J Cancer Res Clin Oncol (2000) 126: 205–211

ORIGINAL PAPER

**Sanja Kapitanović · Senka Radošević · Neda Slade
Milan Kapitanović · Šimun Anđelinović · Željko Ferenčić
Mahvash Tavassoli · Šime Spaventi · Krešimir Pavelić
Radan Spaventi**

Expression of erbB-3 protein in colorectal adenocarcinoma: correlation with poor survival

Received: 7 August 1999 / Accepted: 8 November 1999

Abstract ***Background/aims:*** The family of erbB receptors includes four transmembrane glycoproteins with tyrosine kinase activity. These receptors are widely expressed in normal tissues, but they also have been implicated in the development of several human adenocarcinomas. c-erbB-3/HER-3 has been detected to a greater or lesser extent in many tissues from the digestive, urinary, reproductive and respiratory tracts. The overexpression of c-erbB-3/HER-3 protein has also been shown in 53%–88% of colorectal adenocarcinomas. In this study we investigated the expression of the c-*erbB-3*/*HER-3* gene product in colorectal tumour samples, and compared the results obtained with several clinicopathological parameters, including the survival of patients. ***Methods:*** Paraffin-embedded tissue sections were analysed immunohistochemically, using monoclonal antibody RTJ1 to human erbB-3 protein. Antibody RTJ1 specificity was confirmed by immunoprecipitation followed by Western blotting analysis. Amplification of the *erbB-3* oncogene was tested by dot-blot hybridization. ***Results:*** Adenocarcinomas of the colon were positive for erbB-3 protein in 78% of samples examined. Dot-blot analysis showed no amplification of the *erbB-3* gene in colon adenocarcinomas. Statistical analysis showed that patients with tumours that could not be stained for erbB-3 protein survived significantly longer ($P < 0.05$) than patients with tumours staining positive for the erbB-3 protein. A Cox proportional-hazards model with stepwise variable selection identified age, sex and erbB-3 expression as important prognostic factors. ***Conclusion:*** These findings demonstrate that erbB-3 protein expression could serve as a prognostic factor in colorectal malignancies.

Key words erbB-3 · Colorectal carcinoma · Survival · Prognosis

Abbreviation *EGF-R* epidermal growth factor receptor

S. Kapitanović (✉) · N. Slade · K. Pavelić · R. Spaventi
Division of Molecular Medicine, Ruđer Bošković Institute,
Bijenička c.54, 10 000 Zagreb, Croatia
e-mail: kapitan@rudjer.irb.hr
Tel.: +385-1-4561-108
Fax: +385-1-468 00 94

S. Radošević · Ž. Ferenčić · R. Spaventi
PLIVA Research Institute, Zagreb, Croatia

M. Kapitanović
Department of Surgery, County Hospital Šibenik, Šibenik, Croatia

Š. Anđelinović
Department of Pathology, Clinical Hospital Split, Split, Croatia

M. Tavassoli
RCS Department of Dental Sciences,
King's College School of Medicine and Dentistry,
London, England

Š. Spaventi
Croatian Academy of Sciences and Arts, Zagreb, Croatia

Introduction

The family of type-1 growth factor receptors includes four transmembrane glycoproteins [epidermal growth factor receptor (EGF-R), c-erbB-2/HER-2, c-erbB-3/HER-3 and erbB-4/HER-4] that share a common structure, each comprising a cysteine-rich extracellular area, a transmembrane component and an intracellular region with tyrosine kinase activity (Coussens et al. 1985; Kraus et al. 1989; Plowman et al. 1990, 1993; Yamamoto et al. 1986). These receptors are widely expressed in epithelial, mesenchymal and neuronal tissues, but they have also been implicated, more than other growth factor receptors, in the development of several human adenocarcinomas (Grunt and Huber 1994; Alroy and Yarden 1997; Tzahar and Yarden 1998). Increased expression of EGF-R has been associated with stomach, bladder, lung and breast tumours, while amplification and/or overexpression of the c-*erbB-2*/*HER-2* gene has been correlated with poor prognosis of breast, ovarian, stomach and colorectal cancers (Kay et al. 1994;

Kapitanović et al. 1994, 1997; Slamon et al. 1987, 1989; Yonemura et al. 1997).

c-erbB-3/HER-3 is a 180-kDa glycoprotein encoded by a gene that has been mapped to human chromosome 12q13 (Kraus et al. 1989; Plowman et al. 1990). This protein, at its predicted tyrosine kinase domain, shows 64% amino acid sequence homology to the tyrosine kinase domain of EGF-R and 67% amino acid sequence homology to the tyrosine kinase domain of the c-erbB-2/HER-2 protein (Kraus et al. 1989). c-erbB-3/HER-3 has been detected to a greater or lesser extent in many tissues from the digestive, urinary and respiratory tracts, the circulatory system and the female and male reproductive organs, but not in haematopoeitic tissues. It is overexpressed in more than 20% breast cancers (Lemoine et al. 1992a), in the majority of pancreatic (Lemoine et al. 1992b) and gastric cancers (Sandidas et al. 1993), in a squamous cell carcinoma (Funayama et al. 1998; Ibrahim et al. 1997) as well as in a proportion of other tumour types (Rajkumar et al. 1995; Poller et al. 1992). The overexpression of c-erbB-3/HER-3 protein has also been shown in 53%–88% of colorectal adenocarcinomas (Poller et al. 1992, Ciardiello et al. 1991; Rajkumar et al. 1993).

However, in spite of the large number of studies performed, very little is known about the correlation between c-erbB-3/HER-3 tumour overexpression and clinicopathological parameters, including the survival of patients. The last question has been raised for breast cancer (Lemoine et al. 1992a; Quinn et al. 1994), for which it has been shown that high expression of c-erbB-3/HER-3 is positively associated with the presence of lymph node metastasis, but not with patient survival (Lemoine et al. 1992a). Friess and co-workers (1995) noticed the correlation between enhanced erbB-3 expression in human pancreatic cancer and tumour progression. High c-erbB-3 protein expression is also associated with shorter survival in advanced non-small-cell lung carcinomas (Yi et al. 1997). In this study we investigated the expression of the c-*erbB-3/HER-3* gene product in 55 colorectal tumour samples, and compared the results obtained with several clinicopathological parameters, including the survival of patients. In addition, we have analysed whether the c-*erbB-3/HER-3* gene is amplified in these tumours.

Materials and methods

Patients and tissue specimens

This retrospective study was carried out using specimens of normal colon tissue and benign and malignant colon lesions. All specimens were obtained through routine surgery performed at the medical centres of Šibenik, Split and Zagreb, Croatia. The diagnoses were established by standard diagnostic procedures and confirmed histopathologically. Staging was performed according to Dukes' criteria (Deans et al. 1992; Turnbull et al. 1967). The patients were followed-up according to a standardized protocol that included laboratory tests at 1- to 2-month intervals, chest radiography, ultrasonography, computerized tomographic examination of the liver and endoscopy of the colon at 1-year intervals during the first 3 postoperative years and thereafter at 6- to 12-month intervals. A disease-free interval was defined as the time from resection to the first clinically detectable recurrence of tumour. Causes of death were ascertained from the medical records or autopsy (if performed). Patients who died within 4 weeks of radical surgery were excluded from the analysis. Deaths by other causes were censored observations from the time of death.

None of the patients with colorectal adenocarcinoma underwent preoperative radiation or chemotherapy. The study included 27 men and 28 women ranging in age between 37 and 85 years (mean age, 64.1 years).

Each specimen was routinely fixed in 10% formalin and immersed in melted paraffin. Sections of 4 μm were cut and mounted on glycerine-treated slides. Fresh samples of resected normal colon (more than 5 cm from the tumour) and colon carcinoma, immediately adjacent to the segment of tissue that was fixed in formalin, were snap-frozen in liquid nitrogen and stored in the Human Tumour Bank (Spaventi et al. 1994) at −80 °C until further use. Before inclusion in the study, each specimen was verified by a histopathologist.

Cell line

The human pancreas carcinoma (MiaPaCa-2) cell line was used for immunohistochemistry, DNA isolation and dot-blot hybridization. The cells were cultured in Dulbecco's modified Eagle medium (Gibco) supplemented with 10% fetal calf serum. For immunocytochemical studies, the cells were grown in four-chamber slides (Nunc) overnight in a humidified atmosphere with 5% CO_2 at 37 °C.

Immunohistochemical detection of oncogene proteins

Following deparaffinization in xylene, the slides were rehydrated and washed in phosphate-buffered saline (PBS). The endogenous peroxidase activity was quenched by 15 min incubation in a mixture of 3% hydrogen peroxide solution (of 30%) in 100% methanol (Sigma). After the fixation, slides were cleared with PBS. Nonspecific binding was blocked applying normal rabbit serum in a humidity chamber at a dilution of 1:10 for 30 min at room temperature. Slides were incubated with monoclonal antibody RTJ1 to human erbB-3 protein (the gift of Prof. M.H. Kraus) at 1:10 dilution overnight at +4 °C. Slides were washed three times in PBS containing 3%, 2% and 1% normal human serum. Secondary antibody (rabbit antibody to mouse immunoglobulins; Dakopatts, Denmark), diluted with PBS and normal human serum [40 μl rabbit anti-(mouse IgG) Ab, 100 μl normal human serum and 860 μl PBS] was applied for 1 h at room temperature. Finally, peroxidase/anti-peroxidase (Dakopatts, Denmark) conjugate diluted 1:100 in PBS was applied for 45 min at room temperature. After washing with PBS, slides were kept in diaminobenzidine tetrahydrochloride for 7 min (50 mg in 200 ml PBS with 25 μl 30% H_2O_2) (Sigma), then counterstained with haematoxylin for 30 s, dehydrated and mounted in Canada balsam.

Evaluation of the slides

Each slide was evaluated in the whole tumour area. We assessed the relative level of specific immunostaining and its localization. The intensity of cell immunostaining was evaluated semiquantitatively, so that negative staining was denoted − and positive staining +.

Preparation of the cells for biochemical analysis

The cells were washed with PBS (three times) and resuspended in lysis buffer [150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 10 mM TRIS/HCl,

pH 7.2] with 1 mM phenylmethysulfonyl fluoride and aprotinin (2 μg/ml) as protease inhibitors, and subsequently centrifuged at 15 000*g* in an Eppendorf centrifuge; the supernatant was used in immunoprecipitation.

Immunoprecipitation

Monoclonal antibody RTJ1 at a dilution of 1:1 with 100 μl supernatant (150 mg total protein) was incubated for 1 h on ice with shaking. Immunoprecipitation was followed by the addition of protein-A–Sepharose (Pharmacia, Sweden) overnight. The reaction was stopped by adding cold lysis buffer (150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 10 mM TRIS/HCl, pH 7.2) and the mixture centrifuged in Eppendorf centrifuge for 5 min at 15 000*g*. Immunoprecipitates were washed three more times with cold lysis buffer; 50 μl sample buffer for SDS/polyacrylamide gel electrophoresis (PAGE) was added to each precipitate, and the samples were frozen at −20 °C.

SDS-PAGE

Electrophoresis was run in TRIS/glycine buffer (0.025 M TRIS, 0.19 M glycine, 1% SDS, pH 8.6) on 7.5% polyacrylamide gel according to the method of Laemmli. Molecular masses were calculated by comparison with high-molecular-mass standards (Gibco BRL, USA).

Immunoblotting (Western blotting)

Electrophoretically separated proteins were electroblotted to nitrocellulose membrane (BA 85, 0.45 μm, Schleicher & Schuell, Dusseldorf, Germany) in electrode buffer (25 mM TRIS, 192 mM glycine, pH 8.3) at 150 mA for 45 min with cooling (Midget MultiBlot Electrophoretic Transfer Unit, LKB 2051, Pharmacia, Sweden). Immunodetection of antigen immobilized on nitrocellulose membrane was accomplished by the alkaline phosphatase method. Unoccupied protein-binding sites on the nitrocellulose were blocked with 3% bovine serum albumin (BSA; Sigma Chemical Co., St Louis, USA) and 0.05% Tween 20 (Sigma Chemical Co., St Louis, USA) in PBS for 30 min. The membrane was then incubated with primary monoclonal antibody RTJ1 at a dilution of 1:10 in 0.3% BSA/0.05% Tween 20/PBS overnight at +4 °C with agitation. After washing with same buffer, the membrane was incubated for 1 h with secondary antibody [goat anti-(mouse IgG) conjugated to alkaline phosphatase] at a concentration of 0.2 μg/ml, diluted in same buffer. The blot was washed and developed in alkaline phosphatase substrate solution (100 mM TRIS/HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. The reaction was stopped with stop buffer (20 mM TRIS/HCl, pH 8.0, 5 mM EDTA).

DNA isolation

The positive colorectal adenocarcinoma tissue, frozen in liquid nitrogen, was pulverized mechanically to a fine powder. The powder or MiaPaCa-2 cells were resuspended and DNA was isolated in buffer containing 10 mM TRIS/HCl, pH 8.0, 0.1 M EDTA, pH 8.0, 0.5% SDS and 100 μg/ml proteinase K. RNA was removed by digestion with 20 μg/ml RNase A. DNA was purified by sequential extraction through phenol and chloroform and finally precipitated with two volumes of ethanol at −20 °C.

Dot-blot hybridization

Samples (10 μg) of DNA from the control cell line were obtained, diluted to 50 μl with buffer (10 mM TRIS/HCl, pH 8.0, 1 mM EDTA, pH 8.0), denatured at 100 °C for 10 min, chilled on ice and incubated with 50 μl 1 M NaOH for 20 min at room temparature. An equal volume of neutralization buffer (1 M NaCl, 0.3 M sodium citrate, 0.5 M TRIS/HCl pH 8.0, 1 M HCl) was added to each sample. The samples were blotted onto Hybond nylon membrane (Amersham plc) saturated with 6× standard saline citrate, using a dot-blot apparatus. Reference DNA samples of known copy number were simultaneously blotted. Hybridization was performed under stringent conditions at 68 °C overnight. The probe used in the analyses was a 650-bp *Hin*dIII/*Eco*RI cDNA fragment isolated from recombinant pUC 19 plasmid DNA pE3-HK, containing a fragment of human *erbB3* encoding the carboxyl terminus and a 3′ untranslated sequence (nucleotide position 3568–4189) (Kraus et al. 1989).

Controls

Control staining was performed by omitting the primary antibody. As a positive control in immunohistochemical studies, we used paraffin slides of the malignant insulinoma. In immunocytochemical studies we used MiaPaCa-2 cells as a positive control. As a negative control of amplification in the dot-blot study we also used DNA isolated from Mia PaCa-2 cells.

Statistical analysis

Categorical data were analysed using χ^2 statistics. Quantitative data were analysed with Student's *t*-test and the Mann-Whitney *U*-test. The probability of survival was calculated by the Kaplan-Meier method. Four-week mortality was excluded from the survival curves. Statistical difference was evaluated by Gehan's generalized Wilcoxon test and the Cox's *F*-test. All evaluations were performed using SAS/Stat software and STATISTICA. Multivariate analysis was performed with the Cox's proportional-hazards model with stepwise variable selection. This was used to identify the major prognostic indicators independently associated with survival.

Results

Specificity of antibodies for erbB-3 protein

To verify the specificity of the monoclonal antibodies used in these experiments we tested them immunocytochemically on a human pancreas carcinoma cell line MiaPaCa-2 (Fig. 1A). Antibody specificity was confirmed by immunoprecipitation of the cell lysate and Western blot analysis, which showed precipitation of a single band at 180 kDa. As shown in Fig. 2, erbB-3 protein was detected in lysates of MiaPaCa-2 cells. Immunoprecipitation of a lysate of MiaPaCa-2 cells with the specific anti-erbB-3 antibodies resulted in the precipitation of a single band at 180 kDa (Fig. 2).

erbB-3 protein in colorectal lesions

A total of 85 colon samples (10 samples of normal colon, 10 hyperplastic polyps, 10 adenomas and 55 samples of colon adenocarcinomas) were examined for the presence of erbB-3 oncoprotein. Normal mucosa and benign and malignant lesions of colorectal origin involved in our study clearly differed in erbB-3 immunostaining.

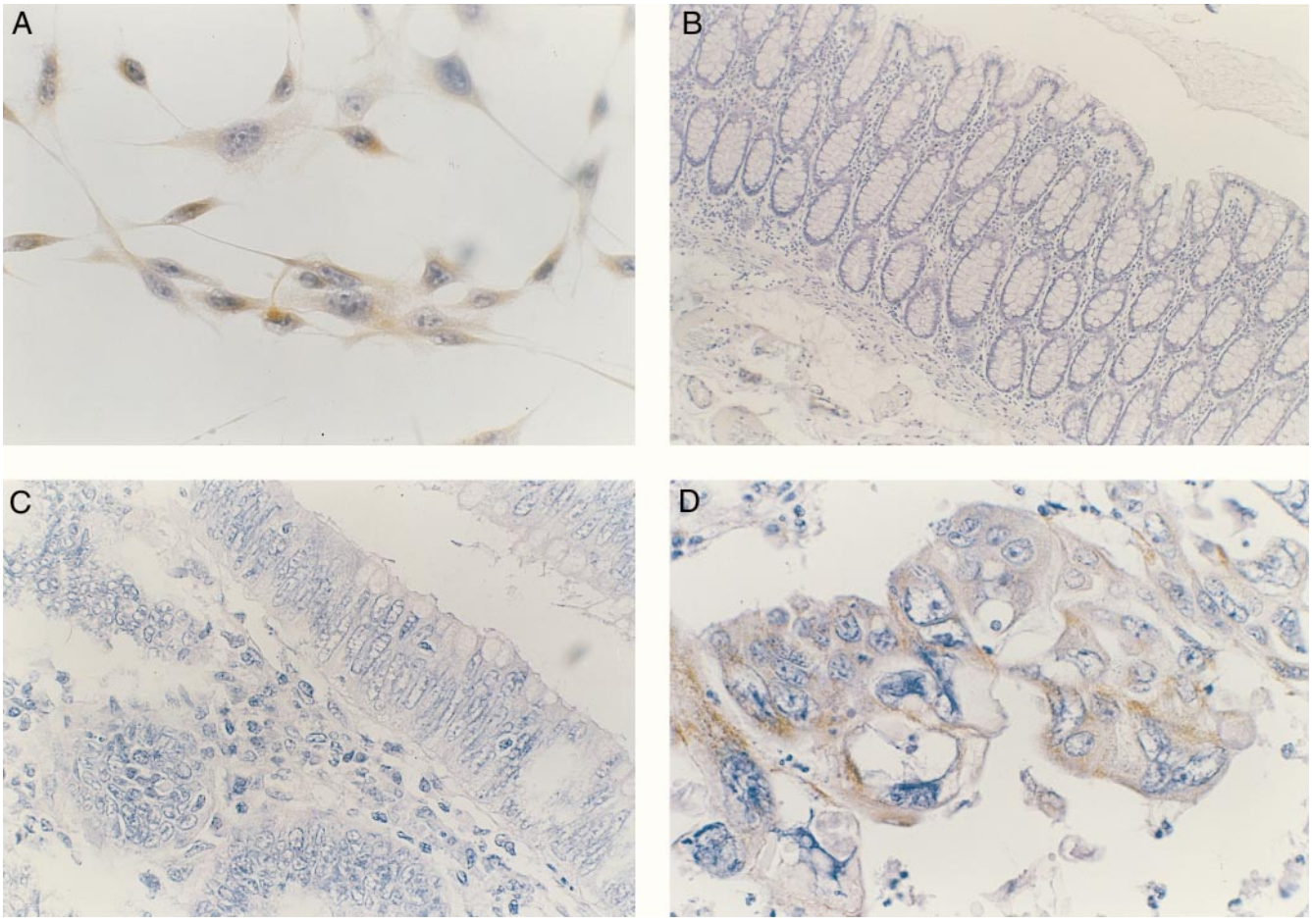

Fig. 1A–D Immunohistochemical staining with RTJ1 antibody. **A** Cultured pancreas carcinoma cells (MiaPaCa-2); note perinuclear cytoplasmic staining. **B, C** Normal colon (**B**) and tubulovillous adenoma (**C**) were negative for erbB-3 protein. **D** Adenocarcinoma of the colon: cytoplasmic perinuclear staining of tumour cells

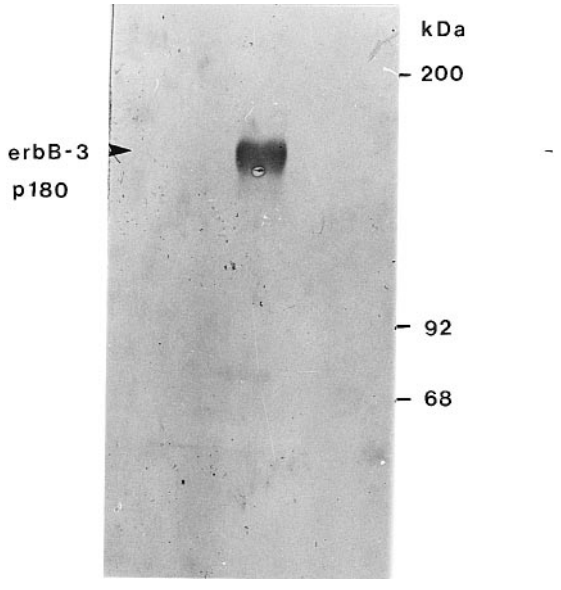

Fig. 2 Immunoprecipitation of cell lysates followed by Western blot analysis with RTJ1 antibodies showed a single band at 180 kDa in a lysate of MiaPaCa-2 cells

All histologically normal colon samples examined were negative for erbB-3 protein (Fig. 1B). Positively stained samples were histologically normal colon mucosa adjacent (within 5 cm of the tumour) to the adenocarcinoma. Luminal epithelial cells with no crypt involvement were also positive.

Hyperplastic polyps examined in this study were all negative for erbB-3 protein. Neoplastic progression in the colon is characterized by the appearance of tumours defined as tubular, tubulovillous and villous adenomas. This study included sections from 10 such lesions. All samples examined, including villous adenomas (Fig. 1C), were negative for erbB-3 protein.

Adenocarcinomas of the colon were positive for erbB-3 protein in 78% of samples examined. The observed staining pattern was essentially identical in all carcinomas and featured the cytoplasmatic perinuclear staining of tumour cells (Fig. 1D). The patients we tested (whose primary tumours were positive for erbB-3 protein) also had erbB-3-positive tumour cells in the regional lymph nodes.

erbB-3 gene amplification

Dot-blot analysis showed no amplification of the *erbB-3* gene in normal colon and colon adenocarcinomas (Fig. 3).

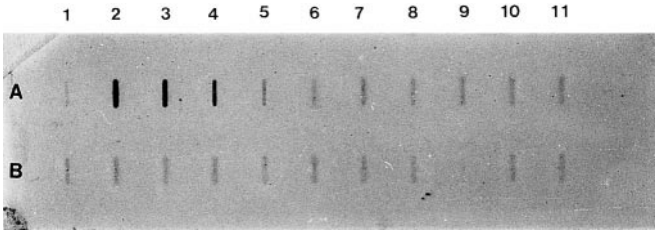

Fig. 3 Dot-blot analysis showed no amplification of the *erbB-3* gene in colon adenocarcinomas. *A1* Negative control, *A2–A4* positive controls, *A5* DNA of MiaPaCa-2 cells, *A6–A11*, *B1–B11* DNA of colon adenocarcinomas

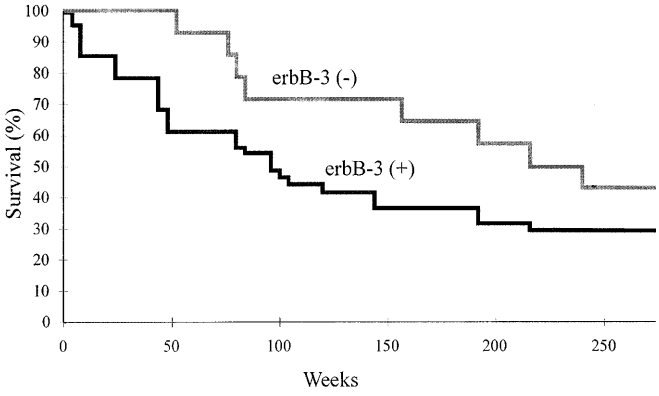

Fig. 4 Overall survival of 55 patients with colon adenocarcinoma according to the presence (+) or absence (−) of positivity for erbB-3 protein in tumour cells

erbB-3 protein expression and clinical parameters

No correlation was found between the positivity of erbB-3 protein expression and the age or sex of patients with positive adenocarcinoma samples. Also no correlation was found between erbB-3 protein expression and tumour size or grade or the Dukes' stage of positive adenocarcinoma samples ($P > 0.05$) (Table 1).

Survival analysis

Exploratory analysis was conducted to correlate the outcome of patients monitored during the 260-week period with the presence of erbB-3 protein, determined immunohistochemically. Survival analysis was performed on 55 patients who survived for more than 4 weeks after surgery. The survival curves according to erbB-3 expression are shown in Fig. 4. The median survival time of the patients with no erbB-3 oncoprotein expression was 181.1 weeks, 43% of them remaining alive at the end of the monitoring period. The median survival time of the patients with erbB-3 oncoprotein expression was 113.9 weeks, 29% of them remaining alive at the end of the monitoring period. Statistical analysis (Cox's *F*-test) showed that patients with tumours that could not be stained for erbB-3 protein survived significantly longer ($P < 0.05$) than patients with erbB-3-protein-positive staining.

Table 1 Clinicopathological features of 55 patients with colorectal cancer stratified by erbB-3 status

Characteristic	erbB-3 staining		
	Positive ($n = 41$)	Negative ($n = 14$)	*P*
Mean age (years)	63.58	65.71	0.575
Sex			
Male	17	10	
Female	24	4	0.053
Tumour size (cm)	4.77	4.68	0.525
Histological grade (differentiation)			
Well	6	1	
Moderate	23	5	
Poor	12	8	0.170
Dukes' stage			
A	7	3	
B	14	3	
C	20	8	0.671

Table 2 Stepwise regression model

Parameter	β	SE	*P*	RR
Sex	−0.827182	0.35115	0.0185	0.437
Age	0.030750	0.01468	0.0362	1.031
erbB-3	0.895234	0.41699	0.0318	2.448

Regression analysis

Stepwise regression analysis, based on the Cox proportional-hazards model, identified age, sex and erbB-3 expression as important prognostic factors (Table 2).

Discussion

In this study we have examined the expression of the erbB-3/HER-3 product in colorectal carcinomas and 78% of carcinomas have shown overexpression of the oncoprotein investigated. The rest of the samples were negative for $p180^{c\text{-}erbB\text{-}3/HER\text{-}3}$. These results are in accordance with previous studies. Ciardiello and coworkers (1991) have demonstrated that the c-erbB-3/HER-3 mRNA is overexpressed in 53.3% of primary colorectal carcinomas and in 55.9% colorectal-carcinoma-derived metastases in the liver. Also, same authors have detected the primary transcript of the c-*erbB-3*/*HER-3* gene in 5/7 colonic adenocarcinoma-derived cell lines. Similar results have been obtained through immunohistochemical analysis of polyclonal (8/9 positive tumour samples; Poller et al. 1992) or monoclonal (11/16 positive tumour samples; Rajkumar et al. 1995) antibodies against $p180^{c\text{-}erbB\text{-}3/HER\text{-}3}$. Maurer and co-workers (1998) showed moderate to strong immunoreactivity for erbB-3 in 89% (31 of 35) of colorectal cancers. As in these studies, the immunoreactivity of the

samples we tested was cytoplasmic in a finely granular perinuclear distribution.

No correlation was found between the protein expression and the histological grade of positive adenocarcinoma samples ($P > 0.05$). Also, no correlation was found between erbB-3 protein expression and the Dukes' stage of positive adenocarcinoma samples ($P > 0.05$).

Survival analysis revealed that patients bearing tumours that expressed c-erbB-3/HER-3 protein survived for significantly shorter periods than patients with c-erbB-3/HER-3-negative tumours. Similar studies on breast cancer could not establish a relationship between c-erbB-3/HER-3 expression and patient survival (Lemoine et al. 1992a; Quinn et al. 1994), although they found that high expression of c-erbB-3/HER-3 is positively associated with the presence of lymph node metastases (Lemoine et al. 1992a). To our knowledge, no similar study has been performed on colorectal cancer.

Unlike other members of the same tyrosine kinase receptor family (EGF-R, c-erbB-2/HER-2), the mechanisms underlying the overexpression of c-erbB-3/HER-3 are still unknown. Since our results, and the results of other studies on breast and pancreatic cancers (Kraus et al. 1989; Lemoine et al. 1992a, b), provide no evidence of gene amplification or rearrangement in human tumours, it is reasonable to assume that these events are not a common feature (if they happen at all) of the c-*erbB-3*/*HER-3* gene. However, it has been shown that the c-*erbB-2*/*HER-2* gene can be activated thorough mechanisms other than gene amplification. They include point mutation in the transmembrane domain of this receptor as well as the control of expression by specific transcription factors (Bargmann et al. 1986, 1988; Hollywood et al. 1993). Further studies will certainly clarify whether similar mechanisms are involved in the activation of the c-*erbB-3*/*HER-3* gene.

Our preliminary data show that expression of erbB-3 protein in tumour cells of colon carcinoma is independently related to survival. Therefore, a study on a larger cohort of patients is necessary to confirm our results.

Acknowledgement This work was supported by the Croatian Ministry for Sciences and Technology, project grant 1104 and program grant 009811.

References

Alroy I, Yarden Y (1997) The erbB signaling network in embryogenesis and oncogenesis: signal diversification through combinatorial ligand-receptor interactions. FEBS Lett 410: 83–86

Bargmann CI, Hung MC, Weinberg RA (1986) Multiple independent activations of the *neu* oncogene by a point mutation altering the transmembrane domain of p185. Cell 45: 649–657

Bargmann CI, Weinberg RA (1988) Oncogenic activation of the *neu*-encoded receptor protein by point mutation and deletion. EMBO J 17: 2043–2052

Ciardiello F, Kim N, Saeki T, Dono R, Persico MG, Plowman GD, Garrigues J, Radke S, Todaro GJ, Salomon DS (1991) Differential expression of epidermal growth factor-related proteins in human colorectal tumors. Proc Natl Acad Sci USA 88: 7792–7796

Coussens L, Yang-Feng YL, Liao Y-C, Chen E, Gray A, McGrath J, Seenburg PH, Levinson A, Ullrich A (1985) Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal localization with *neu* oncogene. Science 230: 1132–1185

Deans GT, Parks TG, Rowlands BJ, Spence RAJ (1992) Prognostic factors in colorectal cancer. Br J Surg 79: 608–613

Friess H, Yamanaka Y, Kobrin MS, Do DA, Buchler MW, Korc M (1995) Enhanced erbB-3 expression in human pancreatic cancer correlates with tumor progression. Clin Cancer Res 1: 1413–1420

Funayama T, Nakanishi T, Takahashi K, Taniguchi S, Takigawa M, Matsumurs T (1998) Overexpression of c-erbB-3 in various stages of human squamous cell carcinomas. Oncology 55: 161–167

Grunt TW, Huber H (1994) The family of c-*erbB* genes: from basic research to clinical oncology. Onkologie 17: 346–357

Hollywood DP, Hurst HC (1993) A novel transcription factor, OB-1, is required for overexpression of the proto-oncogene *c-erbB-2* in mammary tumor lines. EMBO J 12: 2369–2375

Ibrahim SO, Vasstrand EN, Liavang PG, Johannessen AC, Lillehaug JR (1997) Expression of *erbB* proto-oncogene family members in squamous cell carcinoma of the head and neck. Anticancer Res 17: 4539–4546

Kapitanović S, Spaventi R, Poljak L, Kapitanović M, Pavelić ZP, Gluckman JL, Spaventi Š, Pavelić K (1994) High c-erbB-2 protein level in colorectal adenocarcinomas correlates with clinical parameters. Cancer Detect Prev 18: 97–101

Kapitanović S, Radošević S, Kapitanović M, Andelinović, Ferenčić Z, Tavassoli M, Primorac D, Sonicki Z, Spaventi Š, Pavelić K, Spaventi R (1997) The expression of p185HER-2/neu correlates with the stage of disease and survival in colorectal cancer. Gastroenterology 112: 1103–1113

Kay EW, Mulcahy H, Walsh CB, Leader M, O'Donoghue D (1994) Cytoplasmatic c-erbB-2 protein expression correlates with survival in Dukes' B colorectal carcinoma. Histopathology 25: 455–461

Kraus MH, Issing W, Miki T, Popescu NC, Aaronson SA (1989) Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. Proc Natl Acad Sci USA 86: 9193–9197

Lemoine NR, Barnes DM, Hollywood DP, Hughes CM, Smith P, Dublin E, Prigent SA, Gullick WJ, Hurst HC (1992a) Expression of the ERBB3 gene product in breast cancer. Br J Cancer 66: 1116–1121

Lemoine NR, Lobresco M, Leung H, Barton C, Hughes CM, Prigent SA, Gullick WJ, Kleppel G (1992b) The c-*erbB-3* gene in human pancreatic cancer. J Pathol 168: 269–273

Maurer CA, Freiss H, Kretschmann B, Zimmermann A, Stauffer A, Baer HU, Korc M, Buchler MW (1998) Increased expression of erbB3 in colorectal cancer is associated with concomitant increase in the level of erbB2. Hum Pathol 29: 771–777

Plowman GD, Whitney GS, Neubauer MG, Green JM, McDonald VL, Todaro GJ, Shoyab M (1990) Molecular cloning and expression of an additional epidermal growth factor receptor-related gene. Proc Natl Acad Sci USA 87: 4905–4909

Plowman GD, Green JM, Culouscou J-M, Carlton GW, Rothwell VM, Buckley S (1993) Heregulin induces tyrosine phosphorylation of HER4/p180^{erbB4}. Nature 366: 473–475

Poller DN, Spendolve I, Baker C, Church R, Ellis IO, Plowman GD, Mayer RJ (1992) Production and characterization of a polyclonal antibody to the c-erbB-3 protein: examination of c-erbB-3 protein expression in adenocarcinomas. J Pathol 168: 275–280

Quinn CM, Ostrowski JL, Lane SA, Loney DP, Teasdale J, Benson EA (1994) c-*erb*B-3 protein expression in human breast cancer: comparison with other tumour variables and survival. Histopathology 25: 247–252

Rajkumar T, Goden CSR, Lemoine NR, Gullick WJ (1993) Expression of the c-erbB-3 protein in gastrointestinal tract tumours determined by monoclonal antibody RTJ1. J Pathol 170: 271–278

Rajkumar T, Hollywood DP, Hurst HC, Gullick WJ (1995) c-erbB3 expression in breast tumour derived cell lines. Breast 4: 84–91

Sanidas EE, Filipe MI, Linehan J, Lemoine NR, Gullick WJ, Rajkumar T, Levison DA (1993) Expression of the c-erbB-3 gene product in gastric cancer. Int J Cancer 54: 935–940

Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. Science 235: 177–182

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SA, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF (1989) Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. Science 244: 707–712

Spaventi R, Pečur L, Pavelić K, Pavelić Z, Spaventi Š, Stambrook P. (1994) Human tumour bank in Croatia: a possible model for a small bank as part of the future european tumour bank network. Eur J Cancer 30A: 419

Turnbull RB, Kyle K, Watson FR (1967) Cancer of the rectum: the influence of the no-touch isolation technique on survival rates. Ann Surg 166: 420–427

Tzahar E, Yarden Y (1998) The ErbB-2/HER2 oncogenic receptor of adenocarcinomas: from orphanhood to multiple stromal ligands. Biochim Biophys Acta 1377: M25-M37

Yamamoto T, Ikawa S, Akiyama T, Semba K, Nomura N, Miyajima N, Saito T, Toyoshima K (1986) Similarity of protein encoded by human *c-erbB-2* gene to epidermal growth factor receptor. Nature 319: 230–234

Yi ES, Harclerode D, Gondo M, Stephenson M, Brown RW, Younes M, Cagle PT (1997) High c-erbB-3 protein expression is associated with shorter survival in advanced non-small cell lung carcinoma. Mod Pathol 10: 142–148

Yonemura Y, Ninomiya I, Yamaguchi A, Fushida S, Kimura H, Ohoyama S, Miyazaki I, Endou Y, Tanaka M, Sasaki T (1991) Evaluation of immunoreactivity for *erbB-2* protein as a marker of poor short term prognosis is gastric cancer. Cancer Res 51: 1034–1038