Published in final edited form as:
Biofactors. 2010 ; 36(3): 187–195. doi:10.1002/biof.96.

Emergence of Protein Kinase CK2 as a Key Target in Cancer Therapy

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Abstract

Protein kinase CK2, a protein serine/threonine kinase, plays a global role in activities related to cell growth, cell death and cell survival. CK2 has a large number of potential substrates localized in diverse locations in the cell including, e.g., NF-κB as an important downstream target of the kinase. In addition to its involvement in cell growth and proliferation it is also a potent suppressor of apoptosis, raising its key importance in cancer cell phenotype. CK2 interacts with diverse pathways which illustrates the breadth of its impact on the cellular machinery of both cell growth and cell death giving it the status of a “master regulator” in the cell. With respect to cancer, CK2 has been found to be dysregulated in all cancers examined demonstrating increased protein expression levels and nuclear localization in cancer cells compared with their normal counterparts. We originally proposed CK2 as a potentially important target for cancer therapy. Given the ubiquitous and essential for cell survival nature of the kinase, an important consideration would be to target it specifically in cancer cells while sparing normal cells. Towards that end, our design of a tenascin based sub-50 nm (i.e., less than 50 nm size) nanocapsule in which an anti-CK2 therapeutic agent can be packaged is highly promising because this formulation can specifically deliver the cargo intracellularly to the cancer cells *in vivo*. Thus, appropriate strategies to target CK2 especially by molecular approaches may lead to a highly feasible and effective approach to eradication of a given cancer.

Keywords

Protein kinase CK2; casein kinase 2; signaling; cancer; NF-κB; nanocapsule; nanoparticle; tenfibgen; tenascin; antisense; siRNA

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1. Introduction

Protein kinase CK2 (adopted acronym for the inappropriate name casein kinase 2 or II, as it may be noted that casein is not a physiological substrate for CK2) has been extensively studied for more than three decades. The protein kinase CK2 complex is a tetramer comprised of catalytic and regulatory subunits such that the two catalytic subunits are linked *via* two molecules of the beta subunit. The two catalytic subunits α and α′ (~ 42 and 38 kDa, respectively) and the β subunit (~ 28 kDa) form complexes such as $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, and $\alpha'_2\beta_2$ in varying distribution depending on the cell type. A considerable amount of work has been undertaken to delineate basic biochemistry of the kinase and the details of these studies can be found in several review articles [see, e.g., 1-6]. Much work has also been devoted to the biological functions of CK2 and these types of studies have led to identification of a large number of potential substrates localized in diverse compartments in the cell, just as the kinase itself is found in various locales in the nuclear and cytoplasmic compartments. The kinase was originally found to be elevated in rapidly proliferating cells including cancer cells and over time it has become apparent that CK2 is dysregulated by an increase in protein expression in all cancers examined. It has emerged that CK2 plays a global role in control of cell growth and proliferation, and even more interestingly an equally major role in control of cell death [2,3,7-10]. Since the cancer cell phenotype has the consistently remarkable features of deregulated cell growth (elevation) and cell death (suppressed apoptosis) [e.g., 11,12], the observation that CK2 is elevated in cancer cells provides a key link of the kinase to neoplasia. However, it is now becoming apparent that CK2 may be involved in the pathophysiology of many other disease processes; a detailed elegant discussion of CK2 in diverse diseases was presented in a recent publication [7]. In the present review, we will give a brief overview of the development of our understanding of the biological and pathobiological function of CK2, with a specific focus on its functionality in cancer and consideration of its potential as a key target for cancer therapy. We also consider the feasibility of molecular downregulation in a cancer cell specific manner through delivery of the therapeutic agent in a sub-50 nm tenfibgen nanocapsule.

2. General Features of CK2 Activity

CK2 is among the few protein kinases that can utilize both ATP and GTP for transfer of phosphate groups to serine/threonine residues in the proteins harboring the general consensus sequence S/TXXD/E/Y_p/S_p, and it appears that over 300 potential substrates for CK2 may be present in the cell [13]. The question is how CK2 recognizes its substrates in response to diverse signals. An interesting feature of CK2 is that it appears to be constitutively active as its regulation does not follow the general modes of activation commonly observed for protein kinases in the cell. Important insight into the activity of CK2 has been gained by extensive studies on X-ray crystallographic structures of CK2 as has been discussed in detail [see, e.g., 14]. These studies have contributed significantly to the nature of the CK2 structure and aspects of functional activity, although much remains to be learned. These various studies confirmed that the β subunit of CK2 is the linker between the catalytic subunits yielding the $\alpha_2\beta_2$ holoenzyme structure in which the two α subunits do not come into contact with each other. Interestingly, the β subunit harbors a Zn binding motif and it appears that the dimerization of the β subunits requires Zn [14,15]. This dimerization sets the stage for each of the β subunits to bind to a α subunit independently while exhibiting a certain plasticity; the structural details of this interaction have been discussed in detail by Niefind *et al.* [14]. The various crystallographic studies have also provided some insight into the basis of the ability of CK2 to utilize both ATP and GTP for phosphate transfer as well as the nature of the activation state of the catalytic subunit of the kinase [14]. In this regard, the recent observations that the human CK2α structure can adopt partially inactive conformations, whereas the fully active state of CK2α is pre-formed upon binding of CK2β,

are particularly intriguing with respect to the state and regulation of CK2 activity in the cell [14].

3. Aspects of cellular regulation of CK2

The aforementioned structural studies of CK2 highlighted dynamic regions in the kinase that may contribute to functional features of cosubstrate recognition as well as subunit interactions in the cell, and indeed it was proposed that the plasticity of CK2α may reflect a novel mode of regulation of the kinase in the cell [14]. This aspect of CK2 biology has remained an important question over the years. Several modes of intracellular regulation of CK2 function have been postulated. One of the early demonstrations based on studies of androgenic regulation of prostate growth (a physiological normal cell growth and death model) demonstrated dynamic shuttling of CK2 to different intranuclear locations in response to growth stimulus and shuttling out of the nuclear compartment on induction of cell death [e.g., 8،16،17،27]. This view was expanded to suggest that intracellular shuttling of CK2 may represent a general mechanism of its regulation [18]. An important issue that has remained unresolved relates to the nature of CK2 shuttling, i.e., do all the subunits undergo coordinate migration or can the individual subunits migrate independently. Much data indicate that the subunits of CK2 undergo dynamic differential translocation to various intracellular compartments in response to diverse stimuli [3،19]. However, other studies based on a normal cell model suggested the translocation of the holoenzyme rather than individual subunits [20]. The data on individual subunit translocation have generally been derived from use of cancer cell models [19،21،22], and so it is conceivable that the behavior of the kinase in normal *vs* cancer cells may be distinct. Clearly, further studies need to be undertaken to resolve these issues. To account for the range of CK2 cellular functional activity, it has also been suggested that the interaction of CK2 subunits with diverse intracellular molecules may be a contributory factor [23،24]. It has been further proposed that CK2 controls multiple protein kinases through phosphorylation of a protein kinase targeting molecular chaperone Cdc37 [25]. The fact that CK2 does not follow a single specific pathway of action but rather interacts with diverse pathways illustrates the breadth of its reach in the cellular machinery. These aspects of CK2 function in cell growth accord with its apparently global role in regulation of the growth related activities in the cell [26]. The various models of cellular regulation of CK2 mentioned above have common features and may not necessarily be exclusionary, but clearly these various aspects of CK2 function remain under ongoing consideration.

4. Suppression of apoptosis by CK2

While it was known for a long time that CK2 plays a role in cell growth and proliferation in normal and cancer cells, the more recent demonstration that CK2 was also a potent suppressor of apoptosis has squarely placed the functionality of the kinase in the cancer cell phenotype since CK2 has been found to be consistently elevated in all cancers studied [7¯9]. The original possibility of CK2 as a suppressor of apoptosis was suggested in the studies employing androgenic regulation of the prostate gland where it was shown that loss of androgenic growth stimulus resulted in rapid loss of nuclear associated CK2 and preceded the induction of apoptosis [2،16،17،27]. However, a direct compelling demonstration of the ability of CK2 to suppress apoptosis was shown in an experimental model of drug-induced apoptosis where it was shown that prior overexpression of the α subunit of CK2 (but not the β subunit) resulted in potent suppression of apoptosis [28]. Recent studies from various laboratories have further supported the function of CK2 as a suppressor of apoptosis in diverse experimental models [see, e.g., 10]. It now appears that CK2 may exert a broad impact on the apoptotic machinery by influencing the activity of diverse molecules and pathways involved in the regulation of apoptosis. A few of the examples are the PI3K/Akt

pathway [29,30], survivin and other inhibitors of apoptosis proteins (IAPs) [31,32], caspases [33,34], proteins in the Bcl2 pathway, and reactive oxygen species pathways [35-39]. These various observations point to the global impact of CK2 on apoptotic activity in the cell, and further highlight the significance of this function of CK2 in cancer cell biology as discussed subsequently. Finally, in a similar vein, the essential function of CK2 in cell survival is further illustrated by unsuccessful attempts to knockout CK2α and CK2β in yeast and mice [e.g., 40-43]. In mouse embryonic development, disruption of CK2α leads to death mid-gestation with structural defects in heart and neural tube, and disruption of CK2βcauses early lethality at E6.5 [41-43].

5. CK2 and Cancer

The association of CK2 with neoplasia has been known for a long time [e.g., 7-9]. Studies on diverse type of cancers have demonstrated that CK2 is uniformly elevated in all cancers examined. Interestingly, the elevation is noted at the level of protein rather than a significant change at the level of the enzyme message [8]. It is well known that two of the most consistent features of cancer are deregulated proliferation and deregulated apoptotic activity [11,12]. Thus, while CK2 was known to affect proliferation in both normal and cancer cells, the observation that CK2 potently suppressed apoptosis provided a vital link of the kinase to the cancer cell phenotype [10]. In a recent review article we discussed in detail how CK2 function may relate to various key features of cancer cell phenotype [9]. It was recently suggested that a common denominator of diverse cancer cells may be an addiction to CK2 [24]. We propose a further consideration of this appealing concept in view of the following observations. It appears that each tissue has a stable predetermined cellular level of CK2 and this level varies depending on the cell and tissue type. Under normal circumstances, cells resist even a modest upregulation or downregulation of cellular CK2. In the case of cell transformation, it appears that transformed cells acquire a new base level of CK2 and tend to maintain it in a stable manner analogous to that in normal cells, although in this case the cells have a dysregulated level of CK2 compared with that in the original normal cells. This accords with the lack of success in producing stable forced overexpression of CK2 protein in both normal and cancer cells [44,45]. Various observations suggest that a relatively small change in the balance of CK2 expression can have a large impact on cellular homeostasis. This was noted in studies showing that even a modest downregulation of CK2 in the nuclear compartment (chromatin and matrix) leads to induction of widespread cell death by apoptosis. [8,17,46-48]. We have further observed that cancer cells, where the CK2 protein expression is already perturbed, seem to be even more sensitive than normal cells to inhibition of CK2 activity or expression. By the same token, although CK2 by itself is not an oncogene, modest upregulation of CK2 can impart an oncogenic potential to the cells, as observed in experimental animal studies showing the remarkable contributory oncogenicity following increased expression of CK2. For example, overexpression of CK2α in p53 deficient mice or with c-*myc* or *Tal*-1 in transgenic mice resulted in a significant increase in the incidence of leukemia and lymphoma in mice [49-52]. Likewise, incorporation of CK2α with MMTV produced a transgenic mouse model of breast cancer with several features resembling the human disease [51]. In each case, modest overexpression of the CK2α transgene was sufficient to evoke enhanced oncogenic potential in the mice.

6. Importance of CK2 as a Target for Cancer Therapy

Given the dual role of CK2 in cell proliferation and cell death, we originally proposed that CK2 could serve as a key target for cancer therapy [17,47,53]. In developing novel avenues of effective cancer therapy, the ultimate goal is to eradicate all tumor cells in the host and achieve a complete cure of the disease. Thus it is important to consider targeting a gene that is uniquely indispensible for cell survival, as otherwise, tumor cells will escape death by

recruiting an alternate pathway [e.g., 9,55]. Clearly, CK2 is one such gene. Previous efforts at molecular targeting of protein kinases that were not critical for cell survival might limit their utility for clinical translation to cure the disease [e.g., 54,56,57]. This is not the case for this target because CK2 is essential for cell survival. The importance of CK2 as a target for cancer therapy is derived from the following key considerations. First, CK2 appears to be profoundly responsive to modulations of mitogenic signals from numerous initiating events in cells [2,8,16,48,58,59]. Second, downregulation of CK2 expression affects inflammatory, angiogenic, and drug efflux pathways to the benefit of cancer cell elimination [e.g., 60-63]. Third, dysregulated elevation of CK2 in cancer cells reflects the pathologic status of the tumor [8,65-68]. Fourth, CK2 downregulation impacts not only cell growth and proliferation but also apoptotic activity in cancer cells, making its targeting a two-edged sword [7-10,27,28,46,47,69]. Fifth, CK2 is indispensable for cell survival, and as far as we know there appear to be no redundant pathways to compensate for its downregulation [40-43].

Following our proposal, considerable interest in using CK2 as a target for cancer therapy has now emerged. The approaches being proposed are to use small molecule chemical inhibitors of CK2 [70-76], a peptide inhibitor to block CK2 phosphorylation sites in CK2 substrates [77], and molecular downregulation of CK2 using antisense or siRNA [7,9,17,46,47,53,78,80]. Targeting CK2 for cancer therapy raises the issue of its ubiquitous and essential for cell survival nature as being a potential problem for host toxicity. However, it appears that normal cells exhibit relative resistance to induction of apoptosis in response to agents such as antisense CK2α ODN or inhibitors of CK2 relative to cancer cells [46,69]. Despite this, it is important that approaches are devised that will optimally achieve the downregulation of CK2 only in cancer while sparing normal cells. The use of small molecule inhibitors [e.g., 71] or peptides to block CK2 phosphorylation sites [77] *in vivo* is not based on protected or targeted delivery of these agents *in vivo*, and relies on the pharmacologic window; however, their future success remains to be determined with regard to the potential of toxicity to normal cells and also the issue of tumor cell drug resistance, which may contribute to the problem of efficacy of these agents *in vivo*.

Our focus has been on the utilization of molecular downregulation of CK2 with attempts to do so specifically in cancer cells while sparing the normal cells *in vivo*. This approach, if successful, has the clear advantage that it is likely to be highly effective for cell death, and to overcome issues such as drug resistance in tumor cells. Starting with antisense CK2α ODN to downregulate CK2 in cell culture and in prostate cancer (PCa) and head and neck squamous cell carcinoma (HNSCC) xenografts [17,46,69], we originally demonstrated that potent tumor cell death is achieved in these experimental models. More recently, we have now devised novel antisense and siRNA constructs that downregulate both α and α′ subunits of CK2, thus ensuring more complete downregulation of CK2 *in vivo* [78,80].

To achieve the goal of specific molecular downregulation of the targeted signal in tumors, we have developed a novel sub-50 (s-50) nm (i.e., less than 50 nm size) tenfibgen nanocapsule to deliver the CK2 targeting agent specifically to primary and distant tumors *in vivo*. Tenfibgen (TBG), the nanocapsule material, is a subdomain (fibrinogen binding fragment) of tenascin C. The commonly employed experimental methods for nucleic acid delivery have included the use of viral vectors and non-viral vectors such as cationic lipid complexes, polycation complexes, macromolecular conjugates and liposomes. However, none of these have yielded satisfactory cancer cell specific targeting and have not addressed other critical issues essential for satisfactory drug delivery. The novel s-50 nm nanoencapsulation process to date displays many attributes of a potential clinically applicable *in vivo* delivery system [47,53,79,80]. The resulting product is an ultra small neutrally charged particle, with a protective shell of the targeting ligand having a non-ordered surface stabilized by crystallization. For tumor targeting, a protein ligand, tenfibgen

(TBG), enables tumor specific accumulation due to the increased expression of tenascin receptors specifically on the tumor cells, with negligible uptake observed by the reticuloendothelial system (RES) or other organs. The s-50 nanocapsule is also suitable for magnetic resonance imaging (MRI) with demonstrated ability to overcome compartmental boundaries *in vivo*. Furthermore, this system meets the key biological objective challenges for nanomedicine as stipulated by the FDA, NIH and NIST [81]. Our s-50 TBG nanocapsule containing antisense or siRNA directed against CK2 protects the nucleic acid in a tumor cell-specific protein ligand shell during circulation and releases the cargo within the cell following entry *via* the caveolar pathway, thereby bypassing the endosomal trap. Thus, this nanocapsule technology holds enormous promise for the successful molecular targeting of CK2 specifically in tumor cells. Additionally, our data suggest that the s-50 TBG nanocapsule has the ability to target metastases (Fig. 1), including bone metastases (Unger, G., Ahmed, K., et al., unpublished data), which considerably raises its potential for advanced cancer therapy.

How does CK2 downregulation achieve therapeutic goals in cancer? Given that CK2 impacts over 300 potential substrates in the cell and a wide range of pathways that pertain to cell growth and apoptosis [e.g., 13], it is likely that its downregulation would have a vast reach on activities that regulate cell function. Consistent with this, NF-κB is among the various pathways that have received considerable attention pertaining to a link with CK2 signaling. Here we consider the response of this pathway to molecular downregulation of CK2. The following discussion supplements the elegant consideration of NF-κB in development and cancer in reference to CK2 involvement [82]. NF-κB is known to have a broad role in regulation of many genes involved in diverse processes, among which are those relating to cell growth and proliferation, cell death, inflammation, migration, and angiogenesis [e.g., 82¯86]. Aberrant activation of NF-κB has been documented in several cancers including mammary gland, prostate, and head and neck cancer [e.g., 83¯86]. The activation of NF-κB in response to upstream signals is achieved by release of the inhibitory complex with IκBs, whose phosphorylation by various kinases including CK2 results in its degradation. Upon release, NF-κB (e.g., p65/p50) is translocated to the nucleus where it binds to regulatory sites of a variety of genes [82¯84].

Studies on the activation of NF-κB in mammary gland [82،87،88] and head and neck cancer [89] demonstrated the involvement of CK2 in this process. Further, it is of note that CK2 is also involved in the phosphorylation of p65 directly thereby influencing its activity [90]. Interestingly, activation of p65 and a related gene cluster is also linked with repression of tumor suppressor TP53 mRNA and protein expression in a subset of head and neck squamous cell carcinomas retaining wt TP53 genotype (HNSCC) [91،92]. Involvement of CK2 in modulating the activity of TP53 has also been noted [93¯96], thus providing a possible link in these various pathways. Further recent investigations along these lines undertaken in HNSCC have demonstrated differential responses of the NF-κB and TP53 pathways upon modulation of individual subunits of CK2 [80]. Knockdown of individual subunits of CK2 demonstrated a differential decrease of gene expression of not only NF-κB but also cell survival (*BCL-XL*) and cell cycle progression (*CCND1*) genes, whereas an increase of TP53 family genes known to promote growth arrest and apoptosis (*p53* and *Tap63*) was observed. Knockdown of CK2α demonstrated a significant decrease in *ITGA3* and *ITGB4*, while knockdown of CK2α′ resulted in decrease of *ITGA6*. Interestingly, the angiogenic factor *VEGF* was significantly reduced by downregulation of both α and α′ subunits of CK2 [80]. The involvement of CK2 in the process of angiogenesis has also been documented previously [60،61]. Likewise, altered expression of certain integrin genes (*ITG*) involved in HNSCC adhesion and migration has been previously reported [97،98]. Based on the observations that CK2 influences the expression of integrin genes in HNSCC, a further analysis of the effects of downregulation of CK2 subunits α and β on wound healing showed

a marked inhibitory effect [80]; these observations have provided novel information on the important role of CK2 in cell migration.

Studies by Brown *et al.* [80] on the effects of various subunits of CK2 on induction of cell death also demonstrated that downregulation of α but not α′ or β subunit was most prominent in inducing cell death in cultured HNSCC cells, analogous to previous observations in prostate cancer cells [17]. Analysis of cell cycle under these various conditions suggested similar increases in cells arrested in G0/G1 in each case, but specific decreases in S phase by knockdown of CK2α and CK2α′ while that of CK2β resulting in a decrease in cells in G2/M phase [80]. The therapeutic implications of these observations are highlighted by observations that modest downregulation of CK2α in cells sensitizes them to agents such as TRAIL or etoposide [35], and analogous observations by Brown *et al.* [80] have shown that downregulation of CK2 also sensitizes HNSCC to cisplatin. Further, studies were undertaken to examine the *in vivo* effects of downregulation of CK2 by employing anti-CK2α/α′ ODN which target both the CK2α and CK2α′ subunits in the s-50 nm TBG nanocapsule formulation delivered systemically to a xenograft model of HNSCC in mice. The results demonstrated potent induction of apoptosis in tumor cells *in vivo* associated with downregulation of CK2α and CK2α′ expression, a decrease in total NF-κB p65, and decreased p65 serine 536 and serine 529 phosphorylation. Additionally, other genes related to growth and survival such as Cyclin D1, BCL-XL, and BCL2 demonstrated decreased expression while TP53 and p63 were increased. Previous studies have investigated the interaction of CK2 with p53 [e.g., 92¯95]; however, it may be noted also that p53 is not required for induction of apoptosis on downregulation of CK2 [17›99]. The involvement of the downstream pathways relating to NF-κB and TP53 that respond to downregulation of CK2 *in vivo* is illustrated in Fig. 2. Together with previous observations on the therapeutic effectiveness of knocking down CK2α [9›17›46›47›53], our recent studies [78›80] further highlight the importance of targeting both CK2α and CK2α′ as a novel and potentially key strategy for targeted cancer therapy.

7. Concluding remarks

The availability of the s-50 nm TBG nanocapsule to deliver the therapeutic agent (such as antisense, siRNA, or chemical inhibitors for CK2) has important implications as it provides for the first time a means to target cancer cells while sparing normal cells *in vivo*, and thus obviating issues relating to potential host toxicity as a result of CK2 downregulation in general or from the nanocapsule, which includes a normal tissue protein. Given that downregulation of CK2 causes death in diverse types of cancer cells, we postulate that this therapeutic modality could find application in cancers other than prostate cancer and squamous cell carcinoma of head and neck that we have studied. Thus, we propose that the molecular downregulation of CK2 achieved by its targeting *via* the s-50 TBG nanocapsule in a tumor cell specific manner has the potential of successful application to therapy of diverse types of cancers.

Acknowledgments

The original work in the authors laboratories is supported by Department of Veterans Affairs Research Funds and National Cancer Institute (NIH) grant UO1-CA15062 (KA), NIH grant RO1-DK067436 (BK), DOD Contract W81XWH-05-C-0084 (GU), and ZIA-DC-00016 (CVW, ZC).

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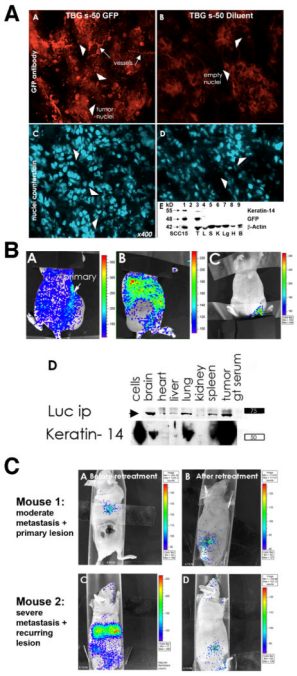

Fig. 1. TBG s-50 nanocapsule tumor-homing and biodistribution in xenograft mice following systemic administration

A. TBG s-50 nanocapsules target tumor cells and tumor-derived microvessels following systemic distribution: *Panels A-D* - Tumor cryosections from mice bearing SCC-15 tongue carcinoma xenografts were immunodetected for nuclear GFP signal 3 weeks following i.p. administration at 0.2 mg/kg of TBG s-50 nanocapsules bearing nuclear-localized GFP plasmid (Panel A) or diluent (Panel B). Positioning of cell nuclei in tumors is depicted by bisbenzamide counterstain (Panels C,D). GFP immunosignal was located in the tumor-derived microvasculature (arrows, Panel A) and clusters of tumor cells (arrowheads, Panel A). Note the lack of nuclear GFP staining in the tumor sections from the TBG s-50 diluent injected mouse. *Panel E* - Immunoblot analysis of tumor and tissue lysates from mice treated with TBG s-50 nuclear GFP or diluent nanocapsules confirms microscopy by another method (only results for GFP treated mice shown). Lysates were subjected to electrophoresis on a 4-12% gradient gel and immunodetected for keratin-14 to identify tumor cells (Panel E-top line), GFP fusion protein (Panel E, middle line), and β-actin as a loading control (Panel E, lower line). The lanes contain the following samples: 1) GFP transfected SCC-15 cell line as positive control, 2) MW marker, 3) tumor, 4) liver, 5) spleen, 6) kidney, 7) lung, 8) heart and 9) brain.

B. TBG s-50 nanocapsules deliver nucleic acid cargo specifically to tumor metastases. *Panels A-D* - A mouse with a large UM-11b laryngeal carcinoma xenograft flank tumor was injected i.p. with TBG s-50 nanocapsules containing 12.5 μg of luciferase plasmid and imaged using the Xenogen method 5 days later to allow for development of luciferase gene expression (Panels A,B). A control mouse, first injected with TBG s-50 nanocapsules bearing trehalose, was injected after 5 days with luciferin substrate and imaged together with the luciferase mouse for background control and comparison (Panel C). Luciferase signal was apparent in the viable rim of the cytolytic tumor mass (Panel A) and throughout the lung and abdominal region (Panel B). Large peritoneal metastases were encountered upon necropsy, but not assayed further. *Panel D* - Tissues from the metastases-burdened mouse were immunoprecipitated for luciferase, and the immunoprecipitates were analyzed by immunoblot along with lysate to correlate luciferase expression with the presence of keratin-14, a marker for head neck cancer. Substantial keratin-14 signal was co-present with

luciferase protein signal in brain, lung, spleen and tumor. The lane labeled “cells” represents UM-11b cells used for xenograft injection.

C. Existing metastases are cleared by TBG s-50 anti-CK2 nanocapsules in mice at ng/kg dosing. *Panels A,C* - Mice with SCC-15 tongue carcinoma xenografts were assayed for tumor burden 6 months following docetaxel/cisplatin treatment by the Xenogen method following i.p. administration of 12.5 μg of TBG s-50 luciferase plasmid. *Panels B,D* - Mice were administered additional tumor-targeted TBG s-50 luciferase plasmid nanocapsules and reimaged 4-6 weeks later following continual TBG s-50 anti-CK2 oligonucleotide nanocapsule treatment. The AB panel series was imaged after 6 weeks of 100 ng/kg q2d i.p. dosing together with topical 3 μg/ml TBG s-50 anti-CK2 oligonucleotide nanocapsule treatment for the last 2 weeks. The CD panel series was reimaged following 4 weeks of 100 ng/kg q2d i.p. treatment with TBG s-50 anti-CK2 oligonucleotide nanocapsules. Following reimaging, mice were treated with surgery (mouse in Panel B) or not (mouse in Panel D) and combination low-dose metronomic chemotherapy (20 μg/kg cisplatin, 1/1000 of murine MTD) with increasing doses of TBG s-50 anti-CK2 oligonucleotide nanocapsules before cessation of treatment. Mice were euthanized six months later at two years of age with no evidence of recurrence by imaging analysis.

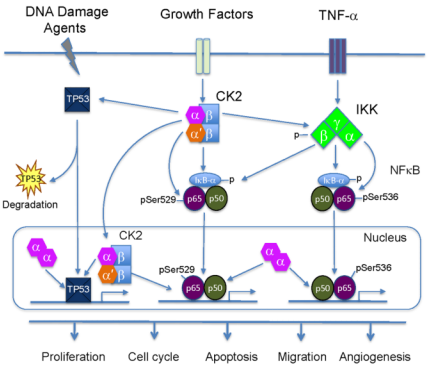

Fig. 2. Proposed model for CK2 modulation of NF-κB and TP53 pathways

Increased external signaling events, such as from TNF-α and other growth factors, bind and activate cell surface receptors, which induce aberrant CK2 and NF-κB activation. CK2 modulates IKKβ and IκBα phosphorylation and degradation, as well as promotes IKK mediated phosphorylation of NF-κB p65 at serine536. In addition, CK2 is able to directly phosphorylate p65 at serine529. At present, it is not clear if these phosphorylations occur on the same or different molecules. Activated NF-κB subunits translocate into the nucleus, bind to the NF-κB target gene promoters, and modulate transcriptional activity. In addition, CK2 is able to regulate TP53 and family members through protein phosphorylation, degradation, and modulation of expression. Abundant CK2α subunits are present in the nucleus of tumor cells and co-regulate NF-κB and TP53 family proteins and target gene expression. Thus, CK2 subunits regulate broad cellular functions affecting proliferation, cell cycle, apoptosis, migration and angiogenesis, which promote tumorigenesis of head and neck or other cancers. TNF-α: Tumor necrosis factor-α; NF-κB: nuclear factor κB; IKK: Inhibitor kappaB kinase; IκBα: Inhibitor κBα