Targeting CK2 for cancer therapy

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Protein kinase CK2 is a highly ubiquitous and conserved protein serine/threonine kinase that has been found to be involved not only in cell growth and proliferation, but also in suppression of apoptosis. CK2 is capable of dynamic intracellular shuttling in response to a variety of signals. It is localized in both the nucleus and cytoplasm in normal cells, but is particularly predominant in the nuclear compartment in cancer cells. CK2 has been found to be uniformly dysregulated in all the cancers that have been examined. Downregulation of CK2 by chemical or molecular methods promotes apoptosis in cells. We have shown that antisense CK2α is particularly potent in inducing apoptosis in cancer cells in culture as well as in xenograft models of cancer such as prostate cancer and squamous cell carcinoma of head and neck. The antisense CK2α oligodeoxynucleotide (ODN) mediates tumor cell death in a dose- and time-dependent manner such that at an appropriate concentration of the antisense, a complete resolution of the xenograft tumor is observed. Interestingly, normal and benign cells (in culture as well as *in vivo*) demonstrate a relative resistance to the antisense CK2α ODN treatment, which raises the possibility of a significant therapeutic window for this therapy. Further, novel approaches such as the delivery of antisense CK2α ODN encapsulated in sub-50-nm tenascin nanocapsules have become available for its targeting specifically in cancer cells. Our studies minimize generally held concerns regarding suitability of CK2 as a target for cancer therapy and provide the first encouraging results for potential future application of this approach for cancer therapy.
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Introduction

A key feature of cancer cells is that they demonstrate a dysregulation not only of growth, but also of apoptosis (programmed cell death). The process of oncogenesis is accompanied by a large number of changes in the biology of cells. Organ-specific features of various cancers add an additional complexity to the pathobiology of these cancers. The vast array of cellular components that are altered in the cancer cells represents the confounding challenge in attempts to devise therapeutic approaches for various types of cancer. Ideally, a cancer therapy target would have features that include (i) ubiquitous dysregulation in cancer cells, (ii) presence only in cancer cells, (iii) essential for cell survival such that targeting of the signal would result in cell death and (iv) the activity of the target in the cells not be redundant, i.e. not be compensated by other molecules upon its targeting. While thus far there is no single target that meets all these conditions, a large number of potential therapeutic targets for cancer therapy are currently being considered. In this context, protein kinase CK2 signal has entered our consideration for such a function only recently [1]. It has generally been thought that CK2, because of its ubiquitous nature, would not be a plausible target for cancer therapy. However, recent studies in the authors' laboratory have yielded encouraging new information to change this view. Here, we provide a brief discussion of the aspects of CK2 that we believe make CK2 a particularly important and potentially useful target that merits consideration for anti-cancer therapy.

General characteristics of protein kinase CK2 signal

Protein kinase CK2 (formerly known as casein kinase 2 or II) is a highly ubiquitous and conserved protein serine/threonine kinase localized in the cytoplasmic and nuclear compartments of the cell. The kinase consists of two catalytic (42-kDa α and 38-kDa α′) subunits and the regulatory (28-kDa β) subunit forming the heterotetrameric configurations $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$ and $\alpha'_2\beta_2$. CK2 is a multifunctional protein kinase; its role in normal and

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abnormal cell growth and proliferation has long been recognized [2–9]. Evidence suggests that CK2 is essential for cell survival and attempts at generating knockout phenotypes have met with failure [10,11]. The growth-related functions of CK2 have been supported by the recognition of a relatively large number of growth-related proteins that have been recognized as substrates for the kinase. These include, for example, proteins and factors involved in nucleic acid synthesis and protein synthesis, transcription factors, oncogenes and tumor-suppressor genes, proteins involved in signal transduction, and viral proteins [3,6,7]. CK2 has been shown to be involved in cell cycle progression [12,13]. Evidence suggests that CK2 undergoes dynamic association with nucleosomes depending on their transcriptional activity [14,15] and in this context it is noteworthy that a detailed analysis of genome-wide expression screens has indicated a global role for CK2 in chromatin remodeling [16].

Cancer and the CK2 signal

CK2 has been found to be dysregulated in all cancers that have been examined [3,6]. While CK2 has long been implicated in cell growth and proliferation in both normal and cancer cells, the nature of its function in the latter was puzzling. At first it was thought that CK2 might simply reflect the proliferative state of the cancer cells. However, it was demonstrated that elevation in CK2 in cancer cells is not simply a reflection of cell proliferation, but rather may relate to the level of dysplasia in different cells [2,17,18]. Further, CK2 is very high in tissues such as the brain and testes [6,19], making it likely that it is involved in other functions in these organs. A new function of CK2 was recognized recently, indicating its key role in suppression of apoptosis [4,5]. It was recognized earlier that removal of androgens in the animal resulted in induction of apoptosis in prostate epithelial cells, which was preceded by rapid loss of nuclear CK2, while androgen administration resulted in suppression of apoptosis and promotion of prostate cell growth associated with early shuttling of CK2 to the nucleus [20–23]. More direct evidence of the suppression of apoptosis by CK2 was provided by us when it was shown that prior overexpression of CK2 resulted in protection of cells from chemically mediated apoptosis such as by etoposide and diethylstilbestrol [24]. More recently, it was shown by us that CK2 can also affect apoptosis mediated via the death receptors in response to various ligands such as tumor necrosis factor (TNF)-α, Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), such that overexpression of CK2 suppresses, while downregulation of CK2 promotes, apoptosis mediated by death receptor ligands [25]. A detailed analysis of the impact of CK2 on TRAIL-mediated apoptosis has been undertaken by us which suggests that CK2 affects this pathway by impacting on several downstream targets (Wang *et al.*, unpublished data). Likewise, apoptosis induced by heat shock and radiation is blocked by CK2 [26–28], and CK2 facilitates repair of chromosomal DNA single-strand breaks [29]. Thus, taken together, it would appear that CK2 may have a global role in impacting on the apoptotic machinery [26] analogous to its global role in chromatin remodeling [16]. These novel observations on the function of CK2 are of particular importance since it has come to be recognized that cancer cells not only exhibit a dysregulation in cell growth and proliferation, but also a dysregulation of apoptotic activity. The involvement of CK2 in suppression of apoptosis may be particularly relevant to its role in the cancer phenotype, where it is known to be elevated, and the importance of CK2 in cancer pathogenesis may relate to its dual function, i.e. in promoting cell growth, but at the same time inhibiting cell death in these cancer cells. It is noteworthy that since the original proposal on the role of CK2 as a suppressor of apoptosis, analogous complementary evidence has been generated to show sensitization of cancer cells to death receptor-mediated cell death in the presence of chemical inhibitors of CK2 [30–33].

Intracellular dynamics of CK2 signal

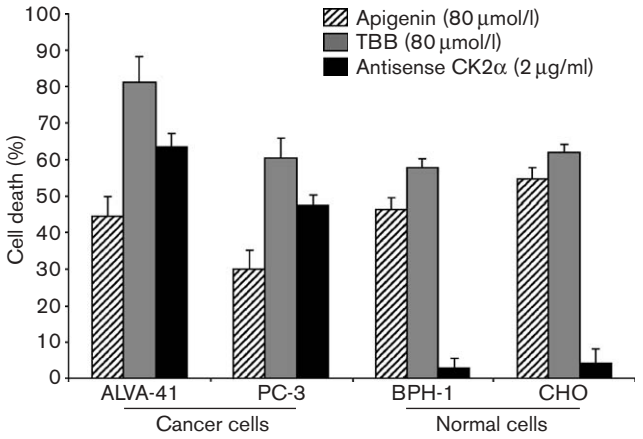
The intracellular dynamics of CK2 suggest that it undergoes shuttling to different compartments of the cell. It was originally recognized that nuclear translocation of CK2 in response to androgenic stimulus in the prostate and its rapid loss from the nuclear compartments (chromatin and nuclear matrix) on androgen removal was a key mode of its intracellular regulation under these conditions [20–22]. Subsequent work suggested that nuclear translocation of CK2 was a common downstream response of cells subjected to diverse types of growth stimuli [34]. It now appears that CK2 can dynamically shuttle to different intracellular compartments, further reinforcing this mechanism as an important means of its functional regulation [2–4,19–22,28,35,36]. Recent studies have also documented that CK2 can undergo dynamic associations with the potential of their integration into different multimolecular functional assemblies [37]. These considerations are of interest as the consequences of upregulation or downregulation of CK2 in the cells, such as those relating to cell growth or apoptosis, are primarily reflected by the dynamic changes in the nuclear (and especially nuclear matrix) associated CK2.

Consequences of downregulation of CK2 signal

We originally demonstrated that antisense CK2α ODN potently induced apoptosis in different types of cancer cells in a dose- and time-dependent manner [38,39]. Of note was the observation that antisense-mediated induction of apoptosis became apparent at a moderate downregulation of the CK2, as evidenced by the activity and immunoreactive enzyme associated with the nuclear

(chromatin and nuclear matrix) compartment. It appeared that downregulation of the chromatin- or nuclear matrix-associated CK2 by 30–40% was sufficient to induce potent apoptosis in cancer cells even when a smaller reduction was noted in CK2 activity in the total lysate [40,41]. Since that time, it has been found that inhibitors of CK2 activity such as apigenin and 4,5,6,7-tetrabromobenzotriazole (TBB) can also induce apoptosis in cells in culture [25,40]. Since CK2 is a ubiquitous signal, it was of interest to determine the effects of the CK2 activity inhibitors and antisense CK2α ODN on normal and benign cells as well. We made the surprising observation that concentrations of the antisense CK2α ODN that induced apoptosis in cancer cells (such as prostate cancer and squamous cell carcinoma of head and neck) had minimal or only a small effect on a number of normal or benign cells in culture [41]. In comparison, the CK2 inhibitors did show the induction of apoptosis in normal and benign cells, although the extent of apoptosis varied depending on the cell type [42]. This point is illustrated in Fig. 1, where we show that inhibitors of CK2 activity (apigenin and TBB) induced significant cell death in both the ALVA-41 and PC-3 prostate cancer cells, and normal CHO and benign BPH-1 cell lines, whereas the antisense CK2α ODN induced potent apoptosis in cancer cells, but minimal cell death in the CHO and BPH-1 cells. These, and our published observations discussed subsequently [41], are the first to suggest the possibility of a pharmacological window in targeting CK2 for induction of apoptosis in cancer cells under conditions that may spare normal cells.

Fig. 1



Comparison of the relative effects of inhibitors of CK2 activity and antisense CK2α ODN in induction of cell death on benign and cancer cells. Normal or benign cell lines (CHO and BPH-1), and prostate cancer cell lines (ALVA-41 and PC-3) were treated with 80 μmol/l apigenin or 80 μmol/l TBB, or 2 μg/ml antisense CK2α ODN for 24 h. Cell viability was determined by employing the WST-1 assay as detailed previously [25].

Targeting CK2 signal in the *in vivo* cancer models

In order to provide proof of principle, we undertook examination of antisense CK2α ODN in an *in vivo* xenograft model of cancer [41]. Experiments were undertaken to study the effect of direct administration of antisense CK2α ODN in human PC3-LN4 prostate cancer xenografts. The results showed that at 5 μg antisense ODN there was a reduction of 67% in the tumor at day 8 following a single intratumoral dose, while at 10 μg of the dose the tumor size was reduced by about 85% at this time. However, at a dose of 20 μg no detectable tumor was present at day 6 following the treatment. The tumor death was due to massive apoptosis in these xenografts, and correlated with extensive downregulation of the CK2α mRNA examined for 10 μg of antisense treatment at the 4-day time point. Under these conditions, no significant corresponding change was noted in the lysate CK2α immunoreactive protein or CK2 activity, but a dramatic decrease in the nuclear matrix-associated CK2α immunoreactive protein and activity was apparent. These observations reinforced the aforementioned results obtained in cell culture models where it was found that downregulation of CK2 in the nuclear matrix compartment was a primary effect of the antisense CK2α ODN and this was reflected in the induction of apoptosis. The fact that the CK2α message level was profoundly reduced would indicate that new CK2 would not be synthesized, and thus the total level of CK2 in the cell would also decline over time. It may be noted that CK2 protein half-life appears to be relatively slow, as was hinted by our earlier observations in different systems (e.g. [20,21]). We further examined the effects of the antisense CK2α ODN on normal cells and to that end we orthotopically injected the antisense CK2α ODN (at 20 μg) into the normal murine prostate. At this high dose, the histological appearance of the murine prostate was not particularly remarkable, and only minimal evidence of apoptosis was detected in the TUNEL stain and essentially little downregulation of the CK2 signal. This result accorded with that observed for the normal or benign cells treated with the antisense CK2α ODN in cell cultures. These various observations were encouraging in that they provided, for the first time, an indication that antisense CK2α ODN therapy may be a viable approach and merited further investigation. Another study employing a pro-apoptotic peptide with the ability to reduce CK2-mediated phosphorylation also reported an anti-tumor effect of downregulation of CK2 activity [43]; however, the *in vivo* results observed by us through the application of the antisense CK2α ODN were significantly more dramatic [41].

The discussion above pointed to the surprising observation that the CK2 signal in normal cells compared with cancer cells is relatively resistant to downregulation of CK2. This accords with the observations that the CK2

signal in cells tends to be relatively 'stable' under normal conditions and cells resist deviations from that state, e.g. as was noted in generating stable clones of CK2 overexpression (at the protein level) (e.g. [3,13]). The remarkable sensitivity of the cancer cells to downregulation of CK2 suggests that in cells where the CK2 signal is already 'dysregulated', these cells are likely to be more susceptible to alterations in the levels of CK2, thereby resulting in cell death. A possibility is also that in cancer cells there may be 'critical' substrates for CK2 that may not be present in normal cells and thus the downregulation of the CK2 signal may evoke a series of events such that the cancer cell cannot survive. Nevertheless, despite the observed pharmacological window using the antisense CK2α ODN, it would be important to devise approaches to target specific delivery of this agent to the cancer cells in the host in a specific manner, as the use of a DNA drug in a non-targeted manner would be less appealing. To that end, we have developed a novel approach for delivery of the antisense ODN to the cancer cells in a target specific manner, as discussed below.

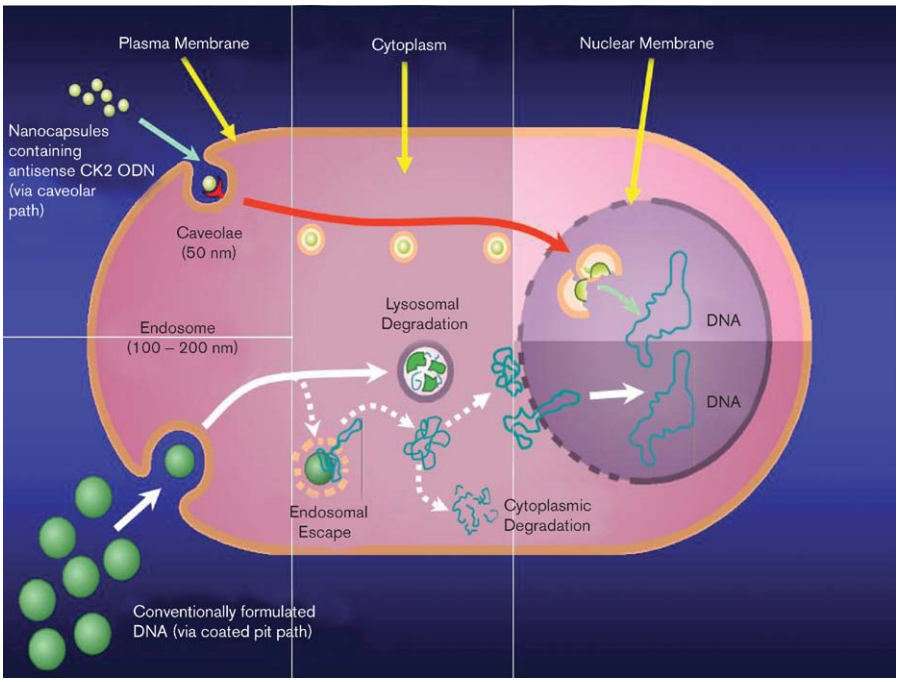
Nanocapsule technology – an approach to delivering antisense CK2 *in vivo*

A key issue in cancer therapy is the confounding problem of delivering the therapy in a target-specific manner (see, e.g. [44–46]). This is particularly critical when the target is present in cancer cells as well as in normal cells. There are several considerations in designing biopharmaceuticals for their specific delivery, e.g. size, fragility and specificity of targeting. If the delivery vehicle is too large, it will most likely utilize the clathrin-coated pit pathway for its entry into the cell. However, if the size of the vehicle is less than 50 nm, it is likely that the preferred route of entry into the cell would be via the caveolar pathway (Fig. 2). The design of the capsule should also take into account the issue of its stability since if it is too fragile, it would not efficiently deliver the cargo to the target. Likewise, the choice of the material for the design of the vehicle should consider its ability to target to cancer cells in preference to normal cells. We have designed a nanocapsule made of tenascin and, more recently, tenfibgen (a domain of tenascin). The nanocapsule is below 50 nm in size and the antisense CK2α ODN is encapsulated in this nanocapsule [42]. The rationale for the choice of the prototype tenascin or tenfibgen material is that it is a protein found in the extracellular matrix of tumors and that its receptors are upregulated in the cancer cell caveoli (e.g. [47,48]). As depicted in Fig. 2, the entry of the antisense CK2α ODN encapsulated in the tenascin-based, sub-50-nm nanocapsules into the cells via the caveolar pathway provides a direct route to the nucleus where the nanocapsule releases its cargo, thereby providing a direct means of blocking CK2α gene expression. The results in Fig. 3 show a comparison of the non-formulated (naked) antisense CK2α ODN and tenfibgen sub-50-nm encapsulated CK2α ODN. A dose of 5 mg/kg was administered via the tail vein to a mouse carrying a PC3-LN4 prostate cancer xenograft and the injection was repeated once after 24 h. At 72 h following the therapy, it was observed that the animal treated with naked antisense showed a reasonably significant response to downregulation of the CK2 signal indicated by a reduction in microvasculature (indicated by the Cd31 signal) and activation of caspase-3 activity. However, the effects on these parameters were much more dramatic when the antisense formulated in the nanocapsule was administered. It is noteworthy that under these conditions there was a significant change in the microvasculature as well as the activation of caspase-3 concomitant with the downregulation of the nuclear CK2 signal. This result clearly indicates that biodistribution of the drug (antisense CK2α ODN) in the tumor was a function of the coat protein, which in this case was tenfibgen (a domain of tenascin). Further, these results suggest that properly administered antisense CK2α ODN may provide an important means of eradicating the tumor. Although most of the experimental models discussed here related to prostate cancer, we obtained similar results when xenograft models of squamous cell carcinoma of head and neck were employed.

Summary and future directions

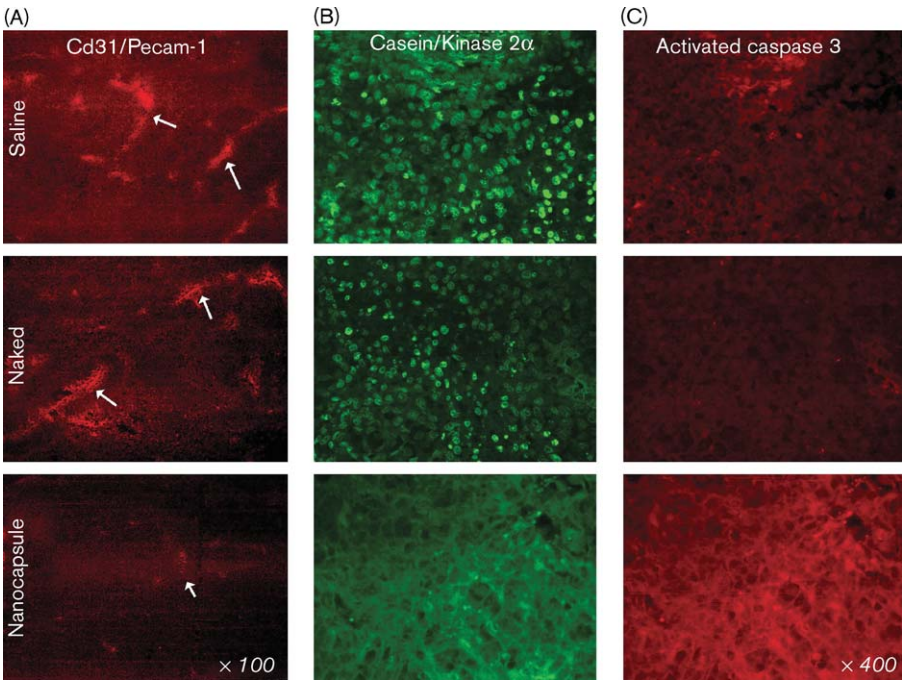
The present discussion points to the potential of CK2 as a particularly effective target for cancer therapy. Up until now, the targeting of CK2 for cancer therapy has not been seriously considered because of its ubiquitous nature, which raised concerns regarding the potentially serious toxicity to the host. However, our results that there may be a differential response of the normal cells compared with cancer cells to downregulation of the CK2 signal raise new hopes for the applicability of this target. An ideal target for cancer therapy should meet a number of important requirements. For example, as mentioned above, it should be uniformly dysregulated in the cancer cells, its targeting should lead to cell death, there should be no redundancy in its activity in the cell and it should be present only in cancer cells. As a target, the CK2 signal meets the first three of these requirements. Thus, despite our observations on the relative resistance of normal cells to downregulation of the CK2 signal, it would be important to target this signal in the cancer cells in a specific manner. To that end, our novel approach utilizing the caveolar pathway for delivery of the drug encapsulated in a sub-50-nm nanocapsule provides a plausible approach to this problem. With respect to prostate cancer therapy, an important consideration is the development of the androgen-insensitive phenotype [49]. Since we have observed that the CK2 signal is equally active in both androgen-sensitive and -insensitive prostate cancer [34], and that the downregulation of this signal in both types of cancer cells is equally effective in inducing apoptosis

Fig. 2



Schematic representation of drug uptake routes into the cell. The two major vesicular pathways for receptor-mediated endocytosis are indicated. The caveolar pathway provides a more direct route for the delivery of the antisense to the nucleus where it blocks the gene of interest.

Fig. 3



Comparison of the effects of unformulated (or naked) antisense CK2α ODN with the formulated antisense CK2α ODN (i.e. antisense encapsulated in tenascin sub-50-nm nanocapsules) on orthotopic prostate cancer tumor in mouse. The antisense treatment involved injection of 5 mg/kg of the unformulated or encapsulated antisense CK2α ODN via the tail vein in the mouse. The tumor size was 8 mm. The animal was sacrificed at 72 h following the treatment and tissue analysis for various markers was undertaken as shown (Ahmad *et al.*, unpublished data).

[38,40], it would seem that this target will be equally effective in treating prostate cancer regardless of the phenotype.

At present our results support the notion that molecular downregulation of the CK2 signal (such as by employing antisense ODN) may be a particularly effective means of inducing cell death; however, as other drugs that may target this signal more specifically become available they would also need to be considered for specific delivery via the nanocapsules since it appears that normal cells are more susceptible to small-molecule inhibitors of CK2 as compared to the use of antisense CK2α ODN. Further investigations along these lines may yield an important new approach to cancer therapy.

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