The Expression of p185<sup>HER-2/neu</sup> Correlates With the Stage of Disease and Survival in Colorectal Cancer

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Background & Aims: HER-2/neu oncogene encodes a transmembrane tyrosine kinase receptor that is amplified and/or overexpressed predominantly in adenocarcinomas. This phenomenon has been most intensively studied in breast carcinoma where its amplification and overexpression correlate with the overall course of disease and poor prognosis. This study was designed to investigate HER-2/neu gene expression in benign and malignant colorectal lesions and to evaluate its prognostic importance in colorectal cancer. Methods: Two hundred twenty-one samples of normal colon, benign lesions, and colorectal adenocarcinomas were studied for expression of HER-2/neu oncoprotein. Immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections of primary tumor and lymph nodes was performed. Immunoprecipitation followed by Western blotting of freshly frozen samples of the same tumors were also performed. Results: Normal colon mucosa, benign lesions, and adenocarcinomas clearly differed in the expression levels and histological distribution of p185<sup>HER-2/neu</sup>. Normal mucosa was mostly negative, but significant number of benign lesions and adenocarcinomas overexpressed HER-2/neu protein. Adenocarcinomas were significantly more positive than benign lesions. The results show significant correlation with the epithelial abnormality degree and clinical parameters including Dukes’ classification and relapse-free and postoperative survival period. Conclusions: The p185<sup>HER-2/neu</sup> rate expression could serve as an independent prognostic factor in patients with p185<sup>HER-2/neu</sup>-positive colorectal malignancies.

Proto-oncogenes, including the HER-2/neu gene (also called c-erbB-2), represent a family of normal cellular genes involved in cell growth and differentiation. There is much evidence that alterations in the structure of these genes or their amplification or overexpression may play a role in the pathogenesis of some human cancers. The HER-2/neu gene encodes a 185-kilodalton transmembrane protein with tyrosine kinase activity that shares approximately 50% of overall homology with epidermal growth factor (EGF) receptor, suggesting a possible role as a membrane receptor.1,2 This proto-oncogene is frequently expressed at low levels in a variety of human adult epithelial cells3 and amplified or overexpressed predominantly in carcinomas of glandular epithelial origin and cell lines derived from them.4–9 This phenomenon has been most intensively studied in breast carcinoma where high levels of HER-2/neu expression have been shown to correlate with the overall clinical course of the disease10 and poor prognosis11,12 and to predict a poorer response to chemotherapy13 and endocrine therapy.14 The expression of HER-2/neu protein has also been described as an important independent prognostic indicator in gastric cancer.15

Recently, we reported a pilot study on a group of 17 patients with HER-2/neu—positive colorectal carcinomas.16 The report showed a positive correlation between HER-2/neu overexpression rate and survival monitored during a 30-month period as well as between overexpression of this oncogene and the time of detection of liver metastases. In the present study, we aimed to determine whether the expression of this protein is related to the progression of disease and whether the intensity of HER-2/neu expression can be a useful prognostic marker of colorectal cancer.

Materials and Methods

Patients and Tissue Specimens

This retrospective study involved 221 specimens of normal colon tissue and benign and malignant colon lesions (Table 1). All specimens were obtained through routine sur-

Abbreviations used in this paper: EGF, epithelial growth factor; MAb, monoclonal antibody; SDS, sodium dodecyl sulfate.

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gery performed at the medical centers of Šibenik and Split, Croatia. The diagnoses were established by standard diagnostic procedures and confirmed histopathologically. Staging was performed by Turnbull's modification of Dukes' staging. The patients were followed up according to a standardized protocol that included laboratory tests (i.e., routine blood count) at 1-2-month intervals, chest radiography, ultrasonography, and computerized tomography, examination of the liver, and endoscopy of the colon at 1-year intervals during the first 3 postoperative years and thereafter in 6-12-month periods. A disease-free interval was defined as the time from resection to the first clinically detectable recurrence of tumor. 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. Treatments by other causes were censored observations from the time of death.

None of the patients underwent preoperative radiation or chemotherapy. The study included 128 men and 89 women with age range between 25 and 88 years (mean age, 63.8 years).

Carcinomembrane antigen estimations were not routinely used and therefore were not analyzed.

Each specimen was routinely fixed in 10% formalin and immersed in melted paraffin. Four micrometer sections were cut and mounted onto glycerine-treated slides. Fresh samples of resected colon carcinoma, immediately adjacent to the segment of tissue that was fixed in formalin, were snap-frozen in liquid nitrogen and stored in a Human Tumor Bank at −80°C until further use. Before inclusion in the study, each specimen was verified by a histopathologist.

**Cell Lines**

Human breast carcinoma (SK-BR-3) and human colon carcinoma (HT-29 and SW-480) cell lines were used. The cells were cultured in Dulbecco's modified Eagle medium (GIBCO, Gaithersburg, MD) supplemented with 10% fetal calf serum. For immunocytochemical studies, the cells were grown in chamber slides (Nunc, Roskilde, Denmark) overnight in a humidified atmosphere with 5% CO2 at 37°C.

**Immunohistochemical Detection of Oncogene Proteins**

After deparaffinization in xylene, the slides were washed in phosphate-buffered saline (PBS) (three times for 3 minutes). The endogenous peroxidase activity was quenched by 5-min incubation in mixture with 3% hydrogen peroxide (Sigma Chemical Co., Deisenhofen, Germany). The fixation slides were then cleaned with PBS. Nonspecific binding was blocked by applying normal rabbit serum to a humidor chamber in a dilution of 1:10 for 30 minutes. Slides were blotted, and the primary monoclonal antibody (MAb) c-myc (Ab-3; Oncogene Science, Uniondale, NY) at a concentration of 2 μg/ml was applied for 15 minutes at room temperature. Slides were washed three times in PBS containing 3%, 2%, and 1% of normal human serum. Secondary antibody (rabbit anti-mouse; Dako, Glostrup, Denmark) diluted with PBS and normal human serum (40 μL rabbit anti-mouse antibody, 50 μL normal human serum, and 910 μL PBS), was added for 1 hour at room temperature. Finally, peroxidase–antiperoxidase (Dako) conjugate diluted 1:100 in PBS was added for 1 hour at room temperature. After washing with PBS, slides were kept in diaminobenzidine tetrahydrochloride for 5 minutes (50 mg in 200 mL PBS with 25 μL of 30% H2O2) (DAB; Sigma Chemical Co.), counterstained with hematoxylin for 30 seconds, immersed in saturated lithium carbonate for 30 seconds, and mounted in PBS-glycerol (1:1).

To verify the immunohistochemical results obtained with c-myc (Ab-3) antibody, we performed an analysis of some samples with a different mouse MAb (C211), which was raised against different sequence in the intracellular domain of p85HER-2/neu. This antibody was a part of the StrAviGen Super sensitive kit (Bio Genex Laboratories, San Ramon, CA). Complete agreement was found between the two antibodies.

**Immunocytochemistry**

Cultured cells were washed in PBS after removing the medium and fixed in methanol. Immunocytochemistry was performed according to the procedure described for the tissue sections.

**Evaluation of Slides**

Each slide was evaluated in the entire tumor area. The concentration of the antigen was assessed by estimating the relative visual intensity of a chromogenic label, and the results were expressed on a 3-point scale as follows: +, weak staining; ++, moderate staining; and +++, strong staining. Each sample was assessed independently by two observers (E.A. and R.S.). There was a 95% internal agreement between them.

**Preparation of Tissue for Biochemical Analysis**

The frozen tissue samples were pulverized to a fine powder in liquid nitrogen. The powder was redissolved in PBS with 1 μmol/L phenylmethylsulfonyl fluoride and aprotinin (2 μg/mL) as protease inhibitors and subsequently centrifuged at 15,000g in an Eppendorf centrifuge (Hamburg, Germany) and ultracentrifuged at 50,000g. The supernatant (S1-cytosol) was used in immunoprecipitation and sodium dodecyl sulfate (SDS) electrophoresis.

The pellet (membranes) was placed in lysis buffer (150 mmol/L NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, and 10 mmol/L Tris-Cl, pH 7.2) and ultracentrifuged at 25,000g. The supernatant (S3,membranes) was used for immunoprecipitation as well.

The cells were redissolved in lysis buffer with 1 μmol/L phenylmethylsulfonyl fluoride and aprotinin (2 μg/mL) and centrifuged at 15,000g in an Eppendorf centrifuge. The supernatant (S3) was used for immunoprecipitation and SDS electrophoresis.

**Immunoprecipitation**

MAb c-myc (S3, Ab-3; Oncogene Science) (1 μg/mL) was incubated with supernatant (1 mg total protein at S3).
S2, or S3) for 1 hour on ice with shaking. Immunoprecipitation was followed with the addition of protein A–Sepharose (Pharmacia, Uppsala, Sweden) for the next 2 hours. Reaction was stopped by adding cold lysis buffer and centrifuged in an Eppendorf centrifuge for 5 minutes at 15,000g. Immunoprecipitates were washed three more times with cold lysis buffer. Sample buffer for SDS–polyacrylamide gel electrophoresis (PAGE), 50 μL, was added to each precipitate, and the samples were frozen at −20°C.

**SDS-PAGE**

Electrophoresis was run in a Tris-glycine buffer (0.025 mol/L Tris, 0.19 mol/L glycine, and 1% SDS, pH 8.6) on 7.5% polyacrylamide gel according to the method of Laemmli. Molecular weights were calculated according to the high-molecular-weight standards (GIBCO BRL).

**Immunoblotting (Western Blotting)**

Electrophoretically separated proteins were electrophoretically heated to nitrocellulose membrane (BA 85, 0.45 μm; Schleicher & Schuell, Dassel, Germany) in electrode buffer (25 mmol/L Tris and 192 mmol/L glycine, pH 8.3) at 150 mA for 45 minutes with a cooling Midget Multiblot Electrophoretic Transfer Unit (LKB 2031; Pharmacia). 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 (Sigma), 0.05% Tween 20 (Sigma), and 5% nonfat milk in PBS for 30 minutes. The membrane was then incubated with primary MAb c–revB-2 (3B5, Ab-3) or P-Tyr (Ab-2) in a concentration of 10 μg/mL in 0.3% bovine serum albumin plus 0.05% Tween 20–PBS for 2 hours at room temperature with agitation. After washing with the same buffer, the membrane was incubated with secondary antibody conjugate in a concentration of 0.2 μg/μL. The reaction was stopped with stop buffer. The membrane was washed in distilled water and dried.

**Controls**

Control staining was performed either by omitting the primary antibody, by the use of nonimmune serum and irrelevant antibodies, or by preincubating the primary antibodies with the peptide antigen (1:10; Oncogene Science). As a positive control in immunohistochemical studies, we used paraffin slides of the invasive breast carcinoma.

In immunocytochemical studies, we used SKBR-3 cells as a positive control c–revB-2–positive breast carcinoma cell line. As a negative control we used colon cancer cell line SW480 (colon cancer that had shown earlier that it does not express c–revB-2 protein).

**Statistical Analysis**

Categorical data were analyzed using χ² statistics. The probability of survival was calculated for the different subgroups by the Kaplan–Meier method. Four-week mortality was excluded from the survival curves. Statistical differences were evaluated by the Mantel–Cox test. All evaluations were performed using BMDP Statistical Software (Cork, Ireland). Multivariate analysis was performed with Cox’s proportional hazards model. A stepwise procedure was used to identify the major prognostic indicators independently associated with survival.

**Results**

**Specificity of Antibodies for p185HER-2/neu**

A series of specific antipeptide antibodies for the HER-2/neu gene product have been described and shown to cross-react with the closely related proteins. However, to verify the specificity of the MAbs used in these experiments, we tested them immunocytochemically on a human breast carcinoma cell line SK-BR-3 and on human colon carcinoma cell lines HT-29 and SW-480 (Figure 1). The cell lines SK-BR-3 (Figure 1A) and HT-29 (Figure 1B) have been previously shown to overexpress HER-2/neu protein. As a negative control, we used a colon adenocarcinoma cell line SW-480 (Figure 1C), in which this protein was not expressed in measurable quantities. Confirmation of the specificity of antibodies was established by immunoprecipitation of cell lysates and Western blot analysis that in both cases showed precipitation of a single band at 185 kilodaltons. As shown in Figure 2, p185HER-2/neu was detected in cell lysates of SK-BR-3 and HT-29 cells but not in SW-480 cells. To verify that the antibodies used did not cross-react with molecules structurally related to p185HER-2/neu, we also performed Western blot analysis with antibodies specific for HER-2/neu, EGFR receptor, and HER-3/erbB-3 product that followed immunoprecipitation of cell lysates with HER-2/neu–specific antibodies. Our results showed that HER-2/neu–specific antibodies do not recognize structurally similar proteins, namely, EGFR receptor and HER-3/erbB-3 (data not shown).

Paraffin slides of invasive breast carcinoma were used as a positive control for immunohistochemistry and showed a strong, predominantly membrane-bound immunoreaction (Figure 1D). Control staining on paraffin sections of colon adenocarcinomas was performed by omitting the primary antibody, with no immunoreaction observed (Figure 1E and F).

**p185HER-2/neu in Colorectal Lesions**

Two hundred twenty-one colon samples (45 samples of normal colon and 176 samples of different colon neoplastic lesions) were examined for the presence of HER-2/neu oncoprotein (Table 1). Normal mucosa, polyps, adenomas, and adenocarcinomas of colorectal origin involved in our study clearly differed in the intensity and
Figure 1. p185HER2 immunostaining in (A) human breast carcinoma cell line SK-BR-3, (B) human colon carcinoma cell line HT-29, and (C) SW480 cells using MAb 611-1 (Ab-3; Oncogene Science). SK-BR-3 cells showed predominantly membrane staining, HT-29 cells showed mixed membrane and cytoplasmic staining, and SW480 cells showed no staining. (D) p185HER2 immunostaining in human breast carcinoma. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue specimen. (E) p185HER2 immunostaining in human colon adenocarcinoma. Tumor cells showed cytoplasmic staining. (F) Negative control of the same specimen. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue specimens (original magnifications: A–C, E, and F, 1000×; D, 400×).
Figure 2. Immunoprecipitation of cell lysates followed by Western blot analysis with an antibody to p185/erbB-2 monoclonal antibody. The antibodies showed a single band at 185 kDa in cell lysates of SKBR3 (lane 1) and HT-29 (lane 2) cells but not in SW480 (lane 3) cells.

Table 1. Immunohistochemical Reactivity of Anti-erbB-2 MAb in Normal Colon, Adenomas, and Adenocarcinomas

<table>
<thead>
<tr>
<th>Histological type</th>
<th>No. positive/ no. tested</th>
<th>Staining (% positive cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Normal colon</td>
<td>5/29</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Hyperplastic polyp</td>
<td>13/16</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Tubular adenoma</td>
<td>15/17</td>
<td>4 (24)</td>
</tr>
<tr>
<td>Tubulovillous adenoma</td>
<td>4/4</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>155/155</td>
<td>25 (16)</td>
</tr>
</tbody>
</table>

NOTE. χ² statistics showed that reactivity (staining) is highly dependent on heterotrophic type of lesion (total χ² = 26.894, P < 0.001, df = 8). *Normal tissue adjacent to carcinoma.
Figure 3. p16INK4A immunostaining in human (A–C) benign colon lesions, (D and E) colon adenocarcinomas, and tumor cells in the (F) submucosa and (G) regional lymph node under the immersion objective using MAb Onco (Ab-5). Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue specimens (original magnifications: A and D, 250×; B, C, E, and F, 1000×).
Figure 4. Immunoprecipitation of the colon carcinoma sample lysates with the anti-p185HER-2/neu antibodies: SKBR-3 cells (lane 1) and the tumor samples that immunohistochemically showed weak (lane 2), moderate (lane 3), and strong (lane 4) positivity.

Figure 5. (A) Immunoprecipitation of the two colon carcinoma samples, cytosolic (C) and membrane (M) proteins with p185HER-2/neu antibodies, (B) followed by Western blot analysis using P-Tyr (Ab-2) antibodies.

Figure 6. Survival of 119 patients with p185HER-2/neu-positive colorectal cancer stratified by the intensity of p185HER-2/neu immunostaining.
Table 2. Relationship of erbB-2/HER-2 Staining to Dukes' Stage

<table>
<thead>
<tr>
<th>Dukes' Stage</th>
<th>No. of cases</th>
<th>erbB-2/HER2 staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>34</td>
<td>13</td>
</tr>
<tr>
<td>B</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>77</td>
<td>10</td>
</tr>
</tbody>
</table>

Values: šχ² statistics showed that staining is highly dependent on Dukes' stage of tumors (total šχ², 26.984; P = 0.001; df = 8).

Table 3. Survival of 80 Patients With Colorectal Cancer Stratified by Clinicopathologic Features

<table>
<thead>
<tr>
<th>Regression analysis</th>
<th>No. of cases</th>
<th>5-yr survival (%)</th>
<th>Relative risk of death</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>70</td>
<td>43</td>
<td>63</td>
<td>1</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>43</td>
<td>42</td>
<td>1</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>≤5</td>
<td>55</td>
<td>44</td>
<td>1</td>
</tr>
<tr>
<td>Histological grade</td>
<td>Well</td>
<td>11</td>
<td>12</td>
<td>0.9</td>
</tr>
<tr>
<td>erbB-2 staining</td>
<td>Weak positive</td>
<td>22</td>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Strong positive</td>
<td>40</td>
<td>46</td>
<td>0.44</td>
</tr>
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</table>

Discussion

Amplification and overexpression of the erbB-2 oncoprotein gene have been reported in adenocarcinomas of various origin, suggesting the importance of this oncoprotein in the neoplastic process. However, despite the large number of studies performed, details on the role of the HER-2 oncoprotein in the carcinogenesis remain vague. In many adenocarcinomas, particularly in breast and stomach, its overexpression closely correlates with the overall clinical course of the disease and survival of patients. For this study, we analyzed the expression of HER-2 oncoprotein in normal human colon tissue and in samples representing different stages of neoplastic progression. We show that normal colon tissue and benign and malignant colon lesions clearly differ in the

Table 4. Stepwise Regression Model

<table>
<thead>
<tr>
<th></th>
<th>i</th>
<th>SE</th>
<th>RR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukes' stage</td>
<td>0.6128</td>
<td>0.219</td>
<td>1.845</td>
<td>0.0052</td>
</tr>
<tr>
<td>erbB-2 staining</td>
<td>0.6153</td>
<td>0.2498</td>
<td>1.845</td>
<td>0.0045</td>
</tr>
</tbody>
</table>

RR: relative risk.
rate of HER-2/new protein expression and its intracellular localization and that, in colorectal cancer, the level of HER-2/new expression correlates with the relapse-free period and postoperative survival time. Furthermore, using the relevant statistical methods, we show that the expression with the age of the patients and Dukes’ stage, the expression of p185HER-2/new is an independent prognostic factor in p185HER-2/new-positive colorectal cancers.

Although some reports suggest that the amplification of HER-2/new gene in colorectal carcinoma is not a very frequent event, 6,22,26 we found that a significant number of adenomas and adenocarcinomas overexpress HER-2/new protein. This difference is not surprising because it is known that HER-2/new overexpression may occur via a number of different mechanisms 21,27,28 and is not only a result of gene amplification. The histological as well as intracellular distribution of HER-2/new product that we observed was similar to that previously described in colorectal lesions. 22 In normal colon tissue and polyps, p185HER-2/new was localized in the luminal surface cells with no crypt involvement. The immunostaining was associated with both cytoplasm and membrane, with the basolateral region of the apical cells staining most intensively. Tubular adenomas showed a similar pattern of immunostaining. However, in these cases, the staining was stronger, less polarity was noted, and the localization of HER-2/new protein extended into the cells of the crypt. Interestingly, a similar picture was also observed in the normal tissue adjacent to carcinoma (but not adjacent to adenoma). D’Emilia et al., 22 who also noted this, analyzed the expression of p185HER-2/new in histologically normal tissue adjacent to carcinoma and showed that such aberrant distribution of the HER-2/new product is restricted only to the regions located 5 cm proximal and distal from the tumor. This finding was confirmed by our results because the samples of the normal mucosa that showed positivity to p185 were those located within 5 cm from the tumor. All these results suggest the existence of alterations associated with compromised mucosal elements in this region, presumably as a result of contact with soluble molecules released from the tumor cells.

In the substantial number of the HER-2/new-positive cases involved in this study, the cells showed membrane but also the intracytoplasmic immunostaining. This result is consistent with the number of other studies on various tumors and normal tissues. 19,20,22,29,30 However, these findings are a matter of numerous discussions because p185HER-2/new has many properties of a “classical” growth factor transmembrane receptor 1,32; therefore, it is expected to be localized predominately on the cell membrane. De Potter et al. 33 suggested that in breast lesions only a membrane staining should be considered as specific, whereas a cytoplasmic one is a result of cross-reactivity with a yet unknown 155-kilodalton protein located on the mitochondrial cristae of normal and malignant cells. However, our results showed that in the colon lesions this is not the case because immunoprecipitation and Western blotting of cell lysates with specific antibodies resulted in detection of a single band at 185 kilodaltons. Furthermore, separate analysis of cytosol and membrane preparations with p185HER-2/new antibodies showed that only one specific band of the same size was present in both fractions. Peptides immunoprecipitated by anti-p185HER-2/new antibodies from both fractions have been also recognized by anti-PTyr antibodies in Western analysis, implicating that in both cases anti-p185HER-2/new antibodies immunoprecipitated a functionally active mature form of this tyrosine kinase receptor. These data are consistent with recent results by Xi and Hung 34 who showed that p185HER-2/new, on stimulation by its ligand, may be translocated to the nucleus where it exerts the transactivating function. Similarly to us, these investigators have shown phosphorylationally that p185HER-2/new could be detected not only on cell membranes but also in the cytoplasm and in the nucleus of stimulated cells.

In agreement with evidence that intracytoplasmic staining is not an artifact is the finding that the intracellular distribution of immunoreactive p185HER-2/new parallels the morphological and functional differences between normal cells of colonic mucosa and adenocarcinoma cells. In epithelial columnar-like cells of normal colonic mucosa and polyps p185HER-2/new was localized in the basolateral regions of cells, but in adenomas, distinction in p185HER-2/new staining pattern between basolateral and other regions of the cells was greatly reduced. Finally, in adenocarcinomas, this distinction is completely lost; the cells are stained evenly and no polarity could be noted. This finding is similar to that described by D’Emilia et al. 22 and agrees with the recent results of Kay et al. 35 who showed only intracytoplasmic c-erbB-2 staining in colon carcinoma as well as those of Campiglio et al. 35 who showed colocalization (and possible physical interaction) of the p185HER-2/new and integrin αβ3 in lung carcinomas.

The behavior of colorectal cancer is believed to be dependent on multiple genetic events throughout the genome. Fearon and Vogelstein 36 have proposed the model of preferential sequence of mutational changes that is widely accepted as one of the dogmas of human tumor genetics. However, even these investigators have not excluded the possibility of existence of other alterations and suggested that accumulation of mutations is more important than the order in which they occur. The ques-
tion remains whether the altered level of HER-2/neu gene expression reflects a primary event in the generation of neoplastic lesions of the colon and, if so, on which stage in the multistep process of the colon tumorgenesis does it become important. Our results showed that overexpression and altered distribution of p185HER-2/neu could be detected in the early stages of the colon tumorigenesis. This result is not unexpected because it has been already shown that HER-2/neu is, when overexpressed, capable of inducing malignant transformation in vitro and in vivo. 

Also, during human breast cancer development, activation of HER-2/neu seems to be one of the earliest events. 

On the other hand, this oncogene product may also play its role in the advanced stages of certain cancers, especially by enhancement of metastasis potential through the induction of metastasis-associated properties of tumor cells. This feature has been suggested by the experimental evidence on B16 cells, 

11 colorectal and non-small cell lung cancer cell lines, as well as transgenic animals. These data have also been supported by our finding that patients bearing colorectal tumors with highly overexpressed p185HER-2/neu had a significantly shorter metastasis-free period than patients with low and moderately p185HER-2/neu-positive tumors. Kudkinsky et al. showed that expression of EGF receptor by human colorectal cancer cells in vitro directly correlated with their ability to produce hepatic metastasis. 

Finally, one cannot exclude the possibility that HER-2/neu activation may also contribute to peritoneal carcinomatosis. In such a scenario, the rate of p185HER-2/neu expression would be that of resistant cell of tumor cells that have been transformed by the sequence of other genetic changes for additional growth-regulatory stimulants.

Regardless of whether the accumulation of the p185HER-2/neu protein is the cause in consequence of the colon cancerogenesis, our results show that the amount of this oncogene in p185HER-2/neu-positive tumors correlates positively with the stage of the disease and patient's survival time. Regression analysis confirmed that intensity of cerb-2 staining was independently related to survival, suggesting a role of cerb-2 expression in colorectal carcinogenesis. These results are in accordance with the recent study of Kay et al., who showed that cytoplasmic HER-2/new protein expression correlates with survival in patients with colorectal cancer. Further studies on the role of this oncogene in colorectal cancer will show whether it can be successfully used as a prognostic marker as well.

References


