Activation of nuclear factor- κ B is linked to resistance to neoadjuvant chemotherapy in breast cancer patients

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Abstract

The nuclear factor (NF)- κ B system is a promising anticancer target due to its role in oncogenesis and chemoresistance in preclinical models. To provide evidence in a clinical setting on the role of NF- κ B in breast cancer, we aimed to study the value of basal NF- κ B/p65 in predicting resistance to neoadjuvant chemotherapy, and to characterise the pharmacodynamic changes in NF- κ B/p65 expression following chemotherapy in patients with locally advanced breast cancer. Pre- and post-chemotherapy tumour specimens from 51 breast cancer patients treated with anthracycline- and/or taxane-containing neoadjuvant chemotherapy were assayed by immunohistochemistry for NF- κ B/p65 subcellular expression. We studied NF- κ B/p65, a well-characterised member of the NF- κ B family that undergoes nuclear translocation when NF- κ B is activated. Activation of NF- κ B (i.e. nuclear NF- κ B/p65 staining in pre-therapy specimens) was linked to chemoresistance. Patients with NF- κ B/p65 nuclear staining in pre-treatment samples had a 20% clinical response rate, while patients with undetected nuclear staining had a 91% response rate to chemotherapy ($P = 0.002$). Notably, four patients achieved a complete histological response and none of them had pre-treatment NF- κ B/p65 nuclear staining. Moreover, the number of patients with NF- κ B/p65 activation increased after chemotherapy exposure. It is concluded that NF- κ B/p65 activation assayed by immunohistochemistry is a predictive factor of resistance to neoadjuvant chemotherapy in breast cancer patients. Moreover, NF- κ B activation was inducible following chemotherapy in a proportion of breast cancer patients. These novel clinical findings strengthen the rationale for the use of NF- κ B inhibitors to prevent or overcome chemoresistance in breast cancer.

Endocrine-Related Cancer (2006) 13 607–616

Introduction

Breast cancer is by far the most frequent cancer in women in the United States and Europe, as well as the second leading cause of death from cancer in women (Greenlee *et al.* 2000). Systemic treatment has long been known to be an essential step to cure or increased survival in patients with breast cancer. However, resistance or recurrence after chemotherapy is frequent. Therefore, understanding

the mechanisms leading to chemoresistance and the development of new drugs and strategies to improve response is a high priority.

Neoadjuvant chemotherapy (also known as pre-operative or primary systemic chemotherapy) has been evaluated in a number of clinical trials and is currently being used in the management of patients with locally advanced breast cancer. To date, anthracycline and/or taxane chemotherapy-based regimens are known to be the most active (Fisher *et al.* 1998,

Smith *et al.* 2002, Bear *et al.* 2003). One of the significant advantages of preoperative chemotherapy is the potential to offer breast conservation to patients who otherwise would need a mastectomy (Bonadonna & Valagussa 1996). However, high interest in neoadjuvant treatment has been further stimulated by the possibility it offers to study the sensitivity of the primary tumour to chemotherapy. In this sense, various studies significantly correlate the clinical and especially the pathological response after neoadjuvant treatment with long-term outcome (Gonzalez-Angulo *et al.* 2005).

The nuclear factor (NF)- κ B family is composed of closely related transcription factors, of which p65 (RelA) is the best characterised. In quiescent cells, the members of the NF- κ B family form heterodimers that are located in the cytoplasm and are rendered inactive by binding to specific inhibitory molecules, the I- κ B proteins (inhibitor-of-NF- κ B) (Ghosh 1999, Li & Karin 2000). Under external stimuli, I- κ B proteins are degraded via the ubiquitin-proteasome pathway, leading to release of the active form of NF- κ B that translocates to the nucleus, where it regulates the expression of genes involved in cell proliferation, survival, adhesion and angiogenesis (Karin *et al.* 2002, 2004). Pre-clinical data support a role for NF- κ B in the development and progression of human malignancies including breast cancer, with special emphasis in HER2 overexpressing tumours (Zhou *et al.* 2000, Pianetti *et al.* 2001, Karin *et al.* 2002, Biswas *et al.* 2004). There is also increasing evidence that NF- κ B plays a role in the development of chemoresistance. In this sense, preclinical studies have shown activation of the NF- κ B pathway by different chemotherapy agents, including anthracyclines and taxanes (Das & White 1997, Bottero *et al.* 2001, Ho *et al.* 2005). This effect appears to be mediated by the activation of anti-apoptotic genes by NF- κ B (Wang *et al.* 1996, 1999, Karin *et al.* 2002). Intense efforts in the development of NF- κ B inhibitors for cancer treatment justify the need for further characterisation and understanding of the NF- κ B pathway in human cancer specimens.

In the present study, we characterised NF- κ B in biopsies from breast cancer patients before and after anthracycline- and/or taxane-based neoadjuvant chemotherapy. We used NF- κ B/p65 nuclear translocation as a surrogate measure of NF- κ B activation, as we and others have recently reported (Biswas *et al.* 2004, Ross *et al.* 2004, Domingo-Domenech *et al.* 2005). The aims of this study were to assess in a clinical setting (a) whether

pre-chemotherapy NF- κ B activation was a predictive factor to resistance to chemotherapy and (b) whether the NF- κ B subcellular pattern of expression was modified by chemotherapy.

Materials and methods

Patients and tissue specimens

We selected a series of 51 patients with locally advanced breast cancer who had been treated with neoadjuvant chemotherapy at the Hospital del Mar. From each patient, we retrieved the tissue blocks that were available from (a) the pre-chemotherapy diagnostic core needle biopsy and (b) the post-chemotherapy surgery specimen (either lumpectomy or mastectomy). This study was performed according to Institutional Guidelines.

Of the 51 patients, 40 had both a pre- and a post-chemotherapy specimen available. Four patients had only the pre-chemotherapy sample and seven had only the post-chemotherapy specimen available. Thus, a pre-chemotherapy sample was available in 44 patients and a post-chemotherapy specimen was available in 47 patients (a total of 91 samples were analysed). Of the 44 available pre-treatment samples, seven non-representative (e.g. damaged or absence of tumour tissue) were excluded from the analysis, leaving a total of 37 pre-therapy specimens, as well as a total of 37 patients with pre- and post-chemotherapy specimens. As for the post-treatment samples, the NF- κ B/p65 tumour immunostaining could not be assessed in 4 patients with pathological complete response, leaving 43 post-therapy specimens.

Pre-treatment tumour specimens were histologically evaluated. As a part of the established routine protocol at Hospital del Mar, oestrogen receptor (ER) was determined by immunohistochemistry (c6F11, DakoCytomation Corp., Carpinteria, CA, USA) using autoclave treatment for antigen retrieval and Envision (DakoCytomation Corp.) for final visualisation. HER2 overexpression was evaluated by immunohistochemistry (Herceptest, Dako), and HER2 amplification was determined by fluorescence *in situ* hybridisation (FISH) using the Pathvision HER2 DNA FISH kit (Vysis Inc, Downers Grove, IL, USA) (Salido *et al.* 2002).

Clinical data and follow-up were obtained from review of the patients' medical records. Pre-treatment patient staging was classified using the American Joint Committee on Cancer (AJCC) staging system for breast cancer (Singletary *et al.* 2002). Clinical

tumour response to primary chemotherapy was evaluated according to the International Union Against Cancer Criteria (Hayward *et al.* 1977). A clinical complete response (cCR) was defined as the disappearance of all detectable malignant disease within the breast by physical examination. A reduction greater than 50% in the product of the two maximum perpendicular diameters of the tumour was classified as clinical partial response (cPR). Clinical progressive disease (cPD) was considered as an increase of at least 25%. Clinical stable disease (cSD) was defined when clinical breast cancer response did not meet the criteria for cCR, cPR or cPD.

Post-chemotherapy specimens were evaluated for pathological response. We used the terms 'pathological response' and 'pathological complete response' to define a change in histopathology after chemotherapy in breast cancer patients (Fisher *et al.* 1998, Smith *et al.* 2002, Bear *et al.* 2003, Gonzalez-Angulo *et al.* 2005). A pathological complete response (pCR) was defined as no histological evidence of invasive disease in the tumour specimen (Fisher *et al.* 1998).

Immunohistochemistry for NF- κ B/p65

Immunohistochemistry was performed at Hospital Clinic according to the methodology previously reported by our group (Domingo-Domenech *et al.* 2005). In brief, formalin-fixed, paraffin-embedded tissue (4 μ m slides) were obtained for immunohistochemical study. Immunohistochemistry was performed on an automated immunostainer (Dako-autostainer; Dakocytomation, Glostrup, Denmark) according to the company's protocols. After deparaffinisation in xylene and graded alcohols, endogenous peroxidase was blocked with 0.03% hydrogen peroxide for 5 min. Slides were then washed with Tris-buffered saline solution containing 0.1% Tween 20 at pH 7.6 and incubated with the primary antibody using an IgG1 class rabbit polyclonal antibody against the carboxy-terminus of the p65/RelA component of the NF- κ B complex (sc-372, C-20; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at a dilution of 1:600, and developed using the Envision signal detection system (DakoCytomation Corp.). Sections were then treated with 3,3'-diaminobenzidine as chromogen for 5 min and counterstained with haematoxylin. Slides were washed in tap water, dehydrated, and mounted with glass coverslips. To confirm the specificity of the primary antibody, immunoreactivity was

blocked by preabsorption of the primary antibody with an excess amount of p65 antigen peptide (sc-372P; Santa Cruz Biotechnology Inc.).

NF- κ B/p65 immunohistochemistry scoring method

NF- κ B/p65 immunohistochemistry was scored independently by two pathologists (BF, JMC) blinded with regard to the clinical–pathological characteristics of the patients. As previously described (Ross *et al.* 2004), a semiquantitative score that considered both NF- κ B/p65 staining intensity and distribution was used to evaluate the cytoplasmic expression. Distribution of NF- κ B/p65 immunostaining was graded as focal ($\leq 10\%$), regional (11–50%) or diffuse ($> 50\%$). Intensity of NF- κ B/p65 staining was evaluated as weak, moderate or intense on a subjective basis. Cytoplasmic NF- κ B/p65 was considered positive when the sample presented intense diffuse, moderate diffuse or intense regional staining. Since NF- κ B activation results in nuclear translocation, nuclear NF- κ B/p65 expression in tumour cells from infiltrating carcinoma was considered as an active state of NF- κ B (Ghosh & Karin 2002, Biswas *et al.* 2004). As a positive internal control, we used NF- κ B/p65 expression in lymphocytes and endothelial cells of the tissue sample (Sen & Baltimore 1986). Concordance between the two evaluations was greater than 85%. A third independent observer (CC) reviewed the discordant cases.

Statistical analysis

Fisher's exact test was used to assess the associations between cytoplasmic/nuclear NF- κ B/p65 expression and other dichotomous clinical–pathological variables, as well as response to therapy. Fisher's exact test was also used to assess the correlation between clinical–pathological variables and response to treatment. Multivariate analysis including binary parameters was performed using the logistic regression model. All the statistical tests were conducted at the two-sided 0.05 level of significance. Statistical analysis was performed with the SPSS Statistical Software, 12.0 version (SPSS, Inc, Chicago, IL, USA).

Results

Patients' characteristics

Fifty-one patients with locally advanced breast cancer previously treated with neoadjuvant chemotherapy

were included in the study. The mean time from the diagnostic biopsy to the beginning of chemotherapy was 22.5 days (range 1–57 days). During that period of time, patients underwent standard clinical and radiological tumour staging. Patients received a median of 4 cycles of chemotherapy (range 2–6 cycles). Forty-six patients (90%) received an anthracycline-containing, 8 patients (16%) a taxane-containing and 3 patients (6%) both an anthracycline- and taxane-containing chemotherapy schedule. The overall clinical response rate (RR) (cCR and cPR) was 75%. After recovering from the effects of chemotherapy, the patients underwent surgery. The mean time between the last dose of chemotherapy and acquisition of the post-chemotherapy specimen from surgery was 31.8 days (range 7–81 days). Four patients (8%) achieved a pathological complete response in the surgery specimen according to histopathological evaluation (Table 1).

NF-κB/p65 subcellular expression and correlation with clinical–pathological parameters in pre-treatment specimens

In pre-treatment specimens, no significant correlation was found between cytoplasmic or nuclear NF-κB/p65 expression and clinical–pathological characteristics of the patients, including tumour size (T1–T2 vs T3–T4), nodal status (positive versus negative), histological type (ductal versus lobular), nuclear tumour grade (1 vs 2 or 3), and ER and HER2 status (data not shown). Eighty per cent of the pre-treatment specimens with nuclear NF-κB/p65 staining had HER2 amplification, while only 31% of the patients with negative nuclear NF-κB/p65 staining showed HER2 amplification, although this difference was not statistically significant ($P = 0.057$).

Pre-chemotherapy NF-κB/p65 subcellular expression and response to chemotherapy in breast cancer patients

There was no association between cytoplasmic NF-κB/p65 expression in pre-treatment samples and response to chemotherapy (Table 2). In contrast, NF-κB/p65 nuclear staining was significantly associated with resistance to neoadjuvant chemotherapy. Only one patient with NF-κB/p65 nuclear staining had a clinical response to chemotherapy (20% RR). However, the clinical response rate in patients with undetected nuclear staining before treatment was 91% ($P = 0.002$) (Table 2). Notably, of the

Table 1 Patients' clinical–pathological characteristics.

Characteristic	No. of patients	%
Total number of patients	51	
Total number of tumour specimens	91	
Pre-treatment	44 (37 analysed)	
Post-treatment	47 (43 analysed)	
Age (years)		
Mean	59	
Range	30–77	
Tumour size		
T1	1	2
T2	9	17
T3	8	16
T4	33	65
Axillary nodal status		
Negative	28	55
Positive	23	45
Nuclear tumour grade		
1	3	7
2	25	58
3	15	35
Not documented	8	
ER status		
Negative	15	31
Positive	34	69
Not available	2	
HER2 status (IHC)		
Negative	24	52
Positive	22	48
Not documented	5	
HER2 status (FISH)		
Non-amplified	32	64
Amplified	18	36
Not documented	1	
Neoadjuvant chemotherapy regimen		
Anthracycline-containing	46	89
Taxane-containing	8	16
Taxane- and anthracycline-containing	3	6
Clinical response		
Complete response (cCR)	8	16
Partial response (cPR)	30	59
Stable disease (cSD)	12	23
Non-measurable	1	2
Pathological complete response (pCR)	4	8

IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridisation.

4 patients who achieved a complete pathological response, none had NF-κB/p65 nuclear staining before chemotherapy.

The correlation between basal clinical–pathological parameters and clinical response to therapy was evaluated. Patients with small tumour size (T1–T2) were more likely to respond to treatment,

Table 2 Subcellular NF- κ B/p65 expression and clinical response to chemotherapy in specimens from locally advanced breast cancer patients before and after treatment.

	Nuclear NF- κ B/p65		Cytoplasmic NF- κ B/p65	
	Presence (activated) ^a	Absence (non activated) ^b	Positive ^c	Negative ^d
Pre-chemotherapy				
specimens (<i>n</i> = 37)	(<i>n</i> = 5)	(<i>n</i> = 32)	(<i>n</i> = 17)	(<i>n</i> = 20)
Clinical response	1	29	15	15
No clinical response	4	3	2	5
Overall response rate	20%	91%	88%	75%
<i>P</i> -value		0.002		0.416
Post-chemotherapy				
specimens (<i>n</i> = 42)*	(<i>n</i> = 9)	(<i>n</i> = 33)	(<i>n</i> = 19)	(<i>n</i> = 23)
Clinical response	4	28	16	16
No clinical response	5	5	3	7
Overall response rate	44%	85%	84%	69%
<i>P</i> -value		0.02		0.305

*One patient lacked clinical response data and was excluded from response calculations.

^aPre-chemotherapy, *n* = 5; post-chemotherapy, *n* = 9. ^bPre-chemotherapy, *n* = 32; post-chemotherapy, *n* = 33. ^cPre-chemotherapy, *n* = 17; post-chemotherapy, *n* = 19. ^dPre-chemotherapy, *n* = 20; post-chemotherapy, *n* = 23.

although this correlation did not reach statistical significance ($P = 0.09$). Clinical nodal status, nuclear tumour grade, ER or HER2 status were not linked to response to chemotherapy. The clinical response to treatment did not differ among the chemotherapy regimens used (Table 3).

When analysing specifically the subset of patients (*n* = 46) treated with an anthracycline-containing regimen, the clinical response was also significantly

lower in patients with NF- κ B/p65 nuclear staining before or after treatment ($P = 0.002$). In the subset of patients treated with taxanes (*n* = 8) the clinical response rate was 100% in those with positive nuclear NF- κ B/p65 staining and 86% in those with negative nuclear NF- κ B/p65 staining ($P = 1$).

In a multivariate analysis designed to assess the possible independent predictive value of NF- κ B/p65 staining to response to chemotherapy, we

Table 3 Clinical–pathological characteristics and clinical response to neoadjuvant chemotherapy in patients with locally advanced breast cancer.

	Response rate (%)	<i>P</i> -value
Tumour size		
T1–T2	100	0.09
T3–T4	70	
Axillary nodal status		
Negative	74	1
Positive	78	
Nuclear tumour grade		
1	100	1
2 or 3	75	
ER status		
Negative	73	1
Positive	76	
HER2 status (FISH)		
Non-amplified	66	0.28
Amplified	84	
Neoadjuvant chemotherapy regimen		
Anthracycline-containing	75	1
Non-anthracycline-containing	80	

FISH, fluorescence *in situ* hybridisation.

Figure 1 NF- κ B immunostaining in breast cancer with the anti-p65 antibody (C-20, sc-372), dilution 1:600, magnification $\times 400$. (A) Breast cancer specimen from a newly diagnosed patient before receiving chemotherapy, with cytoplasmic but no nuclear NF- κ B staining in tumour cells. (B) Breast cancer specimen from the same patient after receiving anthracycline-based neoadjuvant therapy. Nuclear NF- κ B immunoreactivity was detected in tumour cells.

adjusted for ER status and nuclear tumour grade since these are the two best-characterised predictive variables of response to neoadjuvant chemotherapy in locally advanced breast cancer patients. In this multivariate analysis, NF- κ B activation retained its predictive value for resistance to neoadjuvant chemotherapy (hazard ratio = 0.028; 95% confidence interval = 0.002–0.337; $P = 0.005$). ER and tumour grade were not significant independent predictive factors of response to chemotherapy.

Pharmacodynamic changes: immunohistochemical subcellular characterisation of NF- κ B/p65

Forty six per cent ($n = 17$) of the 37 pre-treatment specimens evaluated exhibited positive cytoplasmic expression, and similarly, the percentage of positive cytoplasmic staining in the 43 post-treatment samples was 44% ($n = 19$). The percentage of NF- κ B/p65 nuclear staining was 13% (5 out of 37) in the pre-therapy specimens and 23% (10 out of 43) in the post-treatment samples.

In the subset of 37 patients for whom we had paired pre- and post-chemotherapy samples, NF- κ B/p65 cytoplasmic staining was undetected in both pre- and post-treatment samples in 20 patients and was positive in both pre- and post-treatment samples in 9 patients. In the remaining 8 patients, in 3 the cytoplasmic staining was undetected in pre-treatment and positive in post-treatment specimens, while in 5 cytoplasmic staining was positive in pre-treatment specimens and undetected post-treatment.

With regards to nuclear staining, 30 patients had both pre- and post-treatment samples with undetected NF- κ B/p65 nuclear staining, while 3 patients had both pre- and post-treatment nuclear staining. In the remaining 4 patients, one had undetected pre- and positive post-treatment nuclear staining and the other 3 had a negative pre- and a positive post-treatment nuclear staining sample (Fig. 1).

No significant correlation was found between NF- κ B/p65 nuclear and cytoplasmic staining either in the pre- or post-treatment samples.

Post-chemotherapy NF- κ B/p65 subcellular expression and response to chemotherapy in breast cancer patients

There was no association between cytoplasmic post-treatment NF- κ B/p65 expression and response to chemotherapy (Table 2). In contrast, only 12% of the patients who had a response to chemotherapy showed nuclear NF- κ B/p65 immunoreactivity after treatment, whereas 50% of chemoresistant patients showed NF- κ B/p65 nuclear staining in post-chemotherapy specimens ($P = 0.02$) (Table 2).

Discussion

In the present study, activation of NF- κ B determined by NF- κ B/p65 nuclear immunostaining was significantly correlated with resistance to chemotherapy in breast cancer patients. Furthermore, the number of patients with NF- κ B activation increased after chemotherapy exposure. These novel *in vivo*

data strongly support a role of NF- κ B activation as a cellular mechanism of resistance to anticancer therapy and suggest that NF- κ B is inducible by chemotherapy.

NF- κ B activation has been found to occur in the tumorigenesis of several types of cancer, as we and others have previously reported in prostate cancer (Ross *et al.* 2004, Sweeney *et al.* 2004, Domingo-Domenech *et al.* 2005), and in endometrial (Pallares *et al.* 2004), colorectal (Yu *et al.* 2004), gastric (Sasaki *et al.* 2001), uterine cervix (Nair *et al.* 2003), and head and neck squamous cell carcinoma (Ikebe *et al.* 2004). NF- κ B is also constitutively active in breast cancer cell lines and human breast cancer tissue (Nakshatri *et al.* 1997, Cogswell *et al.* 2000, Karin *et al.* 2002, Biswas *et al.* 2004, Codony-Servat *et al.* 2004). Moreover, it is proposed to be one of the early events in breast oncogenesis, as shown by early NF- κ B DNA-binding in neoplastic transformation of mammary cells (Kim *et al.* 2000). In the present series, 5 out of 37 patients (13%) had constitutive activation of NF- κ B (as assayed by NF- κ B/p65 nuclear staining in pre-treatment biopsies). This result is in line with previous studies that show similar percentages of constitutive activation of NF- κ B/p65 in non-treated breast tumours (Sovak *et al.* 1997, Cogswell *et al.* 2000).

Our data show a higher proportion of NF- κ B/p65 nuclear staining in HER2-positive tumours, although this association was not statistically significant. It is thought that HER2 may be involved in the activation of NF- κ B through the phosphatidylinositol 3-kinase/Akt intracellular pathway, as shown in breast cancer cell lines and transgenic mice (Zhou *et al.* 2000, Pianetti *et al.* 2001, Biswas *et al.* 2004). Moreover, in HER2 overexpressing cells, blocking of the Akt pathway by a dominant-negative Akt leads to inhibition of the NF- κ B pathway (Zhou *et al.* 2000). In the present study, NF- κ B was constitutively active regardless of the hormonal status. Although these results are in accordance with previous data (Cogswell *et al.* 2000), recent studies have suggested a role for NF- κ B in ER-negative breast oncogenesis, especially in those with elevated levels of HER1 or HER2 (Nakshatri *et al.* 1997, Biswas *et al.* 2004). Larger series are needed to address this issue further.

Several studies have addressed the identification of reliable predictive factors of the response to neoadjuvant chemotherapy in locally advanced breast cancer (Kuerer *et al.* 1999, Fisher *et al.* 2002). Classical clinical–pathological parameters that have been studied include tumour size, nodal

status, nuclear tumour grade and ER status (Gonzalez-Angulo *et al.* 2005). Even if the results of the different studies are not always consistent, a relation between response and nuclear tumour grade (Kuerer *et al.* 1999, Fisher *et al.* 2002) and ER status (Kuerer *et al.* 1999) has been suggested in patients treated with neoadjuvant anthracycline-based regimens. It is thought that a combination of some of these tumour markers or the inclusion of molecular markers – such as Ki67 or topoisomerase II α – would lead to a higher predictive value (Burcombe *et al.* 2005). On the other hand, HER2 overexpression is linked to response to adjuvant anthracycline chemotherapy (Baselga *et al.* 2001). Pharmacogenomic studies are also providing very promising results in this field (Pusztai *et al.* 2005). In the present study, tumour size, clinical nodal status, nuclear tumour grade, ER or HER2 status did not distinguish between responders and non-responders to neoadjuvant chemotherapy. Here, we addressed NF- κ B as a novel predictor of chemoresistance in breast cancer. The prognostic value of NF- κ B in several types of cancer has previously been reported by us and others (Lessard *et al.* 2003, Fradet *et al.* 2004, Ross *et al.* 2004, Domingo-Domenech *et al.* 2005).

Antineoplastic agents such as anthracyclines or taxanes have been shown to activate NF- κ B (Das & White 1997, Bottero *et al.* 2001, Ho *et al.* 2005). Although the mechanisms of this activation remain unclear, it has recently been shown that the ataxia telangiectasia-mutated protein plays a pivotal role in the detection of DNA breaks produced by chemotherapy agents, such as topoisomerase inhibitors. The consequent activation of I- κ B kinase may induce I- κ B degradation and NF- κ B activation (Hur *et al.* 2003, Panta *et al.* 2004). On the other hand, the activation of NF- κ B has proved to be a pivotal mechanism of tumour chemoresistance (Wang *et al.* 1999, Baldwin 2001, Karin *et al.* 2002) as supported by the fact that inhibition of NF- κ B sensitises chemoresistant cancer cell lines (Wang *et al.* 1999). The capacity of NF- κ B to induce resistance to cancer therapies is mediated by its role in the regulation of various anti-apoptotic genes such as caspase-8, cellular inhibitors of apoptosis, members of the BCL2 family and cFLIP (Wang *et al.* 1999, Karin *et al.* 2002). In our series, activation of NF- κ B in breast cancer pre-chemotherapy specimens was found to be a predictive factor of chemoresistance, as shown by a 20% response rate in patients with pre-treatment NF- κ B activation compared with a 91% response rate in patients

with undetected active NF- κ B. Moreover, NF- κ B retained its significance as a predictive factor independently of the nuclear tumour grade and ER status. It should be noted that not all the patients included in this study were treated with anthracycline-containing regimens. However, a sub-analysis including only the anthracycline-treated patients showed the same results. Our results also suggest that NF- κ B activation is inducible by chemotherapy, as shown by a higher proportion of NF- κ B nuclear staining after therapy. Furthermore, NF- κ B activation in post-treatment samples was significantly linked to chemoresistance (85% versus 44% response rate in NF- κ B post-chemotherapy activated and non-activated samples respectively).

These observations provide data on a significant link between NF- κ B activation in breast cancer and resistance to chemotherapy. This finding provides a clinical rationale for investigating NF- κ B inhibitors as chemosensitising agents in breast cancer.

Funding

This work was supported by the Spanish Science and Technology Ministry (MCYT), grant number SAF 2003-08181; the Spanish Health Ministry, grant number FIS 01/1519; Red Temática del Cáncer, grant number C03/10 (Instituto de Salud Carlos III), the Asociación Española Contra el Cáncer (AECC)/Catalunya contra el Cáncer and Fundació La Marató de TV3, grant number 053131. CM was supported by a 'Premi Fi de Residencia 2004–2005' research grant from the Hospital Clínic of Barcelona (Spain). AR was supported by a 'Beca de Investigación Oncologica 2002' fellowship from the Fundación Científica de la AECC. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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