Protein kinase CK2 – diverse roles in cancer cell biology and therapeutic promise

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Abstract

The association of protein kinase CK2 (formerly casein kinase II or 2) with cell growth and proliferation in cells was apparent at early stages of its investigation. A cancer-specific role for CK2 remained unclear until it was determined that CK2 was also a potent suppressor of cell death (apoptosis); the latter characteristic differentiated its function in normal versus malignant cells because dysregulation of both cell growth and cell death is a universal feature of cancer cells. Over time, it became evident that CK2 exerts its influence on a diverse range of cell functions in normal as well as in transformed cells. As such, CK2 and its substrates are localized in various compartments of the cell. The dysregulation of CK2 is documented in a wide range of malignancies; notably, by increased CK2 protein and activity levels with relatively moderate change in its RNA abundance. High levels of CK2 are associated with poor prognosis in multiple cancer types, and CK2 is a target for active research and testing for cancer therapy. Aspects of CK2 cellular roles and targeting in cancer are discussed in the present review, with focus on nuclear and mitochondrial functions and prostate, breast and head and neck malignancies.

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Introduction

Protein kinases are profoundly important for post-translational modification of proteins, which concurs with the rather large portion of the translated human genome devoted to the protein kinase complement (referred to as the kinome) [1–3]. One of the protein kinases now known as CK2 (formerly casein kinase II or 2) was possibly the earliest recognized protein kinase activity (e.g., [4]). Subsequent work in various laboratories over the past several decades has identified CK2 as a major regulator of cell function in normal and disease states (e.g., [5, 6]). Among the latter, its involvement in cancer has attracted extensive attention since deregulation of CK2 in cancers is a consistent occurrence (e.g., [7–10]). In the present article, we present a broad overview of CK2 biology and function in cells with particular emphasis on nuclear and mitochondrial roles. Finally, we discuss its involvement and targeting in cancer biology focusing on three specific cancers which are under investigation in our laboratory (prostate, breast, head, and neck cancers).

Basic aspects of CK2 biochemistry

Discovery and investigation into the protein kinase complex that we now call "CK2" began in the 1950s, with descriptions of the enzymatic phosphorylation of proteins [4]. The initial identification of CK2 resulted in the misnomers "casein kinase II or 2" and phosvitin kinase; however, casein and phosvitin are not among the biological substrates for CK2. Over time, four human CK2 genes were identified, three of which encode the proteins enabling CK2 protein-directed kinase activity. The catalytic subunit CK2α is encoded by *CSNK2A1*, located on Chr 20p13. The other catalytic subunit CK2α′ is encoded by *CSNK2A2*, located on Chr 16q21. CK2α (391 AA) and CK2α′ (359 AA) are highly similar proteins, with the majority of divergence contained in the carboxy-termini (81.4% identity, blastp of reference sequences NM_177559.3 and NM_001896.4, [11]). CK2β is the regulatory subunit encoded by *CSNK2B*, located on Chr 6p21. *CSNK2A3* encodes an intronless "pseudogene" that has highest sequence homology with *CSNK2A1* and is located at Chr 11p15 [12, 13]. *CSNK2A3* is proposed to have roles in some cancer types as a protein or potentially as a non-coding RNA [14–17]; overall, investigation of this gene has been limited, and will not be part of further discussion in this review.

The heterotetrameric structure of the CK2 holoenzyme consists of two alpha subunits (molecular mass of α is 45 kDa and of α′ is 41 kDa) linked via two dimerized β subunits (molecular mass is 25 kDa), forming α2β2, or αα′β2, or α′2β2 configurations depending on the cell type (Fig. 1). The presence of the β subunits influences enzyme stability, substrate selectivity, and autophosphorylation [5, 18, 19]. The catalytic subunits are also active as monomers, although the activity is less than that of the tetrameric holoenzyme [20]. CK2 is primarily a S/T (serine/threonine) protein kinase but has been infrequently reported to phosphorylate Y (tyrosine)

residues [18, 21, 22]. Notably, CK2 utilizes both ATP and GTP for catalyzing transfer of phosphate groups to its substrates. Unlike most protein kinases, CK2 exhibits constitutive activation as both a monomeric and tetrameric enzyme, requiring no modification of phosphorylation status or regulated binding of a cofactor [23]; however, as discussed subsequently, specific regulation of its functional activity in response to diverse signals occurs [5]. Several documented post-translational modifications positively and negatively influence CK2 activity. These modifications include phosphorylation, *O*-linked glycosylation, acetylation, stimulation of kinase activity by agents such as polyamines, and association with other proteins (e.g., [24–29]); aspects of these modifications and regulatory events are discussed in more detail in recent reviews [30, 31].

An important feature of CK2 functional biology relates to its requirement for survival during embryonic development. Knockout of either CK2α or CK2β causes embryonic lethality [32–34]. In contrast, knockout of CK2α′ results in viable mice with male infertility [35]. Further, reduced levels of CK2α through heterozygous knockout combined with complete loss of CK2α′ results in developmental and growth abnormalities [36]. Recent work using myoblast cells has further demonstrated that activity from a CK2 catalytic subunit is necessary for cell survival. An attempt was made to generate CK2αα′ knockout C2C12 cells using CRISPR/Cas9 technology; however, it was later determined that surviving cells expressed a truncated form of CK2α′ [37]. There is interdependence among the CK2 subunits for expression levels within cells, including transcriptional feedback mechanisms [38, 39]. In cancer, it is observed that downregulation or knockout of just one of the catalytic subunits causes loss of the β subunit in cultured cells and in xenograft tumors [40–45]. Similarly, overexpression of the CK2α catalytic subunit promotes increased expression of the other subunits in prostate cells [42]. The overall requirement for CK2 expression in cells in conjunction with the consistent reliance of cancer cells on elevated CK2 levels has prompted its consideration as a target for cancer therapy (discussed subsequently).

Much work over the past three decades on the functional biology of CK2 has culminated in the notion that CK2 is a "master regulator" of diverse cellular functions (as reviewed in, e.g., [5–8, 46]). Protein kinase CK2 is ubiquitously expressed at varying levels in tissues and cell types, as well as in the majority of intracellular compartments and organelles. There are close to one thousand substrate phosphosites identified for CK2 (phosphositeplus website [47]), and this wide range of CK2 substrates are localized throughout the cell where they engage in distinct functions [6, 48]. Phosphoproteomics studies have demonstrated the prevalence and malignant growth-promoting effects of CK2 target proteins in cancer and cancer progression [49–52].

CK2 Tetrameric Holoenzyme
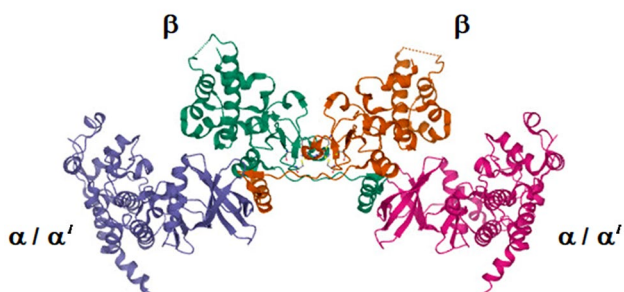
Fig. 1 Crystal structure of the full-length symmetric CK2 holoenzyme. PDB:4MD7 [311]

The far-reaching role of CK2 in proliferative activity is highlighted by the large number of substrates identified in mitosis by quantitative phosphoproteomics; these authors identified 330 CK2 phosphorylation sites on 202 proteins [53]. Given that most cellular proteins harbor CK2 acidophilic phosphorylation sites, it is not surprising that CK2 plays a wide range of roles in normal and disease states; aspects of these functions are discussed subsequently with a primary focus on nuclear and mitochondrial roles for CK2 and the involvement of CK2 in prostate, breast, and head and neck cancers. Other significant aspects of CK2 in cancer biology, such as roles in DNA synthesis and damage repair, immunological aspects, and hematological malignancies are discussed in other recent CK2 reviews [54–56].

Intracellular dynamic shuttling of CK2

The question of how cellular functions of CK2 are controlled has been perplexing due to the intrinsically active status of this kinase. However, this may be an overly simplistic view of the nature of its activity. Nearly 30 years ago, it was recognized that dynamic shuttling of CK2 to the nuclear compartment occurs in response to various growth signals [57–60]. It was noted that prostate cancer cells responsive to androgen presence (such as LNCaP cells) showed a loss of CK2 from the nuclear compartment on androgen deprivation in the culture media; whereas, when androgen was re-introduced to the culture media there was a rapid movement of CK2 from the cytoplasm to the nuclear compartment. Similar results were obtained when androgen was replaced with growth factors (such as EGF). On the other hand, prostate cancer cells not responsive to androgens (such as PC-3 cells) did not show changes in CK2 localization associated with presence or absence of androgen in the culture media. However, removal of growth factors such as EGF from the media of these cells resulted in loss of CK2 from the nuclear compartment which was again reversed on re-addition of growth factors. This shuttling of CK2 to the nuclear compartment in response to growth stimuli suggested involvement of CK2 in regulation of transcriptional activity [61, 62]. Within the nucleus, there is differential localization of CK2 in subnuclear compartments such as nucleoplasm, nuclear matrix, and nucleoli [59, 63–71].

CK2 dynamic localization to and within other intracellular organelles and subdomains such as the Golgi apparatus, endoplasmic reticulum, mitochondria, cytoskeleton, centrosomes, and plasma membrane also occurs in both non-transformed and malignant cells and tissues, hence, emphasizing the potential of its regulated involvement in numerous cellular activities [60, 72–74]. In mitochondria of rat liver, CK2 responds to stimuli by redistribution from the intermembrane space to the inner membrane [75]. CK2

regulation of certain metal ion transport has been explored in both non-malignant and cancer model systems [76–78] and represents one facet of CK2 functions at the plasma membrane. CK2 recruitment, interactions and function at the plasma membrane also occur due to growth factor signaling. As mentioned above, EGF acting via EGFR stimulated localization of CK2 to nuclear compartments in prostate cancer cells and numerous other reports have demonstrated EGF-mediated activation of malignant signaling involving CK2 at the plasma membrane. In one example, EGF-induced ERK2 phosphorylation of CK2α followed by CK2 phosphorylation of α-catenin, thus releasing β-catenin for transactivation to the nucleus [29]. In another example, CK2α colocalized with PAK1 via CKIP-1 at the plasma membrane in response to EGF treatment, whereupon CK2 phosphorylation of PAK1 activates PAK1 malignant functions [79, 80]. BMP2 signaling in non-transformed myoblast cells causes the release of CK2 from BMPR1A, activating SMAD signaling and osteogenesis [81, 82]. This signaling pathway was recently found to be disrupted in osteoporosis [83]. In addition to its shuttling or localization, binding proteins (such as CKIP and Lamin A) may be involved in regulating CK2 activity and function at specific loci, adding another layer of control [24, 84].

The phenomenon of differential intracellular localization of CK2 also varies with disease status. For example, whereas CK2 is diffusely localized in most non-malignant cells, a higher level is concentrated in the nuclear compartment in cancer cells. In another example, after infection in cultured cells CK2 colocalized with the SARS-CoV-2 N protein in filopodial protrusions containing assembled viral particles [85]. Overall, intracellular localization appears to be an important aspect of CK2 functionality and response to cell disease status, and we will describe here information on CK2 activity in the nucleus and the mitochondria.

Nuclear roles for CK2 and connection with cancer

Investigations of the localization and abundance of CK2 in tumors demonstrated that CK2 was high in the proliferating edge of cancer tissue similar to the proliferation marker Ki-67; unlike Ki-67, CK2 levels were also elevated in other parts of the tumor section. These experiments also showed the remarkable increase of CK2 in the nuclear compartment of cancer cells compared with normal cells [86]. Elevated CK2 presence in the nucleus has been shown in numerous cancers, and information for breast, prostate and head and neck cancers are detailed in subsequent sections. Within the nucleus, CK2 is diversely associated with subnuclear sites such as the nucleolus, chromatin, and nuclear matrix where it performs distinct functions [57–59, 61, 64, 67, 68,

87–90]. Given the interactions of CK2 with all three DNA-dependent RNA polymerases and their associated regulatory complexes, CK2 impacts production of all classes of RNA and thus both the structural organization and transcriptional activity of the nucleus [91]. Increased nuclear levels and subnuclear associations of CK2 have implications for several malignant cell characteristics and functions involved in sustaining proliferative signaling and growth, described next.

Various components of the core RNA polymerase II (RNAPII) machinery itself interact with and/or are phosphorylated by CK2 [92]. Dynamic modification of the carboxy-terminal domain (CTD) of the largest RNAPII subunit RPB1, primarily by phosphorylation, regulates the activity of the RNAPII complex according to the phase of the transcriptional cycle and other signals received [93, 94]. CK2 phosphorylates the CTD, and recent work demonstrated that CK2 participates in regulation of CTD phosphorylation status in response to oxidative stress [95–97]. CK2 also influences the activities of TFIIA, TFIIF, and FCP1, among other examples [98–100].

Relating to gene expression, CK2 interacts with and phosphorylates a variety of transcription factors (see, e.g., [101–103]). The effects of these events range from altered localization, stability, activation state, or association with other binding partner molecules. Here we discuss a few examples from the cancer literature. In one example, CK2 phosphorylates the proline rich homeodomain/ hematopoietically expressed homeobox (PRH/HHex) protein, and in prostate cancer cells this was shown to support proliferation and to release a block on migration and invasion [104]. Several groups have also reported on intersection of CK2 activity with the STAT3 and STAT5 transcription factors. Overall, the results demonstrate that inhibition of CK2 hinders STAT3/5 signaling and decreases aggressive phenotypes in multiple cancer types, including breast, glioblastoma, acute myeloid leukemia, multiple myeloma, mantle cell lymphoma, and non-small cell lung cancer [105–110]. CK2 was identified in a screen for molecules that can be targeted to induce senescence in cancer cells [111]. This work identified cross regulation between CK2 and STAT3 in that it was determined that in a PTEN knockout model of prostate tumors, upregulation of CK2 expression occurs via transcriptional activation of the CK2 gene by phospho-STAT3. CK2 involvement with the steroid hormone receptor transcription factors AR, ER, and PR is discussed later in this review in the prostate and breast cancer sections.

CK2 similarly interacts with and modifies numerous splicing factors, as was highlighted in a publication where the authors used a global approach to identify CK2 substrates. The results showed enrichment for splicing machinery component proteins as targets of CK2 [112]. Earlier research had identified specific examples of CK2 involvement with splicing. In the first publication, CK2 phosphorylation of the multi-functional splicing factor RNPS1 increased the formation of spliced mRNA in vivo and also stimulated the exon junction complex activity of RNPS1 [113]. In another publication, CK2 phosphorylated the splicing factor hPrp3p, involved with spliceosome assembly, to influence splicing activity [114]. Further, CK2 phosphorylated protein kinases involved in regulation of splicing, including CDK11 and SRPK1 [96, 115]. Given the oncogenic roles of transcription and splicing factors, CK2 involvement with their functions undoubtedly contributes to the proliferative status and deregulated cellular functions of cancer cells.

CK2 localizes to the nucleolus where it is involved in numerous activities related to the functions within this nuclear body. The nucleolus itself has sub-compartments, serves as the site of rRNA synthesis and ribosomal assembly, and has roles in genome organization, cell cycle control, proliferation, and stress response [116, 117]. CK2 interacts with RNA polymerase I and III complexes, which are responsible for the production of rRNA, tRNA, and 5S rRNA, phosphorylates various components of these machineries, and influences their activity [69, 118–120]. For example, transcriptional repression of RNAPIII due to DNA damage required CK2 [121]. Several laboratories have reported on CK2 phosphorylation of B23/nucleophosmin, including androgenic regulation, cell cycle regulation, and the impact on genes related to protein synthesis, energetic metabolism, and ribosomal biogenesis [122–126]. It was also shown that CK2 and B23 modification status controls compartmentation of the nucleolar processing proteins in the granular component of nucleoli [127]. Another CK2 nucleolar substrate is Nopp140. This protein is concentrated in nucleoli, and CK2 phosphorylation of Nopp140 at numerous sites targets this protein to Cajal Bodies which are associated with nucleoli. Cajal body ribonucleoproteins modify spliceosomal small nuclear RNAs to support snRNP biogenesis and pre-mRNA splicing. CK2-facilitated Nopp140 presence in the Cajal Bodies helps ensure proper snRNA modifications and downstream splicing fidelity [128]. Given the roles nucleolar processes play to support cell growth, increased CK2 presence in this nuclear body would serve to maintain these processes under the demands of transformed cell proliferation.

Epigenetic plasticity enables the development and endurance of cells under the many stressors of malignant growth. Along these lines, numerous examples of CK2 involvement in epigenetic-related processes have emerged. As referenced in the introductory paragraph for this section, growth stimulus in prostate cells resulted in translocation of CK2 to chromatin and nuclear matrix [58, 90]. Stress and death-inducing stimuli similarly cause CK2 movement to and away from nuclear matrix [65, 129, 130]. Chromatin and nuclear matrix are the functional and structural underpinnings of RNA polymerase activity, and the detection and impact of signal responsive movement of CK2 within these components has

been investigated on numerous fronts. Abundant pieces of evidence exist to connect CK2 to chromatin structure modulation via histone and histone-interacting protein regulation, as detailed in the next several paragraphs.

In initial work from our laboratory, CK2 association with and phosphorylation of nucleosomal proteins was shown to vary with transcriptional activity and nucleosomal status; overall, there was higher CK2 activity associated with active compared to non-active nucleosomes from rat liver and prostate [61, 64]. In other work, CK2 co-purified in a complex with SSRP1 and hSpt16 in response to UV irradiation; later, CK2 was found to phosphorylate SSRP1 in multiple domains and alter its DNA binding affinity under different contexts [131–133]. SSRP1 and hSpt16 form the "facilitates chromatin transcription" (FACT) complex that was initially described as a transcriptional elongation factor and is now considered a histone chaperone [134]. Direct CK2 phosphorylation of histone H2A affects transcriptional elongation [21]. Two cancer publications link CK2 with the proto-oncogene *BMI1*, which is implicated in transcriptional repression, stem cell regulation, and DNA damage repair [135]. As a member of the polycomb repressor complex 1 (PRC1), BMI-1 participates in mono-ubiquitination of histone H2A resulting in chromatin remodeling. CK2 stabilizes BMI-1 through phosphorylation, and these two proteins positively correlate with significantly higher expression in high grade serous ovarian cancer [136]. Further, inhibition of CK2 in AML caused downregulation of BMI-1 levels [105]. Finally, CK2 phosphorylation of Che-1 (an RNA polymerase II binding protein involved in maintenance of global histone acetylation) promotes its interaction with histone H3, thereby supporting cell proliferation [137].

Dynamic methylation of histones and DNA is a critical aspect of the shifting spectrum of epigenetic gene expression repression and activation, and its dysregulation contributes to oncogenesis. Interaction of CK2 with methyl group transfer enzymes in cancer cells is a relatively recent area of investigation in the CK2 literature. CK2 phosphorylation of the DNA methyltransferase DNMT3A in osteosarcoma cells reduces its DNA methylation activity, alters CpG methylation patterns, and localizes DNMT3A to heterochromatin [138]. CK2 also phosphorylates lysine-specific demethylase-1 (LSD1, also known as KDM1A), as identified using neuroblastoma cell extracts [139]. In a signal responsive manner, LSD1 promotes demethylation of histone H3 at K3 and K9, resulting in both repressor and activator impact on gene expression; in addition, LSD1 also demethylates non-histone proteins and is involved with DNA damage repair [140]. In this regard, CK2-mediated phospho-LSD1 promotes recruitment of RNF168 and 53BP1 for DNA damage repair [141]. The methylcytosine dioxygenase enzyme TET1 participates in DNA demethylation, and its high expression levels in epithelial

ovarian carcinoma in conjunction with high CK2α levels are associated with poor outcomes for patient survival. These authors demonstrated that TET1-expressing epithelial ovarian cancer cells were sensitive to CK2 inhibition and suggest that inhibition of CK2 activity could represent a promising treatment strategy to block epigenetic reprogramming in early ovarian carcinogenesis [142].

There is also a body of evidence linking CK2 to histone deacetylase activity in cancer. Histone deacetylases (HDACs) remove acetyl modifications from lysine residues on both histone and non-histone proteins. Just over twenty years ago it was first identified that phosphorylation of HDAC1 by CK2 modified its enzymatic activity [143]. Shortly thereafter, HDAC2 was also identified as a substrate for CK2 that promoted enzymatic activity and regulated complex formation such as with Sp1 or Sp3 [144, 145]. Notably, CK2 phosphorylation of HDAC1 and HDAC2 was found to be essential for their dissociation during mitosis [146]. Specific to cancer, CK2 was shown to be a key activator of HDACs in hypoxia-associated tumors [147]. In hepatocellular carcinoma, high CK2α levels in patient tumors were associated with worse overall survival; further, in a subset of these tumors HDAC2 and CK2α were positively correlated. In mechanistic studies, these authors showed that EGF treatment induced increased CK2α, phospho-Akt and HDAC2 levels, and HDAC2 elevation was blocked by expression of dominant negative CK2α [148]. These observations suggest an important link between CK2 and histone deacetylase regulation in cancer.

The DNA binding zinc finger protein called Ikaros is a transcriptional regulator that functions as a tumor suppressor in leukemia by controlling chromatin accessibility and globally regulating formation of enhancers and super-enhancers in gene promoter regions [149]. CK2 directly phosphorylates Ikaros at multiple evolutionarily-conserved sites, impairing its function. Importantly, CK2 inhibition restores Ikaros functionality as evidenced by repression of *BCL2L1* (Bcl-XL) levels and induction of cell death in B-cell acute lymphoblastic leukemia (ALL) cells [150, 151]. It was further demonstrated in T-cell ALL that elevation of CK2 activity contributes to PI3K signaling pathway upregulation in part through impaired Ikaros function [152]. The ability of CK2 levels to subvert the functions of this tumor suppressing epigenetic regulator in leukemia cells emphasizes the impact of elevated CK2 levels in the nucleus of malignant cells.

Together, the data suggest involvement of CK2 with regulation of chromatin structure and expression programs which are key to proliferative and malignant status, ultimately contributing to numerous nuclear-related established and proposed hallmarks and enabling characteristics of cancer cells [153]. CK2 nuclear roles are highlighted in Fig. 2.

Fig. 2 Summary depiction of the nuclear domains and roles of CK2. Created with BioRender.com

Nuclear domains and roles of CK2
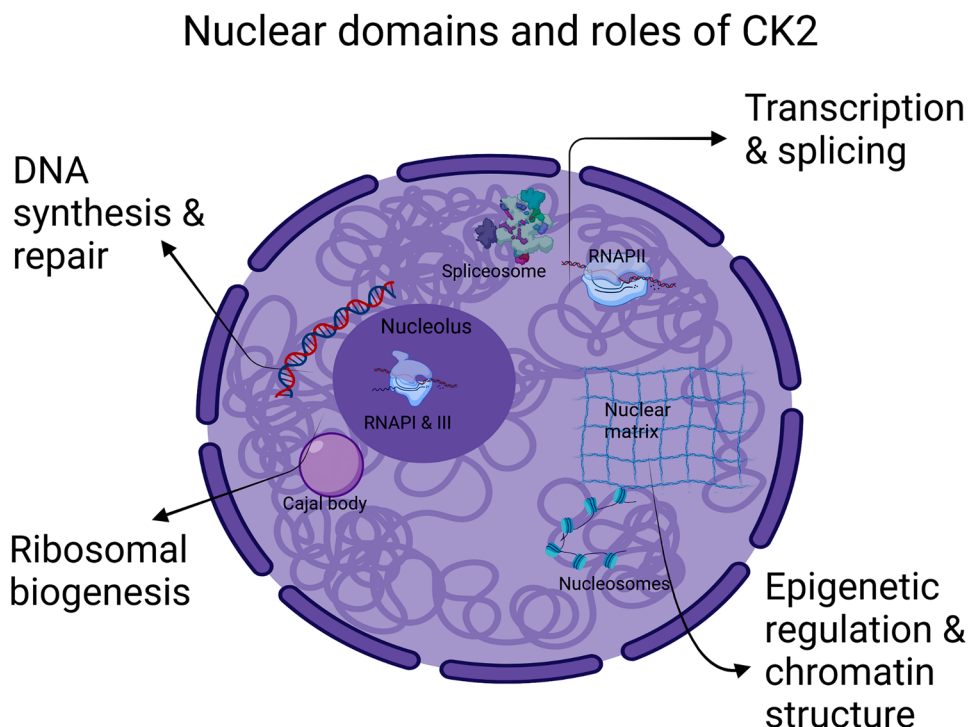
CK2 roles involving mitochondria

The mitochondria are key loci for cancer cell resistance to death and strongly influence cancer cell proliferation and signaling [154]. These organelles produce ATP, host intrinsic apoptosis, manage redox homeostasis, and produce metabolites for biosynthetic processes. Although nuclear DNA encodes the majority of mitochondrial proteins, mitochondria also transcribe and translate numerous genes encoded by mitochondrial DNA. CK2 is localized to mitochondria in mammalian cells but not in yeast. CK2 roles within mitochondria are likely to be numerous, and we are still in the infancy of defining these roles. As described subsequently, studies suggest dynamic shuttling of CK2 to and within mitochondrial compartments and manipulation of cellular CK2 activity produces a marked effect on mitochondrial-related activities.

CK2 was first isolated as a constituent of bovine kidney mitochondria extracts as a kinase activated by spermine or by chromatography on DEAE cellulose or poly(L-lysine)-agarose [155]. It was shown using rat liver mitochondria preparations that CK2 was located in the inner membrane space of quiescent mitochondria, and that spermine treatment mediated the translocation of CK2 to the inner membrane of energized mitochondria [75, 156]. We have demonstrated detection of all three CK2 subunits in mitochondria of multiple human prostate cancer cells [157]. Phosphoproteome analysis using mitochondria from resting human muscle determined 77 phosphoproteins, and bioinformatics

analysis of kinase motifs revealed CK2 as responsible for more than 20% of the activity [158]. Identification of specific mitochondrial CK2 substrates include glycerol-3-phosphate acyltransferase in rat liver, carnitine palmitoyltransferase-I in rat liver, PGC-1- and ERR-induced regulator in muscle 1 (PERM1) in mouse heart, and FUNDC1 in human cervical cancer cells [159–162]. Defined cellular substrates of CK2 are also localized in mitochondria (e.g., AKT-1, NFκB p65), but it is not currently known whether these proteins serve as CK2 regulated substrates in this organelle [163, 164]. Overall, specific targets of CK2 phosphorylation and the functional impact in the mitochondria as related to oncogenic growth remain an under-studied area.

CK2 regulation of cell death involves numerous mechanisms, and original work from this laboratory first demonstrated CK2 impact on the process of cell death mediated by diverse stimuli and machinery [165–169]. The specific connections between CK2 and mitochondrial-orchestrated cancer cell survival and death involve several pathways which continue to be elucidated. Bid, a BH3 domain-only proapoptotic protein, is cleaved by caspase 8 following death receptor activation to form truncated Bid (tBid). tBid amplifies apoptotic signaling via Bax/Bak, ultimately mediating mitochondrial outer membrane permeabilization (MOMP) [170]. CK2 phosphorylation of Bid blocks its proteolytic cleavage and blocking CK2 activity accelerates Bid cleavage and apoptosis [171–173].

In further work on death receptor signaling, an important series of observations from multiple laboratories

documented CK2 activity as a suppressor of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2-L)-mediated cell death in cancer cells. In prostate cancer cells, treatment with the CK2 inhibitor TBB prior to the addition of TRAIL upregulated the pro-apoptotic Bax protein with simultaneous down-regulation of anti-apoptotic proteins Bcl-2 and Bcl-XL. Increased expression of cytochrome *c* in the cytosol coincided with these changes, indicating engagement of the mitochondrial circuitry and sensitization of cells to TRAIL as a result of CK2 inhibition. Conversely, in the same experimental model, TRAIL-induced down-regulation of Bcl-2 and Bcl-XL expression and release of cytochrome c in the cytoplasm was blocked by overexpression of CK2α [168]. A protective role for CK2 against TRAIL-induced cell death was subsequently observed in colon carcinoma, rhabdomyosarcoma, and hepatocellular carcinoma [174–176]. Thus, the response of mitochondrial death circuitry to receptor mediated death signaling was impeded in multiple models due to elevated CK2.

Successive studies in our laboratory identified a striking impact of CK2 activity on mitochondrial viability and intracellular Ca^{2+} pools. The importance of intracellular Ca^{2+} homeostasis in relation to cell viability and mitochondrial/endoplasmic reticulum dynamics has been extensively described (see e.g., [177–180]). In studies using cultured prostate cancer cells, we found that treatment with CK2 inhibitors caused a rapid loss in mitochondrial membrane potential ($\Delta\psi_m$) apparent as early as 2 h. Further investigation suggested that the change in $\Delta\psi_m$ was due to inhibition of the mitochondrial-localized CK2 and that changes in mitochondrial Ca^{2+} levels were a possible early mediator of disrupted $\Delta\psi_m$ [157]. To pinpoint the underlying cause for $\Delta\psi_m$ loss after blocking CK2 activity, we examined Ca^{2+} status in various fractions of cells treated with CK2 inhibitors [181]. Our results revealed that following CK2 inhibition there was a rapid dose-dependent loss in cytosolic Ca^{2+} levels starting within 2 min and reaching a maximal effect at 5–10 min. We observed a concomitant increased detection of Ca^{2+} in the ER and mitochondrial compartments. These results suggested that the rapid change in $\Delta\psi_m$ likely resulted from increased mitochondrial Ca^{2+} levels, thus providing the initial trigger for the unleashing of the mitochondrial apoptotic machinery and uncovering a new mechanism by which CK2 protects cancer cells from cell death [157, 181].

CK2 exerts cancer-supportive roles related to mitochondria via two further potential mechanisms: protein import and mitophagy. In yeast, cytosolic localized CK2 plays a role in mitochondrial protein import. CK2 phosphorylates the precursor forms of translocase of the outer membrane (TOM) protein Tom22 and the import protein Mim1, and CK2 inhibition decreased the abundance of the TOM protein import complex and the Mim1 protein [182]. Mitophagy is

a catabolic process that degrades mitochondria, and CK2 is essential for mitophagy in yeast [183]. Using a conditional mouse skeletal muscle *Csnk2b* knockout model, CK2 phosphorylation of TOMM22 was reduced and was shown to regulate the binding affinity of TOMM22 for mitochondrial precursor proteins. Accumulation of the sensor protein PINK within muscle fibers in the *Csnk2b* knockout model indicated abnormal mitochondria destined for mitophagy. Introduction of a CK2 phosphomimetic form of TOMM22 into the *Csnk2b* knockout muscle cells in vitro and in vivo restored the balance of mitophagy and the function of the mitochondria [184]. In human cervical cancer cells, CK2 phosphorylation of the mitochondrial cargo receptor FUNDC1 reverses induction of mitophagy by the phosphatase PGAM5 [162]. Thus, CK2 appears to play an important role in mitochondrial protein import and overall health in non-transformed cells. It remains to be determined whether elevated CK2 in cancer cells protects mitochondrial viability and/or enables malignant cells to adapt to stress through these protein import and mitophagy pathways.

Here we have described CK2 localization in mitochondria and participation in multiple pathways of mitochondrial homeostasis. These pathways include suppression of death signaling, maintenance of membrane potential, calcium redistribution, participation in protein import, and roles in mitophagy. Much remains to be determined in our understanding of the different functions for CK2 in mitochondria, and how these roles are exploited in malignancy. Of note, it has recently been described that CK2 has a specific role in ER-phagy through phosphorylation mediated interaction of ER-phagy receptor TEX264 and ATG8 proteins, which further points to the diverse nature of CK2 cellular functions [185]. An overview of CK2 functions pertaining to mitochondria are illustrated in Fig. 3.

Overview of CK2 role in oncogenesis and expression in human cancer

As mentioned earlier, elevation of CK2 in a variety of cancers has been observed in numerous studies and appears to be an almost universally consistent feature (see, e.g., [5, 7, 9]). A large part of this observation has been based on immunohistochemical analysis of CK2 proteins in numerous cancer types. Early empirical evidence for a potential oncogenic function of CK2 was produced in studies into the role of this kinase in lymphoproliferation. Transgenic mice were produced expressing *CSNK2A1* in lymphocytes, and from 6 to 15% of mice developed T-cell lymphoma at 6.5 months of age or later [186]. CK2 expression was required for proliferation of these tumor cells, but no increase in CK2α protein or activity was found. These results led to the historical conclusion that transformation required other oncogenic

Fig. 3 Illustration of mitochondrial functions of CK2. Solid arrow lines indicate direct role. Dashed arrow lines indicate unknown or not depicted steps in mechanism. Created with BioRender.com
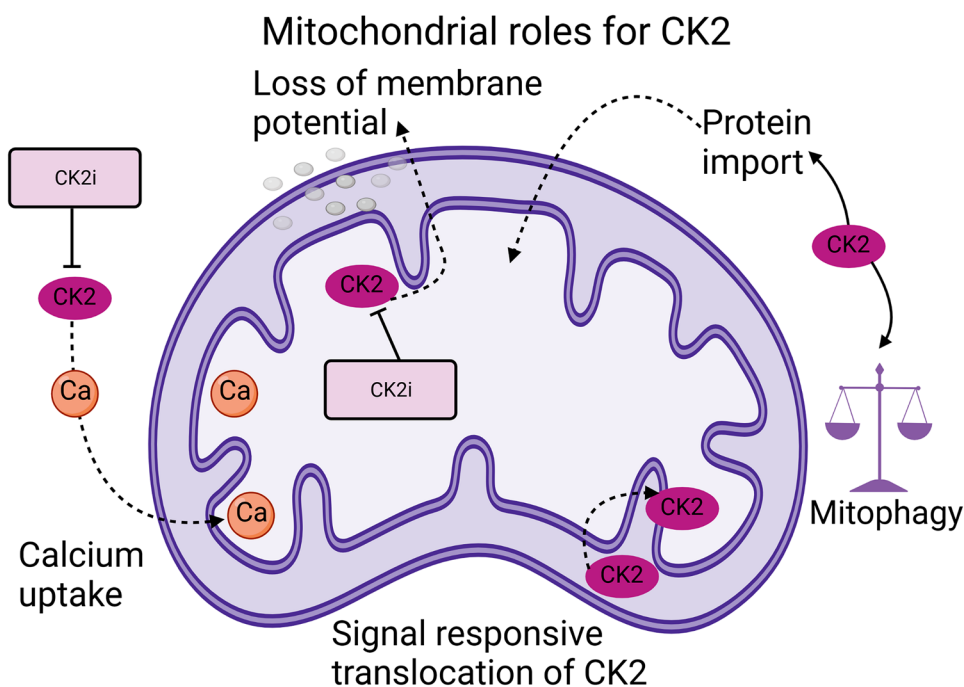
events, and further studies ensued to test this supposition. Doubly transgenic mice for *CSNK2A1* and *MYC* expression in lymphocytes which are bi-transgenic die by postnatal day 3 with acute lymphocytic leukemia [186]. In another study, combination of partial or complete *TP53* deficiency with *CSNK2A1* in lymphocytes caused development of thymic lymphomas significantly faster than in p53-deficient mice alone [187]. Loss of p53 expression appeared to be necessary for the transformation process, and high levels of *MYC* RNA were observed. Finally, transgenic co-expression of *CSNK2A1* and the transcription factor TAL1 increases the rate of lymphoblastic leukemia development in mice [188]. This body of work demonstrated the acceleration of the oncogenic process by elevated CK2 expression in the lymphoid compartment.

A correlation between CK2 expression level and patient survival has also been documented in a variety of cancers [15, 189–191]. Whereas in non-malignant cells CK2 is diffusely located in nuclear and cytoplasmic compartments, its level in the nuclear compartment in cancer cells is distinctly elevated and often associated with poor prognosis [86, 192–196]. As discussed above, the elevation of nuclear CK2 levels may reflect critical survival functions in cancer cells. Alteration in the balance of CK2 subunit expression can also have prognostic implications in cancer [197, 198]. Historically, protein levels and activity measurements formed the foundation for a role for elevated CK2 in cancer. However, the accumulation of microarray and RNA-seq data into publicly available databases has facilitated the determination of CK2 RNA levels in numerous tumor tissues, documenting a widespread though variable expression of

CK2 genes [14, 15]. Generally, significant elevation of CK2 gene expression is observed in malignant compared to non-malignant tissues. However, there are rare instances where significantly decreased CK2 RNA levels are observed, such as in testicular cancer [14].

Therapeutic targeting of CK2 in cancer

Considerable evidence suggests the potential of CK2 as a target for cancer therapy [5, 199–201]. A number of approaches to target CK2 have been explored and include use of small molecule inhibitors of CK2, peptide-based therapy and use of CK2 antisense and RNAi to achieve molecular downregulation of CK2. Since CK2 is a ubiquitous signal in all cells and is essential for survival, its targeting poses challenges with regard to host toxicity, and so its targeting in a cancer cell specific manner would be ideal; however, some preliminary success has been achieved using approaches not selective for malignant cells.

Over the years, the development of numerous small molecule CK2 inhibitors have been useful in studies of CK2 function in various experimental models (see e.g., [202–208]). Despite the development of numerous highly specific inhibitors, thus far only one agent (CX-4945/Silmitasertib) has entered clinical trials [204] (further information on these clinical trials is available at ClinicalTrials.gov). In patients with advanced solid tumors, Phase 1 trials of oral CX-4945/Silmitasertib reported reversible toxicities of diarrhea and hypokalemia and 15% of patients with stable disease for at least 6 months; CX-4945/Silmitasertib was deemed

generally well-tolerated [209, 210]. An inhibitor scaffold was employed to develop a CK2-specific probe called SGC-CK2-1 [211]. This probe was found to be effective in inducing cell death in only a small number of cancer cells which prompted questioning of the "broad cancer essentiality of CK2" [212]. It is surprising that these authors ignored several important points before arriving at such a disingenuous conclusion; this opinion was also shared in a recent publication [213]. The argument that the CK2 probe/inhibitor was not effective in all of the cancer cells tested ignored that the probe was effective in a subset of the cancer cells. That loss of cell viability was not seen in other cancer cells could imply that the inhibitor may not have reached a specific critical location in the cell. It is to be emphasized that a modest loss of CK2 in the nuclear compartment results in induction of cell death even when the cytosolic CK2 level is unaltered [130, 214]. This work indicated that non-cancer cells were more resistant to induction of cell death on downregulation of CK2 levels, which is also the basis of safely using the oral chemical inhibitor CX-4945/Silmitasertib. Other specific potent inhibitors possibly do not afford this type of differentiation, thus engendering toxicity in the host. Furthermore, as discussed subsequently, the remark about CK2 and cancer [212] ignored a large body of work documenting the induction of cell death in diverse cancers (in cell culture and in vivo models) by various non-pharmacological approaches such as RNA interference strategies against CK2. We discuss both pharmacological and non-pharmacological CK2 targeting data for prostate, breast, and head and neck cancer in the next sections of this review.

Another currently active approach to targeting CK2 in cancer employs the use of a peptide to block CK2 phosphorylation of substrates; the peptide has shown efficacy in tumors in vivo although there are possible limitations to its systemic delivery in patients [50, 215–217]. In the early efforts to target CK2 for cancer therapy, a peptide P15 was identified that abrogated phosphorylation sites in CK2 substrates and caused apoptosis. This peptide was conjugated with the cell penetrating peptide Tat and the resulting compound P15-Tat (subsequently named CIGB-300) was found to be active in inducing apoptosis in vitro as well as in vivo [218]. It was later found that CIGB-300 localized to the nuclear compartment in vivo and had a significant effect on the phosphorylation of nucleolar protein B23. Since B23 is a specific substrate of CK2, inhibition of B23 phosphorylation was proposed as a mechanism of CIGB-300 apoptosis-inducing activity; the potential of CIGB-300 in clinical translation originally studied in cervical cancer has been documented in several other cancers [124, 219–222].

A potent and specific means of inducing cancer cell death based on targeting CK2 has relied on the utility of antisense and RNAi against the catalytic subunits of CK2. This has been a focus of studies in our lab for multiple cancer types.

One of the first such studies was to demonstrate induction of apoptosis in prostate cancer cells using antisense oligonucleotides to CK2α [214]. Subsequently, the utility of this approach was also demonstrated in a xenograft model of PCa [130]. Next, a novel nano-encapsulation approach to delivery of CK2 targeting oligonucleotides in a malignant cell-specific manner was developed. This nanoparticle technology utilized incorporation of the oligonucleotides into a tenfibgen-coated nanocapsule. These nanocapsules were less than 50 nm in size and entered the cancer cells via lipid rafts; the specificity was imparted by the presence of elevated tenascin receptors in cancer cells which recognize the tenfibgen subdomain of tenascin. The nanocapsules carrying the oligonucleotide cargo (antisense, double stranded siRNA, or single stranded antisense/siRNA hybrid) targeting CK2 (both α and α′ subunits) were shown to be delivered to the nuclear periphery where the cargo was released. The efficacy of this therapeutic approach was demonstrated in several cancer models [40, 43, 199, 200, 223–230]. Further, a phase I type trial in a large animal model of head and neck cancer (feline oral squamous cell carcinoma) demonstrated the potential applicability of this therapeutic approach for clinical translation warranting further expansion of this line of investigation [231].

In the following is an overview of CK2 involvement in prostate, breast and head and neck cancers. This is not intended to be comprehensive coverage of what is known about CK2 roles and signaling in cancer, as there are more than 1500 papers in this field of research. Other reviews have discussed CK2 in the context of hematological malignancies and solid tumors and potential combination therapy strategies co-targeting CK2 in cancer [232–234].

Prostate cancer

Androgens play a fundamental role in the development of normal prostate, and prostate cancer development is also initially reliant on the continued presence of androgens [235, 236]. One of the earliest observations of CK2 regulation by androgens was observed in prostatic tissue [237–239], and subsequently in prostate cancer and benign neoplasia [192, 240]. These studies suggested that elevated nuclear localization of CK2α was a poor prognostic factor. Additionally, elevated nuclear CK2α was related to higher Gleason score and invasive nature of the disease [192, 240]. It has been observed that pro-survival roles for CK2 in prostate cancer are mediated via maintenance and promotion of androgen receptor (AR) and NF-κB p65 expression; in fact, inhibition of CK2 leads to downregulation of AR-dependent transcriptional activity [42, 241, 242]. It is worth noting that dysregulation of CK2 in prostate cancer is apparent regardless

of AR status or the presence of its variants (see e.g., [42, 201, 243]).

CK2 influences numerous survival pathways in prostate cancer. Inhibition of CK2 has been shown to reduce CYP24A1 expression which enhances 1,25-Dihydroxyvitamin D3 anti-tumor activity in prostate cancer cells [244]. A noteworthy observation relates to the regulation of the tumor suppressor PML by CK2 originally described in lung cancer [245]. Degradation of PML via the ubiquitin/proteasome-mediated pathway depends on direct CK2 phosphorylation of PML S517. PML mutants that are resistant to CK2 phosphorylation exhibit increased tumor suppressive functions, and inhibition of CK2 enhances the PML tumor suppressive property [246]. Similar observations on PML function and regulation by CK2 in PCa have been reported [247]; these investigators documented that inactivation and nuclear exclusion of tumor suppressor FOXO3a with concurrent reduction of active nuclear AKT (pAKT-S473) follow the CK2-mediated disruption of the PML-PHLPP2 signaling axis [247]. Angiogenesis is an important process in cancer, and an earlier observation showed that downregulation of CK2 influenced the microvasculature as indicated by a marked reduction in CD31 signal [199]; more evidence has now accumulated on the involvement of CK2 in angiogenesis (see, e.g., [248]).

Recently, considerable effort has been directed toward the involvement of CK2 in protein networks in prostate cancer. In this regard, an initial study suggested that protein network modeling of prostate gene signatures revealed essential pathways in disease recurrence [249]; these authors followed the multipronged approach of incorporating the gene expression data and protein interactions network which resulted in identifying signature protein pathways in prostate cancer progression. Dissecting posttranslational modifications and their regulation by protein/protein kinase pathways is a key component of prostate cancer research. In this regard, work utilizing biopsy materials identified various protein kinases including CK2 in prostate cancer as drivers of proteomic/phosphoproteomic composition variability in relation to disease progression and patient-based heterogeneity [52, 250, 251]. Recent studies using clinical specimens and database analysis have provided further evidence on the involvement of CK2 in the context of prostate cancer. A phosphoproteomics study using metastatic castrate resistant prostate cancer (CRPC) specimens found CK2 to be among the top seven enriched kinase activities by kinase substrate enrichment analysis (KSEA) [52]. Of note, comparison of patient mRNA levels in metastatic CRPC to mRNA levels from localized PCa using TCGA data identified *CSNK2A1* (CK2α) as an inferred activated kinase [252]. Several of the observed (potentially actionable) signals are also downstream targets of CK2 [49, 52, 250] which further emphasizes the significance of elevated CK2 in cancer.

Examination of CK2 kinase activity in prostate chromatin fractionated and pooled from 6 normal prostate glands, 16 samples of benign prostatic hyperplasia (BPH), and 51 samples of prostatic carcinoma gave an early indication that CK2 levels were elevated in human prostate cancer. Kinase activity was threefold higher in carcinoma relative to normal tissue, and 25-fold higher in BPH tissue [240]. Immunostaining for CK2 in normal, BPH and adenocarcinoma prostate tissue overall matched the kinase activity levels, especially in the nucleus. The pathophysiology of BPH is still incompletely understood, but proposed underlying mechanisms include inflammation and cellular proliferation which are pathways of high CK2 involvement [253]. In a later study of CK2α staining in BPH ($n = 31$) and prostate cancer ($n = 30$) tissues, CK2α nuclear levels were found to be significantly higher in the tumors compared to BPH [254]. A retrospective cohort of 131 prostate adenocarcinoma biopsy tissues were examined by immunohistochemistry for CK2α localization and levels. It was determined that CK2α staining scores were elevated by 2.4-fold in malignant compared to normal prostate glandular cells [192]. Moreover, nuclear CK2α was significantly correlated with more advanced disease characteristics, such as higher Gleason score, locally advanced disease, and invasion into lymphatic and perineural spaces. Human prostate cancer datasets were evaluated using the Oncomine database [15]. This analysis revealed that CK2α RNAs were significantly overexpressed in prostatic carcinoma, adenocarcinoma, and intraepithelial neoplasia. CK2α′ was elevated in in prostatic intraepithelial neoplasia and CK2β was increased in prostate carcinoma.

Several approaches have been applied to molecular downregulation of CK2 expression in prostate cancer xenograft studies. In the first study, antisense (AS) oligodeoxynucleotides (ODN) designed to hybridize with the CK2α-encoding mRNA from the initiating codon through the eighth amino acid were injected directly into PC3-LN4 flank tumors carried by nude mice [130]. Mice received one injection of 5, 10 or 20 µg of AS-CK2α ODN or 20 µg of control ODN. Inhibition of tumor growth was observed over 8 days and directly corresponded with the dose of AS-CK2α ODN injected, and the highest dose completely eliminated the tumor by 7 days post-injection. In AS-CK2α-treated tumors there was evidence for apoptosis and for downregulation of CK2α mRNA and kinase activity. The next study used AS ODN designed to target both CK2α and CK2α′ in a bispecific manner due to concerns over potential compensatory upregulation of CK2α′ after loss of CK2α [229]. Multiple doses and dosing schedules were tested in this study; here we describe the effects of delivering a cumulative dose of 66 µg/kg via 1, 2, or 4 intraperitoneal (ip) injections into mice carrying orthotopic PC3-LN3 tumors. Three of the 4 dosing schedules resulted in significant tumor weight reduction relative to the control AS ODN. CK2αα′ and NFκB

protein levels were decreased in the nuclear matrix, whereas loss of AKT-1 protein was variable at the different dosing schedules.

Subsequent studies used malignant cell-specific tenfibgen (TBG) nanoencapsulated delivery of a chimeric single stranded RNA/DNA oligomer targeting the same bispecific region of CK2αα′ (TBG-RNA-CK2). Mice with orthotopic PC3-LN3 tumors were injected ip with 33 or 330 ng/kg TBG-RNAi-CK2 twice with a 24 h interval [226]. Thirteen days after initiation of treatment for both dose levels, decreased weights and reduced levels for CK2αα′ proteins were observed in primary tumors. Further, reduced distant metastases, retroperitoneal lymph node tumor volumes, and pAKT-1 S129 and NF-kB p65 lymph node tumor signals were found. A dose response study was carried out in flank models of PC3-LN4 and 22Rv1 prostate cancer. In the PC3-LN4 model, dose response comparison was made between TBG nanocapsule intravenous (iv) delivery of single stranded chimeric oligomers versus classic double stranded siRNA oligomers [224]. Both versions of the anti-CK2 nanocapsule significantly reduced tumor volumes. However, the TBG-RNAi-CK2 results were more straightforward and this form of the anti-CK2 nanocapsule was used in the 22Rv1 model where moderate reduction in tumor volumes were achieved. Time course analysis of tumors collected 5, 6, and 7 days post-treatment with TBG-RNAi-CK2 showed early downregulation of CK2αα′β and pNF-kB p65 S529 at day 5 compared to detection of cell death markers such as loss of survivin, Bcl-xL, NF-kB p65, and full length caspase 3 at day 7. In a separate study of an orthotopic 22Rv1 model, TBG-RNAi-CK2 treatment reduced tumor weights, increased the percent of dead tumor tissue, and caused reduced CK2αα′β, NF-kB p65, and AR expression [42]. These studies provide the proof of concept that specifically targeting CK2 in a malignant cell-specific manner is feasible for cancer therapy.

Three studies report on the effects of CK2 small molecule inhibitors on xenograft tumor growth. In the first publication, the ability of CX-4945 to inhibit PC3 xenograft tumor growth was assessed. The authors observed dose responsive tumor growth inhibition along with decreased expression of p21 phosphorylated at T145 and decreased microvessel density [255]. In the second publication, the CK2 small molecule inhibitor TBBz (4,5,6,7-tetrabromo-1H-benzimidazole) was identified in a screen for molecules that regulate CYP24A1 expression. Inhibition of CYP24A1, a member of the cytochrome p450 family, is known to enhance the antiproliferative effects of 1,25-dihydroxyvitamin D3 (1,25D3), a physiologically active form of vitamin D3 [244]. This group evaluated the ability of TBBz alone and in combination with 1,25D3 to block the growth of PC3 xenograft tumors. TBBZ treatment reduced expression of CYP24A1 protein in the tumors and was similarly effective at reducing

tumor growth compared to 1,25D3 alone. Combined treatment with TBBz and 1,25D3 significantly improved tumor growth reduction over either agent alone [244]. Interestingly, in a prospective study, higher hydroxyvitamin D3 concentration in plasma was found to be associated with decreased risk of prostate cancer [256]. The third study used tenfibgen nanoencapsulated DMAT (2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole) in the PC3-LN4 xenograft model.

Data for treatment of prostate cancer xenograft tumors using various anti-CK2 strategies is summarized in Table 1

Breast cancer

A series of publications contributed essential knowledge on functions of CK2 in breast. The Wnt pathway is active during embryogenesis and developmental processes, and reactivation of this pathway is found in numerous cancers including breast cancer. Participation of CK2 in canonical Wnt/β-catenin signaling was described for mouse mammary epithelial cells stably expressing *Wnt1* [257]. Expression of Wnt-1 caused increased cellular proliferation, elevation of CK2α and β-catenin proteins, and increased CK2 kinase activity. CK2 was shown to phosphorylate components of Wnt signaling, including β-catenin, Dv1-2, and Dvl-3 [257, 258]. In mouse and rat mammary tumors induced by the carcinogen DMBA, CK2 was highly expressed relative to normal mammary glands [259–261]. Direct transgenic overexpression of CK2α in the mammary gland caused abnormalities in mammary gland histology in 50% of the female mice, with observations of slowed gland development, incomplete gland regression after pregnancy, and inflammatory and preneoplastic lesions. Thirty percent of CK2α transgenic female mice developed mammary adenocarcinomas (median age 23 months), and elevated β-catenin levels were observed in 55% of tumors tested [259]. Data from CK2α transgenic mice mammary tumors also suggested activation of the NFκB pathway as well as cMYC upregulation [259]. Roles for CK2α in promoting aberrant activation of NFκB were further confirmed in various breast cancer cell lines and CK2α transgenic mouse mammary tumors, including participation in Her2/EGFR2 signaling [262–264]. These studies established a tumorigenic role for increased CK2 expression in breast epithelial cells.

A strong link of CK2 to estrogen signaling has also emerged. In 1985, a report demonstrated characterization of CK2 activity in MCF-7 and MDA-MB-231 breast cancer cells hetero-transplanted into athymic mice [265]. CK2 phosphorylates ERα at several sites, and modification at these amino acids modulates ERα activity [266, 267]. CK2α and ERα or CK2α and pAKT-1 S129 (CK2 site for activation of AKT-1) levels were positively correlated in breast cancer tissues [268]. This group showed that CK2α

Table 1 CK2 targeting in prostate cancer human xenograft models

Model	Treatment	Tumor Effects	Reference
PC3-LN4 flank	AS-CK2α, intra-tumoral, 5 / 10 / 20 μg, once, elapsed treatment time 7 to 10 d	Dose-dependent TGI (85% at 8 days post-treatment at 10 μg)	[130]
		Decreased CK2α protein & CK2 activity in nuclear matrix	
		Apoptosis (TUNEL)	
PC3-LN4 orthotopic	bs-AS-CK2, ip, 66 μg/kg cumulative scheduled as 1 to 4 injections, elapsed treatment time 13 d	Decreased tumor weight (26% of control for 4 dose schedule)	[229]
		Decreased CK2α RNA	
		Decreased CK2αα′β & NFκB p65 protein in nuclear matrix	
PC3 flank	CX-4945, oral bid, 25 / 50 / 75 mg/kg for 32 d	Dose-dependent TGI (86% at 75 mg/kg)	[255]
		Decreased p-p21 T145 & microvessel density	
PC3 flank	TBBz, ip, 15 mg/kg, 3 times weekly, for 2 weeks	Reduced tumor growth & Ki-67 level	[244]
		Suppression of CYP24A1 RNA levels	
PC3 flank	TBBz, ip, 15 mg/kg, + 1,25D3, 15.5 mg/kg, 3 times weekly, for 2 weeks	Significantly reduced tumor growth & Ki-67 level	[244]
		Apoptosis (TUNEL, caspase 3)	
PC3 flank	TBB, unknown dose and regimen	Reduced tumor growth & weight	[309]
		Induction of interferon-γ-inducible	
		protein 10 (IP-10) mRNA	
PC3-LN4 orthotopic	TBG-RNAi-CK2, ip, 33 / 330 ng/kg, twice with 24 h interval, elapsed treatment time 13 d	Decreased primary tumor weight & lymph node tumor volume	[226]
		Decreased CK2αα′ protein in primary tumors	
		Decreased NFκB p65 & pAKT S129 protein in lymph node tumors	
		Decreased distant metastasis	
PC3-LN4 flank	TBG-DMAT, iv & ip, 20 μg/kg, 6 times with 24 h intervals, elapsed treatment time 7 d	Decreased Ki-67 proliferation marker in tumors	[310]
		Decreased CK2αα′ & NFκB p65 protein in tumors	
PC3-LN4 & 22Rv1 flank	TBG-RNAi-CK2 & TBG-siCK2, iv, 0.0001 to 1.0 mg/kg, 3 times with 72 h intervals, elapsed treatment time 10 & 11 d	Reduced tumor volume at multiple doses	[224]
		Reduced CK2αα′β, NFκB p65, pNFκB p65 S529, caspase 3 full length, Bcl-xL, & survivin protein in 4,5,6 d time course tumors	
22Rv1 orthotopic	TBG-RNAi-CK2, iv, 0.02 mg/kg, 3 times with 72 h intervals, elapsed treatment time 8 d	Reduced tumor weight	[42]
		Reduced CK2αα′β, NFκB p65, & AR protein in tumors	

AR, androgen receptor; AS, antisense oligodeoxynucleotide; bid, twice per day; bs-AS, bispecific antisense oligonucleotide targeting CK2αα′; CX-4945, Silmitasertib 5-(3-chlorophenylamino) benzo[c][2,6]naphthyridine-8-carboxylic acid CK2 inhibitor; d, days; h, hours; ip, intraperitoneal; iv, intravenous; TBBz, 4,5,6,7-tetrabromo-1H-benzimidazole CK2 inhibitor; TBG, tenfibgen subdomain of tenascin; TBG-DMAT, nanoencapsulated CK2 inhibitor 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole; TBG-RNAi, single stranded DNA/RNA chimeric oligonucleotide targeting CK2αα′; TBG-siCK2, nanoencapsulated double stranded siRNA targeting CK2αα′; TGI, tumor growth inhibition; TN, tenascin; 1,25D3, 1,25-dihydroxyvitamin D3

expression and activity were upregulated by estrogen treatment in ERα-positive breast cancer cells, and ERα binds to the *CSNK2A1* promoter to activate gene expression in an estrogen-inducible manner. Further data in rodent models demonstrated increased CK2α expression in 4T1 mouse syngeneic breast tumors stably overexpressing ERα and elevation of both ERα and CK2α levels in rat mammary tumors induced by the carcinogen DMBA. These authors proposed that ERα signaling promotes breast tumor growth through increased CK2α expression, resulting in AKT activation

and loss of the tumor suppressor promyelocytic leukemia protein (PML) [268]. A separate group published that inhibition of CK2 activity using the small molecule inhibitor TBCA induced ERα expression in both an ERα-positive and a triple negative breast cancer cell line [269]. The relationship between CK2 and estrogen or ERα-related signaling in breast cancer appears to be complex.

Several other signaling proteins and mechanisms in breast cancer have been identified as pertaining to CK2 function, and here we describe a few examples. The

progesterone receptor B (PR-B) is phosphorylated by CK2, and it was demonstrated that CK2-dependent PR-B phosphorylation influences proliferative transcriptional programs in breast cancer cells [270–272]. CK2 also phosphorylates the BRCA1 tumor suppressor protein [273]. SIRT6 is phosphorylated by CK2α, and multivariate analysis of nuclear CK2α and nuclear SIRT6 indicated significantly shorter overall survival and relapse free survival (RFS) in breast cancer patients [274]. Finally, miR-125b was identified to function as a tumor suppressor in breast cancer cells in part through targeting the CK2α transcript causing reduction of CK2α protein levels [275]. These authors reported that CK2α protein and miR-125b levels demonstrated inverse correlation in breast cancer patient tumors.

Both RNA and protein data from human tumor tissues have established elevated CK2 levels in malignant breast cells and the potential prognostic implications of CK2 dysregulation in breast cancer. An early report showed strong nuclear staining for CK2 protein in breast cancer tissue relative to benign tissue, providing an indication that CK2 expression was altered in human breast tumors [276]. Further studies validated elevation of mRNA and protein levels in breast cancer patient samples for the CK2 genes, with decreased survival associated with high levels of *CSNK2A1* and *CSNK2B* [15, 43, 106, 259]. Using microarray data, high *CSNK2A1* RNA levels were associated with moderately decreased relapse free survival (RFS) in estrogen receptor alpha- (ERα) positive disease [269]. In ER-negative tumors, high *CSNK2A1* expression trended toward shorter RFS. Overexpression of CK2α protein correlated with poor prognosis and metastatic risk in breast cancer patients [277], and the intensity of nucleolar localization of CK2α in breast cancer tumors has been proposed to serve as a marker of poor prognosis [278].

Information gained from the above described studies using clinical samples indicate the potential for therapeutic strategies aimed to block CK2 expression or activity. Data from cultured cells and xenograft studies highlight the efficacy of targeting CK2 in breast cancer model systems. Using both siRNA and small molecule inhibitor approaches, CK2 was shown to be essential for survival of breast cancer cells [43, 106]. Preclinical efficacy studies of intravenous CIGB-300, a peptide that blocks phosphorylation of CK2 target sites, in the mouse F311 syngeneic breast cancer model suggest that it is effective in blocking breast cancer metastatic colonization, and the effects of this therapeutic approach are proposed to be mediated via changes in the CK2-mediated phosphoproteome [50, 279]. Malignant cell-specific molecular downregulation of CK2 using nanoparticle delivery of siRNAs resulted in significant reduction in MDA-MB-231 tumor volume and induction of tumor cell death in a triple negative breast cancer xenograft study [43]. In these tumors, survivin and CDK11 proteins were significantly reduced due to down-regulation of CK2 expression.

Two in vivo studies are published evaluating oral use of the CK2 inhibitor CX-4945/Silmitasertib to treat breast cancer tumors. CX-4945/Silmitasertib treatment at 2 dose levels reduced BT-474 xenograft tumor growth by 88–97% in a HER2-overexpressing breast cancer model [206]. This original determination of CX-4945/Silmitasertib efficacy in breast cancer was performed using a human xenograft tumor in athymic mice. A recent study of CX-4945/Silmitasertib utilized mouse breast cancer models in both immune-compromised and immune-competent mice [280]. First, experiments in multiple cancer-type cell lines and dendritic cells determined that CK2 phosphorylation of programmed death-ligand 1 (PD-L1) prevented PD-L1 proteasomal degradation by disrupting its binding to speckle-type POZ protein (SPOP). Conversely, CK2 inhibition reduced PD-L1 abundance and promoted T-cell activation; moreover, The Cancer Genome Atlas (TCGA) analysis indicated mRNA expression of CK2α was negatively correlated with molecules involved in T-cell activation. Using B16F10 melanoma and EMT6 breast cancer syngeneic tumor models, treatment with CX-4945/Silmitasertib significantly reduced tumor growth in immune-competent mice. The authors also demonstrated that CK2 inhibition activated and expanded tumor-associated dendritic cells in both tumor types. In contrast to the original human xenograft results, mono-therapy with CX-4945/Silmitasertib against the mouse EMT6 tumor in athymic mice did not significantly reduce tumor growth. This group went on to evaluate CX-4945/Silmitasertib in combination with an antibody against T-cell immunoglobulin mucin-3 (Tim-3), which is highly expressed in tumor dendritic cells. Using the syngeneic 4T1 breast cancer model, CX-4945/Silmitasertib plus anti-Tim3 induced more tumor growth inhibition than either single treatment alone; combined treatment also resulted in longer survival.

Bristol Myers Squibb developed selective ATP-competitive CK2 inhibitors [281]. Two of these inhibitors called BMS-211 and BMS-595 were tested by oral administration in multiple mouse-derived tumor models, including the 4T1 breast cancer cell line [282]. Using immune-compromised NOD Skid Gamma (NSG) mice, either BMS-211 or BMS-595 was tested (it is not clear which inhibitor was used) and no significant reduction in tumor growth was observed. Again using the 4T1 model but in immune-competent mice, BMS-211 was tested in combination with the immune checkpoint inhibitor anti-CTLA4-mIgG2a and complete "rejection" was observed in 6 of 8 tumor-inoculated mice followed for more than 30 days. Further in vivo and ex vivo characterization of the effects of BMS-595 using the Lewis lung carcinoma allograft model demonstrated multiple changes in immune cell populations, including blocked differentiation of myeloid cells. The data from different orally

available CK2 inhibitors combined with different immuno-therapy drugs support the notion that targeting CK2 and the immune checkpoint pathway appears to be highly effective as a treatment strategy in cancer [280, 282]. The disparities in the data from these two publications as to how different immune cell populations, especially the myeloid lineage, are altered and whether this is due to differences in the biological effects of the CK2 inhibitors, remains to be clarified.

Data for treatment of breast cancer xenograft and allograft tumors using various anti-CK2 strategies are summarized in Table 2.

Head and neck cancer

Our investigations into CK2 in head neck cancer began with characterization of kinase activity levels in patient head and neck squamous cell carcinoma (HNSCC) tumor samples. First, CK2 kinase activity was measured in cytosolic and nuclear sub-fractions of tumors derived from the larynx,

pyriform sinus, oral cavity, and oropharynx [189]. Comparison of activity in twenty tumors was made to six surgical specimens of normal upper aerodigestive tract mucosa, and significantly higher CK2 activity was found in both nuclear and cytosolic tumor lysates. Cytosolic CK2 activity was associated with aggressive behavior of the disease and significantly worse survival outcome [189]. In a similar study, CK2 kinase activity was measured in nuclear chromatin and cytosol sub-fractions in seven HNSCC tumors compared to six non-malignant oropharyngeal tissue specimens; again, CK2 kinase was significantly higher in chromatin and cytosolic fractions compared to non-cancer tissue [283]. Finally, we examined the immunohistochemical staining pattern of CK2 in HNSCC tumors and normal upper aerodigestive tract tissue and found the CK2 signal concentrated in the nucleus of tumor cells with focal or punctate sub-nuclear sites in contrast to varied staining patterns in other non-malignant tissue cell types [86]. Moreover, CK2 stain co-localized in tumor cells with the marker Ki-67 at the proliferating front of tumors but was also prominent in the nuclei of tumor

Table 2 CK2 targeting in breast cancer human xenograft and mouse syngeneic models

Model	Treatment	Tumor Effects	Reference
BT-474 orthotopic	CX-4945, oral bid, 25 / 75 mg/kg for 31 d	TGI (88% at 25 mg/kg; 97% at 75 mg/kg)	[206]
MDA-MB-231 flank	TBG-siCK2, iv, 0.01 mg/kg, 3 times every 72 h over 10 d	Reduced tumor volume & weight	[43]
		Reduced CK2αα′, survivin & CDK11 protein in tumors	
4T1* flank	BMS-211 or BMS-595, oral, 20 or 60 mg/kg, elapsed treatment ~ 19 d	No significant reduction in tumor growth in immune-compromised (NSG) mice	[282]
4T1* flank	BMS-211, oral, 20 mg/kg; CTLA4-2a antibody, ip, 20 µg, 3 treatments; elapsed treatment time 21 d	Complete rejection of tumor in 6 of 8 immune-competent mice with combined BMS-211/CTLA4-2a antibody	[282]
		Reduced TAM in tumor	
F311* Tail vein	CIGB-300, iv, 10 mg/kg, 5 times with 24 h intervals, elapsed treatment time 21 d	Reduced lung lesions by 45%	[279]
F311* orthotopic with incomplete resection	CIGB-300, iv, 10 mg/kg, 2 cycles of 5 injections with 24 h intervals post-resection, elapsed treatment time 35 d	Reduced lung metastasis by 60%	[279]
F311* orthotopic	CIGB-300, iv, 10 mg/kg, 2 cycles of 5 injections with 24 h intervals, elapsed treatment time 25 d	Reduced lung metastasis by 40%	[279]
EMT6* orthotopic	CX-4945, oral bid, 75 mg/kg, elapsed treatment time ~ 7 d	Significant tumor volume reduction in immune-competent model but not in immune-compromised (athymic) model	[280]
		Expansion of dendritic cells in tumor	
4T1* orthotopic	CX-4945, oral bid, 75 mg/kg;Tim3 antibody, ip, 100 µg, 3 treatments; elapsed treatment time 21 d, survival time 110 d	Significant tumor volume reduction for CX-4945 alone	[280]
		Combined CX-4945 and Tim3 antibody treatment significantly better than CX-4945 alone	
		Significantly improved survival for CX-4945 alone and combined treatment	

*mouse cell lines; bid, twice per day; CIGB-300, peptide that blocks phosphorylation of CK2 target sites; CX-4945, Silmitasertib small molecule inhibitor targeting CK2; d, days h, hours; ip, intraperitoneal; iv, intravenous; TBG, tenfibgen subdomain of tenascin used to coat nanocapsule; TBG-siCK2, nanoencapsulated double stranded siRNA targeting CK2αα′; TGI, tumor growth inhibition; Tim3, T-cell immunoglobulin mucin 3; TAM, tumor-associated macrophages

cells with little or no Ki-67 stain. CK2 kinase activity in these tumors was higher than activity in non-malignant tissue, consistent with previous determinations. Three other publications have shown that CK2 levels and activity are increased in head and neck cancer patient tumors. A study of CK2 kinase activity showed a 3.7-fold increase in neoplastic squamous epithelial tissue ($n = 14$) compared to normal epithelia ($n = 11$) [284]. CK2α and CK2β immunohistochemical staining levels were found to be higher in laryngeal squamous cell carcinoma tissues relative to non-malignant or pre-cancerous tissues [285]. Finally, gain of CK2 gene expression at the mRNA level was documented using RNA-seq data from The Cancer Genome Atlas [286]. Overall, these publications demonstrate that CK2 levels and activity are high in HNSCC tumor cells.

In an initial effort to demonstrate whether downregulation of CK2 kills HNSCC cells, antisense oligonucleotides targeting CK2α were introduced into a gingival squamous cell carcinoma cell line, resulting in reduced CK2 kinase activity and dose-dependent cell growth inhibition [287]. In follow-up work, the antisense oligonucleotide targeting CK2 was packaged into sub-50 nm tenascin coated nanocapsules and delivered topically to mice carrying HNSCC flank tumors. Treatment with antisense to CK2 slowed tumor volume tripling to 17 ± 2.5 days relative to mice receiving control nanocapsules or untreated (8.3 ± 0.7 days and 11.3 ± 1.3, respectively) [169]. The CK2α signal in antisense nanocapsule treated tumors was greatly reduced in the nucleus and overall. Pre-clinical research focused on potential therapeutic targets to selectively downregulate NFκB activation in HNSCC identified CK2 [288]. In follow-up research, it was shown that CK2 subunits are highly expressed in multiple HNSCC cell lines and in tumor sections derived from multiple sites [223]. Knock-down of CK2 in HNSCC cells suppressed NFκB activity and modified expression of numerous NFκB target genes with increased expression of tumor suppressor genes and decreased expression of pro-survival genes. Further, mouse studies were performed using malignant cell-directed tenfibgen-mediated delivery of nanoencapsulated single stranded chimeric RNA interference oligonucleotides targeting CK2α and CK2α'. Using two xenograft HNSCC models, treatment of mice with the anti-CK2 nanocapsules reduced tumor size and induced markers of apoptosis. The treated tumors showed reduction in CK2$\alpha\alpha'$, NFκB p65, pNFκB p65 S529, cyclin D1, Bcl-XL, and Bcl-2. Increased p53 and p63 were also observed. In another study using the RNAi-CK2 nanocapsule delivery approach, significantly reduced tumor volumes and improved survival compared to control treatments was observed out to 200 days in two HNSCC xenograft models [230]. Finally, in a mouse xenograft study using a single model of HNSCC, treatment with the CK2 inhibitor CX-4945 caused very moderate reduction in tumor growth with no improvement in survival,

despite promising results in cultured cells [286]. Based on tumor signaling results, the authors concluded that activation of the MEK/ERK/AP1 pathway was occurring. A combination treatment xenograft study was performed with CX-4945 and the MEK inhibitor PD-0325901, and the results showed significant reduction in tumor volume by PD-0325901 alone which was slightly improved in the combination treatment. Data for treatment of head and neck cancer xenograft tumors in mice using various anti-CK2 strategies is summarized in Table 3.

Research was also carried out in a naturally occurring large animal model of head and neck cancer. First, CK2 gene and protein expression was characterized in feline oral squamous cell carcinoma [289]. It was demonstrated that siRNA-mediated downregulation of CK2 in a feline oral squamous cell carcinoma cell line reduced viability and induced apoptosis. Having established CK2 as a target in feline oral cancer, a small trial was carried out in which nine cats with naturally occurring treatment-naive oral squamous cell carcinoma were treated with a feline-specific version of the RNAi-CK2 tenfibgen nanocapsule [231]. Of the evaluable post-treatment tumors, one third showed decreased CK2α score by immunohistochemical analysis. There was some evidence of efficacy in that three cats had stable disease and one cat had a partial response (Table 3).

Interestingly, it has been shown that CK2 phosphorylates and inhibits the function of p73, a member of the TP53 tumor suppressor family. In this study, inhibition of CK2 caused loss of cancer stem cell-related side population, clonogenic survival, and spheroid formation in HNSCC [290]. Tumor cell motility and invasion are highly linked to dysregulation of the actin cytoskeleton in cancer. The actin-related protein (Arp) 2/3 complex nucleates branched actin networks, and altered activity can drive increased migration, extracellular matrix (ECM) degradation, and cancer progression [291]. CK2 phosphorylation of the actin binding protein cortactin impairs the ability of cortactin to bind Arp2/3 and activate actin nucleation [292]. These authors observed that loss of CK2-mediated cortactin phosphorylation caused decreased invadopodia activity in HNSCC cells. Further, CX-4945/Silmitasertib treatment reduced characteristics of invasion in multiple HNSCC spheroid models and in an orthotopic tongue xenograft tumor mouse model. CK2 is known to interact with the JAK/STAT pathways, and studies of angiogenesis and immunosuppression in HNSCC revealed a connection between JAK2/STAT3 inhibition and CK2. Using an inducible transgenic mouse model for HNSCC, treatment with the JAK2/STAT3 inhibitor AG490 suppressed angiogenesis and decreased the presence of myeloid derived suppressor cells (MDSCs) in the tumor microenvironment. In these tumors and stroma, decreased staining for p-STAT3 was detected as well as decreased CK2 and VEGFA levels, although it is not clear which subunit

Table 3 CK2 targeting in head and neck cancer human xenograft models

Model	Treatment	Tumor Effects	Reference
SCC-15 flank	TN-AS, topical, 200 µg, once, elapsed treatment time 15 d	Reduced tumor volume tripling time by twofold	[169]
		Loss of CK2α signal in tumor cell nuclei	
UM-SCC-11A flank	TBG-RNAi-CK2, ip, 10 µg/kg, 3 times with 72 h intervals, elapsed treatment time 8 d	Reduced tumor size	[223]
		Apoptosis (TUNEL, Caspase 3)	
		Decreased CK2αα′, total NFκB p65, pNFκB p65 S529 & S536, cyclin D1, Bcl-xL, Bcl2 proteins in tumor	
		Increased TP53 and p63 proteins in tumor	
Fadu intradermal	TBG-RNAi-CK2, iv, 10 µg/kg, 2 times with 48 h intervals, elapsed treatment time 7 d	Reduced tumor size	[223]
SCC-15, UM-11B, Fadu intradermal	TBG-RNAi-CK2, iv/ip, various doses from 0.01 to 50 mg/kg, 1 or 2 injections total, time elapsed after treatment 6 mo	Increased survival over controls	[230]
		Decreased metastasis over controls	
UM-SCC-1	CX-4945, oral bid, 25 / 75 mg/kg for 35 d, elapsed treatment time 41 to 48 d	Moderate TGI at 75 mg/kg	[286]
		At 33 d & 75 mg/kg:	
		Reduced pAKT S129 & S308 & S473, pS6 S235/236, pNFκB p65 S529 & S536	
		Apoptosis (TUNEL)	
		Increased Ki-67 and MEK pathways signals	
UM-SCC-1	CX-4945, oral bid, 75 mg/kg + PD-0325901, oral qd, 1.5 mg/kg, for 21 d, elapsed treatment time 40 d	Reduced tumor growth	[286]
		Median survival improvement of 12 d	
		Reduced AKT and MEK signaling	
		Apoptosis (TUNEL)	
Oral SCC Naturally occurring, 9 cats	TBG-fRNAi- CK2αα′, iv, 2 / 20 µg/kg, 6 times total twice weekly, elapsed treatment time 21 d	Reduced CK2α in 2 of 6 evaluable tumors	[231]
		3 cats with stable disease	
		1 cat with partial response	

AS, antisense oligonucleotide; bid, twice per day; CX-4945, Silmitasertib small molecule inhibitor targeting CK2; d, days; h, hours; ip, intraperitoneal; iv, intravenous; PD-0325901, small molecule inhibitor to MEK; SCC, squamous cell carcinoma; TBG, tenfibgen subdomain of tenascin used to coat nanocapsule; TBG-RNAi, single stranded DNA/RNA chimeric oligonucleotide targeting CK2αα′; TBG-fRNAi- CK2αα′, feline-specific form of TBG-RNAi- CK2; TBG-siCK2, nanoencapsulated double stranded siRNA targeting CK2αα′; TBG-DMAT, nanoencapsulated CK2 small molecule inhibitor; TGI, tumor growth inhibition; TGI, tumor growth inhibition; TN, tenascin

of CK2 was examined [293]. Downregulation of CK2 was also demonstrated to induce apoptosis and inhibit migration and invasion of a laryngeal squamous cell carcinoma cell line [294].

It is now well accepted that human papilloma virus (HPV) is a contributory factor in certain subtypes of head and neck cancer, and HPV + and HPV − cancers have distinct biological and clinical aspects [295]. Several attributes of HPV biology are influenced by CK2. The HPV-16 E7 protein inactivates the tumor suppressor pRb and is a substrate of CK2 [296–298]. E7 also binds with the core component of TFIID, the TATA Box Binding Protein (TBP). Phosphorylation of TBP by cellular CK2 increases the affinity with which E7 binds TBP [299, 300]. Further HPV proteins identified as CK2 substrates also include the replication factors E1 and E2 [301, 302]. HPV proteins E2, E4, and E5 are proposed as an alternate oncogenic pathway to that of E6 and E7 expression [303]. CK2 phosphorylation promotes E2

association with the cellular protein DNA Topoisomerase II Binding Protein 1 (TopBP1) and stabilization of E2 during mitosis [302]. In another example of CK2 interaction with HPV activities, phosphorylation of the chromatin-associated bromodomain-containing protein 4 (Brd4) by CK2 has an effect on transcriptional activity of both HPV and the host cell [304, 305]. Noteworthy is a recent demonstration that activity of CK2α but not CK2α′ is required for efficient replication of certain HPV types [301]. We recently showed that interfering with CK2 activity in both HPV(+) and HPV(−) HNSCC cell lines reduced cell viability and induced expression of the tumor suppressor proteins p21 and PDCD4 [41]. In addition, blocking CK2 activity also improved cisplatin response in these two types of HNSCC cells.

As summarized in the initial paragraph for this section, much of what we know about CK2 levels in HNSCC tumors and the association with clinical outcomes derives from data in which HPV status was not determined [86, 189, 283].

Due to the prevalence of HPV infection in head and neck cancers, especially oropharyngeal squamous cell carcinoma (OPSCC), we undertook studies to examine CK2α levels in OPSCC patient tumors with incorporation of the HPV status into our analysis. We found that high levels of CK2α protein are detected in HPV(+) tumors [306]. Unexpectedly, high CK2α levels associated with better overall survival in our patient cohort which was 70.6% HPV(+). Better overall survival for patients with HPV(+) OPSCC disease was also determined, which aligned with other studies [307, 308]. Because the number of HPV(−) patient tumors was small in our study, we turned to publicly available RNA-seq data from The Cancer Genome Atlas (TCGA) to more robustly analyze the possible association of *CSNK2A1* abundance with survival. In HPV(−) patients, the results of the TCGA analysis confirmed previous findings that higher *CSNK2A1* expression levels associate with significantly worse survival outcomes [14, 86, 189, 283, 306]. However, it is worth noting that all HNSCC cell lines evaluated and subjected to cisplatin treatment demonstrated additional cytotoxic response to further treatment with CX-4945 and CK2-siRNA regardless of the HPV status [41].

Overall, it appears that targeting of CK2 for treatment of head and neck cancers is promising. Evidence based on the animal models demonstrates that downregulation of CK2 reduces canonical NFκB-associated signaling, decreases tumor growth, and improves survival. Further investigation is needed to understand differential functions for CK2 in HPV(+) compared to HPV(−) disease and how this might inform therapeutic approaches.

In Fig. 4 we provide an example of some shared response pathways observed in breast, prostate and head and neck xenograft tumors following CK2 downregulation or pharmacologic inhibition. We would like to emphasize that the proteins depicted represent only a few of the proteins and pathways regulated by CK2 in cancer. Further, this diagram does not include immunological responses observed using immune-competent models.

Concluding remarks

The wide array of functions performed by CK2 in cancer cells sum to numerous avenues by which these malignant cells are dependent upon sustained CK2 signaling for survival. We described in this review how roles for CK2 in the nucleus and in the mitochondria likely contribute to this dependency. Further, data gleaned from numerous studies of solid and blood-based cancers have underpinned the promise for therapeutic targeting of CK2 via both single and combined treatment strategies. Here we focused particularly on the cumulative information in prostate, breast, and head and neck cancers. We described three approaches to blocking CK2 function that have been evaluated in model systems and in human and animal clinical studies: small molecule inhibition by predominantly CX-4945/Silmitasertib, peptide-based

Fig. 4 Depiction of response pathways observed in prostate, breast and head & neck tumors following CK2 downregulation or pharmacologic inhibition. We reiterate that the proteins depicted represent only a few of the proteins / pathways regulated by CK2 in cancer. Created with BioRender.com
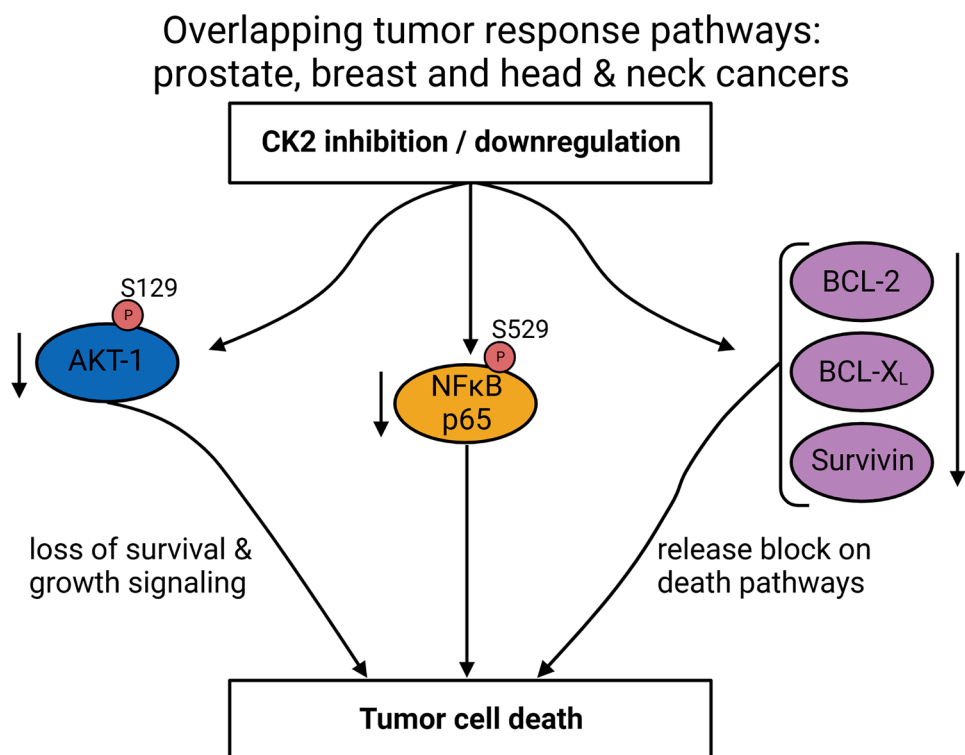
inhibition by CIGB-300, and molecular downregulation using RNAi delivered via nanoparticles. The diverse roles of CK2 in cancer biology continue to grow, emphasizing the need to further evaluate anti-CK2 treatment effects and refine strategies for informed combination therapies, thus optimizing approaches for this promising target.

Declarations

Conflict of interest The authors declare no conflict of interest or competing interests.

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