS, but not GS-R324C, rescued the compromised cell survival caused by GS depletion. In contrast, under glutamine-sufficient conditions, the enzyme-dead mutant GS-R324C acted like its WT counterpart and completely rescued GS depletion-caused inhibition of cell proliferation. Furthermore, we found that the catalytic activity of GS was not only dispensable but also dramatically repressed when cancer cells were under glutamine-sufficient conditions. These findings, in addition to revealing a new function of GS in cancer, addressed a long-standing question in the field of glutamine metabolism. As the most abundant amino acid in plasma, glutamine is central to the metabolic network and is essential in energy metabolism and redox regulation [10]. Therefore, it is unsurprising that actively proliferating cancer cells have increased uptake and reliance on extracellular glutamine, supplied by intratumoral vasculature and cancer-associated fibroblasts [11]. Interestingly, despite the various sources of glutamine from the tumor microenvironment (TME), cancer cells are seemingly capable of self-producing glutamine since it is well-known that GS is frequently overexpressed in cancers [12]. However, glutamine catabolism, by which glutamine is deaminated to glutamate and other metabolites, is also often hyperactive in many types of cancers. Glutaminase (GLS), the enzyme converting glutamine to glutamate, is also usually overexpressed in many types of cancers [13]. Therefore, it seems intriguing and paradoxical that cancer cells require a simultaneous overexpression of both GS and GLS, which may produce a futile cycle. Our results indicated that the overexpressed GS functions as a metabolism-independent driver to guarantee the active proliferation of cancer cells, which goes beyond its role in controlling the balance of glutamine and glutamate.

We next revealed that GS depletion resulted in dramatic cell cycle arrest at the G2/M phase, which is the cellular mechanism of proliferation inhibition caused by GS depletion. Specifically, we found that GS is required for proper and timely metaphase-to-anaphase transition, a key step of mitotic progression. Consistent with its role in cell proliferation, the metabolic function of GS is not involved in the progression of metaphase to anaphase since both WT GS and enzyme-dead GS can rescue the mitotic defect caused by GS depletion. These results ushered in a novel mechanism of how glutamine–glutamate metabolism contributes to cell cycle progression. It is well established that cell division is addicted to sufficient nutrients supplied by different metabolic pathways. During the G1-S transition, glycolysis is activated and required to satisfy the G1 checkpoint; inhibiting glycolysis or lowering the glucose supply impairs the G1-S transition [14]. Similarly, glutaminolysis, driven by GLS, is active and indispensable for the proper progression of both G1-S and S-G2/M transitions simply because the metabolic products of glutamine breakdown are necessary for the production of the macromolecules to fulfill these transitions [15]. Our work found that GS is essential in spinning the wheel of mitosis via a metabolism-independent mechanism when entering into mitosis. Interestingly, GLS is downregulated during mitosis [15], which might lead to a transient accumulation of glutamine during cell division. This transitorily accumulated glutamine may also help inhibit the enzymatic activity of GS and turn on its enzyme-independent and pro-mitotic function.

To reveal the mechanistic insights underlying the non-metabolic functions of GS in mitosis regulation, we conducted an unbiased proteomic analysis to look for novel interacting proteins of GS, hypothesizing that GS may gain new functions simply by coordinating with new partners. It turned out that GS can directly interact with the nuclear pore protein NUP88. The interaction of GS and NUP88 can prevent the binding of NUP88 to CDC20, an essential regulator of the APC/C complex, and as a result, licensing the CDC20-mediated APC/C activation to ensure proper mitotic progression. Importantly, we managed to identify four essential amino acid residues that mediate the interaction between GS and NUP88. Mutating these four residues can effectively abolish the interaction between GS and NUP88, therefore completely abrogating the pro-mitotic function of GS. Interestingly, this GS mutant, while losing the ability to bind to NUP88, remains active in producing glutamine, further suggesting that the catalytic activity of GS does not involve cell cycle progression. NUP88, the binding partner of GS, has been known to regulate mitosis and cell cycle progression beyond its role in cytoplasm-nucleus transport [16]. By forming a NUP88- nucleoporin 98 (NUP98)/ribonucleic acid export 1 (RAE1) complex, NUP88 can prematurely activate APC/C- CDC20 homolog 1 (CDH1) before mitotic onset and cause chromosome separation defects[17]. This function of NUP88 depends on its cytoplasmic localization, where it can interact with NUP98/RAE1 before the breakdown of the nuclear membrane. We found that GS tends to interact with NUP88 after the breakdown of the nuclear membrane. The spatiotemporal difference in the mode of interaction with NUP88 is consistent with our notion that NUP88 could mediate the function of GS via an APC/C-CDH1–independent mechanism. Indeed, the interaction of GS and NUP88 is critical in licensing the binding of CDC20 to CDC27, which guarantees a proper and timely activation of APC/C-CDC20, driving the metaphase-anaphase transition.

GS is an important enzyme to produce a critical amino acid, albeit we found that it also carries out, maybe equally important, moonlighting functions in cancer cells. Considering the special pathological characteristics of cancer, we reason that these dual functions of GS can converge to support cell survival and proliferation in the TME. While glutamine is usually kept at a relatively constant level in the plasma (0.45 ~ 0.8 mM)

[18], the actual concentrations of glutamine in the TME vary and fluctuate. In the periphery of tumors where the level of glutamine is relatively high (due to the vasculature vicinity) [19], GS ensures cell division by its catalytic activity-independent function. However, in the core of tumors where the availability of glutamine is limited [19], the enzymatic activity of GS may mainly fuel glutamine production to support the survival of cancer cells. In other words, the dual functions of GS can cope with fluctuating levels of glutamine across the TME to meet the actual need of cancer cells in a spatial-temporary manner. Furthermore, our findings carry very high significance from the clinic's perspective. Not only is GS overexpressed in human lung cancers, but the high level of GS is also strongly correlated to resistance to microtubule-targeted drugs. The connection between GS and chemoresistance can be well explained by the mechanistic insights into how GS regulates mitosis. Our findings unraveled for the first time that the mitotic progression of cancer cells can be directly governed by a metabolic enzyme of the amino acid pathway, particularly via a metabolic function-independent manner, which can sprout novel strategies for cancer intervention.

Conflict of interest

The authors declare that they have no conflict of interest.

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