

RESEARCH ARTICLE

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Integrative Analysis of the AMPK subunits in Colorectal Adeno Carcinoma

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Abstract

Background: The 5-adenosine monophosphate (AMP)-activated protein kinase (AMPK) is an emerging cancer treatment and therapeutic target. Due to the enzyme's complexity and dual nature, it is a confounding target in the treatment strategy. The study aimed to conduct an integrative analysis of the seven subunits and twelve isoforms of AMPK, which is not reported so far in colorectal adenocarcinoma patients. **Methodology:** The web-based tools UALCAN, Timer 2.0, KM Plotter, cBioPortal, COSMIC, and STRING were used to investigate the differential expression of AMPK subunits, protein-level Expression, promoter methylation status, survival analyses, Enrichment analysis, and protein-protein interaction. **Results:** The mRNA expression of AMPK subunits are upregulated in Colorectal Adeno Carcinoma (COAD), while the protein expression is comparatively reduced in colon tumors. The protein-level expression of $\alpha 2$ and $\beta 2$ is decreased significantly in COAD patients. The $\gamma 3$ subunit in colon tumor is hypermethylated. The study also reports that Liver Kinase B1 mutation in 7% of CRC patients, which might be the reason for downregulation of the gene and the protein expression of AMPK subunits in COAD. **Conclusion:** The Overall analysis of the subunits affirms that AMPK expression is beneficial in cancer.

Keywords: AMPK- Colorectal cancer- differential gene expression- protein expression- survival analysis

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Introduction

Colorectal Cancer (CRC) is the third most commonly diagnosed cancer which, accounts for 10% of global cancer incidence and 9.4% of cancer deaths in 2020, slightly behind lung cancer, which accounts for 18% of fatalities (Lu et al., 2021; Morris et al., 2018). Based on projections of aging, population expansion, and human progress, the global number of new CRC cases is expected to reach 3.2 million in 2040. The increased prevalence of CRC is primarily due to increased exposure to environmental risk factors because of a shift in lifestyle and food towards westernization (WHO, 2020).

The energy homeostasis guardian, 5-adenosine monophosphate (AMP)-activated protein kinase (AMPK), is a well-known serine-threonine kinase family member. All eukaryotes have genes that code for AMPK subunits, making it an evolutionarily conserved set of enzymes. It is a heterotrimeric enzyme complex having catalytic α and regulatory β and γ subunits that are required for the AMPK to function (Guy and Hardie, 1981; Richter and Ruderman, 2010). α Subunit has two isoforms, $\alpha 1$ and $\alpha 2$, encoded by two distinct genes, PRKAA1 (Protein Kinase AMP-Activated Catalytic Subunit Alpha 1) and

PRKAA2 (Protein Kinase AMP-Activated Catalytic Subunit Alpha 2) in mammals. The N-terminus of the α subunit has a conventional Ser/Thr kinase domain (López et al., 2016). The kinase domain has a region called the activation loop or T-loop. The conserved Thr residue in this region should be phosphorylated to activate the complex. The C-terminal region of the α subunit forms a complex with the β and γ subunits (D Grahame Hardie, 2013). β Subunit has two isoforms, $\beta 1$ and $\beta 2$, encoded by different genes PRKAB1 (Protein Kinase AMP-Activated Catalytic Subunit Beta 1) and PRKAB2 (Protein Kinase AMP-Activated Catalytic Subunit Beta 2), respectively. β Subunit is the protein scaffold of the AMPK complex since the C-terminal region of the β Subunit interacts with the α and γ subunits (Grahame Hardie et al., 2012).

There are three isoforms for the γ subunit $\gamma 1$, $\gamma 2$, and $\gamma 3$, encoded by PRKAG1, PRKAG2, and PRKAG3 (Protein Kinase AMP-Activated Catalytic Subunit $\gamma 1$, $\gamma 2$, $\gamma 3$), respectively. γ subunits have four cystathionine β synthase sequences (CBS motifs). CBS1 and CBS3 are the binding sites for ATP, ADP, or AMP which binds competitively, depending on the cellular energy status. CBS2 and CBS4 strongly bind to AMP (Adenosine Mono Phosphate) and play the structural role (Steinberg and

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Carling). AMP, when attached to site 1, leads to activation, and site 3 regulates the phosphorylation state of Thr172 (Hardie and Alessi, 2013).

The study aimed to analyze the expression of AMPK subunits in normal and primary tumors in each stage of Colorectal adenocarcinoma (COAD), expression during nodal metastasis, association with patient weight, and protein-level expression. The study also analyses the Overall Survival of AMPK subunits in Gastric Cancer patients.

Materials and Methods

The publicly available datasets are used for the analysis
Colorectal Cancer Patient Data analysis

The metastatic CRC patient data was collected from cBio Cancer Genomics Portal (<http://www.cbioportal.org>) based on the study of Memorial Sloan Kettering Cancer Center, Gastroenterology 2020. Four seventy-one patient data with unresectable CRC tumors were analyzed (accessed on January 23, 2022).

The Pan-Cancer analysis of AMPK subunits

The Pan-cancer differential expression (DE) of AMPK subunits in COAD and the normal tissues were studied using the TIMER 2.0 (<http://timer.cistrome.org/> accessed on January 18, 2022) (Li et al., 2020). The Gene-DE module in the cancer exploration component was used for the analysis. Both the normal samples (n=41) and the tumor samples (n=451) were analyzed for the significant relation. The DE was generated as a box plot in which the significance was determined using edgeR (Tang et al., 2019; Warriar et al., 2021).

The differential gene and total protein expression analysis of AMPK subunits

UALCAN is a web tool that provides gene expression from The Cancer Genome Atlas (TCGA) which was used for the DE analysis. The protein expression analysis used data from Clinical Proteomic Tumour Analysis Consortium (CPTAC) Confirmatory/Discovery dataset (<http://ualcan.path.uab.edu/index.html>) (Accessed on November 13, 2021). Further analysis was done to investigate the expression in individual cancer stages, the association with the weight of the patient, and the nodal metastatic status. The screening conditions set in this study were “Gene: PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2, PRKAG3”; “Analysis Type: Tumor vs. Normal Analysis”; “Cancer Type: Colorectal Adenocarcinoma”; “Data Type: TCGA and CPTAC dataset. The expression of the genes on average (n=41) was compared to the patient samples (n=286). The sample size for protein expression was normal=100 and primary tumor=97. The analysis was done using the student’s t-test. For each cancer type, Z-values denote standard deviations from the median across samples. CPTAC’s Log2 Spectral Count Ratio data were first standardized within each sample profile, then across samples (Chandrashekar et al., 2017).

Promoter methylation of AMPK subunits

The TCGA module of UALCAN was used to measure

the promoter methylation level of the AMPK subunits. The Box Whisker plot showed the promoter DNA methylation. The average beta value of the TCGA samples is shown in the graph. The significance was calculated using the student’s t-test, which considers the unequal variance. The methylation status was compared to normal (n=37) vs. primary tumor (n=313).

The ratio of methylation probe intensity to the sum of methylated and unmethylated probe intensity is known as beta values. The beta value is a number that ranges from 0 to 1. Unmethylation is shown by a value of zero, while complete methylation is indicated by a value of one. The beta values of CpG probes up to 1500 bp upstream of the gene’s start site are represented in the box plot (Chandrashekar et al., 2017).

Identification and analysis of the association of the top twenty mutated genes in CRC, Gene enrichment, and Protein-protein interaction

The top 20 genes mutated in CRC were taken from the Catalogue Of Somatic Mutations In Cancer (COSMIC) (<https://cancer.sanger.ac.uk/cosmic>) database, which provided the data by tissue type and histology. The query terms were Tissue selection: GIT; Sub tissue selection: includes all; histology also included all types like adenoma, carcinoid-endocrine tumor, carcinoma, and others and sub-histology types included adenocarcinoma, gastrinoma, hamartoma, etc. (Tate et al., 2019).

‘STRING’ (<http://string-db.org>) is a database that gathers, aggregates, and scores publicly available data to investigate possible functional protein interaction networks. The AMPK subunit genes, the prominent upstream and downstream members, and the top 20 mutated genes in CRC were selected to study the protein-protein interaction (Szklarczyk et al., 2019). ‘Metascape’ (<http://metascape.org/gp/index.html#/main/>) is a tool that combines information from more than 40 different knowledge bases with advanced capabilities such as interaction analysis, gene annotation, and member search. The Gene Ontology module was used to analyze the functional involvement of the top 20 mutated genes and the AMPK subunit genes in biological processes (BP), cellular components (CC), and molecular functions (MF), as well as KEGG pathways (Zhou et al., 2019).

Terms with a p-value of less than 0.05, a minimum count of three, and an enrichment factor of more than 1.5 (the enrichment factor is the ratio between the observed counts and the counts expected by chance) were gathered and classified into clusters based on membership commonalities. To account for multiple testing’s, p-values were determined using the accumulative hypergeometric distribution, and q-values were calculated using the Benjamini-Hochberg procedure. When doing hierarchical clustering on the enriched phrases, Kappa score was used as the similarity measure, and sub-trees with a similarity of > 0.3 were considered a cluster. The cluster is represented by the statistically most significant term within it (Szklarczyk et al., 2019).

Survival analysis

Kaplan Meier plotter (KM) (<https://kmplot.com/>)

analysis/), a web-based tool capable of examining the effect of 54,675 genes on survival using 10,461 cancer samples, was used to investigate the prognostic significance of the AMPK subunits. It has 5,143 breast, 1,618 ovarian, 2,437 lung, and 1,065 GC patients with a mean follow-up of 69/40/49/33 months, respectively. The research was based on mRNA expression median values. The patients were categorized based on the high- and low-expression groups. KM Plotter doesn't provide any particular group in CRC. So, the expression and analysis were done in Gastric cancer patients. The log-rank P-value and hazard ratio (HR) with a 95% confidence interval were used to calculate the overall survival (OS) of GC patients.

Results

Patient Data Analysis-cBioPortal

The cancer type details of 471 CRC patients manifest 73.7% of patients have colon adenocarcinoma, 20.4% have rectal adenocarcinoma, 2.8% have colorectal

adenocarcinoma, 2.1% have Mucinous Adenocarcinoma of colon and rectum, and 1.1% percent have Signet Ring Cell Adenocarcinoma of colon and rectum (Figure 1). The tumors were moderately differentiated in 73.2%, poorly differentiated in 25.7%, and well-differentiated in 1.1%. The overall survival rate of CRC patients was 66.0%, with 34% still alive. The primary site of metastasis of CRC is the Liver (63.5%), Lung metastasis ranks the next (38%), Bone metastasis is 7.2%, and metastasis to other sites represents 12.1% (Figure 2).

Pan-Cancer Analysis

The Pan-cancer analysis of the AMPK subunits didn't show any consistent upregulation or downregulation of the genes (Figure 3). The expression was highly variable according to the cancer types. The COAD patients showed significant upregulation in PRKAA1, PRKAA2, PRKAB2, PRKAG1, and PRKAG2 expression. PRKAG3 gene encoding $\gamma 3$ subunit expression was seen only in a few cancer types, including Head and Neck Squamous

Table 1. The Statistical Significance of DE, Promoter Methylation and Protein Expression of AMPK Subunits

Encoding Gene	DE of Subunits	Promoter methylation	Statistical significance protein expression
<i>PRKAA1</i>	4.29E-01	3.13E-06	1.3308E-07888
<i>PRKAA2</i>	1.39E-06	<1E-12	8.25E-19
<i>PRKAB1</i>	3.65E-01	6.20E-03	6.00E-05
<i>PRKAB2</i>	3.73E-02	4.56E-02	4.42E-19
<i>PRKAG1</i>	8.23E-04	2.30E-03	4.30E-05
<i>PRKAG2</i>	2.06E-06	4.47E-10	Not available
<i>PRKAG3</i>	Not expressed	Not expressed	Not available

PRKAA1, protein kinase AMP-activated catalytic subunit alpha 1, PRKAA2, protein kinase AMP-activated catalytic subunit alpha 2, PRKAB1, protein kinase AMP-activated catalytic subunit beta 1; PRKAB2, protein kinase AMP-activated catalytic subunit beta 2; PRKAG1, protein kinase AMP-activated catalytic subunit gamma 1; PRKAG2, protein kinase AMP-activated catalytic subunit gamma 2; PRKAG3: protein kinase AMP-activated catalytic subunit gamma 3

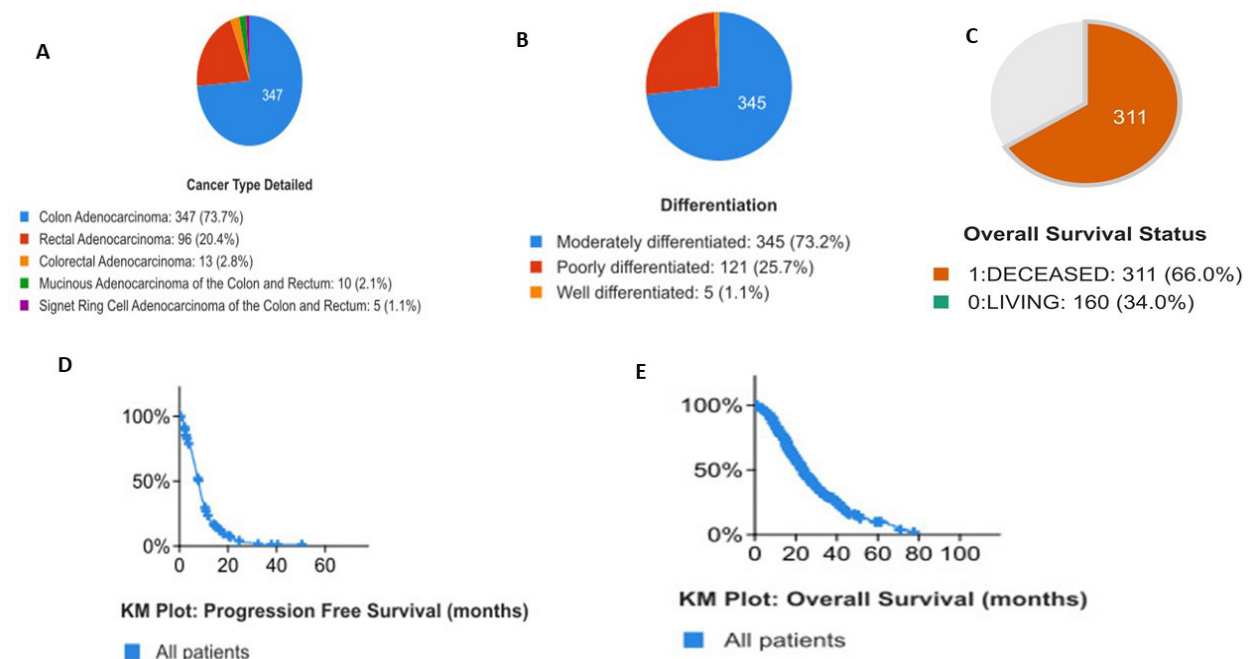


Figure 1. (A), The Types of Colorectal cancer; (B), The pattern of differentiation; (C), Overall Survival (OS) status of patients; (D), KM plots of progression-free survival; and OS (E) of 471 patients from TCGA datasets of cBioPortal.

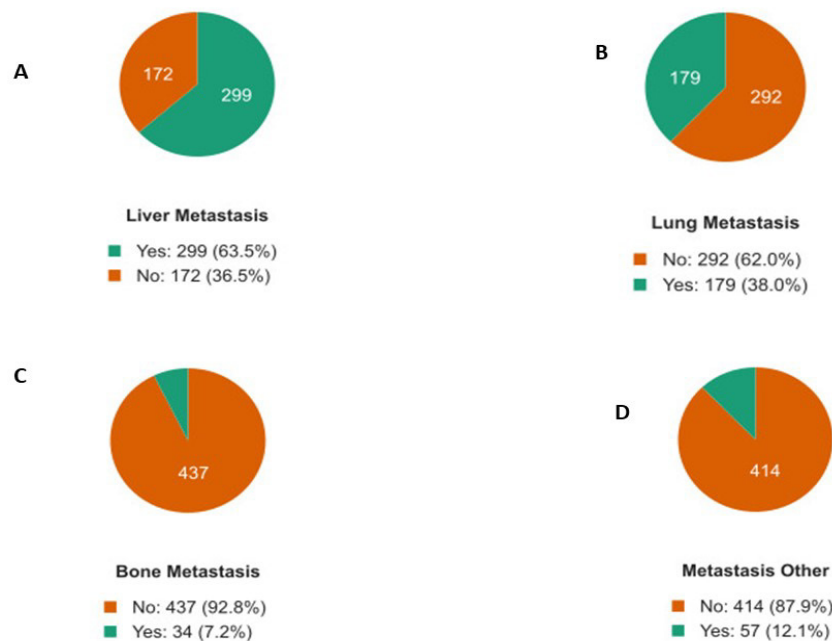


Figure 2. The Pie Diagram Showing the Metastasis Pattern of the 471 Colorectal Cancer Patients from TCGA Datasets.

Table 2. The Median Z-value for Protein Expression of AMPK Subunits

Encoding Gene	Median Z- value (Normal)	Median Z-value (Tumor)
<i>PRKAA1</i>	0.7	-0.043
<i>PRKAA2</i>	1.3	0.003
<i>PRKAB1</i>	0.4	0.029
<i>PRKAB2</i>	1.8	0.08
<i>PRKAG1</i>	0.96	0.015

PRKAA1, protein kinase AMP-activated catalytic subunit alpha 1; PRKAA2, protein kinase AMP-activated catalytic subunit alpha 2; PRKAB1, protein kinase AMP-activated catalytic subunit beta 1; PRKAB2, protein kinase AMP-activated catalytic subunit beta 2; PRKAG1, protein kinase AMP-activated catalytic subunit gamma 1; PRKAG2, protein kinase AMP-activated catalytic subunit gamma 2; PRKAG3, protein kinase AMP-activated catalytic subunit gamma 3

Cell Carcinoma (HNSC) (Figure 3E). The expression of the $\beta 2$ subunit was non-significant in COAD patients.

Differential Gene Expression of AMPK subunits

PRKAA1 gene expression was not affected by the tumor condition. The individual stages of cancer also showed a consistent gene expression level. The nodal metastasis also didn't affect the expression of the gene. The analysis of the DE based on the patient weight showed a significant result; the highly obese patients showed downregulation of the gene. The PRKAA2 gene expression was significantly reduced compared to normal. A consistent result was shown in individual stages of cancer. The expression increases as the cancer advances and as obesity increases in patients. N1 and N2 levels showed a comparatively increased expression of the gene. The PRKAB1 and PRKAB2 expression didn't differ in normal and tumor tissues. PRKAG1 expression was increased in primary and individual cancer stages. A slight increase in the expression was seen as the patient's

weight increased, and the same pattern was seen in the nodal metastasis. PRKAG2 expression was decreased compared to the normal tissues, and down-regulation is observed in individual cancer stages and on various stages of nodal metastasis. The expression is slightly reduced in patients with average weight and obese patients (Figure 4).

Protein Expression and Promoter methylation

PRKAG3 gene, which codes for the AMPK $\gamma 3$ subunits, was not expressed in normal and COAD since the subunit is solely expressed in muscular tissues. All the other genes coding the subunits were statistically significant ($p > 0.05$) compared to the expression of the gene in a normal colon.

The protein-level expression decreased in all subunits compared to the normal (Figure 5). The $\gamma 2$ and $\gamma 3$ subunits were not reported to be expressed in colon cancer. Z values represent standard deviation from median across samples. The expression of $\alpha 1$ and $\beta 2$ is significantly reduced (normal=1.3, tumor=0.003), (median normal=1.8, tumor=0.08) respectively (Table 2).

Analyzing the promoter methylation level of the AMPK subunits, only the AMPK $\gamma 2$ in both normal and primary tumors was hypomethylated, and $\gamma 3$ in tumor conditions was hypermethylated. Still, the hypomethylation did not affect the gene expression and protein expression (Table 1, Figure 6).

Prognostic role of AMPK subunits

KM Plotter assessed the prognostic significance of AMPK subunits in GC. Survival curves were plotted for GC patients. KM plot for the AMPK subunits exhibited the median survival in months for both low expression and high expression cohorts of the subunits. High expression cohorts of PKAA1, PRKAA2, PRKAB1, PRKAB2 has shown improved OS than the low expression cohorts

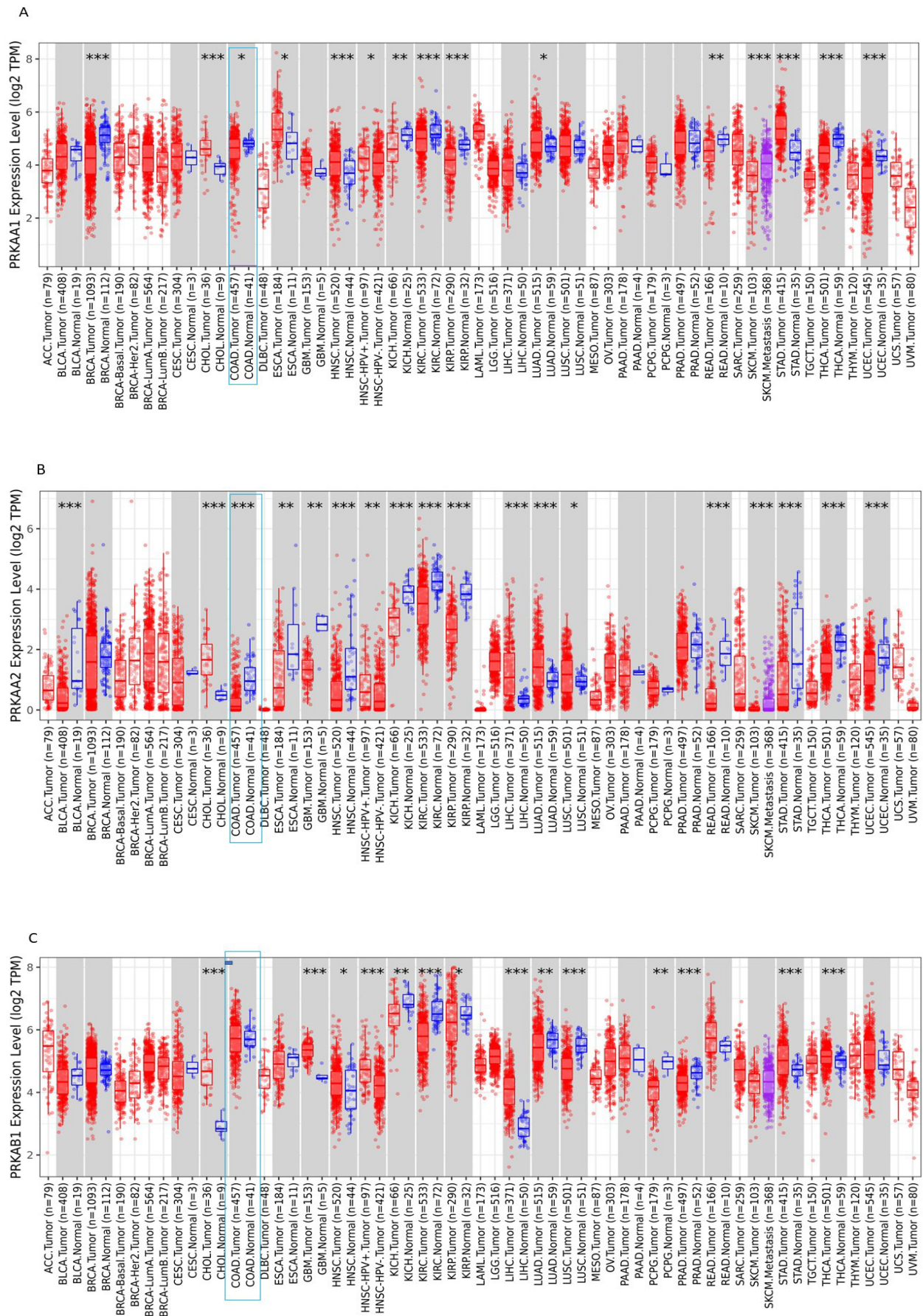


Figure 3. The PAN CANCER Analysis of AMPK Subunits (A-C) in Normal and Colon Tumors across the TCGA Datasets. The p- values are also represented as stars

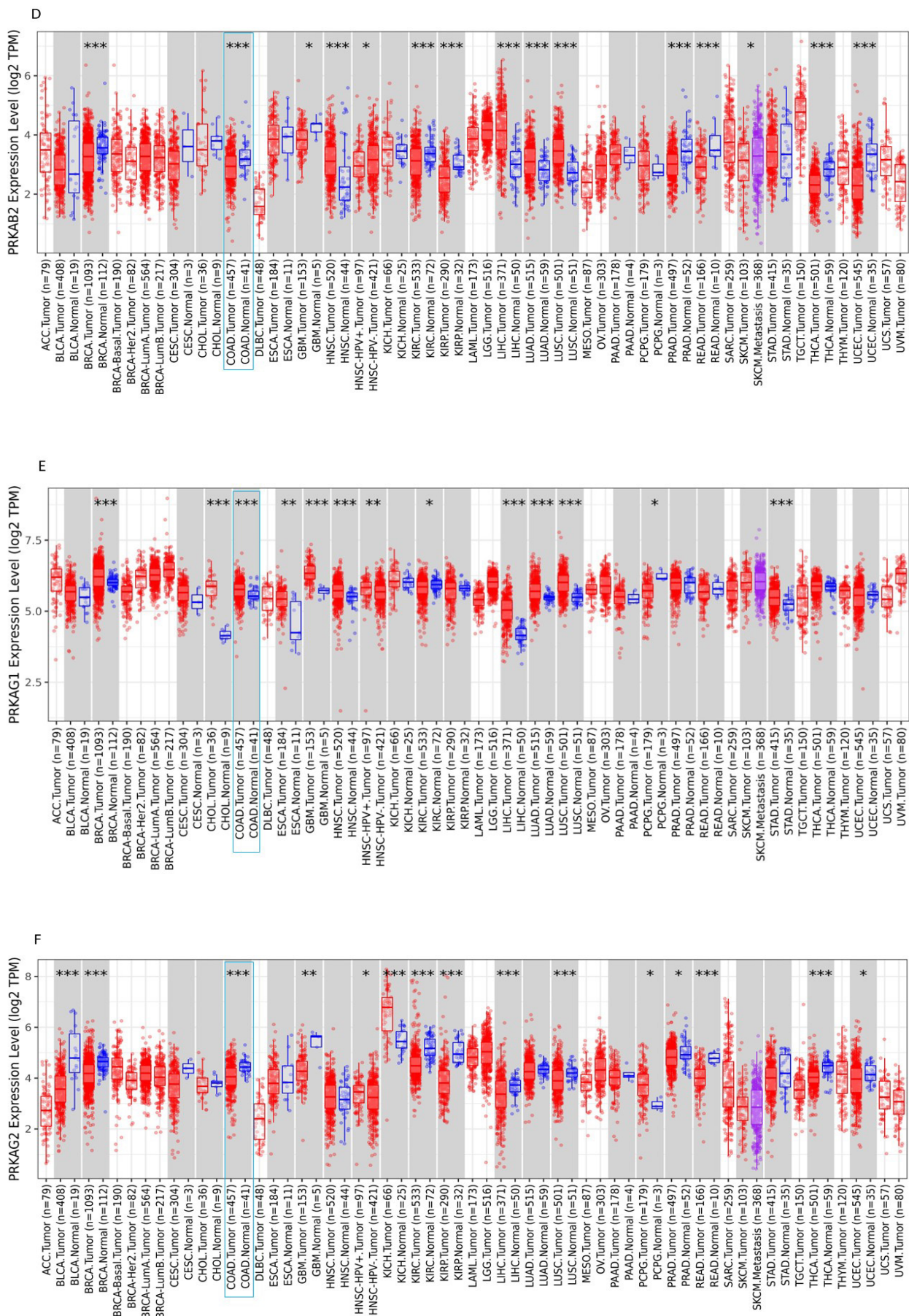


Figure 3. The PAN CANCER Analysis of AMPK Subunits (D-F) in Normal and Colon Tumors across the TCGA Datasets. The p- values are also represented as stars

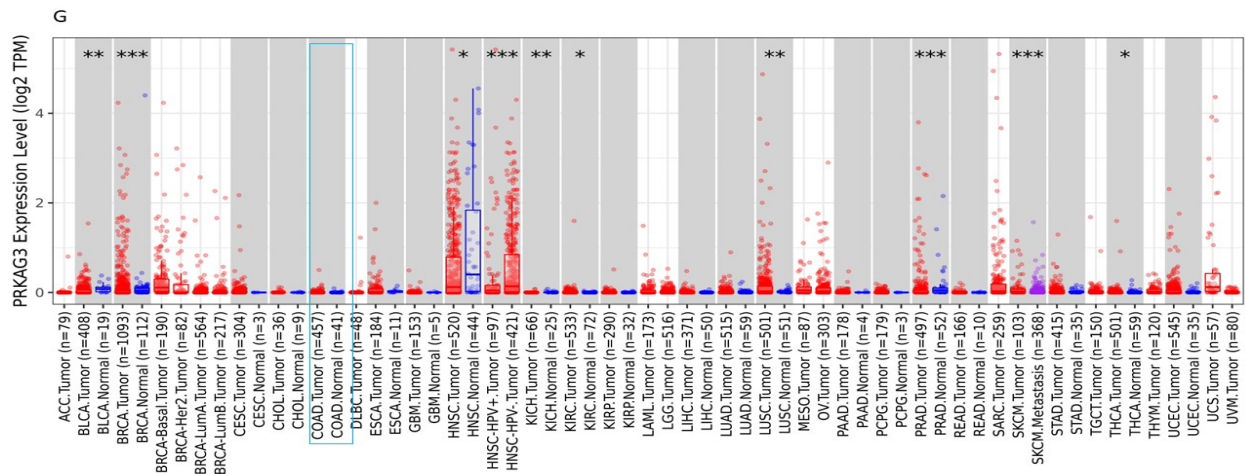


Figure 3. The PAN CANCER Analysis of AMPK Subunits (G) in Normal and Colon Tumors across the TCGA Data-sets. The p- values are also represented as stars

(Figure 7). The p- value for the above is statistically significant, whereas the PRKAG1 and PRKAG2 were not significant and are not related to the OS of the patients (Figure 7).

Protein-Protein Interaction and network analysis

The significant genes mutated in CRC were taken from the COSMIC database. The genes were KRAS (Kirsten rat sarcoma virus), TP53 (Tumor Protein), PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) CDKN2A (cyclin-dependent kinase inhibitor 2A), MEN1 (Multiple endocrine neoplasia, type 1), NOTCH1 (Neurogenic locus notch homolog protein 1), ARID1A (AT-Rich Interaction Domain 1A), FAT1, APC (Adenomatous polyposis coli), NRG1 (Neuregulin 1), KMT2D (Histone-lysine N-methyltransferase 2D), STK11 (Serine/threonine kinase 11), SMAD4 (Suppressor of Mothers Against Decapentaplegic 2), ERBB4 (Erb-B2 Receptor Tyrosine Kinase 4), ERBB3 (Erb-B2 Receptor Tyrosine Kinase 3), ATM (Ataxia-telangiectasia), MSH6 (mismatch repair protein), RECQL4 (RecQ Like Helicase 4), BRAF (B-Raf murine sarcoma viral oncogene homolog B), and FBXW7 (F-box and WD repeat domain containing 7). Along with the 20 mutated genes PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, and mTOR (Mechanistic Target Of Rapamycin Kinase) were also included to analyze the PPI using STRING. The analysis showed that the genes that code for AMPK interact with STK11, TP53, PIK3CA, mTOR, and ULK1 (Unc-51-like autophagy activating kinase). All the other interactions were through mTOR. The upstream kinase of AMPK, STK11, strongly associates with mTOR, ERBB4, NOTCH, and ATM (Figure 8).

Discussion

The involvement of AMPK in cancer is debatable (Richter and Ruderman, 2010), even though new research has proven that AMPK is a tumor suppressor (Steinberg and Carling, 2019). AMPK is reported to have a dual

nature in cancer mostly due to its complex structure-function and the context-dependent roles (Chuang et al., 2014).

The tumor suppressor role of AMPK is believed to be mediated via LKB1. A recent study on B cells supports the tumor suppressive role, which has only one catalytic subunit, AMPK- $\alpha 1$. This has been knocked out, which accelerated the development of lymphomas in transgenic mice overexpressing c-Myc in the B cells. The tumor suppressor role of AMPK is mainly due to cell cycle arrest. It is associated with the stabilization of p53 and the cyclin-dependent kinase inhibitors p21WAF1 and p27CIP1. It also inhibits the anabolism of all the macromolecules and inhibits cell growth. The arrest in cell proliferation is by inhibiting mTORC1 phosphorylation. This leads to the 'anti-Warburg' effect by inhibiting hypoxia-inducible factor-1 α (HIF-1 α) (Baba et al., 2010).

The present study analyzes the DE and protein expression of AMPK subunits using web tools. The subunit expression of AMPK is studied in different cancers, including CRC. The reported studies align with the current research that genes coding AMPK subunits are expressed in the tumor and normal tissues, with $\beta 2$ and $\gamma 3$ subunits an exemption. TCGA data from UALCAN reports that $\alpha 2$ expression is downregulated in COAD, but the data is not supported by the TIMER 2.0 data. According to TIMER 2.0 data, AMPK $\beta 1$ is downregulated in COAD patients. Even though the mRNA is expressed in tumor samples, the protein level expression is decreased in COAD patients. As per the literature survey, the cancer cells downregulate the expression of AMPK through LKB1 mutation. 30% of non-small cell lung cancers and 20% of cervical cancers, and 10% of cutaneous melanomas report this mutation.

There are shreds of evidence that AMPK activity is reduced in cancer. Reports show that enhanced tumor cell growth reduced the expression of the AMPK- $\alpha 2$ subunits in some cases of hepatocellular carcinoma. B-Raf mutation in melanoma cells also reported reduced AMPK activation. A more prominent mechanism of AMPK inhibition is phosphorylation at Ser 485 by Akt. This has been hypothesized to prevent the activating

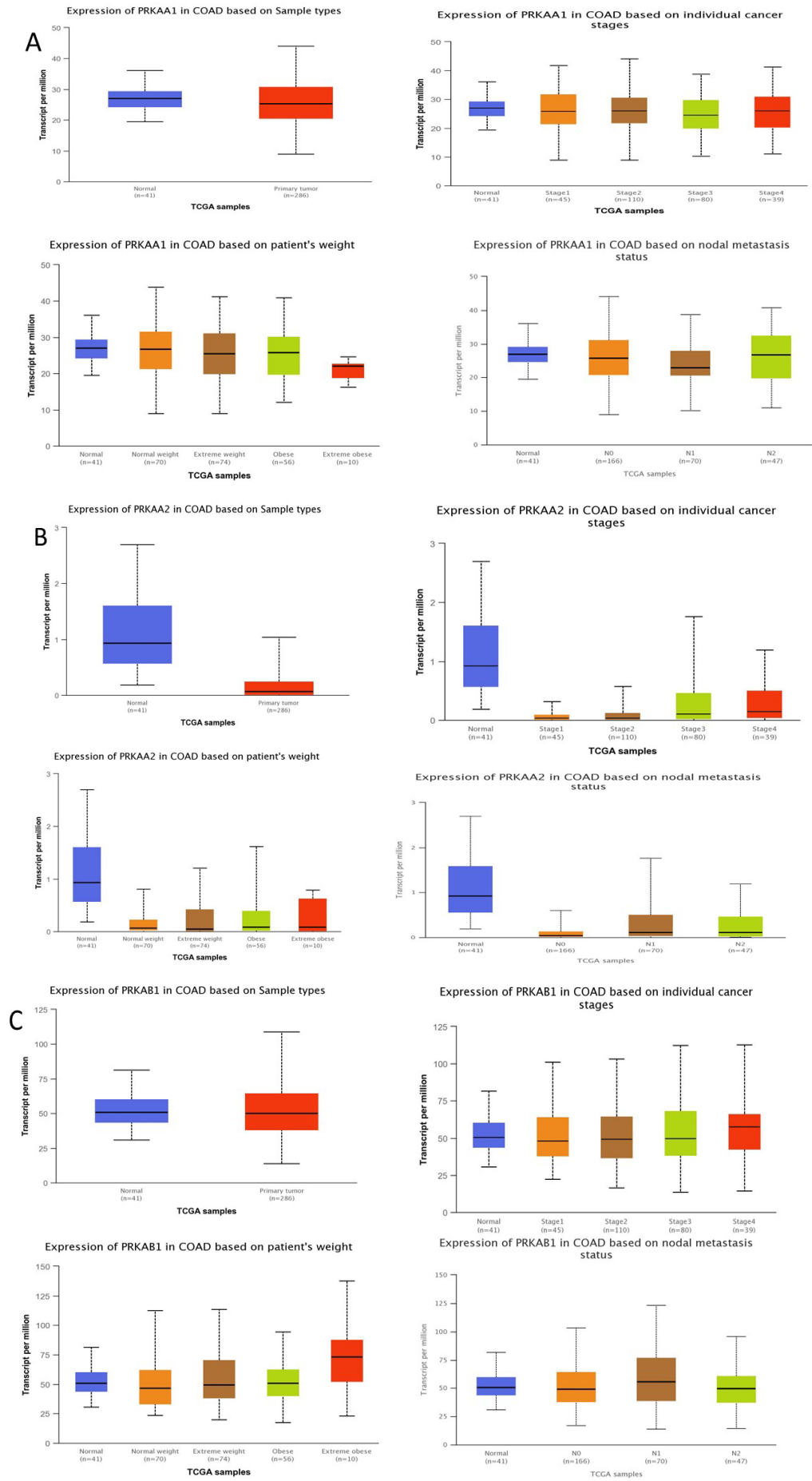


Figure 4. The Differential Expression of the Gene is Given across Tumor vs Normal Tissues, Individual Stages of Cancer, based on Patient Weight, and based on the Nodal Metastatic Status of Cancer. A: PRKAA1, B: PRKAA2, C: PRKAB1

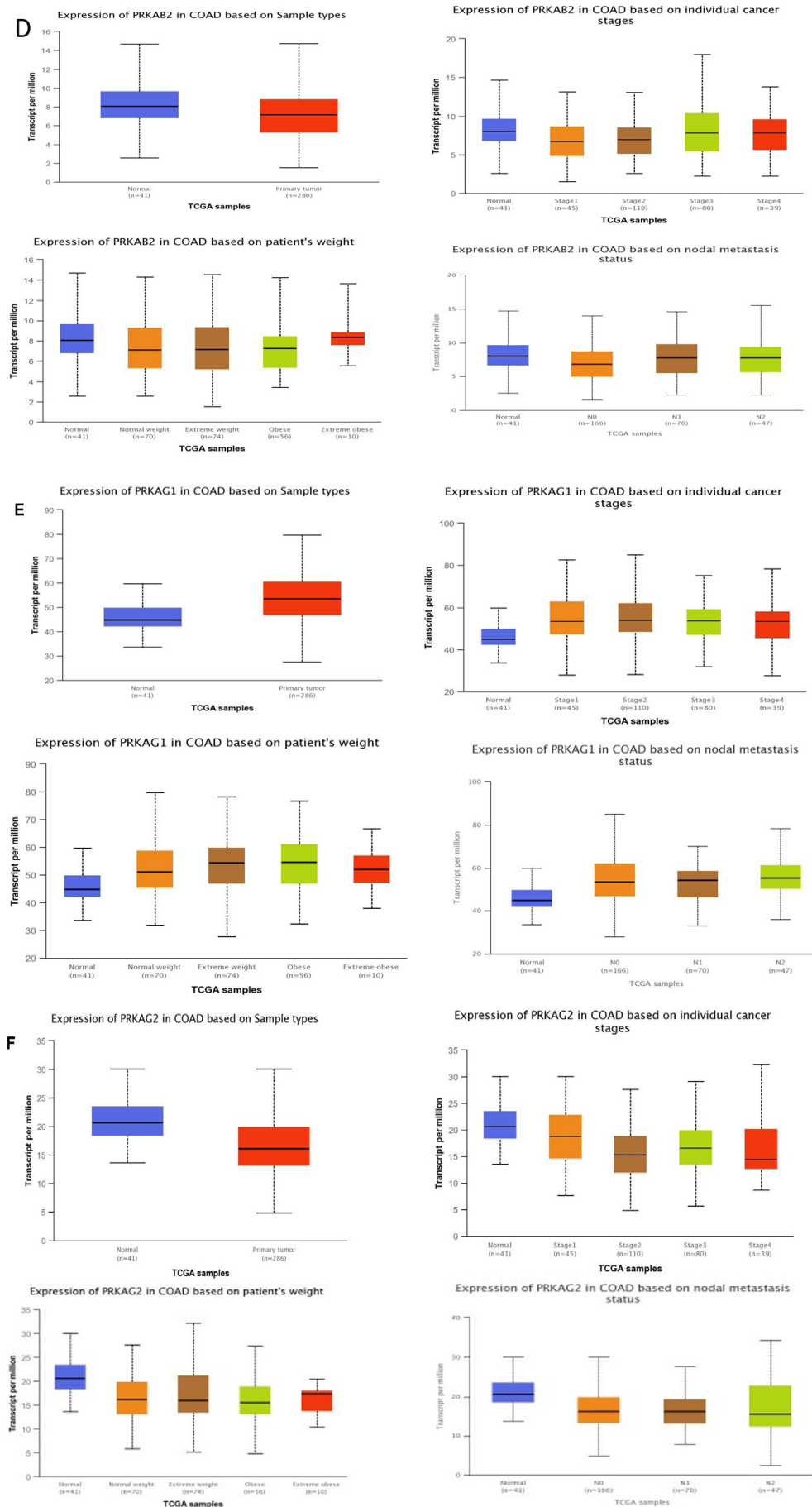


Figure 4. The Differential Expression of the Gene is Given across Tumor vs Normal Tissues, Individual Stages of Cancer, based on Patient Weight, and based on the Nodal Metastatic Status of Cancer. D, PRKAB2, E: PRKAG1, F: PRKAG2

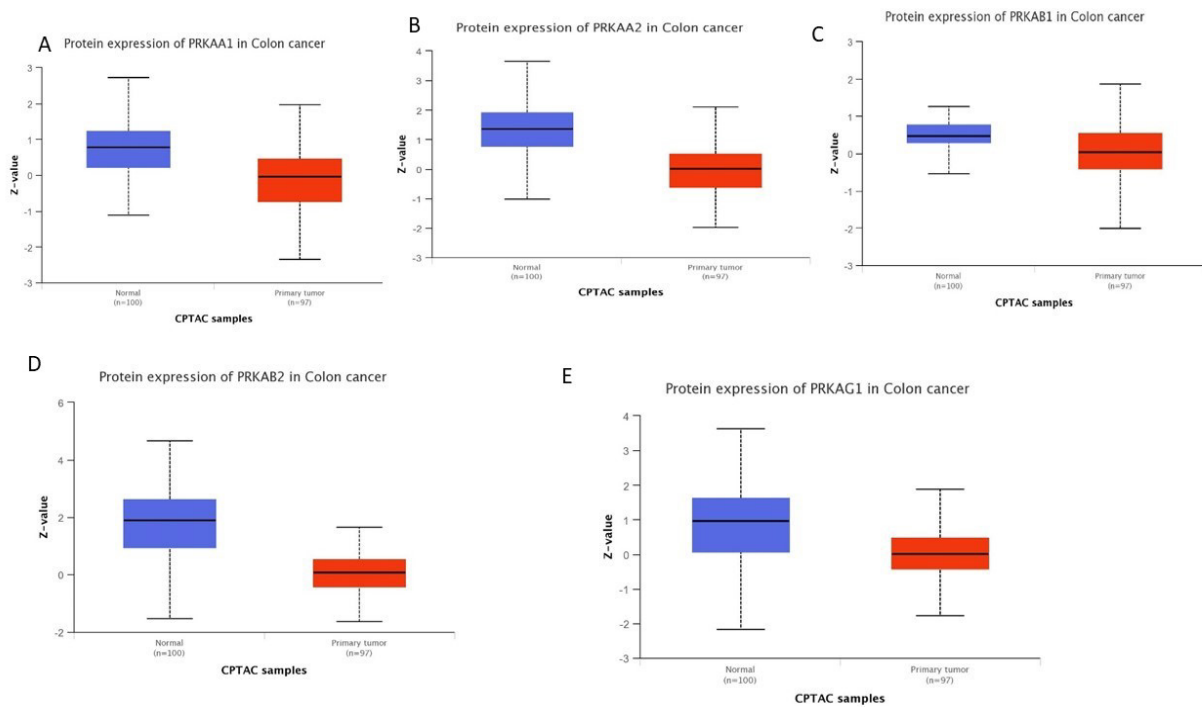


Figure 5. The Figure Shows CPTAC Samples Used to Compare the Protein-Level Expression of AMPK Subunits in Normal and Tumor Tissues. A: PRKAA1, B: PRKAA2, C: PRKAB1, D: PRKAB2, E: PRKAG1

phosphorylation at Thr172, which is not yet studied in cancer. The possible hypothesis of downregulation of AMPK in cancer is its context-dependent role (Hardie, 2011; Shackelford and Shaw, 2009). But the analysis of the study reports that AMPK subunits are expressed at the mRNA level. At the same time, the protein expression is reduced, which indicates that the AMPK complex formation and its functioning in the tumor microenvironment are to be studied in different types of cancer. AMPK is considered to be a target in obesity and metabolic syndrome. AMPK α 1 expression is increased in patients with obesity and CRC. But the highly obese conditions inhibited the subunit expression, which needs further studies to unveil the mechanism.

The gene expression of AMPK α 2 was significantly reduced in COAD patients. Protein level expression of the subunits is also reduced in the tumor, which may be attributed to the peculiarities of AMPK like tissue specificity, Subcellular localization, tumor microenvironment, etc. The downregulation of gene and protein expression might be due to the mutation of the upstream kinase LKB1. The COSMIC data analysis for the major mutations in CRC shows that 7% of the CRCs manifest mutation of the STK11 gene encoding LKB1 in CRC patients. The string analysis shows that the genes encoding the AMPK subunits are associated with P53 and PIK3CA genes which are also mutated in CRC. The gene enrichment analysis using metaspape presents the significant functions of AMPK subunits and the top 20 genes mutated genes in CRC, which include positive regulation of biological processes, metabolic process, response to stimulus, growth, developmental process, signaling, rhythmic and cellular process, etc. This also provides the possible diseases associated with similar

genes: Head and Neck Squamous cell carcinoma and their involvement in integrated breast cancer pathway, response to radiation, Cushing syndrome, and DNA recombination.

Epigenetic modifications have a definite role in tumor formation and progression. DNA methylation is one of the significant epigenetic modifications that have a crucial role in cancer. DNA global hypomethylation is an early event in colorectal tumorigenesis, and the progression of the disease is associated with an increase in DNA methyltransferase activity (Habib et al., 2000). Global DNA hypomethylation and promoter-specific DNA methylation have been linked to genomic instability and tumor initiation in CRCs (Paz et al., 2005). The promoter methylation of the AMPK subunits is analyzed in normal and tumor tissues. The promoter DNA sequence of the γ 3 subunits is hypermethylated, and this may not have any significance in CRC because the subunit is only expressed in muscle cells. The γ 2 subunit is hypomethylated in the normal colon and tumor tissues.

Many studies have shown that AMPK is an effective target in the therapy of CRC (Honari et al., 2019; Hu et al., 2019; La et al., 2017; Li et al., 2015). Metformin's usage in CRC has been studied extensively in vitro, in vivo, and in preclinical investigations (Bradley et al., 2018; Meng et al., 2017; Mogavero et al., 2017). The analysis conducted in the present study also affirms that AMPK activation benefits CRC patients' overall survival. KM Plotter was used to analyze the predictive importance of AMPK subunits in GC. The survival study reveals that the PRKAB2 expression is not associated with the patient's overall survival. However, overexpression of all other subunits has been linked to a better prognosis in CRC patients. Earlier studies also report that AMPK α 1 expression is associated with an improved

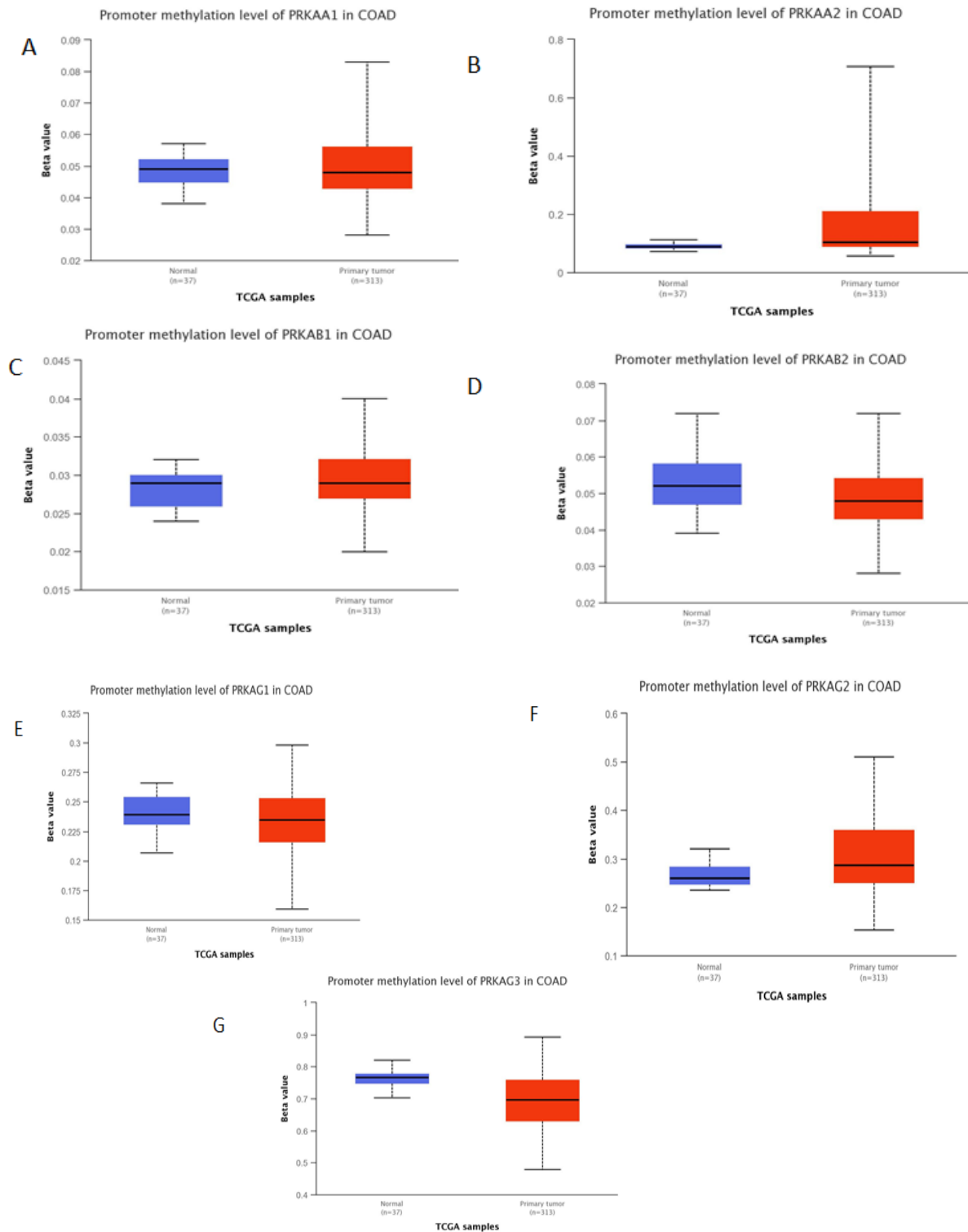


Figure 6. The Figure Represents the Promoter Methylation Level of the Subunits of AMPK. The data used to compare the samples are collected from TCGA. A: PRKAA1, B: PRKAA2, C: PRKAB1, D: PRKAB2, E: PRKAG1, F: PRKAG2, G: PRKAG3

OS in patients. In 37 renal cell carcinoma patients, total AMPK α 1/ α 2 protein expression was linked with improved Progress Free Survival. Recent research suggests that overexpression of the genes encoding for AMPK α 1, α 2, β 1, β 2, and γ 1 subunits was also related to improved OS in 417 clear-cell renal cell carcinoma patients, the study was based on the data from Cancer Genome Atlas (TCGA). Hoffman and colleagues found a link between higher expression of the genes encoding the regulatory AMPK α 1

and α 2 subunits and improved 5-year survival ($P = 0.001$ and 0.021 , respectively) in diffuse large B-cell lymphoma patients using the Oncomine database (Zadra et al., 2015).

As a concluding note, the genes encoding the AMPK subunits are upregulated in COAD, while the protein expression is comparatively reduced in colon tumors. The γ 2 subunit in normal and colon tissue are hypomethylated, and the γ 3 subunit in colon tumor is hypermethylated. The OS analysis of the subunits affirms that AMPK expression

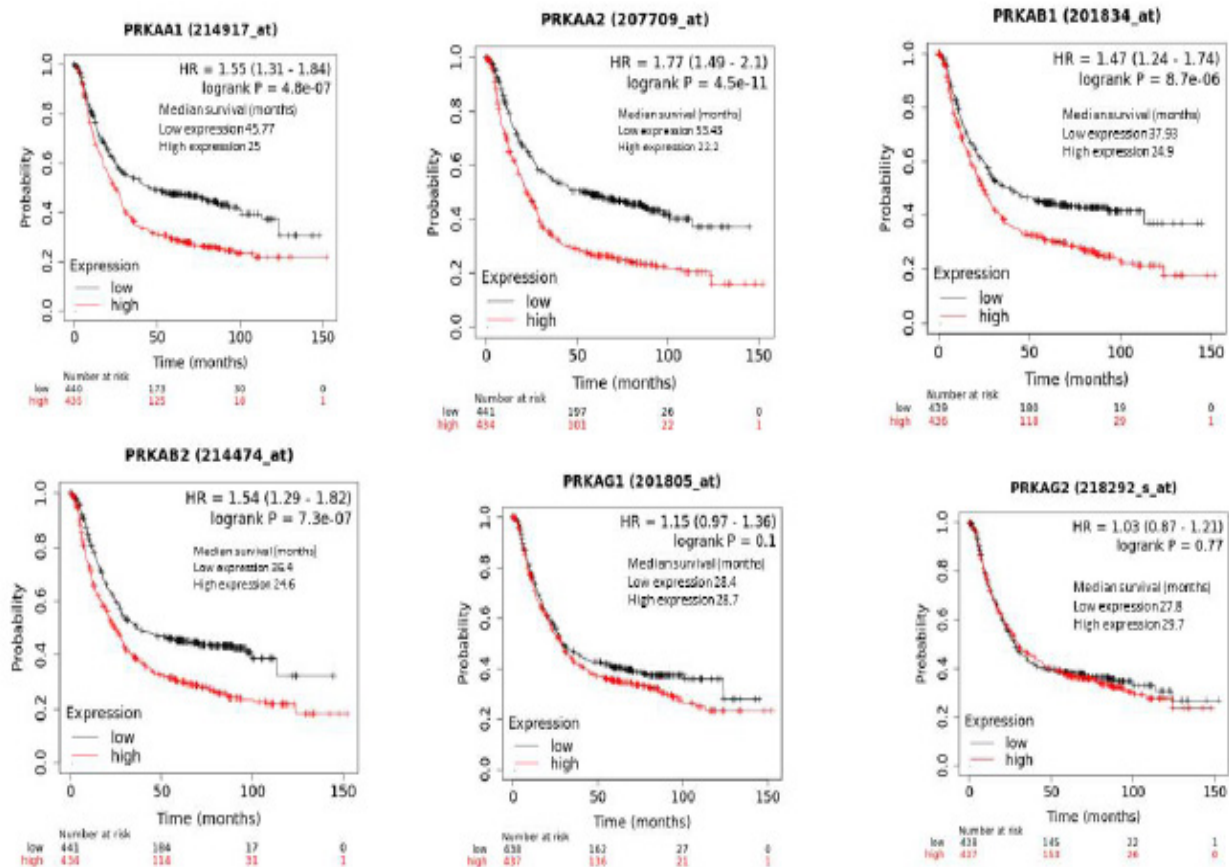


Figure 7. The KM Plot of the AMPK Subunits. The figure depicts the OS of the patients in lower and higher expression of the subunits. HR: Hazard Ratio

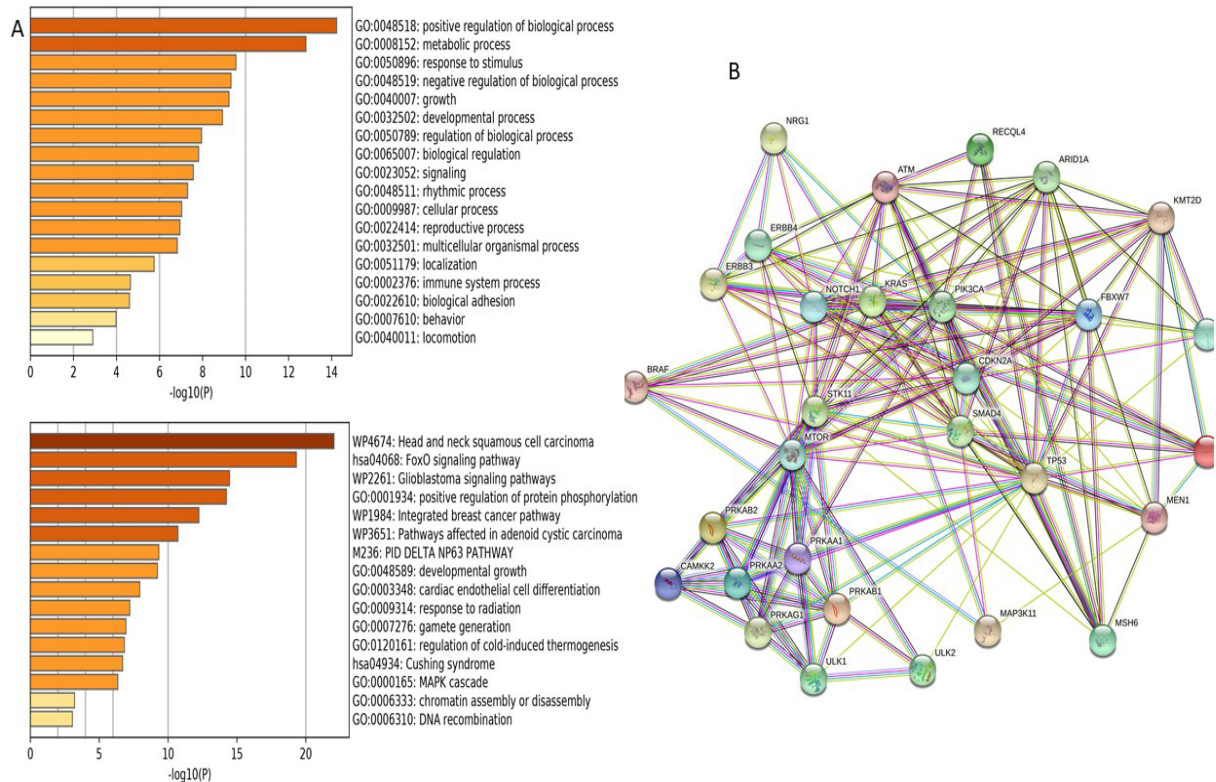


Figure 8. GO Analysis of AMPK (A) PPI Network analysis (B) by String of the most Mutated 20 Genes in COAD Patients.

is beneficial in cancer. The downregulation of the gene and the protein of AMPK expression might be due to the mutation of the upstream kinase LKB1.

Author Contribution Statement

Study conception and design by KBH, JMA, and BTB. BTB and SE collected and processed the data. BTB has done the analysis. BTB prepared the first manuscript. All the authors commented on and revised the first draft of the manuscript. All the authors approved the final draft.

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Ethical approval

No human or animal participation is involved in the study so the ethical approval doesn't apply.

Availability of Data

The data that are extracted for the study is included in the current study.

Conflict of Interest

The authors declare no competing interest financially and non-financially.

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