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RESEARCH ARTICLE

Functional network analysis reveals potential repurposing of β-blocker atenolol for pancreatic cancer therapy

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Abstract

Background The survival rate of patients with pancreatic cancer is low; therefore, continuous discovery and development of novel pancreatic cancer drugs are required. Functional network analysis is an integrated bioinformatics approach based on gene, target, and disease networks interaction, and it is extensively used in drug discovery and development.

Objective This study aimed to identify if atenolol, a selective adrenergic inhibitor, can be repurposed for the treatment of pancreatic cancer using functional network analysis.

Methods Direct target proteins (DTPs) and indirect target proteins (ITPs) were obtained from STITCH and STRING databases, respectively. Atenolol-mediated proteins (AMPs) were collected from DTPs and ITPs and further analyzed for gene ontology, KEGG pathway enrichment, genetic alterations, overall survival, and molecular docking.

Results We obtained 176 AMPs that consisted of 10 DTPs and 166 ITPs. Among the AMPs involved in the pancreatic cancer pathways, several AMPs such as MAPK1, RELA, MAPK8, STAT1, and STAT3 were identified. Genetic alterations in seven AMPs were identified in 0.9%–16% of patients. Patients with high mRNA levels of *MAPK1*, *RELA*, *STAT3*, *GNB1*, and *MMP9* had significantly worse overall survival rates compared with patients with low expression. Molecular docking studies showed that RELA and MMP9 are potential target candidates of atenolol in the treatment of patients with pancreatic cancer.

Conclusion In conclusion, atenolol can potentially be repurposed to target pancreatic cancer cells by modulating MMP9 and NF-κB signaling. The results of this study need to be further validated in vitro and in vivo.

Keywords Functional network · Atenolol · Drug repurposing · Pancreatic cancer

Introduction

In 2018, pancreatic cancer was estimated to be the seventh leading cause of cancer deaths in the world [1]. The overall five-year survival rate for this disease is only 2%–9% [2]. The mortality rate of pancreatic cancer is high, partly because of inaccurate diagnosis and limited treatment options [3]. Current treatments for pancreatic cancer are surgery and adjuvant chemotherapy [3]. However, the response of patients to chemotherapy is low because of chemoresistance, metabolic aberrations, invasion, and metastasis [4]. Therefore, targeted therapies need to be developed for the effective treatment of pancreatic cancer.

Only a few effective and safe anticancer agents have been successfully developed worldwide. Furthermore, the research, development, and marketing of these drugs are expensive and time consuming [5]. Drug repurposing, which is the use of approved drugs for new medical indications, is a strategy to

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accelerate the discovery and development of anticancer drugs. The development of information technology and the use of big data enable drug development to be efficient and cost-effective [6]. The use of omics-based technology, improvements in data storage, data mining, and machine learning have provided adequate sources of information about diseases, molecular mechanisms, and drugs [7]. Functional network analysis is an integrated bioinformatics approach based on gene, target, and disease networks interaction, and it is extensively used in drug discovery and development [8].

Atenolol (Fig. 1a) may be develop as anticancer agent on the basis of the drug re-purposing approach using functional network analysis. Atenolol is a β-blocker that inhibits β-adrenergic 1 and 2 receptors (ADRB1 and ADRB2) [9]. Atenolol is used to control blood pressure in patients with angina pectoris, hypertension [6], and ischemic heart disease [10]. A previous study have shown that atenolol is also effective as an anticancer agent against infantile hemangioma, which is a benign vascular tumor derived from blood vessel cell types [11]. In addition, the combination of atenolol with metformin effectively eradicates breast cancer tumors and their cellular microenvironment [12].

Activation of ADRB2 signaling promotes the progression of pancreatic ductal carcinoma [13] and pancreatic cancer microenvironment [14], thus, blocking ADRB2 signaling is a promising strategy for pancreatic cancer therapy. Atenolol is known to block β-1 and β-2 adrenergic receptor [15]. Propranolol, an ADRB antagonist, was found to inhibit pancreatic cancer cell proliferation [16]. Nevertheless, the use of atenolol, which has longer action and fewer side effects than propranolol [17], for the treatment of patients with pancreatic cancer has never been explored yet.

Using functional network analysis, we explored the potential of repurposing atenolol in pancreatic cancer. Direct target proteins (DTPs) and indirect target proteins (ITPs) were

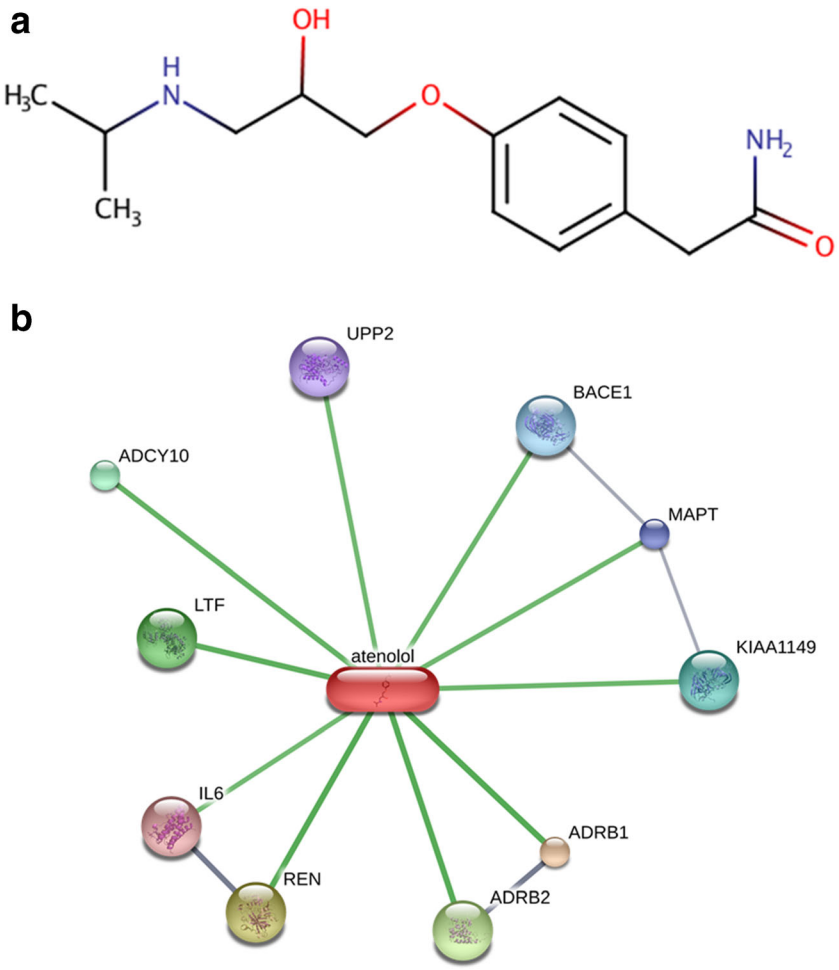

Fig. 1 **a** Structure of atenolol. **b** Atenolol interaction with direct target proteins (DTPs). **c** PPI network of proteins related to atenolol-mediated proteins (AMPs)

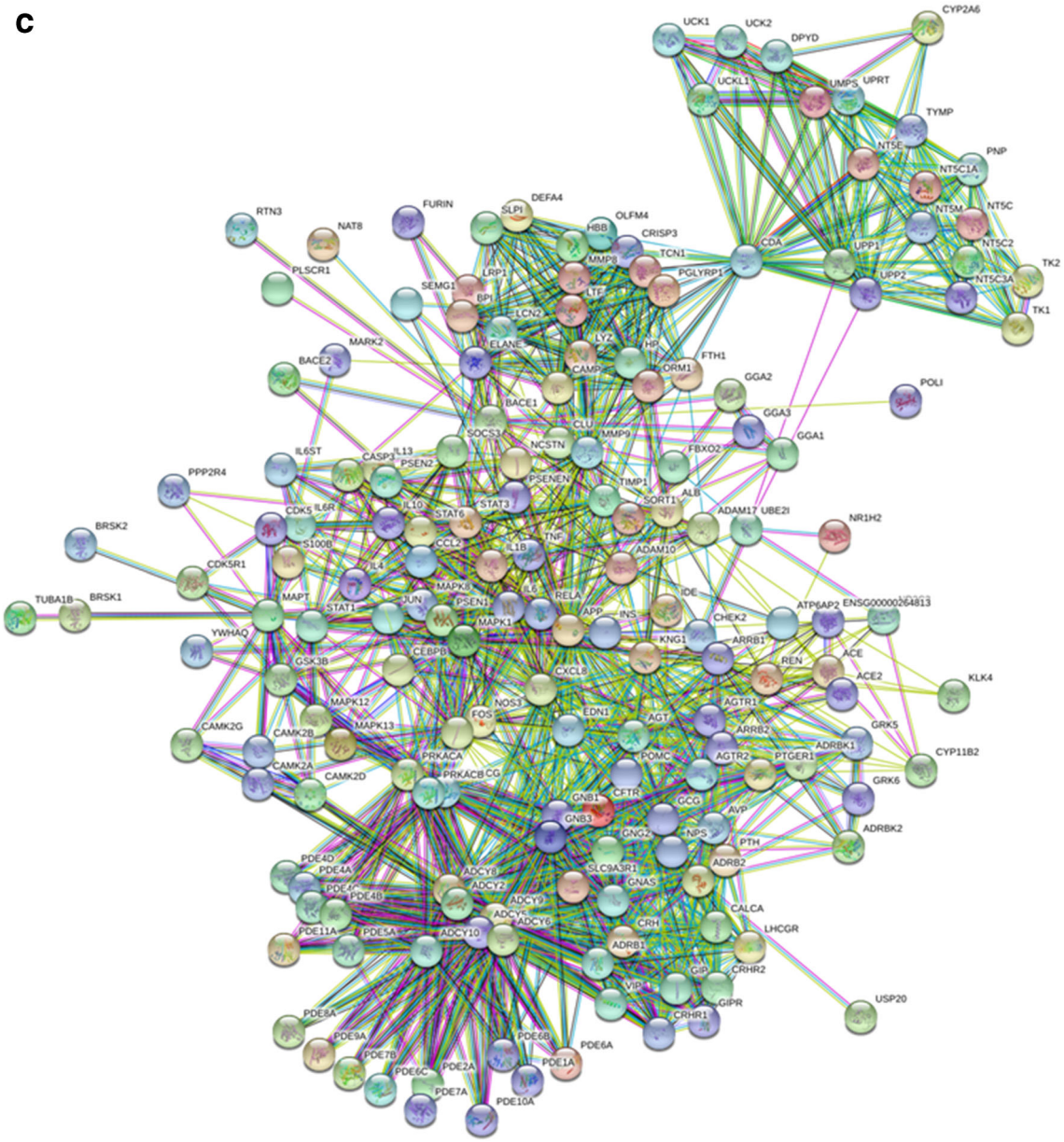

Fig. 1 (continued)

obtained from the STITCH and STRING databases, respectively. Atenolol-mediated proteins (AMPs) were collected from DTPs and ITPs. AMPs were further analyzed for gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. A protein–protein interaction (PPI) network of AMPs was constructed using the STRING database, and hub proteins were selected based on their degree score. Genetic alterations were analyzed using cBioportal. Molecular docking studies were performed to identify the potential interaction between atenolol and the target proteins. We identified a possible molecular mechanism of atenolol using integrated bioinformatics analysis, which suggested that RELA and MMP9 could be potential targets of atenolol in pancreatic cancer therapy.

Material and methods

DTP acquisition

DTPs are the target protein of a compound that possesses as agonist, antagonist, or inhibitor towards the DTPs. DTPs of atenolol were obtained from the STITCH database (http://

stitch.embl.de) [18]. The results were then used for subsequent analysis.

ITP acquisition

The proteins that are interacted with the DTPs were called Indirect target proteins (ITPs). The ITPs of each DTP were obtained from the STRING database (https://string-db.org) [19], with a minimum interaction score of 0.4 and maximum number of interactors of 20. The ITPs of all DTPs were generated after removing repetitive proteins. A total of DTPs and ITPs were further considered as atenolol-mediated proteins (AMPs).

PPI network

PPI networks were constructed with STRING-DB v11.0 [19], with confidence scores >0.7 and visualized by Cytoscape software [20]. Genes with a degree score of more than 10 analyzed by CytoHubba plugin were selected as hub genes [21].

GO and KEGG pathway enrichment

GO and KEGG pathway enrichment analyses were conducted by The DAVID v6.8 [22]. $p < 0.05$ was selected as the cutoff value.

Analysis of genetic alterations among hub genes

The genetic alterations in selected genes were analyzed using cBioPortal (http://www.cbioportal.org) [23, 24]. In the present study, the genes MAPK1, RELA, STAT3, ADCY8, GNB1, and MMP9 were screened for genetic alterations in all pancreatic studies available in the cBioportal database. The pancreatic cancer study with the highest frequency of genetic alterations was chosen for further connectivity analysis.

Gene expression profile and Kaplan–Meier survival analysis

Gene expression profiles and the prognostic value of the hub genes across pancreatic adenocarcinoma samples were evaluated using GEPIA [25] and Kaplan–Meier survival curves (http://kmplot.com) [26], respectively, by log-rank test. $p < 0.05$ was selected as the cutoff value.

Molecular docking

Docking simulation was conducted to predict the binding properties of atenolol with MMP9 and IKK. All computational simulations were generated on the Windows 10 Operating System, with Intel Core i5-7th Gen as a processor and 4 GB of RAM. PDB ID 4H3X contained a non-selective MMP hydroxamic acid derivative representing the model of a compound bound to the catalytic site of MMP9, while PDB ID 2OVX embedded barbiturate inhibitor RO-206-0222, depicting the model of a compound bound to MMP9 with the inactive E402Q mutant [27, 28]. The model of IKK was represented by the non-canonical NFκB pathway IKKα (5EBZ) and canonical NFκB pathway IKKβ (4KIK) on the basis of the presence of the known inhibitors, including IKK inhibitor XII and staurosporine, respectively [29, 30]. MOE-Dock program on MOE 2010 (licensed from the Faculty of Pharmacy, UGM) was used for docking simulation, RMSD calculation, and visualization of the binding interaction. The structure of atenolol was drawn in ChemDraw software and subjected to a conformational search that was minimized in MOE using the energy minimization module. The calculation allowed an induced fit using the rigid backbone for the conformation of the template protein with Amber10: EHT force field, triangle matcher as placement, and GBVI/WSA dG (kcal/mol) as the scoring function. The default settings were used in each application unless any further explanation was available. The results of the analysis were used to identify the conformation that produced the lowest energy state when atenolol was bound to the target protein.

Results and discussion

DTP and ITP acquisition

This study explored the potential of repurposing atenolol for the treatment of pancreatic cancer. We searched the DTPs of atenolol in the STITCH database, which identified 10 DTPs of atenolol (Table 1). The interactions between atenolol and DTPs were also analyzed (Fig. 1b).

Table 1 Direct protein targets (DTPs) of atenolol

No	Protein symbol	Protein name
1	UPP2	Uridine phosphorylase 2
2	BACE1	Beta-secretase 1
3	MAPT	Microtubule-associated protein tau
4	ADCY10	Adenylate cyclase type 10
5	LTF	Lactotransferrin
6	KIAA1149	Beta-secretase 1
7	IL6	Interleukin-6
8	REN	Renin
9	ADRB2	Beta-2 adrenergic receptor
10	ADRB1	Beta-1 adrenergic receptor

PPI networks

We then generated DTP-related proteins using the STRING database, and the results are summarized in Supplementary Table 1. Using STITCH and STRING, we retrieved 176 AMPs that consisted of 10 DTPs and 166 ITPs (Supplementary Table 1). The AMPs were then constructed into a PPI network, which consisted of 173 nodes, 1470 edges, an average node degree of 17, and a high confidence interaction (Fig. 2)change to Fig. 1c. Furthermore, hub proteins were selected from the PPI network based on a specific degree score (Table 2).

Among the hub proteins, ADRB2 was the only DTP with the highest degree score of 40. This result indicated that the biological effect of atenolol was strongly correlated with ADRB2. GO analysis showed that AMPs were involved in the adenylate cyclase-activating G protein-coupled receptor signaling pathway, peptidyl-threonine phosphorylation, and negative regulation of apoptosis. The β-2 adrenergic receptor is a member of GPCR, which is involved in prostate cancer progression [31]. The AMPs are located in the cytosol, extracellular matrix, and cytoplasm. Matrix metalloproteinases (MMPs) are key extracellular matrix enzymes and targets for anticancer drugs [32]. Moreover, the AMPs play a molecular function in modulating MAP kinase activity and DNA binding. Activation of the MAPK signaling pathway plays an important role in human pancreatic cancer [33].

GO and KEGG pathway enrichment analysis

GO analysis of AMPs was classified into three groups, namely, biological process, cellular component, and molecular function (Supplementary Table 2). Pathway enrichment by KEGG of the AMPs (Supplementary Table 3) showed the regulation of ~94 pathways. Many of the proteins, such as MAPK1, RELA, MAPK8, STAT1, and STAT3, were found to be involved in pancreatic cancer. Hub protein selection based on degree score showed that ADRB2 was the only DTP with a high degree score. These findings highlight the potential importance of ADRB2 in pancreatic cancer progression.

Analysis of genetic alterations among hub proteins

Seven AMPs were analyzed using cBioportal to explore their genomic alterations in pancreatic cancer. These AMPs consisted of three genes involved in the pancreatic cancer pathway (*MAPK1*, *RELA*, and *STAT3*), three genes with the highest degree scores (*ADCY8*, *GNB1*, and *MMP9*), and *ADRB2*, which is the only DTP with a high degree score. Among ten pancreatic cancer studies, the highest frequency of genetic alterations (40%) was found in a study by Witkiewicz et al. (2015) [34], Fig. 3should be Fig. 2a), which was selected for further analysis. Oncoprint analysis showed genetic alterations in seven AMPs ranging from 0.9% to 16%

Table 2 Top 20 hub proteins based on degree score

No	Protein symbol	Protein name	Degree score
1	APP	Amyloid-beta A4 protein	56
2	ADCY8	Adenylate cyclase type 8	53
3	ADCY2	Adenylate cyclase type 2	51
4	ADCY5	Adenylate cyclase type 5	48
5	ADCY9	Adenylate cyclase type 9	48
6	ADCY6	Adenylate cyclase type 6	48
7	GNB1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	45
8	GNB3	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3	42
9	AGT	Angiotensinogen	40
10	ADRB2	Beta-2 adrenergic receptor	40
11	EDN1	Endothelin-1	39
12	GNG2	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2	39
13	IL6	Interleukin-6	38
14	CXCL8	Interleukin-8	38
15	POMC	Pro-opiomelanocortin	37
16	MAPK1	Mitogen-activated protein kinase 1	37
17	AVP	Vasopressin-neurophysin 2-copeptin	37
18	INS	Insulin	36
19	MMP9	Matrix metalloproteinase-9	35
20	GCG	Glucagon	35

(Fig. 4change to Fig. 2b). Furthermore, amplification was the most common gene alteration.

This result was supported by previous studies on *ADCY8*, *GNB1*, *MMP9*, and *RELA* genetic alterations in cancer progression. For example, a mutation in *ADCY8* is associated with decreased expression of tumoral PD-L1 in lung squamous cell carcinoma [35]. An activating mutation in *GNB1* is associated with resistance to tyrosine kinase inhibitors in ETV6-ABL1-positive leukemia cells [36]. Genetic polymorphisms in *MMP9* are associated with breast cancer risk in the Chinese Han population [37]. In addition, high expression of RELA is associated with the activation of NF-κB signaling and poor prognosis in patients with pancreatic cancer [38].

MAPK1 is a member of the MAPK family, which regulates various cellular processes, such as proliferation, differentiation, invasion, and metastasis [39]. Pancreatic cancer is characterized by constitutive activation of the MAPK1 pathway [40]. Moreover, increased MAPK1 activation is found in patients with pancreatic ductal adenocarcinoma with liver metastasis [41]. Thus, targeting MAPK1 may be a strategic way to treat pancreatic cancer.

Signal transducer and activator of transcription 3 (STAT3) is a member of the STAT family, which is activated by phosphorylation, regulates transcriptional activity, and is involved in various human tumors [42]. Activation of the STAT3 signaling pathway enhances the migration and invasion of

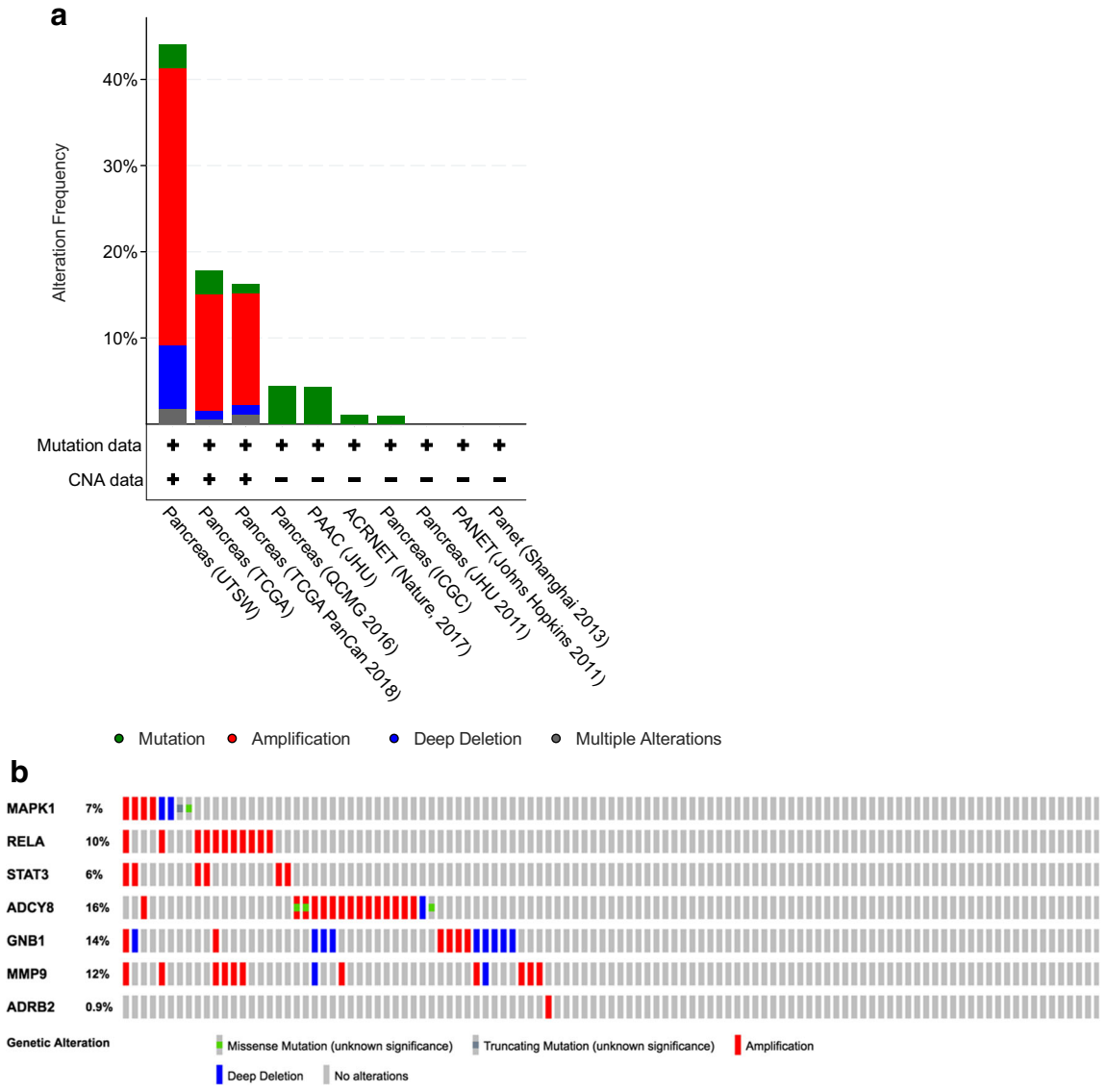

Fig. 2 **a** Overview of the changes in *MAPK1*, *RELA*, *STAT3*, *ADCY8*, *GNB1*, *ADRB2*, and *MMP9* in genomic datasets from nine studies of pancreatic cancer. **b** Summary of the alterations in *MAPK1*, *RELA*, *STAT3*, *ADCY8*, *GNB1*, *ADRB2*, and *MMP9* across pancreatic cancer samples (based on a study by Witkiewicz et al., 2015). **c** Gene network and **d** drug–gene network connected to *MAPK1*, *RELA*, *STAT3*, *ADCY8*, *GNB1*, and *MMP9* across pancreatic cancer samples (based on a study by Witkiewicz et al., 2015)

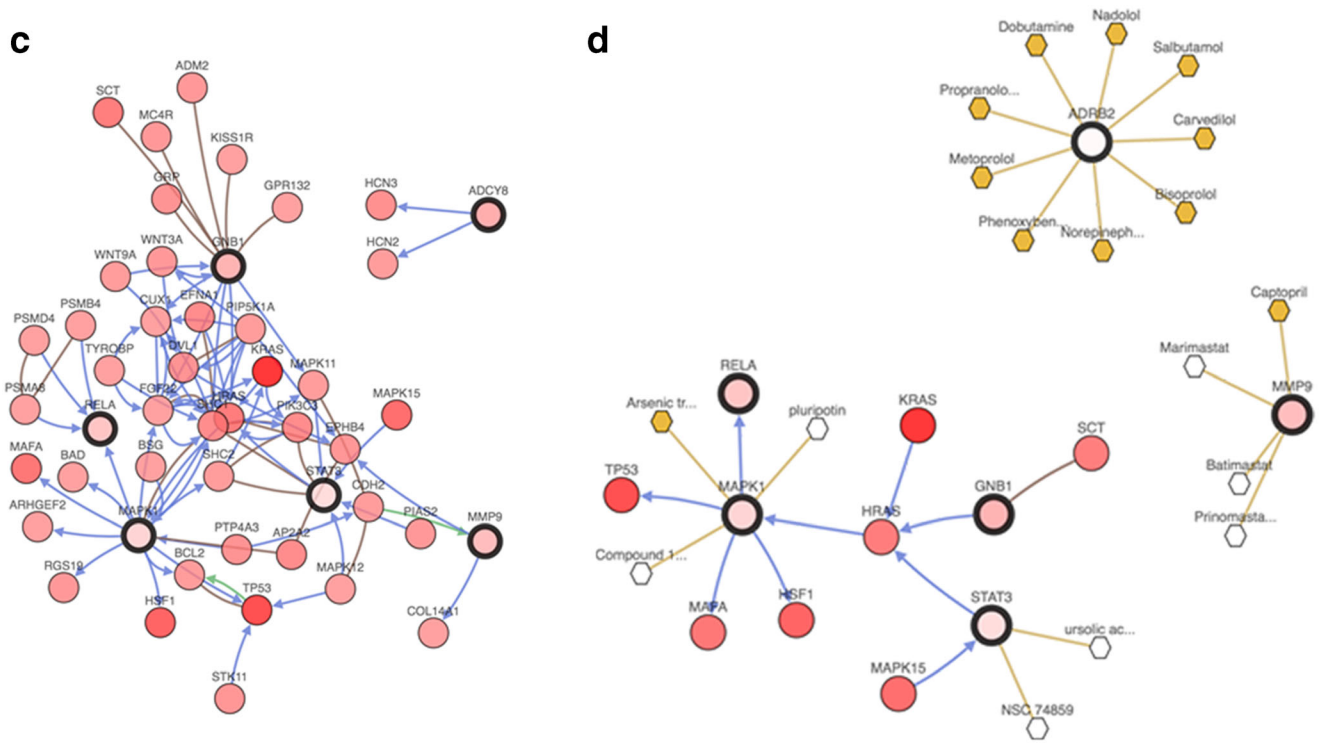

Fig. 2 (continued)

pancreatic ductal adenocarcinoma cells [43]. Furthermore, suppression of STAT3 by emodin increases the sensitivity of human pancreatic cancer cells to EGFR inhibitors, such as erlotinib, gefitinib, afatinib, and cetuximab [44].

ADCY8 encodes adenylate cyclase, an enzyme that catalyzes cyclic AMP formation from ATP. ADCY8 is expressed in pancreatic β-cells and plays an important role in insulin secretion [45]. Another study revealed that genetic polymorphism of ADCY8 is associated with glioma risk in female patients with type I neurofibromatosis [46]. In addition, ADCY8 has been shown to play a role as a tumor suppressor gene in cervical cancer in which promoter methylation of ADCY8 is correlated with poor prognosis in patients [47].

Guanine nucleotide-binding protein beta 1 (GNB1) or the β subunit of heterotrimeric G proteins is a regulator of neurodevelopment [48]. GNB1 regulates the mTOR-induced antiapoptotic pathway in human breast cancer [49]. Furthermore, the downregulation of GNB1 is associated with poor prognosis in patients with clear cell renal cell carcinoma and is related to the VEGF signaling pathway [50]. Therefore, targeted therapy against GNB1 may be a promising candidate in pancreatic cancer treatment.

ADRB2 encodes the β-2 adrenergic receptor (also known as ADRB2), a member of the G protein-coupled receptor family, which regulates the cardiovascular system [51]. To date, the role of ADRB2 in carcinogenesis is not well understood. Adrenergic signaling plays an important role in tumor development [52]. The ADRB2 blocker propranolol inhibits migration in MDA-MB 231 breast cancer cells [53]. Another study showed that blocking of ADRB2 reduces pancreatic nerve growth factor expression, which accelerates tumor development in mice [54]. Thus, the study of ADRB2 as a target of atenolol in pancreatic cancer cells is an interesting topic.

Mutual exclusivity analysis showed that only three gene pairs (*RELA-MMP9*, *RELA-STAT3*, and *GNB1-MMP9*) exhibited significant ($p < 0.05$) co-occurrence in pancreatic samples from the UTSW study (Table 3). Subsequently, we explored the interactive relationship between seven selected genes and altered genes in the UTSW study. The results showed that a network contained six query and 45 neighbor genes (Fig. 5Achange to Fig. 2c). To reduce network complexity, we filtered out neighbor genes with 25% alterations. The results showed that only HRAS with the highest alterations remained among neighbor genes (Fig. 5B). Moreover, ADRB2, MAPK1, and MMP9 were the main targets of most cancer drugs, which indicated the potential of those proteins to be a potential atenolol target in pancreatic cancer treatment.

The co-occurrence of *MMP9*, *RELA*, and three other genes revealed an essential role of MMP9 and RELA in the

mechanisms involved in atenolol treatment. MMPs play an important role in cancer initiation, tumor growth, and metastasis [55]. A previous study showed that the expression of MMP9 is increased and associated with vascular invasion, lymph node invasion, liver metastases, and TNM stage in patients with pancreatic cancer [56]. The results of this study highlighted the potency of atenolol as an inhibitor of MMP9 in pancreatic cancer cells. These results were supported by a recent study, which demonstrated that inhibition of MMP9 with an antibody leads to increased tumor-associated IL-28 and decreased stromal markers and vimentin, thereby enhancing the efficacy of chemotherapy in pancreatic cancer [57].

Gene expression profile and Kaplan–Meier survival analysis

We explored the gene expression level of seven AMPs among patients with pancreatic adenocarcinoma using the KM Plotter database. The expression of *MAPK1*, *RELA*, *STAT3*, *GNB1*, and *MMP9* was significantly higher in pancreatic cancer samples than in normal samples (Fig. 6change to Fig. 3a). Additionally, *ADRB2* levels were higher in pancreatic cancer samples than in normal samples. There was no difference in the level of *ADCY8* between normal and pancreatic cancer samples. A Kaplan–Meier plot for the overall survival of

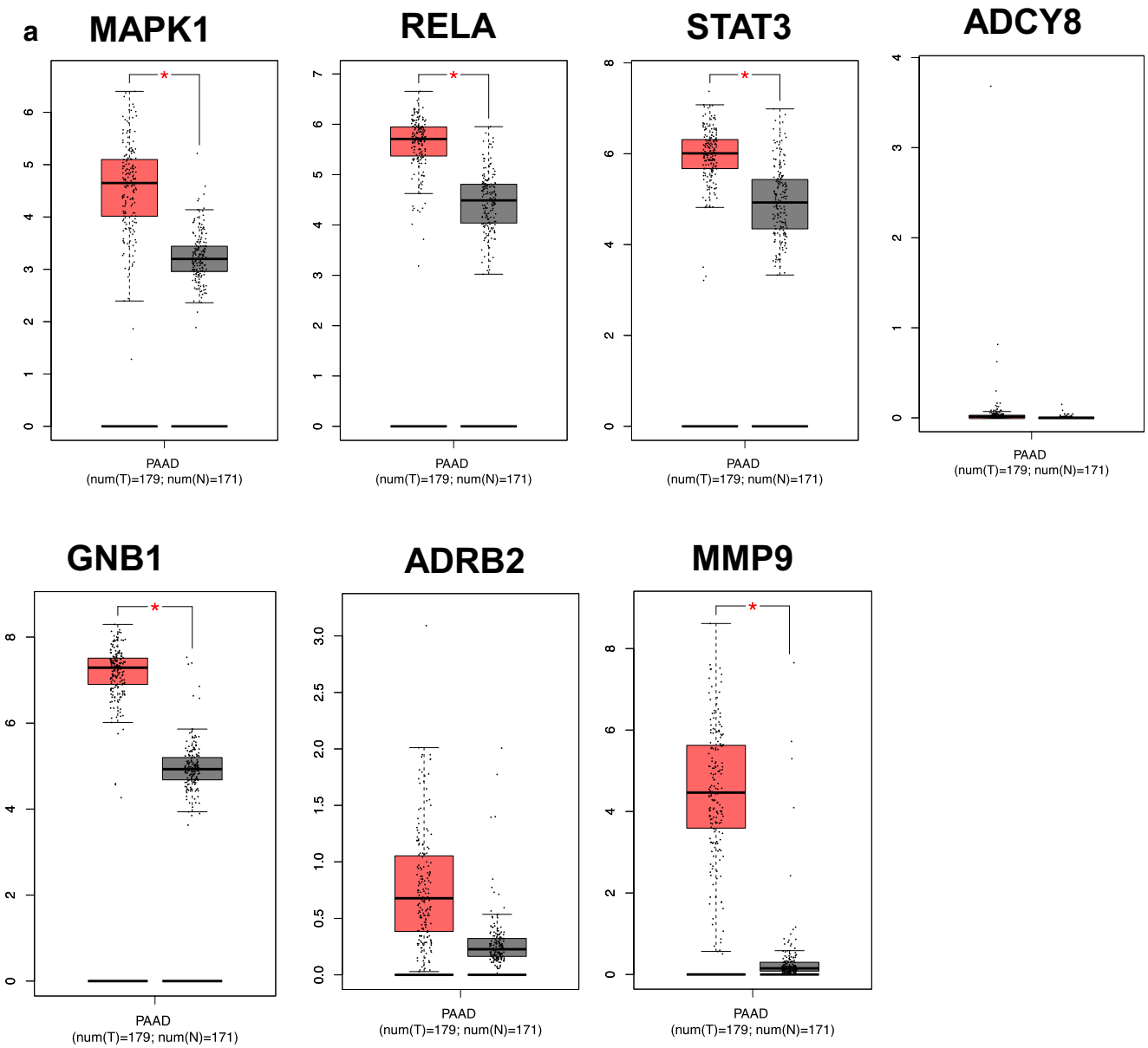

Fig. 3 **a** Gene expression profile of *MAPK1*, *RELA*, *STAT3*, *ADCY8*, *GNB1*, and *MMP9* across pancreatic adenocarcinoma (analyzed by GEPIA). **b** Kaplan–Meier survival related to the expression of *MAPK1*, *RELA*, *STAT3*, *ADCY8*, *GNB1*, and *MMP9* across pancreatic adenocarcinoma (analyzed by KM Plotter)

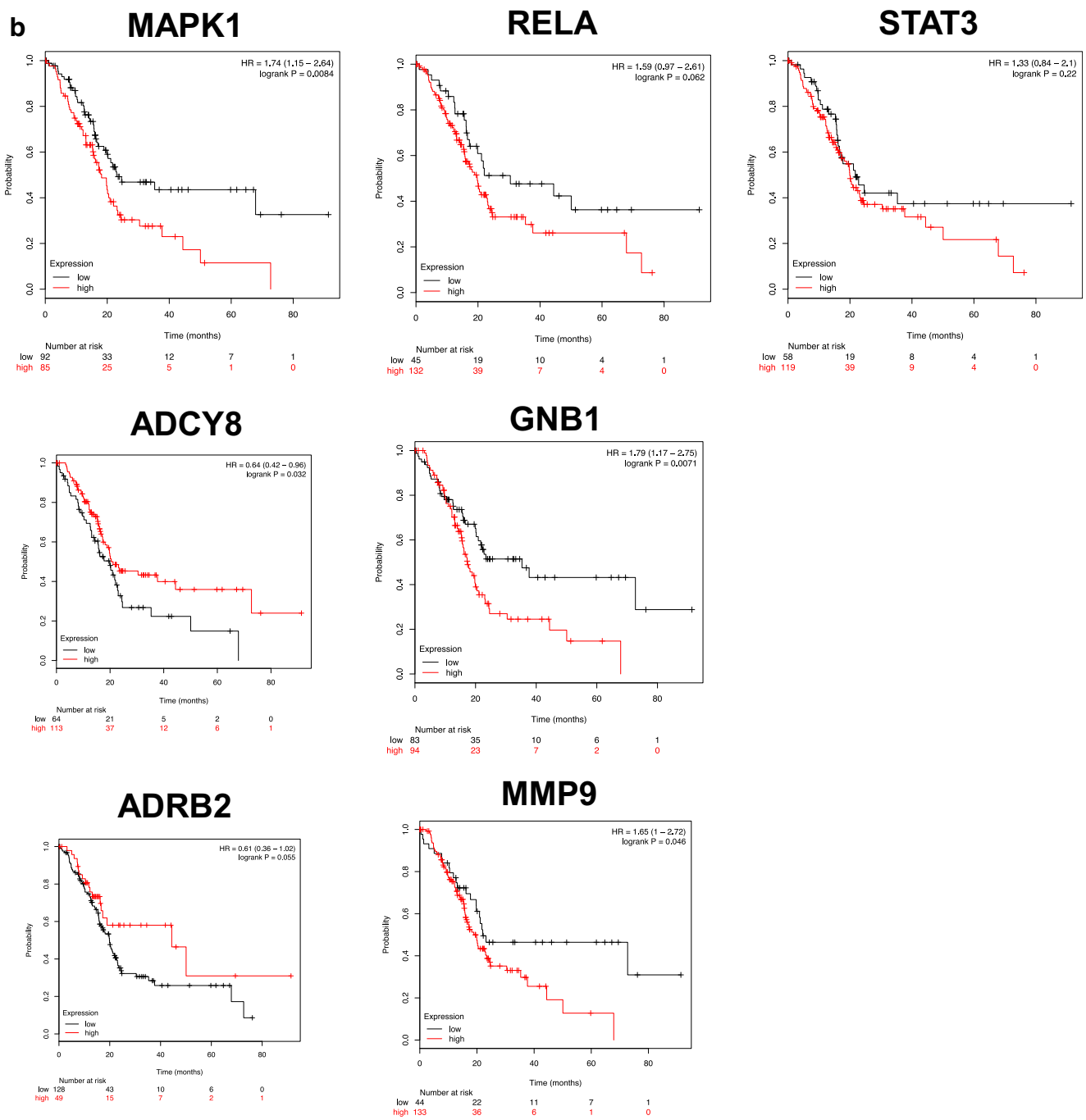

Fig. 3 (continued)

patients with pancreatic cancer was obtained based on the low and high expression levels of each gene. Patients with high mRNA expression of *MAPK1* ($p = 0.0084$), *RELA* ($p = 0.062$), *STAT3* ($p = 0.22$), *GNB1* ($p = 0.0071$), and *MMP9* ($p = 0.046$) had significantly worse overall survival rates than those in the low expression level group (Fig. 7change to Fig. 3b). Moreover, patients with increased mRNA levels of *ADCY8* ($p = 0.032$) and *ADRB2* ($p = 0.055$) had better overall survival rates than those in the low expression level group.

Molecular docking

The inhibition of RELA/NFκB signaling and MMPs can be used as a treatment strategy for pancreatic cancer cell therapy. In this study, we performed molecular docking analysis to predict the possible atenolol-mediated inhibition of regulatory proteins in pancreatic cancer cells. Docking simulation and ligand–protein binding visualization were generated by Molecular Operating Environment (MOE) software. The

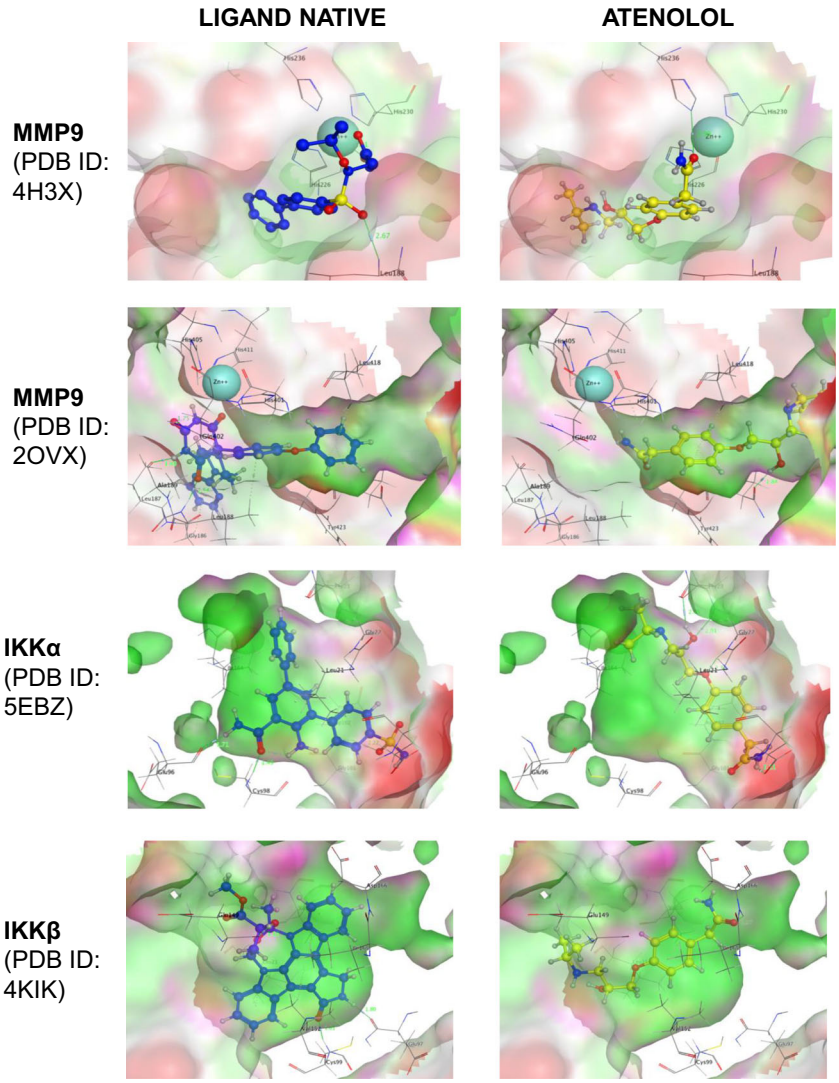

Fig. 4 Visualization of ligand interactions with MMP9, IKKα, and IKKβ using MOE

protein targets of IKK and MMP9 were selected from KEGG pathway enrichment analysis, the top 50 genes with the highest degree score, the Kaplan–Meier plot, and networks of atenolol-associated genes in pancreatic cancer. We used two PDB IDs for each protein to validate the binding properties of atenolol. Our molecular docking study using a 4H3X model found that atenolol exhibited a lower docking score than a hydroxamic acid derivative, indicating potent binding affinity (Table 4). The visualization of the binding interaction data revealed that the hydrophilic amide group of atenolol formed an H-bond with His236, rather than forming a direct metal bond with Zn^{2+} and an H-bond with Leu188, which was observed with the hydroxamic acid derivative (Fig. 8)change to Fig. 4. Using a 2OVX model, atenolol had a comparable docking score with RO-206-0222. The comparable binding affinity was mediated by forming an H-bond between the hydroxyl group and Arg424 and an Arene-H bond between the Cα atom of Atenolol and His401. These bonds were stabilized by another Arene-H bond with Leu418, Arg424, and Tyr423, resulting in the hydrophobic binding properties of RO-206-0222 (Fig. 8change to Fig. 4).

Given the instability of full-length MMP9, every molecular docking approach requires several types of crystal structure models to validate the binding properties of a certain compound [28]. In our study, we used two different PDB IDs representing the wild type and mutant catalytic sites in MMP9 for validation. Our analysis of the wild-type catalytic site in MMP9 revealed that the hydrophilic amide group of atenolol contributed to the H-bond binding with His236 and one of three histidine residues coordinated with the catalytic ion Zn^{2+} responsible for substrate proteolysis in the active enzyme [58]. The interruption of the catalytic ion Zn^{2+} also appeared between Cα of atenolol and His401 with the E402Q mutant, highlighting the importance of the hydrophilic amide group on the binding interaction. In addition, a similar hydrophobic binding pattern was observed with the strong MMP9 inhibitor RO-206-0222, suggesting the strong inhibitory activity of atenolol on the MMP9-E402Q mutant [28]. Atenolol

Table 3 Mutual exclusivity analysis of selected genes in pancreatic cancer study

A	B	Log2 odds ratio	*p* Value	Tendency
RELA	*MMP9*	>3	<0.001	Co-occurrence
RELA	*STAT3*	>3	0.013	Co-occurrence
GNB1	*MMP9*	2.426	0.017	Co-occurrence
MAPK1	*STAT3*	>3	0.061	Co-occurrence
RELA	*ADCY8*	<-3	0.14	Mutual exclusivity
MAPK1	*RELA*	1.769	0.185	Co-occurrence
STAT3	*GNB1*	1.791	0.191	Co-occurrence
MAPK1	*MMP9*	1.447	0.243	Co-occurrence
MAPK1	*GNB1*	1.174	0.303	Co-occurrence
STAT3	*ADCY8*	<-3	0.352	Mutual exclusivity
ADCY8	*GNB1*	0.515	0.426	Co-occurrence
RELA	*GNB1*	0.539	0.467	Co-occurrence
STAT3	*MMP9*	0.601	0.542	Co-occurrence
MAPK1	*ADCY8*	−0.398	0.637	Mutual exclusivity
ADCY8	*MMP9*	−0.026	0.672	Mutual exclusivity
ADCY8	*ADRB2*	<-3	0.844	Mutual exclusivity
GNB1	*ADRB2*	<-3	0.862	Mutual exclusivity
MMP9	*ADRB2*	<-3	0.881	Mutual exclusivity
RELA	*ADRB2*	<-3	0.899	Mutual exclusivity
MAPK1	*ADRB2*	<-3	0.927	Mutual exclusivity
STAT3	*ADRB2*	<-3	0.945	Mutual exclusivity

bound to the catalytic site of MMP9 by interrupting the ion Zn^{2+} catalytic site and was predicted to possess strong inhibitory activity.

The molecular docking study of the ATP-binding site of IKKα and IKKβ demonstrated that atenolol had comparable binding affinity with IKK inhibitor XII but lower binding affinity than staurosporine (Table 4). The three H-bonds represented by the binding between the amide group with Glu19, hydroxyl group with Thr23, and ether group with Gly22 contributed to the comparable binding affinity of atenolol with IKKα (Fig. 8)should be Fig. 4. The low affinity of atenolol compared with that of staurosporine and IKKβ was caused by a reduced number of amino acid bindings, which only formed one H-bond between the amide group and Asp166 and one Arene-H bond between benzene and Val29. Meanwhile, the staurosporine interaction possessed three H-bonds with a short distance and four Arene-H bonds (Fig. 8)should be Fig. 4. In summary, atenolol interacted with MMP9 on its wild type or E402Q mutant catalytic site and with IKK through the ATP-binding site.

NF-κB is a transcription factor that contributes to cancer development [59]. RELA (also known as p65) is a subunit of the NF-κB transcription factor complex, together with p50 [60]. Activation of NF-κB signaling leads to phosphorylation of IkBα and subsequent translocation of the p65/p50 complex into the nucleus [61]. NF-κB signaling contributes to

Table 4 Molecular docking results of atenolol against the protein targets MMP9, IKKα, and IKKβ

Protein	Ligand native						Atenolol					
	Docking score (kcal/mol)	RMSD (Å)	Ligand atom	Amino acid	Binding type	Distance	Docking score (kcal/mol)	RMSD (Å)	Ligand atom	Amino acid	Binding type	Distance
MMP9 (PDB ID: 4H3X)	−9.60	0.945	O	Leu188	H-bond	2.67	−11.99	1.904	O	His236	H-bond	2.91
			O	Zn^{2+}	Metal Cont-act	1.01						
MMP9 (PDB ID: 2OVX)	−11.63	0.626	O	Leu188	H-bond	1.54	−11.45	1.771	H	Arg424	H-bond	1.84
			H	Ala189	H-bond	1.49			H	His401	Arene-H	
			O	Gln402	H-bond	1.75			C	Leu418	Arene-H	
			C	Gly186	Arene-H				C	Arg424	Arene-H	
			C	Leu188	Arene-H				C	Tyr423	Arene-H	
			C	Leu418	Arene-H							
			C	Arg424	Arene-H							
			C	Tyr423	Arene-H							
IKKα (PDB ID: 5EBZ)	−10.92	0.828	H	Glu96	H-bond	1.71	−10.88	0.924	O	Thr23	H-bond	2.01
			O	Cys98	H-bond	1.49			H	Thr23	H-bond	2.05
			O	Asp102	H-bond	2.22			O	Gly22	H-bond	2.31
			C	Asp102	Arene-H				H	Glu19	H-bond	2.11
			C	Ile164	Arene-H							
			C	Leu21	Arene-H							
IKKβ (PDB ID: 4KIK)	−14.07	0.399	H	Glu149	H-bond	1.52	−10.17	0.733	O	Asp166	H-bond	1.97
			H	Glu97	H-bond	1.80			C	Val29	Arene-H	
			H	Cys99	H-bond	1.61						
			C	Val152	Arene-H							
			C	Ile165	Arene-H							
			C	Val29	Arene-H							
			C	Leu21	Arene-H							

pancreatic cancer development. Furthermore, high expression of RELA is associated with the activation of NF-κB signaling and poor prognosis in patients with pancreatic cancer [38]. Activation of NF-κB/p65 signaling stimulates anti-apoptotic responses in pancreatic cancer cells [62]. The inhibition of NF-kB signaling induces apoptosis [63] and inhibits angiogenesis in pancreatic cancer cells [64]. Taken together, targeting NF-κB/RELA may be a promising strategy in pancreatic cancer therapy.

IKK is a component of NF-κB signaling proteins and a multiprotein complex consisting of two kinase subunits. The first is IKKα, which is responsible for adaptive immunity, and the second is IKKβ, which is essential for innate immunity and inflammation [65]. Our molecular docking data on IKKα demonstrated the important role of the hydroxyl and ether groups beside the amide group in forming an H-bond. The hydroxyl group interacted with Thr23, the target binding site of Akt for phosphorylation, whereas the ether group bound to Gly22, an important residue on the glycine-rich loop [66]. The amide group and the benzene ring were two important functional groups for atenolol binding to IKKβ. Although the binding affinity of atenolol was lower than that of staurosporine, the interaction of the amide group from atenolol with Asp166 was similar to compound NSC-719177, a strong IKKβ inhibitor that is effective at low concentrations [67]. The hydrophobic binding between the benzene ring with Val29 was also similar to the other selective IKKβ kinase inhibitors, such as 4-phenyl-7-azaindoles, thiophenecarboxamide, and 2-amino-3-cyano-4-alkyl-6-(2-hydroxyphenyl) pyridine derivatives [68–70]. This study highlighted the key structure of atenolol responsible for its interaction with the ATP-binding site of IKK. The findings suggested that atenolol possibly inhibited its activity through the canonical or non-canonical pathway of NF-kB signaling.

The results of this study highlighted the potential of atenolol for pancreatic cancer therapy. RELA and MMP9 are potential target candidates of atenolol in the treatment of patients with pancreatic cancer. These target candidates greatly complement other genomics data and provide essential information for further research on atenolol, as well as the development of pancreatic cancer drugs. This study used bioinformatics approaches, and the results must be further validated in vitro and in vivo. In addition, the potential drug repurposing of atenolol for use in other types of cancer should be explored.

Conclusion

In conclusion, we performed functional network analysis using the databases STITCH; STRING; Database for Annotation, Visualization and Integrated Discovery (DAVID); cBioportal; and KMPlotter to investigate the potential of atenolol for drug repurposing in pancreatic cancer. A molecular docking study revealed that atenolol may inhibit MMP9 and NF-κB signaling in pancreatic cancer cells. Therefore, atenolol has the potential to be repurposed in pancreatic cancer therapy by targeting MMP9 and NF-κB signaling. The results of this study need to be further validated in vitro and in vivo.

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Data availability All data generated or analysed during this study are included in the Additional Files of this article.

Compliance with ethical standards

Competing of interests The authors declare that they have no no competing interests.

Ethical approval Not applicable.

Consent for publication All authors agree to publish our manuscript.

Abbreviations *ADRB1,* β-Adrenergic 1 Receptor; *ADRB2,* β-Adrenergic 2 Receptor; *AMPs,* Atenolol-mediated proteins; *DAVID,* Database for Annotation, Visualization and Integrated Discovery; *DTPs,* Direct target proteins; *GO,* Gene Ontology; *ITPs,* Indirect target proteins; *KEGG,* Kyoto Encyclopedia of Genes and Genomes; *MMP,* Matrix metalloproteinase; *PPI,* Protein–Protein Interaction; *SNP,* Single-Nucleotide Polymorphism; *STAT3,* Signal Transducer and Activator of Transcription 3

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