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CK2 Signaling in Androgen-Dependent and -Independent Prostate Cancer

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Abstract Protein serine/threonine kinase casein kinase 2 (CK2) is a key player in cell growth and proliferation but is also a potent suppressor of apoptosis. CK2 has been found to be dysregulated in all the cancers that have been examined, including prostate cancer. Investigations of CK2 signaling in the prostate were originally initiated in this laboratory, and these studies have identified significant functional activities of CK2 in relation to normal prostate growth and to the pathobiology of androgen-dependent and -independent prostate cancer. We present a brief overview of these developments in the context of prostate biology. An important outcome of these studies is the emerging concept that CK2 can be effectively targeted for cancer therapy. J. Cell. Biochem. 99: 382–391, 2006. © 2006 Wiley-Liss, Inc.

Key words: CK2 (formerly casein kinase 2); apoptosis; signaling; nuclear matrix; chromatin; prostate; androgen; growth factors; cancer

It is well known that a large number of signals are dysregulated in various neoplasias. Some of these dysregulated signals may be more prominent in specific types of cancer, and search for cancer-specific signals remains a major area of scientific endeavor. This applies to prostate cancer as well. However, not withstanding the importance of search for novel cancer-specific signals, various commonly known signals are also worthy of attention as they may play critical roles in the pathobiology of cancer cells. Among such signals is the highly conserved and ubiquitous protein kinase CK2 (acronym for the former name casein kinase 2) that has come to be recognized as a key player in cell growth and proliferation as well in regulation of apoptotic activity in cells. Importantly, it has become apparent that CK2 is uniformly dysregulated in all the cancers that have been examined, including prostate neoplasia [Guerra and Issinger, 1999; Tawfic et al., 2001]. This laboratory was the first to initiate studies of the CK2 signal in the prostate [Ahmed and Ishida, 1971], and this effort has culminated in the delineation of its significant roles in androgen-dependent and -independent prostate cancer growth, as well as its recently recognized function as a suppressor of apoptosis [see e.g., Ahmed, 1994, 1999; Ahmed et al., 2000, 2002; Guo et al., 2001; Tawfic et al., 2001; Wang et al., 2001; Yu et al., 2001; Unger et al., 2004; Ahmad et al., 2005]. Here we provide a brief overview of these aspects in the context of the functional activity of CK2 in androgen-dependent and -independent prostate cancer. We also discuss evidence that points to its strong potential as a target for prostate cancer therapy.

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PROTEIN KINASE CK2 SIGNAL

CK2 is a most highly conserved and ubiquitous protein serine/threonine kinase localized in both the cytoplasm and nucleus where it engages in many functions, including roles in normal and abnormal cell growth and proliferation. Several recent review articles have given a detailed account of general features of CK2 [Ahmed, 1999; Guerra and Issinger, 1999; Xu et al., 1999; Ahmed et al., 2000, 2002; Tawfic et al., 2001; Pinna, 2002; Litchfield, 2003; Pyerin and Ackermann, 2003]. Here, we present only a brief account of some of the general characteristics of this signal. The heterotetrameric structure of CK2 consists of two catalytic subunits (~42 kDa α and ~38 kDa α′) and two regulatory subunits (~28 kDa β) existing as $\alpha_2\beta_2$, or $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$ configurations; the relative distribution of the catalytic subunits varies depending on the cell type. The heterotetrameric structure is formed by linking of the two catalytic subunits through the β subunits. Although the catalytic subunit exhibits some activity, formation of the tetrameric structure involving β subunits imparts maximal activity. There are no known ligands for CK2 to regulate its activity although it appears that polyamines are stimulatory. Since CK2 is localized in both the cytoplasm and nucleus, our studies have suggested that the β subunits may promote linkage with the nuclear matrix structure which, along with chromatin, is a key locus for CK2 signaling in the nucleus [see e.g., Ahmed, 1999; Ahmed et al., 2000; Tawfic et al., 2001].

The regulation of CK2 is not fully understood, as it appears to be constitutively active, and it does not appear to be an early response gene [Ahmed et al., 1993a]. However, much evidence suggests that shuttling of CK2 in and out of the nuclear compartments is a major means of its functional regulation in response to diverse stimuli, including those which promote growth and those that promote cell death [Tawfic et al., 1996; Ahmed et al., 2000; Guo et al., 2001; Yu et al., 2001]. Indeed, dynamic shuttling of CK2 to different compartments in the cell has been proposed to represent a mechanism of its functional regulation in different cellular loci [e.g., Faust and Montenarh, 2000]. Germane to these considerations are also the observations that subunits of CK2 are in a dynamic state in the cell and may enter into diverse interactions [Allende and Allende, 1998; Filhol et al., 2004; Olsten et al., 2005]. The status of CK2 distribution is also distinct in normal versus cancer cells; CK2 in normal or benign cells is diffusely localized in various compartments of the cell while in cancer cells it appears to be more heavily concentrated in the nuclear compartment [Faust et al., 1999]. Evidence also suggests that CK2 may be related to the aggressive behavior of a tumor and may serve as a prognostic indicator [Gapany et al., 1995; Faust et al., 1996].

The growth-related functions of CK2 are reinforced by its involvement in the phosphorylation of numerous substrates in the cell, many of which are nuclear-associated and are involved in gene expression and cell growth [see e.g., Meggio and Pinna, 2003]. In Figure 1, we present a representative list of some of the substrates of CK2. The number and kind of proteins and genes that are potential targets for CK2 suggests the vast reach of CK2 functionality in the cell including that related to cell growth and regulation of apoptosis [see e.g., Guerra and Issinger, 1999; Guo et al., 1999a; Tawfic et al., 2001; Sun et al., 2002; Barz et al., 2003; Meggio and Pinna, 2003; Loizou et al., 2004; Olsten et al., 2005; Wang et al., 2006]. CK2 is essential for cell survival [e.g., Padmanabha et al., 1990], and attempts to produce CK2α- and CK2β-knockout mice have been unsuccessful [e.g., Bouchou et al., 2003]. With respect to the dysregulation of CK2 in cancer cells, it may be pointed out that CK2 by itself is not oncogenic but its dysregulation appears to co-operate with other molecules thereby enhancing the oncogenic potential in the cells [see e.g., Xu et al., 1999].

PROSTATE CANCER : SOME GENERAL FEATURES

In recent years, several excellent review articles have been devoted to the topic of prostate cancer [see e.g., Chatterjee, 2003; Nelson et al., 2003; Heinlein and Chang, 2004; Chung et al., 2005; Scher and Sawyer, 2005; Tindall and Dehm, 2005; Uzgare and Isaacs, 2005]. Since the focus of this review is primarily on CK2 signal in the prostate we will only briefly consider a few general aspects of prostate cancer pathobiology so as to link it to CK2 signaling. Prostate cancer is a hormonal cancer, that is, androgens and androgen receptor play essential role in its etiology although the precise mechanisms are

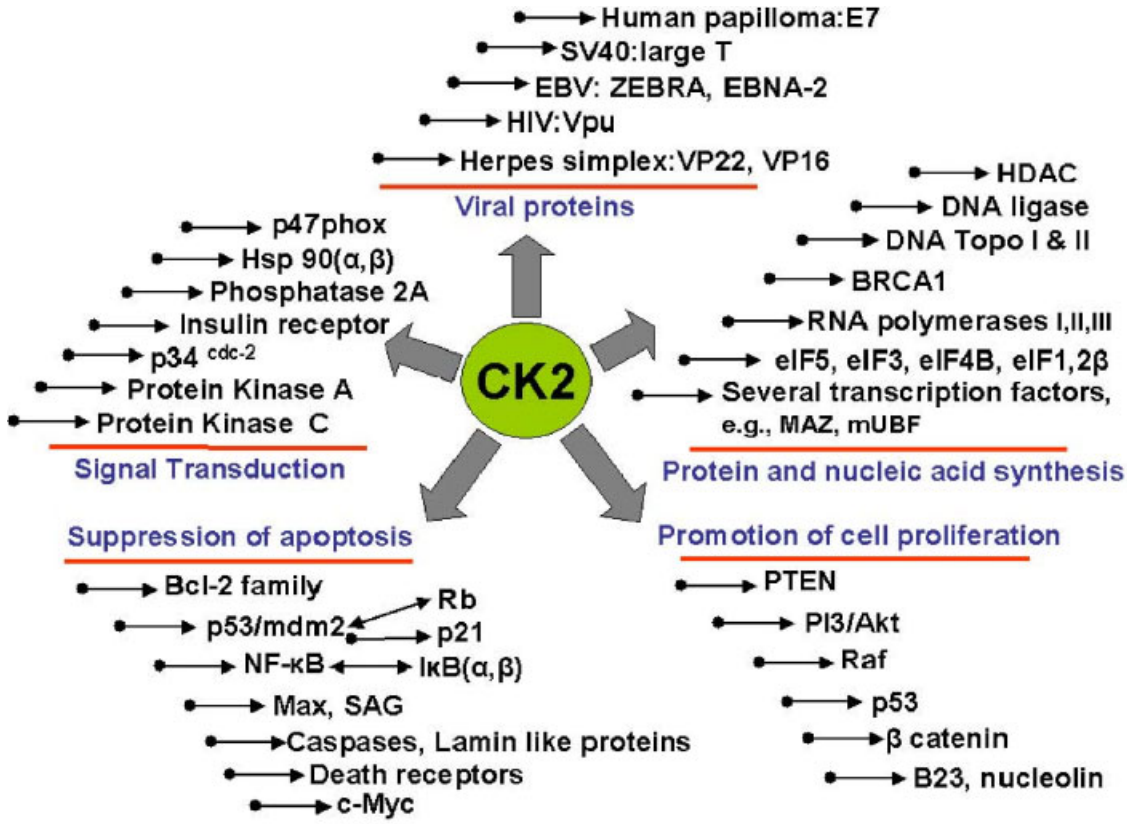

Fig. 1. A brief listing of functional targets of CK2 in the cell. CK2 impacts on diverse activities in the cell including those related to cell growth and proliferation and those related to cell death [for details, see e.g., Guerra and Issinger, 1999; Guo et al., 1999a; Tawfic et al., 2001; Ahmed et al., 2002; Meggio and Pinna, 2003; Olsten et al., 2005]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

not fully understood. Just as the growth and function in normal prostate are dependent on androgen action, the emerging prostate cancer also demonstrates reliance on androgens for growth. This is the basis of the initial therapeutic intervention for prostate cancer so that androgen ablation results in significant regression of the tumor. However, the tumor re-emerges in a form that is no longer responsive to further androgenic manipulations and is not responsive to any of the available therapies. This form of prostate cancer that develops subsequent to androgen ablation therapy has generally been referred to as androgen-independent or hormone-refractory although such a terminology may not be entirely appropriate as the androgen receptor appears to be functional in most cases of prostate cancer. Rather, androgen receptor under these conditions becomes sensitized to low levels of androgens on androgen deprivation, and/or is activated by cross-talk mechanisms through the action of a variety of paracrine factors including growth factors and cytokines. It has also been proposed that small changes in overexpression of the androgen receptor can profoundly influence resistance to androgen deprivation therapy [see e.g., Tindall and Dehm, 2005]. Another important factor in the progression of prostate cancer relates to the consideration of the tumor-associated stroma that has been proposed to actively promote progression of prostate cancer from localized growth to distant metastases [see e.g., Chung et al., 2005]. Such a mode of prostate cancer progression could also play a role in the generation of various phenotypes including cells that do not express androgen receptor. It may also be noted that for experimental purposes both androgen receptor expressing (e.g., androgen-sensitive LNCaP) and androgen receptor negative (e.g., androgen-insensitive PC-3) cell lines have been usefully employed for a wide range of laboratory investigations of the biology of these cells.

Investigations from different laboratories have identified the numerous signaling pathways that appear to be dysregulated in prostate cancer. For details of these studies the reader is directed to recent reviews on prostate cancer [see e.g., Chatterjee, 2003; Nelson et al., 2003; Heinlein and Chang, 2004; Chung et al., 2005; Tindall and Dehm, 2005; Uzgare and Isaacs, 2005]. Like most cancers, prostate cancer demonstrates dysregulation of both the growth- and apoptosis-related pathways; this information is summarized briefly as follows. A variety of growth factors and cytokines (e.g., EGF, bFGF, IGF1, KGF, VEGF, TGFα, TGFβ, IL4, IL6) play a significant role in prostate cancer progression and may engage in cross-talk with the androgen receptor [see e.g., Djakiew, 2000; Chatterjee, 2003; Nelson et al., 2003; Heinlein and Chang, 2004; Scher and Sawyer, 2005; Tindall and Dehm, 2005; Uzgare and Isaacs, 2005]. Growth factors and cytokines can interact with the cells through their cognate receptors and lead to cascades of downstream activities such as activation of MAPK, ERK, and PI3K/Akt pathways. PTEN/PI3K/Akt signaling has been found to be dysregulated in a large number of prostate cancers [see e.g., Heinlein and Chang, 2004; Scher and Sawyer, 2005]. GSTP1 and NKX3.1 have also been implicated as contributors to development of prostate cancer [Abate-Shen and Shen, 2000; Nelson et al., 2003]. Involvement of NF-κB mediated via the action of cytokines has also been proposed. Proteins involved in regulation of apoptosis such as members of Bcl-2 family, IAP, and caspases have been found to be dysregulated in prostate cancer [see e.g., Heinlein and Chang, 2004; Uzgare and Isaacs, 2005]. Alterations in tumor suppressor genes such as Rb, BRCA1/BRCA2 have also been implicated in prostate cancer, and downregulation of $p27^{kip}$ has been suggested to play a key role in prostate cancer cell cycle [see e.g., Nelson et al., 2003; Heinlein and Chang, 2004]. Ras/MAPK pathway and other MAP kinases have been proposed to be involved in both the androgen-sensitive and -insensitive prostate cancer [see e.g., Heinlein and Chang, 2004; Gioeli, 2005]. To conclude, it is clear that many of the pathways involved in cell growth and apoptosis have been found to be dysregulated in prostate cancer, and there is considerable evidence that androgen receptor may interact with these pathways by various mechanisms. It has been commented that there is a considerable redundancy in activities that regulate growth and apoptosis in prostate cancer [Uzgare and Isaacs, 2005], although it is unlikely that this is a unique situation for prostate cancer being most likely analogous to other cancers.

CK2 SIGNAL IN NORMAL PROSTATE AND PROSTATE CANCER

In this section we aim to discuss the work on CK2 signaling in the context of the normal and cancerous prostate growth. We will bring forth the latest observations that suggest that CK2 may be a particularly important potential target for therapy of prostate cancer and other cancers. We originated the studies on the protein kinase signaling in the normal prostate in response to androgen action. These studies demonstrated the rapid loss of phosphorylation of certain nuclear proteins in the rat ventral prostate in response to androgen deprivation in the animal (and associated with prostate epithelial cell death), and very rapid phosphorylation of these prostatic nuclear proteins when castrated rats were given a single injection of 5α-DHT [Ahmed and Ishida, 1971]. It was established that CK2 was a key enzyme that was responsive to androgenic manipulations in the prostate and played a role in the phosphorylation of the nuclear proteins as mentioned above [Goueli et al., 1980; Goueli and Ahmed, 1991]. Because of its androgen sensitivity, we initially thought that the enzyme in the prostate was distinct from that in other tissues; however, with the molecular cloning of cDNAs of CK2 from diverse sources it became apparent that it was a highly conserved protein kinase [Guerra and Issinger, 1999; Tawfic et al., 2001; Litchfield, 2003]. We also noted that the basis of the rapid change in phosphorylation of the nuclear proteins mentioned above was not owing to a rapid androgen-stimulated early expression of the CK2 genes [Ahmed et al., 1993a]. It then became apparent that the aforementioned rapid alterations in the nuclear protein phosphorylation in the prostate in response to altered androgenic status related to the rapid shuttling of CK2 to and from the nuclear compartments such as chromatin and nuclear matrix [Ahmed et al., 1993b; Tawfic and Ahmed, 1994]. As mentioned earlier, this aspect of CK2 function represents an important mechanism of its functional activity in response to diverse signals.

Considering the dynamics of CK2 in normal growth and death in rat prostate in response to altered androgens, we decided to examine whether CK2 functioned in an analogous manner in prostate cancer cell lines. For this, we studied LNCaP as a model of androgen-sensitive cell line and PC-3 as a model of androgen-insensitive cell line [Guo et al., 1999b]. We observed that CK2 shuttling to the nuclear matrix occurred in response to androgenic stimulation of LNCaP cells while androgen deprivation resulted in a loss of CK2 from the nuclear compartments. This was clearly analogous to what we had observed in studies on the androgenic regulation of rat prostate, described earlier. On the other hand, PC-3 cells did not respond to androgenic growth stimulus and there was no change in the nuclear CK2 in these cells under these conditions. However, PC-3 cells subjected to the growth factor stimulus demonstrated rapid shuttling of CK2 to the nuclear matrix and removal of growth factors from the medium resulted in loss of nuclear CK2. This was observed in the presence of several growth factors (such as EGF, KGF, TGFα). It was equally noteworthy that LNCaP cells which respond to both the androgen and growth factor stimuli behaved in an analogous manner in the presence of various growth factors or androgen with respect to CK2 dynamics. These observations provided for the first time an evidence that CK2 dynamics in response to growth stimuli were similar regardless of the nature of prostate cancer cell phenotype (i.e., those responsive to androgen and growth factors and those responsive to growth factors only) [Guo et al., 1999b]. Thus, CK2 demonstrated shuttling to the nuclear compartments in response to growth stimuli and shuttling out of the nucleus on removal of growth stimuli regardless of the phenotype of prostate cancer.

Relation of CK2 to the cancer phenotype has been an important issue for a long time. As mentioned earlier, CK2 has been found to be elevated in all the cancers that have been examined. Since CK2 is also elevated in proliferating normal cells, it had generally been thought that CK2 elevation in cancers was simply a reflection of the rapid proliferation of cancer cells. However, comparison of immunohistochemical analysis of Ki-67 antibody staining with CK2α antibody staining of the same tumor section clearly demonstrated that while Ki-67 staining was apparent mostly in the proliferating edge of the tumor the staining for CK2 antibody though present in the proliferating edge of the tumor was also spread extensively in the tissue section. This suggested that elevation of CK2 reflected the pathological status of the tumor rather than simply being an indicator of proliferation status [Faust et al., 1999].

A second observation that provided an important link of CK2 to the cancer phenotype related to our demonstration that CK2 acts as a potent suppressor of chemical-mediated apoptosis in prostate (and other) cancer cells [Guo et al., 2001]. This would be particularly important since CK2 is elevated in cancer cells and thus it would not only promote stimulation of growth and proliferation but also act as a suppressor of cell death (apoptosis). Dysregulation of apoptosis is regarded as a particularly important characteristic of cancer cell phenotype. Thus, a significance of our observation on CK2 as suppressor of apoptosis is that it provides an important link of CK2 to the cancer phenotype. In our earlier studies on androgenic regulation of rat prostate, we noted that on androgen withdrawal in the animal there was a rapid loss of CK2 from the nuclear compartments which on further analysis would appear to be an early event relating to impending apoptosis in the rat prostate epithelial cells [Yu et al., 2001]. For example, an analysis of percent of prostatic glandular cells dying per day via programmed death in response to androgen deprivation suggested that there were 3.0% of these cells at 24 h, and 21% at 48 h [Berges et al., 1993]. In an analogous analysis of the changes in rat prostate nuclear matrix-associated CK2 we observed that CK2 was reduced by 80% at 24 h and by 92% at 48 h following androgen deprivation. This would suggest that loss of CK2 from the nuclear matrix preceded induction of apoptosis in these cells following androgen deprivation [Yu et al., 2001]. Conversely, in these animals, administration of a single dose of androgen resulted in rapid and extensive shuttling of CK2 to the nuclear matrix associated with cell growth and suppression of apoptosis [Yu et al., 2001]. CK2 also suppresses apoptosis in prostate and other cells in response to heat shock and radiation [Ahmed et al., 2002; Davis et al., 2002; Yamane and Kinsella, 2005]. More recently, we and others have demonstrated that CK2 can suppress receptor-mediated apoptosis

such as that mediated by interaction of TNF-α, TRAIL, and FasL with the death receptors in prostate cancer cells [Wang et al., 2005a, 2006] and other cancers [Ravi and Bedi, 2002; Izeradjene et al., 2005]. In further analysis of TRAIL action in prostate cancer cells we have demonstrated that both androgen-insensitive (PC-3) and -sensitive (ALVA-41) prostate cancer cells are sensitized to TRAIL by chemical inhibition of CK2 using its specific inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB). Additionally, overexpression of CK2α using pcDNA6-CK2α suppresses TRAIL-mediated apoptosis in these prostatic cancer cells by affecting various activities associated with this process. These include effects on activation of caspases, DNA fragmentation, and downstream cleavage of lamin A. The overexpression of CK2 also blocked the mitochondrial apoptosis machinery engaged by TRAIL. These findings further define the important role of CK2 in TRAIL signaling in androgen-sensitive and -insensitive prostatic carcinoma cells [Wang et al., 2006]. Based on these various observations, we have suggested that CK2 has a broad effect on apoptotic activity as it can suppress apoptosis mediated by chemicals, loss of growth or survival stimuli, and death receptors, not only in androgen-sensitive and -insensitive prostate cancer, but also in other cancers [Ahmad et al., 2005; Wang et al., 2005a, 2006].

A third important development in studies of CK2 relates to the effects of its molecular downregulation on cell viability. We originally observed that treatment of cells in culture with antisense CK2 ODN resulted in rapid induction of apoptosis in a dose- and time-dependent manner in both the androgen-sensitive and -insensitive prostate cancer cells [Wang et al., 2001]. It may be noted that a reduction of CK2 activity in the nuclear matrix of these cells by about 30–40% was sufficient to evoke the apoptotic response. In order to determine that antisense CK2α ODN would be effective in the tumor tissue, we investigated the effect of antisense CK2α ODN on prostate cancer xenograft in vivo (generated from PC3-LN4) [Slaton et al., 2004]. Our results showed that a single injection of antisense CK2α ODN induced a dose- and time-dependent tumor death such that the tumor was completely resolved at the higher tested dose. Cell death was due to apoptosis and correlated with a potent downregulation of the CK2α message and a loss of nuclear matrix-associated CK2 in the xenograft tissue. Interestingly, under these conditions the CK2 measured in the cell lysates was not significantly altered which accords with its relatively slow turnover and is analogous to our several observations that loss of CK2 from the nuclear matrix is an initial key event pertinent to induction of apoptosis [Yu et al., 2001]. Of note, validation of CK2 as an oncological target has also been undertaken in other types of cancer cells [Guerra and Issinger, 1999; Seeber et al., 2005].

The above observations provided the "proof of principle" evidence on the ability of antisense CK2 to induce apoptosis in prostate tumor cells in vivo. However, these observations also raised the important question as to whether such a strategy would be feasible for translational purposes since, as mentioned earlier, CK2 is a ubiquitous enzyme and is essential for cell survival. Accordingly, serious concerns may be raised for its consideration as a cancer therapy target because of potential serious toxicity to the host. To address these issues, we decided to examine the effects of molecular downregulation of CK2 in benign and normal cells also [Slaton et al., 2004]. Interestingly, we observed that normal and non-cancer cells compared with cancer cells demonstrated a relative resistance to the effect of antisense ODN. For example, at a dose of 5 μg/ml for 24 h there was about 50% apoptosis in ALVA-41 and PC3-LN4 cells without any significant effect on benign or normal cells such as BPH-1, PrEC, normal human dermal fibroblasts, and normal human epithelial keratinocytes under the same conditions. Further, a single high dose orthotopic injection of the antisense CK2α ODN (at a concentration which potently induced apoptosis in cancer xenograft) had a minimal effect on the normal gland [Slaton et al., 2004]. The molecular basis of this differential response of cancer versus normal cells to antisense CK2 is unclear at present although a number of potential explanations are possible as discussed previously [Ahmad et al., 2005]. Nonetheless, these observations suggest that there may be a therapeutic window for the use of this approach (i.e., molecular downregulation of CK2 using antisense CK2 ODN or siRNA) for cancer therapy [Wang et al., 2005b].

Chemical inhibition of CK2 also results in induction of apoptosis in different types of cancer cells including prostate cancer cells [Pinna, 2002; Wang et al., 2005a,b]. It would

also be appealing to make use of small molecule inhibitors of this kinase for therapy. Interestingly, however, the differential response of normal and cancer cells to chemical inhibitors of CK2 is not as pronounced. This implies that the mechanism of action of antisense CK2α ODN can distinguish between cancer cells and normal cells while the chemical inhibition of CK2 is analogous in both type of cells [Ahmad et al., 2005]. For various considerations, it would seem more advantageous to deliver the antisense CK2 more specifically to the tumor cells in vivo, and likewise, if small molecule inhibitors of CK2 could be delivered to the tumors directly they may also be useful for consideration as anticancer agents. To further refine the targeting of CK2 through molecular (or chemical) downregulation we are employing a novel technology based on delivery of the targeting molecule to be carried as a cargo in tenfibgen-based sub 50-nm nanocapsules that enter the tumor cells via the caveolar pathway resulting in intracellular delivery of the drug. Systemic delivery of antisense CK2α ODN encapsulated in sub 50-nm tenfibgen nanocapsules induces potent apoptosis in a xenograft model of prostate cancer; these studies are currently being pursued in our laboratory [Ahmad et al., 2005; Wang et al., 2005b].

CONCLUSIONS AND PERSPECTIVES

We have provided a brief overview of the role of CK2 in the context of androgen-sensitive and -insensitive prostate cell growth and cell death. It is often commented that CK2 is constitutively active and as such its regulation is unclear. However, the constitutive activity of CK2 in itself could be regarded as being important for a kinase that is involved in many key activities including cell survival. The readers are directed to the elegant discussion of this aspect in a recent review [Pinna, 2002]. As discussed earlier, intracellular localization of CK2 appears to be an important mode of its functional activity, as may be noted by its differential localization in cancer versus normal cells [Faust et al., 1999], and its dynamic shuttling to different loci in response to various stimuli [Ahmed, 1999; Ahmed et al., 2000; Faust and Montenarh, 2000]. While CK2 does not fit a clear pathway in the various schemes of signaling cascades, its importance cannot be ignored in light of the extensive amount of work that has been undertaken by us and others in the context of cancer biology. As discussed in the foregoing, evidence from our laboratory and from others is mounting to support that CK2 is a key modulator of apoptosis mediated by diverse type of agents [Ahmed et al., 2002; Litchfield, 2003; Wang et al., 2005a]. We submit that several of the molecules in the apoptosis pathways in prostate cancer as outlined recently [Nelson et al., 2003; Uzgare and Isaacs, 2005; McKenzie and Kyprianou, 2006] are impacted by CK2 (Fig. 1). We realize that much further work is needed to determine the various sites of CK2 action on the apoptosis machinery; however, several studies have pointed to loci such as the Bcl-2 family, caspases, mitochondria, p53, NF-κB, IAPs, and DNA repair [e.g., Keller et al., 2001; Tawfic et al., 2001; Vazquez et al., 2001; Ahmed et al., 2002; Li et al., 2002; Loizou et al., 2004; Di Maira et al., 2005; Wang et al., 2005a; Wang et al., 2006].

An important issue regarding any therapeutic approach to prostate cancer (and other cancers) is that cancer cells tend to have ability to develop redundant pathways to evade the therapeutic agents. Thus, even though a particular molecular target is amenable to therapeutic intervention, cancer cells tend to escape death by utilizing alternate or redundant pathways. Proposed approaches to prostate cancer therapy include targeting androgen receptor, growth factor pathways, and apoptosis pathways [see e.g., Chatterjee, 2003; Tindall and Dehm, 2005; Uzgare and Isaacs, 2005; Mimeault and Batra, 2006]. Though interesting, these approaches cannot preclude the escape of prostate cancer cells with different phenotypes that do not respond to the therapy. In this context, it has been proposed that combinatorial therapies may be a more effective means of tackling this problem [Uzgare and Isaacs, 2005]. A concern with this approach would be an even increased risk of potential host toxicity.

We suggest the following characteristics of a signal that would be particularly promising for consideration as a therapeutic target. First, it is not redundant and is essential for cell survival; second, it is consistently altered in cancer cells; and third, it is functional in prostate cancer cells regardless of their phenotype. These features apply to the CK2 signal in the prostate, supported by several studies from our laboratory [Tawfic et al., 2001; Wang et al., 2001;

Slaton et al., 2004; Unger et al., 2004; Ahmad et al., 2005; Wang et al., 2005a,b; Wang et al., 2006]. Considering the functional biology of CK2, interruption of this signal should impact not only on cell growth but also on apoptosis, and thus would become a "two-edged sword." It may be noted that antisense CK2α ODN treatment is fully effective in inducing apoptosis in xenograft tumors such as PC3-LN4 and SCC15 (a head and neck squamous cell carcinoma) that are highly resistant to other forms of therapy [Ahmad et al., 2005; Wang et al., 2005b].

The only concern regarding CK2 as a potential target thus far has been the fact that it is a ubiquitous signal present in both the normal and cancer cells raising the issue that considerable toxicity may result if it is interrupted in normal cells. However, as mentioned above, there are a number of observations that would tend to obviate these concerns. For example, although physical distribution of CK2 in normal and cancer cells is distinct [Faust et al., 1999], there also appears to be some distinct biological difference in the CK2 signal in normal versus cancer cells. This pertains to the response of cancer cells compared with normal cells to molecular downregulation of CK2. While profound apoptotic effect in cancer cells is observed on molecular downregulation of CK2, there is only a minimal effect in normal cells under the same conditions. This is observed not only in cell culture models but also in the animal model where injection of antisense CK2α ODN orthotopically into mouse prostate produced only a minimal response [Slaton et al., 2004]. Another interesting feature of CK2 targeting is that a single injection of the antisense CK2α ODN at an appropriate dose can result in eradication of the tumor in experimental studies on the xenograft tumors, thus obviating the need for extended administration of the drug which should also minimize concerns regarding in vivo toxicity [Slaton et al., 2004]. Besides these encouraging observations, we suggest that further refinement in delivery of this type of agent (antisense CK2α ODN) by utilizing delivery vehicle such as the nanocapsules described by us should provide an even more useful approach to prostate cancer therapy [Ahmad et al., 2005].

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