

Expression of c-erbB-2 Oncoprotein in Primary Human Tumors : An Immunohistochemistry Study

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c-erbB-2 is a proto-oncogene that codes for a putative membrane receptor which has structural similarity to epidermal growth factor receptor. This c-erbB-2 oncoprotein has subsequently been reported to be demonstrated in cell lines of tumors of the mammary gland, intestine, lung, liver and thyroid in rodents, leading to the hypothesis that the gene is transcriptionally active in different tissue at different stages of development and is sensitive to mutation at these stages.¹

Surveys of human tumors have shown c-erbB-2 oncoprotein amplification in tumors from a number of organ sites, but restricted to adenocarcinoma.²⁻⁴ Monoclonal and polyclonal antisera against the protein product have shown expression of this oncoprotein in a variety of tissues and tumors by immunohistochemistry,⁵⁻⁷ including formalin-fixed and paraffin-embedded material.^{6,8}

The authors undertook this study to investigate the extent and pattern of staining of c-erbB-2 oncoprotein in variety of tumors from Thai patients using a specific monoclonal antibody known to be

SUMMARY An immunohistochemical study was performed with 130 primary malignant human tumors of breast (n=55), colon/rectum (n=16), stomach (n=19), esophagus (n=14), lung (n=15) and liver (n=11) using the 21N c-erbB-2 specific monoclonal antibody to identify the tumors that over-expressed the c-erbB-2 oncoprotein. Positivity appeared as an intense brown granular staining located predominantly at the cell membrane. This occurred in 41.8% of breast carcinomas, 12.5% of colorectal adenocarcinomas. None of the gastric adenocarcinomas, squamous cell carcinomas of the esophagus, small cell lung carcinomas or hepatocellular carcinomas were positive for the oncoprotein. The result of this study suggests that over-expression of the c-erbB-2 oncoprotein is common in breast cancer and relatively rare in other malignancies examined.

effective in formalin-fixed, paraffin-embedded material.

MATERIALS AND METHODS

Specimens

On hundred and thirty primary human tumor specimens were included in this study. They were primary tumor of breast (n=55), colon/rectum (n=16), stomach (n=19), esophagus (n=14), lung (n=15) and liver (n=11). These tumors were of varying sizes and grading. The tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections from these tumors were stained with hematoxylin and eosin for histopathological study. A represen-

tative block from each primary tumor was selected for immunohistochemical study.

Immunohistochemical study

The 5 μ m sections of each specimen were deparaffinized, rehydrated, washed in distilled water, followed by 5-minute incubation in 3% H₂O₂ solution at room temperature to block any endogenous peroxidase, washed in phosphate-

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buffered saline (PBS) and incubated with normal serum as blocking reagent to minimize nonspecific binding. A 1:200 dilution of the affinity-purified monoclonal antibody specific for the 185-kDa c-erbB-2 protein (Biosciences, California) was applied to the specimens and incubated for 30 minutes, then incubated with a second biotinylated antibody (Biosciences, California) followed by the avidin-biotin complex reagent (Biosciences, California). The peroxidase reaction was developed using diaminobenzidine as chromagen. The specimens were counterstained with hematoxylin and mounted. Negative control slides consisted of specimens which were incubated in PBS instead of the specific monoclonal antibody and were run concurrently. Each run also included a positive control slide shown previously to give a strong positive reaction with the c-erbB-2 oncoprotein. Only tumors that showed distinct cell membrane staining were considered positive for over-expression of the c-erbB-2 oncoprotein. Sections that had equivocal staining were scored as negative.

RESULTS

No staining was seen in normal, metaplastic or dysplastic adjacent mucosa. Positive staining was seen in malignant epithelium only, appearing as heterogeneous, intense brown granular staining located predominantly at the cell membrane (Fig. 1).

The positive staining specimens were obtained in 41.8% (23/55) of the invasive ductal carcinoma of the breast, 12.5% (2/16) of the colorectal adenocarcinoma. None of the gastric adenocarcinomas, squamous cell carcinomas of esophagus, small cell lung carcinomas or hepatocellular carcinomas were positive for the oncoprotein (Table 1). All 55 cases of breast cancer had lymph node metastases. No component of ductal carcinoma *in situ*

was seen in these tumors. Two colorectal tumors that stained positively were well-differentiated adenocarcinomas with lymph nodes metastases.

DISCUSSION

The overall tumor cell membrane staining positive for c-erbB-2 oncoprotein was demonstrated in

only 19.2% (25/130) of tumors in this study. Compared to 24% (35/405) overall positivity by McCann⁸ who studied 405 primary malignant human tumors, and showed positivity for the c-erbB-2 oncoprotein in 32/191 (17%) of the breast carcinomas, 1/23 (4%) of the colorectal tumors, 1/48 (2%) of bladder carcinomas and 1/84 (1%) of non small

Table 1. c-erbB-2 oncoprotein expression in human tumors.

Tumor type	No. of c-erbB-2 positive/total no. of specimens stained (%)
Invasive ductal carcinoma of the breast	23/55 (41.8)
Colorectal adenocarcinoma	2/16 (12.5)
Gastric adenocarcinoma	0/19 (0)
Squamous cell carcinoma of esophagus	0/14 (0)
Small cell lung carcinoma	0/15 (0)
Hepatocellular carcinoma	0/11 (0)

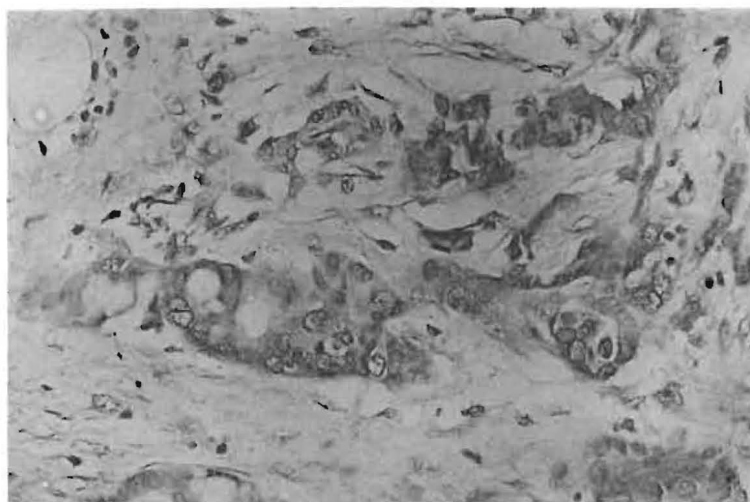


Fig. 1 Invasive ductal carcinoma of the breast demonstrating typical pattern of positivity as strong reactivity in cytoplasmic membrane ($\times 400$).

cell lung cancer. No staining was recognized in small cell lung cancer, prostatic carcinoma and malignant melanoma. Slamon⁹ demonstrated that 28% of invasive ductal carcinomas had overexpression of c-erbB-2 oncogene. Numerous factors may be responsible for the difference in results of these studies, Nadji¹⁰ had experience concerning the quality of fixation of tissue which is one of the most important factors in determining the outcome of an immunoperoxidase reaction. They found that delayed fixation, inadequate fixation and poor fixation may produce false-positive or false-negative results. Optimal fixation time is also important. Prolonged fixation causes loss of cellular antigens. However, this problem can be overcome by using frozen tissue samples.¹¹ The advantage of immunoperoxidase stain is its ability to specifically assess the status of the protein in tumor cells and it was not subjected to dilution effects introduced by surrounding non-malignant cells and/or stromal proteins. Schimmelpenning¹² showed evidence that c-erbB-2 oncoprotein expression in invasive ductal carcinoma will markedly elevated in aneuploid tumors compared to the tumors containing diploid DNA. Unfortunately, the nuclear DNA distribution pattern in this series was not studied. According to Allred¹³ and other studies, most overexpression of c-erbB-2 oncoprotein in breast carcinoma was associated with the subset of invasive ductal carcinoma combined with ductal carcinoma *in situ* more than invasive ductal carcinoma alone. In this study, all breast tumors were invasive ductal carcinomas without an intraductal component. Muller¹⁴ had shown that the development of carcinoma associated with "neu" overexpression appeared to be restricted to breast tissue. These studies motivated many others within the past 3 years, showing that amplification and/or

overexpression of c-erbB-2 is common in human breast cancer,^{5,7-9} and rare or absent in sarcomas as well as in small round-cell tumors of childhood.¹⁵

Positivity for the c-erbB-2 oncoprotein was easily identifiable as an intense brown staining localized predominantly at the cell membrane. This supported the view that the c-erbB-2 oncoprotein is a transmembrane molecule. Most of the positive staining tumors revealed the heterogeneous distribution of staining which suggested that small populations of tumor cells that have elevated levels of the c-erbB-2 oncoprotein can be indicated by staining.

Wright¹⁶ indicated that overexpression of c-erbB-2 oncoprotein was associated with a poor clinical outcome, the survival rate of patients being decreased. In our study we were unable to obtain adequate follow-up data, so the investigation of the prognostic significance of staining was not possible. But this is an issue which deserves further study.

In conclusion, our results indicated that overexpression of c-erbB-2 oncoprotein is more common in breast cancer than other cancers. This can be identified by an immunohistochemical technique in paraffin-embedded tissue. The prognostic significance of c-erbB-2 oncoprotein expression of these tumors in Thai patients requires further investigation.

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