Virchows Archiv A Pathol Anat (1992) 421:393–400

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistochemical localization of c-*erb*B-2 protein and epidermal growth factor receptor in normal surface epithelium, surface inclusion cysts, and common epithelial tumours of the ovary

Da-peng Wang[1], Ikuo Konishi[1], Masafumi Koshiyama[1], Yoshihiko Nanbu[1], Toshiko Iwai[1], Hirofumi Nonogaki[1], Takahide Mori[1], and Shingo Fujii[2]

[1] Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan
[2] Department of Obstetrics and Gynecology, Shinshu University School of Medicine, Matsumoto, Japan

Received May 18, 1992 / Received after revision July 6, 1992 / Accepted July 14, 1992

Summary. The c-*erb*B-2 (HER-2/*neu*) protein is a membrane glycoprotein growth factor receptor showing molecular homology with the epidermal growth factor receptor (EGFR). We examined the immunohistochemical reactivity of monoclonal antibodies against both of these proteins in normal surface epithelium, surface inclusion cysts, and common epithelial tumours of the ovary. The ovarian tumours were classified as benign (16), borderline malignant (2), and malignant (19). Normal surface ovarian epithelium was weakly positve for both c-*erb*B-2 protein and EGFR. In surface inclusion cysts, however, the epithelial cells lining the lumen exhibited stronger staining for c-*erb*B-2 protein, but no staining for EGFR. All 16 benign ovarian tumours and the 2 borderline malignant ovarian tumours were positive for c-*erb*B-2 protein and negative for EGFR. Of the ovarian carcinomas, 13 of the 19 (68.4%) were positive for c-*erb*B-2 protein and negative for EGFR, while 4 showed positivity for both c-*erb*B-2 protein and EGFR. Two cases were negative for both proteins. Expression of both c-*erb*B-2 protein and EGFR was found in endometrioid carcinoma with squamous differentiation and in clinically advanced poorly differentiated serous carcinomas. Expression of c-*erb*B-2 protein appears to be increased and that of EGFR is reduced in the early stage of epithelial ovarian oncogenesis. The expression of EGFR with c-*erb*B-2 protein in ovarian carcinoma is related both to histological differentiation and/or advanced clinical stage.

Key words: c-*erb*B-2 protein – Epidermal growth factor receptor – Ovarian tumour – Oncogenesis

Introduction

The human c-*erb*B-2 or HER-2/*neu* proto-oncogene encodes a 185 kDa transmembrane glycoprotein, c-*erb*B-2 protein, which has molecular homology with epidermal growth factor receptor (EGFR) (Coussens et al. 1985; Yamamoto et al. 1986). The inner domain of c-*erb*B-2 protein has tyrosine kinase activity which is also demonstrated in EGFR and the c-*erb*B-2 protein is considered to be a growth factor receptor. The ligand for c-*erb*B-2 protein has recently been identified as a 30 kDa factor (gp30) (Lupu et al. 1990). In breast carcinomas, amplification of c-*erb*B-2 gene and/or over-expression of this gene product in tumour tissues have been reported to correlate with poor prognosis (Slamon et al. 1987). The physiological function of c-*erb*B-2 protein in normal human tissues remains uncertain, although immunohistochemical expression of this protein has been demonstrated both in fetal tissues (Mori et al. 1989) and in adult tissues including the epidermis, oral mucosa, kidney, and female genital tract (Press et al. 1990; Wang et al. 1992). An inverse relationship between the expression of c-*erb*B-2 protein and EGFR has been reported in the differentiation of keratinocytes in normal adult skin (Maguire et al. 1989) and in the differentiation and function of cells in the mullerian-derived female genital tract and placenta (Wang et al. 1992), and in oncogenesis in the kidney (Weidner et al. 1990). These findings suggest that the switching of expression of the two receptor proteins, c-*erb*B-2 protein and EGFR, is associated with differentiation or transformation of cells.

The common epithelial tumours of the ovary are believed to arise in the ovarian surface epithelium via the formation of surface inclusion cysts, in which the surface epithelial cells differentiate into cells resembling the epithelium of mullerian-derived genital tract (Parmley 1987). Although amplification of the c-*erb*B-2 gene and/or over-expression of c-*erb*B-2 protein have been reported in ovarian carcinoma (Slamon et al. 1987; Berchuck et al. 1990), the relationship between the expression of c-*erb*B-2 protein and EGFR in the early stage of oncogenesis is unknown. To elucidate this relationship, we investigated the immunohistochemical localization of the two proteins, c-*erb*B-2 protein and EGFR, in normal surface epithelium, surface inclusion cysts, and common epithelial tumours of the ovary.

Correspondence to: I. Konishi

Materials and methods

Fresh surgical specimens of ovarian tumours were obtained from 37 women who underwent bilateral salpingo-oophorectomy, with or without hysterectomy. Of the 37 tumours, 16 were histologically benign, 2 were of borderline malignancy and 19 were malignant. Eight cases of endometrial (endometriotic) ovarian cysts were included in the benign tumour group. Using the classification of the International Federation of Gynecology and Obstetrics (FIGO), of the 19 cases of ovarian carcinoma, 10 were stage I, 2 were stage II, 4 were stage III, and 3 were stage IV. Histological type and grade of differentiation were determined using the WHO classification (Serov and Scully 1973). The contralateral ovaries of 20 of the ovarian tumour patients were free of tumour and were also used for the study. In addition, we obtained samples of normal ovaries from 7 patients undergoing bilateral salpingo-oophorectomy and hysterectomy for uterine myoma. Examination of apparently normal ovaries disclosed the presence of surface inclusion cysts in 18 of the 27 specimens. The materials were obtained immediately after the surgical procedure, and were snap-frozen in OCT compound (Ames, Elkhart, Ind., USA) and stored at −70° C. Serial cryostat sections were stained with haematoxylin and eosin of light microscopy.

Immunostaining for c-*erb*B-2 protein and EGFR was performed on cryostat sections by the avidin-biotin-peroxidase complex method, using a Histscan Monoclonal Detector Kit (Biomeda, Foster, Calif., USA). In brief, 4 μm cryostat sections were fixed in cold acetone for 5 min, treated with 0.3% hydrogen peroxide, and incubated with normal goat serum to block non-specific binding. Serial sections were then incubated with mouse monoclonal antibody for the external domain of c-*erb*B-2 protein (diluted 1:25, Triton Diagnostics, Alameda, Calif., USA) or with mouse monoclonal antibody for the external domain of EGFR (Ab-1) (diluted 1:80, Oncogene Science, Uniondale, N.Y., USA), or with control normal mouse serum at 4° C overnight. The sections were then treated with biotinylated goat anti-mouse IgG, followed by treatment with avidin-biotin-peroxidase complex. They were then stained with 3-amino-9-ethylcarbazole solution containing 0.15% hydrogen peroxide. Counterstaining was performed with haematoxylin. For positive controls, we used cryostat sections of breast carcinomas for c-*erb*B-2 protein, and sections of squamous cell carcinoma of the cervix for EGFR.

The antibody for the external domain of c-*erb*B-2 protein used in this study stains the gene product not only in a manner that correlates with gene amplification but also in the absence of gene amplification in both breast carcinoma and in normal tissues (Iglehart et al. 1990). The intensity of staining was graded as (−) for no immunostaining, (+) for weak staining, (++) for moderate staining, and (+++) for very intense staining, by the evaluation of two observers.

Results

Specific staining with anti-c-*erb*B-2 protein antibody and anti-EGFR antibody was observed mainly on the cell membrane. The immunohistochemical localization of the proteins in the lesions examined is summarized in Table 1. FIGO stage, histological type, histological differentiation, and immunohistochemical expression of c-*erb*B-2 protein and EGFR in ovarian cancer cases are listed in Table 2.

Table 1. Immunohistochemical expression of c-*erb*B-2 protein and epidermal growth factor receptor (EGFR) in normal surface epithelium, surface inclusion cysts, and common epithelial ovarian tumours

	c-*erb*B-2 protein	EGFR
Normal surface epithelium	+	+
Surface inclusion cysts	++	−
Common epithelial ovarian tumours		
Benign	++	−
Borderline malignant	++	−
Malignant	++/+++	−/++

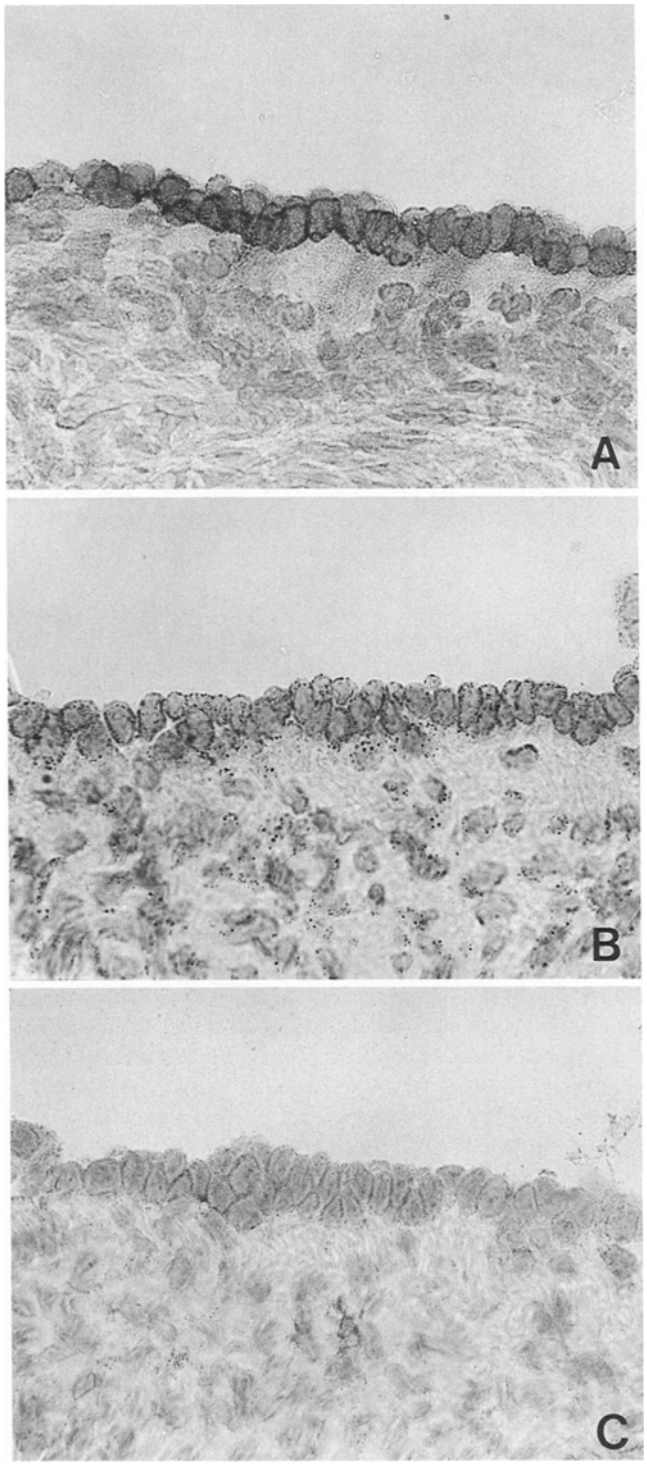

Fig. 1. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and epidermal growth factor receptor (EGFR) (**B**) in normal surface epithelium of the ovary. Surface epithelial cells are weakly positive for both c-*erb*B-2 protein and EGFR. **C** Negative control. ×800

Table 2. FIGO stage, histological type, histological differentiation, and immunohistochemical expression of c-*erb*B-2 protein and EGFR in epithelial ovarian carcinomas

Case	Age	FIGO stage	Histological type	Histological differentiation	c-*erb*B-2 protein	EGFR
1	62	Ia	Serous	Well diff.	++	−
2	62	Ia	Serous	Well diff.	+++	−
3	35	Ia	Mucinous	Mod. diff.	++	−
4	17	Ia	Mucinous	Well diff.	+++	−
5	52	Ia	Endometrioid	Mod. diff.	+++	++[a]
6	59	Ic	Endometrioid	Well diff.	+++	−
7	48	Ic	Endometrioid	Well diff.	++	−
8	39	Ic	Clear cell	Mod. diff.	+++	−
9	51	Ic	Clear cell	Mod. diff.	−	−
10	47	Ic	Transitional	Mod. diff.	−	−
11	87	IIb	Mucinous	Well diff.	+++	−
12	68	IIc	Endometrioid	Mod. diff.	+++	−
13	39	IIIc	Serous	Mod. diff.	++	−
14	68	IIIc	Serous	Poorly diff.	+++	−
15	58	IIIc	Serous	Poorly diff.	+++	++
16	59	IIIc	Clear cell	Mod. diff.	++	−
17	46	IV	Serous	Poorly diff.	+++	++
18	75	IV	Serous	Poorly diff.	+++	++
19	49	IV	Serous	Mod. diff.	+++	−

[a] A case of endometrioid carcinoma with squamous differentiation

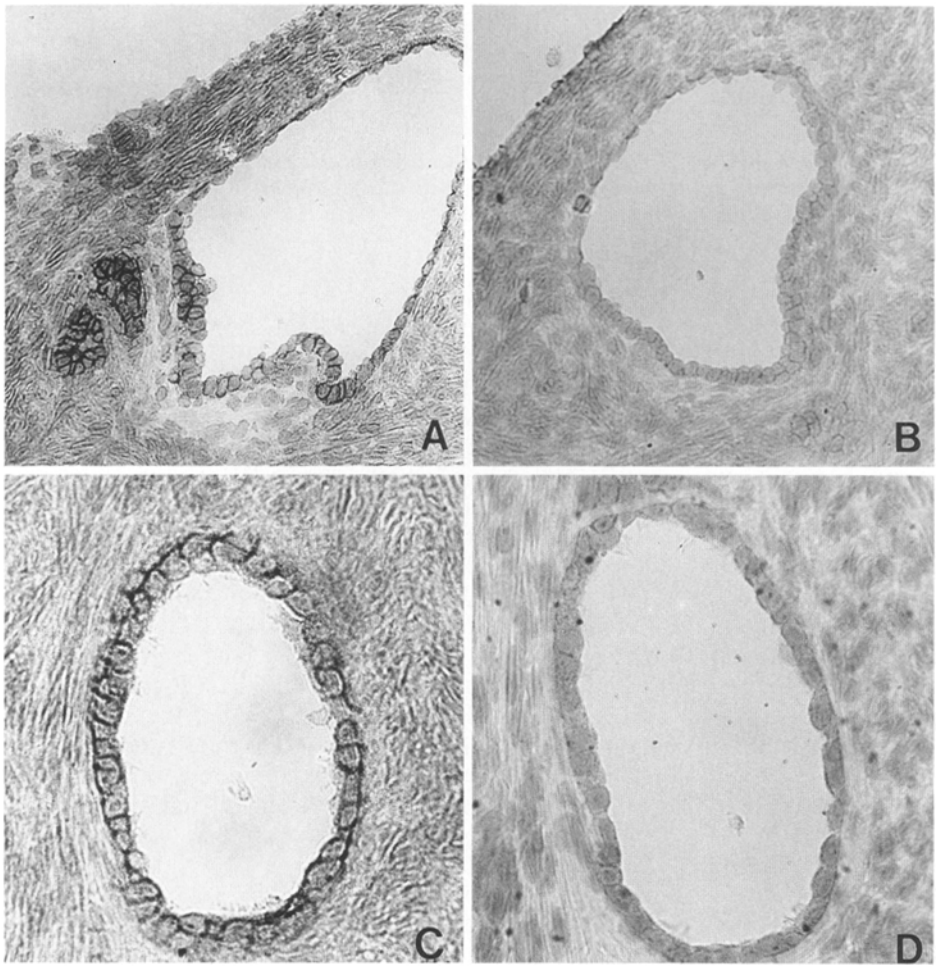

Fig. 2. Immunohistochemical localization of c-*erb*B-2 protein (**A, C**) and EGFR (**B, D**) in surface inclusion cysts of the ovary. Cuboidal epithelial cells, including ciliated cells, are positive for c-*erb*B-2 protein, but negative for EGFR. **A, B** ×400; **C, D** ×800

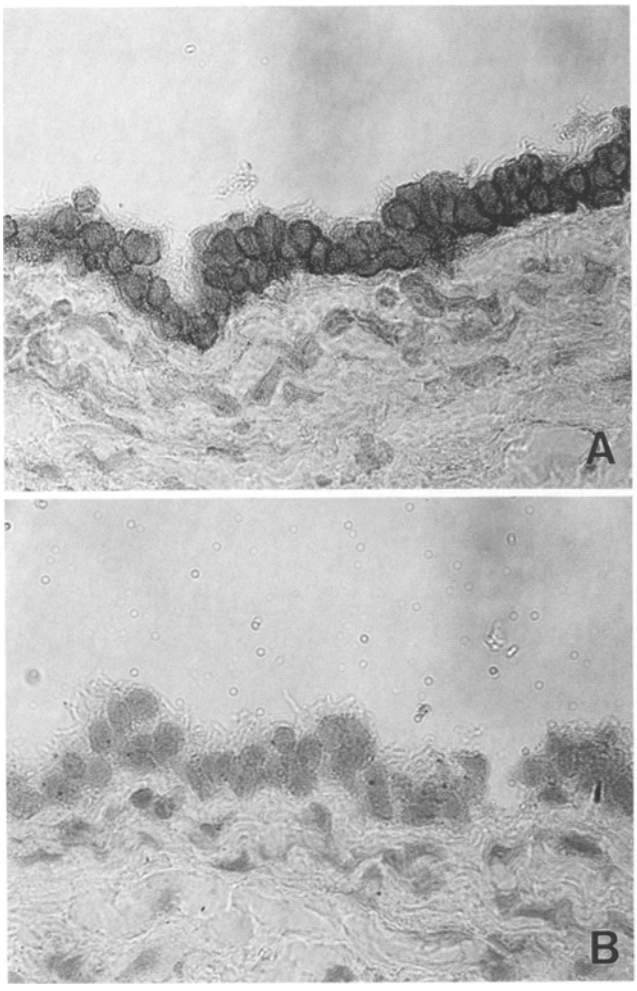

Fig. 3. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in serous cystadenoma of the ovary. The tumour cells are positive for c-*erb*B-2 protein and negative for EGFR. ×800

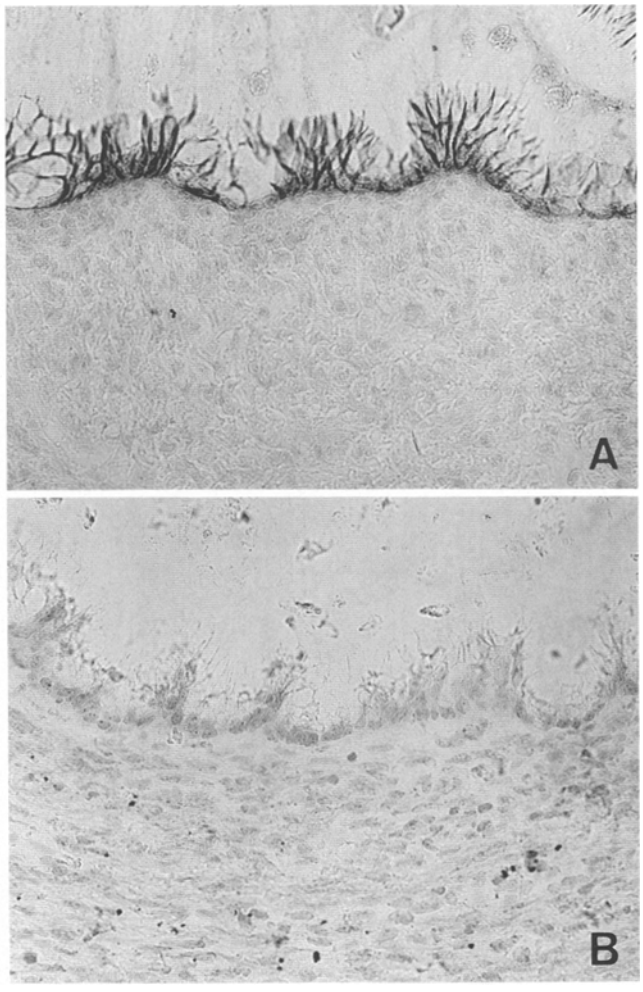

Fig. 4. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in mucinous cystadenoma of the ovary. The tumour cells are positive for c-*erb*B-2 protein and negative for EGFR. ×400

In histologically normal ovaries, surface epithelial cells were weakly stained for both c-*erb*B-2 protein and EGFR (Fig. 1). In the surface inclusion cysts of the ovary, however, both ciliated cuboidal cells, resembling tubal epithelial cells, and columnar cells, resembling endometrial glandular cells, exhibited positive staining for c-*erb*B-2 protein, but no reaction for EGFR (Fig. 2). The staining intensity for c-*erb*B-2 protein in the cells of surface inclusion cysts was stronger than that in the cells of normal surface epithelium.

Of the 16 benign tumours, 3 were serous cystadenomas, 5 were mucinous cystadenomas, and 8 were endometrial (endometriotic) cysts. The epithelial cells of all tumours were positive for c-*erb*B-2 protein and negative for EGFR (Figs. 3, 4). There were no apparent differences in the staining intensity for c-*erb*B-2 protein among the different histological types of ovarian tumour. Both of the serous and mucinous tumours of borderline malignancy showed positive staining for c-*erb*B-2 protein, but were negative for EGFR.

In the ovarian carcinomas, c-*erb*B-2 protein positivity was observed in 17 of the 19 cases (89.5%), while EGFR positivity was observed in 4 of the 19 (21.5%). With respect to the clinical stage, c-*erb*B-2 protein positivity was observed in 10 of 12 stage I or II cases, and in all 7 stage III or IV cases. In contrast, EGFR positivity was found in only 1 of 12 (8.3%) stage I or stage II cases, but in 3 of 7 (42.9%) stage III or stage IV cases.

The 19 carcinomas were histologically classified as serous (8), mucinous (3), endometrioid (4), clear cell (3), and transitional cell (1) carcinoma. Five of the 8 serous tumours showed moderate to intense staining for c-*erb*B-2 protein and no staining for EGFR (Fig. 5); however, the remaining 3 were positive for both c-*erb*B-2 protein and EGFR (Fig. 6). All of the 3 EGFR-positive cases were poorly differentiated, whereas 4 of the 5 cases with no reaction for EGFR were well to moderately differentiated. All 3 cases of mucinous carcinoma were histologically well to moderately differentiated, and were strongly positive for c-*erb*B-2 protein and negative for EGFR. The 4 endometrioid carcinomas were well to moderately differentiated; of these, 3 were c-*erb*B-2 protein positive and EGFR negative. In the remaining endometrioid carcinoma, most tumour cells were c-*erb*B-2 protein positive

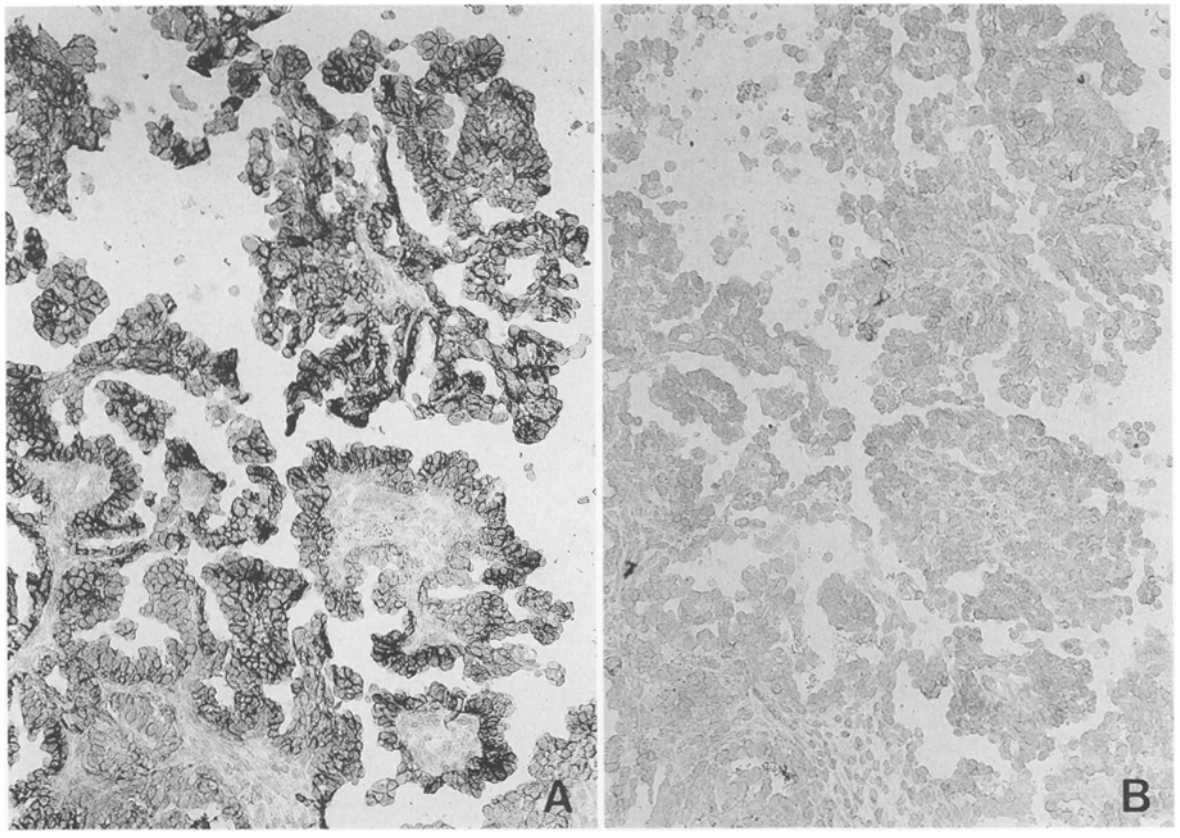

Fig. 5. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in serous cystadenocarcinoma of the ovary. Malignant tumour cells show strong staining for c-*erb*B-2 protein, but are negative for EGFR. ×400

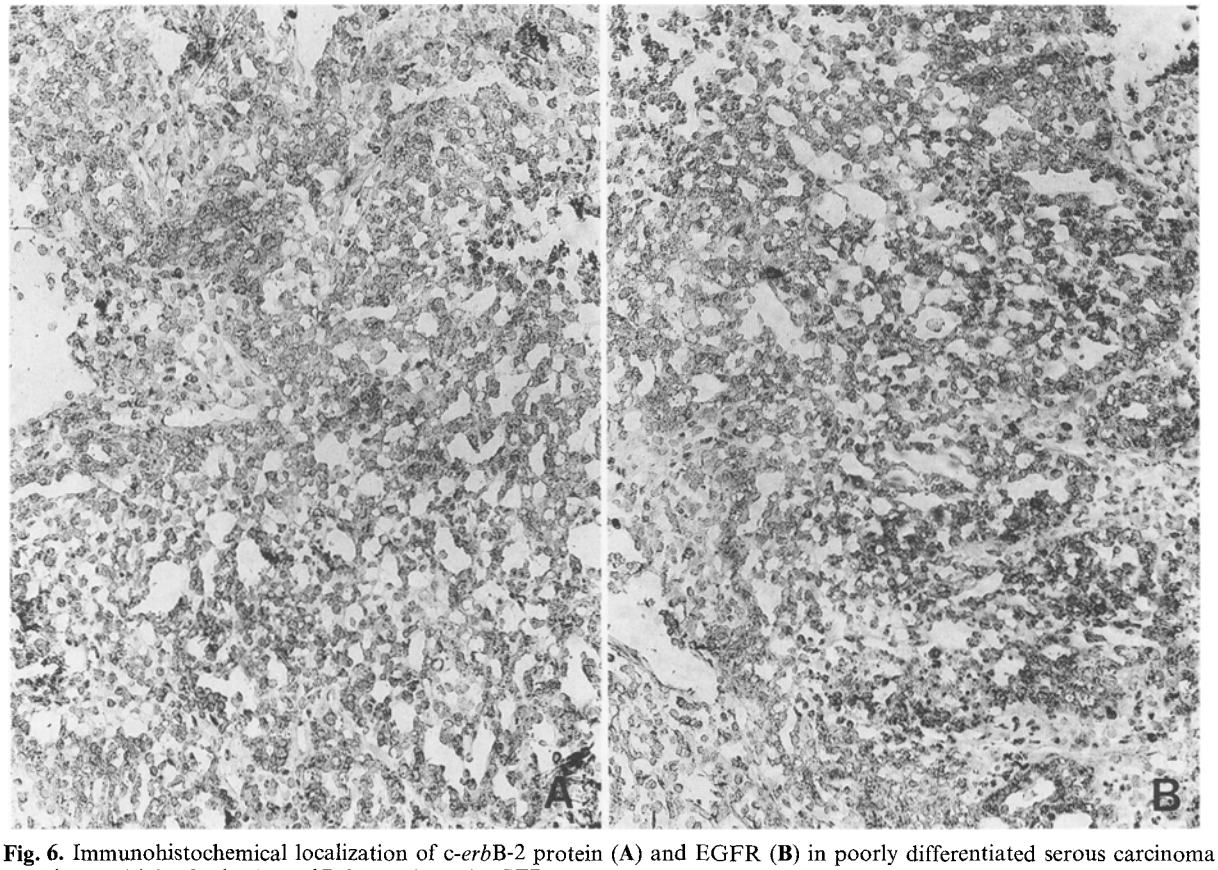

Fig. 6. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in poorly differentiated serous carcinoma of the ovary, showing positivity for both c-*erb*B-2 protein and EGFR. ×400

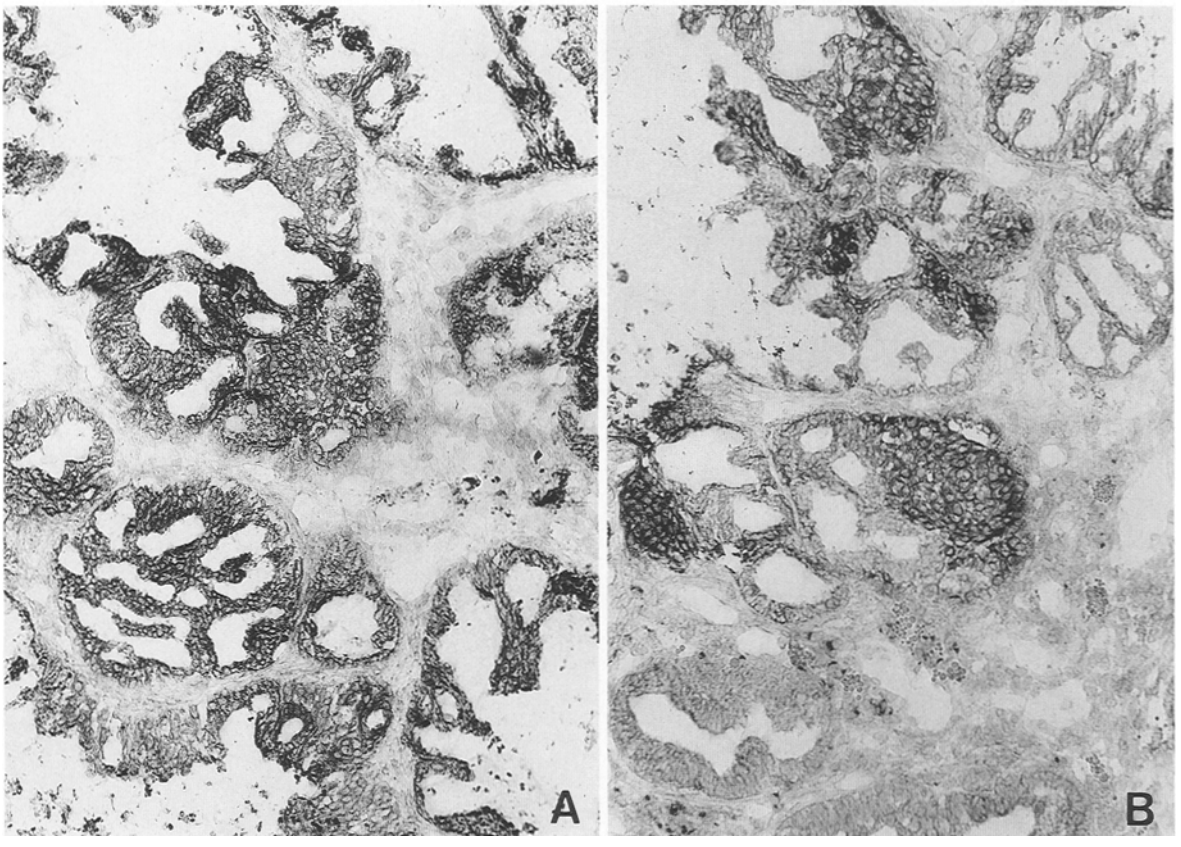

Fig. 7. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in an endometrioid carcinoma of the ovary. Well to moderately differentiated tumour cells with lumen formation are positive only for c-*erb*B-2 protein, but tumour cells with squamous differentiation are positive for both c-*erb*B-2 protein and EGFR. ×400

and EGFR negative, but tumour cells with squamous differentiation showed positivity for both c-*erb*B-2 protein and EGFR (Fig. 7). Two of the 3 clear cell carcinomas were c-*erb*B-2 protein positive and EGFR negative. The remaining case of clear cell carcinoma and the transitional cell carcinoma were negative for both c-*erb*B-2 protein and EGFR.

Collectively, 13 of the 19 ovarian carcinomas (68.4%) were c-*erb*B-2 protein positive and EGFR negative. Four of the 19 (21.1%) were positive for both c-*erb*B-2 protein and EGFR; EGFR was expressed in 3 cases of stage III or IV poorly differentiated serous carcinoma, and 1 case of stage I endometrioid carcinoma with squamous differentiation.

Discussion

Our study showed that normal ovarian surface epithelium was weakly positive for both c-*erb*B-2 protein and EGFR. This is consistent with previous immunohistochemical studies of c-*erb*B-2 protein expression (Berchuck et al. 1990; Press et al. 1990), and with the presence of specific binding capacity for epidermal growth factor (Rodriguez et al. 1991). However, we have demonstrated that the epithelial cells lining the surface inclusion cysts were positive for c-*erb*B-2 protein, but negative for EGFR. Moreover, the c-*erb*B-2 protein staining intensity of these cells was stronger than that of normal ovarian surface epithelium. In addition, all 16 benign epithelial tumours and 2 borderline malignant lesions of the ovary were positive for c-*erb*B-2 protein and negative for EGFR, irrespective of histological type. It is generally believed that surface inclusion cysts arise from cortical invaginations of the ovarian surface epithelium that have lost their connection with the surface, and that these inclusion cysts give rise to the majority of common epithelial tumours of the ovary (Clement 1987). Our immunohistochemical results suggest that the expression of c-*erb*B-2 protein is increased and the expression of EGFR is reduced in the surface epithelial cells during the early stage of ovarian oncogenesis.

Although the ovary is not of mullerian origin, the surface epithelium is derived from the coelomic epithelium, which gives rise to the mullerian ducts in the embryo. The latter form the fallopian tubes, uterine body, and cervix, with their large variety of epithelia. Epithelial ovarian tumours that were classified as serous (tubal-related), endometrioid (endometrial-related), and mucinous (endocervical-related) are generally considered to reflect various directions of mullerian differentiation of the ovarian surface epithelium (Parmley 1987). Our previous immunohistochemical study has revealed that mullerian-derived epithelial cells in the fallopian tube, endometrium, and endocervix are positive for c-*erb*B-2 protein and usually negative for EGFR, whereas the sur-

rounding stromal cells express EGFR, but not c-*erb*B-2 protein. In addition, the endometrial glandular cells in the proliferative phase express both c-*erb*B-2 protein and EGFR, but those in the secretory phase show increased expression of c-*erb*B-2 protein, associated with reduced expression of EGFR (Wang et al. 1992). In the ovarian surface epithelium, we have found analogous changes of expression of these receptor proteins in the cells during the formation of surface inclusion cysts. Thus, the changes of the expression of c-*erb*B-2 protein and EGFR may be related to the differentiation of ovarian surface epithelium into the mullerian-type epithelium.

Most epithelial ovarian carcinomas showed a pattern of expression for both proteins that was similar to the pattern observed in benign and borderline malignant ovarian tumours. However, the staining intensity of c-*erb*B-2 protein in most ovarian carcinomas was stronger than that in surface inclusion cysts and in benign ovarian tumours. Over-expression of c-*erb*B-2 protein in ovarian carcinomas may be associated with amplification of the c-*erb*B-2 proto-oncogene; this has been reported to correlate with poor survival in ovarian cancer patients (Slamon et al. 1987; Berchuck et al. 1990), although some studies have not supported an adverse prognostic effect of c-*erb*B-2 protein in ovarian cancer (Haldane et al. 1990; Wilkinson et al. 1991).

Expression of both c-*erb*B-2 protein and EGFR was found in 4 of the 19 (21.1%) ovarian carcinomas. Previous immunohistochemical studies using different monoclonal antibodies have found EGFR positivity in ovarian cancer, ranging from 20% (Gullick et al. 1986) to 77% (Berchuck et al. 1991). EGFR positivity determined by measuring the binding of radiolabelled epidermal growth factor to epithelial ovarian cancer tissue homogenates has been reported to be 35.7% by Bauknecht et al. (1988) and 39.7% by Owens et al. (1991). In our cases, the immunohistochemical expression of EGFR was observed in the poorly differentiated serous carcinomas and in endometrioid carcinoma with squamous differentiation. Since EGFR is usually expressed in the basal cells of normal squamous epithelium (Wang et al. 1992) and is strongly expressed in squamous carcinomas (Gullick et al. 1986), it is likely that ovarian endometrioid carcinoma with squamous differentiation does express EGFR. Other EGFR-positive tumours were poorly differentiated serous carcinomas, all of which were clinically advanced (stage III or IV). EGFR expression was not observed in well to moderately differentiated carcinomas or in stage I and II, except in a case of endometrioid carcinoma. These findings suggest that EGFR expression in ovarian carcinomas is related to the differentiation state of the tumour and/or advanced clinical stage. In addition, these EGFR-positive cases were also strongly positive for c-*erb*B-2 protein. Several lines of evidence have recently indicated synergic interaction between c-*erb*B-2 protein and EGFR in the development and maintenance of malignant cell phenotype (Qian et al. 1992). A possible association between EGFR expression in ovarian cancer and poor patient survival has also been reported (Berchuck et al. 1991). Therefore, the expression of EGFR, in addition to c-*erb*B-2 protein, seems to be a late event that is linked with the progression of ovarian carcinomas, rather than with the early stage of carcinogenesis.

In summary, we have found changes of expression of c-*erb*B-2 protein and EGFR in epithelial ovarian oncogenesis; increased expression of c-*erb*B-2 protein and reduced expression of EGFR is observed in the cells of inclusion cysts and in benign and borderline malignant tumours, which is presumably related to mullerian differentiation of ovarian surface epithelium. Most ovarian carcinomas express c-*erb*B-2 protein strongly, but not EGFR. The expression of EGFR, in addition to that of c-*erb*B-2 protein, is related to histological differentiation and/or advanced clinical stage. Peptide growth factors have recently been suggested to play an important role in the neoplastic transformation, growth, and metastasis of ovarian cancers (Malik and Balkwill 1991). The changes in the expression of both these receptor proteins, c-*erb*B-2 protein and EGFR, may represent one of the many steps in ovarian oncogenesis.

References

Bauknecht T, Runge M, Schwall M, Pfleiderer A (1988) Occurrence of epidermal growth factor receptors in human adnexal tumors and their prognostic value in advanced ovarian carcinomas. Gynecol Oncol 29:147–157

Berchuck A, Kamel A, Whitaker R, Kerns B, Olt G, Kinney R, Soper JT, Dodge R, Clarke-Pearson DL, Marks P, McKenzie S, Yin S, Bast RC (1990) Overexpression of HER-2/*neu* is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res 50:4087–4091

Berchuck A, Rodriguez GC, Kamel A, Dodge RK, Soper JT, Clarke-Pearson DL, Bast RC (1991) Epidermal growth factor in normal ovarian epithelium and ovarian cancer. I. Correlation of receptor expression with prognostic factors in patients with ovarian cancer. Am J Obstet Gynecol 164:669–674

Clement PB (1987) Nonneoplastic lesions of the ovary. In: Kurman RJ (ed) Blaustein's pathology of the female genital tract, 3rd edn Springer, Berlin Heidelberg New York, pp 471

Coussens L, Yang-Feng TL, Liao YU, Chen L, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J, Francke U, Levinson A, Ullrich A (1985) Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with *neu* oncogene. Science 230:1132–1139

Gullick WJ, Marsden JJ, Whittle N, Ward B, Bobrow L, Waterfield MD (1986) Expression of epidermal growth factor receptors on human cervical, ovarian, and vulvar carcinomas. Cancer Res 46:285–292

Haldane JS, Hird V, Hughes CM, Gullick WJ (1990) c-*erb*B-2 oncogene expression in ovarian cancer. J Pathol 162:231–237

Iglehart JD, Kraus MH, Langton BC, Huper G, Kerns BJ, Marks JR (1990) Increased *erb*B-2 gene copies and expression in multiple stages of breast cancer. Cancer Res 50:6701–6707

Lupu R, Colomer R, Zugmaier G, Sarup J, Shepard M, Slamon D, Lippman ME (1990) Direct interaction of a ligand for the *erb*B2 oncogene product with the EGF receptor and p185^{erbB}2. Science 249:1552–1555

Maguire HC, Jaworsky C, Cohen JA, Hellman M, Weiner DB, Greene MI (1989) Distribution of *neu* (c-*erb*B-2) protein in human skin. J Invest Dermatol 89:786–790

Malik S, Balkwill F (1991) Epithelial ovarian cancer: a cytokine propelled disease? Br J Cancer 64:617–620

Mori S, Akiyama T, Yamada Y, Morishita Y, Sugawara I, Toyoshima K, Yamamoto T (1989) C-*erb*B-2 gene product, a membrane

protein commonly expressed on human fetal epithelial cells. Lab Invest 61:93–97

Owens OJ, Stewart C, Brown I, Leake RE (1991) Epidermal growth factor receptors (EGFR) in human ovarian cancer. Br J Cancer 64:907–910

Parmley T (1987) Embryology of the female genital tract. In: Kurman RJ (ed) Blaustein's pathology of the female genital tract, 3rd edn Springer, Berlin Heidelberg New York, p 1

Press MF, Cordon-Cardo C, Slamon DJ (1990) Expression of the HER-2/*neu* proto-oncogene in normal human adult and fetal tissues. Oncogene 5:953–962

Qian X, Decker SJ, Greene MI (1992) $p185^{c\text{-}neu}$ and epidermal growth factor receptor associate into a structure of activated kinases. Proc Natl Acad Sci USA 89:1330–1334

Rodriguez GC, Berchuck A, Whitaker RS, Schlossman D, Clarke-Pearson DL, Bast RC (1991) Epidermal growth factor expression in normal ovarian epithelium and ovarian cancer. II. Relationship between receptor expression and response to epidermal growth factor. Am J Obstet Gynecol 164:745–750

Serov SF, Scully RE (1973) Histological typing of ovarian tumors. World Health Organization, Geneva

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF (1987) Studies of the HER-2/*neu* protooncogene in human breast and ovarian cancer. Science 244:707–712

Wang DP, Fujii S, Konishi I, Nanbu Y, Iwai T, Nonogaki H, Mori T (1992) Expression of c-*erb*B-2 protein and epidermal growth factor receptor in the normal tissues of the female genital tract and the placenta. Virchows Arch [A] 420:385–393

Weidner U, Peter S, Strohmeyer T, Hussnatter R, Ackermann R, Sies H (1990) Inverse relationship of epidermal growth factor receptor and HER2/*neu* gene expression in human renal cell carcinoma. Cancer Res 50:4504–4509

Wilkinson N, Todd N, Buckley CH, Gusterson BA, Fox H (1991) An immunohistochemical study of the incidence and significance of c-*erb*B-2 oncoprotein overexpression in ovarian neoplasia. Int J Gynecol Cancer 1:285–289

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