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Mining mutations for protein kinase CK2 across several cancer types

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MINING MUTATIONS FOR PROTEIN KINASE CK2

ACROSS SEVERAL CANCER TYPES

by

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ABSTRACT

While the death toll of cancer has declined worldwide, the incidence is at an all-time high. Protein Casein kinase II (CKII) is a protein serine/threonine kinase that is essential to many cellular and biological functions in the mammalian organism. CK2 works as a heterotetramer protein consisting of two kinase and two regulatory subunits. Mutations in *CK2* genes that lead to increased CK2 activity are associated with several human cancer types. To further our understanding of CK2's relationship with cancer, we set to create and analyze a mutational profile for *CK2* genes using a publicly available ATCG dataset via cBioPortal. For this, a collection of genomic data from patients with different cancer types was pooled from cBioPortal, allowing singular analysis of CK2 genes throughout different cancer types. Additionally, Kaplan-Meier plots assessed the effect of overall alterations in each CK2 gene and combinations of them. We found amino acid residue mutational hotspots in diverse cancer types. We also found some correlation between overall CK2 gene mutation and cancer patient disease-free survival and disease progression. Although our analysis is limited in its scope and specifics, however, it begs further investigation into the role of CK2 mutations in human malignancies.

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LIST OF ABBREVIATIONS

ACS .... American Cancer Society

BU .... Boston University

CDC .... Center of Disease and Control

CK2 .... Casein Kinase 2

CML .... Chronic Myeloid Leukemia

DFG .... Aspartic-Phenylalanine-Glycine triplet

EGFR .... Epidermal Growth Factor Receptor

EMT .... Epithelial-Mesenchymal Transition

HPV .... Human Papillomavirus

NCI .... National Cancer Institute

NIAAA .... National Institute on Alcohol Abuse and Alcoholism

NIGMS .... National Institute of Graduate Medical Sciences

PER2 .... Period Circadian Regulator 2

pHi .... Intracellular pH

SHH .... Sonic Hedgehog

SNP .... Single Nucleotide Polymorphism

TRAIL .... Tumor Necrosis Factor-Related Apoptosis

INTRODUCTION

Cancer Prevalence and gene mutations

Incidence of cancer is a major concern worldwide and in the United States. An extreme public health concern, cancer acts as the second leading cause of death in the United States[1]. The ten cancers that have the highest incidence in the United States are breast, prostate, lung, colon and rectum, corpus and uterus, melanoma, urinary, non-Hodgkin lymphoma, kidney and renal, and thyroid. Among these, the top three cancers with the highest death rate include lung, breast, and prostate. While there has been a decline in the cancer death rate by 21% since 1991, the rate of incidence is still unchanged, and poses a great health threat to society (Figure 1).

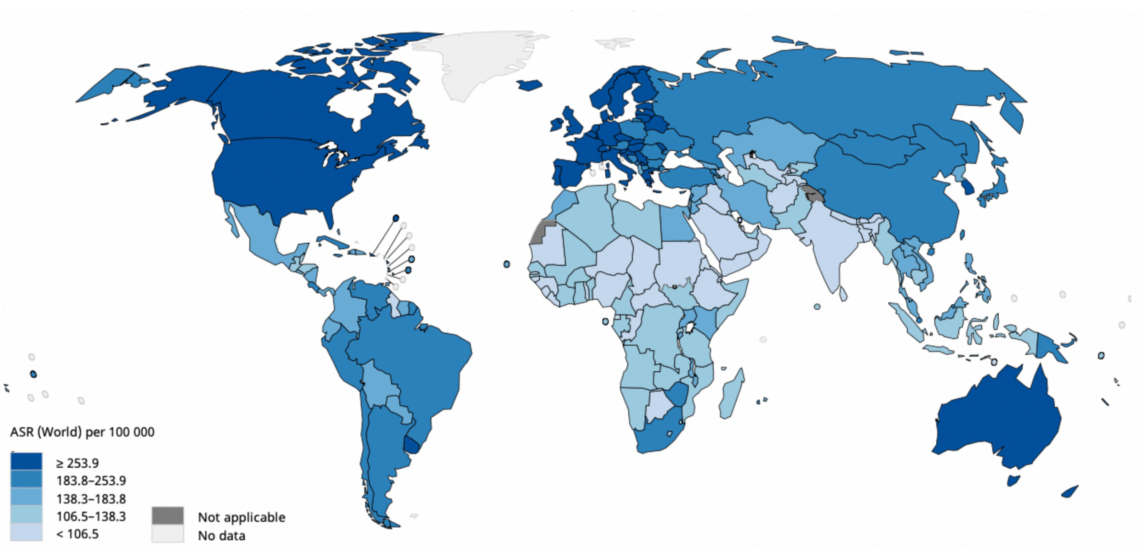


Figure 1. Estimated Age-Standardized Incidence Rates in All Cancers. In this map, cancer incidence rates are estimated and standardized world-wide using both males and females, and all ages. Higher cancer incidence rates are indicated by darker shades of blue.

Cancer arises from oncogene activation and tumor suppressor gene inhibition. These two hallmarks of cancer are instrumental in contributing to the tumorigenesis of cells[2]. This disease of the genome results from a wide array of faulty genetic events, which include amplifications, deletions, frame-shift mutations, insertions, translocations, nonsense and missense mutations. Advancements in computational analytics, as well as cancer tissue sampling has led to the identification of many mutational signatures[3]. Cancers will result from driver mutations, which promote a growth advantage for the cell[3,4]. Passenger mutations, however, do not confer the same advantage, might accompany certain cancer types, and it is important to distinguish whether they actually contribute to the cancer or are just along for the ride. The existence of cancer genome databases, has revolutionized the approach to how cancer is studied, as well as modernized therapies. Indeed, oncology is currently facing a revolutionary shift from the common, but very toxic forms of therapy such as chemo and radiotherapy. Much more advance and precise forms of therapy have emerged such as targeting specific proteins and immunotherapies. Treatment of cancer through the augmentation of the body's immune response has shown to be consistently successful in many phase III clinical trials, therefore, immunotherapy poses to be a future blueprint to combat many forms of cancer[5,6].

Protein kinase dysregulations and mutations are linked to a multitude of cancers. The development of new targeted therapies that inhibit faulty protein kinases has been promising as a cancer therapeutic approach. The targeting of these critical regulators in malignancy comprise of the second largest group of drug targets, accounting for 20-30% of current drug discovery[7,8].

CK2: Cellular and Biological Function

Protein Kinase CK2 is classified as a protein serine/threonine kinase. CK2 is a widely expressed protein and is ubiquitously conserved among eukaryotic organisms. CK2 plays an important role in many crucial cellular processes. In this respect, CK2 acts to regulate transcription,[9,10] translation,[11–13] cell cycle progression,[14,15] cell survival[16,17]. It is highly evident that CK2 plays a role during cellular proliferation. Genetic studies in yeast have shown the dependence of the cell on CK2 to get through the G_1/S and G_2/M transitions[18]. CK2 has associations with the mitotic spindle and in the mitotic phosphorylated form CK2 will interact with Pin1, which is a key regulator of cell division[19,20]. As an active player in cellular processes, it is no surprise that CK2 is essential for cell survival and apoptosis. Induced expression of inactive CK2α and CK2α' compromise cell proliferation in mammalian cells[21]. Phosphorylation by CK2 protects certain proteins from caspase-mediated degradation. In this regard, it is important to note that there remains a striking similarity between the consensus motif for phosphorylation by CK2, and the caspase degradation recognition sequences[22,23].

On top of these vital cellular functions, CK2 dictates many biological mechanisms as well. Inhibition of CK2 is correlated with a decrease in endothelial cell proliferation and migration[24]. Promotion of angiogenesis by CK2 is a key biological function during the development of cancer, and inhibition of CK2 during induced endometrial lesions is reduces vascularization[24,25]. Due to its phosphorylation sites on the protein PER2, CK2 acts as a regulator of circadian rhythms, which is an essential physiological process. Additionally, inhibition of CK2 resulted in oscillations in circadian rhythm gene expression[26]. Published studies also indicate the role CK2 has in embryogenesis. Knockout of CK2α leads to embryonic lethality and defects in organ formation that is in part due to decreased proliferation[27]. Knockout of CK2α' leads to male infertility that is in part due to altered sperm differentiation[28]. Knockout of CK2β and RNAi mediated knockdown in mice both lead to a failure in development during gastrulation[29]. In addition to organogenesis during embryonic development, CK2 is seen to play a significant role in organ diseases as well[30]. HPV infection is highly prevalent worldwide and is correlated to cervical, head and neck, anal and penile cancers. Interestingly, CK2 has been found to partake as an agonist in HPV related cancer development. As such, studies comparing HPV infected keratinocytes versus normal keratinocytes found CK2 activity elevated in HPV-immortalized cells. Further analysis shows CK2 as a regulator for HPV through the HPV-16 E7 viral oncoprotein[31,32].

CK2: genes, proteins, expression levels, activity and localization

The CK2 kinase family consists of two isoforms: CK2α and CK2α'. There has been substantial evidence suggesting that CK2 isoforms can exist and function as monomeric kinases *in vivo*[29,33]. In addition, they can form a heterotetrameric holoenzyme consisting of two kinase and two regulatory subunits[29,33]. CK2α, and CK2α' being the two kinase subunits, meanwhile the regulatory units are denoted CK2β. The CK2α gene (CSNK2A1) is located on chromosome 20, while the gene for CK2α' (CSNK2A2) is on chromosome 16. The gene for the CK2β regulatory unit (CSNK2B) is on human chromosome 6. Additionally, there exists a pseudogene of CK2α, which is deemed CK2αP (CSNK2A3). This pseudogene is located on chromosome 11 in humans. Although intronless, this pseudogene is expressed in mammalian organisms as a transcript and plays some oncogenic role in cancer development[34,35].

Csnk2a1 protein have elevated levels compared to CK2β in mouse tissue[28]. The different proteins of CK2 can affect the levels of their counterparts, which can play a role in CK2's downstream functions. For example, phosphorylation of CK2β by CK2α stabilized CK2β[36]. A plethora of CK2 substrates have been identified, with many phosphorylated by CK2α and CK2α'[37]. CK2β does not exert strict control over enzymatic activity, which is typically seen in other kinase regulatory units. However, CK2β does impact substrate specificity and other properties of the holoenzyme such as stability[38,39]. Unique to CK2 is the lack of autophosphorylation events that regulate its activity, which supports the idea that CK2 is constitutively active. CK2 proteins can be found in both nucleus and cytosol of cells within an organism, although at different levels[40].

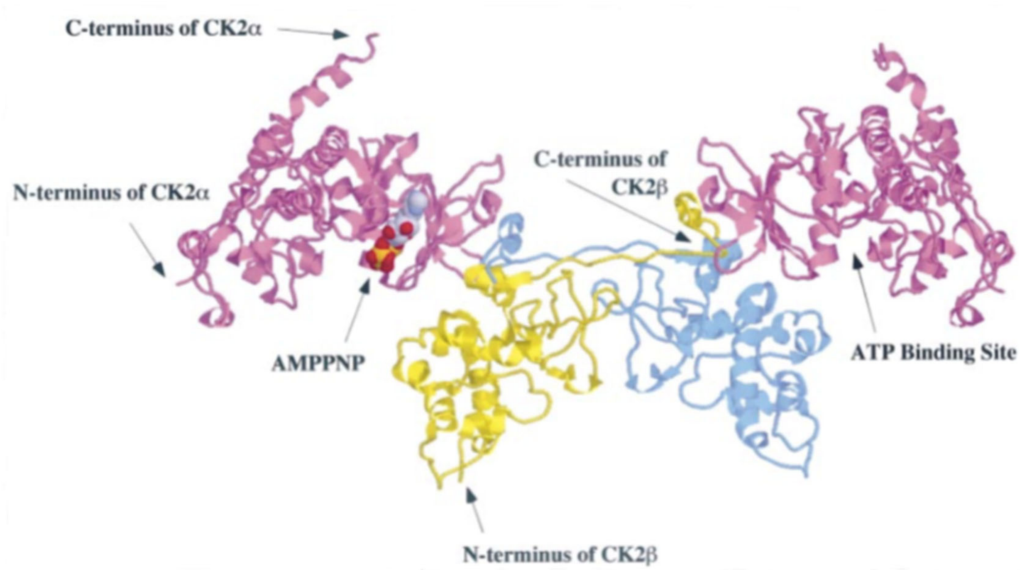


Figure 2. Tetrameric Protein CK2 Structure. Image taken from *Litchfield, 2003* shows crystallized structure of tetrameric protein CK2. Magenta colored units represent the catalytic CK2α, and the blue and yellow are the regulatory units CK2β. Region in magenta that is labeled and highlighted shows the ATP binding site in the CK2α catalytic protein.

CK2 isoforms are considered to be quite unique due to being intrinsically active, the ability to use both ATP and GTP as a substrate, and the target phosphorylation site (Serine and Threonine) has to be surrounded by acidic amino acid residues[22]. In comparison to most protein kinases, CK2 shares many features structurally, however there are some key differences that lead to its characteristic functions/form, which are a result of their amino acid sequence (figure 3)[41]. It is important to note that there are five common amino acid residues conserved among all protein kinases. On CK2α, these are Lys68, which is responsible for α and β phosphate stabilization of the ATP substrate; salt bridging with the Lys68 is accompanied by Glu81; Asp156, and Asn161 is found in the catalytic loop, which aids in substrate residue deprotonation; Finally, Asp175, will orient the primary divalent cation and is a part of the Aspartic-Phenylalanine-Glycine (DFG) conserved triplet in the CK2α (figure 4)[22].

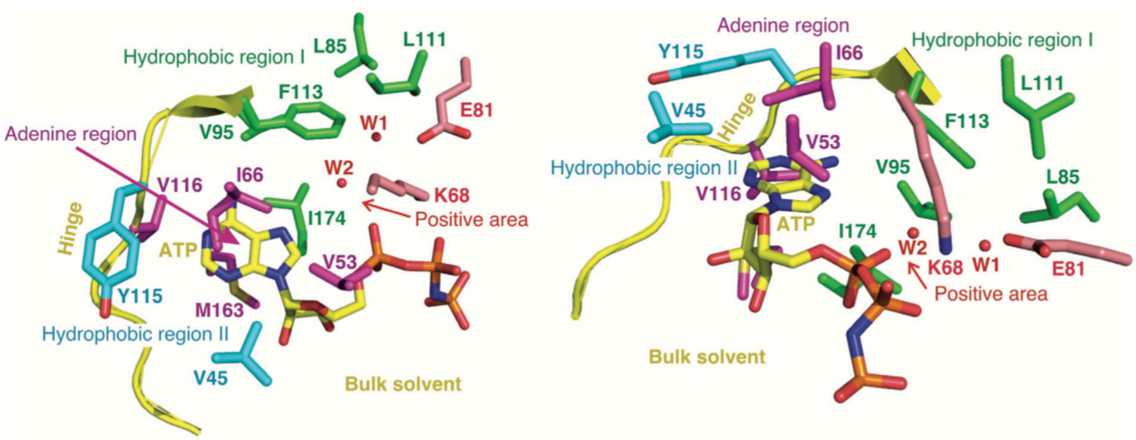


Figure 3: ATP Binding in CK2α. Thanks to the amazing anaylsis of CK2 that came from *Battistutta, 2009* the importance of specific residues are seen in ATP stabilization. In green are the three residues mentioned that are seen in all kinases. Each image represents a different view point-the left image show from the top of the ATP molecule when binding with CK2α, and the right panel shows the side view from the phosphate chain.

Even though CK2 displays a similar sequence to most protein kinases, the differences are in regions important for interactions with substrates.

- There is a conserved activation loop in protein kinases consisting of Asp-Phe-Gly, however in CK2 tryptophan replaces phenylalanine at position 176 (figure 4), which alters the region slightly contributing to substrate binding and recognition[22].

- There is also the presence of a basic cluster of amino acids at residues 74-80, which are responsible for the phosphorylation of acidic amino acids during substrate recognition[22].

- At the gatekeeper position of CK2α has Phe113 (figure 4). It is known as the gatekeeper due to the residues side chain contributing to the size of the hydrophobic pocket. A majority of Ser/Thr kinases have Threonine, which is more susceptible to tyrosine kinase inhibitors[22]. Alterations in this region can contribute to CK2's resilience towards protein targeted therapies[22,33].

- Lastly, exists a stretch of residues spanning from Leu70 to Lys76 on the helix of CK2α that are less characterized, and not really known what their function is[22].

Understanding the structure and nature of CK2 and its numerous substrate interactions is imperative in understanding its potential role in cancer pathogenesis.

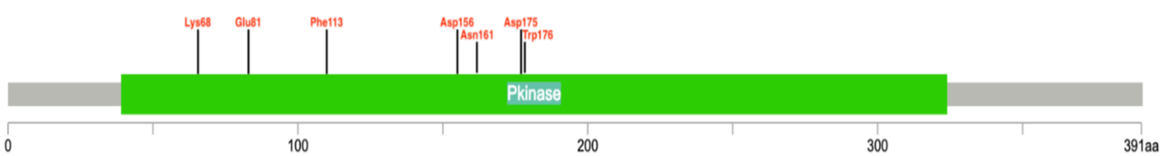


Figure 4: Residue Hallmarks of Catalytic CK2α. This is a visual representation of the CK2α protein and the residues that are critical for its catalytic functions and interactions with the other CK2 proteins.

The function of these residues in CK2 are well studied and known. Mutational studies have helped illuminate the necessity of certain residues mentioned previously. For example, series of point mutations throughout the known activation loop disrupts CK2's ability to provide a fully active conformation to the catalytic isoforms[42].

Cancer mutation

The majority of onco-kinases acquire their pathogenic ability because of mutations. Additionally, loss of tumor suppressor gene activity will contribute to cancer pathogenesis. Lowering or inhibiting the activity of many of these onco-kinases is imperative to halt cancer activity. Examples of this can be seen across many different cancers. One famous example of creating a therapeutic that targets a kinase domain is the Imatinib. This was used for the BCR-ABL gene in chronic myeloid leukemia (CML). However, a series of novel missense mutations helped spawn Imatinib resistant CML[43]. Luckily, with a lot of developments new drugs were placed on the market that addressed this resistance. It is important to understand that even in the face of targeting an onco-kinase, it is critical that all different mutation types are understood, which will help lead to the best possible therapeutic outcome.

CK2 in cancer

Due to its regulatory involvement in many critical cellular and biological processes there is strong evidence suggesting CK2's role in cancer pathogenesis[16,38,40,44,45]. Atypical levels of protein kinase CK2 have been observed in several cancer types such as lung, prostate, breast, head and neck, and more[36,40]. Overexpression of CK2 transcripts has been found in human cancers[34,45]. In addition to elevated protein and transcript expression levels in human cancer cells, CK2 stimulates transformation in transgenic mouse models[40,46]. For example, CK2 is overexpressed in human breast cancers, and is upregulated in rat carcinogen-induced breast cancers[47]. Additionally, CK2 overexpression is present in nearly every hematological cancer, and its overexpression in mice leads to lymphoid tumors[40,48]. Excess CK2 expression can lead to tumorigenesis through dysregulation of key pathways[49].

The majority of onco-kinases contribute their pathogenic effects due to increased enzymatic activity when they would normally be inactive. Because of CK2's constitutive activity, for a period of time it was not considered as a therapeutic target. However, current trials in non-small cell lung carcinoma have shown some success when using CK2 inhibitors[25]. While overexpression of CK2 is typically linked with cancer prognosis, it is not necessarily definitive. Elevated protein kinase CK2 expression levels in several cancer types correlate with lower overall patient survival[34,45]. However, current studies that also show underexpression of CK2 transcripts in certain cancer types, as well as CK2 transcript overexpression correlating with higher patient survival, which is contrary to the prevalent model[34,45].

As we mentioned above, both overexpression and mutations in genes are at the heart of carcinogenesis. However, in the case of CK2, there has been little to no analysis on mutational profiles and CK2 in different cancer types. Our goal in this study was to describe the mutational profiles in CK2α, CK2α', CK2αP, and CK2β. For this we used human genomics data pooled from cBioPortal, we analyzed mutational profiles, and the trends of across several cancer types. We particularly focus on single point mutations as there was more data for these. With these mutational profiles we analyzed correlations of the specific mutational types along these genes to specific cancer types. Additionally, we analyzed how the severity of mutations in CK2α, CK2α', CK2αP, and CK2β affect mortality as well as cancer disease progression.

METHODS

Information regarding cancer types, prevalence, and treatment

Data, treatment modalities and all other information regarding the different cancers we studied were obtained through the NCI (http://www.cancer.gov), ACS (http://www.cancer.org) and CDC (http://www.cdc.gov) websites.

cBioPortal Analysis

Mutational profiles of CK2 genes and pseudogene (CSNK2A1, CSNK2A2, CSNK2A3, and CSNK2B) was analyzed using the cBioPortal cancer genomics database. Originally developed at Memorial Sloan Kettering Cancer Center, cBioPortal is hosted by the Center for Molecular Oncology, and works in collaboration with Dana Farber Cancer Institute, Princess Margaret Cancer Centre in Toronto, Children's Hospital of Philadelphia, The Hyve in the Netherlands and Bilkent University in Ankara, Turkey. cBioPortal is a web resource that aids in analyzing and exploring multifaceted cancer genomics data. It provides molecular profiling of cancer tissues depending on the cancer type and specific genes of inquiry. The portal gives table summaries of all the studies/samples and their respective mutations and or alterations to the specific gene of interest. It also provides graphical summaries of gene levels, survival analysis and co-occurrence/mutual exclusivity data of different genes. The Mutual exclusivity of genes in different cancer types is given in a table representation, and provides the p-value via a Bonferroni statistical test. We selected $p < 0.5$ as a threshold for significance.

Survival information is given in two components-the overall survival, and disease/progression. Both are through Kaplan-Meier estimates which utilize a logrank test P-value, we selected $p<0.05$ as statistical significance.

cBIOPortal	**Definition**
Amplification	This indicates more copies, often focalized increase in protein
Deep Deletion	This indicates a deep loss, homozygous deletion
Mutations	Classified as a collection of all possible mutations-which comprise single point mutations
Fusion	Combination of two previously separate genes
Multiple Alterations	This includes having more than one of the above together

Table 1. cBioPortal Mutation Classification. This table lists the gene alteration type with the classical definition of what they are.

Phosphosite Analysis

Phosphosite provides information pooled from all published data regarding Phosphorylation sites for genes of your inquiry. They are supported and funded through the National Cancer Institute (NCI), National Institute of Alcohol Abuse and Alcoholism (NIAAA), and National Institute of General Medical Sciences (NIGMS). The proteins of inquiry (CK2α, CK2α', and CK2β) that were analyzed for kinase domains, and phosphorylation, acetylation, and ubiquitylation sites along the sequence. Additionally, modification sites, common sites of mutations and their effects were analyzed. Phosphosite also includes known somatic cancer mutations and germline disease single nucleotide-polymorphisms (SNP's) per gene.

RESULTS

Mutational Analysis of CK2α

CSNK2A1 was used to query the cBioPortal database. Analysis of cBioPortal showed alterations in CSNK2A1 to be due to amplification, mutation, deep deletion, fusions or multiple alterations. Among further look at all the cancer types analyzed, amplifications and mutations were not only the predominate form of alteration, they appeared in almost all cancer types. The only exception seen were sarcomas which contained only amplifications, and adrenocortical carcinomas which contained only deep deletions (figure 4). The five cancers that contained the highest alteration frequency for CSNK2A1 were Bladder/urinary tract cancer, nerve sheath tumor, cancers of unknown primary (metastasized cancer), ovarian cancer, and ovarian epithelial tumors. Among these five, amplifications and/or mutations held the dominant form of alteration type, followed by deep deletions.

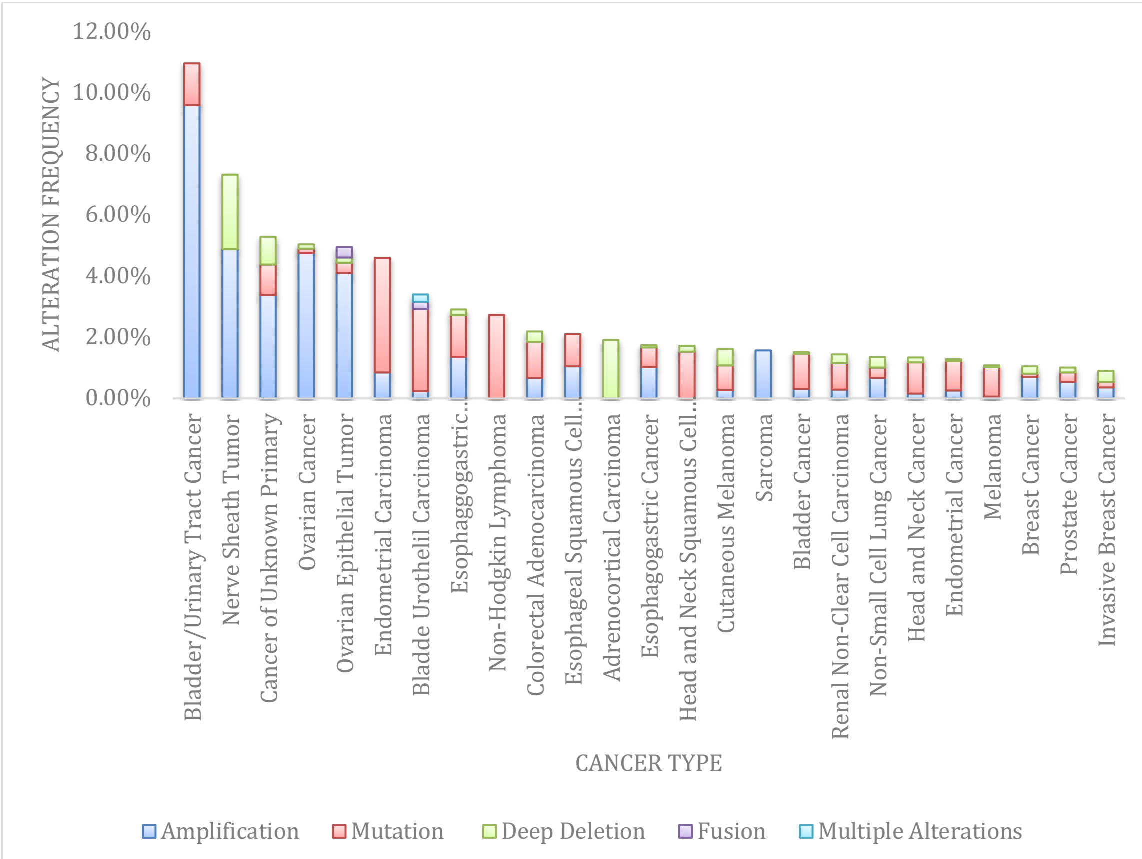


Figure 5. Alteration Frequency of CK2α in Several Cancer Types. Compilation of alteration frequency of CK2α gathered from cBioPortal graphically to show which cancer types contain the highest alteration to the CK2α gene. Change in CK2α gene is divided into amplification, mutation, deep deletion, fusion and multiple alterations.

Mutations are scattered throughout most of the CSNK2A1 gene, and the amino acid residues affected varied depending on the individual cancer, and there were no specific cluster of mutations in the CSNK2A1 sequence in any cancer type. The majority of mutations among all cancer types were missense mutations. There is a total of 201 missense, 70 truncating which result in nonsense mutations, and 4 inframe shifts which result in inframe deletions. The protein kinase domain spans from residue 39-324, and contains a multitude of mutations. However, there were four specific amino acid residues that were mutated more frequently than others: Arginine 80, Histidine 236, Arginine 280, and Arginine 306. These four hot spots contained the highest number of mutations across several cancer types (figure 5).

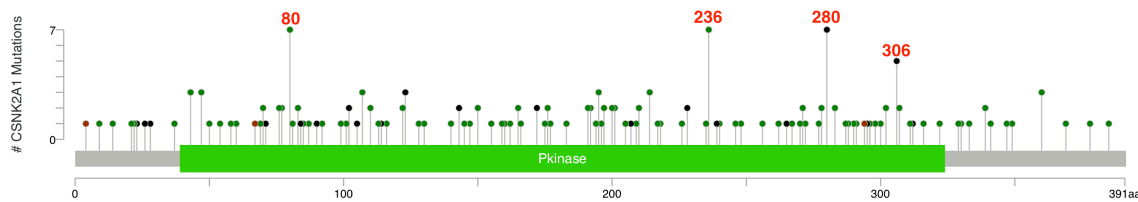


Figure 6. Mutation Map of CK2α Gene. Pooled from cBioPortal is a visual representation of the CK2α gene and all the mutations that have been reported among several cancer types. Residues that contained the highest number of mutations in different cancer types were highlighted and labeled. These four residues are referenced as hot spots for mutations in CSNK2A1.

Position 80: Residue 80 is a part of the 74-80 basic cluster in the kinase domain responsible for substrate recognition and responsible for the aid of phosphorylation in highly acidic substrates. Mutations at this residue are all missense and include: R80C (colon adenocarcinoma; Colorectal adenocarcinoma, Germinal center B-cell type cancer); and R80H (Uterine corpus endometrial carcinoma; Diffuse large B-cell lymphoma; medulloblastoma; prostate adenocarcinoma)(see table 2). Their individual action within each cancer case cannot be determined, however should be further investigated.

Position 236: Residue 236 also sees a variation in mutation among several distinct cancer types: H236R in head and neck squamous cell carcinoma, lung squamous cell carcinoma, medulloblastoma, prostate adenocarcinoma, cutaneous melanoma, and melanoma. Amino acid residue 236 is not really known to function for any specific purposes.

Position 280: Residue 280 lies within the kinase domain, R280* nonsense mutation was found in samples from Uterine serous carcinoma, uterine endometrioid carcinoma, breast invasive ductal carcinoma, prostate adenocarcinoma and head and neck squamous cell carcinoma. Papillary renal carcinoma showed an R280Q missense mutation. In prostate adenocarcinoma, the samples that contained a nonsense mutation resulted in a shallow deletion of the CK2α transcript. There is no known importance for this residue.

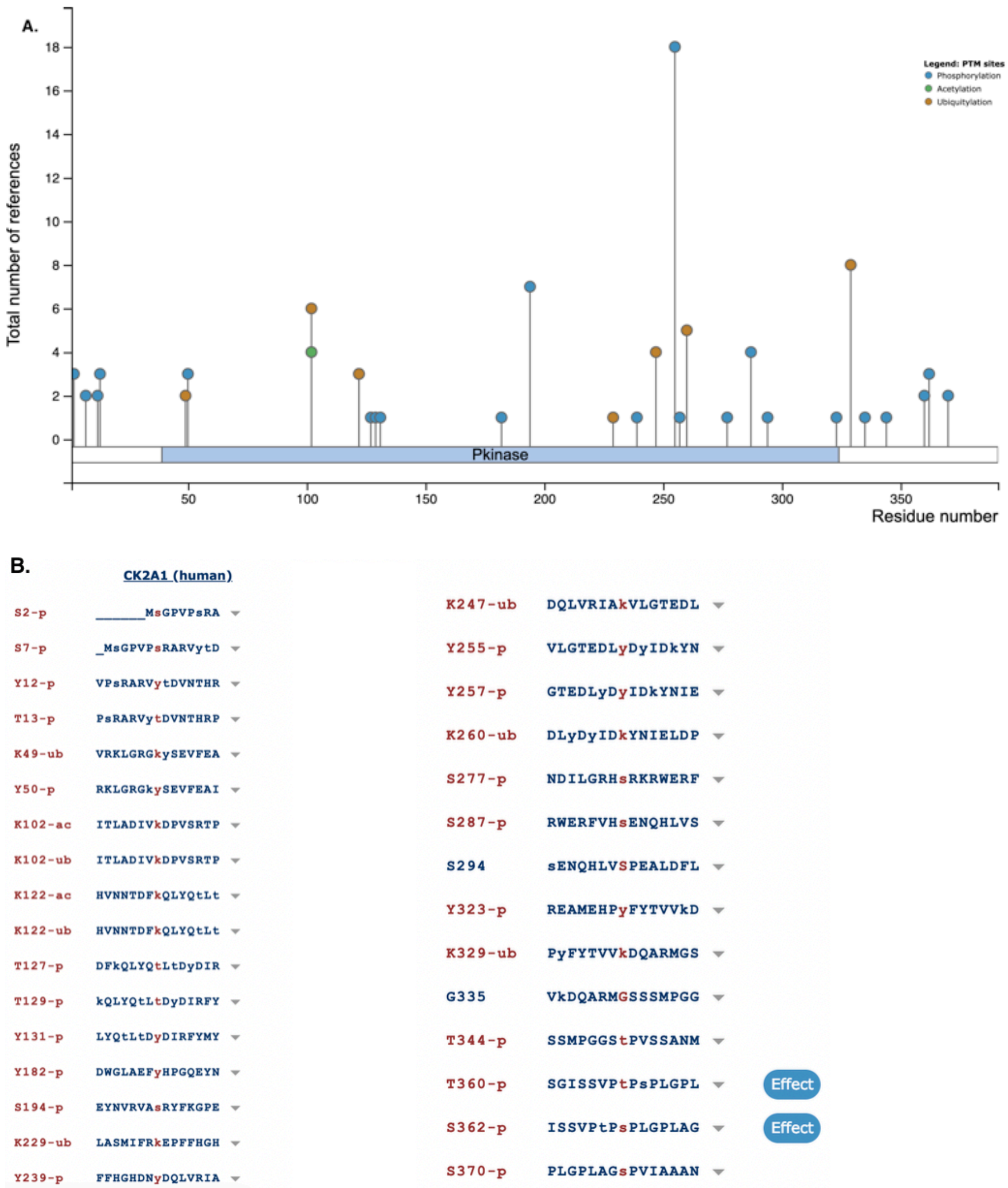
Arginine 306: The final hot spot of mutations along the CK2α transcript is at Arginine 306. All of the recorded mutations from cBioPortal show a nonsense mutation. The cancers involved are Uterine Endometrioid Carcinoma, Uterine Carcinosarcoma, hepatocellular carcinoma, Head and Neck squamous cell carcinoma, and cervical squamous cell carcinoma.

It is important to note that mutations in 3 of 4 of these hotspots are prevalent for Head and neck squamous cell carcinoma, Prostate adenocarcinoma, and uterine endometrioid carcinoma.

Cancer Type	R80	H236	R280	R306
Breast Invasive Ductal Carcinoma			R280*	
Cervical Squamous Cell Carcinoma				R306*
Colon Adenocarcinoma	R80C			
Colorectal Adenocarcinoma	R80C			
Cutaneous Melanoma		H236R		
Diffuse Large B-Cell Lymphoma	R80H			
Germinal B-Cell Type	R80C			
Head and Neck Squamous Cell Carcinoma		H236R	R280*	R306*
Hepatocellular Carcinoma				R306*
Lung Squamous Cell Carcinoma		H236R		
Medulloblastoma	R80H	H236R		
Melanoma		H236R		
Papillary Renal Cell Carcinoma			R280Q	
Prostate Adenocarcinoma	R80H	H236R	R280*	
Uterine Endometrioid Carcinoma	R80H		R280*	R306*
Uterine Serous Carcinoma			R280*	
Uterine Carcinosarcoma				R306*

Table 2. Amino Acid Residue Changes in CK2α. This table houses a list of all the cancer types that contain one of the four major residue changes that were found to occur in CK2α.

CK2α was used to query the Phosphosite database. The amino acid residues that can be phosphorylated on CK2α was compiled and pooled (figure 6). Among these phosphorylation sites, there are two recorded that when altered can cause CK2α activity to be elevated. Both of these sites Threonine 360, and Serine 362 are outside of the kinase domain. Induced activity is seen in both of these phosphorylation sites[50–52]**.** Positions 47, 50, 175, and 198 have all been reported mutated spots for germ line mutations are associated with neurodevelopmental disorders[53].



Site	Sequence	
S2-p	______MsGPVPsRA	
S7-p	_MsGPVPsRARVytD	
Y12-p	VPsRARVytDVNTHR	
T13-p	PsRARVytDVNTHRP	
K49-ub	VRKLGRGkySEVFEA	
Y50-p	RKLGRGkySEVFEAI	
K102-ac	ITLADIVkDPVSRTP	
K102-ub	ITLADIVkDPVSRTP	
K122-ac	HVNNTDFkQLYQtLt	
K122-ub	HVNNTDFkQLYQtLt	
T127-p	DFkQLYQtLtDyDIR	
T129-p	kQLYQtLtDyDIRFY	
Y131-p	LYQtLtDyDIRFYMY	
Y182-p	DWGLAEFyHPGQEYN	
S194-p	EYNVRVAsRYFKGPE	
K229-ub	LASMIFRkEPFFHGH	
Y239-p	FFHGHDNyDQLVRIA	
K247-ub	DQLVRIAkVLGTEDL	
Y255-p	VLGTEDLyDyIDkYN	
Y257-p	GTEDLyDyIDkYNIE	
K260-ub	DLyDyIDkYNIELDP	
S277-p	NDILGRHsRKRWERF	
S287-p	RWERFVHsENQHLVS	
S294	sENQHLVSPEALDFL	
Y323-p	REAMEHPyFYTVVkD	
K329-ub	PyFYTVVkDQARMGS	
G335	VkDQARMGSSSMPGG	
T344-p	SSMPGGStPVSSANM	
T360-p	SGISSVPtPsPLGPL	Effect
S362-p	ISSVPtPsPLGPLAG	Effect
S370-p	PLGPLAGsPVIAAAN	

Figure 7. Phosphorylation Mapping of CK2α. A.) A gene construct of CK2α from PhosphoSite displays all the phosphorylation (blue), acetylation (green), and ubiquitylation (brown) sites along the gene. B.) Additionally, a table is listed showing all the amino acid residue sequences where all phosphorylation (p), acetylation (ac), and ubiquitylation (ub) sites are. The two residues S360 and S362 are area seen in literature that lead to an effect when mutated.

Mutational Analysis of CK2α':

Analysis of cBioPortal showed alterations in CSNK2A2 due to mutation, deep deletions and amplification. Among the genetic alterations, mutations and deep deletions are the most prevalent and prevailing in alteration frequency for each cancer type. There are a few exceptions, such as colon cancer, undifferentiated stomach adenocarcinoma, medulloblastoma SHH, and non-melanoma skin cancer contained only mutational alterations; seminoma contained only amplifications; cervical adenocarcinoma, and Non-Hodgkin lymphoma alteration were a result of deep deletions. (figure 7). The five cancers that contained the highest frequency of CK2α' alterations were colon Cancer, undifferentiated stomach adenocarcinoma, endometrial carcinoma, cancer of unknown primary (metastatic cancer), and medulloblastoma sonic hedgehog (SHH).

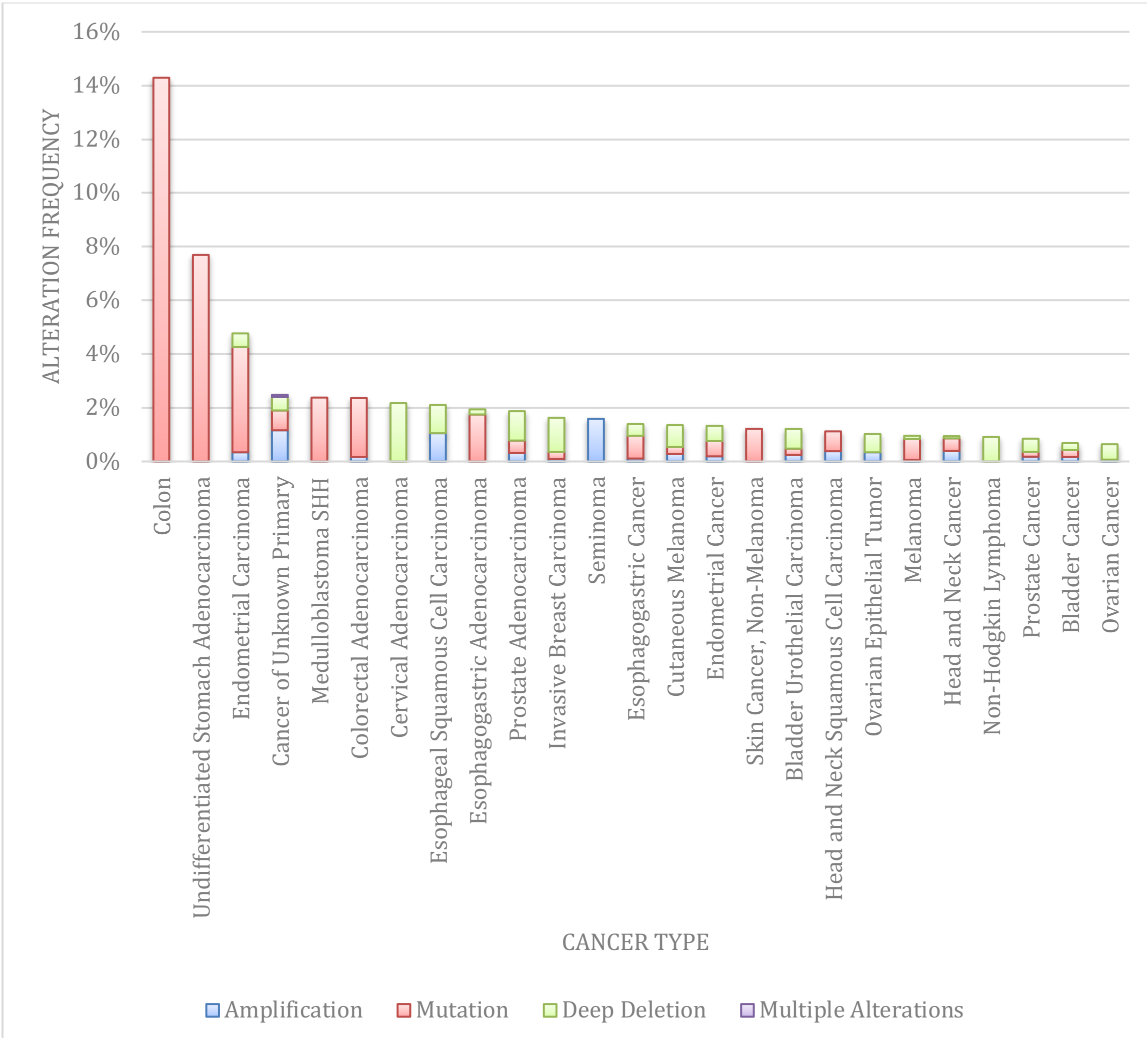


Figure 8. Alteration Frequency of CK2α' in Several Cancer Types. Compilation of alteration frequency of CK2α' gathered from cBioPortal graphically to show which cancer types contain the highest alteration to the CK2α' gene. Change in CK2α' gene is divided into amplification, mutation, deep deletion and multiple alterations.

Mutations in CSNK2A2 are scattered throughout the gene, with no prominent visual clusters depending on the cancer type, but there are some grouped together. The majority of the mutations were missense mutations. There is a total of 141 missense, and 45 truncating mutations, which result in either nonsense, splice, frameshift deletion or insertions. The protein kinase domain for CK2α' starts at residue 40 and ends at 325. The kinase domain contains the multitude of mutations. There were four residues with a higher number of mutations than the rest on the kinase domain: Arginine 90, lysine 171, Arginine 229, and Arginine 245. There is a large variation of mutation types among the residue hotspots for CK2α' and result in a collection of missense, functional deletion, splice, and nonsense mutation.

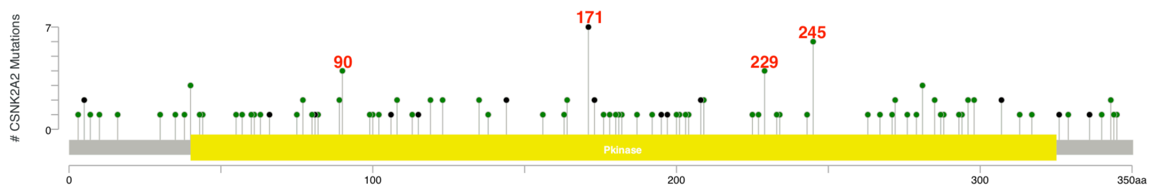


Figure 9. Mutational Map of CK2α' Gene. Pooled from cBioPortal is a visual representation of the CK2α' gene and all the mutations that have been reported on it among several cancer types. Residues that contained multiple mutations in different cancer types were highlighted and labeled. These four residues are referenced as the mutational hotspots for CK2α'

Arginine 90: The first hot spot of mutations takes place towards the beginning of the kinase domain at Arginine 90. The cancer types from the cBioPortal data base that contained mutations at this spot were prostate adenocarcinoma (from two separate studies), cutaneous melanoma, and mixed cancer types which came from a study that utilized cell lines from 36 different tumor types. The mutations in all of these studies and different cancer types resulted in a missense mutation that changed Arginine into Histidine. As a result of this missense mutation, CK2α' experienced a shallow deletion in prostate adenocarcinoma, and an amplification for cutaneous melanoma.

Lysine 171: The second mutation hot spot on the kinase domain of CK2α' is located at Lysine 171. Four separate studies that collected Stomach adenocarcinoma samples found frame shift deletions for CK2α' as Lysine 171. Two separate sample collections for Head and Neck Squamous cell carcinoma that resulted in a slicing at K171. Missense mutations were found for samples coming from a mixed cancer types, the missense was K171R. In this case, a conservative substitution is made replacing lysine (a positively charged basic amino acid) for another basic amino acid. In rectal adenocarcinoma samples there is a slicing at K171, but no fundamental change to CK2α'. Lastly, a cBioPortal collection of uterine endometrioid carcinoma contained frame shift insertions at K171.

Arginine 229: Arginine 229 is the third spot on the kinase domain in CK2α' with a collection of mutations. A nonsense mutation R229* (stop codon) in mixed cancer types and uterine endometrioid carcinoma. Missense mutation R229Q was found in both uterine endometrioid carcinoma and bladder urothelial carcinoma. A missense mutation changing the basic arginine to glutamine can result in a decrease in phosphorylation events.

Arginine 245: The last major site of mutations, all missense, is on the kinase domain is near the C-terminal at Arginine 245. Missense mutations R245P were found in invasive breast carcinoma and breast invasive lobular carcinoma. In both of these carcinomas CK2α' received a frameshift deletion, which result in truncation.

Missense mutation of R245C was found in esophageal adenocarcinoma. Uterine Carcinoma/uterine mixed Mullerian tumor samples, signet ring cell carcinoma of the stomach, and colon adenocarcinoma contained missense mutation R245H. Uterine carcinosarcoma also had a missense mutation of R245H. Finally, Bladder urothelial carcinoma contained missense mutation R245S.

Among all these cancer types, only one showed more than one hotspot mutation: bladder urothelial carcinoma.

Cancer Type	R90	K171	R229	R245
Bladder Urothelial Carcinoma			R229Q	X
Breast Invasive Lobular Carcinoma				R245*
Colon Adenocarcinoma				R245H
Cutaneous Melanoma	R90H			
Esophageal Adenocarcinoma				R245C
Head and Neck Squamous Cell Carcinoma		X		
Invasive Breast Carcinoma				R245
Mixed Cancer Types	R90H	X	R229*	
Prostate Adenocarcinoma	R90H			
Rectal Adenocarcinoma		X		
Signet Ring Cell Carcinoma of the Stomach				R245H
Stomach Adenocarcinoma		X		
Uterine Carcinosarcoma				R245H
Uterine Endometrioid Carcinoma		X	R229Q /R229*	
Uterine Carcinoma				R245H

Table 3. Amino Acid Residue Changes in CK2α'. This table houses a list of all the cancer types that contain one of the four major residue changes that were found to occur in CK2α'.

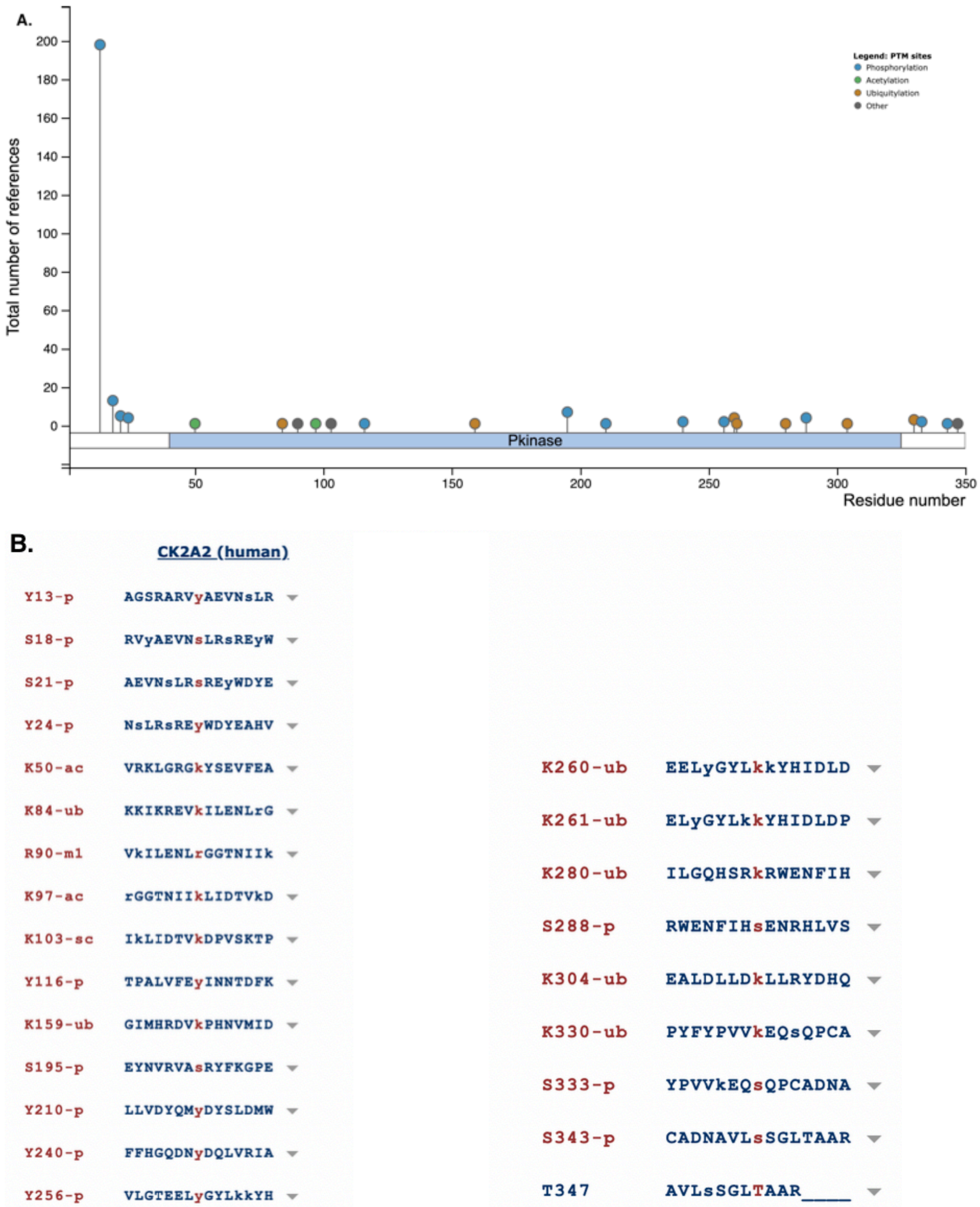


Figure 10. Phosphorylation Mapping of CK2α'. A.) A gene construct of CK2α' from PhosphoSite displays all the phosphorylation (blue), acetylation (green), and ubiquitylation (brown) sites along the gene. **B.)** Additionally, a table is listed showing all the amino acid residue sequences where all phosphorylation (p), acetylation (ac), and ubiquitylation (ub) sites are.

CK2α' was used to query the Phosphosite database. The amino acid residues that can be phosphorylated on CK2α' was compiled and pooled (figure 9). Using this database, there are no recorded amino acid residues that have any inductive effect or that are altered as a result of a germ line mutation.

Mutational Analysis of CK2αP:

cBioPortal analysis showed alterations in the CK2α pseudo gene CK2αP (CSNK2A3) to be a result of amplifications, mutations, deep deletions, or multiple alterations. Further look at the cancer types presented showed mutations and deep deletions to be the predominate alteration form, as well as amplifications being largely pronounced in bladder/urinary tract cancer. Of the 20 cancer types represented, 5 of them showed exclusively mutations.: small cell lung cancer, Non-Hodgkin Lymphoma, Colorectal Adenocarcinoma, Esophageal Squamous Cell Carcinoma, Hepatocellular Carcinoma, and Cervical Squamous Cell Carcinoma (See figure 10). The five cancers with the highest alteration frequency were Bladder/Urinary tract cancer, Endometrial Carcinoma, Diffuse Glioma, Small Cell Lung Cancer, and Esophagogastric Adenocarcinoma. Among these five cancers mutations were the highest cause for gene alteration.

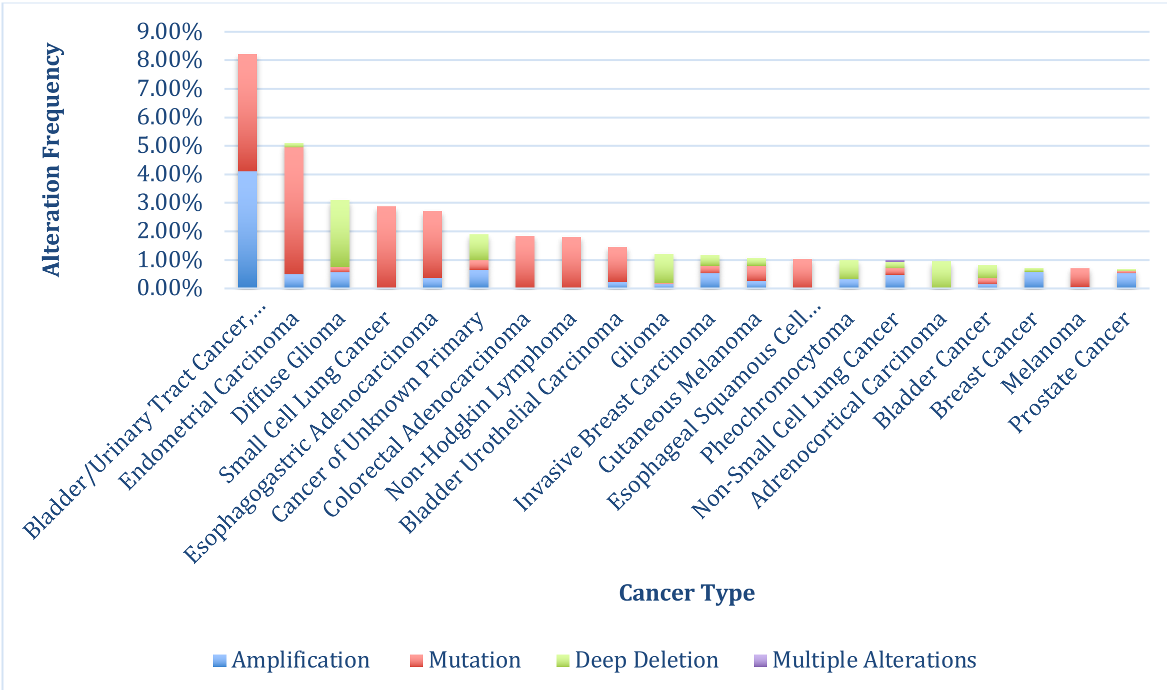


Figure 11. Alteration Frequency of CK2αP in Several Cancer Types. Compilation of alteration frequency of CK2αP gathered from cBioPortal graphically to show which cancer types contain the highest alteration to the CK2α' gene. Change in CK2αP gene is divided into amplification, mutation, deep deletion and multiple alterations.

There is no specific cluster of mutations, however most of them are scattered throughout most of the pseudogene sequence (figure 11). These mutations would exist if the mRNA was translated for CK2αP. The majority of mutations among all cancer types were missense mutations and frame shift deletions. There is a total of 149 missense mutations, 26 truncations, and 2 inframe mutations.

There were 2 specific residues that were seen to be mutation more frequently than others: Arginine 80, and Serine 287. All of which would exist if the mRNA transcript of CK2αP was translated.

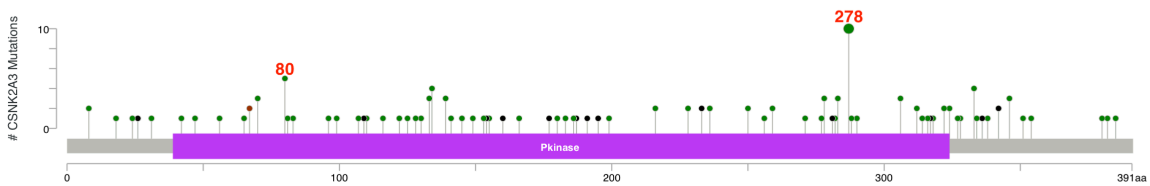


Figure 12. Mutational Map of Hypothetical Translated CK2αP Gene. Pooled from cBioPortal is a visual representation of the CK2αP gene and all the mutations that have been reported on it among several cancer types. Residues that contained multiple mutations in different cancer types were highlighted and labeled. These four residues are referenced as the mutational hotspots for CK2αP

Hypothetically, if that mRNA was translated, position 80 and 287 will be in the kinase domain (see table 4). It is important to note that of the different cancer types found mutations in the bases that hypothetically would code for 80 and Serine 287, stomach adenocarcinoma is found to contain mutations at both of these residues in multiple studies collected in cBioPortal (see table 4). The rest of the cancer types contain only one of two regions mutated

Cancer Type	"R80"	"S287"
Colon Adenocarcinoma	X	
Small Cell Lung Cancer		X
Stomach Adenocarcinoma	X	X
Tubular Stomach Adenocarcinoma	X	
Uterine Endometrioid Carcinoma	X	
Uterine Serous Carcinoma	X	

Table 4. Amino Acid Residue Changes of Hypothetical translated CK2αP. This table houses a list of all the cancer types that contain one of the four major residue changes that were found to occur in CK2αP in the case that is hypothetically translated from its mRNA.

Mutational Analysis of CK2β:

Analysis of cBioPortal showed alterations in CK2β to be due mostly due to amplification, followed by deep deletions and mutation. Indeed, of the 26 cancer types pooled from cBioPortal 11 of them showed solely amplifications. These cancers include bladder/urinary tract cancer, Ovarian Epithelial Tumor, Ocular Melanoma, Cholangiocarcinoma, Pancreatic Adenocarcinoma, Hepatocellular Carcinoma, Cervical Cancer, Cervical Squamous Cell Carcinoma, Esophageal Squamous Cell Carcinoma, Hepatobiliary Cancer, Endometrial Cancer, and Head and Neck Squamous Cell Carcinoma. All other cancer types contained mutations, and or deep deletions (figure 12). The five cancers that contained the highest alteration frequency for CK2β were Cancer of unknown primary (metastasized cancer samples), cutaneous melanoma, Non-Hodgkin Lymphoma, Bladder/Urinary Tract Cancer NOS, and Ovarian Cancer. Among these five, amplifications held the dominant form of alteration type, followed by either deep deletions and mutations.

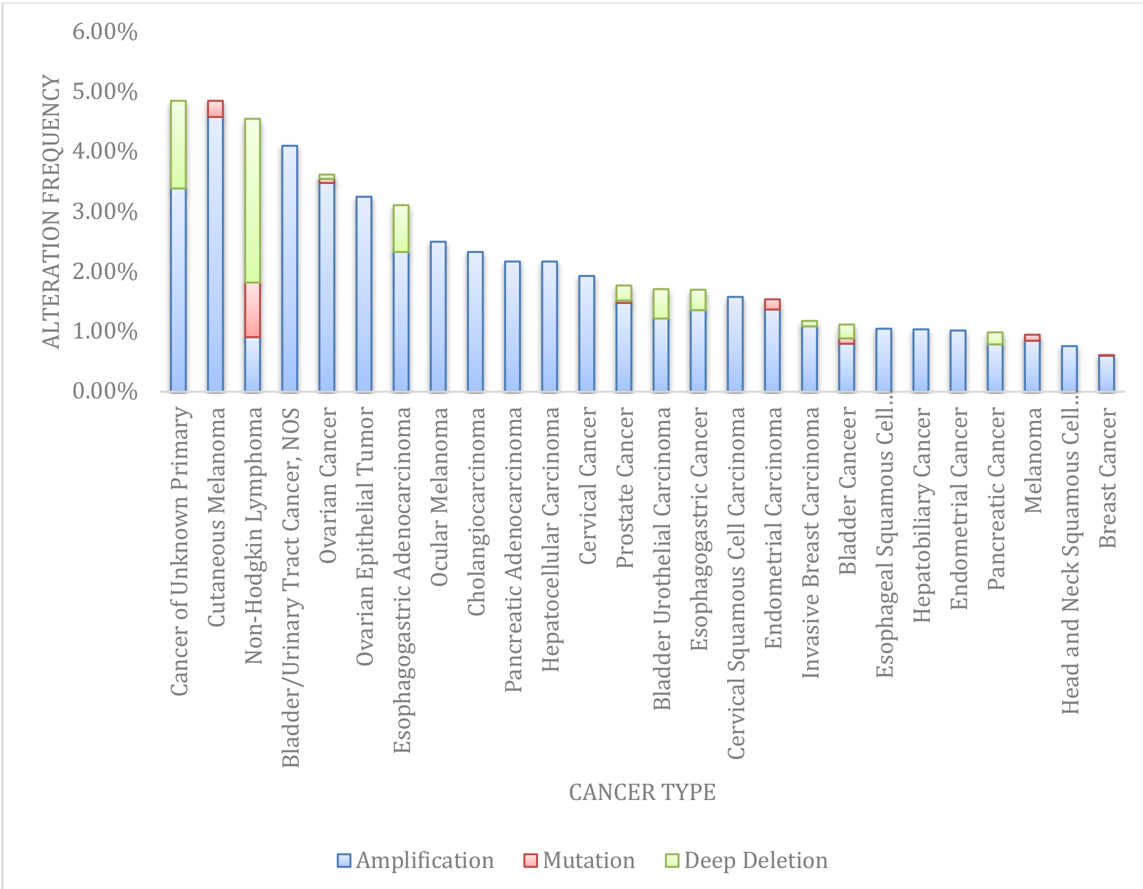


Figure 13. Alteration Frequency of CK2β in Several Cancer Types. Compilation of alteration frequency of CK2β gathered from cBioPortal graphically to show which cancer types contain the highest alteration to the CK2β gene. Change in CK2β gene is divided into amplification, mutation, deep deletion and multiple alterations.

The mutations are mostly clustered in pockets throughout the gene (figure 13). The majority of mutations among all cancer types are missense mutations. There is a total of 23 missense mutations, and 6 truncating mutations, which are either nonsense mutations or frameshift deletions. The important domain for the regulatory beta gene spans from residue 2-216, and contains a multitude of mutations. However, only single mutations were seen at most residues, there was only one residue that was mutated more frequently than others, which is glutamine 182.

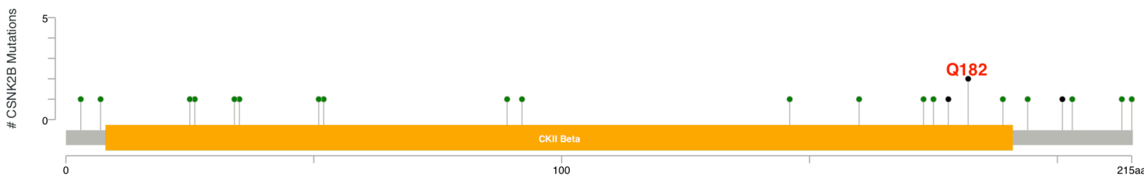


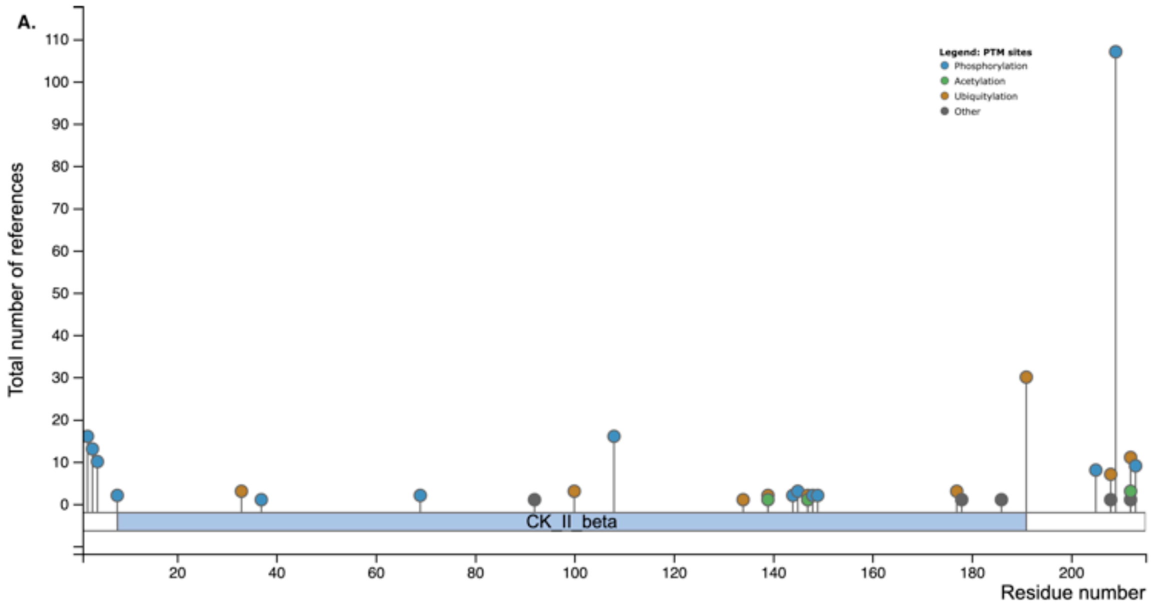
Figure 14. Mutational Map of CK2β Gene. Pooled from cBioPortal is a visual representation of the CK2β gene and all the mutations that have been reported on it among several cancer types. Residues that contained multiple mutations in different cancer types were highlighted and labeled. The one residue is referenced as the mutational hotspots for CK2β

Position 182 is part of the gene's 2-216 regulatory region, which contributes to CK2β protein interactions and formation of the holoenzyme. Mutations at this site were nonsense (Q182*) and were found in glioblastoma multiforme and Diffuse Large B-Cell Lymphoma also having Q182* (Table 5). There individual action within each cancer case cannot be determined, however should be further investigated.

Cancer Type:	Q182
Glioblastoma Multiforme	Q182*
Diffuse Large B-Cell Lymphoma	Q182*

Table 5. Amino Acid Residue Changes in CK2β. This table houses a list of all the cancer types that contain one of the four major residue changes that were found to occur in CK2β.

A.



B.

CK2B (human)

Site	Sequence		Site	Sequence
-	gap		T145-p	PkCMDVytPkssRHH
-	gap		K147-ub	CMDVytPkssRHHHT
S2-p	_______MsssEEVsW	Effect	K147-ac	CMDVytPkssRHHHT
S3-p	______MsssEEVsWI	Effect	S148-p	MDVytPkssRHHHTD
S4-p	_____MsssEEVsWIS	Effect	S149-p	DVytPkssRHHHTDG
S8-p	MsssEEVsWISWFCG		K177-ub	VHPEYRPkrPANQFV
K33-ub	DEDYIQDkFNLtGLN		R178-m1	HPEYRPkrPANQFVP
T37-p	IQDkFNLtGLNEQVP		R186-m1	PANQFVPrLYGFkIH
S69-p	LEDNPNQsDLIEQAA		K191-ub	VPrLYGFkIHPMAYQ
R92-m1	ARYILTNrGIAQMLE		S205-p	QLQLQAAsNFksPVk
K100-ub	GIAQMLEkYQQGDFG		K208-ub	LQAAsNFksPVktIR
Y108-p	YQQGDFGyCPRVYCE		K208-m1	LQAAsNFksPVktIR
K134-ub	IPGEAMVkLYCPkCM		S209-p	QAAsNFksPVktIR_
K139-ub	MVkLYCPkCMDVytP		K212-ub	sNFksPVktIR_____
K139-ac	MVkLYCPkCMDVytP		K212-sm	sNFksPVktIR_____
Y144-p	CPkCMDVytPkssRH		K212-ac	sNFksPVktIR_____
			T213-p	NFksPVktIR______

Figure 15. Phosphorylation Mapping of CK2β. A.) A gene construct of CK2β from PhosphoSite displays all the phosphorylation (blue), acetylation (green), and ubiquitylation (brown) sites along the gene. Additionally, somatic and germ mutations are reported along the kinase domain. **B.**) Additionally, a table is listed showing all the amino acid residue sequences where all phosphorylation (p), acetylation (ac), and ubiquitylation (ub) sites are. The three residues S2, S3, and S4 are all phosphorylated residues seen in literature that lead to an effect when mutated.

CK2β was used to query the Phosphosite database. The amino acid residues that can be phosphorylated on CK2β was compiled and pooled (figure 14). Based on literature reports phosphorylation to residues serine 2, 3 and 4 promote elevated activity of CK2β. At residue serine 2, phosphorylation results in induced enzymatic activity, and protein stabilization[51,54]. Phosphorylation at serine 3, and 4 will also result in an induced effect of protein stability[51,54]. There was no reported germ line or somatic mutations reported on the CK2β gene in this database.

Kaplan-Meier Survival Analysis:

Kaplan-Meier survival plots were generated and extracted from cBioPortal in order to visually analyze the impact mutations on the different CK2 genes had on survival. Kaplan-Meier plots were analyzed for individual genes alone versus pairs or in triplets of CK2 genes. This analysis in cBioPortal did not allow for testing of the individual mutations, but all of the mutations together for all the cancers in one single gene.

First, when looking at the genes individually, mutations in CK2α, CK2α', CK2αP, or CK2β did not show statistical significance in the Kaplan-Meier plot analysis for either overall survival or disease-free progression. However, when we analyzed the impact that mutations had on survival when pairing the genes together the results vary. No significant correlation for mutation and survival is found in the following gene pairs: CK2α and CK2α'; CK2α and CK2αP; CK2α' and CK2αP; and

CK2α’ and CK2β. There is however statistical significance in the correlation between mutations in CK2α and CK2β or CK2αP and CK2β and cancer disease progression (figures 15 and 16). When we analyzed the genes in triplet pairs, there is no statistical findings that mutations correlate with overall survival, or disease/progression.

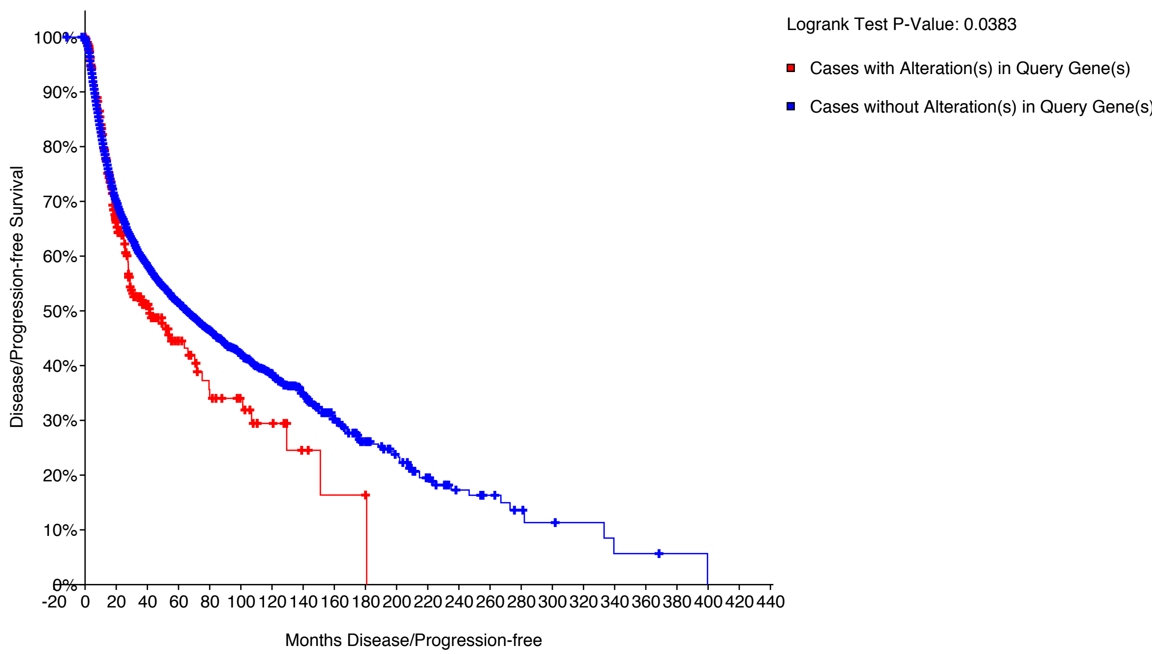


Figure 16. Mutation in CK2αP and CK2β Effect on Cancer Disease Progression. Kaplan-Meier plots from cBioPortal pooling data from combinational mutations in CK2αP and CK2β show that alterations in both of these genes leads to further disease progression when compared among all cancer types. (P=0.038)

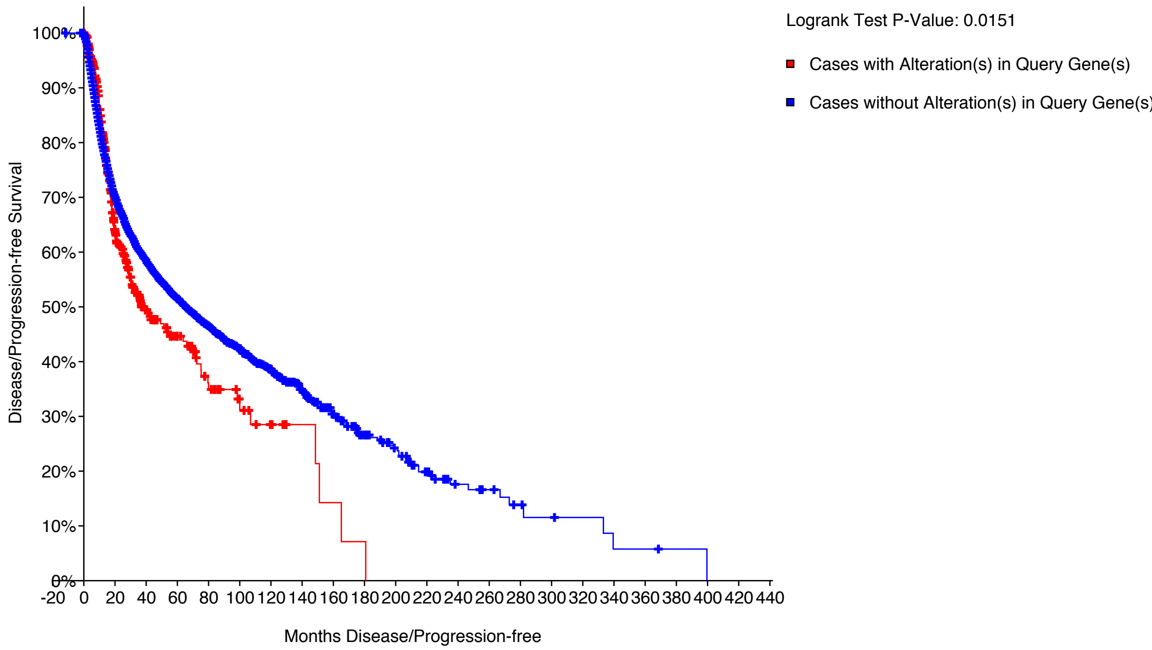


Figure 17. Mutation in CK2α and CK2β Effect on Cancer Disease Progression. Kaplan-Meier plots from cBioPortal pooling data from combinational mutations in CK2α and CK2β show that alterations in both of these genes leads to further disease progression when compared among all cancer types. (P=0.0151)

CK2α	CK2α’	CK2β
R→C	R→C	Q→*
R→H	R→H	
R→*	R→Q	
H→R	R→*	

Table 6. Summary CK2 Gene Residue Change for the 3 CK2 proteins. The collection of missense and nonsense mutations for CK2α and CK2α’ are listed in order to visually represent the similar missense and nonsense mutations both genes contain. Additionally, nonsense mutation for CK2β is listed.

DISCUSSION

For CK2 proteins, a number of studies discovered amino acid mutations. Some of these mutations are important for disease, including missense mutations in CK2 that are a leading cause in disorders such as Okur-Chung neurodevelopmental syndrome[55]. Therefore, analyzing mutations on CK2 proteins in cancer is essential to determine their role in carcinogenesis. Here we described CK2 mutations across different cancers. Mutations that don't change the overall protein structure/function will likely not affect protein activity, therefore should have no effect in carcinogenesis. However, missense mutations, which change an amino acid due to a codon change, or nonsense mutations which code for a stop codon and can ultimately cause protein truncation could be very important for carcinogenesis. Therefore, we discuss these below.

Mutations in CK2 kinase proteins

When looking at a change in amino acid level for both CK2α and CK2α' across several cancer types we see change in arginine to histidine, which is conservative. Interestingly enough, it has been shown that throughout several cancer types mutation signatures mainly contain arginine to histidine missense mutations[56,57]. Arginine to histidine mutations are elevated in driver mutations when compared to passenger mutations, stressing the importance of this type of mutation in-driving tumorigenesis[58]. In the case of the tumor suppressor p53, the extraordinary loss of arginine throughout several cancer types is pertinent in the functioning of metabolism and oxidative stress for cells[57]. In addition to Histidine, this study found arginine to be replaced also with cysteine or tryptophan in over 18,000 cancer samples[57]. The authors proposed that the gain of amino acids such as histidine aids in cancer development *via* alleviation of oxidative stress. Indeed, cysteine, histidine and tryptophan increase total antioxidant and metal-binding capacity of the proteome of cancer cells[57]. Additionally, changing amino acid Arg to His critically impacts the cell and its relationship with the environment. The switch from Arg to His causes a gain in pH sensing inside the cell, which gives cancer cells an advantage in increased intracellular pH (pHi) due to altered protein binding and activity at increased pHi[59]. An increase in pHi is commonly seen in most cancer types[59,60]. Both CK2α and CK2α' have arginine to histidine mutations across very diverse cancer types. There are no existing studies that explore the effect that these amino acid changes have either on driving cancer, change in oxidation stress or pH sensing. In the future, it will be important to study the possible physiological implications of Arg to His mutations in CK2 kinase proteins will have for the cancer cell.

Additionally, in both CK2α and CK2α' there were some arginine to cysteine missense mutations. This change is another common missense mutation described in many cancers[57,61]. Arg -> Cys, unlike Arg -> His will change the net charge of the protein, as well as the protein dynamics with a loss of a positively charged residue and cysteine, which can make disulfide bridge interactions[62]. A study surveying the protein-protein interactions of glioblastoma found an elevated level of arginine to cysteine mutations[61]. However, future studies testing Arg to His mutations in CK2 kinase proteins will determine the *in vivo* and *in vitro* impact they have in the protein, the cell and carcinogenesis.

Our analysis found CK2α and CK2α' nonsense mutations, which result in protein truncation. Truncations in kinase domains for proteins like the epidermal growth factor receptor (EGFR) render the receptor tyrosine kinase constitutively active[63]. CK2 kinase activity can be elevated over its basal level by agonists such as Wnt[64]. Suggesting that it is plausible that these truncating mutations lead to elevated activity. Even though we did not focus on mutations outside the kinase domain, truncation in the N-terminus would abolish the constitutive activity[42]. In addition, a study of kinases with truncation mutations in or outside the kinase domains identified these types of mutations as driver mutations[63]. Future studies testing nonsense mutations in CK2 kinase proteins will determine whether they can contribute to kinase activity alterations or whether they would be passenger mutations or driver mutations contributing to cancer pathogenesis. In addition, while CK2α can function as a monomeric kinase, it also binds CK2β in a holoenzyme form. Therefore, mutations in CK2 kinases can also affect the formation and activity of the holoenzyme.

Mutations in CK2β

Our results showed nonsense mutations from the loss of glutamine, which led to protein truncation. In addition to CK2α, CK2β interacts with other protein kinases. In the case of human checkpoint kinase Chk1 the interaction gives an advantage to tumor cells[65]. Typically causing cell cycle arrest in the G_2 stage, however when Chk1 is activated by CK2β if promotes cell cycle progression and preserves genome integrity[65]. Formation of the CK2 holoenzyme is largely dependent on CK2β and its interaction with the catalytic CK2α. The truncations of CK2β we described might lead to elevated or decreased levels in CK2 holoenzyme formation or altered protein interactions with other protein kinases. In the future, it will be important to determine all the binding regions for kinases on CK2β and how these mutation changes the interaction with these proteins, and whether they have a regulatory significance or contribute to any specific cancer development[65].

Potential effect of the CK2 mutations in different cancer types

Studies have shown CK2α's role in phosphorylating SIRT 6, which is seen in progressive breast carcinoma[66]. Additionally, inhibition of CK2α has been shown to help amplify the effects of antitumor drugs in breast carcinoma.

Head and Neck Squamous Cell Carcinoma

Head and neck squamous cell carcinoma show CK2α and CK2α' mutations due to missense, nonsense and splicing mutations. Importantly, malignant transformation of upper aerodigestive tract mucosa to head and neck squamous cell carcinoma has been consistently associated with altered CK2α activity[67–69]. In addition, an increase in transcript levels for CK2α and CK2α' are consistent in head and neck cancers-specifically head and neck squamous cell carcinoma[45,67]. Here we show that there are CK2α mutations in H23, R280, R306 or K171 in head and neck squamous cell carcinoma. These mutations are in the kinase domains of both CK2α and CK2α'. However, there is no report of mutations in H23, R280, R306 or K171 contributing to head and neck squamous cell carcinoma.

Uterine Endometrioid Carcinoma

We found multiple residue changes across both CK2α, CK2α', and hypothetical CK2αP in uterine endometrioid carcinoma. Endometrioid carcinoma is among the most common cancer types seen in premenopausal women. However, there has been little research looking at CK2's role in endometrioid carcinoma, and no research in mutational profiles. One study found that CK2 inhibition promotes apoptosis in endometrial carcinoma cells[70]. Suggesting that mutations that elevate CK2 kinase activity may protect from apoptosis. A study looking at tumor necrosis factor-related apoptosis (TRAIL) as an antineoplastic agent found its efficacy to be greatly reduced in endometrial carcinoma cells due to resistance caused by elevated FLICE-inhibitor protein levels. When CK2 inhibition occurs, sensitization of endometrial carcinoma cells to TRAIL occurs[70].

Not only is uterine endometrioid carcinoma present in mutations in CK2α, CK2α', and CK2αP; it's also associated with multiple different residue changes in each of these genes-whether the result of missense, nonsense or deletion mutations. Such prevalence warrants further investigation of each of these genes and their perspective mutational roles in endometrioid carcinoma.

Colon Adenocarcinoma

Another cancer type that was seen in mutations in all three genes CK2α, CK2α', and CK2αP is colon adenocarcinoma. Heightened CK2 expression is known to protect human colon carcinoma cells from TRAIL induced apoptosis. Similar to what is seen with endometrial carcinoma cells, inhibition and loss of function of the CK2 genes leads to high apoptotic rates of these carcinoma cells[70,71]. Further exploration is needed on the specific residues that were mutated that were found in colon adenocarcinoma. CK2α is a known gene that is found in many different carcinomas. However, this is almost always due to overexpression. Some of these mutations that we analyzed resulted in nonsense mutations and missense mutations. It is entirely possible that these mutations might result in an increase in CK2 activity. What is most interesting from the results is the diversity of carcinoma types seen in the mutated CK2α and CK2α' genes, as well as the locations of these different cancers.

With the abundance of adenocarcinoma cases from the results for CK2α it would be interesting to explore whether any of these particular mutations aid in the epithelial-mesenchymal transition (EMT). It is known that enhanced epithelial-mesenchymal transition leads to tumorigenesis with metastatic ability[72]. CK2α overexpression a driver of EMT. Studies have found RNA silencing of CK2α to lower EMT in cancer cells. Additionally, TGF-β pathway which plays a huge role in EMT, is seen to lower the activity of CK2β creating an imbalance between CK2α and CK2β activity which can lead to EMT tumorigeneses[73,74]. Due to the limitations of our study and the data contained in cBioPortal, it would be interesting to further analyze some of these carcinoma cases seen in CK2α and see if they resulted due to a direct gene expression enhancement, or whether there is a CK2β mutation working in concert causing its expression to be diminished promoting that imbalance that leads to EMT. CK2α is also known to phosphorylate Snail1 protein which is a regulator of target genes directly responsible for EMT and cell survival[73]. It is possible that missense mutations in the kinase domain of CK2α that lead to overexpression might drive EMT with heightened phosphorylation of Snail1.

Diffuse Large B-Cell Lymphoma

Although, there were not many mutational residue hot spots in CK2β as there were in CK2α, one shared cancer between the two is diffuse large B-cell lymphoma. Diffuse large B-cell lymphoma makes up 30-40% of the non-Hodgkin Lymphomas, and is the most common amongst them[75]. Only one residue mutation was correlated with this cancer type in both CK2α and CK2β. CK2 is highly expressed and crucial for proliferation and survival in non-Hodgkin Lymphoma. This finding further illuminates the uniqueness behind the tetrameric enzyme CK2. The two cancer types that arose from the two different residue hot spots are very unique from the multitude of carcinomas that came from mutated CK2α, CK2α', and CK2αP. This warrants further research on CK2β's role in lymphatic cancers, and whether it is acting alone or partnered with a CK2α mutation.

Limitations of the study

cBioPortal provided an abundance of information in regards to CK2 and its presence in different cancer types. However, there were still many limitations to what we could access and conclusions that can be made in regards to this data. While mutations were listed, allele frequency for these mutations in cancer patients were also listed but not compared with their frequency among the normal populace. Therefore, no proper analysis of these specific mutations could be compared. Additionally, limited information was provided by cBioPortal on cooccurrence of mutations in CK2 kinase proteins and CK2β. While they listed mutual exclusivity, all of their findings were of no statistical significance, so mutations in CK2 and cooccurrence remains to be determined. In the future, access to whether these specific mutations from our results were a contributor to the significant Kaplan-

Meier plots would pose as a very promising finding. As this would indicate specific mutations lower mortality in specific cancer types. Our findings of CK2α and CK2β, as well as CK2αP and CK2β mutations positively correlating with cancer disease progression is consistent with other Kaplan-Meier analysis of CK2.[45]

CONCLUSION

Future studies need to study specific gene mutations in the residues we reported and see if there is any true correlation with arising cancer types. Additionally, with this information a more refined Kaplan-Meier plot looking at one specific mutation (such as missense, or nonsense mutations) would illuminate the role a mutated CK2 has on mortality across several cancer types.

APPENDIX

APPENDIX 1. DEFINITIONS

Point Mutation (base substitution)	Altered codon corresponds to	Effect on function
missense	Different amino acid	No effect to non-functional protein
nonsense	Stop codon	Shortened or nonfunctional protein
silent	No change	None
Multiple alterations		
	Amino acid biochemical properties	Effect
Conservative substitution	similar	Lesser
Radical / Non-conservative substitution	different	Larger to deleterious

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