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Gene expression changes of isocitrate dehydrogenase 1 and isocitrate dehydrogenase 2 affect carcinogenesis and survival probability

İzositrat dehidrojenaz 1 ve izositrat dehidrojenaz 2 genlerinin gen ekspresyon değişiklikleri karsinojenezi ve hayatta kalma olasılığını etkiler

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Abstract

Isocitrate dehydrogenase (IDH) is an essential metabolic enzyme in the regulation of cellular metabolism. IDH gene encodes three protein isoforms, IDH1, IDH2, and IDH3, and the expression level of isoforms is altered in human cancer types. Examining the gene expression level of IDH is a therapeutic advantage that could help find a new target to use in cancer metabolism. The present study aimed to explore the gene expression level of IDH1 and IDH2 isoforms in the ten common human cancers using bioinformatic tools. In addition, the effect of gene expression changes on IDH1 and IDH2 on carcinogenesis and survival probability was examined in publicly available data deposited in the TCGA database. The results showed that the expression of IDH isoforms showed tissue-specific differences. IDH1 expression increased in esophageal and lung squamous cell carcinoma and lung and stomach adenocarcinoma tumors. Bladder urothelial, breast urothelial, and lung squamous cell carcinoma, colon, and lung adenocarcinoma displayed a significant upregulation of IDH2 expression. There was a direct relationship between the expression of IDH isoforms and the progression of various cancer types. High IDH1 expression led to decreased survival probability in esophageal carcinoma, lung, and stomach adenocarcinoma. Elevated IDH2 expression level led to decreased survival probability in bladder urothelial, breast urothelial, and lung squamous cell carcinoma and colon adenocarcinoma. In conclusion, all data showed that IDH1 could be a biomarker for esophageal carcinoma, lung and stomach adenocarcinoma, and IDH2 for bladder urothelial, breast urothelial, and lung squamous cell carcinoma, and colon adenocarcinoma.

Keywords: Bioinformatic, Carcinogenesis, Isocitrate dehydrogenase 1, Isocitrate dehydrogenase 2

Öz

İzositrat dehidrojenaz (IDH), hücre metabolizmasının düzenlenmesinde önemli bir metabolik enzimdir. IDH geni, IDH1, IDH2 ve IDH3 olmak üzere üç protein izoformunu kodlar ve izoformların ekspresyon seviyesi, insan kanser türlerinde değişiklik gösterir. IDH'nin gen ekspresyon seviyesinin incelenmesi, kanser metabolizması alanında kullanılacak yeni bir hedef bulmaya yardımcı olabilecek terapötik bir avantajdır. Bu çalışmanın amacı, biyoinformatik araçlar kullanarak on yaygın insan kanserinde IDH1 ve IDH2 izoformlarının gen ekspresyon seviyesini araştırmaktır. Ek olarak, IDH1 ve IDH2'nin gen ekspresyon seviyesindeki değişikliklerin karsinogenez ve hayatta kalma olasılığı üzerindeki etkisi TCGA veri tabanında depolanan halka açık veriler üzerinde incelenmiştir. Elde edilen sonuçlar, IDH izoformlarının ekspresyonunun dokuya özgü farklılıklar ve heterojen özellik sergilediğini gösterdi. IDH1 ekspresyonu özofagus ve akciğer skuamöz hücreli karsinom ile akciğer ve mide adenokarsinomu tümörlerinde arttı. Mesane ürotelyal, meme ürotelyal ve akciğer skuamöz hücreli karsinomu ve kolon ve akciğer adenokarsinomu, IDH2 ekspresyonunda önemli bir artış sergiledi. IDH izoformlarının ekspresyonu ile çeşitli kanser türlerinin ilerlemesi arasında doğrudan bir ilişki olduğu bulundu. Yüksek IDH1 ekspresyonu, özofagus karsinomu, akciğer ve mide adenokarsinomunda hayatta kalma olasılığının azalmasına yol açtı. Yüksek IDH2 ekspresyon seviyesi, mesane ürotelyal, meme ürotelyal ve akciğer skuamöz hücreli karsinom ve kolon adenokarsinomunda hayatta kalma olasılığının azalmasına yol açtı. Sonuç olarak, tüm veriler IDH1'in özofagus karsinomu, akciğer ve mide adenokarsinomu için ve IDH2'nin mesane ürotelyal, meme ürotelyal ve akciğer skuamöz hücreli karsinom ve kolon adenokarsinomu için bir biyobelirteç olabileceğini gösterdi.

***Anahtar kelimeler**: Biyoinformatik, Karsinogenez, İzositrat dehidrojenaz 1, İzositrat dehidrojenaz 2*

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1. Introduction

Cellular energy changes and metabolic disorders are some of the characteristic biological features of cancer cells (Hanahan, 2022). The TCA cycle is widely used for energy production and macromolecule synthesis in cancer cells, where metabolic rearrangement occurs for rapid proliferation and survival (Anderson et al., 2018). The tricarboxylic acid (TCA) cycle is crucial for cell energy production, as well as for producing precursors for biosynthetic pathways and maintaining redox balance. Some metabolites involved in the TCA cycle connect the mitochondria and the nucleus. These metabolites are crucial in cancer development and progression as they affect various cellular activities, including metabolism and signaling (Martínez-Reyes & Chandel, 2020). Mutation of some enzymes roles in the TCA cycle, such as *fumarate hydratase* (*FH*), *isocitrate dehydrogenase* (*IDH*), and *succinate dehydrogenase* (*SDH*), affects the integrity of the TCA cycle because it leads to irregularity in the levels of some metabolites (Eniafe & Jiang, 2021). In addition, changes in mRNA or protein expression levels of these enzymes also affect metabolite levels (Koseki et al., 2015).

IDH is one of the key regulator enzymes that has a central role in the TCA cycle (D'Adamo & Haft, 1965). Three IDH isoforms (*IDH1*, *IDH2*, and *IDH3*) have been identified within eukaryotic cells. Subcellular localization, using cofactors and showing holoenzyme organization of IDH isoforms are different. $NADP^+$-dependent *IDH1* and *IDH2* enzymes are homodimeric, and the reactions catalyzed by IDH1 and IDH2 are reversible. *IDH1* carries out the reductive carboxylation of α-Ketoglutarate (α-KG) to isocitrate by NADPH and CO_2 in the cytoplasm, whereas *IDH2* catalyzes the same reaction in the mitochondria (Dalziel, 1980; Ramachandran & Colman, 1980). In addition, *IDH2* and *IDH1* are isozymes with significant sequence identity (70% similarity in humans) (Gabriel et al., 1986; Pollard & Ratcliffe, 2009). *IDH1* roles the generation of nonmitochondrial NADPH and plays a part in promoting the activity of several cytoplasmic and nuclear dioxygenases (Pollard & Ratcliffe, 2009). On the other hand, the NAD^+-dependent *IDH3* is a heterotetrameric enzyme that catalyzes the irreversible reaction in the mitochondrial matrix (Ramachandran & Colman, 1980; Gabriel et al., 1986). Furthermore, *IDH3* catalyzes one of the rate-limiting steps in the TCA cycle and acts as a part of the mitochondrial respiratory system (Barnes et al., 1971). The oxidative decarboxylation and reductive carboxylation catalyzed by *IDH* have an essential impact on lipogenesis, redox homeostasis, and cell proliferation (Koh et al., 2004; Wise et al., 2011; Jiang et al., 2016; Du et al., 2016). For this reason, studies about the discovery of a biomarker in cancer have focused on *IDH* in recent years (Atalay et al., 2023; Aljohani et al., 2020; Zarei et al., 2023; Chen et al., 2017; Kong et al., 2018; Li et al., 2023).

Cancer is a significant disease responsible for a large number of deaths worldwide It is known that there are more than 100 types of cancer. Breast, lung, cervix, colon, liver, prostate, esophageal, and stomach cancer are the most common cancers that cause death for men and women in 2022. The incidence of cancer is increasing day by day. The reported data showed that cancer led to approximately 10 million deaths in 2020 (Chhikara et al., 2023; Siegel et al., 2023). One of the most effective ways to prevent or reduce the number of deaths caused by cancer is early detection. In recent years, scientists focused on cancer metabolism. Especially some metabolic enzymes (*IDH, SDH, FH*) that cause transcriptional changes closely related to tumor development were studied, and their expression level was important in finding new biomarkers (Nadhan et al., 2023). Atalay and Kayali found that the mRNA levels of the *IDH1* and *IDH2* genes were significantly altered in primary and metastatic cell lines compared to colonic epithelial cell lines (Atalay & Kayalı, 2022). However, the effect of gene expression changes of *IDH1* and *IDH2* genes on carcinogenesis and survival probability has not been studied in the most common cancer types. The first aim of the present study was to explore the gene expression level of *IDH1* and *IDH2* isoforms in the ten common human cancers including BLCA (Bladder Urothelial Carcinoma), BRCA (Breast Invasive Carcinoma), COAD (Colon adenocarcinoma), ESCA (Esophageal carcinoma), KIRC (kidney renal clear cell carcinoma), LUAD (Lung Adenocarcinoma), LUSC (Lung Squamous Cell Carcinoma), PAAD (Pancreas adenocarcinoma), PRAD (Prostate Adenocarcinoma), and STAD (Stomach adenocarcinoma) by using bioinformatic tools. In the second part, the effect of gene expression changes of *IDH1* and *IDH2* on carcinogenesis and survival probability was examined in publicly available data. Our data indicated that the expression of *IDH* isoforms showed tissue-specific differences and heterogeneity. High *IDH1* and *IDH2* expression cause decreased survival probability in bladder urothelial, breast urothelial, esophageal, and lung squamous cell carcinoma, and lung, stomach, and colon adenocarcinoma.

2. Equipment and method

2.1. The level of *IDH1* and *IDH2* expression in ten common cancer tissues

The Cancer Genome Atlas (TCGA) data in tumor (4616) and normal (458) samples were analyzed via the UALCAN web portal (Chandrashekar et al., 2017). to determine the *IDH1* and *IDH2* expression levels in the 10 most common 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). Figure 1 was generated using the GraphPad Prism software, version 8.0 (GraphPad Software, USA), and p values less than 0.05 were considered statistically significant.

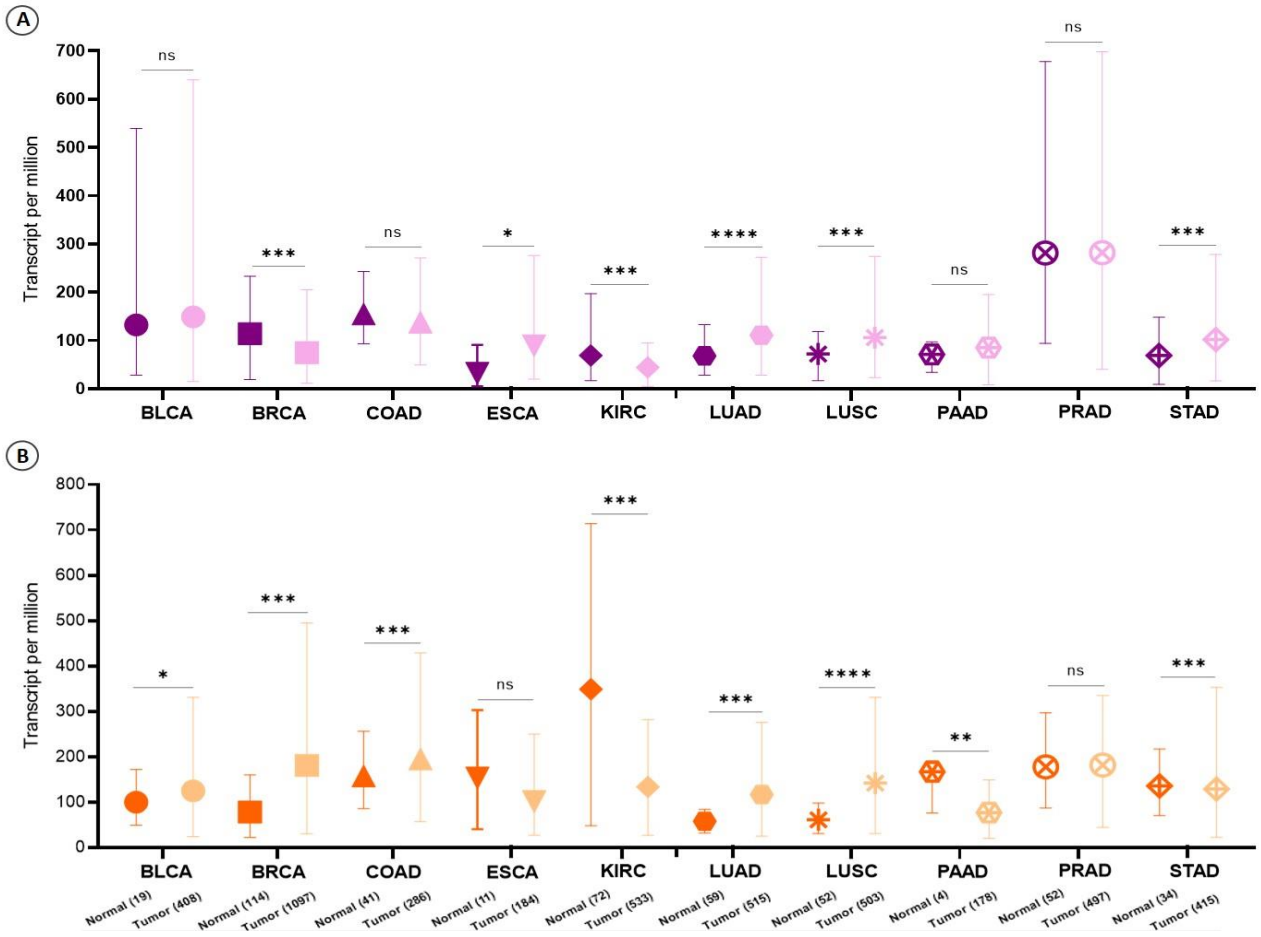

Figure 1. *IDH* isoforms (*IDH1* and *IDH2*) expression in the ten common human cancers. Box-Whisker plot showing the mRNA expression levels of *IDH1* (A) and *IDH2* (B) in normal and primary tumor tissues of most common human cancers. The *IDH* isoform expressions were collected from the UALCAN web portal. The cancer types are represented on the x-axis. The expression values (transcript per million) are represented on the y-axis. *IDH1* and *IDH2* (A-B) mRNA levels were analyzed through the UALCAN web portal (https://ualcan.path.uab.edu/) *p <0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: non-significant.

We constructed the Heatmap (Figure 2) which includes the expression of all subunits only in tumor data from the TCGA project using the "Multiple Gene Comparison" function in GEPIA (Tang et al., 2019).The level of *IDH1* and *IDH2* expressions based on individual cancer stages in the most common cancer types was also investigated in the TCGA dataset via the UALCAN web portal (Chandrashekar et al., 2017). All results were combined in Figure 3. The normal and tumor tissue numbers *mentioned* in Figure 1 and Figure 3 are given in Table 1.

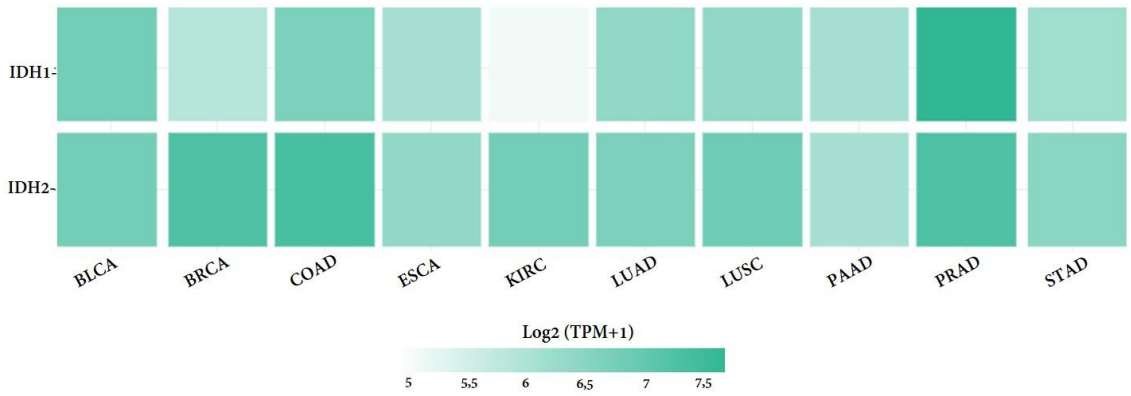

Figure 2. mRNA levels of two *IDH* isoforms (*IDH1* and *IDH2*) in most common tumor tissues. Heatmap showing the *IDH1* and *IDH2* mRNA levels in BLCA, BRCA, COAD, ESCA, KIRC, LUSC, LUAD, PAAD, PRAD, and STAD tumor tissues. The figure was generated by the GEPIA web tool (https://gepia.cancer-pku.cn/). The vertical axis represents *IDH* isoforms, and the horizontal axis represents cancer types.

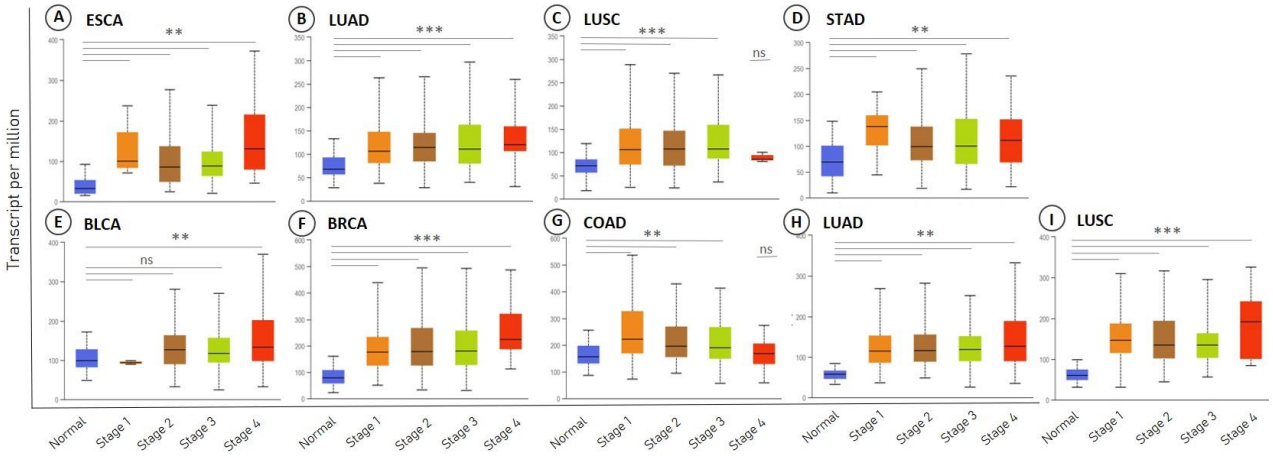

Figure 3. The expressions of *IDH1* and *IDH2* in different cancer stages. IDH1 (A-D) and IDH2 (E-I) mRNA levels were analyzed through the UALCAN web tool. The cancer stages and normal tissue are represented on the x-axis. The expression values (transcript per million) are represented on the y-axis **p<0.01, ***p<0.001, ns: non-significant.

Table 1. The normal and tumor tissue numbers for ten common human cancer

Tumor type	Tissue number	
	Normal	**Tumor**
BLCA	19	408
BRCA	114	1097
COAD	41	286
ESCA	11	184
KIRC	72	533
LUAD	59	515
LUSC	52	503
PAAD	4	178
PRAD	52	497
STAD	34	415

2.2. Investigation of patient survival probability

The "Survival" function was used in the UALCAN web portal (Chandrashekar et al., 2017) for the ten most common cancer types, and all results that contain a poor prognosis were combined in Figure 4. Therefore, the effect of elevated *IDH1* and *IDH2* expression on the survival probability was investigated.

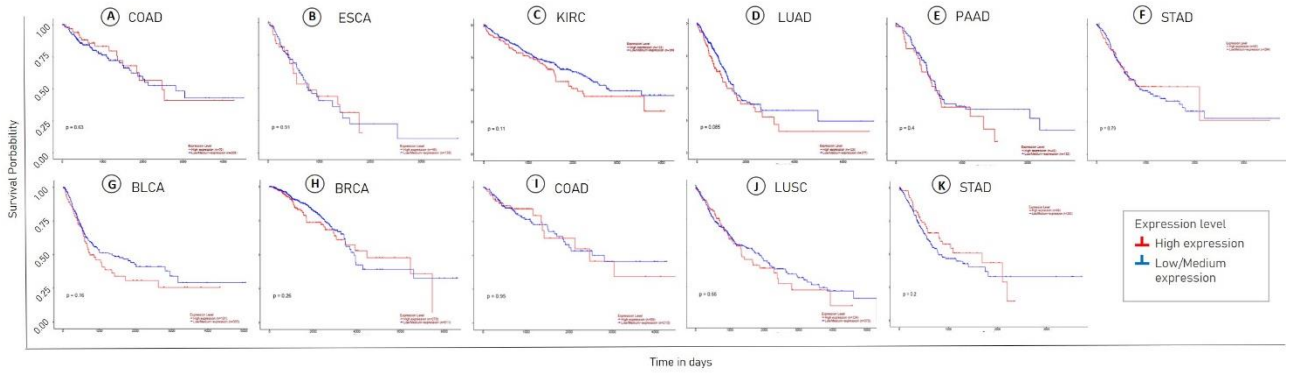

Figure 4. The effect of *IDH* isoform expressions on patient survival status. Kaplan-Meier plot shows cancer patients' survival status in high and low expression of *IDH1* (A-F) and *IDH2* (G-K). The survival probability data were collected from the UALCAN web portal. The x-axis represents days, and the y-axis represents the survival probability.

3. Results

3.1. Expression status of *IDH* isoforms *(IDH1* and *IDH2*) in ten common human cancer types

In the first step, the expression of *IDH1* and *IDH2* was examined by using The Cancer Genome Atlas (TCGA) data via the UALCAN web portal for ten common human cancers, including bladder urothelial, breast urothelial, esophageal, kidney renal clear cell, lung squamous cell carcinoma, and colon, lung, pancreas, prostate, and stomach adenocarcinoma. Figure 1 was generated using the GraphPad Prism software, version 8.0 (GraphPad Software, USA), and p values less than 0.05 were considered statistically significant.

According to the TCGA data, the expression of *IDH1* isoform increased in esophageal carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, and stomach adenocarcinoma tumor tissues. In contrast, it decreased in breast urothelial carcinoma and kidney renal clear cell carcinoma compared to normal tissue (Figure 1A). Bladder urothelial, breast urothelial, and lung squamous cell carcinoma and colon and lung adenocarcinoma displayed a significant upregulation of *IDH2* expression, whereas kidney renal clear cell carcinoma and pancreas and stomach adenocarcinoma displayed a significant downregulation (Figure 1B). The expression of multiple *IDH* isoforms in all cancer tissues was examined in the Heatmap constructed in GEPIA. It was found that the expression of *IDH* isoforms showed tissue-specific differences and heterogeneity (Figure 2). In addition, expression of *IDH1* and *IDH2* isoforms was decreased only in kidney cancer types, whereas they only increased in lung squamous cell cancer types (Figure 1).

In the next step, we investigated how the expression of *IDH* isoforms changes as cancer progresses in cancer types that displayed upregulation for this isoform. The *IDH1* was highly expressed in esophageal and lung squamous cell carcinoma and lung and stomach adenocarcinoma as the tumor progressed (Figure 3A-D). Only in lung squamous cell carcinoma, there were no significant changes in *IDH1* expression at stage 4 compared to normal tissue (Figure 3C). The expression of *IDH2* was increased in breast urothelial and lung squamous cell carcinoma and colon and lung adenocarcinoma, along with the advancement of the tumor stage (Figure 3E-I). The bladder urothelial carcinoma displayed significantly high *IDH2* expression only in Stage 4 (Figure 3E), and there was no significant increase in *IDH2* mRNA level at Stage 4 in colon adenocarcinoma (Figure 3G). The results show a direct relationship between the expression of *IDH* isoforms (*IDH1* and *IDH2*) and cancer progression.

3.2. The effect of *IDH* isoform expression on patient survival probability

The next step is to examine how the increased expression of *IDH* isoforms (*IDH1/2*) affects survival probability. Among cancer types in which the *IDH1* isoform is overexpressed, high *IDH1* expression led to decreased survival status of esophageal carcinoma, lung, and stomach adenocarcinoma patients (Figure 4B, D, G). The high *IDH1* expression caused reduced survival probability in colon adenocarcinoma and kidney renal cell carcinoma, whose expression was decreased (Figure 4A, C). In addition, elevated *IDH1* expression caused survival probability in pancreas and prostate adenocarcinoma, whose expression was not significantly altered (Figure 4E, F). High *IDH2* expression decreased survival probability in bladder urothelial, breast urothelial, and lung squamous cell carcinoma and colon adenocarcinoma (Figure 4H, I, J, K). In addition, the high *IDH2* expression caused reduced survival probability in stomach adenocarcinoma whose expression decreased (Figure 4L).

4. Discussion

Cells undergoing metabolic and behavioral changes during carcinogenesis exhibit metabolic changes to obtain essential nutrients from the environment and use these nutrients to form new biomass (Hanahan, 2022). The TCA cycle intermediates are essential for cells because they cause epigenetic changes that affect the development or progression of cancer. In addition, the cofactors (NAD(P)H and FADH2) produced in the TCA cycle are used in the OXPHOS to get high ATP production for cancer cells which rapidly proliferate (Anderson et al., 2018). Examining gene expressions of enzymes directly involved in producing these metabolites may provide new strategies to prevent tumor growth. It is known that the elevated or reduced expression level of *IDH1* or *IDH2* has a significant effect on the metabolism of different cancers (He et al., 2022). In the literature, it was found that the *IDH1/2* expression was overexpressed in lung and breast cancer, and it was downregulated in kidney cancer (Al-Amodi et al., 2018; Li et al., 2018; Peng et al., 2019), similar to our results (Figure 1). In our study, ten common human cancer types were evaluated, and it was found that the expression of *IDH* isoforms showed tissue-specific differences and heterogeneity (Figure 1 and Figure 2). Next, the expression of *IDH* isoforms was investigated as the cancer progressed. The results show that there is a direct relationship between the expression of *IDH* isoforms (*IDH1* or *IDH2*) and the progression of breast urothelial, esophageal, and lung squamous cell carcinoma and colon, lung, and stomach adenocarcinoma (Figure 3E-I).

The studies about *IDH* were focused on its potential catabolic role in the TCA cycle (D'Adamo & Haft, 1965). Converting isocitrate into α-KG by *IDH* is a crucial control point for the TCA cycle. All *IDH*-mediated reactions *that* result from α-KG, NADH, NADPH, or isocitrate production have an essential impact on lipogenesis, redox homeostasis, and cell proliferation (Koh et al., 2004; Wise et al., 2011; Jiang et al., 2016; Du et al., 2016). α-KG is an important intermediate in other metabolic processes and acts as a cofactor of enzymes role in epigenetic modification (Matilainen et al., 2017). Studies show that the effect of *IDH* on lipid synthesis is associated with cancer development. Rapidly proliferating cancer cells need new membranes; fatty acids are vital to forming new membrane processes. NADPH and citrate produced from reductive carboxylation carried out by *IDH* participate in the fatty acid synthesis pathway (Koh et al., 2004). In addition, NADPH is an important antioxidant that helps cells protect from oxidative damage caused by various cellular stresses (Minard & McAlister-Henn, 1999). On the other hand, the reversible catalyzing of the reactions by *IDH1* and *IDH2* has multiple effects on the cells due to the consumption and production of NADPH and the release and binding of CO_2 (He et al., 2022).

One of the potential roles of *IDH* in the metabolism is driving tumor progression (Nadhan et al., 2023). The roles of *IDH1/2* isoforms in promoting tumor proliferation were studied in different cancer types (colon, lung, esophageal, breast, and pancreas) (Atalay et al., 2023; Špačková et al., 2021; Li et al., 2023; Chen et al., 2017; Zarei et al., 2023). However, studies of *IDH1/2* expression on carcinogenesis and probability of survival have been limited to some types of cancer (He et al., 2022). The study with more cancer types helps understand the biological characteristics of *IDH* isoforms to improve treatment. Our results showed that there is a direct relationship between the elevated *IDH1/2* isoforms and the progression of cancers in bladder urothelial, breast urothelial, esophageal, and lung squamous cell carcinoma and colon, lung, and stomach adenocarcinoma (Figure 3). Next, we found that survival probability decreased in some cancers such as bladder urothelial, breast urothelial, lung squamous cell and esophageal carcinoma, and colon, lung, and stomach adenocarcinoma which have elevated *IDH1* or *IDH2* expression (Figure 4).

5. Conclusion

In conclusion, a detailed study of the gene expressions of metabolic enzymes involved in producing metabolites that have an essential effect on cancer progression may provide new strategies to prevent tumor growth. In the present study, different isoforms of *IDH* were investigated specifically for cancer types, and it was found that *IDH1* could be a biomarker for esophageal, lung, and stomach and *IDH2* for bladder, breast, colon, and lung cancers. In the future, it is planned to study target cancer types to investigate the reorganized metabolic pathways.

Author contribution

E. Bulut Atalay contributed to the study conception/design, interpretation of the data, the literature search, and writing – the original draft.

Declaration of ethical code

The authors declare that the materials and methods parts of this study do not require ethical committee approval and/or legal-specific permission.

Conflicts of interest

The author declares that there is no conflict of interest.

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