EUROPEAN JOURNAL OF CANCER 43 (2007) 928–934

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Nuclear localization of protein kinase CK2 catalytic subunit (CK2α) is associated with poor prognostic factors in human prostate cancer ☆

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ARTICLE INFO

Article history:
Received 16 August 2006
Received in revised form
7 November 2006
Accepted 13 November 2006
Available online 30 January 2007

Keywords:
CK2
Nuclear localization
Biomarker
Prognostic factors
Prostate cancer

ABSTRACT

Many genomic abnormalities have been identified in various subsets of prostate cancer, but until now, few genes have been associated with the progression of this cancer. High activity of protein serine/threonine kinase CK2 has been observed in various solid tumours and this alteration has been linked both to growth-related functions and to suppression of cellular apoptosis. Here, we provide the first evidence for a strong association between a nuclear localization of CK2α, evaluated by immunohistochemistry, and poor prognostic factors in a retrospective cohort of 131 human prostate adenocarcinomas. Nuclear CK2α localization is significantly correlated with higher Gleason score, more locally advanced disease (cT3–T4) and more perineural or lymphatic invasion ($p < 0.0019$ to 0.046). In contrast, despite a strong trend, no significant relationship was found between higher initial PSA and nuclear CK2α localization. Thus, this previously undescribed molecular heterogeneity is the first step in defining CK2 as both a potential biomarker and a promising target in human prostate cancer.

1. Introduction

Prostate cancer is a leading cause of illness and death among men in the United States and Western Europe. It represents the most frequent cancer in man up to 50 years of age and the second cause of death due to cancer in western countries. In France, prostate cancer is diagnosed each year in 40,000 men and annually 10,000 deaths are linked to this disease. Despite the use of well established prognostic factors (e.g. Gleason score, TNM classification, Prostate-specific Antigen (PSA) at time of diagnosis) and post-operative pathological stage, some patients will develop PSA relapse or metastatic disease. There is a lack of accurate markers of clinical outcome, particularly for predicting aggressiveness of prostate carcinoma and in defining high risk patients. Improved prognostic markers are therefore needed to distinguish aggressive tumours from more indolent prostate cancer.

Protein kinase CK2 is a highly conserved and ubiquitous serine/threonine kinase. The kinase consists of two catalytic (42-kDa α and 38-kDa α′) subunits associated with a regulatory

☆ This study was supported by grants from the Ligue Nationale Contre le Cancer.
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doi:10.1016/j.ejca.2006.11.021

(28-kDa β) subunit forming heterotetrameric complexes. Disruption of both *Saccharomyces cerevisiae genes* encoding CK2 catalytic subunits is a lethal event,[1] and the demonstration that disruption of the gene encoding the regulatory CK2β subunit in mammals is also lethal[2] reinforces the importance of CK2 in the maintenance of cell viability in normal cell life and during embryogenesis. Recent studies using live-cell fluorescent imaging demonstrated that CK2 can dynamically shuttle to different intracellular compartments suggesting this mechanism as an important means of its functional regulation.[3,4] CK2 activity is elevated in tissues and cells with a high mitotic index, including cancer cells.[5–7] Furthermore, enforced CK2α expression in transgenic mice is sufficient to induce T-cell lymphomas[8] and breast cancer.[9] The consequence of the altered activity of CK2 observed in various solid tumours has so far been linked both to growth-related functions and to suppression of cellular apoptosis, aiding tumour resistance to radiation and anti-cancer drug treatments.[10–12] Complementary evidence shows sensitization of cancer cells to death receptor-mediated cell death in the presence of chemical inhibitors of CK2. For instance, inhibition of CK2 in breast or colon cancer cell lines induces apoptosis mediated by the Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL).[13,14] Moreover, antisense RNA targeting CK2α induces tumour shrinkage in a human prostate cancer xenograft model.[15] Taken together, these results suggest that the importance of CK2 in prostate cancer pathogenesis may relate to its key role in growth control and down regulation of apoptotic pathways.[12]

Due to the lack of suitable CK2 antibodies, immunohistochemical studies aimed to evaluate CK2 expression in human cancers are very few. To determine the global levels of CK2 in prostatic tissues obtained from patients, we performed immunohistochemistry (IHC) with a highly specific antibody directed against CK2α. Herein, we report the first data demonstrating a strong association between CK2α staining pattern assessed by IHC and poor prognostic factors in a retrospective cohort of 131 prostate adenocarcinomas.

2. Patients and methods

2.1. Antibody

The polyclonal anti-CK2α antibody (αCOC) has been obtained by immunization of New Zealand White rabbits against a 13 amino-acid peptide coupled to Keyhole limpet hemocyanin. After sodium sulfate precipitation, antibodies were immuno-affinity purified and stored at a protein concentration of 1.7 mg/ml. For antibody depletion experiments, immuno-affinity purified anti-CK2α antibody was prepared at a 1/250 dilution in Dako diluent (Dako) and incubated overnight at 4 °C with 270 μg of either GST or GST-CK2α immobilized on Glutathione Sepharose beads (Amersham Biosciences). After centrifugation, the supernatants were removed and used for subsequent immunofluorescent or IHC staining.

2.2. Proteins, cell extracts and western blotting

Human recombinant histidine-tagged CK2α was expressed in *Escherichia coli* (BL21) and purified at a final concentration of 4 mg/ml. Cell extracts were obtained from HeLa or murine ES cells cultured in DMEM medium. Cells were washed in PBS and lysed in TDG lysis buffer (50 mM Tris-HCl, pH 7.4, 0,5 M NaCl, 0.5%, Triton X-100, 1 mM 4(2-aminoethyl)-benzenesulfonylfluoride (AEBSF), 25 μg/ml each of leupeptin, aprotinin, 1 mM DTT, 2% glycerol. Then, lysates were centrifugated at 14,000*g* for 15 min. Proteins were resolved by 12% SDS-PAGE and transferred electrophoretically onto a polyvinylidine difluoride membrane (Roche diagnostic). Residual binding sites on the membranes were blocked for 3 h at 22 °C in PBS buffer containing 0.05% Tween 20 (PBS-T) and 5% powdered skim milk, followed by overnight incubation at 4 °C with the αCOC antibody at a 1/1000 dilution in blocking buffer. Membranes were then washed three times with PBS-T and incubated with horseradish peroxidase-labeled anti-rabbit IgG antibody for 1 h at room temperature. The CK2α expression was revealed with the ECL system (Luminol, PerkinElmer) according to the manufacturer's protocols.

2.3. siRNA

Knockdown of CK2α expression was performed with siRNA purchased from Upstate Cell Signaling (CSNK2A1 smart pool). NIH3T3 or MCF-10A cells were cultured on coverslips in 24-well plates and transfected at 30–40% confluence by adding Oligofectamine (Invitrogen) complexed with siRNA (final 22.7 nM). After 72 h, the efficiency of transfection was determined by immunoblot, yielding >60% down-modulation of CK2α expression.

2.4. Immunofluorescence

Control or siRNA-treated NIH 3T3 or MCF-10A cells were fixed for 20 min in 4% paraformaldehyde and permeabilized for 10 min at 22 °C in PBS buffer, 0.5% Triton X-100. Residual binding sites were blocked with 5% foetal calf serum (FCS) in PBS-T for 1 h at 22 °C. Cells were then incubated overnight at 4 °C with primary αCOC antibody in blocking buffer. Samples were washed and incubated with Cy3-labeled rabbit secondary antibody for 1 h at 22 °C in the dark and counterstained with Hoechst 33342. Coverslips were mounted with Vectashield (AbCys). Microscopic analysis was performed on Axiovert® 200 M, using Axiocam® MRm CCD captor and Axiovision® software (Zeiss).

2.5. Immunohistochemistry

Prostate biopsies were fixed in AFA buffer (75% alcohol, 2% formol, 5% acetic acid, 18% pure water) and included in paraffin. Sections of 3 μm were cut with a microtome (Microm®) and mounted on poly-L-lysine-coated slides. After deparaffinization in xylene, the sections were rehydratated in graded alcohols. Endogenous peroxydase was quenched at room temperature in perhydrol buffer containing 2% methanol. Antigen retrieval was obtained by diving slides for 2 min in a 95 °C solution of 0.01 M sodium citrate buffer pH 6.0. Slides were then processed with a semi-automatic revelation system (Coverplate®), using a revelation kit (Histostain Plus®, Zymed) containing a blocking solution, a multi-species second antibody and an enzymatic complex. The primary rabbit

αCOC polyclonal antibody was applied for 1 h at 22 °C at a 1/500 dilution. Detection was accomplished with the AEC Vector® kit (Abcys), following manufacturer's instructions. Identical sections stained in the absence of the primary antibody were used as negative controls. Semiquantitative assessment of antibody staining of the slides was graded using the following score: 0 = no staining; 1+ = weak staining; 2+ = moderate staining; 3+ = strong staining. Nuclear and cytoplasmic stainings were both scored, as well as normal glands (if present), as an internal control. The overall score for each sample represents a consensus of scores by two of us (DP and ML), who were blinded to all clinicopathological variables.

2.6. Patients

Prostate biopsies were performed at the Grenoble University Hospital's Urology Department during the 2003–2004 period. Samples were excluded of the study if there were (i) a lack of material (tumour less than 1 mm or present in just one core), (ii) a diagnosis other than adenocarcinoma (neuroendocrine tumour, benign adenoma, prostate infection). In total, 131 men were identified using database of the Grenoble University Hospital's Anatomopathology Laboratory. For each patient, a single biopsy (the most representative) was analysed. The clinical and pathological information and prognostic factors about prostate cancer samples were collected in the patient database. An additional set of 6 patients with transurethral resection and a diagnosis of benign prostate hypertrophy were assessed for comparison.

2.7. Statistical analysis

The associations between the categorical variables were assessed by means of the χ^2 tests. The 95% CIs for significance were estimated, and a $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Specificity of the anti-CK2α antibody

The specificity of the αCOC antibody has been demonstrated by (*a*) its high titer against human recombinant CK2α (data not shown); (*b*) its ability to recognize human recombinant CK2α by Western blot analysis, (Fig. 1A, first lane), and to detect CK2α as a single 42 kDa protein in cellular extracts of ES or HeLa cells (Fig. 1A, lane 2 and 3, respectively); (*c*) its ability, but not the preimmune immunoglobulin from the same rabbit to detect CK2α by indirect immunofluorescence on fixed cells (Fig. 1B); (*d*) the extinction of the immunofluorescence signal upon incubation of the αCOC antibody with recombinant CK2α (Fig. 1C and D); (*e*) the strong decrease of the immunofluorescence staining in CK2α siRNA-treated cells (Fig. 1E and F); (*f*) the extinction of the IHC staining upon incubation of the αCOC antibody with recombinant CK2α (Fig. 1G and H).

Taken together, these results showed that the αCOC antibody is specific and useful in IHC staining of human prostate tissue.

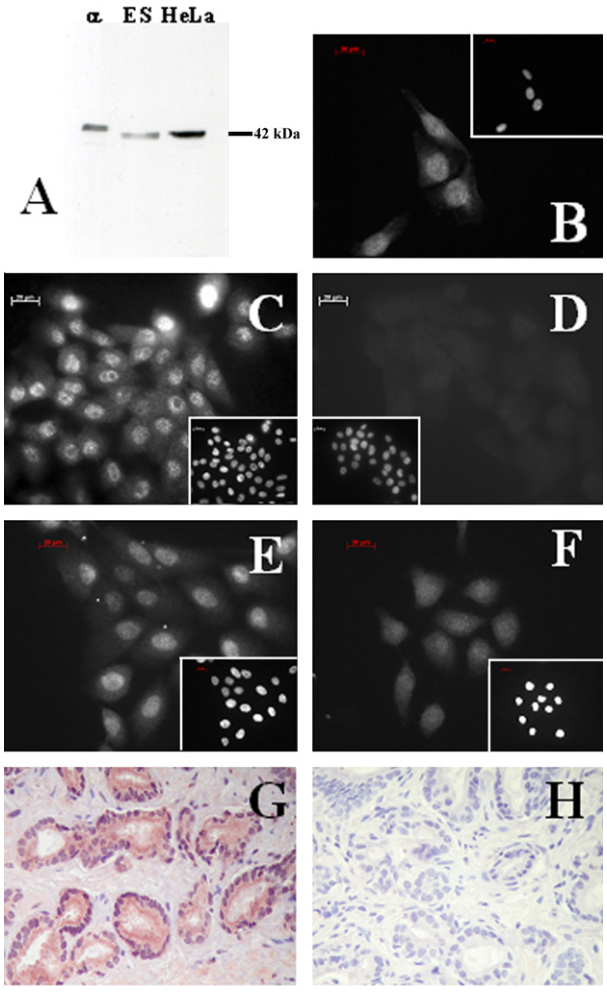


Fig. 1 – Validation of the αCOC antibody. A: Western blot analysis. α: His-tagged human recombinant CK2α; ES: cellular extract of murine embryonic stem cells; HeLa: cellular extract of HeLa cells. αCOC antibody dilution: 1/1000. B, C, D, E, and F: immunofluorescence analysis on cultured cells. Immunostaining of HeLa cells (B); immunostaining of MCF-10A cells with the antibody solution incubated with GST (C) or GST-CK2α coupled beads (D); immunostaining of MCF-10A cells treated with control siRNA (E) or CK2α siRNA (F). αCOC antibody dilution: 1/500. Small insert: nuclear staining with Hoechst. G and H: immunohistochemical analysis on human prostate adenocarcinoma. Immunostaining of a prostate cancer biopsy with the antibody solution incubated with GST (G) or CK2α coupled beads (H). αCOC antibody dilution: 1/500.

3.2. Immunohistochemistry of prostatic glandular epithelium

To take into account the ubiquitous and nucleo-cytoplasmic distribution of CK2α, nuclear and cytoplasmic staining have been both scored, in normal and malignant prostatic cells. Intensity of staining ranges from 0 to 3+, and sum of the two scores represents the total level of CK2α expression. For example, a patient sample with 1+ in the nucleus and 3+ in the cytoplasm has a total of 4+. Different representative patterns are showed in Figs. 2 and 3.

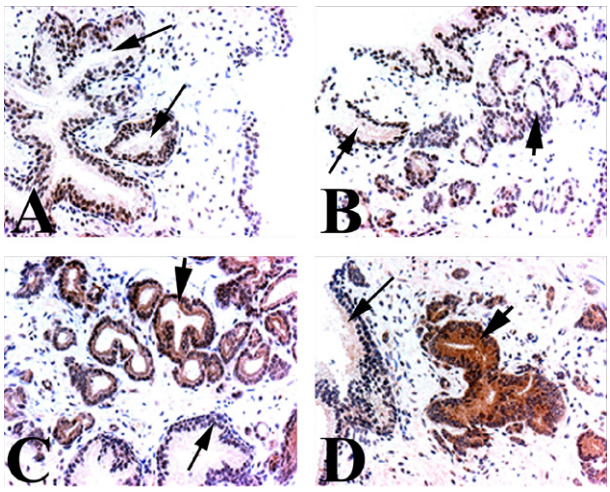


Fig. 2 – Expression of CK2α in normal and tumoral prostate biopsies. (A): normal tissue, staining score: 1+; (B): tumoral tissue, staining score: 1+; (C): tumoral tissue, staining score: 2+; (D): tumoral tissue, staining score: 3+. Magnification × 10. Long and short arrows point normal and tumoral glands, respectively. Note the mainly cytoplasmic staining of CK2α.

3.3. *CK2α is overexpressed in malignant prostate glandular cells*

Patient specimens in this study were obtained at the Grenoble University Hospital during 2003 and 2004. One hundred seventy patients were identified, but 39 have been excluded for reasons described in Materials and Methods. A total of 131 patients (i.e. 131 different biopsies) have been analyzed for CK2α expression and localization. Clinicopathological characteristics of the 131 patients are collected in Table 1. Noteworthy, 111 samples showed normal prostatic glands adjacent to carcinoma. They were used as internal controls, to evaluate expression level and localization of CK2α in normal tissue. CK2α is mainly expressed in the cytoplasm both in normal and in tumour cells. For example, in tumoral glands, mean IHC staining scores are 1.39 (CI95 = 1.26–1.52) and 0.56 (CI95 = 0.43–0.69) in the nucleus and the cytoplasm, respectively ($p < 0.001$). Results are similar in normal glands (data not shown). Moreover, IHC staining of prostatic tissue samples clearly showed that although the level of CK2α differs considerably between individual tissues, the kinase is consistently over-expressed in malignant prostate glandular cells. Mean total IHC staining scores (i.e. nuclear score plus cytoplasm score) are 1.95 (CI95 = 1.76–2.14) and 0.8 (CI95 = 0.65–0.95), in malignant and normal prostate glandular cells respectively ($p < 0.001$). These data support the hypothesis that CK2 is conspicuously overexpressed in prostate cancer.

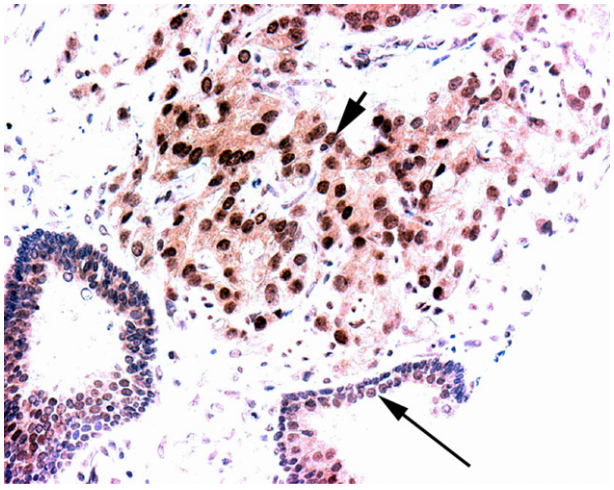


Fig. 3 – Example of nuclear CK2α immunostaining in human prostate adenocarcinoma. Total immunostaining score: 5+ (nucleus = 3+, cytoplasm = 2+). Long and short arrows point to normal and tumoral glands, respectively.

Table 1 – Clinicopathologic characteristics of the 131 Patients with prostate adenocarcinoma

Median age	70 (51–93)
Median PSA (ng/ml) ($n = 104$)	12.3 (1.8–10,400)
Gleason score ($n = 131$) (%)	
⩽6	60 (45.8)
⩾7	71 (54.2)
cTNM staging ($n = 131$) (%)	
cT1c	61 (46)
cT2	43 (33)
cT3–T4	24 (18.6)
cTx	3 (2.4)
M1	8
Lymphatic/perineural invasion ($n = 131$) (%)	
Absent	68 (51.9)
Present	63 (48.1)
Tumoral glands present	131
Normal glands present	111

3.4. *Nuclear localization of CK2α is correlated with high-grade tumours*

To examine whether CK2α expression is associated with a particular disease phenotype, we first determined the relationship between levels of CK2α expression and established prognostic factors (i.e. initial PSA, Gleason score, initial cTNM classification, lymphatic or perineural invasion). Total or cytoplasmic IHC staining scores show no significant correlation with any of these factors (data not shown, see Appendix 1 and 2). However, we noticed the presence of CK2α in the nucleus in a subset of prostate cancers (Fig. 3). Patients were therefore stratified into those with no nuclear staining in tumour cells (group Nuc–, $n = 77$, 58.7%), and those with a nuclear staining score of 1+ or more (group Nuc+, $n = 54$, 41.3%). In this last subset of patients, 38 (70.4%) have a Gleason score of 7 or more, as compared with 33 (42.9%) in group Nuc– (χ^2test, $p = 0.0019$). A positive correlation of nuclear positive target cells with the cT stages has also been identified: in 52 evaluable Nuc+ patients, 23 (44.3%), 14 (26.9%) and 15 (28.8%) have a cT1c, cT2 and cT3–T4 disease respectively. In 76 evaluable Nuc– patients, 38 (50%), 29 (38.1%) and 9 (11.9%) have a cT1c, cT2 and cT3–T4 tumour grade respectively. This difference is statistically significant (χ^2 test, $p = 0.046$). Patients with lymph node invasion or metastasis were under-represented, so there are no statistically significant differences between

Table 2 – Characteristics and prognostic factors in Nuc⁻ and Nuc⁺ patients

	Nuc⁻ (n = 77)	Nuc⁺ (n = 54)	
Median age	69 (52–88)	72 (51–93)	
Median PSA (ng/ml)	8.9 (1.8–1500)	**27.5 (2.5–10400)**	
Gleason score (%)			
⩽6	44 (57.1)	16 (29.6)	(**p = 0.019**)
⩾7	33 (42.9)	**38 (70.4)**	
cTNM staging (%) (Tx excluded)			
cT1c	38 (50)	23 (44.3)	(**p = 0.046**)
cT2	29 (38.1)	14 (26.9)	
cT3–T4	9 (11.9)	**15 (28.8)**	
Lymphatic/perineural invasion (%)			
Absent	46 (59.7)	22 (40.7)	(**p = 0.032**)
Present	31 (40.3)	**32 (59.3)**	

the Nuc– and Nuc+ subgroups for these two items. However, Nuc+ patients have more locally aggressive tumours: 32 (59.3%) have lymphatic or perineural invasion, as compared with 31 Nuc– patients (40.3%, χ^2 test, $p = 0.032$). Thus, increased nuclear staining of CK2α in a subset of prostate cancers strongly correlated with poor prognostic factors. A differential nuclear staining was also observed on the basis of initial PSA. Median and mean initial PSA are 27.5 ng/ml (2.5–10,400) and 466.1 ng/ml (CI95 = 57.8–874.1) in Nuc+ patients and 8.9 ng/ml (1.8–1500) and 57.5 ng/ml (CI95 = 10.8–104.1) in Nuc– patients respectively. Despite a strong trend, these differences are not statistically significant. Characteristics and results are collected in Table 2. Due to lack of sufficient follow-up, survival data have not been generated.

4. Discussion

Elevated CK2 activity in human breast tumour specimens[9] and in head and neck cancers has been reported.[16,17] Interestingly, using global gene expression profiling, the CK2α gene has been identified as a prognostic marker in patients with squamous cell carcinoma of the lung.[18] However, despite these studies, clinical data dealing with the specific expression of CK2 at the protein level are scarce. Consequently, the prognostic value of CK2 remains uncertain. To our knowledge, there are only two published studies using immunohistochemistry for evaluating CK2α expression in human cancers. One concerned a cohort of 10 head and neck cancers[19] and the other investigated CK2α immunostaining in a limited series of prostate tissue samples.[7] With a cohort of 131 prostate tumour specimens, the scope of our study is more consistent. Moreover, the anti-CK2α polyclonal antibody used in our study to evaluate the expression and the localization of CK2α in prostate cancer biopsies shows a strong specificity for CK2α in Western blot as well as in immunofluorescence and IHC analysis. Transrectal prostate biopsies are interesting, because in most of them, normal prostatic glandular epithelium is also present allowing easy comparison between tumour and normal tissue. Globally, IHC staining pattern for CK2α in prostate biopsies shows a nucleo-cytoplasmic distribution, consistent with other findings,[7] and appears highly heterogeneous in different patient samples. In most prostate cancers, CK2α is overexpressed predominantly in the cytoplasm but this cytoplasmic staining does not correlate with respect to the stage of disease or degree of tumour differentiation. By contrast, and interestingly, patients with CK2α nuclear staining (even weak) have high-grade and poorly differentiated tumour (Gleason score ⩾7), more locally aggressive tumour (cT3–4) and more potential capsular involvement (lymphatic or perineural invasion). Initial PSA tends to be higher in Nuc+ patients, but differences are not statistically significant. Collectivelly, our data support the notion that the nuclear localization of CK2α could be an adverse prognostic marker in this pathology. CK2 immunostaining was also assessed in 6 patients with benign prostate hypertrophy (BPH). Although the level of CK2α differs considerably between individual samples, the kinase seems to be slightly overexpressed, mainly in the cytoplasm. In addition, a subset of BHP glandular cells exhibit an increased CK2α nuclear staining (see Fig. 1 in Appendix 3). This nucleo-cytoplasmic staining in a non precancerous disease characterized as a benign cell proliferation, is not surprising since CK2 has long been implicated in cell growth and proliferation,[20] and its nuclear localization appears as a hallmark of proliferating cells.[21,22] CK2 activity has also been found up-regulated in many cancers but its exact role in the biology of malignant transformation remains largely unclear. For this reason, considerable effort has been directed towards elucidation of its mechanism of regulation. An important consideration is the observation that CK2 is localized to many distinct sites and interacts with and phosphorylates a large number of cellular proteins raising the possibility that cells may contain many discrete subpopulations of CK2 subjected to independent local regulation. In addition, data from both the crystal structure and live cell imaging studies are providing strong evidence that the holoenzyme formation is transient.[3,4] Indeed, CK2α and CK2β subunits are not consistently co-expressed to similar levels in different tissues and free populations of each subunit partitioned among signalling complexes by different interacting proteins have been identified.[3,6] It is therefore conceivable that different signals can selectively activate a CK2 sub-fraction to achieve a specific response.

Our study demonstrates for the first time a strong association between CK2α nuclear localization and tumour aggressiveness. The mechanistic basis of CK2α nuclear accumulation is currently unclear but its continuous presence in the nucleus might not only be related to the increased

proliferative capacity of dedifferentiated tumour cells but also to their marked resistance to apoptotic signals. Indeed, a new function of CK2 was identified recently, indicating its key role in suppression of apoptosis.[11] Inhibition or down-regulation of CK2 leads to apoptosis in several cancer cell lines including prostate cancer cells. For instance, CK2 determines TRAIL sensitivity: CK2 activity is low in TRAIL-sensitive cancer cell lines but high in TRAIL-resistant cancer cell lines.[23] It was recently shown that both androgen-insensitive and androgen-sensitive prostate cancer cells are sensitised to TRAIL by chemical inhibition of CK2 whereas over-expression of the CK2α subunit protected prostatic cancer cells from TRAIL-mediated apoptosis.[24] This is consistent with the observations that antisense-mediated growth arrest and induction of apoptosis in prostate cancer cells as well as in an *in vivo* xenograft model of prostate cancer, became apparent at a moderate down-regulation of nuclear-associated CK2α.[15,25] What could be the functional consequence of a nuclear localization of CK2 in dedifferentiated cancer cells? Interesting clues are provided by an elegant study showing that CK2 both phosphorylates and promotes the proteasome-mediated degradation of the promyelocytic leukaemia protein (PML). Importantly, an inverse correlation between CK2 activity and PML protein level could be demonstrated in primary lung cancer specimens.[26] Since PML is a nuclear-matrix-associated protein, a nuclear accumulation of CK2α may be functionally relevant to inactivate the tumour-suppressive functions of PML. In the same vein, CK2 has been recently described as a key regulator of the tumour suppressor NKX3.1 in prostate cancer cells. Interestingly, it has been suggested that phosphorylation by the free CK2α' isoform protects NKX3.1 from degradation.[27] It is conceivable that the nuclear CK2α accumulation described in our study, could be coupled to a diminished free CK2α' expression accounting for the loss of NKX3.1 expression during prostate cancer progression.[27] However, the level of CK2α' expression could not be evaluated in the present study given that our antibody is specific for CK2α.

Since it has been observed that the downregulation of the CK2 signal is equally effective in inducing apoptosis in both androgen-sensitive and –insensitive prostate cancer,[24] targeting CK2 may be particularly effective in treating prostate cancer regardless of the phenotype.[12] Taken together, these data implicate CK2 in prostate cancer progression and our observations provided the first evidence for a strong correlation between CK2α expression pattern and tumour aggressiveness. Although clinical relevance of CK2 localization in human prostate cancer needs to be further investigated, CK2 could be potentially used to predict clinical outcome, especially in clinically localized prostate cancer. In this situation, pretreatment nomograms with clinical (cT), histological (Gleason score) and biological (initial PSA) parameters are widely used, but none of them include biomarkers.[28,29] In other cancers, such biomarkers are now well established, fostering changes in disease's management (e.g. c-erbB2 in breast cancer). Prospective studies are now required to firmly establish CK2 nuclear localization as an independent prognostic factor associated with distinct clinical outcomes and as a useful biomarker to select patients for enrolment in clinical trials. Expended trials in a larger-scale prospective and multicentric study will also allow the evaluation of CK2 status in term of survival, for clinically localized prostate cancer. To the extent that CK2 is a reporter or an integral component of aggressive disease, new therapeutic approaches based on the alteration of its activity or amount could be especially effective.

Conflict of interest statement

None declared.

Acknowledgements

We are grateful to Olivier Blond, Françoise Vigier and Mireille Cuzel for technical assistance. We thank Prs Edmond Chambaz and Jean-Yves Cahn for their critical review and comments and Pr Michel Bolla for his encouragement. ML was supported by grants from the Association pour le Recherche sur le Cancer and the Faculté de Médecine de Grenoble (Eckstein-Gershony). This work was supported by grants from the Ligue Nationale Contre le Cancer (équipe labellisée 2004), the Institut National de la Santé et de la Recherche Médicale, and the Commissariat à l'Energie Atomique.

Appendix 1. Relationship between prognostic factors and total CK2α immunostaining score (0/1+, 2+, 3+ or more)

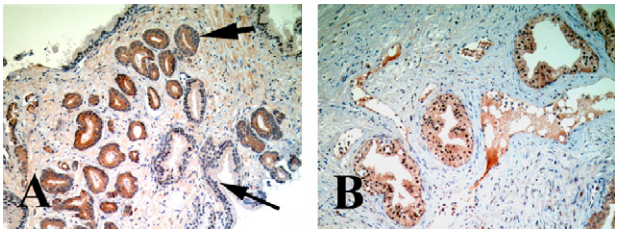
	0/1+ (n = 54)	2+ (n = 40)	3+ (n = 37)	
Gleason score (%)				
$\leqslant$6	33 (61)	15 (37.5)	12 (32.4)	**(0/1+ vs 2+ p = 0.023)**
$\geqslant$7	21 (39)	25 (62.5)	25 (67.6)	(2+ vs 3+ p = 0.64)
				(0/1+ vs 3+ p = 0.0072)
cTNM staging (%) (Tx excluded)				
cT1c	30 (58.8)	17 (42.5)	14 (38.9)	**(0/1+ vs 2+ p = 0.15)**
cT2	17 (33.3)	15 (37.5.9)	11 (30.5)	(2+ vs 3+ p = 0.55)
cT3–T4	4 (7.9)	8 (20)	11 (30.5)	**(0/1+ vs 3+ p = 0.017)**
Lymphatic/perineural invasion (%)				
Absent	34 (62.9)	22 (40.7)	18 (43.2)	**(0/1+ vs 2+ p = 0.083)**
Present	20 (37.1)	32 (59.3)	22 (56.8)	(2+ vs 3+ p = 0.87)
				(0/1+ vs 3+ p = 0.63)

Appendix 2 Relationship between prognostic factors and cytoplasmic CK2α immunostaining score (0/1+ or 2+/3+)

	0/1+(n = 54)	2+/3+(n = 40)	
Gleason score (%)			
⩽6	38 (46.9)	22 (44)	(p = 0.74)
⩾7	43 (53.1)	28 (56)	
cTNM staging (%) (Tx excluded)			
cT1c	39 (51.3)	22 (44)	(p = 0.39)
cT2	26 (34.2)	16 (32)	
cT3–T4	11 (14.5)	12 (24)	
Lymphatic/perineural invasion (%)			
Absent	38 (47)	25 (50)	(p = 0.73)
Present	43 (53)	25 (50)	

Appendix 3

Biopsy and transurethral resection of prostate. A: biopsy showing CK2α immunostaining in normal (long arrow) and tumoral (short arrow) prostatic tissue. B: transurethral resection of a benign prostate hypertrophy showing a nucleocytoplasmic CK2α staining in glandular cells.



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