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HYPOTHESIS

# Induction of IMPDH-Based Cytoophidia by a Probable IMP-Dependent ARL13B–IMPDH Interaction

**Najva Alijani[1] and Razieh Yazdanparast[1,a]***

[1]*Institute of Biochemistry and Biophysics, University of Tehran, 13145-1384 Tehran, Iran*
[a]*e-mail: ryazdan@ut.ac.ir*

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**Abstract**—Inosine Monophosphate Dehydrogenase (IMPDH) catalyzes rate-limiting step of the reaction converting inosine monophosphate (IMP) to guanine nucleotides. IMPDH is up-regulated in the healthy proliferating cells and also in tumor cells to meet their elevated demand for guanine nucleotides. An exclusive regulatory mechanism for this enzyme is filamentation, through which IMPDH can resist allosteric inhibition by the end product, GTP. It has been proven that intracellular IMP, which rises during the proliferative state, potentially promotes IMPDH filamentation. On the other hand, interaction of IMPDH with ADP-ribosylation factor-like protein 13B (ARL13B) directs guanine biosynthesis toward the *de novo* pathway. However, ARL13B is not localized in the IMPDH-based cytoophidia, super structures composed of bundled IMPDH filaments and other proteins. Here, we hypothesized that ARL13B could increase availability of the *de novo*-produced IMP for IMPDH by interacting with the IMP-free IMPDH and microtubules adjacent to the purinosome. Following IMP-binding, IMPDH would be released from ARL13B and preferentially associated with its cytoophidia. Considering clinical side effects of catalytic inhibitors of IMPDH and their ability to induce IMPDH cytoophidia, we suggest that combination of proper doses of IMPDH catalytic inhibitors and inhibitors of the *de novo* IMP biosynthesis could be more effective in controlling cell proliferation.

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*Keywords*: ARL13B–IMPDH interaction, IMP, cytoophidia, purine biosynthesis

## INTRODUCTION

Inosine monophosphate dehydrogenase (IMPDH), a critical enzyme in purine metabolism, catalyzes rate-limiting step in the *de novo* biosynthesis of guanine nucleotide by conversion of inosine-5′-monophosphate (IMP) to xanthosine-5′-monophosphate (XMP) [1]. While its inhibitors are clinically used as immunosuppressive, antiviral, and antimicrobial drugs, they have not yet been approved for cancer treatment. This is mainly due to severe side effects at high clinical doses and inconsistent responses [2], which highlights the need for further IMPDH research.

In vertebrates, octameric units of IMPDH can reversibly assemble into single-stranded filaments, which, in turn, can associate to create intricate structures known as “cytoophidia”, also known as “rods and rings” (RR) structures because of their shapes [3-5]. This fascinating ability adds an extra level to the enzyme activity regulation, facilitating resistance to allosteric inhibition by guanosine-5′-triphosphate (GTP) [6]. Some conditions, such as a high substrate-to-product (IMP/GTP) ratio [6, 7], cell treatments with IMPDH inhibitors such as Mycophenolic acid (MPA) and/or Ribavirin [3, 4], and deficit of essential purine biosynthetic precursors such as glutamine and folate [8, 9], promote cytoophidia formation. On the other hand, some highly proliferative cells, such as induced pluripotent stem cells (iPSCs), mouse embryonic stem cells (ESCs), and mitogen-activated T-cells robustly form cytoophidia in rich media without external treatments [4, 7, 10], probably due to the elevated IMP level resulting from upregulated proliferation signals [6].

*Abbreviations*: ARL13B, ADP-ribosylation factor-like protein 13B; GTP, Guanosine-5′-triphosphate; IMP, inosine-5′-monophosphate; IMPDH, inosine monophosphate dehydrogenase.

* To whom correspondence should be addressed.

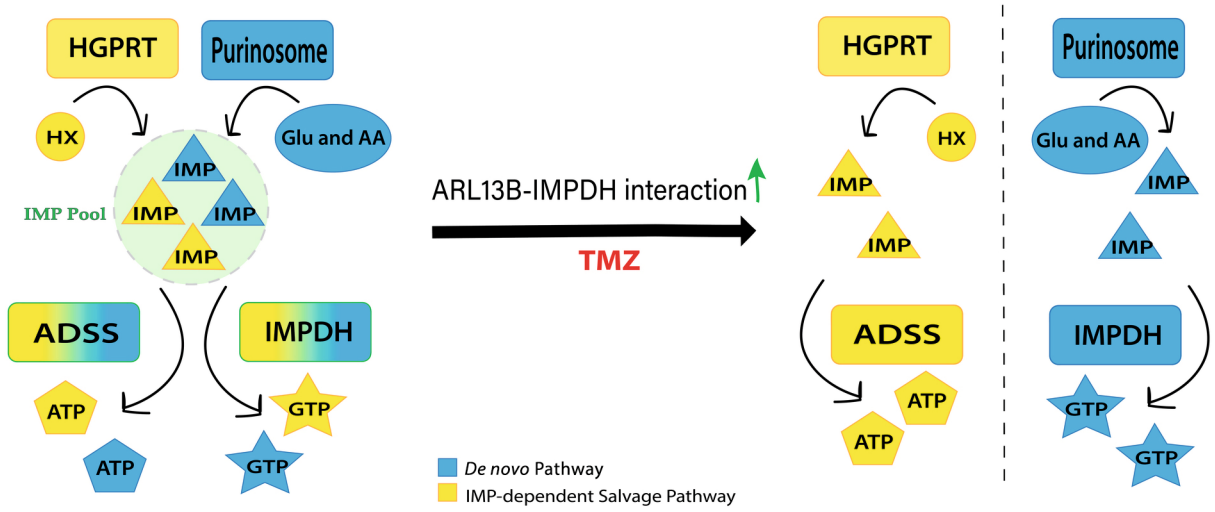
## HYPOTHESIS AND SUPPORTING DATA

In tumor cells, the rate of GTP metabolism undergoes more extensive changes than that of ATP. While ATP levels show a modest rise of approximately 20% in tumor cells, GTP levels significantly increase by nearly 200% relative to normal cells [11, 12]. Interestingly, upregulated GTP is redirected toward nucleolus for tRNA and rRNA synthesis, the most abundant RNA species in the cell, which have an indispensable role in protein synthesis, resulting in nucleolar transformation and cell proliferation [13]. Dramatic upregulation of GTP in the tumor cells could suggest preferential direction of IMP toward GTP biosynthesis rather than ATP production. We speculate that the GTP channeling toward nucleolus as well as a probable direction of IMP toward IMPDH would increase the IMP/GTP ratio in the surrounding environment of the IMPDH and therefore, could lead to IMPDH filamentation. Moreover, constitutive formation of IMPDH-based cytoophidia in some highly proliferative cells might be a consequence of not only a balance between IMP production and GTP consumption but an equilibrium between the probable direction of IMP toward IMPDH and GTP channeling toward nucleolus.

While IMP biosynthesis in quiescent cells is low and is realized through the salvage pathway, the highly proliferative cells mainly meet their high demand for purine nucleotides via the *de novo* synthesis pathway [14, 15]. Notably, interaction of IMPDH with the small GTPase from the ARF family, named ADP-ribosylation factor-like protein 13B (ARL13B), shifts purine biosynthesis from the salvage pathways toward the *de novo* pathways [16]. Despite the enrichment of ARL13B in primary cilia as the cilium marker, literature also documents presence of Arl13B in cytoplasm associated with biological functions [17, 18]. Cytoplasmic ARL13B colocalizes and cooperates with some cytoskeleton components involved in endocytic recycling traffic such as Arf6, Rab22, CD1a, actin, and also microtubules [17]. Interaction of the cytoplasmic ARL13B with cytoskeleton components also regulates cell migration [18]. Distribution of ARL13B between the cilia and cytoplasm as well as its stability and functions could be affected by various factors. For instance, lipid modifications, especially palmitoylation, are vital for ciliary localization, function, and stability of ARL13B [19]. Based on the present literature data on disappearance of the cilia body structure from the cell surface during the processes of cell division and migration [20-22], it is assumed that the ARL13B content could probably end up in the cytoplasm. Interestingly enough, our preliminary data confirmed this prediction.

Shireman et al. showed that the 96-h treatment of cells with Temozolomide (TMZ), an alkylating agent, increases ARL13B expression and its interaction with IMPDH outside of the primary cilia in glioblastoma tumor cells [16]. It was also determined that the effects of TMZ on purine metabolism are mediated by the ARL13B–IMPDH interaction and are independent of the Sonic hedgehog signaling (Shh) and primary cilia. The authors hypothesized that by shifting purine metabolism from the salvage pathway to the *de novo* pathway, this interaction could prevent the cell from using alkylated GTP and maintain cell survival [16]. However, it is unclear how the ARL13B–IMPDH interaction could cause this switching in the purine biosynthetic pathway. It can be seen from the particular results of this study that the 96-h treatment with TMZ can increase incorporation of the radio-labeled hypoxanthine and glycine into DNA and RNA. This means that the ARL13B–IMPDH interaction could increase incorporation of the precursors belonging to two IMP-dependent pathways of purine metabolism: the hypoxanthine-dependent salvage pathway and the *de novo* pathway (Fig. 5E from [16]). However, TMZ treatment, and subsequent rise in the ARL13B–IMPDH interaction, decrease the GMP production from the hypoxanthine-dependent salvage pathway in the tumor sample (Fig. 5F from [16]). These data suggest that the TMZ-induced increase in incorporation of $^{3}$H-hypoxanthine into nucleic acids (Fig. 5E from [16]) is probably due to the increased conversion of hypoxanthine to AMP only and not to GMP. This suggestion is confirmed by the increase in the downstream product of AMP catabolism, hypoxanthine, in the TMZ-treated samples (Fig. 5F from [16]). Decrease in the fractional enrichment of hypoxanthine-derived IMP and GMP in the TMZ-treated samples (Fig. 5G from [16]) also suggests direction of the hypoxanthine-derived IMP toward the AMP synthesis and not GTP. Our conclusions from Fig. 5 of [16] are summarized in Scheme 1.

Accordingly, increase in the ARL13B–IMPDH interaction directs the *de novo*-produced IMP toward IMPDH, theoretically supporting cytoophidia formation. In this scenario, ARL13B–IMPDH interaction and cytoophidia formation, as two probable aspects of GTP metabolic reprogramming in proliferating cells, could occur in the same spatiotemporal pattern. Our laboratory has detected increase of the ARL13B–IMPDH interaction in the cytoophidia-forming cells and its positive correlation with the cell viability. We believe that the ARL13B–IMPDH interaction could change orientation and proximity of IMPDH toward purinosome, a metabolon consisting of the enzymes responsible for the *de novo* purine biosynthetic pathway. This assumption emerged from the summation of three independent observations. First, purinosome moves along the microtubule toward mitochondria and localizes in the mitochondria-microtubule interface at the peak of purine demand [23]. Second, there is evidence of ARL13B




**Scheme 1.** Separation of IMPDH from the IMP-dependent salvage pathway of purine biosynthesis and increasing its access to the *de novo*-produced IMP after 96-h TMZ treatment of glioblastoma cells (GBMs). Purinosome, a metabolon of the enzymes participating in the *de novo* purine biosynthetic pathway (blue pathway), and hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme responsible for recycling purine from hypoxanthine (yellow pathway) and guanine (not shown here), provide the cellular IMP pool. IMPDH and adenylosuccinate synthase (ADSS) jointly use this IMP resource to produce GMP and AMP, respectively. Increased ARL13B–IMPDH interaction following 96-h TMZ treatment drives IMPDH to the *de novo*-produced IMP and prevents its access to the salvage-produced IMP. This means a decreased role of the IMPDH's competitor, ADSS, in using the *de novo*-produced IMP and its greater role in using the salvage-produced IMP. ADSS, adenylosuccinate synthase; Glu and AA, glucose and amino acids as precursors of *de novo* biosynthesis of IMP; HX, hypoxanthine; HGPRT, hypoxanthine-guanine phosphoribosyltransferase.

and microtubule association in the cytoplasm, though lower than in cilia. ARL13B is a membrane-associated and anchor-like protein whose interaction with axoneme tubulin is vital for the uniform distribution of the ciliary membrane proteins [24]. ARL13B marginally co-localizes with microtubules in the cytoplasm and can participate in endosomal recycling along microtubules through interaction with actin [17]. Furthermore, some studies demonstrated participation of ARL13Bs in other microtubule-associated processes such as cell migration and proliferation [18, 20], which typically happen in the temporary absence of cilia [20-22]. Third, cytoophidia is associated with some intracellular membranes such as mitochondrial and endoplasmic reticulum (ER) membranes [25]. Moreover, another consistent component of the IMPDH-based cytoophidia, ADP-ribosylation factor-like protein 2 (ARL2), is involved in both αβ-tubulin biogenesis and mitochondrial fusion [25]. These findings could imply a relationship between the location of cytoophidia and purinosome in the cells or even their co-localization in the proximity of intracellular membranes with the emergence of high demand for GTP. This relation becomes feasible with the help of tubulin-interacting small GTPases like ARL2 and ARL13B. In our model, the ARL13B–IMPDH interaction anchors IMPDH to the microtubule and in proximity to purinosome. Since it was reported in the previous study that ARL13B is not localized in the IMPDH-based cytoophidia [25], we believe that ARL13B only interacts with soluble and IMP-free-IMPDHs. IMP binding triggers dissociation of IMPDH from ARL13B, making it free to form cytoophidia. This model could explain the role of ARL13B–IMPDH interaction in the preferential direction of the *de novo*-synthesized IMP toward IMPDH for robust GTP production.

Induction of the ARL13B expression and ARL13B–IMPDH interaction by TMZ [16] as an alkylating agent suggests that the recognized GTP deficit might increase the ARL13B–IMPDH interaction. As two GTP-binding proteins, ARL13B and/or IMPDH could directly sense the level of GTP. Other GTP-sensing proteins such as phosphatidylinositol 5-phosphate 4-kinase β (PI5P4Kβ), a cytoplasmic membrane-bound protein with a unique GTP-dependent kinase activity [26], could also participate in any step of IMP channeling toward IMPDH upon GTP depletion. However, according to the above assumption, IMPDH inhibitors could induce the ARL13B-IMPDH interaction by reducing GTP levels. The level of this interaction would depend on the amount of IMPDH proteins with an empty active site and, therefore, on the inhibitor concentration. Lower concentrations of IMPDH inhibitors, with sufficient enzymes having empty active sites, could induce ARL13B–IMPDH interaction and cytoophidia formation. In this case, they can compensate for GTP reduction and even induce cell growth by producing a high level of GTP. In contrast, lethal doses of inhibitors could decrease the ARL13B–IMPDH interaction by depleting the enzyme population with an empty

active site. Although lethal doses could be successful for cancer control *in vitro*, their clinical use is not feasible due to severe side effects. Hence, it seems that inhibitors of the *de novo* IMP biosynthesis, which are theoretically cytoophidia-dissembling agents, could be useful in this case. They could not only decrease the level of the IMPDH substrate, but could also decrease cytoophidia formation. Thus, a combination therapy of these two types of inhibitors could be more effective in controlling tumor cells than the IMPDH inhibitors alone. This hypothesis is under investigation in our laboratory and the results will be published soon.

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**Contributions.** Both authors contributed to the study's conception and design. N.A. performed material preparation, data collection, and analyses. N.A. also prepared the first draft of the manuscript, which was edited by R.Y. Both authors read and approved the final draft to be submitted.

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**Ethics declarations.** This work does not contain any studies involving human and animal subjects. The authors of this work declare that they have no conflicts of interest.

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