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Isocitrate Dehydrogenase: Three Subunits in Different Cancer Types—Changes in Gene Expression, Mutation Status, and Cancer Progression.

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Research Article

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ABSTRACT

The isocitrate dehydrogenase (IDH) gene encodes three protein isoforms (IDH1, IDH2 and IDH3). IDH3 exists in three subunits (IDH3A, IDH3B, and IDH3G). Examining the gene expression level and mutation status of IDH3 subunits could help find new diagnoses or treatment options for specific cancer types. Until now, studies on the IDH3 enzyme have been focused on the IDH3A subunit and a few types of cancer. In our study, the effect of IDH3A, IDH3B, and IDH3G expression changes on cancer progression and survival in the ten major cancer types were investigated using bioinformatic tools. Then, the impact of mutation status on cancer progression was examined in the TCGA database. The expression level of IDH3 subunits increased as the bladder, breast, colon, esophageal, lung, and stomach cancers progressed. The elevated expression level of all IDH3 subunit expressions leads to poor prognosis in colon, esophageal, lung, prostate, and stomach cancers. The cumulative alteration rates revealed low-frequency (0.3-5.9 %) mutations in all IDH3 subunits. IDH3A, IDH3B, and IDH3G expression was significantly altered after mutation among all types of cancer studied except the prostate. Our data showed that there may be correlation between the mutation status of the IDH3 subunits and breast, esophageal, lung and stomach cancer progression.

Keywords: Cancer, Biomarker, IDH3A, IDH3B, IDH3G.

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Introduction

Isocitrate dehydrogenase (IDH) is one of the critical regulator enzymes in the tricarboxylic acid (TCA cycle). IDH plays a role in the α-KG (α-ketoglutarate) production from isocitrate with the formation of NADH or NADPH, which is essential for the metabolism of normal mammalian cells [1]. There are three IDH isoforms, IDH1, IDH2, and IDH3, and their molecular structure, subcellular localization, catalytic mechanism, and cofactor requirement are different [2]. $NADP^+$-dependent IDH1 and IDH2 enzymes are homodimeric, and the reactions catalyzed by IDH1 and IDH2 are reversible [3]. IDH1 catalyzes the oxidative decarboxylation of isocitrate to α-KG in the cytoplasm and peroxisomes, whereas IDH2 carries out the same reaction in the mitochondria [2]. On the other hand, the NAD^+-dependent IDH3 enzyme has structurally different heterotetrameric chemistry. IDH3 catalyzes the irreversible conversion of isocitrate to α-KG, one of the rate-limiting steps in the TCA cycle, acts as a part of the mitochondrial respiratory system [4]. This enzyme has multiple subunits: α, β, and γ. In mammals, IDH3 consists of the αβ- and αγ-subunits, which together form α2βγ heterotetramers [5]. The α subunit is catalytic, the β subunit plays a structural role, and the γ subunit has the regulatory role of activating the holoenzyme. To show complete activity, IDH3 needs the assembly and synergistic function of αβ and αγ heterodimers [6]. The IDH3A gene (chromosome 15q25.1), IDH3B gene (chromosome 20p13), and IDH3G gene (chromosome Xq28) abundantly encode the α, β-and γ-subunits in the mitochondria-rich tissues, respectively [7.] Hartong et al. (2008) found that IDH3B is required for IDH3 activity in most tissues [8]. In another study, loss of the IDH3B in the mice led to isocitrate accumulation in the TCA cycle [9]. It is known that IDH3 is a disease-causing gene, and IDH3A and IDH3B subunits are significantly related to different diseases such as pseudo-coloboma, epileptic encephalopathy, and retinal degeneration [8,10]. The expression level of IDH subunits is significantly altered in human cancer types. The studies about IDH3's role in tumorigenesis are deficient and generally focus on IDH3A [11].

Cancer is derived from changes resulting from mutations in processes such as cell division and metabolism. The mutations cause cells to undergo atypical metabolic changes in energy production reactions [12]. According to GLOBOCAN estimations, 19.3 million new cancer cases were observed worldwide in 2020; unfortunately, 9.96 million patients died [13]. Recently, the diagnosis and treatment of cancer disease have been studied with increasing interest. In addition to protooncogenes and oncogenes, mutations in metabolic enzymes such as IDH and oncometabolite that cause transcriptional changes are also very important in the development and progression of cancer [14]. IDH1 is an atypical gene that acts as a tumor suppressor in cases where mutation causes loss of function and as an

oncogene where mutation causes gain of function [2]. In the literature, it is known that wild-type IDH isoforms (IDH1 and IDH2) have a role in the growth and progression of different tumors (breast, colon, esophageal, lung, and pancreas [15-19]. However, the expression levels of IDH3 subunits (IDH3A, IDH3B, and IDH3G) in different cancer types and the role of mutations in cancer progression have not been given adequate research attention.

It has been determined that there are more than 200 types of cancer [13]. Breast, lung, cervix, colon, and stomach cancer are the most common types of cancer that cause death in women. The six most common cancers that lead to death for men were lung, liver, stomach, prostate, esophageal, and colon cancer in 2022 [20]. Knowing the gene that is explicitly overexpressed for the cancer type is vital. The specific expression of IDH isoforms or subunits to cancer type and the expression level change as cancer progresses cause metabolic rearrangement. This situation can potentially be used to diagnose and treat various cancers [14]. In the literature, Iscan et al. (2021) investigated the expression profile of two isoforms of the TP73 gene in the most common human cancers by using publicly available data. They found that TAp73α is overexpressed in lung squamous and breast invasive carcinoma, whereas the second isoform DNp73α is downregulated in these cancer types [21]. In another study, Guneri-Sozeri and Erkek-Özhan (2022) studied one of the frequently mutated genes (ELF3) in bladder cancer. They provide essential information about gene expression changes in all cancer types in the TCGA Gen Atlas Project [22].

In the present study, the effect of expression changes of IDH3A, IDH3B, and IDH3G genes on cancer progression and survival probability was analyzed in the bladder, breast, colon, esophageal, kidney, lung, pancreas, prostate, and stomach cancer types by using bioinformatic tools such as TSVdb, cBioPortal for Cancer Genomics, and UALCAN. Then, the mutation frequency of these subunits and the impact of mutation status on cancer progression were investigated. All results showed that IDH3A, IDH3B, and IDH3G might be potential targets for diagnosing and treating colon, esophageal, lung, prostate, and stomach carcinomas.

Experimental Methods

The Level of IDH3A, IDH3B, IDH3G Expression in ten Major Cancer Tissues

The analysis of genomic data was performed in tumor and normal samples using TCGA data via the UALCAN web portal [23] to determine the level of IDH3A, IDH3B, and IDH3G expression based on samples types in the ten major cancers including bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), kidney renal clear cell carcinoma (KIRC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pancreas adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), and stomach adenocarcinoma (STAD). 4616 tumors and 458 non-tumor tissues were analyzed. Fig. 1 and Supplementary Fig. 2 was generated using the GraphPad Prism software, version 8.0 (GraphPad Software, USA). p values less than 0.05 were considered statistically significant and marked on the Figures.

IDH3A, IDH3B, and IDH3G expression based on individual cancer stages in the most common cancer types were also investigated in the TCGA dataset via the UALCAN web portal [23]. The “Expression” and “Individual Cancer Stages” functions were used, respectively, and the cancer stages were defined. All results were combined in Fig. 2.

Our previous report [24] described how to construct the heatmap used in Supplementary Fig. 1.

The Protein Level of IDH3A, IDH3B, IDH3G Expression in five Major Cancer Tissues

To investigate the protein level of IDH3A, IDH3B, and IDH3G expression, the "Proteomics" function was used in UALCAN web portal [23] for the five major cancer types including BRCA (Normal n = 18, Tumor n = 125), COAD (Normal n = 100, Tumor n =97), LUAD (Normal n = 111, Tumor n = 111), LUSC (Normal n = 102, Tumor n = 110), PAAD (Normal n = 74, Tumor n = 137) and Supplementary Fig. 2 was generated.

Investigation of Patient Survival Probability

To investigate the relationship between elevated expression of IDH3 subunits and clinical outcomes, the "Survival" function of the UALCAN web portal [23] was used. All results with a poor prognosis were combined in Fig. 3.

Analyze the Mutation Status

The alteration status of IDH3A, IDH3B, and IDH3G was examined on the cBioPortal for Cancer Genomics using TCGA PanCancer Atlas data of the ten major cancer types. Genetic alterations of the IDH3 subunits were analyzed for both mutations and copy numbers and finally, we achieved the cumulative alteration rates (Fig. 4; Supplementary Fig. 5). BLCA, BRCA, COAD, ESCA, KIRC, LUAD, LUSC, PAAD, PRAD, and STAD samples in the TCGA PanCancer Atlas were chosen as sample queries. In addition, the mutation types and frequencies were also determined using cBioPortal for Cancer Genomics web tool [25] (Supplementary Fig. 3; Supplementary Fig. 4).

Gene Expression Analysis According to Mutation Status

The effect of mutations on the gene expression of IDH3 subunits (IDH3A, IDH3B, IDH3G) was examined using the "Plot" function of the cBioPortal for Cancer Genomics web tool [25]. Boxplots were combined in Fig. 5.

Statistical Analysis:

Statistical analysis was carried out by GraphPad Prism 8.0 (GraphPad Software, CA, USA). One-way ANOVA was used to compare differences between two or more

groups, followed by Tukey's multiple comparison test. Asterisks (* for p<0.05, ** for p<0.01, *** for p<0.001, **** for p<0.0001) are used on the graph to indicate statistical significance.

Results and Discussion

Expression Status of IDH3 Subunits (IDH3A, IDH3B, and IDH3G) in the ten Major Types of Cancer

IDH enzymes that catalyze the rate-limiting step in the TCA cycle dramatically affect cell metabolism [4]. Wild-type IDH1/2 enzymes are overexpressed in some cancer tissues compared to healthy tissue to meet the increased energy and substrate needs of the cancer cell [15].

In the first step, the expression level of IDH3A, IDH3B, and IDH3G was investigated by using TCGA data via UALCAN web portal for the ten major cancer types, including BLCA, BRCA, COAD, ESCA, KIRC, LUAD, LUSC, PAAD, PRAD, and STAD. According to the TCGA data, the expression of IDH3A increased in esophageal, lung, and stomach tumor tissues. In contrast, it decreased in bladder, colon, kidney, and prostate tumor tissues compared to normal tissue (Fig. 1A). The bladder, esophageal, lung, and stomach cancer types displayed a significant upregulation of IDH3B. In contrast, breast and kidney cancers showed significant downregulation (Fig. 1B). IDH3G's expression level is elevated in all tumor tissues except kidney and pancreas tumors (Fig. 1C). The expression of all subunits in only cancer tissues was examined in the Heatmap constructed in GEPIA. Interestingly, the expression of IDH3A, IDH3B, and IDH3G showed specific differences and heterogeneity (Supplementary Fig. 1). In addition, the expression levels of all subunits only increased in esophageal, lung, and stomach tumor tissues and decreased in kidney cancer types according to control tissue (Fig. 1A-C). It was found that IDH3 subunit expressions (IDH3A, IDH3B, and IDH3G) were high in the different tumor types according to normal tissue, and their expression showed heterogeneity.

To corroborate the findings about mRNA expression, protein expression analysis was curated by using TCGA data via UALCAN web portal for the five major cancer types, including BRCA, COAD, LUAD, LUSC, and PAAD. According to the TCGA data, the protein expression of IDH3A decreased in breast, colon, and prostate primary tumor tissues compared to normal tissue (Supplementary Fig. 2A). The lung cancer displayed a significant upregulation of IDH3B protein level. In contrast, breast, colon, and prostate cancers showed significant downregulation (Supplementary Fig. 2B). IDH3G's protein level is decreased in breast, colon, and lung primary tumor tissues (Supplementary Fig. 2C). The IDH3A protein level in colon cancer and IDH3B protein level in the breast and lung showed similarity with the mRNA expression level.

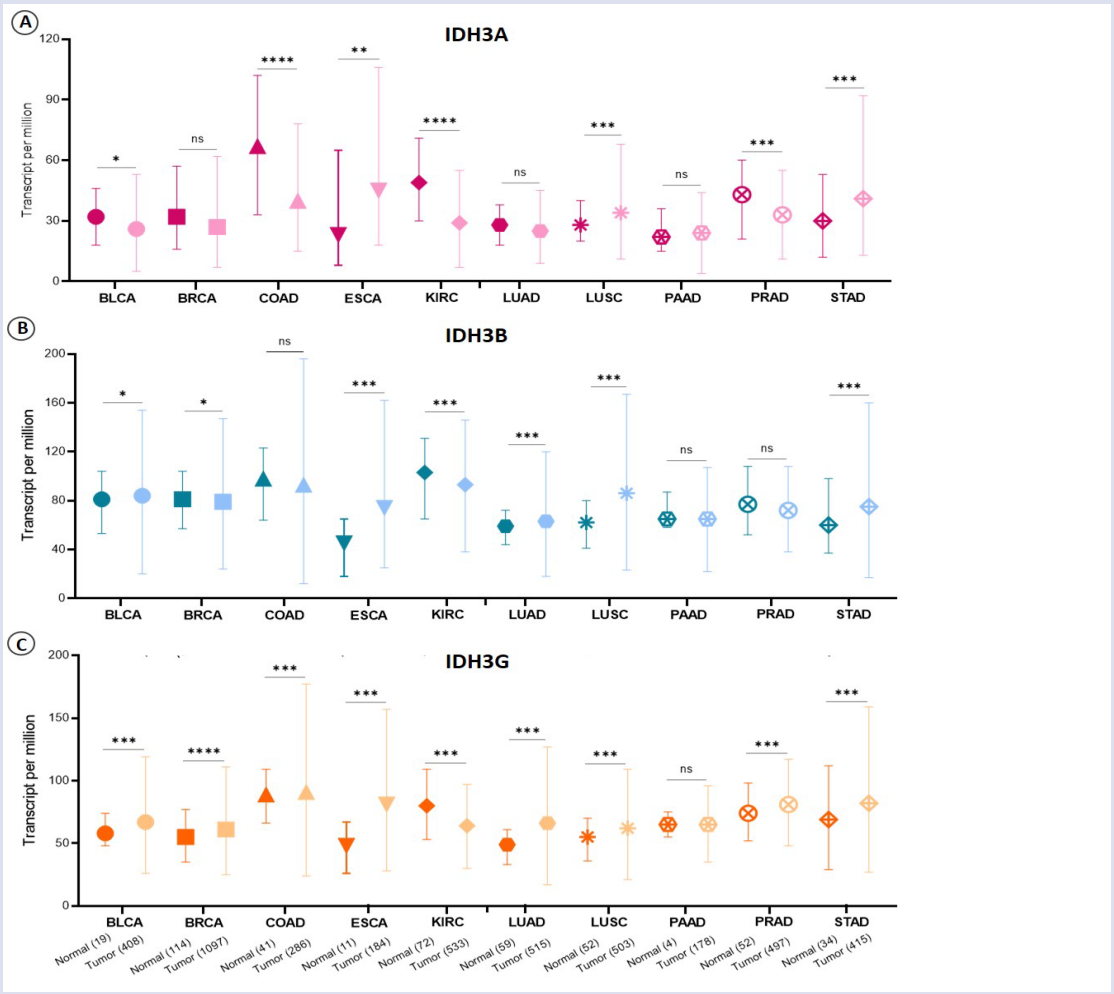

Figure 1. IDH3 subunits (IDH3A, IDH3B, IDH3G) gene expression in the ten most common cancer types.

Box-Whisker plot showing the mRNA expression levels of IDH3A (A), IDH3B (B), and IDH3G (C) in normal and primary tumor tissues of most common human cancers. The IDH3 subunits expressions were collected from UALCAN web portal for BLCA (Normal n = 19, Tumor n = 408), BRCA (Normal n =114, Tumor n = 1097 tumors), COAD (Normal n = 41, Tumor n =286), ESCA (Normal n = 11, Tumor n = 184), KIRC (Normal n = 72, Tumor n = 533), LUAD (Normal n = 59, Tumor n = 515), LUSC (Normal n = 52, Tumor n = 503), PAAD (Normal n = 4, Tumor n = 178), PRAD (Normal n = 52, Tumor n = 497), and STAD (Normal n = 34, Tumor n = 415) tumors. The cancer types are represented on the x-axis. The expression values (transcript per million) are represented on the y-axis. The mRNA levels of IDH3A, IDH3B, and IDH3G (A-C) were analyzed through the UALCAN web portal (http://ualcan.path.uab.edu/).

The responses to treatment are closely associated with tumor progression [26]. In the next step, we investigated how the expression of IDH3 subunits changes as cancer progresses in cancer types that displayed upregulation for this subunit. The expression level of IDH3A was highly elevated in esophageal, lung squamous cell, and stomach cancer types as the tumor progressed (Fig. 2A, B, C). There was no significant increase in the mRNA level of IDH3A in stage 4 of LUSC according to normal tissue (Fig. 2B). The expression level of IDH3B was increased in bladder, esophageal, lung, lung cell squamous, and stomach cancer types along with the tumor advancement (Fig. 2D, E, F, G, H). The mRNA level of IDH3B was not changed at stage 1 of BLCA according to normal tissue (Fig. 2M). The expression IDH3G was highly expressed in bladder, breast, colorectal, esophageal, lung, lung squamous, and stomach cancer types as the tumor progressed (Fig. 2I, J, K, L, M, N, O). There was no significant increase in IDH3G mRNA level at Stage 1 in BLCA and COAD (Fig. 2I, K) and Stage 4 in LUSC according to normal tissue (Fig. 2N).

The stage-specific results showed slight variations in the expression of IDH3 subunits based on individual cancer stages. On the other hand, all IDH3 subunits were highly expressed along with advancing tumor stages in different cancer types.

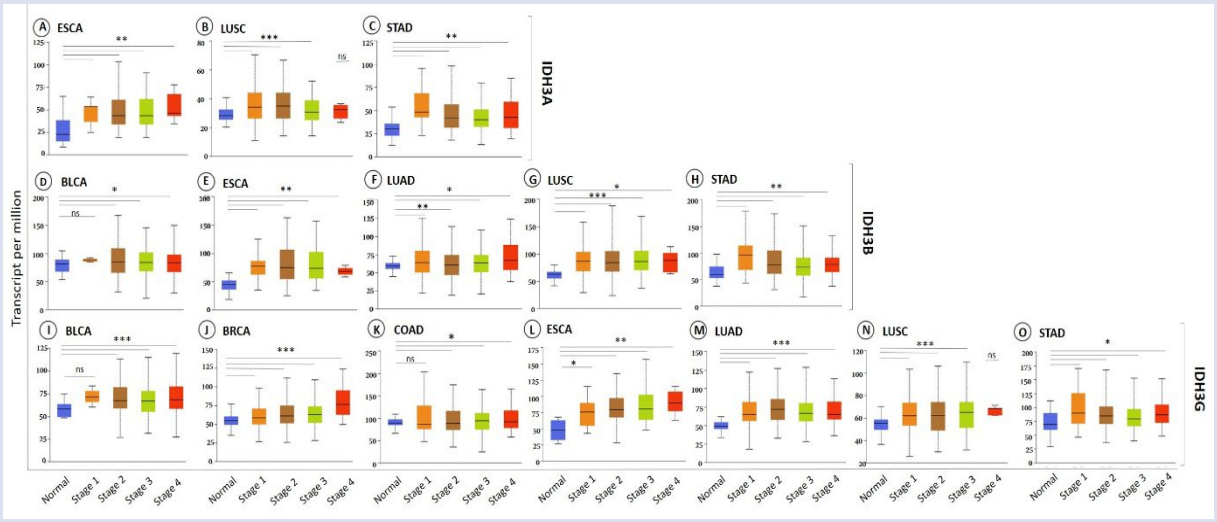

Figure 2. The expression level of IDH3 subunits based on individual cancer stages.

The mRNA level of IDH3A (A-C), IDH3B (D-H), and IDH3G (I-O) was analyzed through the UALCAN web tool. The number of samples for each stages are Normal n = 19, Stage 1 n = 2, Stage 2 n = 129, Stage 3 n = 137, Stage 4 n = 132 in BLCA, Normal n = 114, Stage 1 n = 183, Stage 2 n = 615, Stage 3 n = 247, Stage 4 n = 20 in BRCA, Normal n = 41, Stage 1 n = 45, Stage 2 n = 110, Stage 3 n = 80, Stage 4 n = 39 in COAD, Normal n = 11, Stage 1 n = 13, Stage 2 n = 78, Stage 3 n = 55, Stage 4 n = 9 in ESCA, Normal n = 59, Stage 1 n = 277, Stage 2 n = 125, Stage 3 n = 85, Stage 4 n = 28 in LUAD, Normal n = 52, Stage 1 n = 243, Stage 2 n = 157, Stage 3 n = 85, Stage 4 n = 7 in LUSC, Normal n = 34, Stage 1 n = 18, Stage 2 n = 123, Stage 3 n = 169, Stage 4 n = 41 in STAD. The cancer stages and normal tissue are represented on the x-axis. The expression values (transcript per million) are represented on the y-axis.

The Effect of IDH3 Subunit Expression Level on Patient Survival Probability

In order to be a biomarker, it is not sufficient to provide information about disease progression. This gene should also significantly affect the treatment response. Information about the relevance of the target gene to the cancer patient survival allows reaching points that cannot be achieved with the gene expression analysis results of the biomarker [26]. The next step is to examine how the elevated expression of IDH3 subunits affects survival probability as cancer progresses. It is known that high expression levels of wild-type IDH isoforms (IDH1, IDH2) in tumor tissues lead to poor prognosis [33]. Among the cancer types in which IDH3A is overexpressed, high IDH3A expression caused decreased survival probability in the bladder, esophagus, lung squamous cell, and stomach carcinoma (Fig. 3A, C, D, E). In addition, the high

expression of IDH3A caused reduced survival probability in breast cancer whose expression was not significantly altered (Fig. 3B). High IDH3B expression led to decreased survival probability in the bladder, lung squamous cell, and stomach carcinoma (Fig. 3F, H, J). The expression of IDH3B was increased in colon and prostate cancer types where the expression of IDH3B was not significantly altered (Fig. 3G, I). The elevated IDH3G expression led to a decreased survival probability in colon, prostate, and stomach cancers (Fig. 3K, L, M).

The clinical significance, critical in determining a biomarker, was examined using survival probability analysis. The results showed that IDH3A has clinical significance for the colon, IDH3B for the prostate, and IDH3G for liver cancer.

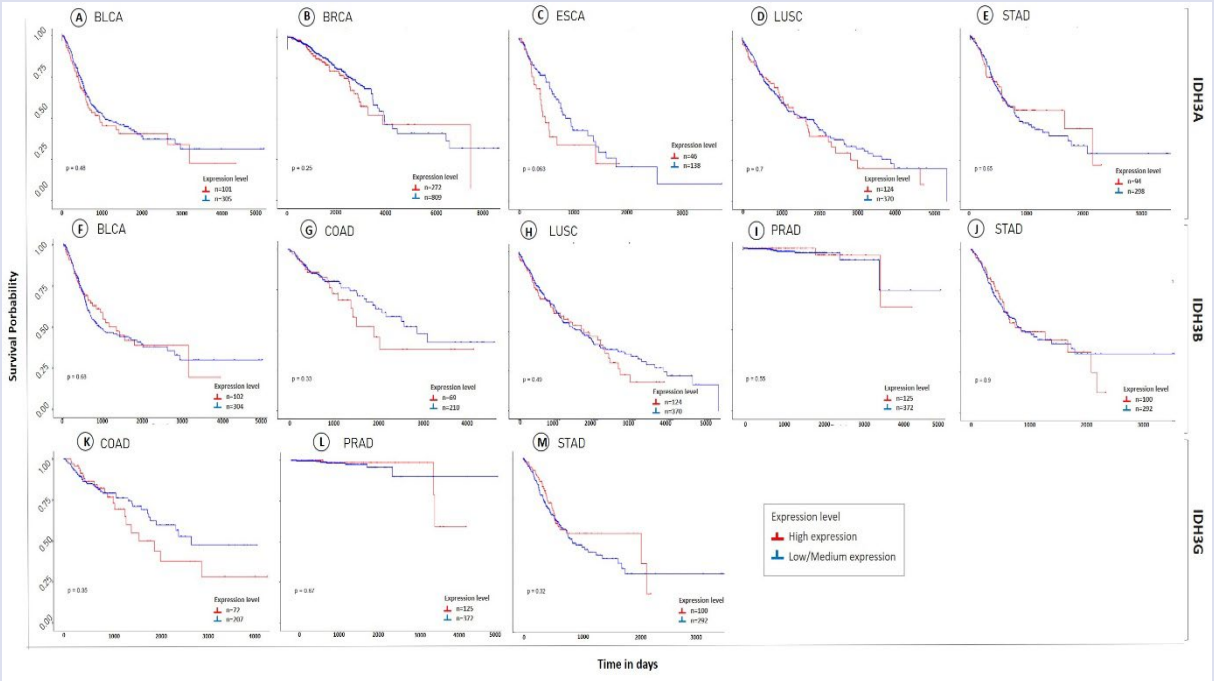

Figure 3. The effect of IDH3 subunits expressions on patient survival status.

Kaplan Meier plot showing the survival status of cancer patients in high and low gene expression of IDH3A (A-E), IDH3B (F-J), and IDH3G (K-M). The x-axis represents days and the y-axis represents the survival probability. The survival probability data were collected from the UALCAN web portal.

The Mutations in all IDH3 Subunits were low Frequency in the ten Major Cancer Types

Mutations in the metabolic enzymes have an essential role in the carcinogenesis. The effect of D-2-HG oncometabolite, which is produced with mutant IDH 1/2 enzymes, has mainly been studied in different types of cancer progression, such as breast and colon [15,17]. However, the mutation status in IDH3 subunits and the effect of these mutations on gene expression are not very well known in the ten major cancer types. The alteration status of IDH3A, IDH3B, and IDH3G was examined on PanCancer Atlas data of the ten major cancer types by cBioPortal for Cancer Genomics [25]. The analysis included both mutations and copy numbers in 4954 samples. Mutation types and locations on IDH3A, IDH3B, and IDH3G genes are presented in Supplementary Fig. 3 and Supplementary Fig. 4.

The cumulative alteration rates revealed low-frequency mutations in all IDH3 subunits (Fig. 4). Among all subunits, the highest mutation and copy number alterations were observed in the IDH3G, with a cumulative alteration rate of 5.9 % in STAD (Fig. 4C). Afterwards, cumulative alteration frequency was observed in the IDH3B in BRCA (2.46 %) (Fig. 4B). The lowest cumulative alteration frequency was observed in the IHD3A. The highest IDH3A mutation and copy number alterations were observed in LUSC (1.66 %) (Fig. 4A). Focusing on only mutation frequency, the highest mutation rate of the IDH3G gene is 1.5 %, and they mostly contain missense mutations (23) (Supplementary Fig. 3; Supplementary Fig. 4C; Supplementary Fig. 5C). The highest mutation rate of IDH3A and IDH3B genes are 1.1 % in COAD and ESCA, respectively (Supplementary Fig. 5A, B). There is no mutation in prostate cancer in all IDH3 subunits (Supplementary Fig. 5). Genetic alterations of mutated IDH3A and IDH3B are missense, truncating, and splice mutations (Supplementary Fig. 3). However, the frequencies differ for genes (Supplementary Fig. 4A, B). In addition, inframe mutations are also observed in mutated IDH3G (Supplementary Fig. 4C). The locations of mutations in all IDH3 subunits are also included in Supplementary Fig. 3, and the results showed that there have been mutational overlaps in all IDH3 subunits.

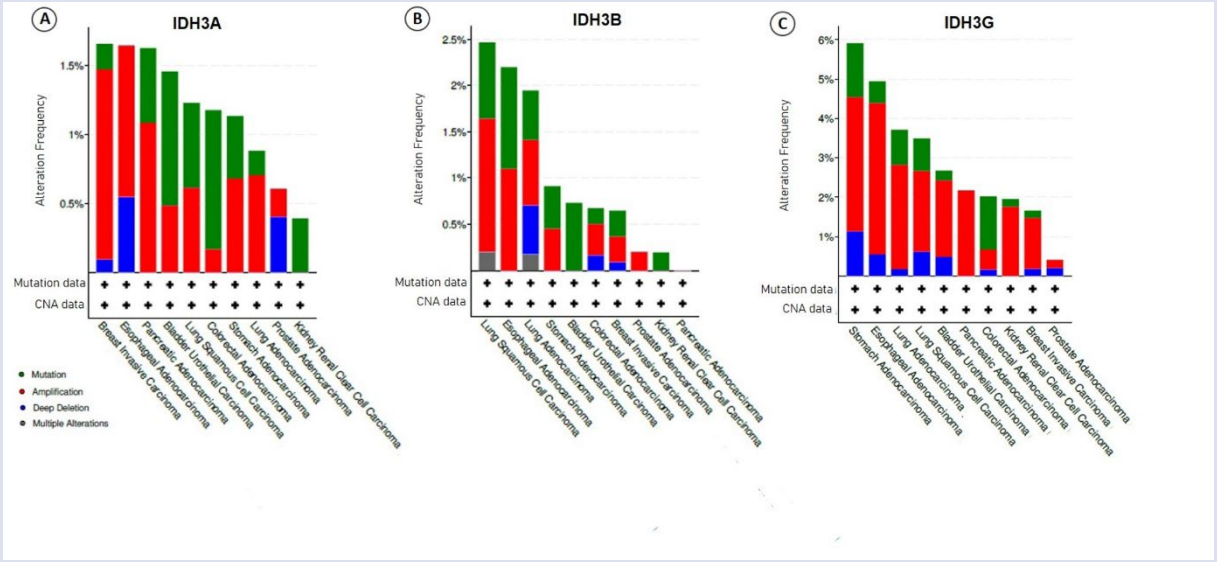

Figure 4. Alteration frequency of IDH3 subunits in TCGA PanCancer datasets.

The cumulative mutation rate of IDH3A (A), IDH3B (B), and IDH3G (C) were analyzed through the cBioPortal web tool by using samples (n=4954) in the TCGA PanCancer Atlas Study. In addition to mutation, amplification, deep deletion, and multiple alterations in IDH3 subunits are observed in the ten most common cancer types (A-C).

The Mutations in the IDH3 Subunits Directly Affect the Gene Expression

After investigating the mutation status of IDH3 subunits, the effect of mutations on the gene expression of IDH3 subunits was examined through the cBioPortal for Cancer Genomics [25]. Mutations in the IDH3A caused a moderate decrease in gene expression in BLCA (Fig. 5A). The IDH3A expression was highly decreased after mutations in KIRC, LUSC, and STAD (Fig. 5D, F, H) and highly increased in BRCA, COAD, LUAD, and PAAD (Fig. 5B, C, E, G). Mutations in the IDH3B and IDH3G generally caused a decrease in gene expression in all cancer types. The IDH3B expression was moderately decreased after mutations only in the COAD (Fig. 5K) and highly decreased in the BLCA, ESCA, KIRC, LUAD, and LUSC (Fig. 5I, L, M, N, O). It was moderately and highly increased in BRCA and STAD after mutation, respectively (Fig. 5J, P). Mutations in the IDH3G did not affect the expression of the IDH3G gene in BLCA (Fig. 5R). Mutations in the IDH3A caused a moderate decrease in gene expression in BRCA, KIRC, and LUAD (Fig. 5S, V, Y), and it caused a slight decrease in COAD, ESCA, LUSC, and STAD carcinoma (Fig. 5T, U, Z, X).

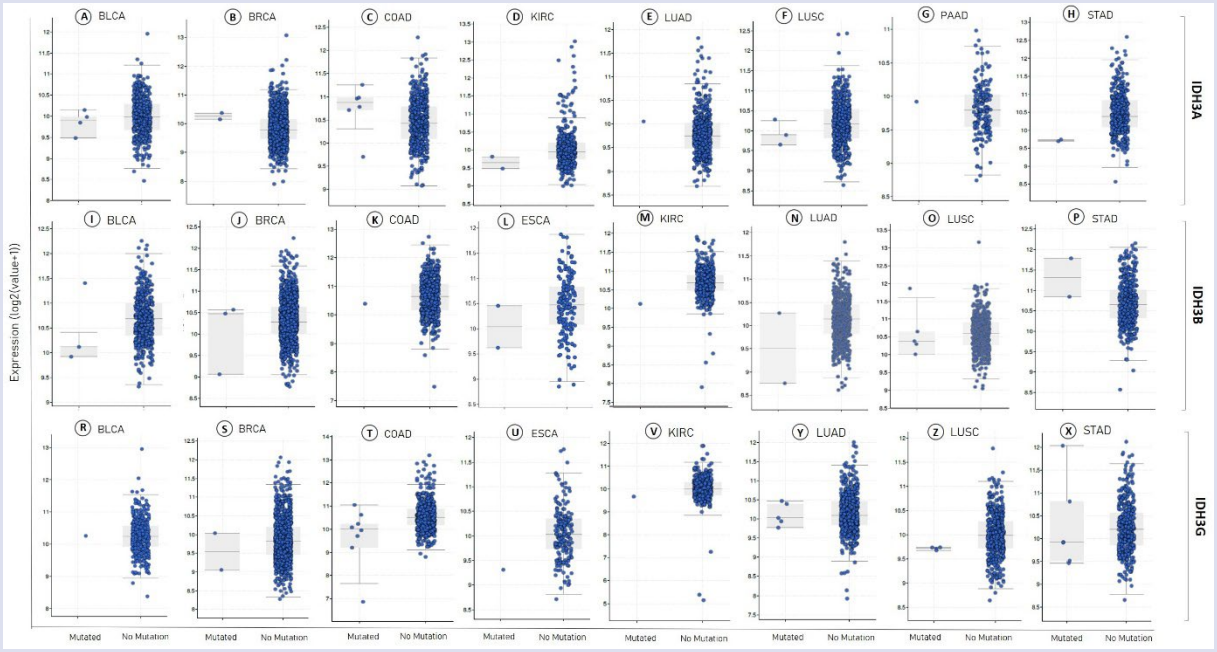

Figure 5. The IDH3A, IDH3B, and IDH3G gene mutations directly affect gene expression.

Box plots exhibit the expression level of IDH3A (A-H), IDH3B (I-P), and IDH3G (R-X) in IDH3A (A-H), IDH3B (I-P), and IDH3G (R-X) mutant and non-mutant in the bladder, breast, colorectal, esophageal, kidney, lung, pancreas, prostate, and stomach cancers.

The most up-to-date sources should be used in the discussion

Discussion

The IDH enzyme was first isolated in 1972 from *E.coli* [27], and later studies focused on its potential catabolic role in the TCA cycle [1]. It is essential for the metabolism of normal mammalian cells because it is located at the key branching point in carbohydrate metabolism and carries out the reaction of the rate-limiting step [14, 41]. All IDH-mediated reactions that result from α-KG, NADH, NADPH, or isocitrate production are of great moment for tumor cells. IDH serves a diverse biological function, which role in cellular defence against oxidative damage, determines the $NADP^+$/NADPH ratio, and participates in lipid metabolism [28, 29]. In addition to their roles, IDH1/2 isoforms promote tumor proliferation in different cancer types [14-19]. However, the studies about IDH3 are limited [30-31], and no studies have investigated the effect of gene expression profiles in the IDH3 subunits on the various cancer progressions and the relation between mutation frequencies of IDH3 subunits and cancer progression.

As known, a biomarker gene should be associated with disease progression and treatment response [26]. It was found that the expression level of IDH3A, IDH3B, and IDH3G is higher in esophageal, lung squamous cell, and colon tumors according to normal tissues, respectively (Fig. 1). The expression level of IDH3 subunits increased as the bladder, breast, colon, esophageal, lung, and stomach cancers progressed (Fig. 2). High IDH3 subunit expression led to decreased survival probability in colon, esophageal, lung, prostate, and stomach carcinomas (Fig. 3). All results are combined in Supplementary Fig. 6. IDH3A showed higher gene expression and lower survival probability for esophageal, lung, and stomach (Supplementary Fig. 6 A, B, C), IDH3B showed higher gene expression and lower survival probability for lung and stomach (Supplementary Fig. 6 D, E), and IDH3G showed higher gene expression and lower survival probability for colon, prostate, and stomach cancers (Supplementary Fig. 6 F, G, H). All the data indicate that IDH subunits are predictive value for cancer detection.

Investigating how the expression of IDH isoforms changes according to cancer stages is essential for pathophysiological studies. Obtaining cancer stage-specific data has clinical implications in various aspects, such as early diagnosis of cancer and improving personalized treatment [32]. In our study, target gene expression levels were investigated in the different cancer stages. We found that the expression of IDH3A, IDH3B, and IDH3G interestingly showed specific differences and heterogeneity in the ten major types of cancer (Fig. 1; Fig. 2; Supplementary Fig. 1). Then, mRNA levels of IDH3A, IDH3B, and IDH3G based on tumor grade were examined, and the data showed a direct relationship between the expression of IDH subunits and the progression of cancer (Fig. 2). In our study, the gene expression of three subunits of IDH3 was also changed in the same tumor types. Another study found that IDH3A is associated with tumorigenesis of hepatocellular carcinoma and glioblastoma [30-31]. However, no study is related to the expression level of IDH3 subunits in the different cancer types. We have examined the effect of elevated IDH3 expression on survival probability, and it was found that high expression of IDH3 subunits in the bladder, colon, esophageal, lung, prostate, and stomach tumor tissues leads to a poor prognosis (Fig. 3), similar to a previous study [11].

Wild-type IDH1 and IDH2 are essential enzymes that have a role in the growth and progression of different kinds of tumors. In addition to wild-type isoforms, the mutant IDH1/2 enzymes perform oncogenic activities [33]. Some tumor types, the mutation frequencies of the IDH1 and IDH2 genes are very high [34]. In contrast to its isoforms IDH1 and IDH2, the mutation in the IDH3 does not lead to 2-hydroxyglutarate production [14]. Our study found that cumulative alteration rates were low frequency in all IDH3 subunits (Fig. 4). However, it has been dramatically affecting gene expression in some cancer types such as the bladder (Fig. 5I), breast (Fig. 5B), colon (Fig. 5C), esophageal (Fig. 5L, U), kidney (Fig. 5M), lung (Fig. 5E, F, N), and stomach (Fig. 5H, P). To comprehend the spatial expression profiles of the subunits and their role in carcinogenesis, the correlation between the mutation frequencies of IDH3 subunits and cancer progression is examined and results were combined in Supplementary Fig. 7. The highest mutation frequency of IDH3A was observed in breast invasive carcinoma and esophageal adenocarcinoma, and a relationship was observed between mutation frequency and cancer progression (Supplementary Fig. 7A). The relation between high mutation frequency of IDH3B and cancer progression was observed in lung squamous cell carcinoma and esophageal adenocarcinoma (Supplementary Fig. 7B). The relation between high mutation frequency of IDH3G and cancer progression was observed in stomach and esophageal adenocarcinoma (Supplementary Fig. 7C). In the literature, most studies related to mutations in IDH3 subunits focus on diseases such as pseudo-coloboma, epileptic encephalopathy, and retinitis pigmentosa [9-10, 35-36]. Krell et al. (2011) found that IDH3 mutations were not glioblastoma-associated in cancer-related studies [37]. However, our data showed that there may be a relation between the mutation of IDH3 subunits and different kinds of cancer progression.

In the present study, cancer-specific IDH3 subunits were determined for the ten major cancer types, and we found target cancer types for IDH3A, IDH3B, and IDH3G. Until now, studies on the role of IDH3 subunits in tumor development and progression are limited to a few tumors. Zeng et al. (2015) find that IDH3A can be a therapeutic

target for breast and lung cancers [11]. Wu et al. (2019) studied the role of IDH3B in esophageal cell proliferation *in vitro* and *in vivo* [38]. The results demonstrated that overexpression of IDH3B contributes to esophageal cell proliferation and is related to poor prognosis in patients with ESCC. In another study, it was found that the level of IDH3G was higher in gastric cancer tumor tissue than in normal tissue [39]. Knockdown of IDH3G leads to the sensitization of gastric cancer cells to drugs (epirubicin, cisplatin) [40]. In another way, some inhibitors (Vorasidenib and ivosidenib) are using for mutant IDH1 and IDH2 enzymes to combat with different type of cancers [42, 43].

In conclusion, our data showed that there might be a relation between the mutation of IDH3 subunits and breast, esophageal, lung, and stomach cancer progression. The results demonstrated that strategies targeting IDH3A and IDH3B for lung and stomach cancers, IDH3A for esophageal cancer, and IDH3G for colon, prostate, and stomach cancers might greatly benefit cancer management. However, these results were obtained only as a result of bioinformatics analyses and in vitro experiments were not performed. It is planned to study the IDH3 subunits in their related cancer types to find the effect of mutation on cancer progression by *in vitro* methods.

Conflict of interest

There are no conflict of interest in this work.

Acknowledge

The authors reported that there is no funding associated with the work.

Ethical Approval Statement

Any human subjects and animals are not used in this study.

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