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Nanoencapsulated anti-CK2 small molecule drug or siRNA specifically targets malignant cancer but not benign cells

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ABSTRACT

CK2, a pleiotropic Ser/Thr kinase, is an important target for cancer therapy. We tested our novel tenfibgen-based nanocapsule for delivery of the inhibitor 2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole (DMAT) and an siRNA directed against both CK2α and α′ catalytic subunits to prostate cancer cells. We present data on the TBG nanocapsule itself and on CK2 inhibition or downregulation in treated cells, including effects on Nuclear Factor-kappa B (NF-κB) p65. By direct comparison of two CK2-directed cargos, our data provide proof that the TBG encapsulation design for delivery of drugs specifically to cancer cells has strong potential for small molecule- and nucleic acid–based cancer therapy.

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1. Introduction

Among the many protein kinases proposed as anti-cancer targets, CK2 (formerly casein kinase II/2) is one of the most consistently elevated protein kinases associated with the oncogenic phenotype across various cancer types [1,2]. CK2 is a protein serine/threonine kinase existing as a heterotetramer consisting of two catalytic subunits α and α′ linked through two regulatory β subunits. The kinase is localized both to the nuclear and cytoplasmic compartment and may be constitutively active; several modes of its regulation have been proposed, but their precise nature is not fully understood [2,3]. Through its phosphorylation of numerous substrates, CK2 functions to influence the various biochemical and metabolic processes of cell growth and proliferation, as well as cell death and inflammation

Abbreviations: CK2, official acronym for former casein kinase 2 or II; d, day(s); DMAT, 2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole; h, hour(s); HNSCC, head and neck squamous cell carcinoma; FBS, fetal bovine serum; i.p., intraperitoneal; i.v., intravenous; ID, injected dose; IHC, immunohistochemical or immunohistochemistry; kDa, kiloDalton; min, minutes; NF-κB, Nuclear Factor-kappa B; NP, NP-40; OGN, oligonucleotide; PBS, phosphate buffered saline; PCa, prostate cancer; PCR, polymerase chain reaction; P-Ser529, phosphorylated at serine residue 529; Q-RT-PCR, quantitative reverse-transcriptase PCR; RFP, red fluorescent protein; s.c., subcutaneous; Ser, serine; siCK2, siRNA to CK2αα′; siRNA, small interfering RNA; TBG, tenfibgen; Thr, threonine; TN-C, tenascin-C.

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and stress signaling, among other processes. This kinase is a very highly conserved protein in nature, and cells or organisms cannot survive without CK2 expression [4,5]. We originally proposed that the essential nature of CK2 for cell survival renders it a particularly attractive target for the goal of eradicating tumor cells by inhibition of CK2 activity or molecular downregulation of CK2 expression [6].

CK2 steady-state expression levels are distinct for various cell types, and its expression in different tissues under normal quiescent conditions remains stable. However, it has been found to be upregulated in all cancers that have been examined. In this regard, CK2 can be considered a "non-oncogene" whose dysregulated expression is critical for the maintenance of the cancer cell malignant phenotype [1,7,8]. This increase in CK2 expression in cancer cells is generally evident at the protein and activity levels and correlates with increased nuclear localization [1,7]. Elevated CK2 expression, at either protein or RNA levels, or activity has been noted in human leukemias, breast, colorectal, gastric, head and neck, kidney, lung, multiple myeloma, and prostate cancers [1,2,9–11]. Also significant are the observations that CK2 upregulation in neoplasia reflects a complex state of dysplasia, not simply the enhanced proliferative state of the tumor cells [9]. In addition, dysregulation of CK2 expression may relate to the severity of disease and serve as a prognostic indicator [1,2,11–14]. The more recently established function of CK2 in suppressing apoptosis supports its role in cell survival and firmly ties CK2 upregulation and function to the cancer cell phenotype [7,15]. It is noteworthy that several lines of evidence point to nuclear associated CK2 as the most sensitive subcellular fraction in responding to alterations in cell growth and death [6,16]. Thus, the combined pleiotropic functions of CK2 and the general dependence of cancer cells on increased CK2 expression offer a strong impetus for the development of anti-CK2 cancer therapeutics [6].

Downregulation of CK2 in numerous human cancer cell lines using siRNAs and antisense oligonucleotides directed against CK2 subunits has generally resulted in reduced cell viability or cell death [6,10,11,16–20]. Xenograft tumor studies in nude mice have verified that antisense-mediated downregulation of CK2 expression promotes rapid and early loss of CK2 from the nuclear matrix and is associated with induction of apoptosis in both prostate and head and neck squamous cell carcinoma (HNSCC) tumors *in vivo* [2,21–23]. Likewise, numerous small molecule inhibitors of CK2 have been developed over the past 30 years. In cultured human cancer cells, these inhibitors effectively reduce cell viability and cause cell death [10,11,19,20,24–27]. Distinctive features of the CK2 activation and catalytic site suggest that these characteristics of CK2 can be exploited for the design of inhibitors [28,29]. There are four published studies to date using small molecule CK2 inhibitors in animal models of cancer and retinal neovascularization [30–32]. There are also animal and human studies utilizing a novel peptide that impairs CK2 substrate phosphorylation and demonstrates an anti-neoplastic effect in several cancers [33].

Based on the above information, it is clear that CK2 can be targeted using either small molecule inhibitors to affect kinase activity or using antisense and siRNA-mediated molecular downregulation of RNA and protein expression; however, as discussed subsequently, there are positive and negative aspects to both of these approaches. Regardless of whether a pharmacologic or molecular approach is used, a particularly important issue that must be addressed concerns the ubiquitous and essential nature of the CK2 signal. In order to avoid unwanted toxic side-effects in the host, it would be highly advantageous to administer the anti-CK2 drug in a delivery vehicle designed to specifically enter malignant cells while sparing the normal. Currently available delivery methods have certain limitations including *in vivo* protection of the cargo and bioavailability and specific targeting to tumor cells [34]; these limitations are overcome by our novel delivery technology, designated sub-50 nm (i.e., less than 50 nm size) nanocapsules (or s50 nanocapsules). As described subsequently, the s50 nanocapsule is composed entirely of a protein ligand (tenfibgen or TBG) designed to form a shell around the cargo (such as a small molecule inhibitor or condensed antisense or siRNA).

Here we have tested the TBG nanoencapsulated anti-CK2 small molecule inhibitor DMAT (2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole) as well as TBG nanoencapsulated siRNA directed against both catalytic subunits of CK2 α and α' for their effect on the proliferation and viability of malignant prostate cancer cells compared with that of benign prostate cells. We present characterization data for our unique s50 TBG nanocapsule, including size, charge and morphology. We demonstrate the efficacy of CK2 inhibition or molecular downregulation in the cells and the resulting effects on the downstream CK2 target NF-κB p65. We show that TBG nanocapsules carrying anti-CK2 drug specifically target cancer cells and not the normal cells. Significantly, these data also provide proof, by direct comparison of two types of anti-CK2 cargos, that the TBG encapsulation design for delivery of drugs specifically to cancer cells has utility for both small molecule- and nucleic acid–based drugs, thus reinforcing the utility of CK2 as an important anti-cancer target.

2. Materials and methods

2.1. Cell lines and cell culture

PC3-LN4 cells, obtained as described [21], were maintained in monolayer culture containing modified Eagle's MEM or RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and penicillin–streptomycin [35]. Immortalized BPH-1 cells were obtained from Dr. Simon Hayward (Vanderbilt University, Nashville, TN). BPH-1 cells were maintained in monolayer culture containing RPMI 1640 supplemented with 10% heat-inactivated FBS and 2 mM L-glutamine [36]. The human prostate epithelial cells (PrECs) were obtained along with their specific medium from Lonza (Walkersville, MD). C4-2 cells were obtained from MD Anderson Cancer Center and were maintained in monolayer culture containing RPMI 1640 supplemented with 10% heat-inactivated FBS and 2 mM L-glutamine. Cells are grown in an