Evaluation of protein kinase CK2 as a therapeutic target for squamous cell carcinoma of cats

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OBJECTIVE
To investigate protein kinase CK2 (CK2) expression in squamous cell carcinoma (SCC) of cats and to examine effects of CK2 downregulation on in vitro apoptosis and viability in SCC.

SAMPLE
Biopsy specimens of oral mucosa and testis and blood samples from clinically normal cats, biopsy specimens of oral SCC from cats, and feline SCC (SCCF1) and mammary gland carcinoma (K12) cell lines.

PROCEDURES
Immunohistochemical labeling for CK2α was performed on biopsy specimens. Sequences of the CK2α subunit gene and CK2α′ subunit gene in feline blood and feline cancer cell lines were determined by use of PCR and reverse-transcription PCR assays followed by direct Sanger sequencing. Specific small interfering RNAs (siRNAs) were developed for feline CK2α and CK2α′. The SCCF1 cells were treated with siRNA and assessed 72 hours later for CK2α and CK2α′ expression and makers of apoptosis (via western blot analysis) and for viability (via 3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H-tetrazolium assays).

RESULTS
CK2α was expressed in all feline oral mucosa samples and 7 of 8 oral SCC samples. Expression of CK2α and CK2α′ was successfully downregulated in SCCF1 cells by use of siRNAs, which resulted in decreased viability and induction of apoptosis.

CONCLUSIONS AND CLINICAL RELEVANCE
In this study, CK2 appeared to be a promising therapeutic target for SCCs of cats. A possible treatment strategy for SCCs of cats would be RNA interference that targets CK2. (Am J Vet Res 2017;78:946–955)

Protein kinase CK2 is a predominantly serine-threonine protein kinase consisting of 2 catalytic subunits (CK2α and CK2α′) and 2 regulatory β subunits. Protein kinase CK2 is involved in cell growth and proliferation, is essential for cell survival, and is ubiquitously expressed in tissues. However, CK2 does not appear to have notable direct oncogenic potential; rather, it supports other actions responsible for tumorigenesis. Functions of CK2 that promote a protumor environment include activation of the nuclear factor κB, Wnt–β-catenin, and phosphatidylinositide 3-kinase–Akt pathways; inactivation of tumor suppressors such as phosphatase and tensin homolog; and suppression of apoptosis.

Although CK2 is constitutively active and ubiquitously expressed, it appears to be functionally regulated as determined on the basis of subcellular localization. Compared with results for physiologically normal tissues, there is increased expression or nuclear localization (or both) of CK2 protein in many tumors of humans, including prostatic, head and neck, lung, breast, colorectal, and renal tumors; mesothelioma; glioblastoma; and hematopoietic malignancies such as multiple myeloma and leukemia. Such alterations in CK2 expression are commonly associated with a negative prognosis. Such alterations in CK2 expression are commonly associated with a negative prognosis. Functions of CK2 in cell survival, suppression of apoptosis, and support of tumorigenic pathways, along with increased expression in multiple tumors, make it an attractive target for new cancer treatments. Targeting CK2 with RNAi or small molecule inhibitors has potential therapeutic benefit for squamous cell carcinoma of cats.
shown promise in mice with experimentally induced cancers, and such treatments currently are being used in preclinical or early-stage clinical trials. The ubiquity of CK2 expression and its constitutively active nature imply that treatment with inhibitors of CK2 has the potential to adversely affect physiologically normal cells. When compared with results for corresponding physiologically normal cells, cancer cells appear to be relatively sensitive to CK2 inhibition, which implies that there may be a pharmacological window to target CK2 in cancer cells and spare physiologically normal cells. Strategies to improve delivery of anti-CK2 treatments specifically to tumors have also been investigated, such as use of tenfibgen-based nanocapsules, which has resulted in minimal toxicosis in physiologically normal tissues of mice with experimentally induced tumors.

Although CK2 has been evaluated as a therapeutic target in cancers of humans, to the authors’ knowledge, it has not been evaluated in naturally occurring cancer in other species. The purpose of the study reported here was to assess this potential target in SCC of cats. Given the similarities in biological behavior, potential etiology, and molecular alterations between head and neck cancer of humans and oral SCC of cats and the role of CK2 in head and neck cancer of humans, we hypothesized that CK2 would be expressed in oral SCC of cats and that inhibition of CK2 via RNAi would result in decreased viability and increased apoptosis in a feline SCC cell line.

Materials and Methods

Sample

Paired biopsy specimens of oral SCC and visibly normal oral mucosa were obtained from 6 client-owned cats examined at the University of Minnesota Veterinary Medical Center or the University of Tennessee John and Ann Tickle Small Animal Hospital. A blood sample (2 mL) was collected from each of 2 client-owned clinically normal adult domestic short-haired cats examined at the University of Minnesota Veterinary Medical Center. Informed consent of the owners and approval by an institutional animal care and use committee at each university were obtained for use of these tissue specimens and blood samples. Surgically discarded testicular tissue following routine castration of a clinically normal young adult domestic short-haired cat was also obtained for use. A feline SCC cell line (SCCF1) and 2 oral SCC biopsy specimens from cats were obtained. A feline mammary gland carcinoma cell line (K12) was also obtained for use. The SCCF1 cells were maintained in Williams E medium supplemented with 10% fetal bovine serum, 10 mM HEPES, and 1% penicillin-streptomycin. The K12 cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin.

IHC analysis

Tumor and tissue specimens were fixed in neutral-buffered 10% formalin, routinely processed, and embedded in paraffin. Histologic sections were cut at a thickness of 4 μm and stained with H&E stain or prepared for IHC analysis. Formalin-fixed paraffin-embedded sections of tissue were deparaffinized and rehydrated. Antigen retrieval was performed by use of 10mM citrate buffer (pH, 6.0) in a steam device. Endogenous peroxidase was blocked, and a protein block then was applied. The IHC analysis for CK2α was performed with an anti-CK2α rabbit monoclonal primary antibody (dilution, 1:100) on an automated slide stainer. Detection was achieved by use of a commercial detection system with diaminobenzidine as the chromogen. Sections were counterstained with Mayer hematoxylin. Feline testis was used as a positive control tissue because of the high expression of CK2 in testicular tissue of other species. For negative control slides, an appropriate isotype control antibody was used in place of the primary antibody. Images of tissue sections were obtained at 200X and 400X magnification by use of a microscope, camera, and associated software. Images of each section were obtained by use of the same settings for white balance and shading correction. Image sharpness was uniformly adjusted and minor background blemishes were removed with commercial software.

All paired SCC and visibly normal oral mucosa samples were stained in the same assay, and each sample was scored for CK2α expression by 2 veterinary pathologists (MGO and IC). A semiquantitative scoring system (scale, 0 to 4) was used; the score for each sample was assigned on the basis of the number of immunoreactive cells (rare, occasional, moderate, or large numbers), overall intensity (weak, moderate, or strong) of nuclear and cytoplasmic labeling, and intensity of labeling in the most immunopositive and immunonegative regions of the section. Final scores for each sample were arrived at by consensus of the 2 investigators.

The rabbit anti-CK2α monoclonal primary antibody had not previously been validated for use on feline tissues. Therefore, specificity of this antibody against feline CK2α was verified by use of a western blot containing lysates of the SCCF1 cell line.

PCR and RT-PCR assays for gene expression of feline CK2

Adherent SCCF1 and K12 cells were collected by use of 0.25% trypsin-EDTA. Genomic DNA was isolated from cells and from blood of clinically normal cats with a commercial kit used in accordance with the manufacturer’s protocols. On-column DNase digestion and total RNA isolation from cells was performed with a commercial kit used in accordance with the manufacturer’s protocols.

Sequences of CSNK2A1 and CSNK2A2 were identified in the published feline genome by use of a basic local alignment search tool. Two matching sequences for CSNK2A1 were identified on chromosome A3 (reference sequence database No. NC_018725.1). One match was in exon 5 of CSNK2A1 (No. 101084186),
and the other match was in a region annotated as intergenic. Primers were designed to span both of these regions. One sequence for CSNK2A2 was found on chromosome E2 (NC_018737.1) in the region annotated as CSNK2A2 (gene No. 101093065), and primers were designed to span this region. For RT-PCR assays, additional primers were designed on the basis of the predicted mRNA sequences of feline CSNK2A1 (RNA XM_003983674.2) and CSNK2A2 (XM_003998102.1). All primers were obtained from a commercial company (Appendix 1).

The PCR assays were performed by use of a commercial kit (used in accordance with the manufacturer’s protocols) with 100 ng of template DNA in a thermal cycler. Primer sets 1, 2, and 3 were used for PCR assays. The cDNA was synthesized and amplified via RT-PCR assays from 0.4 µg of RNA by use of a commercial kit used in accordance with the manufacturer’s protocols. Primer sets 2, 4, and 5 were used for RT-PCR assays. Samples that contained no template or for which the RT step was omitted were used as negative control samples. An aliquot (5 µL) of the PCR-amplified genomic DNA or cDNA for each sample was loaded onto a 2% agarose gel with ethidium bromide (0.5 µg/mL), and gel electrophoresis was performed. A DNA ladder was used as a reference. Images of gels were obtained by use of an imaging system.

Sequencing of feline CSNK2A1 and CSNK2A2

The PCR-amplified genomic DNA and cDNA were purified by use of a PCR purification filter unit. Direct Sanger sequencing was then performed at the University of Minnesota Biomedical and Genomics Center with the same primers used for the PCR and RT-PCR assays. Sequencing of CSNK2A1 and CSNK2A2 was conducted on 2 feline cancer cell lines of different tissue origins (SCCF1 and K12 cell lines) and blood samples from 2 clinically normal cats to ensure that the siRNAs would be specific for CK2α and CK2α′ in the cell line of interest as well as to screen for mutations that may have occurred in immortalized cells compared to clinically normal cats.

siRNA

Sequences from PCR and RT-PCR products were used to design siRNAs to target regions of interest in feline CSNK2A1 and CSNK2A2 (Appendix 2). Regions of interest were determined on the basis of those targeted successfully with RNAi in human cancer cells. Anti-CK2 and control siRNAs were obtained from a commercial source.

Cell transfection and CK2 knockdown

The SCCF1 cells were plated onto 6-cm cell culture plates for 24 hours and were at approximately 50% confluence at the time of transfection. The SCCF1 cells were transfected with 30nM anti-CK2α siRNA, 30nM anti-CK2α′ siRNA, 30nM control siRNA, or 15nM anti-CK2α siRNA in combination with 15nM anti-CK2α′ siRNA by use of a commercial transfection reagent. Untreated cells and cells treated with transfection reagent alone served as additional control samples. Cells were collected by use of 0.25% trypsin-EDTA at 72 hours after initiation of transfection and frozen at –20°C until further analysis.

Figure 1—Photograph of western blots and results for an antibody that specifically detects feline CK2α in SCCF1 cells (A) and photomicrographs of CK2α expression (positive control sample; B) and lack of expression (negative control sample; C) in testicular tissue obtained from a clinically normal cat. In panel A, actin was included as a loading control sample, and lanes were as follows: 1, untreated; 2 and 3, control siRNA; 4, anti-CK2α siRNA and anti-CK2α′ siRNA; 5, anti-CK2α′ siRNA; and 6, anti-CK2α siRNA. Notice the band of the expected size, except in lanes 4 and 6 for cells treated with a CK2α-specific siRNA. Cells treated with anti-CK2α′ siRNA alone had bands for detection of CK2α but not CK2α′, which indicates specificity of the antibody for the α subunit. In panel B, notice that CK2α is expressed in feline testis and that labeling is notably increased in the actively proliferating cells in the seminiferous tubules (asterisk), compared with labeling in the supporting stromal tissue (dagger). Anti-CK2α rabbit monoclonal primary antibody (panel B) or an isotype appropriate control antibody (panel C); diaminobenzidine and Mayer hematoxylin counterstain; bar = 50 µm.
**Western blot analysis**

Frozen cells were lysed by use of a lysis buffer with protease and phosphatase inhibitors as described elsewhere. Samples were loaded in 10% or 12% tris-glycine SDS-polyacrylamide gels. An aliquot (50 µg) of total protein was loaded for each sample, and a 10-µL ladder was included as a reference. Gels underwent electrophoresis and then were transferred to nitrocellulose membranes, which were blocked by incubation with 5% nonfat dry milk in TBS-T for 30 minutes. Primary antibodies in 5% nonfat dry milk in TBS-T were added, and membranes were incubated overnight at 4°C, washed in TBS-T, and then incubated with secondary antibody in 5% nonfat dry milk in TBS-T for 1 hour.

Expression of CK2 was assessed by use of a combination of rabbit anti-CK2α and rabbit anti-CK2α′ primary antibodies (dilution of each, 1:3,000), and apoptosis was assessed by use of rabbit anti-cleaved caspase 3 and rabbit anti-lamin A/C primary antibodies (dilution of each, 1:1,000). Actin expression as a loading control sample was assessed by use of goat anti-actin (dilution, 1:1,000). Secondary antibodies were goat anti-rabbit (dilution, 1:40,000) or bovine anti-goat (dilution, 1:1,000). Membranes were imaged via autoradiography by use of chemiluminescent substrates, and densitometry was performed by use of open-source image-processing software. Change in CK2α and CK2α′ expression was determined by use of densitometry normalized to actin for treated versus untreated or control-treated cells. Eight western blot analyses were performed to generate at least 3 replicates for each protein and each condition.

**MTS assay**

The SCCF1 cells were plated onto 96-well plates (density, 5,000 cells/well). Cells were allowed to adhere overnight and then were transfected with 30nM anti-CK2α siRNA or a combination of 15nM anti-CK2α siRNA and 15nM anti-CK2α′ siRNA. Control samples were processed as described previously. Each condition was created in triplicate or quadruplicate in each plate. After cultures were incubated for 72 hours, cell viability was assessed by use of an MTS assay, which was used as recommended by the manufacturer. Experiments were repeated 3 times to ensure reproducibility. Viability of siRNA-treated cells was normalized to that for cells treated only with transfection reagent.

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**Figure 2**—Photomicrographs of representative oral SCCs (A and B) and visibly normal oral mucosa (C and D) tissues obtained from cats and used for IHC analysis (scored on a scale of 0 to 4). The oral SCCs have IHC scores of 2 (A) and 3 (B), whereas the normal oral mucosa obtained from the cat in panel A has an IHC score of 1 (C), and the normal oral mucosa obtained from the cat in panel B has an IHC score of 3 (D). Nuclear labeling of CK2α is greater than cytoplasmic labeling in all positive-staining tissues. Anti-CK2α rabbit monoclonal primary antibody followed by diaminobenzidine and Mayer hematoxylin counterstain; bar = 50 µm.
Mean viability for the 3 experiments was determined for each condition and compared among conditions by use of a 2-tailed *t* test. Significance was set at *P* < 0.05.

**Results**

**Western blot validation of IHC monoclonal antibody**

A single band for CK2α protein was detected in...
control lysates, and the CK2α band was not detected after transfection with an siRNA specific for CK2α (Figure 1). Expression of CK2α was detected in feline testis, with labeling most prominent in proliferating cells of the seminiferous tubules, compared with labeling of interstitial cells. No nuclear or cytoplasmic immunoreactivity was seen in the negative control sample.

**CK2α expression in visibly normal oral mucosa and oral SCC**

Expression of CK2α was detected in 7 of 8 oral SCCs and all 6 of the visibly normal oral mucosa samples. There was variability among subjects in IHC scores for both oral SCCs and visibly normal oral mucosa (Figure 2). Expression of CK2α was detected in both nuclear and cytoplasmic compartments in all expression-positive tissues. Nuclear labeling for CK2α was consistently of greater intensity than was cytoplasmic labeling in both tumor and visibly normal mucosa. The CK2α labeling in SCC samples was greater in neoplastic epithelial cells than in tumor stroma. In visibly normal oral mucosa, CK2α labeling in the nuclear compartment was uniform throughout the epithelial layers, whereas cytoplasmic labeling was not apparent in the most superficial epithelial layers. These findings, along with those for feline testis, were consistent with data for humans that indicated CK2 activity is correlated with increased cell proliferation.3,55 When IHC scores were compared for paired oral SCC and visibly normal oral mucosa samples obtained from 6 cats, tumors had higher scores than visibly normal tissues for 3 cats, had the same scores for both tissues for 2 cats, and had a lower score than normal tissue for 1 cat.

**Sequence analysis of CSNK2A1 and CSNK2A2**

The PCR and RT-PCR assays successfully amplified products of the expected size for all primer sets (Figure 3). Sequence analysis of PCR products for CSNK2A1 primers matched the predicted genomic DNA sequences. The RT-PCR assay conducted with the CSNK2A1 primers amplified the same sequence with both primer sets, which also matched the predicted CSNK2A1 mRNA sequence. This indicated that although there were 2 potential genomic matches to the targeted CSNK2A1 sequence, only the predicted CSNK2A1 sequence, and not that on the intergenic region, was present in the transcript pool. The PCR and RT-PCR primers for CSNK2A2 amplified products of the predicted size for CSNK2A2 DNA and RNA.

The DNA and cDNA sequences generated by use of PCR and RT-PCR assays for feline CSNK2A1 and CSNK2A2 were identical between blood from clinically normal cats and SCCF1 cells (Figure 4). This was not unexpected because it appears that altered CK2 expression in cancers of humans is not generally driven by CK2 mutations.35 There were 2 base pair mismatches in the region of interest (that which is successfully targeted with RNAi in human cancer cells) between feline CSNK2A1 and CSNK2A2. In this region of interest, feline CSNK2A2 was an exact match to the corresponding human sequence and feline CSNK2A1 contained 2 base pair mismatches.

**siRNA targeting of CK2α and CK2α′ in SCCF1 cells**

Immunoblot analysis with all antibodies used in the present study revealed bands of the expected size in SCCF1 cells (Figure 5). The anti-cleaved caspase 3 antibody detected an appropriately sized band in cells treated with anti-CK2α siRNA but not in untreated cells. Treatment of SCCF1 cells with siRNAs specific for feline CK2α and CK2α′ resulted in successful down-regulation of the target proteins. Transfection with anti-CK2α siRNA resulted in markedly decreased expression of CK2α with concurrently decreased expression of CK2α′, although the latter was less pronounced. Transfection with anti-CK2α′ siRNA resulted in markedly
specific siRNAs successfully suppressed expression of CK2α and CK2α′ proteins in SCCF1 cells, which resulted in decreased viability and induction of apoptosis. As has been observed for human cancer cells, decreased expression of CK2α with little effect on expression of CK2α′. Downregulation of CK2 subunits was associated with increased cleavage of caspase 3 and lamin A/C, which indicated induction of apoptosis. This was most notable when CK2α expression was lowest. Treatment of SCCF1 cells with anti-CK2α siRNA alone or in combination with anti-CK2α′ siRNA resulted in a significant decrease in viability, compared with results for control samples (Figure 6).

Discussion

Protein kinase CK2 is a highly conserved and ubiquitous protein kinase and, as would be expected, was detected in feline tissues in the study reported here. As has been observed in studies of human cancer, downregulation of CK2 in feline cancer cells resulted in induction of apoptosis and reduced viability. The roles of CK2 in cell survival and suppression of apoptosis along with altered expression and activity in human tumors make it an attractive target for cancer treatment. Inhibition of CK2 via RNAi or small molecule inhibitors in human cancers in vitro and in rodent xenograft tumors results in antitumor effects (reduced viability, induction of apoptosis, and inhibition of tumor growth). Given the clinical similarity between head and neck cancer of humans and oral SCC of cats, positive results for CK2 inhibition in head and neck cancer of humans, and the grave prognosis for cats with oral SCC, we elected to investigate CK2 expression and inhibition in SCC of cats to determine whether CK2 may be a rational target for treatment of SCC in cats. Protein kinase CK2α is frequently expressed in oral SCC of cats, and in 3 of 6 cats in the present study, expression was increased in the tumor, compared with expression in visibly normal oral mucosa. One possible explanation for the fact that CK2α expression was not increased in tumor versus morphologically normal tissue in all cases in the study reported here is that morphologically “normal” tissue may have increased proliferative activity, as has been shown by increased cyclin D1 and Ki67 in a study of oral SCC and adjacent “normal” tissue of humans. Thus, it is reasonable to also expect increased CK2 expression, associated with increased cell proliferation, in such areas. There may also be some intrinsic variability in CK2 expression in oral SCC of cats. Given that CK2 expression may impact response to CK2 inhibition in human cancer cells, confirming baseline tumor expression of CK2 in clinically affected patients prior to treatment is likely to be important. The authors recommend that future clinical studies of anti-CK2 treatments for oral SCC of cats include baseline determination of the amount of CK2 expression in tumor and evaluation of this as a potential determinant of response to treatment.

Specific siRNAs successfully suppressed expression of CK2α and CK2α′ proteins in SCCF1 cells, which resulted in decreased viability and induction of apoptosis. As has been observed for human cancer.
cells, the greatest induction of apoptosis in the present study was seen for cells treated with anti-CK2α siRNA (with or without concurrent treatment with anti-CK2β′ siRNA). Therefore, CK2α may be the most important therapeutic target. However, it has been observed that specific downregulation of one subunit can result in compensatory upregulation of the other subunit, so inhibition of both CK2α and CK2α′ is desirable. In cultured human cells and xenograft tumors, this has been achieved with a single oligonucleotide perfectly matched to human CSNK2A1 and containing 1 mismatch to human CSNK2A2. Feline anti-CK2α siRNA also downregulated CK2α′, but not to the degree seen in human cells. This likely was because there were 2 mismatched residues between the targeted CSNK2A1 and CSNK2A2 sequences in cats, compared with 1 mismatch in humans. For cats, it may be more effective to target both subunits with separate, perfectly matched, oligonucleotides.

The authors acknowledge that CK2 inhibition was evaluated in only 1 feline cell line in the present study. However, considered together with observations for human cancer cells, we believe the results justify further evaluation of CK2-targeted treatments in cats with oral SCC. Expression of CK2 was effectively suppressed in feline cells by the use of specific oligonucleotides, and suppression caused reduced cell viability and apoptosis. Performing the same experiments in the few additional feline SCC cell lines that are currently available would be unlikely to add materially to the data in terms of predicting efficacy or tumor sensitivity in naturally occurring oral SCC of cats. Results of the study reported here established that feline SCC cells can be effectively inhibited by targeting of CK2, and identification of clinical cases that would be more likely or less likely to benefit from CK2-targeted treatments (eg, dependent on the amount of expression of the target in the tumor) would require much more information than can be gleaned from the currently available cell lines. We believe that the data from the present study supported development of RNAi-based targeting of CK2 as a treatment strategy for SCC of cats. Given the broad potential applications of CK2-targeted treatments for multiple tumor types of humans, evaluating CK2 expression and inhibition in other tumors of cats as well as naturally occurring tumors of other species is also warranted.

The study reported here was the first in which CK2 expression in visibly normal tissues and oral SCC of cats and antigrowth effects associated with CK2 inhibition in feline SCC in vitro have been evaluated. Results of this study provide support for future studies conducted to evaluate CK2-targeted treatments for cats with naturally occurring SCC.

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Footnotes

a. Provided by Dr. Tom Rosol, Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.
b. Provided by Dr. Jaime Modiano, Department of Veterinary Clinical Sciences, College of Veterinary Medicine; Department of Laboratory Medicine and Pathology, School of Medicine; and Masonic Cancer Center, University of Minnesota, Minneapolis, Minn.
c. Gibco, Life Technologies, Grand Island, NY.
d. Atlanta Biologicals, Lawrenceville, Ga.
e. Lonza, Basel, Switzerland.
g. Dako, Glostrup, Denmark.
h. Envision+ anti-rabbit-HRP detection system, Dako, Glostrup, Denmark.
j. EB00M microscope, Nikon Instruments, Melville, NY.
k. DSR12 camera, Nikon Instruments, Melville, NY.
l. Elements D, version 4, Nikon Instruments, Melville, NY.
m. Photoshop Elements, version 11, Adobe Systems, San Jose, Calif.

n. RNase QIAeasy blood and tissue kit, Qiagen, Valencia, Calif.
o. RNase QIAeasy mini kit, Qiagen, Valencia, Calif.
q. Integrated DNA Technologies, Coralville, Iowa.
r. Expand high-fidelity PCR system, Qiagen, Valencia, Calif.
s. PTC-100 thermal cycler, BioRad, Hercules, Calif.
t. OneStep RT-PCR kit, Qiagen, Valencia, Calif.
v. Gel Logic system, CareStream, Rochester, NY.
w. Montage, EMD Millipore, Billerica, Mass.
y. siGENOME RISC-free siRNA, Thermo Fisher Scientific, Waltham, Mass.

aa. RIPA lysis and extraction buffer, Thermo Fisher Scientific, Waltham, Mass.
dd. A300-197A, Bethyl Laboratories, Montgomery, Tex.
ee. A300-199A, Bethyl Laboratories, Montgomery, Tex.
ff. 9661, Cell Signaling Technology, Danvers, Mass.
gg. 2032, Cell Signaling Technology, Danvers, Mass.
hh. sc-1616, Santa Cruz Biotechnology, Santa Cruz, Calif.
References


by antisense CK2 in human prostate cancer xenograft model. 


### Appendix 1

Primers used to amplify regions of interest for feline CSNK2A1 and CSNK2A2 by use of PCR and RT-PCR assays.

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<th>Target</th>
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### Appendix 2

Sequences for siRNAs targeting feline CK2α and CK2α′.

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