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Expression of c-*erb*B-2 protein and epidermal growth factor receptor in normal tissues of the female genital tract and in the placenta

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Summary. The c-*erb*B-2 (HER-2/*neu*) protein is a membrane glycoprotein growth factor receptor that has molecular homology with the epidermal growth factor receptor (EGFR). To investigate the relationship between the expression of c-*erb*B-2 protein and EGFR in the tissues of the human female genital tract and in the placenta, we examined the immunohistochemical reactivity of monoclonal antibodies against both of these proteins. In the müllerian-derived genital tract, epithelial cells of the fallopian tube, endometrium, and endocervix showed reactivity for c-*erb*B-2 protein, whereas reactivity for EGFR was distributed mainly in the stromal cells throughout the menstrual cycle and during pregnancy. In addition, the staining intensity for EGFR in the endometrial stroma increased with its decidualization. In the exocervical squamous epithelium, basal cells were c-*erb*B-2 protein-negative and EGFR-positive, but the more differentiated squamous cells of the intermediate layer were c-*erb*B-2 protein-positive and EGFR-negative. In the placental tissues, cytotrophoblasts and syncytiotrophoblasts of the chorionic villi were c-*erb*B-2 protein-negative and EGFR-positive. In contrast, intermediate trophoblasts in the extravillous space were c-*erb*B-2 protein-positive and EGFR-negative. Thus, there is an inverse relationship between the expression of c-*erb*B-2 protein and EGFR in the tissues of the female genital tract and in the placenta. This suggests that there may be a regulatory mechanism(s) for the expression of both proteins that is associated with the differentiation and/or function of cells in the female genital tract and the placenta.

Key words: c-*erb*B-2 protein – Epidermal growth factor receptor – Female genital tract – Placenta

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Introduction

The *neu* oncogene was first isolated from chemically induced neonatal rat neuroglioblastomas (Schechter et al. 1984). The human homologue of this gene was subsequently identified (Coussens et al. 1985; King et al. 1985; Semba et al. 1985), and because of its homology to the human epidermal growth factor receptor (HER) or c-*erb*B-1 proto-oncogene, it was referred to as HER-2 or c-*erb*B-2. The c-*erb*B-2 gene encodes a 185 kDa transmembrane glycoprotein, c-*erb*B-2 protein, which has molecular homology with epidermal growth factor receptor (EGFR) (Yamamoto et al. 1986). Since the inner domain of c-*erb*B-2 protein has a tyrosine kinase activity which is also demonstrated in EGFR, the c-*erb*B-2 protein is considered to be a growth factor receptor that may participate in some, as yet unknown, functions of the human cell. Amplification of the c-*erb*B-2 gene and/or over-expression of c-*erb*B-2 protein have been shown in carcinomas of the breast (King et al. 1985), stomach (Fukushige et al. 1986), colon (Cohen et al. 1989), salivary gland (Semba et al. 1985), endometrium (Berchuck et al. 1991), and ovary (Tyson et al. 1988). In breast and ovarian carcinomas, especially, high amplification of c-*erb*B-2 and/or over-expression of this gene product in the tumour tissues have been reported to correlate with poor patient prognosis (Slamon et al. 1987; Berchuck et al. 1990). Thus, the c-*erb*B-2 gene is considered to play an important role in the biological behaviour and/or pathogenesis of several types of human carcinomas, although there is a report which does not support an adverse prognostic effect of c-*erb*B-2 in ovarian cancer (Haldane et al. 1990).

The physiological significance of c-*erb*B-2 protein in normal human tissues remains unclear, although the function of EGFR has been extensively studied (Oberg et al. 1990). However, the recent generation of monoclonal and polyclonal antibodies against c-*erb*B-2 protein (Gullick et al. 1987; Mori et al. 1987) has revealed that this protein is expressed in human fetal tissues, mainly in the gastrointestinal tract (Mori et al. 1989),

as well as in human adult tissues such as skin epidermis, oral mucosa, kidney, and tissues of the female genital tract (Press et al. 1990). This suggests that c-*erb*B-2 protein is a normal membrane constituent of a variety of epithelial cell types.

Moreover, in normal human skin, differing distributions of c-*erb*B-2 protein and EGFR in the squamous epithelium have been reported, and an inverse relationship between the expression of the two receptor proteins has been suggested in the differentiation of keratinocytes (Maguire et al. 1989). In addition, in the kidney, normal tissues have been reported to show high expression of the c-*erb*B-2 gene and low expression of the EGFR gene, whereas in renal cell carcinomas, the pattern is reversed; there is high expression of the EGFR gene and low expression of the c-*erb*B-2 gene (Weidner et al. 1990). In the normal tissues of the female genital tract, however, the relationship between the expression of c-*erb*B-2 protein and EGFR remains unknown. In addition, the distribution of c-*erb*B-2 protein in placental tissues has not yet been elucidated. We therefore investigated the immunohistochemical localization of the two proteins, c-*erb*B-2 protein and EGFR, in normal tissues of the female genital tract and in the placenta.

Materials and methods

Fresh surgical specimens of fallopian tube, endometrium, and cervix were obtained from 46 women with benign gynaecological diseases, who had undergone hysterectomy and salpingo-oophorectomy. Of the 46 patients, 32 had regular menstrual cycles. At the time of operation, 16 of these were in the proliferative phase and 16 were in the secretory phase; 8 patients were pregnant; the remaining 6 patients were post-menopausal. Immediately after the surgical procedure, ampullary portions of the fallopian tubes, endometrial samples from the mid-fundal area of the uterine cavity, and cervical samples were obtained, snap-frozen in OCT compound (Ames, Elkhart, Ind., USA), and stored at −70° C. Serial cryostat sections were stained with haematoxylin and eosin for light microscopy. The menstrual cycle of the patient was estimated by endometrial dating according to the method of Noyes et al. (1950).

Placental tissues, including the chorion and decidua of a first-trimester gestation (8 cases), and the placenta with membranes of a second-trimester gestation (4 cases) and a third-trimester gestation (3 cases), were obtained at legal therapeutic abortions, spontaneous deliveries, and caesarean sections.

Immunostaining for c-*erb*B-2 protein and EGFR on the cryostat sections was performed by the avidin-biotin-peroxidase complex method, using a Histscan monoclonal detector kit (Biomeda, Foster, Calif., USA). In brief, thin cryostat sections were fixed in cold acetone for 5 min, treated with 0.3% hydrogen peroxide, and incubated with normal goat serum for blocking non-specific binding. The serial sections were then incubated with mouse monoclonal antibody for the external domain of c-*erb*B-2 protein (diluted 1:25, Triton Bioscience, Alameda, Calif., USA), or mouse monoclonal antibody for the external domain of EGFR (diluted 1:80, Oncogene Science, Manhasset, N.Y., USA), or 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, and stained with 3-amino-9-ethylcarbazole solution with 0.15% hydrogen peroxide. Counterstaining was performed with haematoxylin. For positive controls, we used cryostat sections of ovarian 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 mammary carcinoma (Iglehart et al. 1990) and normal tissues (Press et al. 1990). The antibody for the external domain of EGFR used in this study, which inhibits EGF binding to its receptor, is an antagonist of in vivo EGF-stimulated tyrosine kinase activity, and is thought to be specific for the EGFR, stains not only malignant tissues but also benign tissues (Berchuck et al. 1989).

Tissues were grouped into one of the following expression categories depending on the amount of membrane immunostaining: negative (−), weak, distinct (+), moderate (++), and strong/intense (+++). We do not report a percentage of immunoreactive cells, since relatively homogeneous immunostaining was observed in specific cell types throughout frozen sections of normal tissues (approximately 90% of the cells of a particular type showing similar immunostaining properties). Similar observations were reported in frozen sections of normal adult and fetal tissues by Press et al. (1990) and of human uterine tissues by Berchuck et al. (1989).

Results

Specific staining with anti-c-*erb*B-2 protein antibody and anti-EGFR antibody was observed mainly along the cell membrane.

The immunohistochemical localization of c-*erb*B-2 protein and EGFR in normal tissues of the female genital tract is summarized in Tables 1 and 2.

Table 1. Immunohistochemical localization of c-*erb*B-2 protein and epidermal growth factor receptor (EGFR) in the female genital tract

Tissues	c-*erb*B-2 protein		EGFR	
	Glandular epithelium	Stroma	Glandular epithelium	Stroma
Fallopian tube	++	−	−	++
Endometrium				
Basal layer	+	−	+	+
Functional layer				
(proliferative phase)	++	−	+	+
(secretory phase)				
early secretory	+++	−	+/−	+
mid-secretory	+++	−	−	+
late secretory	++	−	−	+++
(during pregnancy)	+	−	−	+++
(postmenopause)	+	−	+/−	+
Endocervix	++	−	−	++

Table 2. Immunohistochemical localization of c-*erb*B-2 protein and EGFR in exocervical squamous epithelium

	c-*erb*B-2	EGFR
Basal cell	−	++
Parabasal cell	+/−	+
Squamous cell of intermediate layer	++	+/−
Superficial layer	−	−

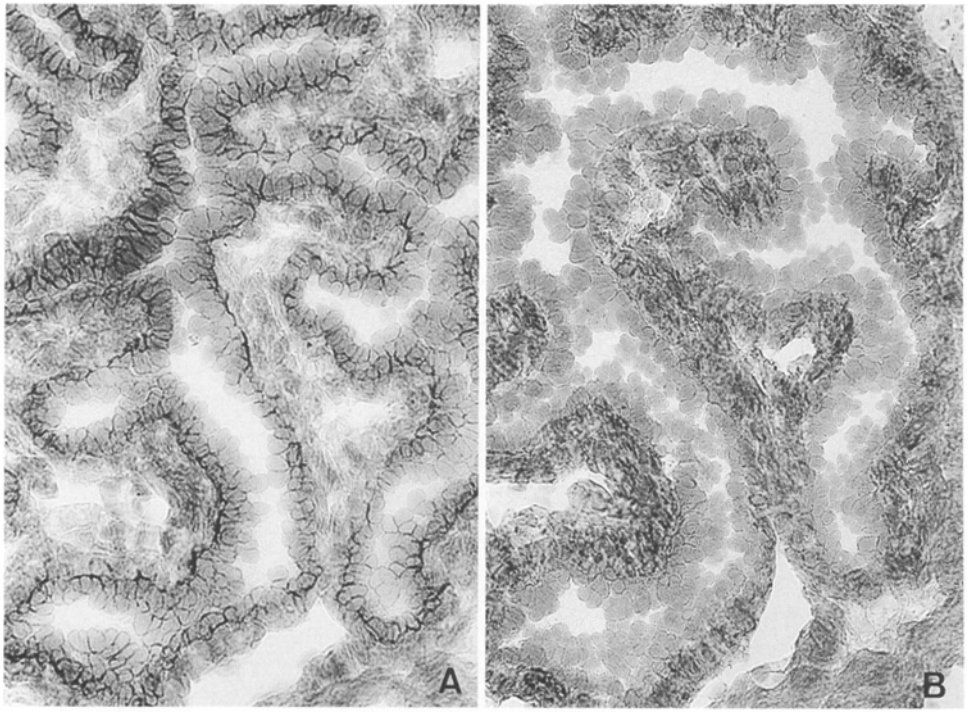


Fig. 1. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and epidermal growth factor receptor (EGFR) (**B**) in the fallopian tube. Epithelial cells are positive for c-*erb*B-2 protein, whereas stromal cells are positive for EGFR. ×400

In the fallopian tube epithelial cells were weakly stained for c-*erb*B-2 protein and were negative for EGFR, irrespective of the menstrual phase (Fig. 1A, B). In contrast, fibroblast-like cells of the sub-epithelial stroma were negative for c-*erbB*-2 protein but positive for EGFR throughout the menstrual cycle (Fig. 1A, B). The variations in c-*erb*B-2 protein staining intensity in the epithelial cells and EGFR staining intensity in the stromal cells were not found in the tubal specimens.

In the basal layer of the endometrium, immunoreactivity for c-*erb*B-2 protein was localized in the glandular epithelium, while immunoreactivity for EGFR was observed in both the glandular epithelium and stroma. Variations in the staining intensity of the two proteins during the menstrual cycle were not distinct in the basal layer. Myometrial smooth muscle cells and endothelial cells of the spiral arterioles were negative for c-*erb*B-2 protein, but weakly positive for EGFR.

In the functional layer, the staining intensity for c-*erb*B-2 protein and EGFR fluctuated during the menstrual cycle and during pregnancy. Endometrial glandular cells during the proliferative phase were weakly positive for c-*erb*B-2 protein and also weakly positive for EGFR (Fig. 2A, B). In the secretory phase, however, reactivity for c-*erb*B-2 protein in the glandular epithelium was stronger than in the proliferative phase, whereas reactivity for EGFR was not observed (Fig. 3A, B). Glandular cells during pregnancy were weakly positive for c-*erb*B-2 protein, but negative for EGFR (Fig. 4A, B). Endometrial stromal cells were negative for c-*erb*B-2 protein but positive for EGFR throughout the menstrual cycle and during pregnancy (Figs. 2–4). In addition, the staining intensity for EGFR in the endometrial stroma of the secretory phase was stronger than that of the proliferative phase (Figs. 2, 3). Decidualized stromal cells during pregnancy showed the most intense staining for EGFR (Fig. 4B).

In the post-menopausal endometrium, atrophic glands were weakly positive for c-*erb*B-2 protein. Reactivity for EGFR in the post-menopausal glands varied among the specimens examined. Weak and sporadic reactivity for EGFR was identified in 4 cases, but the remaining 2 cases showed no reactivity for EGFR. Post-menopausal endometrial stroma was also negative for c-*erb*B-2 protein but positive for EGFR.

Endocervical glandular cells were strongly positive for c-*erb*B-2 protein and negative for EGFR. Fibroblast-like stromal cells in the cervix were negative for c-*erb*B-2 protein, but weakly positive for EGFR (Fig. 5A, B). The staining intensity of c-*erb*B-2 protein in the cervical epithelium and that of EGFR in the cervical stroma did not change during the menstrual cycle.

In the stratified squamous epithelium of the exocervix, basal cells were negative for c-*erb*B-2 protein, but strongly positive for EGFR (Fig. 6A, B). Parabasal cells were negative or weakly positive for c-*erb*B-2 protein, but positive for EGFR. In the squamous cells of the intermediate layer, cells were positive for c-*erb*B-2 protein, and its intensity gradually increased from the lower layer to the upper layer. In contrast, the staining intensity for EGFR in the squamous cells of the intermediate layer gradually decreased with stratification. The superficial cells of the exocervical epithelium were negative for both proteins.

Immunohistochemical localization of c-*erb*B-2 protein and EGFR in the placental tissues is summarized in Table 3.

Both types of chorionic villi trophoblasts, cytotro-

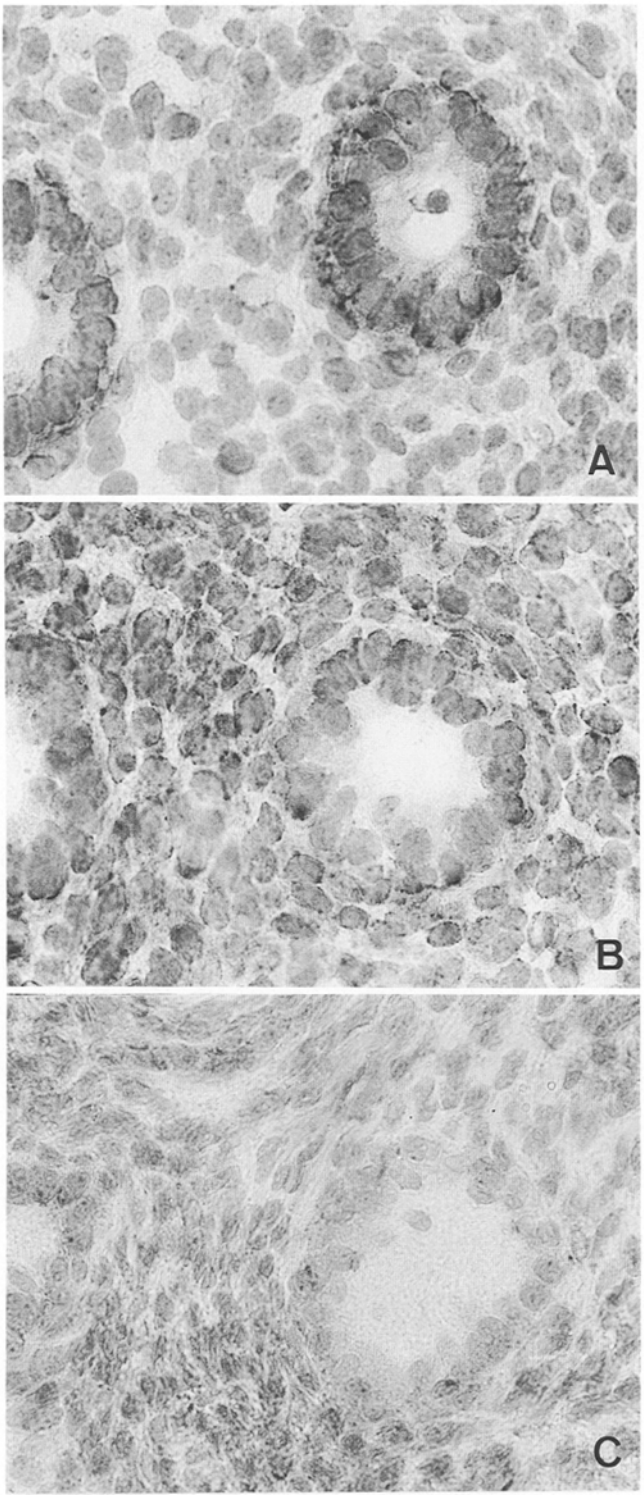


Fig. 2. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in the endometrium of early proliferative phase. EGFR reactivity is observed in both glandular epithelial cells and stromal cells, but c-*erb*B-2 protein is localized only in the epithelial cells. **C** Negative control. ×800

phoblasts and syncytiotrophoblasts were negative for c-*erb*B-2 protein, but positive for EGFR (Fig. 7A, B). In contrast, extravillous intermediate trophoblasts, which were observed either as cell clusters being attached to the chorionic villi or as isolated cells scattered in the decidua, were strongly positive for c-*erb*B-2 protein and negative for EGFR (Fig. 8A, B). Since the surrounding decidual cells were negative for c-*erb*B-2 protein, the presence of intermediate trophoblasts in the decidua was clearly identified. However, a few multinucleated giant trophoblasts in the decidual tissue were negative for c-*erb*B-2 protein positive for EGFR.

During the second and third trimester the syncytiotrophoblasts of the chorionic villi were negative for c-*erb*B-2 protein but strongly positive for EGFR. Cytotrophoblasts were rarely observed in the placental villi at this stage of gestation. Intermediate trophoblasts in the extravillous space were strongly positive for c-*erb*B-2 protein but negative for EGFR.

Amniotic cells were negative for c-*erb*B-2 protein but strongly positive for EGFR.

Discussion

Our study has demonstrated the immunohistochemical localization of c-*erb*B-2 protein and EGFR in normal tissues of the adult female genital tract and the placenta. In the exocervical squamous epithelium, reactivity for c-*erb*B-2 protein was negative in the basal cells, weakly positive in the parabasal cells, and strongly positive in the squamous cells of the intermediate layer. In contrast, basal cells were strongly positive for EGFR, and the more differentiated squamous cells of the intermediate layer showed less expression of EGFR, that is, we observed an inverse relationship between the distribution of c-*erb*B-2 protein and EGFR in the exocervical squamous epithelium. This inverse relationship between the distribution of c-*erb*B-2 protein and EGFR in squamous epithelium has also been described in human adult skin (Maguire et al. 1989). The switching of expression of these receptors, therefore, appears to be necessary for the differentiation of normal squamous epithelium.

In the female genital tract of müllerian origin, in fallopian tubes, endometrium, and endocervix, a clear-cut difference between the distribution of c-*erb*B-2 protein and EGFR was found in the epithelium and stroma. c-*erb*B-2 protein was localized in the epithelial cells, whereas EGFR was localized mainly in the stromal cells. Embryologically, both the epithelium and the stroma of the müllerian ducts are derived from the embryonic mesoderm. Undifferentiated embryonic mesoderm cells differentiate into epithelial-like cells lining the coelomic cavity and into sub-coelomic mesenchymal cells (Parmley 1987). In addition, not only during embryogenesis but also in adult life, the epithelium of the female genital tract is believed to be generated from its accompanying undifferentiated mesenchymal cells (Lawrence and Shingleton 1980). In the human fetus, c-*erb*B-2 protein was also found to be localized in the müllerian-derived epithelia (Press et al. 1990), and immunoreactivity for EGFR has been found in the stroma of the fetal tube and uterus (unpublished observation). Thus, the distinct distribution of EGFR in the stromal cells and c-*erb*B-2 protein in the epithelial cells of the female genital tract

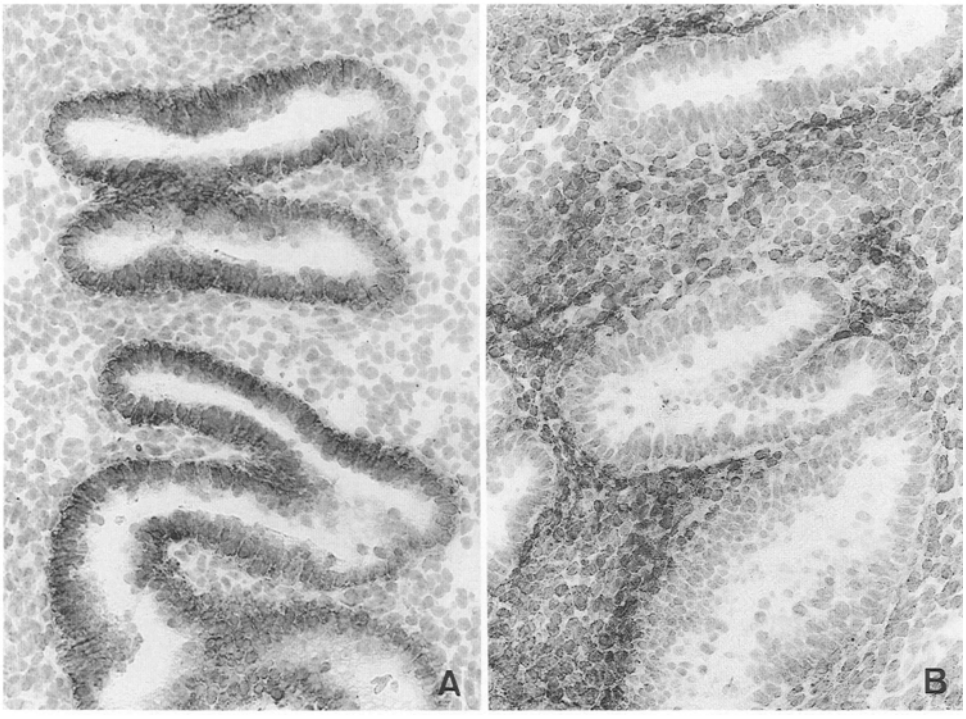


Fig. 3. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in the functional layer of the endometrium in the early secretory phase. Glandular epithelial cells are positive for c-*erb*B-2 protein, while the stromal cells are positive for EGFR. ×400

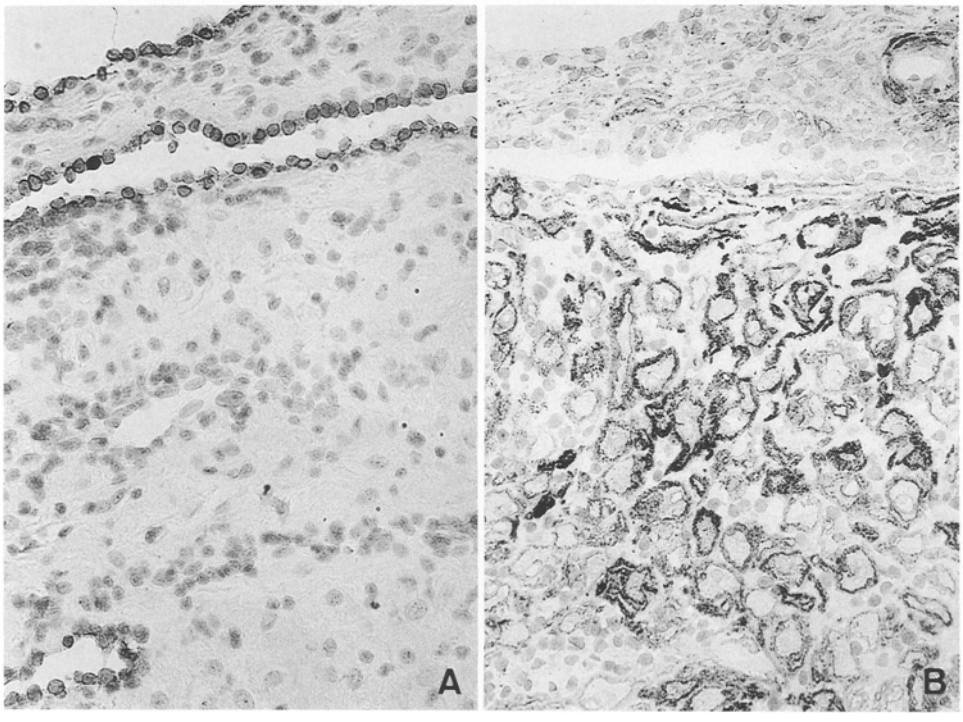


Fig. 4. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in the endometrium at 8 weeks of gestation. Glandular epithelium shows weak c-*erb*B-2 protein positivity, while the decidual cells are strongly positive for EGFR. ×400

suggests that EGFR is primarily expressed in the mesoderm, but that switching of expression from EGFR to c-*erb*B-2 protein may be elicited in the epithelial cells during their differentiation from the mesoderm.

Observations in the adult endometrium, which shows dynamic morphological changes during the menstrual cycle and is a representative model for the study of proliferation and differentiation, also support this assumption. In the proliferative phase, both glandular epithelium and stromal cells were positive for EGFR, but c-*erb*B-2 protein reactivity was confined to the glandular epithelium. However, in the secretory phase, only the stromal cells showed reactivity for EGFR, with the staining intensity for EGFR being strongest in the decidualized stromal cells. In addition, positivity for c-*erb*B-2 protein was also confined to the glandular epithelium

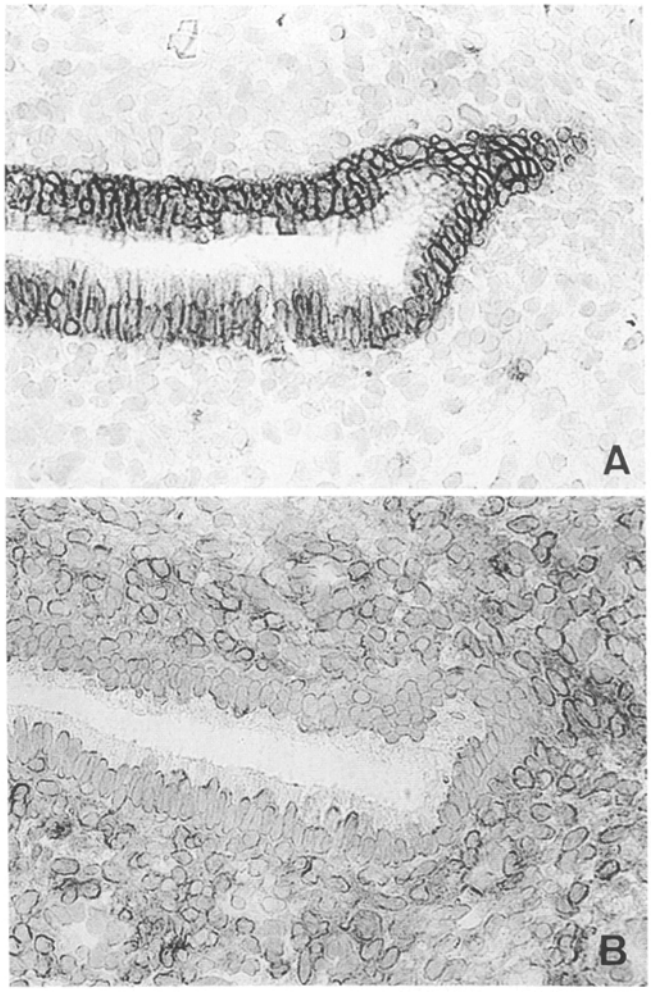


Fig. 5. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in the endocervix. Glandular epithelial cells are positive for c-*erb*B-2 protein, while the stromal cells are positive for EGFR. ×400

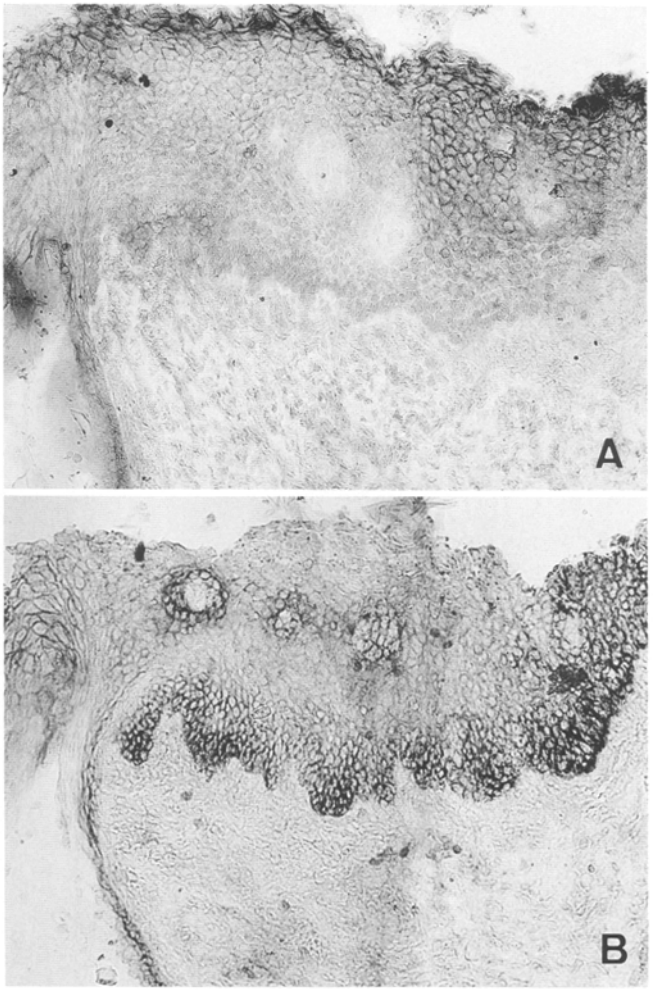
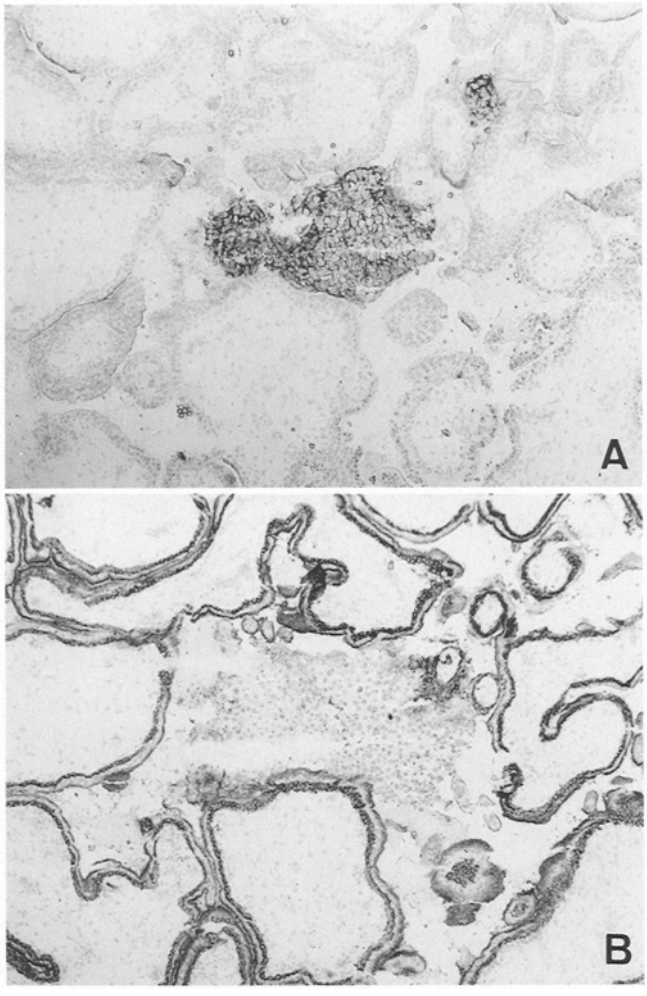


Fig. 6. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in exocervical squamous epithelium. The c-*erb*B-2 protein is negative in the basal cells, but positive in the differentiated squamous cells of the intermediate layer. In contrast, EGFR is strongly positive in the basal cells, but weakly positive or negative in the cells of the intermediate layer. ×200

Table 3. Immunohistochemical localization of c-*erb*B-2 protein and EGFR in the placenta

Gestational weeks	First trimester		Second trimester		Third trimester	
	c-*erb*B-2 protein	EGFR	c-*erb*B-2 protein	EGFR	c-*erb*B-2 protein	EGFR
Chorionic villi						
Cytotrophoblast	−	+++	−	+	[a]	[a]
Syncytiotrophoblast	−	++	−	++	−	++
Extravillous space						
Intermediate trophoblast	+++	−	++	−	++	−
Multinuclear giant trophoblast	−	++	−	++	−	++
Amnion	−	+	−	+	−	+
Decidual cells	−	++	−	+	−	+

[a] Cytotrophoblasts were not detected in the villi of the third-trimester placenta



in the secretory phase. Thus, the endometrial glandular epithelium seems to lose EGFR expression according to the stage of differentiation. There have been several reports on EGFR in the adult endometrium. Biochemical studies have demonstrated specific EGF-binding activity in endometrial tissues, but with conflicting results regarding variations of this activity during different phases of the menstrual cycle (Sheets et al. 1985; Taketani and Mizuno 1988). A recent study on EGFR immunoreactivity in normal and malignant endometrium in which authors used a monoclonal antibody (mAb528) against EGFR protein, has described reactivity for EGFR in both gland and stroma of normal endometrium throughout the menstrual cycle (Berchuck et al. 1989). However, an autoradiographic study, carried out with ^{125}I-EGF in the human uterus (Chegini et al. 1986), demonstrated more numerous grains in the stroma than in the glandular epithelium, smooth muscle, and arteriolar endothelium; this is consistent with our results that EGFR reactivity was observed mainly in the stroma. In addition, an important EGF role has been suggested in decidualization and/or decidual function (Yamamoto et al. 1989). This is consistent with our results showing that expression of EGFR in the endometrial stroma increased with its decidualization. Furthermore, endometrial glandular cells reacted for c-*erb*B-2 protein throughout the menstrual cycle, and the staining intensity was

Fig. 7. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in the chorionic villi at 7 weeks of gestation. Cytotrophoblasts and syncytiotrophoblasts are negative for c-*erb*B-2 protein, but strongly positive for EGFR. ×80

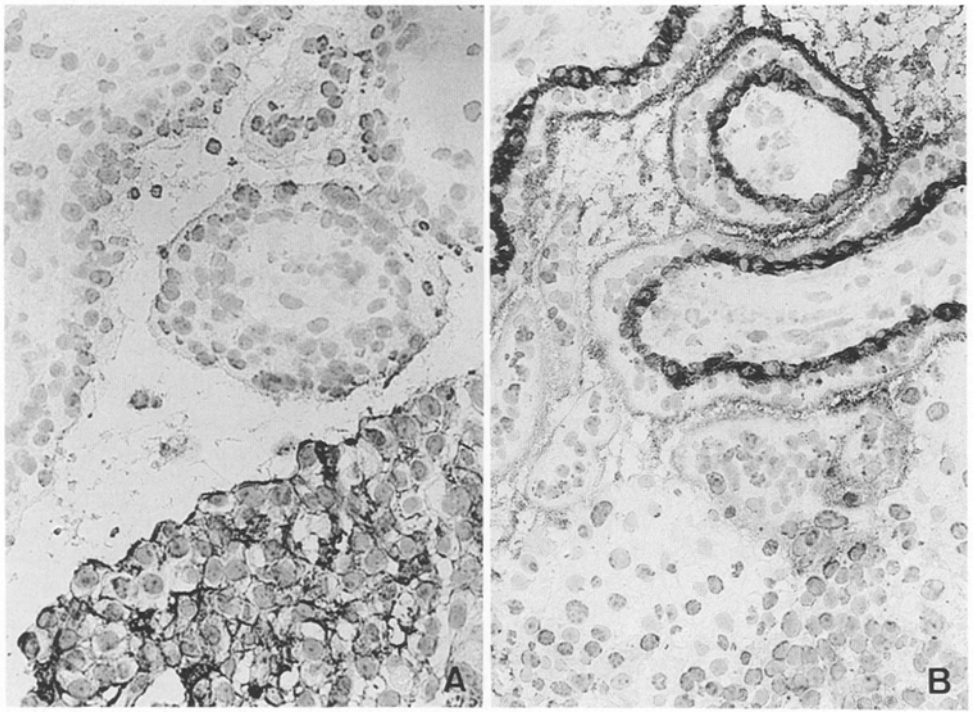


Fig. 8. Immunohistochemical localization of c-*erb*B-2 protein (**A**) and EGFR (**B**) in the placental extravillous space at 8 weeks of gestation. Intermediate trophoblasts are positive for c-*erb*B-2 protein but negative for EGFR. ×800

most intense in the secretory phase. These results suggest that the expression of both c-*erb*B-2 protein and EGFR is associated with functional changes of the endometrium introduced by the hormonal milieu during the menstrual cycle.

In the placental tissues, immunohistochemical reactivity for EGFR was localized in the villous trophoblasts of both cytotrophoblasts and syncytiotrophoblasts, whereas c-*erb*B-2 protein was expressed in the intermediate trophoblasts in the extravillous space. Although immunohistochemical loaclization of EGFR in the villous trophoblasts and specific EGF-binding activity of chorionic tissue have been reported (Carson et al. 1983; Rao et al. 1985; Maruo and Mochizuki 1987), to our knowledge, this is the first description of the expression of c-*erb*B-2 protein in the intermediate trophoblasts. The term "intermediate trophoblast" was coined by Kurman et al. (1984) to described the specialized trophoblasts in the extravillous space, which have been also described as "X cells" (Benirschke and Kaufman 1990). Immunohistochemically, villous syncytiotrophoblasts are positive for both human chorionic gonadotropin (hCG) and human placental latogen (hPL), while intermediate trophoblasts have shown only hPL to be present (Gosseye and Fox 1984; Kurman et al. 1984). Among the extravillous trophoblasts, however, multinucleated giant trophoblasts have been reported to be positive for both hCG and hPL (Kurman et al. 1984). Interestingly, in this study we found that the multinucleated giant cells in the decidua were c-*erb*B-2 protein-negative and EGFR-positive, and were distinguished from intermediate trophoblasts. Thus, the inverse relationship between the expression of c-*erb*B-2 protein and EGFR was also observed in the placenta, and this relationship may have a close association with trophoblast function.

In summary, there is a clear-cut difference between the immunohistochemical localization of c-*erb*B-2 protein and EGFR in normal tissues of the female genital tract and in placental tissues. The inverse relationship between the expression of c-*erb*B-2 protein and EGFR, which have molecular homology as growth factor receptors, suggests that there may be a regulatory mechanism(s) for the expression of both proteins that is associated with the differentiation and/or function of cells in the female genital tract and the placenta.

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