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Protein kinase CK2α subunit over-expression correlates with metastatic risk in breast carcinomas: Quantitative immunohistochemistry in tissue microarrays

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ABSTRACT

Background: CK2α is a signalling molecule that participates in major events in solid tumour progression. The aim of this study was to evaluate the prognostic significance of the immunohistochemical expression of CK2α in breast carcinomas.

Methods: Quantitative measurements of immunohistochemical expression of 33 biomarkers using high-throughput densitometry, assessed on digitised microscopic tissue microarray images were correlated with clinical outcome in 1000 breast carcinomas using univariate and multivariate analyses.

Results: In univariate analysis, CK2α was a significant prognostic indicator ($p < 0.001$). Moreover, a multivariable model allowed the selection of the best combination of the 33 biomarkers to predict patients' outcome through logistic regression. A nine-marker signature highly predictive of metastatic risk, associating SHARP-2, STAT1, eIF4E, pmap-KAPk-2, pAKT, caveolin, VEGF, FGF-1 and CK2α permitted to classify well 82.32% of patients (specificity 81.59%, sensitivity 92.55%, area under ROC curve 0.939). Importantly, in a node negative subset of patients an even more (86%) clinically relevant association of eleven markers was found predictive of poor outcome.

Conclusion: A strong quantitative CK2α immunohistochemical expression in breast carcinomas is individually a significant indicator of poor prognosis. Moreover, an immunohistochemical signature of 11 markers including CK2α accurately (86%) well classifies node negative patients in good and poor outcome subsets. Our results suggest that CK2α evaluation together with key downstream CK2 targets might be a useful tool to identify patients at high risk of distant metastases and that CK2 can be considered as a relevant target for potential specific therapy.

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1. Introduction

There is a growing consensus that the outcome of patients diagnosed with cancer may be significantly improved by personalised medicine. Thus biomarker discovery from clinically relevant specimens is critically needed to predict response based on underlying molecular biology. Protein kinase-mediated phosphorylation is an important post-translational modification regulating key cellular processes.[1] Tight regulation of phosphorylation events is crucial to the correct function of many cellular signalling pathways and loss of regulation of these pathways underlies many human diseases, including cancer.[1,2]

Protein kinase CK2, formerly referred to as Casein Kinase II, is a multi-subunit enzyme consisting of two catalytic α and α′ subunits associated with a dimer of regulatory β subunits.[3] CK2 is a multifunctional and pleiotropic ser/thr kinase that plays major roles in cell cycle progression, apoptosis, cell differentiation and transcription processes.[1–3] As a signalling protein, CK2 can be targeted to different cellular compartments in response to various stresses such as hypoxia, heat shock, DNA damage.[1–3] In addition, CK2α is overexpressed and its activity is enhanced in multiple forms of human cancers[4] including prostate,[5] endometrium[6] and breast.[7–9] Thus, CK2 is now regarded as a potential target for specific therapy in human malignancies.[9–11] It is also postulated that this enzyme could contribute to breast carcinoma development because an enforced overexpression of CK2α in the mammary gland of transgenic mice promotes hyperplasia and neoplasia of the mammary gland.[7] Moreover, an upregulation of CK2 protein and activity was observed during the development of DMBA-induced mammary tumours, suggesting a pathologic relationship between CK2 expression and mammary tumourigenesis.[7] Primary breast cancer samples from patients as well as breast cancer cell lines also display increased CK2 activity. In addition, a strong CK2α staining, as assessed by immunohistochemistry (IHC) in a small series of patients was observed in prostate tumours compared to normal tissue.[12]

However, up to now, no study has shown that CK2α subunit expression in breast carcinoma, alone or along with other proteins involved in major signalling pathways, could be a prognostic indicator when correlated with patients' outcome.

In the present study our goal was, using in situ high throughput methods, to investigate CK2α expression by a quantitative immunohistochemical analysis on digitised TMA of a large cohort of breast carcinomas ($n = 1000$). This analysis was correlated with overall and metastasis-free patients' survival and along with a panel of 32 other biomarkers.

2. Materials and methods

2.1. Patients

A consecutive series of 1000 patients with invasive breast carcinomas were operated from 1998 to 2000 (mean follow-up, 112 months) at Marseille s' *Centre Hospitalo-Universitaire* in Genecology Oncology Departments. Surgery was in all cases the first treatment. For the first step of initial diagnosis and treatment, patients' management was handled by the same group of surgeons and by three senior pathologists (C. Charpin, S. Garcia and S. Giusiano). Conservative treatment, mastectomy and node resection (complete or sentinel) were applied according to current European recommendations. Likewise, radiotherapy, chemotherapy and hormone therapy were applied according to criteria currently used at that time.

The 2007 follow-up data showed that 206 patients were metastatic and 53 patients deceased.

Analysis of the distribution of the series by age, histological type and grade, and nodal status before TMA construction revealed a usual distribution of breast carcinomas series and no bias in tumour selection, as compared to literature data. Due to technical difficulties in performing immunocytochemical tests on many serial paraffin sections of a TMA to evaluate the 33 different markers, complete data for all markers were finally obtained for 905 patients out of the initial series of 1000.

Our study focused mainly on correlation of quantitative immunohistochemical data with patients' outcome. Current histoprognostic criteria on H&E staining were not retained for statistical analysis for two reasons: (i) mainly to limit the burden of data, and (ii) to focus on the statistical analysis on continuous variables (numerical values of measurements) homogeneously obtained by densitometric measurements of immunoprecipitates with the image analyser.

2.2. Immunohistochemistry

2.2.1. Tissues

Tumour fragments were sampled large and thick enough to allow further TMA construction. Tissue samples were all taken from surgical specimens after formalin fixation. Attention was paid to optimise consistent tissue-handling procedures, including fast immersion in buffered formalin in an appropriate container by pathologists trained in the procedure. Duration of fixation was 24 h for smaller samples (<5 cm) and 48 h for larger ones, to improve formalin penetration, before specimen dissection at room temperature. After fixation, paraffin pre-embedding and embedding were performed in currently available automated devices.

Paraffin blocks prepared from 1998 to 2000 were stored in the same room, where temperature was controlled and maintained at 20 °C prior to TMA construction.

2.2.2. TMA construction

The procedure for construction of TMAs was as previously described.[13–16] Briefly, cores were punched from the selected 1000 contributive paraffin blocks, distributed in four new blocks including two cores for each tumour (500 cases per block, a total of 2000 cores) of 0.6 mm diameter. In order to avoid false positive staining that might result from stromal or inflammatory cells that could react with the antibodies tested, the tumour areas selected for the TMA punches were dense carcinomatous areas with minimal stroma containing some vessels and few fibroblasts.

All the new TMA blocks were stored at 4 °C and 4 μm thick sections were prepared for each marker to be examined by IHC.

2.2.3. *Immunohistochemical procedures*

Immunoperoxidase procedure was performed using an automated Ventana Benchmark XT device and Ventana kits on serial tissue sections that were prepared and stored at 4 °C, 24 h before immunohistochemical processing, as previously reported.[13–17]

Markers used were some of those reported in the literature as involved in tumour cell spreading, angiogenesis, and involved in main signalling pathways (Table 1). Markers were detected using commercially documented antibodies (Table 2). The selection also hinged upon our experience of immunostaining quality in pre-tests using commercially available antibodies on frozen tissue and current full paraffin sections, prior to high-throughput immunodetection on TMAs. Dilutions of antibodies were determined by a pre-screening analysis on the usual full paraffin 4 μm thick sections prior to use on TMA sections.

Specificity of antibodies was documented by the suppliers, and anti CK2α antibody characteristics were previously described along with a screening of prostate carcinomas.[12,17] Antibodies recognising phosphorylated molecules are identified with 'p' sign such as pmTOR or pAKT. In contrast, those simply indicated as STAT1, FYN, FAK, recognise non-phosphorylated molecules (Table 2).

2.2.4. *Image analysis*

Automated densitometric measurements of immunoprecipitates in cores were assessed for each marker antibody in each core individually identified after digitisation and image cropping of the slides, as previously reported.[13,14,16–18] Briefly, TMA analysis with a SAMBA 2050 automated device (SAMBA/TRIBVN, Châtillon 92320, France)[16–18] was performed according to the following protocol. First, an image of the entire slide was built up using low-power magnification (×2). This image was made up of a mosaic of images acquired along a rectangular grid with contiguous fields. Second, the area of the slide containing the TMA cores was automatically delineated and scanned at higher magnification (×20, pixel dimension: 7.4 μm). Third, after autofocusing, the images were acquired with an overlap greater than the largest mechanical positioning error. Using the image contents, a matching algorithm determined precisely the relative position of each image with respect to its neighbours. Calculated overlap was removed from images to produce a new set of higher-magnification images, thus covering accurately the cores of interest. A specially developed tool referred to as TMA crop then allowed superposition of the TMA grid onto the reduced image and precise alignment of each node of the grid with the core location within the image. The final step was performed automatically using the core image contents to ensure pixel precision of the match. From the images acquired with ×20 magnification, a new set of images was next computed, one for each core. For colour analysis of the core images, the SAMBA appropriate software was applied as previously reported[13,14] in usual full tissue sections.

In the present study, we correlated the patients' follow-up parameters with a quantitative score combining the surface stained and the intensity of staining[16–18] computed by the SAMBA 'immuno-software'.

2.3. *Statistical analysis*

Immunohistochemical expression of each marker was first correlated with patients' disease-free survival using NCSS and Statistica statistical softwares.

In univariate analysis, the prognostic significance was determined by log rank tests (Kaplan–Meier curves). The appropriate threshold of prognostic significance for a given marker was determined as previously described[18] and recommended[16,17] for univariate analysis.

Supervised hierarchical clustering of significant prognostic indicators in the series provided qualitative data to be compared with previously reported research results documenting the relationship and the role played by these molecules in the process of cancer metastasis.[1416,17]

Importantly, we used logistic regression and ROC curves to identify the combination of markers (signature) with the best sensitivity and specificity for prognosis prediction. Multivariable fractional polynomials model of logistic regression was used to correlate the outcome variable with the quantitative IHC expression of markers quantitatively evaluated as continuous variables without predetermined cut-points.[19,20]

2.4. *Western blot analysis*

Protein extracts were prepared by homogenising frozen powdered breast tumours in RIPA buffer containing a cocktail of protease and phosphatase inhibitors (50 mM Tris–HCl, pH 7.5, 1% Triton X-100, 150 mM NaCl, 1 mM NaF, 1 mM Na_3VO_4, 10 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin and 1 μg/ml leupeptin), followed by centrifugation at 14,000*g* to remove debris. Western blotting was performed according to conventional protocols. Briefly, samples of tumour extracts (20 μg protein) were separated by SDS–PAGE on 4–12% acrylamide-gradient gel and transferred on nitrocellulose. The membranes were blocked in PBS containing 5% milk and 0.05% Tween-20 and incubated overnight with CK2α subunit specific antibodies (CK2α COC 1/1000). A goat anti-rabbit antibody coupled to horseradish peroxidase was used at a dilution of 1:10,000. Antigens were vis-

Table 1 – Markers and groups of markers determined by logistic regression, evaluated by quantitative IHC in 905 breast carcinomas.

Group of markers	Immunohistochemical markers
Signalling pathways	PI3K, pAKT, pmapkapk-2, pmTOR, P38, MAPKinase, $p4^E$-BP-1, eIF4E, FOXO3a, FAK, JAK, FYN, STAT-1, STAT-3, SHARP-2, P42 MAPKinase
Angiogenesis	HIF-1α, VEGF, CD146, CD34, FGFR-1, CA-IX
Tumour spreading	cMet, caveolin, moesin, β-catenin, MMP7, MMP11, cKit, P-cadherin

Table 2 – Antibodies used in 1000 breast carcinoma TMA, selected according to literature data and our previous study showing the prognostic relevance of corresponding markers (29) (Ventana Benchmark XT automated device, immunoperoxidase).

	Antibodies	Supplier	Source[a]	Clone
1	MMP7	Abcam	Rpab	
2	MMP11	Abcam	Rmab	EP1259Y
3	eIF4E	Cell signalling	Rmab	C46H6
4	P70 S6 kinase	Cell signalling	Rmab	49D7
5	FOXO3a	Cell signalling	Rpab	
6	P 42-MAP-kinase (ERK-2)	Cell signalling	Rpab	
7	Phospho-mTOR (Ser2448)	Cell signalling	Rmab	49F9
8	VEGF	R&D Systems	Mmab	26503
9	Phospho-4^{E}-BP-1(Thr37/46)	Cell signalling	Rmab	236B4
10	HIF-1α	Gift[b]	Mmab	729T3
11	β-Catenin	Novocastra	Mmab	17C2
12	FGFR-1 Flg (C-15)	Santa Cruz	Rpab	
13	Maspin	BD Pharmingen	Mmab	G167-70
14	MET	Chemicon/Abcys	Mmab	4AT44
15	P-Cadherin	Novocastra	Mmab	56C1
16	Ezrin (p81, 80k, cytovillin)	Neomarkers	Mmab	3C12
17	phospho-AKT (Ser473)	Cell Signalling	Rmab	587F11
18	CD 44v6	Novocastra	Mmab	VFF-7
19	**CK2α**	Homemade	Rpab	αCOC
20	Moesin	Neomarkers	Mmab	38/87
21	phospho-STAT-3(Tyr705)	Cell Signalling	Rmab	D3A7
22	phospho-MAPKAPK-2	Cell Signalling	Rmab	(Thr334)
23	STAT-1	Cell Signalling	Mmab	9H2
24	FAK	Cell Signalling	Rpab	
25	P38 MAP-Kinase	Cell Signalling	Rpab	
26	SHARP 2	Abcam	Rpab	
27	FYN	Abcam	Mmab	1S
28	Carbonic anhydrase IX	Abcam	Rpab	
29	CD 117/KIT	Dako	Rpab	
30	PI3 Kinase	Cell Signalling	Rpab	
31	JAK 1	Cell Signalling	Rpab	
32	Caveolin 1	Santa Cruz	Rpab	
33	CD-146	Novocastra	Mmab	N1238

[a] Mmab, mouse monoclonal antibody; Rpab, rabbit polyclonal antibody
[b] Kindly provided by Dr. J. Pouyssegur. CNRS-UMR 6543 (Nice, France).

ualised using enhanced chemiluminescence (Perkin–Elmer) and Fusion FX acquisition systems. Verification of equal loading was done by immunodetection of HSP90 (Stressgen clone13F1 1/1000).

2.5. *CK2 kinase activity*

CK2 kinase assays were performed in a final assay volume of 18 μl containing 3 μl of tumour extract (3 μg protein) and a mixture containing 167 μM of peptide substrate (RRREDEESDDEE), 20 mM $MgCl_2$ and 1 μCi of (γ-^{32}P) ATP (6000 Ci/mmol). The final concentration of ATP was 25 μM. Assays were performed under linear kinetic conditions for 5 min at 22 °C before termination by the addition of 60 μl of 4% trichloroacetic acid.

3. Results

3.1. *Distribution of positive CK2α staining within tumour cells*

Immunohistochemical analysis of individual slides showed that CK2α staining was observed both in cell nucleus and cytoplasm (Fig. 1). Measurements of this pancellular distribution of CK2α were assessed on digitised microscopic images enclosing all spots in the individual slide. After 'cropping' of the images, densitometry was assessed by the analyser in each spot identified using a software specifically developed for immunohistochemically stained sections. The degree and extent of immunostaining were automatically evaluated and used to compute quantitative scores (QS) consequently defined by the analyser software.

3.2. *Univariate analysis*

On the basis of literature data on breast carcinoma biomarkers enrolled in metastatic and angiogenic processes, together with molecules of major signalling pathways of tumour cell machinery, 32 biomarkers were selected (Table 1) and screened along with the expression of CK2α in TMAs containing tumours from disease-free patients and from patients with metastases and recurrent disease.

The first step in assessing prognostic value consisted in comparison of mean quantitative scores in relation to the number of positive and negative patients in the disease-free and diseased categories (Mann–Whitney and chi-squared

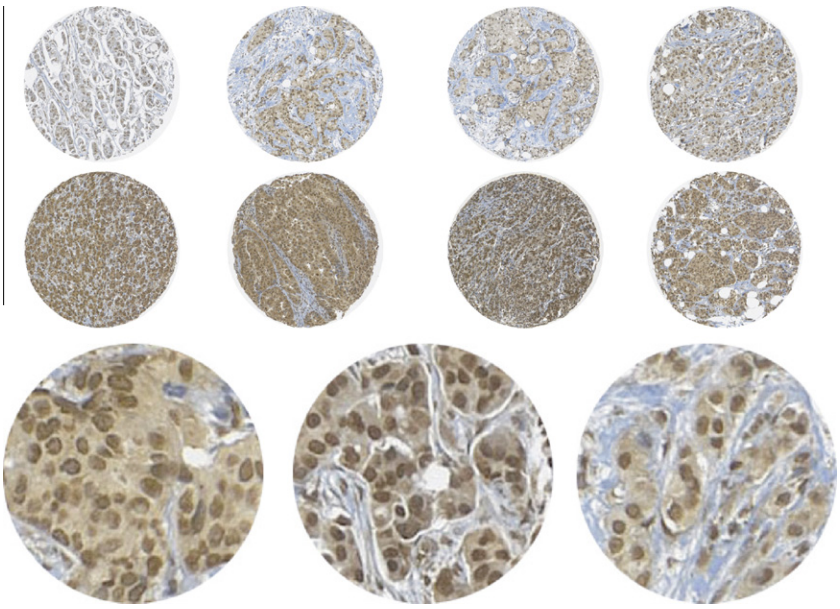

Fig. 1 – TMA cores of node negative breast carcinomas. Immunostaining is predominantly observed in nuclei and to a less extent in cytoplasm. In positive tumours the positive immunoreactivity concerns a variable number of tumour cells and the staining intensity ranges from weak to strong (with various values of mean optical density measured by image analyser).

tests). Mean CK2α quantitative scores in patients with metastases, were significantly greater than those with disease-free survival. The prognostic significance of markers was further individually evaluated by a univariate log rank test (Kaplan–Meier survival curves). There was a significant difference ($p < 0.001$) in median DFS between low and high CK2α-expressing patients (Fig.2 lower panel).

The threshold of staining of prognostic significance was first determined according to the p value curves from univariate analysis (log rank), as reported by Altman et al.[18] and previously used[14–17] as shown for CK2α (Fig. 2 upper panel) ($p < 0.001$).

To validate the IHC analysis, we performed a biochemical analysis of CK2α expression and activity on a selected subset of 20 tumour samples exhibiting a wide range of IHC scores (1–84). Western blotting analysis was done to determine the level of CK2α catalytic protein. As shown in Fig. 3, CK2α expression in tumour samples correlated with the quantitative immunohistochemical scores. Similarly, CK2 kinase activity paralleled with the CK2α expression level, indicating that the increase in CK2 activity might be largely attributable to upregulation of CK2α expression. Thus, this biochemical analysis validated our quantitative immunostaining analysis.

Fig. 4 illustrates in supervised hierarchical clustering the relationship between CK2α and significant prognostic markers in the log rank test. This method separated patients into two groups, illustrating the respective distribution of markers with regard to the metastatic clinical events. Dendograms, established according to the expression of individual markers with regard to favourable or poor outcome patients, show the close relationships between groups of markers, suggesting that they may play similar roles in metastasis development that impact on patients' outcome. Importantly, cells from

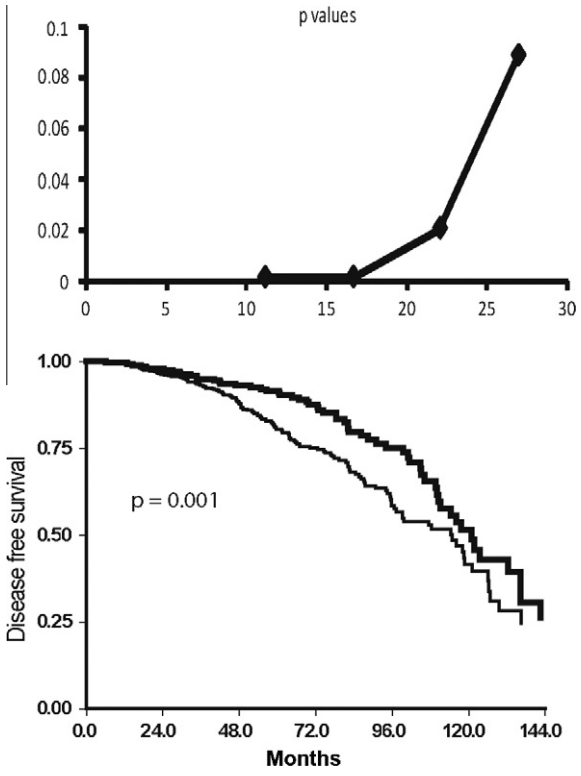

Fig. 2 – (Upper panel) The p value curve indicates the relevance and reliability of the threshold used to compared subsets with low and high CK2α expression. Lower panel on disease-free survival (DFS). Median DFS in patients with high CK2α was x months, compared with y months in patients with low CK2α ($p = 0.001$).

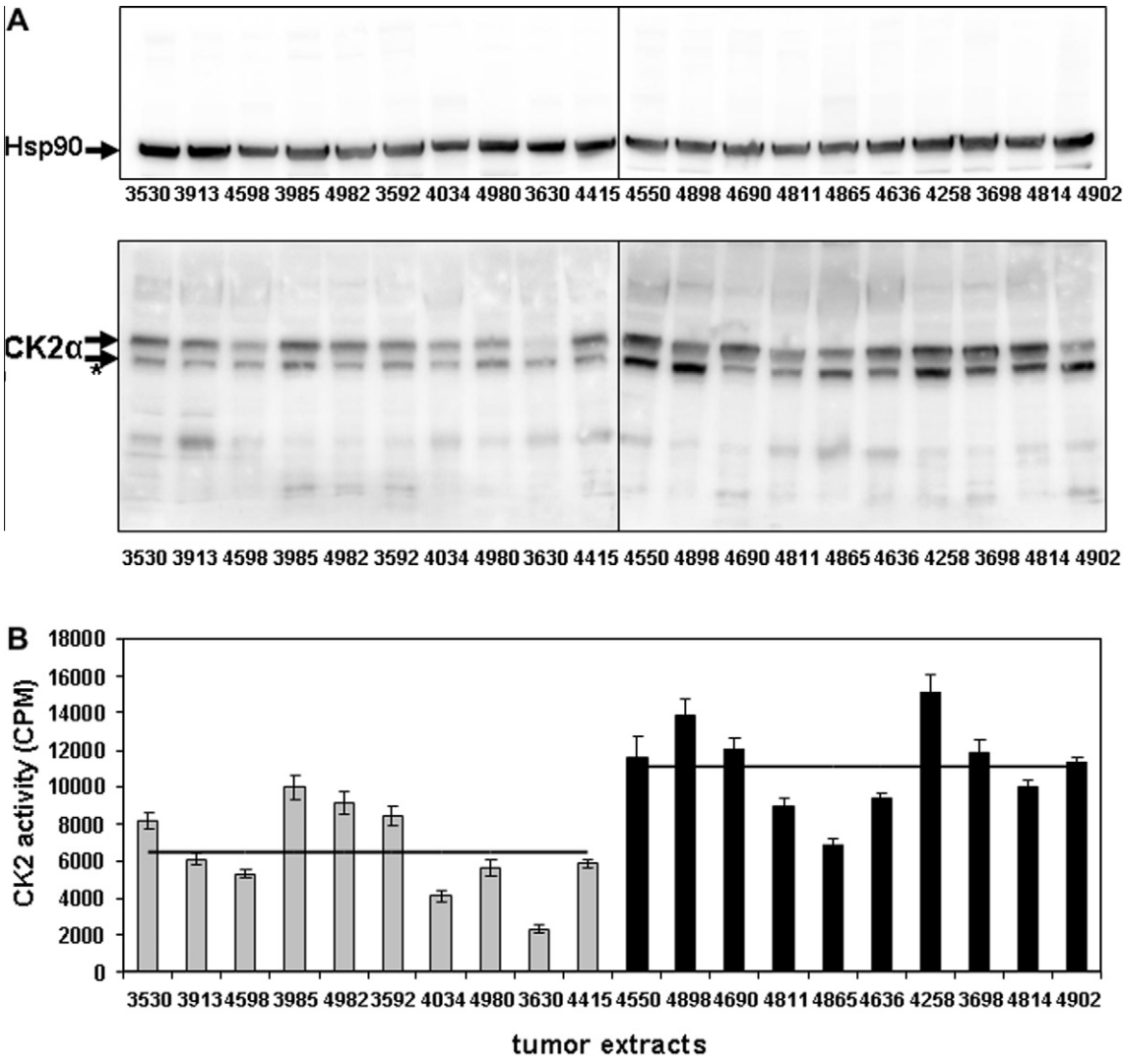

Fig. 3 – CK2α expression and CK2 activity in 20 selected tumour samples. Extracts from 20 selected tumour samples exhibiting low or high IHC scores were analysed for CK2α expression by Western blot and hsp90 was used as a loading control (upper panel). CK2 activity was determined in the same samples that were divided in two groups corresponding to low and high scores (grey and black, respectively). Results are shown as the mean of assays run in triplicate with the error bars representing the standard deviation with a significance of $p < 0.01$.

the patient group with the highest risk of metastasis stained positive for all the markers tested. Thus, this combination of multiple markers strongly correlated with clinicopathological parameters.

3.3. *Multivariate analysis*

3.3.1. *Logistic regression (ROC curves)*

The relationship between groups of predictive markers and the patients' outcome variable was then evaluated by the multivariable fractional polynomials model of logistic regression.[19,20] In this method no cut-point was predetermined for regression and marker immunostaining values were considered as continuous variables.

The optimal combination computerised from the image analysis data bank for the markers in the series of breast carcinomas is shown in Table 2.

When CK2α was associated with markers of main signalling pathways of angiogenesis and of tumour metastatic progression, the multivariable fractional polynomials regression showed that CK2α was included in an optimal combination of nine markers predictive of high metastatic risk (Table 1). The analysis shown in Fig. 5 classified well patients in this category of poor prognosis (lower panel) in 82.32% of the cases (specificity 168/20, 81.5% and sensitivity 577/699, 82.55%, area under ROC/receiving operating characteristic curve = 0.939). This combination included SHARP2, STAT1, eIF4E, pAKT, caveolin, CK2α, pMAPK, VEGF, FGF-1, in which SHARP2 ranked as first, FGF-1 as ninth, and CK2α as sixth.

According to the estimated regression model, the probability of metastasis was computed from quantitative scores obtained after densitometry measurements for each marker, and with the following logit = 0.6156 + 1.9654E–02.Cav + 0.2301E–02.CK2α + 0.04850E–02.eIF4E + 5.9821E–04.FgFR-1 +

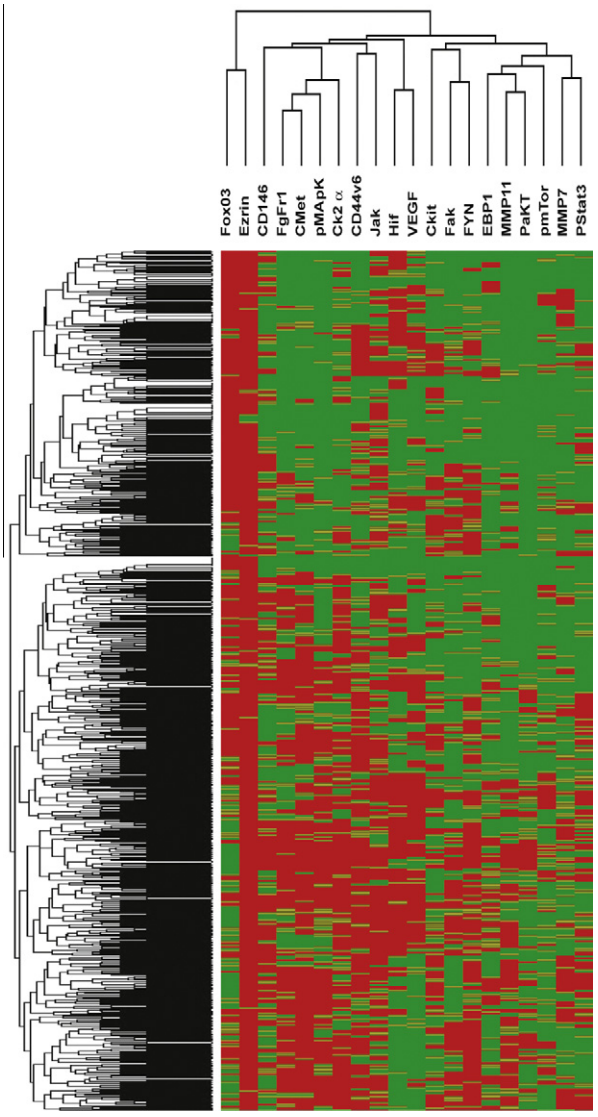

Fig. 4 – Supervised hierarchical clustering of the markers with prognostic significance (metastatic risk) in the log rank test, established with quantitative densitometry of immunohistochemical assays on TMA (*n* = 572 patients with node-negative breast carcinomas) and quantitative score cut-points for each marker, as determined by log rank test. Each column represents a patient. Each row represents a marker staining as indicated on the right side. Green colour represents weak marker staining, red colour represents strong marker staining. This analysis separated patients into two groups and dendrograms show the close or distant relationships of markers acting on metastasis development and on patients' outcome. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

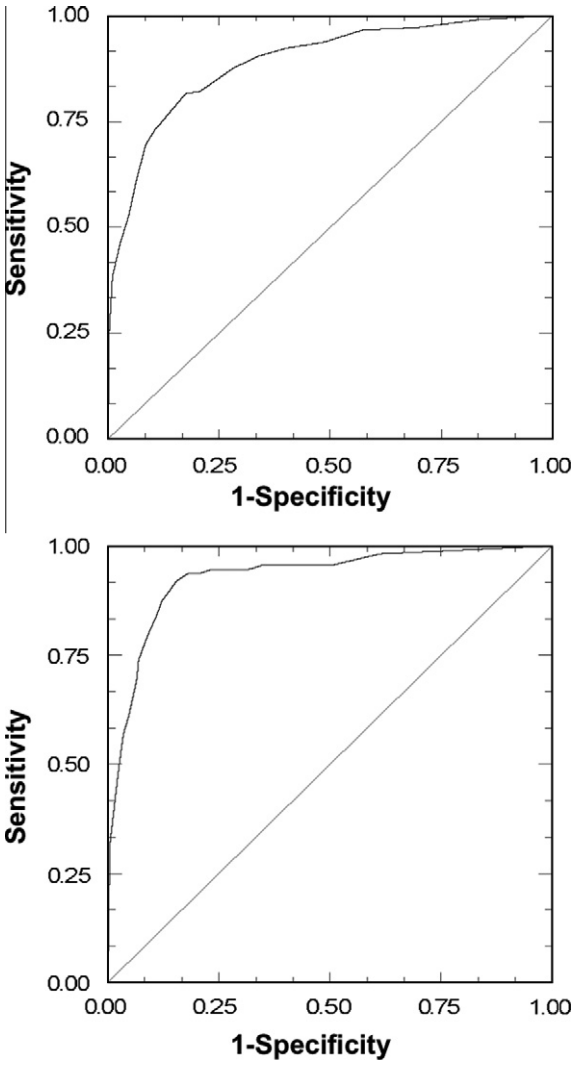

Fig. 5 – Logistic regression and ROC curves determined the immunohistochemical signature (SHARP2, STAT1, eIF4E, pmapkapk-2, pAKT, caveolin, CK2α, VEGF and FGF-1), that correctly classified patients whatever their node status and also interestingly node-negative patients: 82.32% with nine markers (top), and 86.01% with eleven markers (bottom). The larger the surface under the ROC curve, the greater the probability of prediction accuracy.

9.0567E–03.pAKT + 3.1445E-03.pMAPK + 4.59525E–03.SHARP2 + 0.13057E–03.STAT1 + 8.23921E–03.VEGF.

This model estimates 'B' for a specific group (in the present analysis the group of patients with metastases) where logit (*Y*) = *XB* and X is densitometric quantitative score for each marker.

To calculate the probability of classifying in the correct category of outcome, the logit is transformed using Prob = exp(–logit)/1 + exp(–logit)] or Prob = exp(–*XB*)/[1 + exp (–*XB*)].

Interestingly, in the node negative patients' subset (*n* = 572), CK2α was also included in another optimal combination of 11 markers, classifying well patients in 86.01% (sensitivity 102/111, 91.89% and specificity 390/461, 84.6%, area under ROC curve = 0.928). In this combination that contains HIF1-α, SHARP2, eIF4E, pMAPK, MMP7, pAKT, CK2α, FGF-1, pmTOR, Fyn and JAK, HIF1-α ranked as first, CK2α as seventh and JAK as last, with a logit = 3.8297E–02 + 2.2727E–03.CK2α + 6.3130E–03.eIF4E + 7.1550E–03.FgFR1 + 2.2779.FYN + .18505E–02.HIF1-α + .6530–03.JAK + 6.1954E–03.MMP7 + .12559E–02.pakt + 7.5238E–03.pmapk + 24276E–03.pmTOR + 5.9089E–03.SHARP2.

3.3.2. *Validation sets*

Validation sets were established for the 905 series with one-third ($n = 302$) and two-thirds ($n = 603$) of the total number of cases showing similar results, with 80.5% and 83.7% of patients correctly classified, respectively.

For the node negative set, well-classified patients accounted for 83.2% and 84.3% for one-third and two-thirds of the entire 572 patients' set, respectively.

3.4. *Spearman's correlation coefficient*

In an attempt to better document CK2α interactions with other markers in breast carcinoma metastatic process, Spearman's correlation coefficient was computed according to the localisation of markers on dendrograms (Fig. 4) and to literature data.

A significant ($p < 0.001$) correlation was observed between CK2α and PTEN ($\rho = 0.55$), and pAKT ($\rho = 0.53$), whereas Spearman's coefficient was weaker ($p < 0.001$) with PI3K ($\rho = 0.37$), E-cadherin ($\rho = 0.31$), β-catenin ($\rho = 0.26$), and even weaker with HIF-1α ($\rho = 0.13$) and with other markers ($\rho < 0.15$), individually.

4. Discussion

The classification of breast cancers into subgroups on the basis of gene expression patterns is regarded as a method of choice, but widespread use of gene-expression profiling in clinical setting remains limited. Consequently, there is a strong need and interest in using immunohistochemical markers to classify breast cancer into subtypes that are biologically distinct and behave differently. The aim of this study was to evaluate the prognostic significance of CK2α expression in association with various clinicopathological parameters in 905 patients with breast tumours. Elevated CK2 activity in human breast tumour specimens has been reported.[7] However, clinical data dealing with the specific expression of CK2α at the protein level are scarce. Consequently, the prognostic value of CK2α in breast cancer remains uncertain. With a cohort of 905 breast tumour specimens, the scope of our study is consistent. Using high-throughput densitometry of digitised microscopic tissue micro-array images, quantitative measurements of IHC expression of 33 biomarkers were correlated with clinical outcome in univariate analysis and with logistic regression. Our study demonstrates for the first time in breast cancer, a strong association between CK2α over-expression and tumour aggressiveness suggesting that this kinase could be an adverse prognostic marker. Moreover, when CK2α was associated with markers of main signalling pathways, involved in angiogenesis and tumour metastatic progression, a multivariable analysis showed that CK2α ranked favourably in an optimal combination of nine markers predictive of high metastatic risk. Collectively, our data support the notion that high CK2α allows an accurate classification of patients in this category of poor prognosis. Indeed, deregulated expression of CK2 in cells can be oncogenic as transgenic expression of CK2α can promote mammary gland tumourogenesis.[7] However, the mechanistic basis of CK2α deregulation is currently unclear. CK2α plays a key role in promoting cell survival under stress conditions. Therefore, its over-expression in breast cancer cells might not only be related to the increased proliferative capacity of dedifferentiated tumour cells suggesting that CK2 activity is required for ongoing tumour growth, but also to their marked resistance to apoptotic signals. CK2 activity has been implicated in transcriptional regulation, differentiation and development, DNA damage signalling and cell survival.[1,21,22] This broad range of cellular response is orchestrated through CK2-dependent regulation of key signalling molecules in the PI3K/AKT/mTOR, NF_KB, Wnt and hypoxia pathways.

For example, it was demonstrated that primary breast cancer samples from patients or from carcinogen-induced rodent models as well as breast cancer cell lines display increased CK2 activity and aberrant activation of NF_KB which in turn, modulates the survival and the transformed phenotype of breast cancers.[7] Moreover, CK2 also participates in Wnt/β-catenin signalling in mammary epithelial cells.[23]

Our analysis shows a positive relationship between CK2α and a group of markers predictive of patients' outcome, such as SHARP2, STAT1, eIF4E, pAKT, caveolin, pMAPK, VEGF and FGF-1. Several components of the PTEN/PI3K/AKT signalling pathway that are involved in cell survival have been described as causal forces in cancer and breast cancers are particularly reliant on this signalling pathway. Interestingly, CK2 acts as a 'lateral' regulator in the PI3K/AKT/mTOR transduction pathway.[22] For example, the tumour suppressor PTEN is a phosphatidylinositol D3-phosphatase that acts as a negative regulator of this pro-survival pathway. Multiple kinases include CK2 target PTEN providing a negative regulation of its activity and thereby triggering a robust activation of the PI3K/AKT pathway.[24,25] Dephosphorylation and inactivation of AKT is counteracted by CK2, reinforcing the activation of this survival pathway.[26,27] Altogether, these observations are in accordance with the significant correlation that we observed between CK2α, PTEN and pAKT assessed by Spearman correlation analysis.

Activation of signal transducers and activators of transcription (STATs) plays an important role in the maintenance of growth and differentiation. Constitutively activated STATs facilitate neoplastic behaviours of a variety of cancers. It was reported that STAT1 is a downstream target of CK2 and Serine-phosphorylated STAT1 plays a critical role in the pathogenesis of Wilms' tumour and possibly other neoplasms with similar STAT1 phosphorylation patterns.[28]

A potential functional link between CK2 and translational initiation/repression has been described. CK2 phosphorylates the proline-rich homeodomain protein (PRH/Hex) that regulates mRNA transport by binding to translational initiation factor eIF4E.[29]

HIF-1α and VEGF are mechanistically linked to intratumour hypoxia and play important role in angiogenesis and tumour progression.[30] Indeed, it has been observed that CK2 activity is increased in hypoxic conditions and participates to the upregulation of HIF-1α transactivation activity.[31] Since intratumour hypoxia increases the tumourigenicity of cancer cells by selecting more aggressive and metastatic clones, over-expression of CK2 in breast cancer could participate in the molecular circuits that propagate the cancer phenotype through the HIF-1α/VEGF interplay.

Altogether, high expression level of CK2 in breast cancer may generate a favourable cellular environment for the progression of a tumour towards an aggressive phenotype.

Importantly, a recent study has identified the CK2α gene as one of the 186 genes forming an 'invasiveness gene signature' predictive of metastasis and poor survival in breast cancer.[32] Many individual molecular prognostic factors have been identified in patients with primary breast cancer, but few have individually played a significant role in disease management, whereas a multiple marker approach identifying active signalling pathways has begun to guide the prediction of prognosis or treatment response.

In accordance, our data support the notion that CK2α has a clear biological relevance for the prognosis in node-negative breast carcinoma. The incorporation of CK2α into prognostic tools currently used in clinical practice might be useful to reliably identify patients at high-risk of disease recurrence and distant metastases development.

Conflict of interest statement

None declared.

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