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Original Article

Unlocking the diagnostic, prognostic roles, and immune implications of BAX gene expression in pan-cancer analysis

Mostafa A Abdel-Maksoud[1], Sajid Ullah[2], Amun Nadeem[3], Aisha Shaikh[4], Muhammad Khurram Zia[5], Adel M Zakri[6], Taghreed N Almanaa[1], Akram A Alfuraydi[1], Ayman Mubarak[1], Yasir Hameed[7]

[1]Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; [2]Badr Al Samma Hospital, Barka 320, Oman; [3]Department of Pathology, Gujranwala Medical College Teaching Hospital Gujranwala, Pakistan; [4]SMBB Medical University, Larkana, Pakistan; [5]Department of Surgery, Liaquat College of Medicine and Dentistry and Darul Sehat Hospital, Karachi, Pakistan; [6]Department of Plant Production, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia; [7]Department of Biotechnology, Institute of Biochemistry, Biotechnology and Bioinformatics, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

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Abstract: Objectives: Cancer, a formidable disease, continues to challenge our understanding and therapeutic approaches. This study delves into the pan-cancer analysis of BCL2 Associated X (BAX) gene expression, seeking to unravel its significance in cancer development, prognosis, and potential therapeutic strategies. Methods: A combination of bioinformatics and molecular experiments. Results: Our pan-cancer investigation into BAX expression encompassed 33 distinct cancer types, revealing a remarkable and uniform increase in BAX expression. This groundbreaking finding emphasizes the potential universality of BAX's role in cancer development and progression. Further, our study explored the prognostic implications of BAX expression, highlighting a consistent association between up-regulated BAX and poor overall survival (OS) in Liver Hepatocellular Carcinoma (LIHC) and Skin Cutaneous Melanoma (SKCM). These results suggest that BAX may serve as an adverse prognostic indicator in these malignancies, emphasizing the importance of personalized treatment strategies. Epigenetic and genetic analyses of BAX provided valuable insights. Hypomethylation of the BAX promoter region was evident in LIHC and SKCM, which likely contributes to the up-regulation of BAX, while genetic mutations in the BAX gene itself were infrequent in these cancers. Our exploration of BAX-associated signaling pathways and the correlation between BAX expression and CD8+ T cell infiltration shed light on the intricate molecular landscape of cancer. BAX's interaction with key apoptotic and immune-related pathways reinforces its role as a central player in tumor development and the immune microenvironment. Moreover, our drug prediction analysis identified potential therapeutic agents for modulating BAX expression in the context of LIHC and SKCM, bridging the gap between research and clinical application. Conclusion: In sum, our comprehensive BAX study not only enhances our understanding of its significance as a biomarker gene but also offers novel avenues for therapeutic interventions, contributing to the ongoing quest for more effective cancer treatments and improved patient care.

Keywords: Cancer, BAX, diagnosis, prognosis

Introduction

Cancer, a complex and multifaceted group of diseases, continues to be a global health challenge with profound implications for both patients and healthcare systems [1-3]. Defined by the uncontrolled growth and spread of abnormal cells, cancer is characterized by its heterogeneity, affecting virtually every tissue and organ within the human body [4-7]. With over 100 different types of cancer, each with its unique molecular and clinical features, understanding the underlying mechanisms and potential therapeutic targets has become a paramount goal of biomedical research.

The intricate interplays of genetic, environmental, and lifestyle factors underpin the development of cancer [8-11]. Genetic abnormalities, in particular, have been identified as key drivers

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in the transformation of normal cells into cancerous ones [12-14]. These abnormalities may disrupt the regulatory pathways responsible for cell growth, differentiation, and apoptosis, ultimately leading to the emergence of a tumor [15].

One such gene that has garnered considerable attention in the context of cancer is BCL2 Associated X (BAX). BAX, a pro-apoptotic member of the BCL-2 family, plays a central role in regulating programmed cell death, or apoptosis [16, 17]. Its primary function is to promote apoptosis by activating the mitochondrial pathway, thereby facilitating the release of cytochrome c and other pro-apoptotic factors [18]. BAX accomplishes this by permeabilizing the mitochondrial outer membrane, a critical step in the intrinsic apoptotic pathway [19]. Dysregulation of BAX expression or function has been implicated in various cancer types, contributing to uncontrolled cell proliferation and resistance to apoptosis [20-22].

As a central player in the apoptotic cascade, BAX has been the focus of extensive research aimed at elucidating its role in cancer development, progression, and response to therapy [23]. Understanding how BAX expression and activity are modulated in different cancer types is essential for uncovering potential therapeutic strategies and prognostic markers. The integration of bioinformatics tools with experimental validation offers a comprehensive approach to investigate the multifaceted role of BAX in cancer.

In this study, we present a thorough analysis of BAX gene expression and its implications in a pan-cancer context. Leveraging large-scale genomic datasets and experimental validation, we explore the significance of BAX in the development and progression of various cancer types. Our investigation spans different facets of BAX's involvement in cancer, including its expression profiles, prognostic value, mutational status, and potential impact on the tumor microenvironment. The combination of bioinformatics and experimental approaches will provide a more comprehensive understanding of BAX in cancer, potentially uncovering novel insights that can inform future diagnostic and therapeutic strategies.

Methodology

Analysis of BAX expression in pan-cancer view point

UALCAN is a powerful and user-friendly web resource designed to facilitate the exploration of gene expression in cancer [24]. This platform provides an invaluable tool for researchers seeking to unravel the expression profiles of genes like BAX. In our study, UALCAN played a pivotal role by enabling us to conduct a comprehensive pan-cancer analysis of BAX gene expression. This analysis allowed us to assess BAX's expression patterns across a diverse spectrum of cancer types, shedding light on its potential roles in tumor development and progression.

Prognostic analysis of BAX in pan-cancer view point

GEPIA2 (Gene Expression Profiling Interactive Analysis 2) is a web-based tool that empowers researchers for in-depth analysis of gene expression and survival data using The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) datasets [25]. In our study, we harnessed the capabilities of GEPIA to conduct comprehensive pan-cancer prognosis analysis of the BAX gene. This invaluable resource allowed us to assess the impact of BAX expression on overall survival across a wide range of cancers.

Relevance of BAX expression with clinical variables

UALCAN is a powerful platform for in-depth exploration of cancer-related gene expression [26]. In our study, UALCAN played a pivotal role in elucidating the expression profile of BAX expression across different clinical variables within specified cancer types.

Methylome analysis of BAX

OncoDB is a valuable resource for researchers delving into epigenetic alterations in cancer [27]. In our study, we harnessed the capabilities of OncoDB to perform promoter methylation analysis of the BAX gene within specified cancer types. This platform provided us with access to extensive datasets and analytical

tools to explore the methylation status of the BAX gene's promoter region.

Genetic alteration analysis of BAX

cBioPortal is a robust and user-friendly platform widely utilized for exploring genetic alterations in various cancers [28]. In our study, we harnessed the power of cBioPortal to conduct mutational analysis of the BAX gene across specified cancer types. This invaluable tool allowed us to investigate alterations in BAX, including mutations, copy number variations, and structural variants. Such insights are crucial for understanding the role of BAX in cancer development and progression, facilitating our quest to unravel the genetic underpinnings of this critical gene in the context of oncology.

TIMER2

TIMER2 is a robust and widely used database that has played a pivotal role in the realm of immunogenomics [24]. This valuable resource provides a comprehensive collection of immune infiltration data across multiple cancer types, enabling researchers to delve into the complex interplay between tumor-infiltrating lymphocytes and cancer progression. In a recent study, TIMER2 was employed to investigate the Pearson correlation between BAX, a critical regulator of apoptosis, and the infiltration level of CD8+ T cells in various tumor microenvironments.

Pathway analysis of BAX-related genes

STRING, a powerful bioinformatics tool [29], enabled us to unravel the complex web of interactions between BAX and its related genes. Through this platform, we constructed a Protein-Protein Interaction (PPI) network, providing a comprehensive view of the molecular partners influencing BAX's functionality.

Next, we harnessed the DAVID tool [30] to unravel the functional significance of BAX-related genes. This platform allowed us to conduct comprehensive pathway analysis, revealing the intricate biological processes influenced by these genes.

Drug prediction analysis of BAX

DrugBank proves indispensable for cancer researchers, providing an accessible interface for drug prediction analysis [31]. In our investigation, we harnessed the capabilities of DrugBank to delve into the realm of drug prediction analysis concerning the BAX gene.

Validation of BAX gene expression through quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis

We acquired 15 pairs of fresh LIHC tissue samples and corresponding adjacent noncancerous tissues from 15 patients. These specimens were collected by highly skilled surgeons and meticulously examined by experienced pathologists at the Nishtar Medical College, Multan, Pakistan, during the period spanning from January to June in 2023. We ensured the ethical considerations by obtaining written informed consent from all patients or their legal guardians. The collected tissue samples were promptly frozen upon retrieval and safely stored at -80°C for subsequent analysis, preserving their integrity for research purposes.

To validate the findings of our bioinformatics analysis related to the BAX gene, we performed RT-qPCR to measure BAX gene expression in LIHC samples and control samples. Following the manufacturer's protocol, total RNA was extracted from LIHC tissues using RNAiso Plus (Takara, China). Subsequently, cDNA was synthesized from the total RNA using the transcriptor cDNA synthesis kit (Roche, Germany). We assessed the relative mRNA levels of BAX using the Stormstar SybrGreen qPCR Master Mix (DBI, Germany). To ensure accurate measurements, we normalized the expression levels of BAX to the internal reference gene GAPDH. Relative mRNA expression levels were calculated using the 2-ΔΔct method, and all PCR assays were conducted in triplicate. The primer sequences employed for gene expression validation through RT-qPCR are presented below.

GAPDH-F 5'-ACCCACTCCTCCACCTTTGAC-3', GAPDH-R 5'-CTGTTGCTGTAGCCAAATTCG-3'; BAX-F 5'-TTTGCTTCAGGGTTTCATCC-3', BAX-R 5'-CAGTTGAAGTTGCCGTCAGA-3'.

Statistics details

For enrichment analysis, we used Fisher's exact test for computing statistical difference. Correlational analyses were carried out using Pearson method. For comparisons, a student

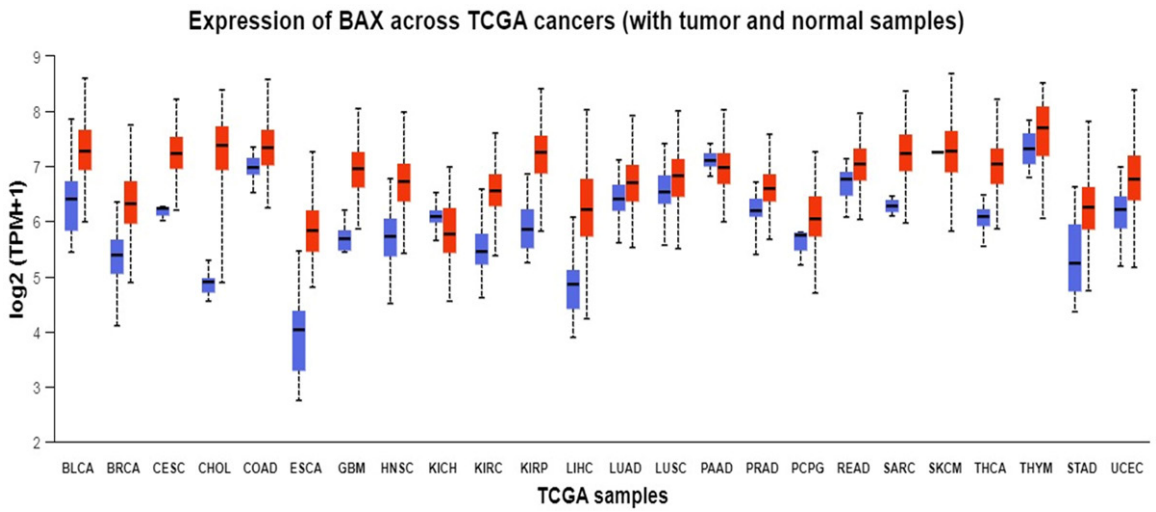

Figure 1. The mRNA-level pan-cancer expression analysis of the BAX gene conducted using UALCAN. Significance was determined with a *p*-value <0.05. In the figure, the blue boxplot depicts BAX expression in normal samples, while the red boxplot illustrates BAX expression in cancerous samples. BAX = BCL2 Associated X.

t-test was adopted in the current study. All the analyses were carried out in R version 3.6.3 software.

Results

Analysis of BAX expression in pan-cancer view point

During our extensive pan-cancer investigation into the expression of the BAX gene, we noted a noteworthy and uniform increase in all 33 cancer types we examined (as depicted in **Figure 1**). This significant discovery highlights a broad surge in BAX gene expression in diverse cancer types, emphasizing its potential importance within the realm of cancer development.

Prognostic analysis of BAX in pan-cancer view point

In our comprehensive pan-cancer prognostic analysis of the BAX gene, we made a notable observation concerning its impact on overall survival (OS). Our results indicated a consistent and intriguing association between up-regulated BAX gene expression and poor OS in two specific cancer types, namely Liver Hepatocellular Carcinoma (LIHC) and Skin Cutaneous Melanoma (SKCM) within the context of all 33 cancer types we examined (**Figure 2**). This finding underscores the potential significance of BAX as a prognostic marker in these particular cancers, suggesting that its overexpression may serve as an adverse prognostic indicator for patients.

Relevance of BAX expression with clinical variables

Subsequently, we conducted an expression analysis of the BAX gene in patients diagnosed with LIHC and SKCMS, stratified by various clinical variables such as cancer stage, race, and gender. Our findings revealed a significant up-regulation of BAX expression in LIHC and SKCM patients across different cancer stages, racial backgrounds, and genders when compared to control samples (**Figure 3**). This consistent up-regulation of BAX underscores its potential role as a key player in the molecular landscape of these cancers, irrespective of the clinical parameters we assessed. Further investigation is required to elucidate the precise implications of these findings for disease progression.

Methylome and genetic alteration analysis of BAX

In our study, we advanced our research by conducting a comprehensive analysis of the promoter methylation and genetic mutational status of the BAX gene. To achieve this, we utilized the OcoDB and cBioportal databases, renowned resources for accessing genetic and epigenetic information. Our findings in this analysis revealed intriguing insights into the methylation

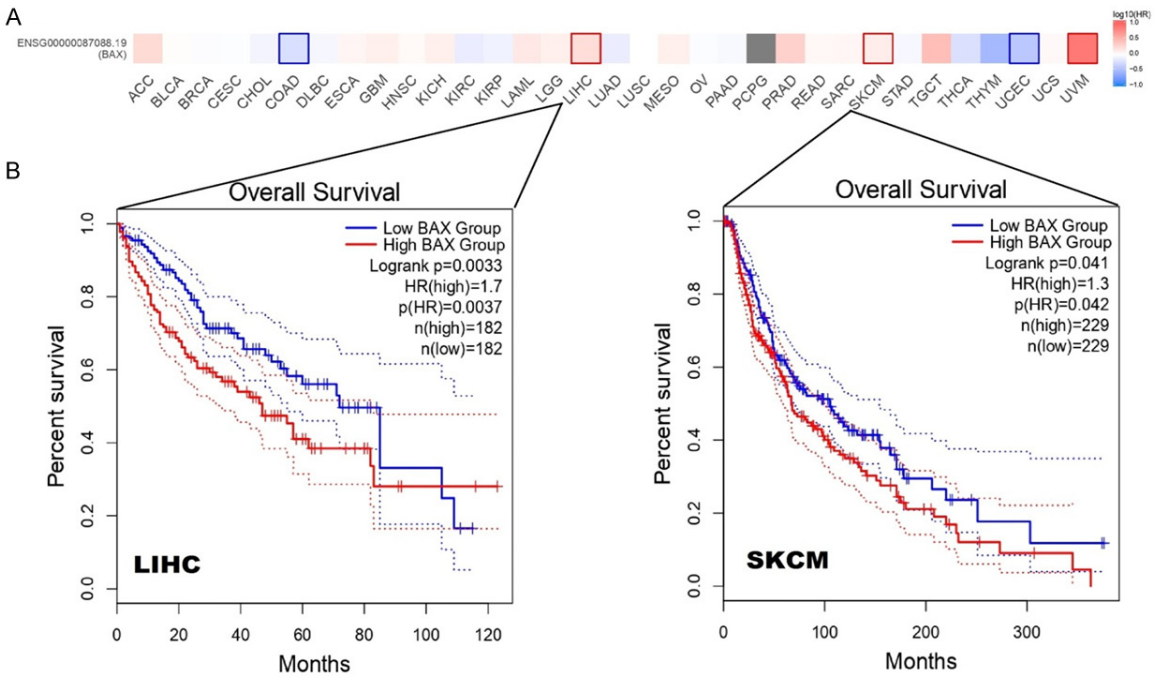

Figure 2. The pan-cancer analysis of overall survival (OS) outcomes associated with the BAX gene, conducted using GEPIA2. A. A survival map depicting BAX gene outcomes across 33 cancer types. B. Survival graphs displaying BAX gene outcomes in LIHC and SKCM. The significance level was established with a *p*-value <0.05. BAX = BCL2 Associated X, LIHC = Liver Hepatocellular Carcinoma, SKCM = Skin Cutaneous Melanoma.

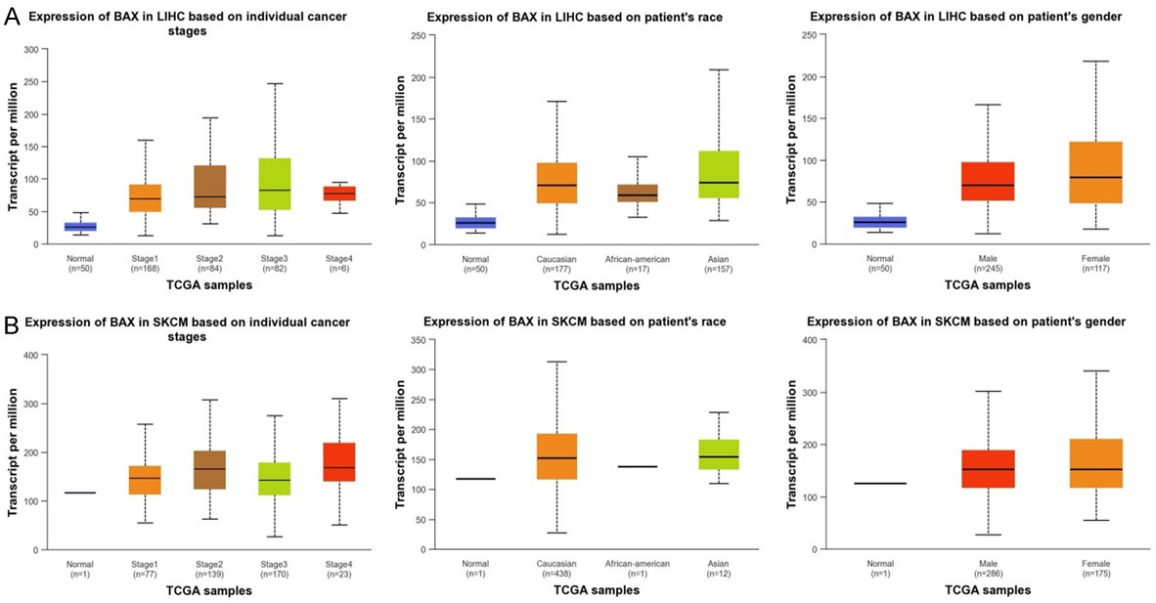

Figure 3. Assessment of BAX gene expression across LIHC and SKCM patients stratified by various clinical parameters. A. The BAX gene expression among LIHC patients with different clinical variables. B. The BAX gene expression among SKCM patients with diverse clinical parameters. Significance was determined using a *p*-value <0.05. BAX = BCL2 Associated X, LIHC = Liver Hepatocellular Carcinoma, SKCM = Skin Cutaneous Melanoma.

status of the BAX gene in LIHC and SKCM patients when compared to control samples. Promoter methylation analysis outcomes demonstrated that the BAX gene exhibited a

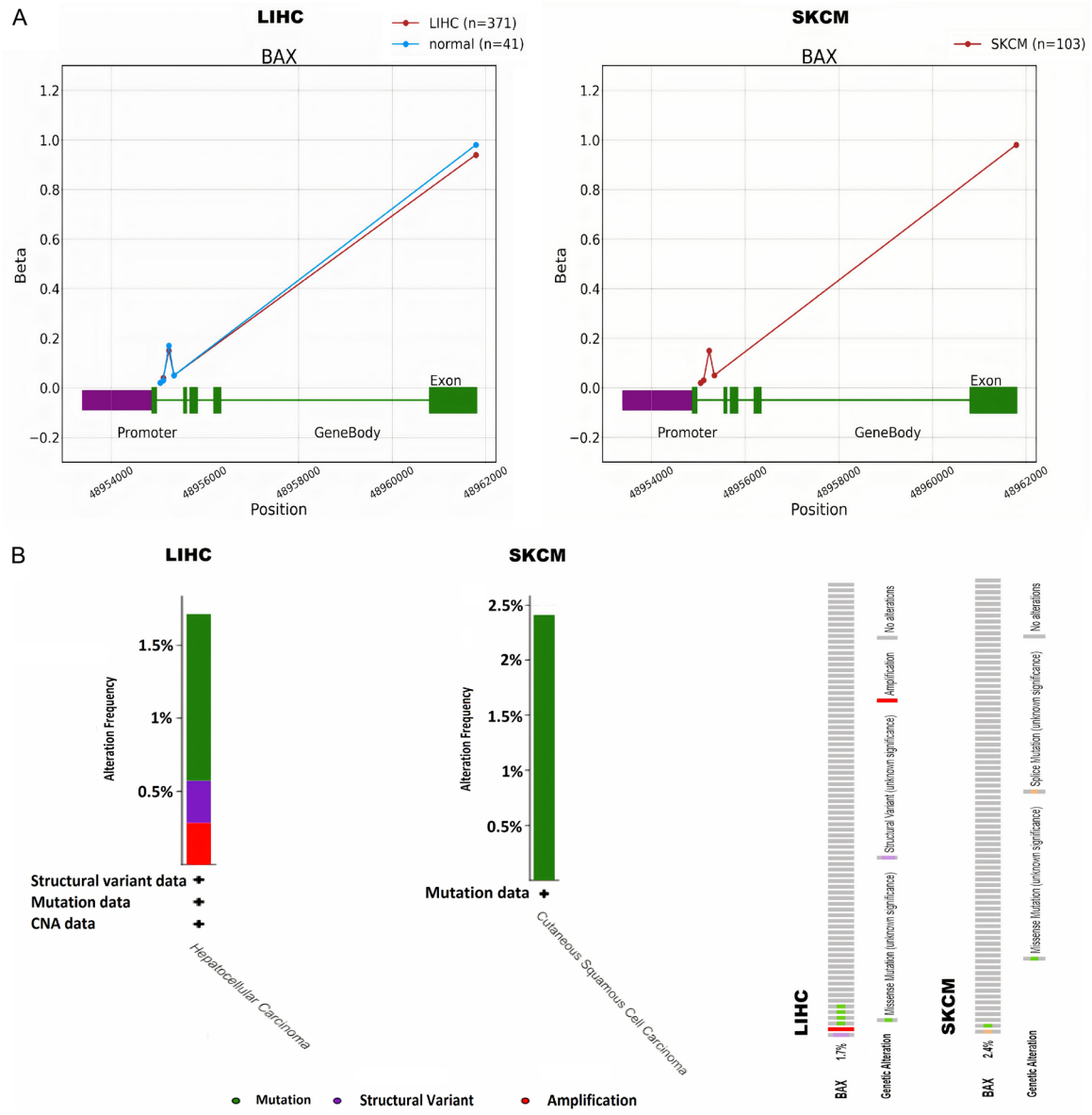

Figure 4. Results from the analysis of promoter methylation and genetic alterations in the BAX gene among LIHC and SKCM patients. A. Findings from the promoter methylation analysis of the BAX gene employing OncoDB. B. Outcomes of the genetic alteration analysis of the BAX gene using cBioPortal. Significance was determined using a *p*-value <0.05. BAX = BCL2 Associated X, LIHC = Liver Hepatocellular Carcinoma, SKCM = Skin Cutaneous Melanoma.

reduced level of methylation in the promoter region of the BAX gene in these cancer types (**Figure 4A**). Hypomethylation of the promoter region often correlates with increased gene expression [32], which could help explain our earlier findings of up-regulated BAX expression in LIHC and SKCM.

Furthermore, our genetic mutational analysis did not reveal a high prevalence of genetic mutations in the BAX gene in LIHC (1.7%) and SKCM (2.35%) (**Figure 4B**). This suggests that while promoter hypomethylation may be a contributing factor to BAX up-regulation in these cancer types, genetic mutations in the BAX gene itself may not be a common driver of oncogenesis in LIHC and SKCM. These combined results shed light on the complex interplay of epigenetic and genetic factors in the regulation of BAX and provide valuable insights into the molecular landscape of LIHC and SKCM.

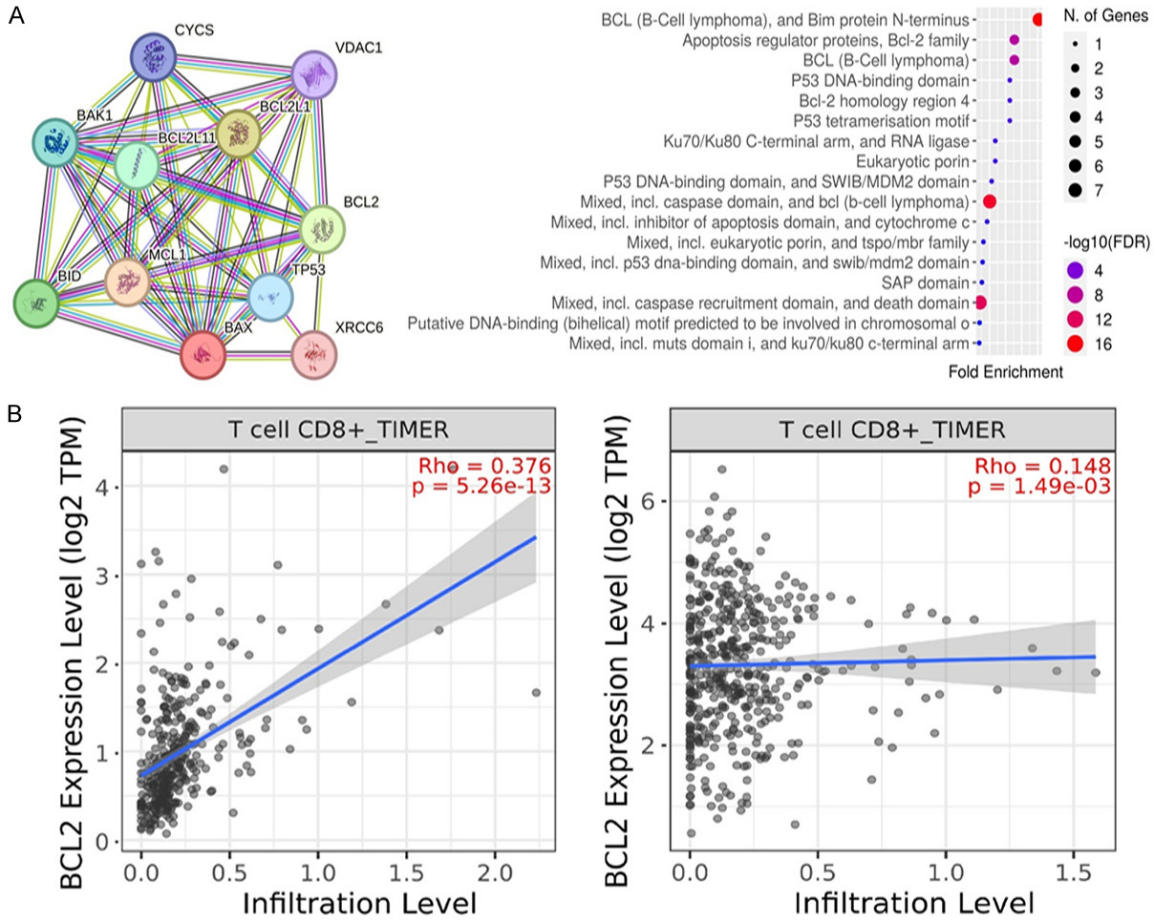

Figure 5. Investigation into pathways and examination of the Pearson correlation between BAX expression and the infiltration level of CD8+ T immune cells in LIHC and SKCM. A. Protein-Protein Interaction (PPI) network featuring proteins associated with BAX and pathway terms linked to BAX. B. Pearson correlation results of BAX with the infiltration level of CD8+ T immune cells in LIHC and SKCM. Significance was determined using a *p*-value <0.05. BAX = BCL2 Associated X, LIHC = Liver Hepatocellular Carcinoma, SKCM = Skin Cutaneous Melanoma.

Exploration of BAX-associated signaling pathways

Continuing our investigation, we extended our analysis by constructing a PPI network for the BAX gene using data from the STRING database. The PPI network unveiled nine distinct proteins that interact with BAX (**Figure 5A**). These interactions are pivotal in understanding the functional role of BAX and its involvement in cellular pathways. Subsequently, we conducted a Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the BAX gene and its interacting partners using the DAVID tool. The results of this analysis demonstrated that these genes collectively participate in intriguing pathways, including pathways "BCL (B cell lymphoma) and BIM protein N-terminus, apoptosis regulator proteins, BCL (B cell lymphoma), P53 DNA binding domain, bcl-2 homology region 2, and P53 tetrameristion, etc. (**Figure 5A**)". These findings provide a deeper understanding of the functional context of BAX and its associated proteins, shedding light on the intricate cellular pathways where BAX plays a crucial role.

Correlation between BAX expression and infiltration level of CD8+ T cells

In the subsequent phase of our study, we harnessed the power of the TIMER2 database to delve into the Pearson correlation between BAX gene expression and the infiltration level of CD8+ T cells in LIHC and SKCM. The analysis yielded compelling results, highlighting a notable and positive correlation between BAX over-

Table 1. DrugBank-based BAX-associated drugs

Sr. No	Hub gene	Drug name	Effect	Reference	Group
1	BAX	Celecoxib	Decrease expression of BAX mRNA	A20938	Approved
		Dronabinol		A22087	
		Cyclosporine		A20661	
		Doxorubicin		A21894	
		Calcifediol		A22310	
		Resveratrol		A23864	

BAX = BCL2 Associated X.

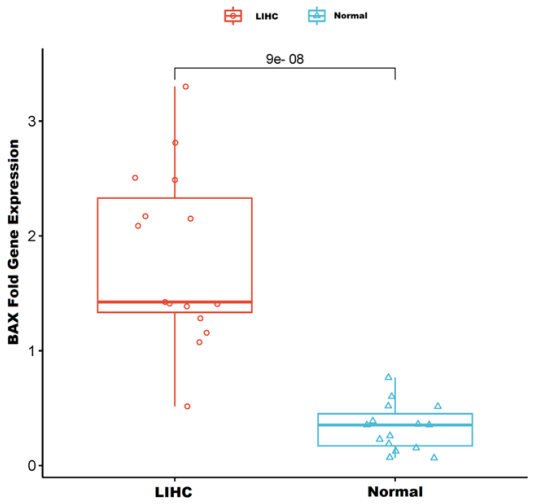

Figure 6. Evaluation of BAX gene expression in LIHC samples paired with adjacent controls using RT-qPCR. Significance was determined using a *p*-value <0.05. BAX = BCL2 Associated X, LIHC = Liver Hepatocellular Carcinoma.

expression and the infiltration level of CD8+ T cells in both LIHC and SKCM (**Figure 5B**). This positive correlation emphasized the potential significance of BAX in the immune microenvironment of these cancer types.

Drug prediction analysis of BAX

In the subsequent phase of our study, we conducted a drug prediction analysis targeting the BAX gene with the aim of identifying experimentally approved drugs that could potentially reverse the expression of BAX in the treatment of LIHC and SKCM. Our results unveiled a list of promising drug candidates, including Celecoxib, Dronabinol, Cyclosporine, Doxorubicin, Calcifediol, and Resveratrol, which demonstrated the capability to reduce the expression of BAX in the context of LIHC and SKCM (**Table 1**). These findings hold significant promise for potential therapeutic interventions, as they provide insights into pharmaceutical agents that may play a pivotal role in modulating BAX expression. Further research and validation studies are necessary to explore the efficacy and safety of these drugs in clinical settings.

Validation of BAX gene expression through RT-qPCR

Lastly, to validate BAX gene expression, we performed RT-qPCR analysis of this gene using 15 LIHC samples paired with adjacent controls. The outcomes of our RT-qPCR analysis unequivocally demonstrated a significant and consistent increase in BAX gene expression within the LIHC samples when compared to the adjacent control tissues (**Figure 6**). This finding stands as a pivotal validation of the initial observations derived from our bioinformatics analysis, which strongly indicated an up-regulation of the BAX gene in the context of LIHC.

Discussion

Cancer, a multifaceted and life-threatening disease, has been a subject of intense scientific scrutiny for decades [33]. It encompasses a diverse group of malignancies characterized by uncontrolled cell growth, which can lead to devastating consequences [34]. The understanding of cancer has evolved, highlighting the need for a comprehensive approach to identify shared molecular features across various cancer types. The pan-cancer analysis of BAX gene expression was conducted to elucidate its potential role as a universal player in cancer development, prognosis, and therapy.

The most striking findings of our study are the uniform increase in BAX gene expression across 33 cancer types examined in pan-cancer view. This observation emphasized the potential importance of BAX in the development and progression of diverse cancer types. BAX, a pro-apoptotic gene, is typically associated with promoting programmed cell death, which is often disrupted in cancer [35, 36]. The consistent up-regulation of BAX in various can-

cers suggests that it may be harnessed by cancer cells to evade apoptosis and promote their uncontrolled growth.

In addition to the pan-cancer analysis, our study focused on the prognostic implications of BAX gene expression. Notably, we observed a consistent association between up-regulated BAX and poor OS in LIHC and SKCM patients out of analyzed 33 cancer types. This finding suggests that BAX may serve as an adverse prognostic indicator for patients with these specific cancer types. Previous studies also suggested that overexpressed BAX is prognostic indicator of worst OS in cancer patients [37-39]. However, no study deciphers the underlying mechanism of BAX relevance with poor OS of cancer patients and there has been no research that elucidates the fundamental mechanism responsible for the connection between BAX and poor OS of the cancer patients. Therefore, understanding the mechanisms by which increased BAX expression influences disease progression in these cancers is critical. It may involve the suppression of apoptosis, contributing to treatment resistance and aggressive tumor growth. These findings can guide personalized treatment strategies and clinical decision-making for patients with LIHC and SKCM.

To gain a more comprehensive understanding of BAX regulation, we examined both the promoter methylation and genetic mutational status of the BAX gene. In LIHC and SKCM, we found hypomethylation of the BAX gene's promoter region, which often correlates with increased gene expression [40, 41]. This may explain the observed up-regulation of BAX in these cancer types, supporting the idea that epigenetic modifications contribute to its overexpression [42, 43]. In contrast, our genetic mutational analysis revealed a relatively low prevalence of BAX gene mutations in LIHC and SKCM. In previously published research, it has been noted that mutations in the BAX gene are infrequent among individuals with cancer [44, 45]. This scenario suggests that while epigenetic factors, particularly promoter hypomethylation, may contribute to BAX up-regulation, genetic mutations in the BAX gene itself are not a common driver of oncogenesis in LIHC and SKCM. These results highlight the complex interplay of epigenetic and genetic factors in the regulation of BAX expression.

The exploration of BAX-associated signaling pathways using a PPI network and KEGG analysis revealed participation of BAX and BAX-associated genes in various pathways, including those related to apoptosis regulation and the P53 pathway [46, 47]. These findings deepen our understanding of how BAX functions in the intricate landscape of cellular processes.

Our analysis of the correlation between BAX expression and the infiltration level of CD8+ T cells in LIHC and SKCM showed a notable and positive correlation. This suggests that BAX may play a role in shaping the immune microenvironment of these cancers. The presence of CD8+ T cells in the tumor microenvironment is often associated with a better prognosis, as they are key effectors of the anti-tumor immune response [48, 49]. The positive correlation observed between BAX and CD8+ T cell infiltration, on the other hand, yielded contrasting outcomes, as the presence of CD8+ T immune cells did not lead to improved prognoses for patients with LIHC and SKCM.

Our drug prediction analysis identified a list of experimentally approved drugs (Celecoxib, Dronabinol, Cyclosporine, Doxorubicin, Calcifediol, and Resveratrol) with the potential to reverse the expression of BAX in LIHC and SKCM. These findings open the door to potential therapeutic interventions that could target BAX expression to improve patient outcomes. However, these predictions require further validation and testing in preclinical and clinical settings to determine their safety and efficacy.

Conclusion

In conclusion, our study on BAX gene expression and its implications in various cancer types sheds light on the importance of BAX in cancer development, prognosis, and potential therapeutic strategies. The uniform increase in BAX expression across diverse cancer types emphasizes the need for further research into the mechanisms responsible for this phenomenon. The prognostic implications of BAX in LIHC and SKCM suggest a role for BAX in disease progression and treatment response, necessitating personalized approaches. The interplay of epigenetic and genetic factors in BAX regulation, its involvement in signaling pathways, and its impact on the immune microenvironment all contribute to a more compre-

hensive understanding of the role of BAX in cancer. Furthermore, the drug predictions provide promising avenues for targeted therapy, but these require rigorous validation.

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Disclosure of conflict of interest

None.

Address correspondence to: Mostafa A Abdel-Maksoud, Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia. E-mail: Mabdmaksoud@ksu.edu.sa; Yasir Hameed, Department of Biotechnology, Institute of Biochemistry, Biotechnology and Bioinformatics, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan. E-mail: Yasirhameed2011@gmail.com

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