IDH1, a metabolic enzyme or its mutant migrate to nucleus in a KPNA-dependent manner

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Abstract

Cancerous cells show altered expression of several genes which involves activation of transcription factors and/or inactivation of transcriptional regulators. Several metabolites are known to aid cancerous growth. Isocitrate dehydrogenases (IDH-1 and IDH-2) have been found to be mutated in cancers like gliomas and chondrosarcomas. These enzymes are known to affect the transcription profile of cancerous cells. However, how a metabolic enzyme mediates the transcriptional regulation is an area open for investigation. Here we report a possible mechanism of movement of a metabolic enzyme from cytosol to the nucleus to alter the transcription profile of a cancerous cell.

Keywords: isocitrate dehydrogenase, KPNA, nuclear transport, glioma

DOI: 10.48047/ECB/2023.12.8.586

Introduction:

Isocitrate dehydrogenases (IDH1 and IDH2) are metabolic enzymes that convert isocitrate to α-ketoglutarate (α-KG) in cells. In gliomas, chondrosarcomas and a subset of cancers, IDH1 or IDH2 are mutated in the hotspot arginine at R132 of IDH1 and R140 of IDH2. Mutation of these residues leads to gain of function, wherein the mutant enzymes produce D-2 hydroxy glutarate (D-2HG) from α-KG in tumor cells. The overproduced D-2HG is a structural congener of α-KG, thus it inhibits diverse α-KG utilizing enzymes such as TET family dioxygenases, Jumnoji (histone demethylases) and DNA demeythalases [1]. Hence, the methylation

landscape of mutant IDH expressing cells undergo marked transformation, eventually leading to a shift in gene expressions. However, unlike other oncogenes such as RAS, Myc or Akt, focus on IDH1-linked research is more on its metabolite D-2HG rather than the cellular roles of IDH1 protein [2].

Metabolic enzymes tune gene expressions in tumour cells either by locally producing metabolites or associating with transcription factors in the nucleus [3, 4]. Acetyl-CoA a high energy metabolite is produced by cytoplasmic ATP citrate lyase (ACLY), Carnitine O-Acetyltransferase (CRAT) and mitochondrial PDC enzymes in the nucleus to acetylate histones [5-7]. Apart from producing nuclear acetyl-CoA, in glucose deficient cells, Acyl-coenzyme A synthetase 2 (ACCS2) interacts with transcription factor EB and activates lysosomal and autophagy genes [8]. Further, the glycolytic enzyme, PKM2 modulates transcriptional program in the nucleus by associating with transcription factors such as STAT3, HIF-1, Oct4 and β-catenin [9-12]. In tumour cells, replete with glucose phosphofructokinase, transcriptional cofactors migrate to nucleus and cooperate with YAP/TAZ to activate growth promoting genes [13]. Thus, metabolic enzymes exhibit their canonical functions by generating metabolites or non-canonical roles by binding to transcription factors in the nucleus. α-KG/D-2HG, produced by IDH1 or its mutant, either activates or inhibits enzymes in the nucleus[14]. However, whether IDH1 translocates to the nucleus to locally produce these metabolites or associates with any transcription factor is unknown.

IDH1 is known to be localized in peroxisomes and cytoplasm; however clinical studies indicate nuclear staining of mutant IDH1. Cytoplasmic and weak nuclear staining was found in 122 primary brain tumour tissues stained with mutant IDH1-specific antibody [15]. In another study, immunohistochemistry (IHC) analysis of 57 diffuse glioma tissues reveals that mutant IDH1 (mIDH1) was found in cytoplasm and nucleus [16]. Further, mutant IDH1 was found in the nucleus of secondary glioblastoma and anaplastic astrocytoma tissues [17]. In two separate studies of a cohort of 57 and 211 glioma samples, mIDH1 was found in cytoplasm and nucleus [18]. As these studies aimed to detect mIDH1 in gliomas by IHC, not much is known about wild-type IDH1 localization in these tissues. Therefore, it is imperative to decipher the underlying mechanisms that contribute to nuclear localization of IDH1.

Materials and Methods:

Cell Culture

HeLa and HEK 293T cell lines were ATCC sourced. Cells were grown in DMEM with 10% fetal bovine serum and antibiotics in 5% CO_2. Sub-confluent populations of cells were split and grown in dishes or six-well plates for the assays. After attaining 70% confluency, the cells were used for transfection.

Transfection of DNA in Mammalian Cells

HEK 293T cells were seeded in 60 mm dishes and next day after attainining ~70% confluency, were transfected with respective plasmids. Transfections were performed for about 4h using polyethylenimine (PEI) in serum-free media and cells were allowed to grow for another 20-24 h in the complete medium [19,20]. The protein lysates were resolved by SDS-PAGE and the protein levels were determined using appropriate antibodies [21].

Co-immunoprecipitations

For immunoprecipitations, HEK293T cells were washed with ice-cold PBS and lysed in lysis buffer. The cell lysates were cleared by centrifugation and supernatants were used for immunoprecipitation with 2 mg of appropriate antibodies for 8 hours at 4˚C. The immunoprecipitated proteins were washed three times with wash buffer, eluted by boiling in SDS sample buffer and resolved by SDS-PAGE. The proteins were transferred onto nitrocellulose membrane for western blot analysis.

SDS-PAGE and Western Blotting

Protein detection was done using resolving the lysates in SDS-PAGE followed by western blotting according to the standard protocols as described previously [32].

Results:

IDH1 and its mutant localize to nucleus in G2 phase

In clinical samples of glioma, mutant IDH1 shows a strong cytoplasmic and nuclear staining, these results were corroborated in various studies that evaluated the clinical efficacy of the mutant IDH1 specific antibody [15, 16, 18, 22, 23]. To check whether IDH1 or its mutant localizes to nucleus in cancer cells, we performed nuclear/cytoplasmic fractionation experiments. As IDH1 and IDH2 protein levels accumulate in G2/M phase of the cell cycle, we checked whether nuclear IDH1 levels depend on cell cycle. HeLa cells were treated with various inhibitors that block cells in a stage specific manner, olomocine- G1 phase, hydroxy urea- S phase, and RO3306- G2 phase [24, 25]. After fractionating cell lysates, Immunoblot analysis revealed that IDH1 shows nuclear accumulation in G2 phase (Fig. 1A). Next, we checked whether the mitochondrial resident, IDH2 localizes to nucleus in cancer cells, we analyzed nuclear IDH2 levels after fractionation. Unlike IDH1, IDH2 protein was found in the cytoplasmic fraction of HeLa cells under various conditions of treatments (Fig. 1B). As mutant IDH1 was found to localize in nucleus of glioma cells, we analyzed for its localization in cancer cell lines. Similar to wild-type IDH1, the mutant IDH1(R132H) was equally distributed between cytoplasm and nucleus in G2 phase, whereas arresting cells in G1 or S-phase did not trigger any nuclear localization of IDH1 (Fig.1C). Overall, our results suggest that IDH1 or its mutant is found in nucleus in G2-arrested cells.

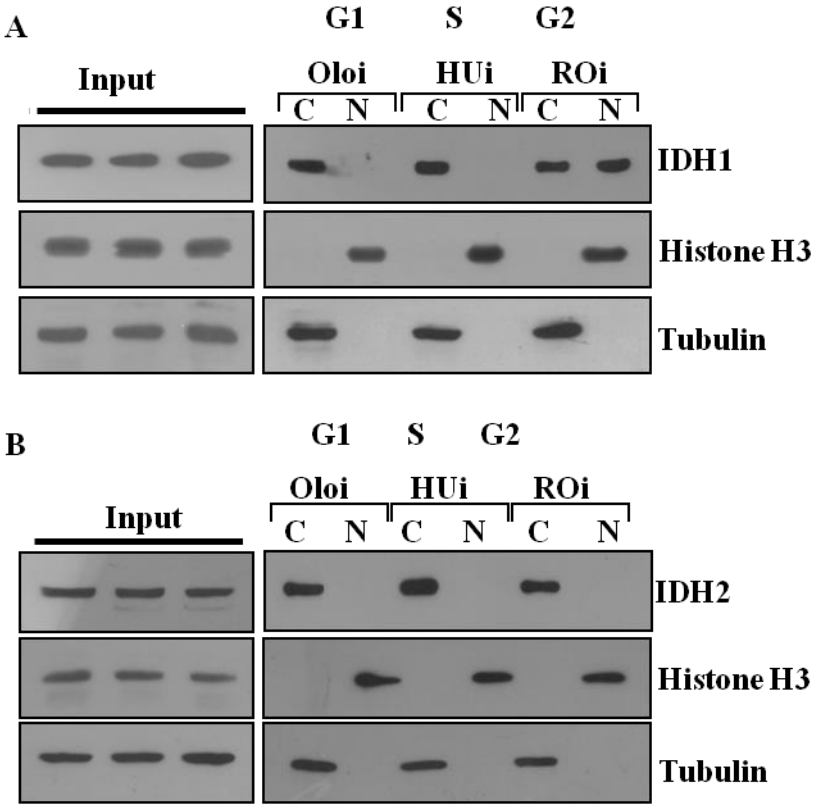

Figure 1 IDH1 and its mutant are found in nucleus in G2 phase. A, HeLa cells were treated with different phase specific inhibitors, Olomoucine- G1(20ng/ml), hydroxy urea-S (6mM), and RO3306-G2 (8mM) For 24 h, and later subjected to nuclear/cytoplasmic fractionation by using Reap buffer (1x PBS, protease inhibitor cocktail, 0.1% NP-40). Immunoblot analysis performed for IDH1, to check the purity of the fractions Histone H3 was used as a nuclear protein marker and tubulin protein designates the cytoplasmic fraction. **B,** IDH2 was analyzed similar to **A.**

IDH1 and its mutant interact with KPNA-5

Cytoplasmic resident proteins are transported into nucleus in a KPNA dependent manner. Here KPNA-1-5 bind to various cargo proteins through the nuclear localization signals. Then, importin-β binds to this complex and guides the cytoplasmic protein to the nucleus. As IDH1 or mutant IDH1 are found in nucleus in G2 arrested cells, we checked whether they harbour a nuclear localization signal. We performed bioinformatics analysis using the cNLS-mapper software and determined the putative NLS (Fig. 2A) [26]. IDH1 harbours bipartite NLS in the N-terminal 69-122 amino acids long, similarly mutant IDH1 also possess a NLS

upstream to R132H mutation. To check whether IDH1 or its mutant bind to any of the KPNAs1-5, immunoprecipitation assays were performed wherein FLAG-IDH1 or its mutant was co-transfected with T-7-tagged KPNA 1-5 in HEK293T cells. Notably, IDH1 and its mutant strongly and specifically interacted with KPNA-5(Fig. 2B-C). In summary, KPNA-5 binds to IDH1 or its mutant possibly through the NLS in IDH1 protein.

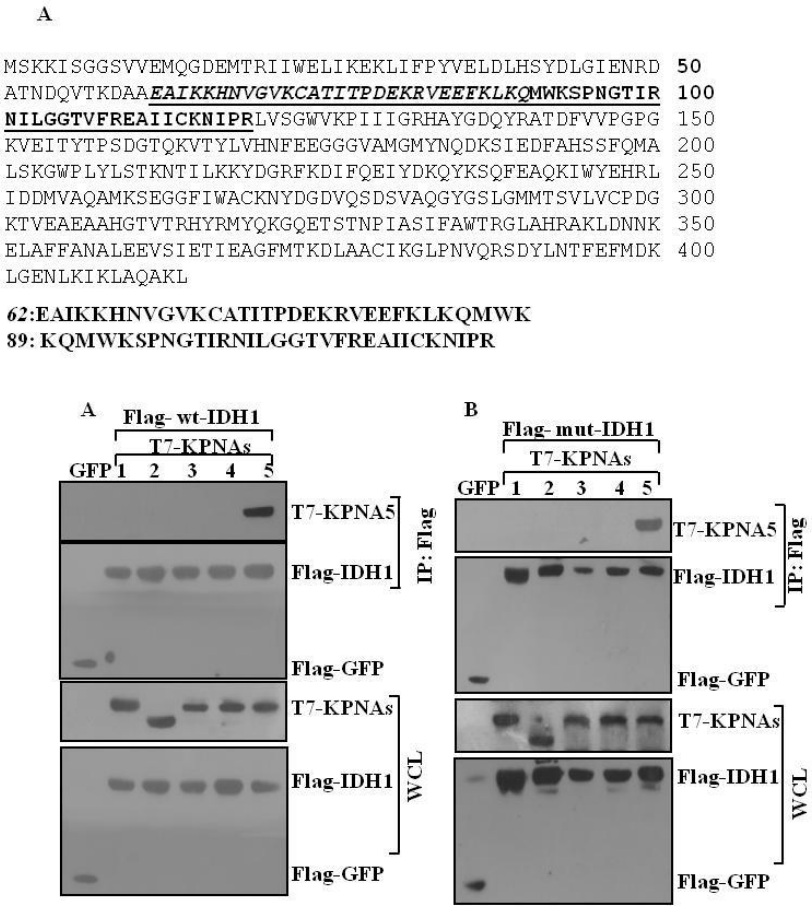

Figure 2 Importin-6-alpha interacts with wild type and mutant IDH1. A, Bioinformatics analysis of complete protein coding region of IDH1 by using cNLS mapper and predicted bipartite nuclear localization signal in the N-terminus of the IDH1. **B and C,** Flag-IDH1 or its mutant protein were co-transfected with different KPNA plasmids and subjected to immunoprecipitation using Flag-M2 beads. Immunoblot analysis was performed for Flag and T7 tagged proteins using specific antibodies.

Discussion:

In this study we found that IDH1 and its mutant localizes to nucleus in G2 phase of cell cycle, whereas blocking cells in G1 or S-phase did not trigger IDH1 nuclear localization. Although IDH1 and IDH2 harbor ~70% of sequence identity, they differ in cellular localizations. Irrespective of cell cycle, IDH2 was always found in cytoplasmic fractions. Bioinformatics analysis of IDH1 revealed a strong bipartite nuclear localization signal lodged in the N-terminal of IDH1 and its mutant. In contrast, IDH2 does not harbor any NLS like sequence in the entire region. IDH1 and its mutant strongly interact with KPNA-5, whereas KPNAs-1-4 do not bind to IDH1 proteins.

Many metabolic enzymes moonlight to the nucleus to affect gene expressions for example, PKM2 is a glycolytic enzyme that catalyses the conversion of phosphoenol pyruvate to pyruvate in tumor cells, lower activity of PKM2 supports cell survival and proliferation. Moreover, decreased enzyme activity of PKM2, leads to the supply of essential metabolites to other pathways such as one-carbon and lipid synthesis. Interestingly, PKM2 migrates to nucleus and functions as protein kinase, wherein it phosphorylates histone3, STAT3 and H2AX to promote proliferation or enhance cellular response to stress [10, 27, 28]. Similarly, IDH3α a protein of the heterotetrameric IDH3 enzyme harbors a canonical and evolutionarily conserved nuclear localization signal in amino acid 124 [29]. In S-phase, IDH3α is found in nuclear periphery and interacts with cSHMT2 to regulate serine metabolism [30]. Also, in zygote development IDH3α is found in the nucleus to supply essential metabolites [31]. Therefore, it is likely that IDH3α participates in promoting cellular progression in S-phase, whereas IDH1 supports gene expressions in G2. Hence to fully comprehend IDH1 signalling it is imperative to understand the metabolic function of IDH1 in concert with its protein-protein interactions in G2/M.

In conclusion, IDH1 or its mutant migrates to nucleus in a KPNA dependent manner.

Declarations

Ethical approval: No human and/or animal studies are involved in this study. **Competing interests:** The authors do not have any competing interests of a financial or personal nature related to this work.

Authors' contributions: KB: Conceptualization, Methodology and Writing original draft. HD: Methodology and Validation. VV: Writing, review & editing. NJ: Supervision, review & editing, funding acquisition.

Funding: K.B thanks UGC for the senior research fellowship and H.D. thanks DST-INSPIRE, India for the senior research fellowship. We thank Director, CSIR-IICT for providing all the required facilities to carry out the work (Ms. No. IICT/Pubs./2021/299). Funding: This work was supported by grants from the Council of Scientific and Industrial Research (CSIR-IICT/MLP0068), India to N.J.

Availability of data and materials: Request for reagents and supportive data can be directed to N.J.

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