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Original Article

Angiotensinogen, a promising gene signature for rectum and stomach adenocarcinoma patients

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Abstract: Objectives: Angiotensinogen (AGT), as a component of the renin-angiotensin system (RAS), is one of the major risk factors for cancer development. To date, there has not been a systematic pan-cancer analysis of AGT. Methods: This pan-cancer study comprehensively investigated AGT in 24 different cancers based on the UALCAN, KM plotter, GENT2, HPA, MEXPRESS, cBioportal, STRING, TIMER, and CTD databases. Results: The results showed that AGT was highly expressed in most tumors, and AGT overexpression may be related to the worst survival of Rectum adenocarcinoma (READ) and Stomach Adenocarcinoma (STAD) patients only. Furthermore, pathway analysis indicated that AGT-associated genes are involved in six critical pathways. Moreover, the higher expression of AGT was found to be detrimental to the promoter methylation level ($P<0.05$), immune cells infiltration ($P<0.05$), and genetic alterations. We have also predicted various chemotherapeutic drugs contributing to the expression regulation of AGT. Conclusion: Our results together support that AGT is a possible biomarker for READ and STAD.

Keywords: AGT, diagnostic, prognostic, biomarker, READ, STAD

Introduction

Cancer is a broad term that represents a large group of diseases that can damage multiple organs of the body [1]. This disease has been recognized as the 2nd leading cause of death following cardiovascular diseases worldwide and accounts for a total of 13% worlds' death burden [1]. It is estimated that a total of 18 million cancer cases and approximately 9.6 million cancer-associated deaths may occur in 2022 [2].

Smoking is the most common risk factor for cancer development, accounting for approximately 22% of all cancer cases [3]. Viral infections, which cause approximately 25% of cancers in low and middle-income countries, also contribute to the development of this disease [3]. Despite recent advances in reducing cancer risk factors and improving cancer detection and treatment methods, cancer incidence, morbidity, and mortality are still increasing at an alarming rate [4]. Thus, cancer-causing potential mechanisms and underlying pathways, still need to be investigated thoroughly.

The AGT gene encodes a protein known as angiotensinogen [5]. This protein is important in the renin-angiotensin system, which regulates blood pressure and maintains fluid and salt balance in the body. Initially, in the renin-angiotensin system, the angiotensinogen is converted into angiotensin I. Then, angiotensin I is converted into angiotensin II, which narrows down (constricts) the blood vessels to raise blood pressure (BP). Angiotensin II is also involved in the production of the aldosterone hormone, which stimulates the salt and water absorption in the kidneys and, therefore, increases the amount of body fluids [5]. In addition, angiotensin II also plays an essential role in the develop-

ment of the kidney by activating different growth factors involvingin the kidneys' structure development [6]. To date, not many studies have reported the roles of AGT in breast and endometrial cancers. For instance, one study reported the up-regulation of AGT in breast tumor and suggested that AGT silencing in breast tumor cells can improve the tumor response to various immune checkpoint inhibitors [7]. Similarly, *Rhodes et al.* ranked AGT gene as one among the highly overexpressed genes in patients with breast cancer using the microarray technique [8]. However, the decrease in AGT expression across breast cancer via the action of cytosolic mRNA-binding proteins hass also been reported previously [9]. *Pringle et al.* reported the high prevalence of AGT polymorphism (rs5186) and linked it with the overexpression of AGT in endometrial cancer [10]. To our knowledge, the expression profiling of AGT via pan-cancer analysis has not been done yet.

In our research, the expression of AGT and further correlations between its expression and different other parameters across different cancers have been explored via publicly accessible authentic databases and Bioinformatics tools.

Methods

Ethics statement

Because the current study strictly followed the online database publication guidelines, approval from an ethics committee was not required.

Ualcan

The UALCAN database [11] was searched in this study to document the mRNA expression of the AGT across various human cancers.

KM plotter

The AGT gene was quarried in the KM plotter tool [12] tool to obtain the overall survival (OS) curves across different cancers. Hazard ratios (HR) with 95% confidence intervals (CI) were also determined and showed over the graphs.

GENT2 and GEPIA

GENT2 and GEPIA databases [13, 14] were searched in the current study for further validating the expression of AGT in different cancers.

Human protein atlas (HPA)

The HPA database [15] aims to provide cancer multi-omics data across several cancer subtypes. In this study, HPA was queried to analyze AGT expression levels at the protein level in different cancer tissues paired with normal control.

Mexpress

The MEXPRESS database focuses on analyzing associations among promoter methylation and gene expression levels [16]. Via this database, the correlations among AGT promoter methylation and mRNA expression levels were explored across different cancers.

cBioportal

The cBioPortal database is a repository of multi-omics data obtained from nearly 240 cancer-related pieces of research [17]. In this study, via this tool, AGT genetic mutations were evaluated using TCGA project-based datasets of different cancers.

A PPI network and enrichment analysis

The STRING database focuses on constructing PPI networks [18]. Via this database, we obtained a PPI network of the AGT enriched genes in this study. Furthermore, the obtained PPI network was also visualized using Cytoscape [19] and the pathway analysis of the PPI network genes was carried out using DAVID [20].

Gene-immune analysis

The TIMER database [21] was queried in this study to figure out correlations using Spearman analysis between the levels of infiltrating CD8+ T immune cells and AGT expression across different cancers.

Screening of AGT-associated chemotherapeutic drugs

AGT was queried in the Comparative Toxicogenomics Database (CTD, http://ctdbase.org/) [22] during the current study to identify various chemotherapeutic drugs capable of altering the expression of AGT.

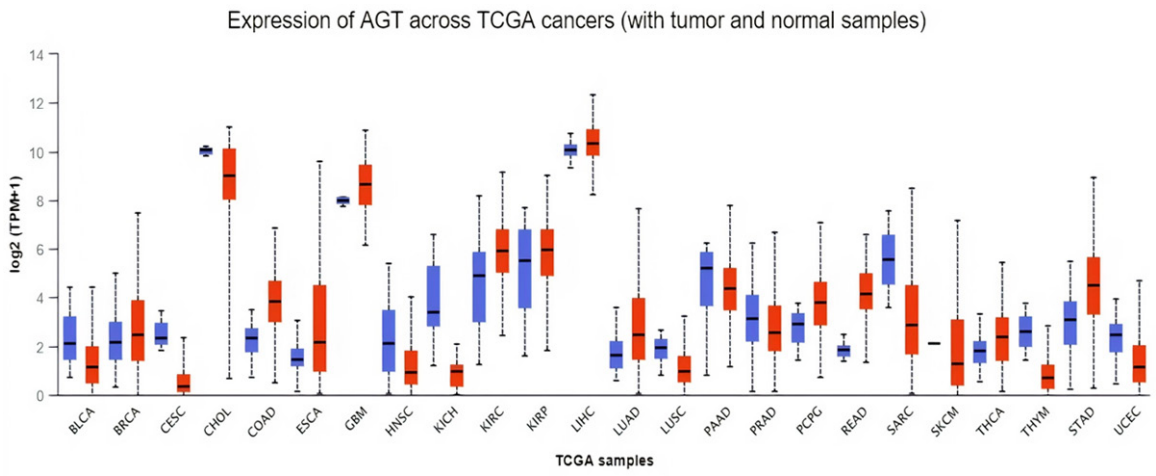

Figure 1. The pattern of AGT expression in different human cancers via UALCAN. A *p*-value <0.05 was used to indicate the significant scores.

Statistical analysis

A t-test was used to evaluate differences in AGT expression in normal and cancer tissues via UALCAN. The Mann-Whitney U test was implemented for IHC results analysis via HPA database. The correlation of gene expression was analyzed using Spearman's correlation. What's more, P<0.05 was considered statistically significant.

Results

Expression profiling of AGT

In 24 types of tumor tissues paired with normal controls, the expression profiling of AGT was done using UALCAN. In view of the analysis results, AGT expression was enriched in all the analyzed cancer tissues and found to be significantly (P<0.05) up-regulated in these cancer tissues than the normal controls, including Rectum adenocarcinoma (READ) and Stomach Adenocarcinoma (STAD) (**Figure 1**).

AGT effect on the survival

As shown in **Figure 2**, AGT overexpression was found to be obviously associated with the worst OS durations of the READ (HR = 2.38, 95% CI: 1.1-3.36, P = 0.024), and STAD (HR = 1.62, 95% CI: 1.17-2.25, P = 0.0033-04) patients out of the analyzed 24 total cancers. Altogether, this data suggests that AGT might have a significant contribution to the development and progression of READ and STAD.

Correlations between pathological variables and AGT

The AGT expression data related to different pathological variables of READ and STAD was taken from UALCAN. The retrieved data revealed that AGT was also significantly (P<0.05) overexpressed in READ and STAD patients stratified based on patient gender, nodal metastasis, and patient body weight relative to controls (**Figure 3**). A clinicopathological characteristics-wise READ and STAD sample classification is summarized in **Tables 1** and **2**.

Verification of AGT expression in new independent cohorts

In order to further verify the up-regulation of AGT in READ and STAD, we analyzed the AGT expression in READ, STAD, and normal samples of new independent cohorts via GENT2 and GEPIA. The results of this analysis were consistent with those of UALCAN, highlighting the significant (P<0.05) overexpression of AGT in READ and STAD samples relative to the corresponding controls (**Figure 4**).

Status of AGT expression at the protein level

Following AGT expression profiling at the mRNA level in READ and STAD, its expression at protein the level was measured with the help of the HPA database. The retrieved data from HPA showed that AGT was not detected in normal rectum and stomach tissues, whereas its low expression was found in the cancer tissues of

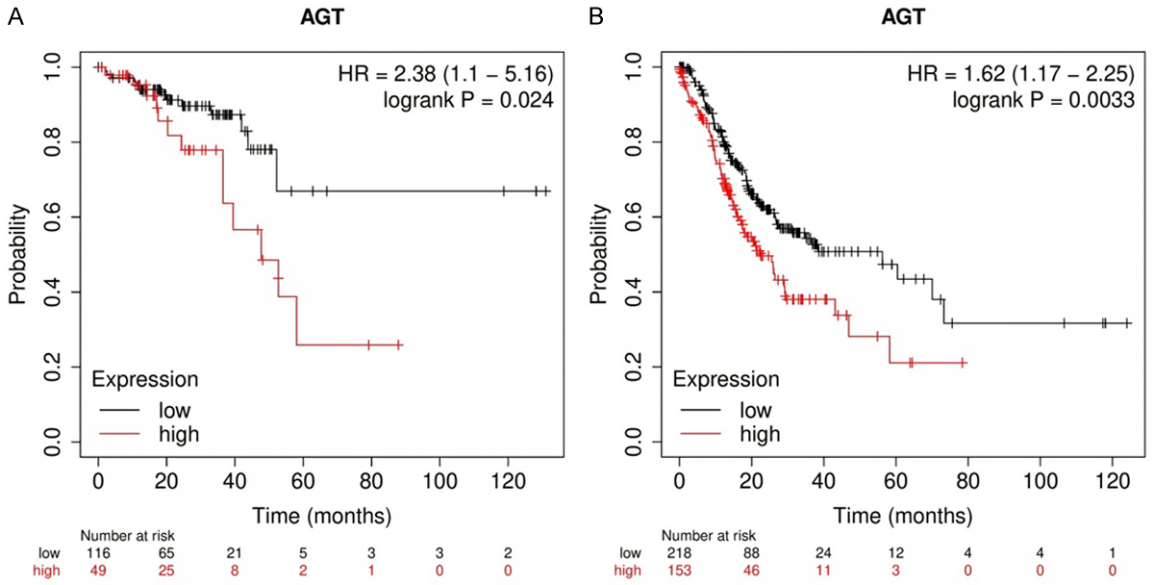

Figure 2. KM plotter based OS analysis of the AGT in distinct types of cancers. (A) in READ, and (B) in STAD. A *p*-value <0.05 was used to indicate the significant scores.

the rectum and stomach relative to the normal controls (**Figure 5**). Collectively, these results also suggested the slight overexpression of AGT protein in the READ and STAD samples.

Promoter methylation analysis of the AGT

According to earlier studies, abnormal methylation of the promoter regions of functional genes can cause cancer [23]. Therefore, to explore the hinge of AGT expression, we investigated AGT promoter methylation status in RAED and STAD samples paired with controls using MEXPRESS. According to the results, the obtained probe-specific methylation values in RAED and STAD samples have shown significant (P<0.05) negative correlations between AGT expression and its promoter methylation levels (**Figure 6**). Therefore, it is speculated that AGT is hypomethylated in the READ and STAD samples.

Genetic mutations analysis of AGT

Information related to AGT genetic alterations including (amplification, deletion, mutation, and fusion) in READ and STAD was obtained from two different TCGA datasets; Rectum Adenocarcinoma, and Stomach Adenocarcinoma. The retrieved information revealed that AGT mutate in a small proportion (1.7% and 1.1% samples) of the analyzed READ and STAD samples, respectively, having deep amplification as the major genetic abnormality (**Figure 7**).

A PPI network and enrichment analysis

The obtained PPI network of AGT via STRING revealed that there are a total of 10 genes that physically interact with AGT (**Figure 10**). Further pathway analysis of these AGT-associated genes via DAVID tool revealed that most of these genes were significantly involved in different critical pathways including Renin-angiotensin system, Renin secretion, and Adrenergic signaling in cardiomyocytes (**Figure 8**; **Table 3**).

Gene-immune analysis

The discovery of correlations between CD8+ T immune cell infiltration and gene expression has paved the way for better cancer immunotherapies [24]. So, in our study, we used TIMER to determine the relationships between CD8+ T immune cell infiltration and AGT in READ and STAD. Results revealed significant (P<0.05) positive correlations between the CD8+ T immune cells infiltration and mRNA expression of AGT in READ and STAD (**Figure 9**).

Screening of AGT-associated chemotherapeutic drugs

We discovered that AGT expression can be regulated by a variety of drugs based on the gene-

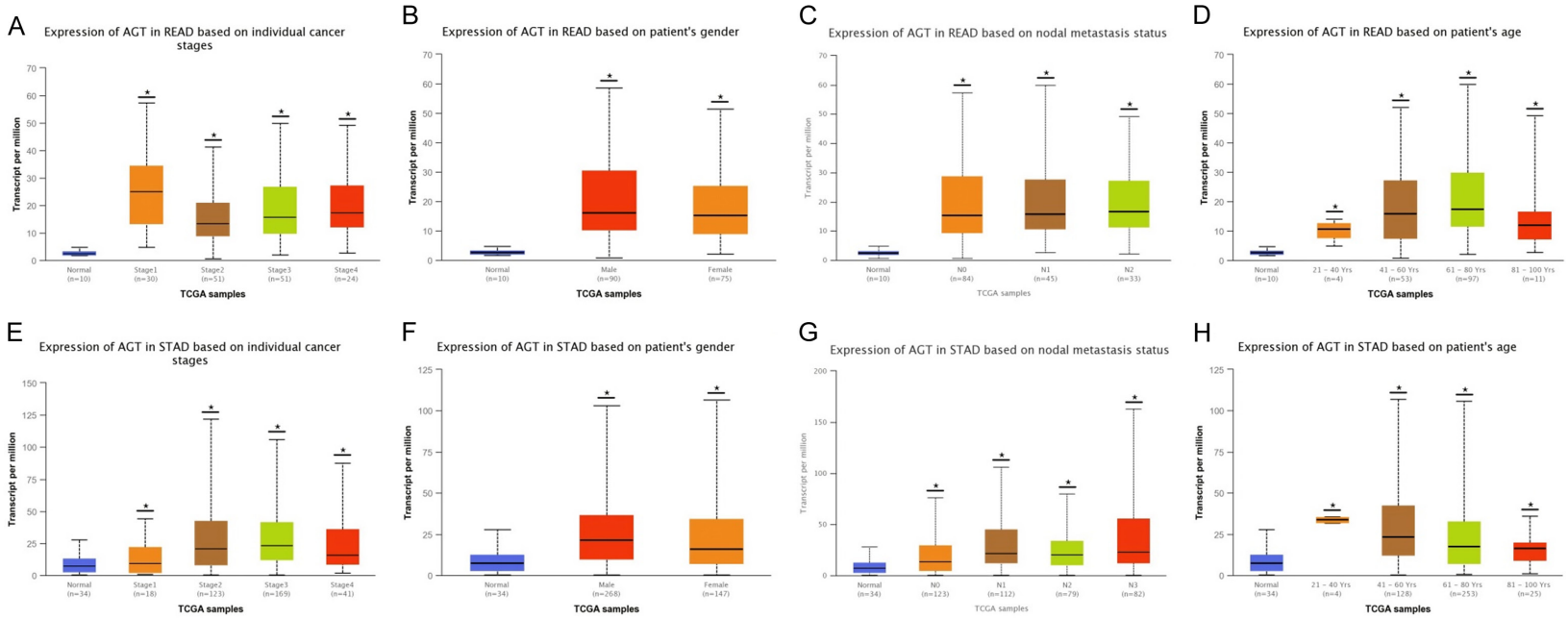

Figure 3. Different clinicopathological features specific expression analysis of the AGT in distinct types of cancers. (A) Individual cancer stage specific expression analysis of the AGT in READ, (B) Patient's gender specific expression analysis of the AGT in READ, (C) Nodal metastasis specific expression analysis of the AGT in READ, (D), Patient's age specific expression analysis of the AGT in READ, (E) Individual cancer stage specific expression analysis of the AGT in STAD, (F) Patient's gender specific expression analysis of the AGT in STAD, (G) Nodal metastasis specific expression analysis of the AGT in STAD, and (H), Patient's age specific expression analysis of the AGT in STAD. A *p*-value <0.05 was used to indicate the significant scores.

Table 1. Clinicopathological variable-wise distribution of READ samples

Sr. No.	Parameter	No. of samples per parameter	Sum of samples	Sum of excluded samples having no details	Total number no of samples undertaken analysis
1	Cancer stage distribution		165	109 (n) or (66 %)	56 (n) or (44%)
	Stage 1	30			
	Stage 2	51			
	Stage 3	51			
	Stage 4	24			
2	Gender distribution			0 (n) or (0%)	165 (n) or (100%)
	Male	90			
	Female	75			
3	Nodal metastasis status distribution			4 (n) or (2.4%)	161 (n) or (97.6%)
	N0	84			
	N1	45			
	N2	33			
4	Body weight based distribution			0 (n) or (0%)	165 (n) or (100%)
	20-40 years	04			
	41-60 years	53			
	61-80 years	97			
	81-100 years	11			

Table 2. Clinicopathological variable-wise distribution of STAD samples

Sr. No.	Parameter	No. of samples per parameter	Sum of samples	Sum of excluded samples having no details	Total number no of samples undertaken analysis
1	Cancer stage distribution		415	64 (n) or (15.4%)	351 (n) or (84.6%)
	Stage 1	18			
	Stage 2	123			
	Stage 3	169			
	Stage 4	41			
2	Gender distribution			0 (n) or (0%)	415 (n) or (100%)
	Male	268			
	Female	147			
3	Nodal metastasis status distribution			11 (n) or (2.6%)	404 (n) or (97.4%)
	N0	123			
	N1	112			
	N2	169			
4	Body weight based distribution			05 (n) or (1.2%)	410 (n) or (98.8%)
	20-40 years	04			
	41-60 years	128			
	61-80 years	253			
	81-100 years	25			

drug interaction network created using CTD and Cytoscape. For example, bisphenol A and aldosterone can elevate AGT expression, while biopterin and cyclosporine can reduce AGT expression level (**Figure 10**).

Discussion

Cancer is a multifactorial disease, and its distinguish features are better described as cancer hallmarks [25]. Earlier studies have report-

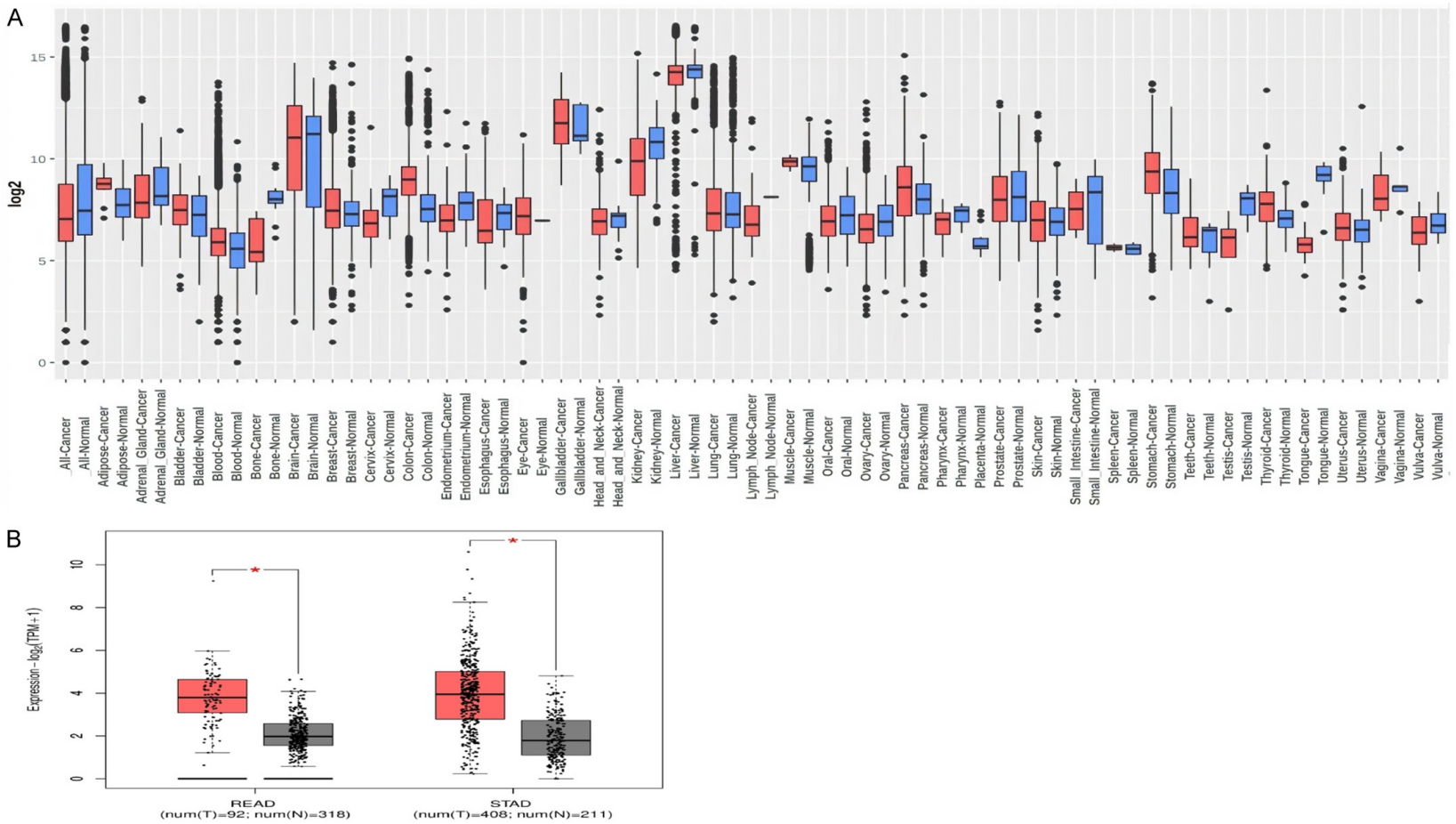

Figure 4. Transcription expression level validation of AGT using independent READ and STAD cohorts via GENT2 and GEPIA database. (A) Via GENT2, and (B) Via GEPIA. A *p*-value <0.05 was used to indicate the significant scores.

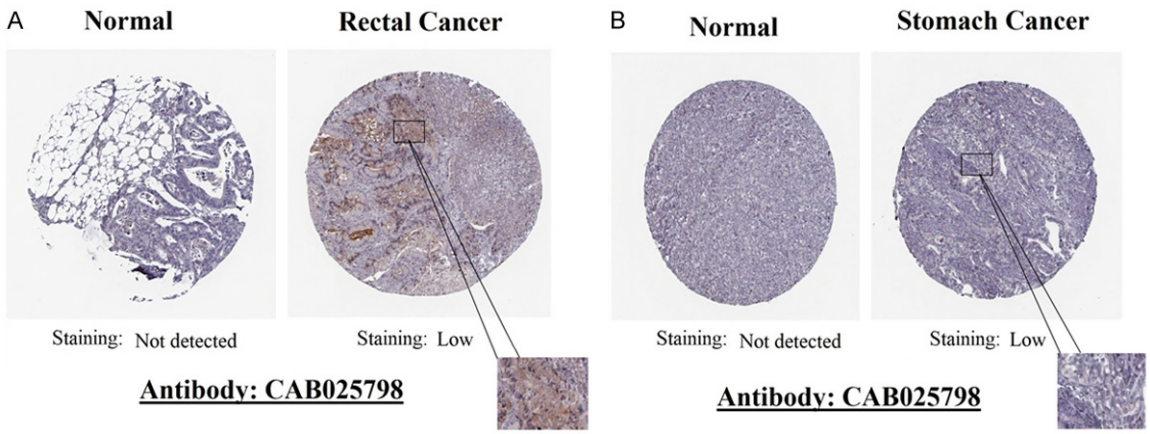

Figure 5. Translation expression of AGT across rectal cancer, stomach cancer, and normal controls taken from the Human Protein Atlas (HPA) database (× 200). (A) rectal cancer, and (B) stomach cancer.

ed six different types of cancer-associated hallmarks, including [25]: (i) cancer cells stimulate their growth, (ii) they thwart inhibitory signals that would harm their growth, (iii) they avoid programmed cell death, (iv) they promote the growth of blood vessels that provide them nutrients to grow further, (v) they are capable of multiplying, and (vi) move out to other body organs to spread the disease. Additionally, four new characteristics have also been identified that are associated with cancer cells [26]. These are: (i) cancer-associated signaling pathways regulate abnormally, (ii) cancerous cells survive the immune system, (iii) structural and numerical abnormalities occur in the chromosomes across cancer cells, and (iv) there is inflammation at the cancer site. In order to divide and spread out, a cancer cell has to acquire functional capabilities through the activation of different hallmarks during cancer progression [27].

Cancer remains a major threat to human survival worldwide, despite significant advances in early detection and accurate treatment. As a result, the discovery of common biomarkers that could be used for cancer detection, prognosis prediction, and treatment without serious complications has been the major objective for physicians and scientists in this field [28].

ATG belongs to the very diverse renin-angiotensin system (RAS) which is responsible for regulating blood pressure. Earlier, only a few studies have reported the AGT's role in a limited number of cancers. For example, *Shimomoto et al.* predicted its role in CRC metastasis [29]. Another study has reported the up-regulation of AGT in breast tumor and suggested it as a good chemotherapeutic target to improve survival of the breast tumor patients [7]. Similarly, *Rhodes et al.* have demonstrated that AGT gene is one among the highly overexpressed genes in patients with breast cancer [8]. Moreover, the decrease in AGT expression across breast cancer via the action of cytosolic mRNA-binding proteins has also been reported earlier [9]. To our knowledge, not many studies have been studied regarding AGT's role in distinct other major types of human cancers. In this study, we examined AGT expression in 24 main types of human cancers to identify a few AGT-associated specific types.

AGT was found to be up-regulated in the majority of many types fo human cancers. We then investigated the relationship between AGT expression and prognostic values in various cancers, and our findings showed that AGT overexpression was significantly ($P<0.05$) correlated with the worst OS durations in READ and STAD patients. Taken together, these findings depict that AGT might have played a crucial role in the initiation and progression of READ and STAD. More analysis also revealed that AGT was significantly ($P<0.05$) up-regulated in READ and STAD patients of various clinicopathological features, including individual cancer stages, patient's gender, nodal metastasis, and patient's body weight relative to the normal controls.

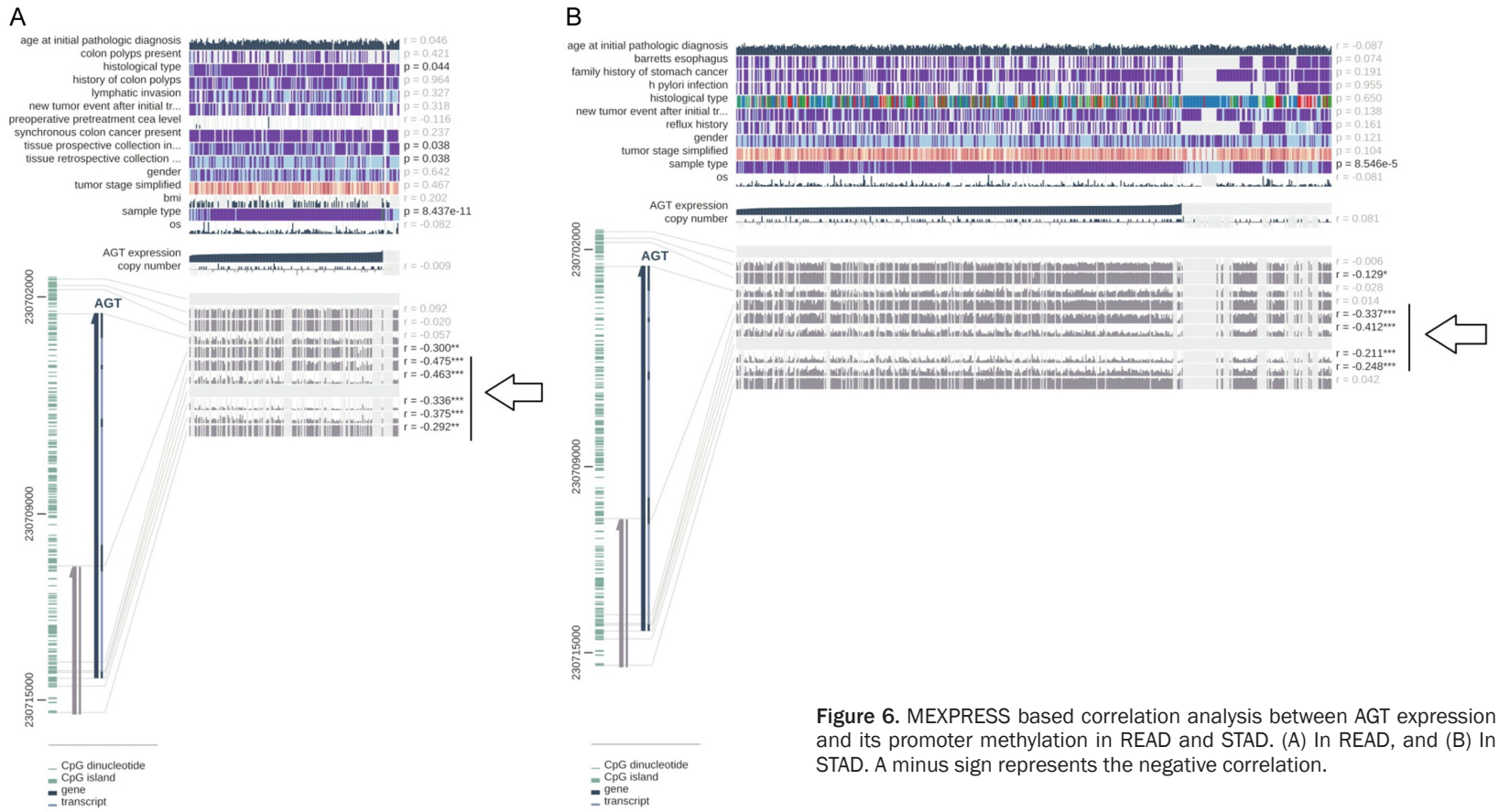

Figure 6. MEXPRESS based correlation analysis between AGT expression and its promoter methylation in READ and STAD. (A) In READ, and (B) In STAD. A minus sign represents the negative correlation.

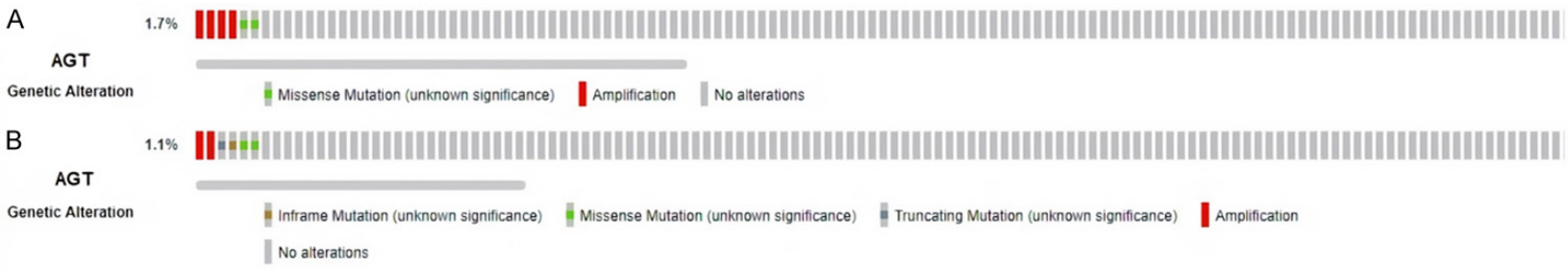

Figure 7. Genetic alterations analysis of the AGT in TCGA READ, and STAD datasets, (A) AGT genetic alterations analysis in the TCGA READ dataset, and (B) AGT genetic alterations analysis in the TCGA STAD dataset.

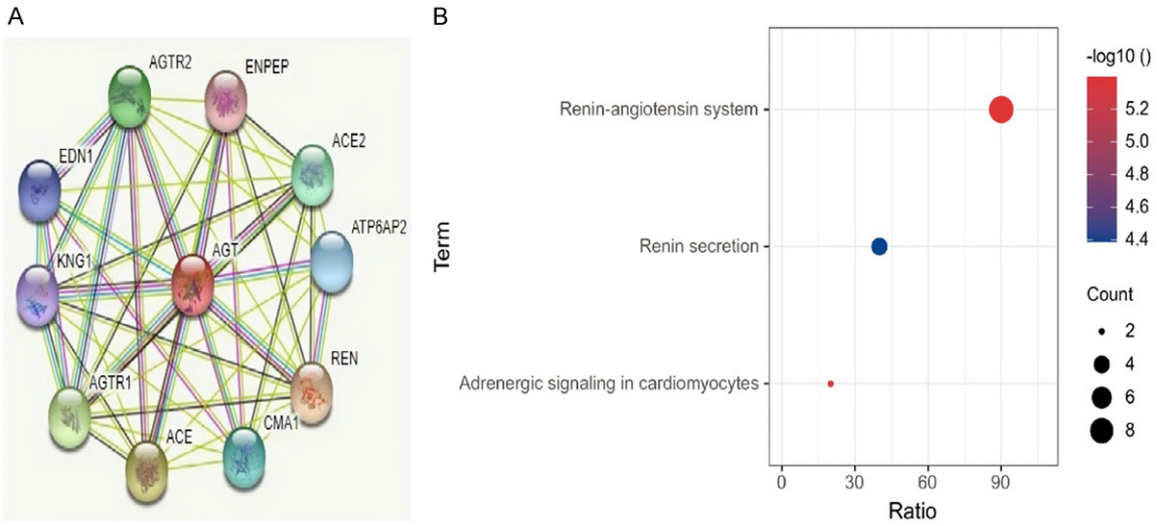

Figure 8. A PPI network and pathway analysis of AGT-associated genes. (A) A demonstration of the PPI network, and (B) A heatmap of AGT-associated genes enriched pathways.

Concerning the causes of AGT overexpression in READ and STAD, we evaluated the correlations between AGT overexpression and its promoter methylation level, CNVs, and genetic mutations in READ and STAD. AGT was found to be enriched in the deep amplification abnormality in an insignificant proportion of READ (1.1%) and STAD (1.2%). Hence, it is obvious that genetic alterations are not responsible to disturb the expression of AGT in READ and STAD patients. Furthermore, the results of AGT promoter methylation analysis revealed a negative correlation between AGT expression and promoter hypomethylation. Hence, we speculate that promoter hypomethylation is the main cause of AGT up-regulation in READ and STAD.

Various molecular biomarkers have been studied to date for the detection and prognosis of READ including expression and mutational profiling of APC, KRAS, TP53, CTNNB1, SRC, SMAD4, SMAD2, DCC, hMESH2, hMLH1, hPMS1, hPMS2, hMSH6, MSI, and IGF-1 genes in the serum and stool specimens of the READ patients [30]. However, to our knowledge, none of these and any other biomarker has been explored so far that could be generalized in the RAED patients of various clinicopathological features. In the present study, we showed the significant ($P<0.05$) up-regulation of AGT expression in READ patients with various clinicopathological features as compared to the normal controls. Furthermore, AGT promoter OS information has also proven its useful values as a novel potential prognostic biomarker of READ patients.

Currently, expression variations in different genes like the up-regulation of ESR1, CLTC, TP53, HUWE1, EWSR1, PCMT1, and HDAC1 genes have been suggested as the reliable diagnostic and prognostic STAD biomarkers [31]. Moreover, the serum up-regulation of miR-125b-5p and miR-196a-5p has also been suggested as good diagnostic biomarkers of STAD by *Chen at al.* [32]. Similar to READ, none of these or any other biomarker has been explored so far that could be generalized in the STAD patients of various clinicopathological features. However, in the present study, we revealed the significant ($P<0.05$) up-regulation of AGT expression in STAD patients with various clinicopathological features (individual cancer stage, patient gender, nodal metastasis, and patient body weight) as compared to the normal controls. We have also shown that AGT overexpression was associated with the decreased OS of STAD patients. This data has also supported AGT up-regulation as a novel diagnostic and prognostic biomarker of STAD.

CD8+ T immune cells infiltration are the most essential part of the immunotherapy [33, 34]. The observed correlations between these cells, and AGT could lead to new treatment options for READ and STAD patients. Furthermore, the pathway enrichment analysis of AGT-associated genes revealed their significant involvement

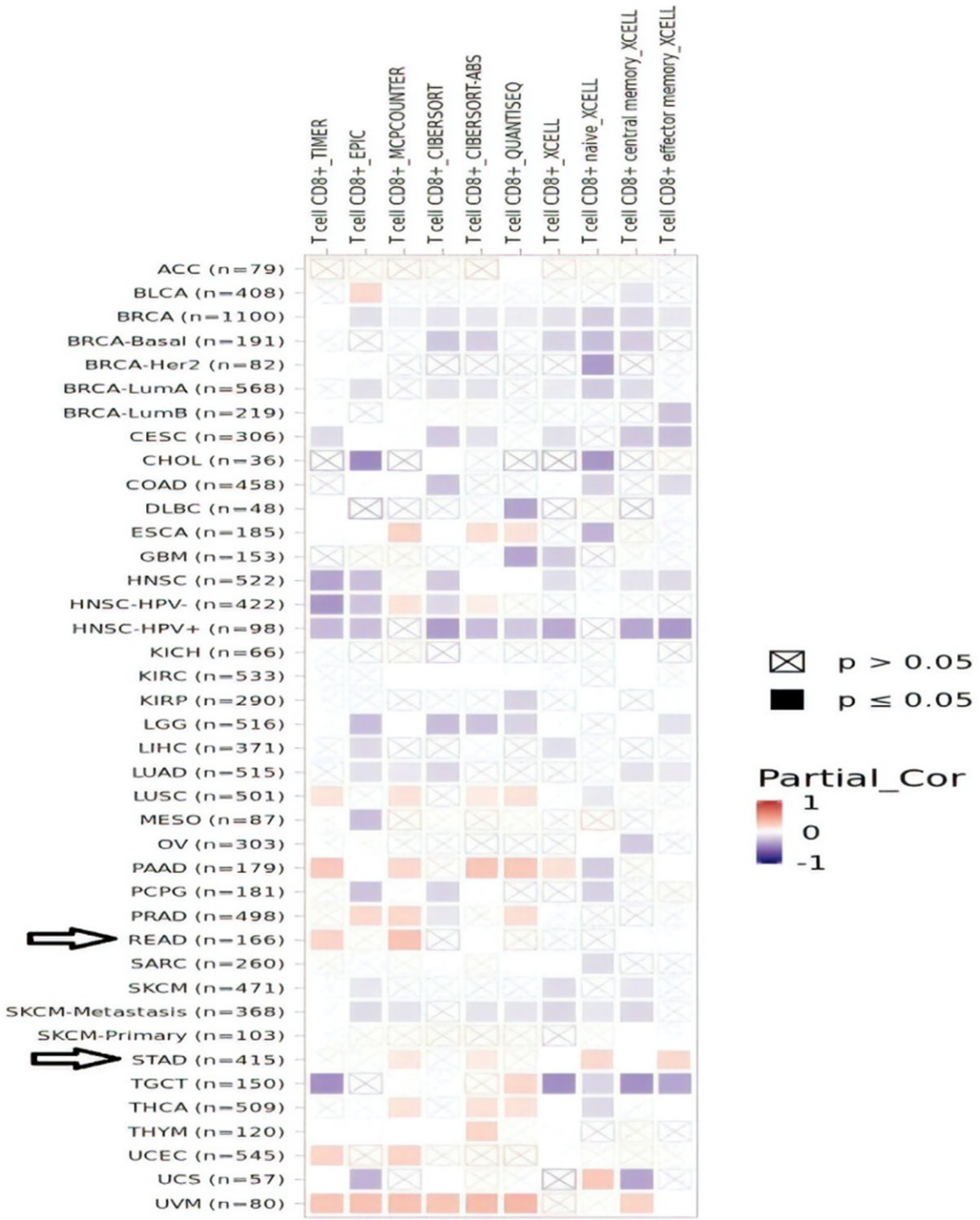

Figure 9. TIMER based Spearman correlational analysis between the AGT expression and CD8+ T immune cells level in READ and STAD. A *p*-value <0.05 was used to indicate the significant scores.

in various signaling pathways, including "Renin-angiotensin system", "Renin secretion, and "Adrenergic signaling in cardiomyocytes". Finally, this study also explored a few AGT-associated potential drugs for the treatment of READ and STAD.

Conclusion

In conclusion, we analyzed and validated the overexpression of AGT in READ and STAD that would be helpful in diagnosing and predicting the prognosis of READ and STAD patients with

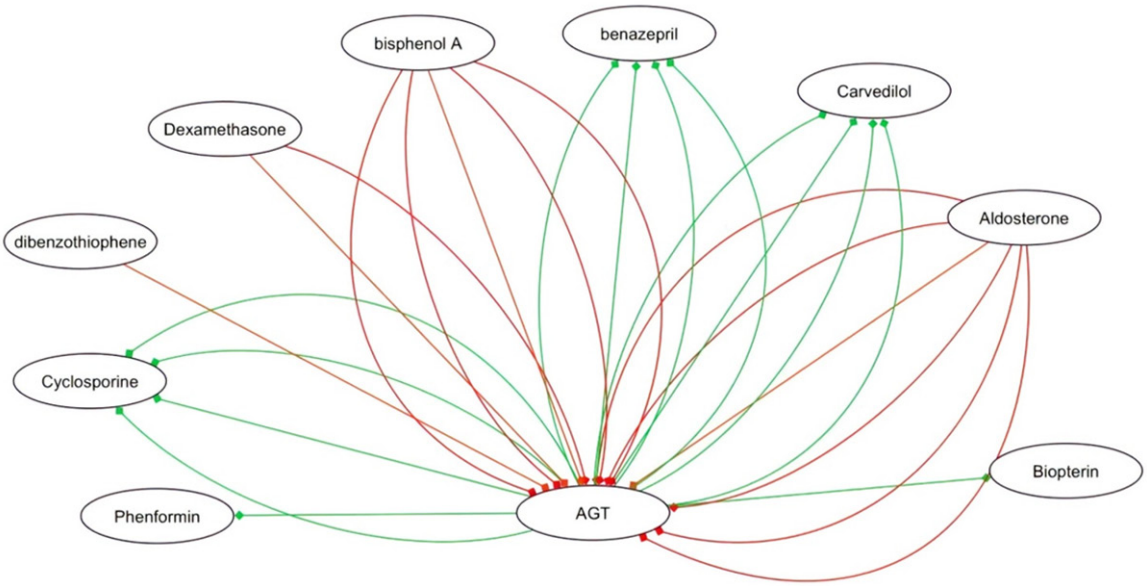

Figure 10. Gene-drug interaction network of AGT. Red arrows: chemotherapeutic agents that can increase the expression of AGT; green arrows: chemotherapeutic agents that can decrease the expression of AGT. The count of arrows in this network between chemotherapeutic drug and gene represents the number of researches that have supported the interaction.

Table 3. AGT enriched genes pathway analysis

Pathway ID	Pathway Name	Gene count	*P*-value	Gene name
hsa04614	Renin-angiotensin system	9	<0.05	ACE2, ENPEP, ACE, CMA1, ATP6AP2, AGTR1, REN, AGTR2, AGT
hsa04924	Renin secretion	4	<0.05	ACE, AGTR1, REN, AGT
hsa04261	Adrenergic signaling in cardiomyocytes	2	<0.05	AGTR1, AGTR2

different clinicopathological features. However, additional large-scale testing is recommended before clinical application.

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Disclosure of conflict of interest

None.

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