Expression and localization of epidermal growth factor receptors and ras oncogene products in gynecologic tumors

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Summary: We have investigated the expression of the products of two well-characterized oncogenes, *erbB* and Ha-*ras,* in the primary cultures of human gynecologic tumor cells by indirect immunofluorescent analysis. Epidermal growth factor receptor (c-*erbB* gene product) and p21 (*ras* gene product) were highly expressed in 21 out of 26 cases (81%) and 14 out of 18 cases (78%), respectively. Furthermore, they were frequently co-localized in the same region of the cells. These results suggest that these two oncogene products may play a part in gynecologic tumorigenesis and that they can interact with each other.

Key words: epidermal growth factor receptor; *c-erbB*; *ras*; oncogene; gynecologic tumors.

INTRODUCTION

A number of reports have so far suggested the involvement of some oncogenes in carcinogenesis of gynecologic tumors. At a genetic level, c-Ki-*ras*2 and N-*ras* oncogenes were activated in less than 10% of ovarian cancers ([1]) and amplification of c-Ki-*ras*, c-*myc* and HER-2/*neu* genes was found in 8-25% ([2-4]), 20% ([5]) and 26% ([6]), respectively, of ovarian malignancies. Furthermore, overexpression of mRNAs of c-*erbB* and c-Ha-*ras* was observed in more than 80% ovarian tumors ([7, 8]). High expression of *ras* oncogene product (p21) was found in 52% of squamous cell carcinomas of the uterine cervix ([9]) and in almost all of ovarian carcinomas and in grade 2 and 3 endometrial adenocarcinomas ([10, 11]). Taken together, it is possible that unusual expression of oncogene products might be closely related to gynecologic tumorigenesis.

In the present study, the expression of c-*erbB* gene product (epidermal growth factor-receptor, EGF-R) and c-Ha-*ras* p21 was investigated in the primary cultures of gynecologic tumor cells. The results showed that EGF-R and p21 were highly expressed in most but not all of the tumor cells, suggesting that these oncogene products are frequently involved in the development of gynecologic tumors.

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MATERIALS AND METHODS

All gynecologic cancer tissue were obtained at the time of surgery and were immediately dissected into small pieces with a razor. The cells were suspended in TC199 medium supplemented with 10% calf serum, plated in a Lab-Tek chamber (Miles Scientific) and cultured at 37 °C for 3 or 4 days.

Indirect immunofluorescent staining of cultured cells was carried out as described previously ([12]). The first antibodies used were mouse anti-EGF-R monoclonal antibody (Transformation Research Inc.) and rabbit anti-Ha-*ras* polyclonal antibody, HAS 2, which had been prepared by immunizing rabbits with a synthetic peptide corresponding to the region between positions 160 and 179 of c-Ha-*ras* p21 ([10, 13]). Second antibodies were fluorescein-conjugated anti-mouse IgG and rhodamine-conjugated goat anti-rabbit IgG (Zymed Laboratory Inc.). The cells were examined with fluorescent microscopy (Zeiss).

RESULTS

The expression of EGF-R and p21 was examined in 26 gynecologic tumors which included 13 endometrial cancers, 6 cervical cancers, 6 ovarian cancers and one rectum cancer (Table 1). Relative levels of expression of EGF-R in each tumor cell

Table 1. – *Relative expression of EGF-R and p21 in gynecologic tumor cells.*

Patient no.	Cancer type	Histhological type	Relative expression [1] EGF-R	p21
1	Endometrial cancer	Well differentiated adenocarcinoma	–	ND[2]
2	Endometrial cancer	Well differentiated adenocarcinoma	++	ND
3	Endometrial cancer	Well differentiated adenocarcinoma	++	ND
4	Endometrial cancer	Well differentiated adenocarcinoma	–	–
5	Endometrial cancer	Well differentiated adenocarcinoma	+++	++
6	Endometrial cancer	Well differentiated adenocarcinoma	+++	+
7	Endometrial cancer	Well differentiated adenocarcinoma	++	–
8	Endometrial cancer	Well differentiated adenocarcinoma	+++	+
9	Endometrial cancer	Well differentiated adenocarcinoma	++	++
10	Endometrial cancer	Moderately differentiated adenocarcinoma	+++	ND
11	Endometrial cancer	Moderately differentiated adenocarcinoma	++	–
12	Endometrial cancer	Moderately differentiated adenocarcinoma	++	+
13	Endometrial cancer	Poorly differentiated adenocarcinoma	–	+
14	Cervical cancer	Squamous cell carcinoma	++	ND
15	Cervical cancer	Squamous cell carcinoma	+	ND
16	Cervical cancer	Squamous cell carcinoma	+	+++
17	Cervical cancer	Squamous cell carcinoma	+	+++
18	Cervical cancer	Adenocarcinoma	+++	–
19	Cervical cancer	Adenocarcinoma	++	++
20	Ovarian cancer	Endometrioid carcinoma	++	ND
21	Ovarian cancer	Endometrioid carcinoma	+++	+++
22	Ovarian cancer	Endometrioid carcinoma	+	+
23	Ovarian cancer	Adenocarcinoma	++	++
24	Ovarian cancer	Adenocarcinoma	+	+++
25	Ovarian cancer	Mucinous cystadenocarcinoma	–	+
26	Rectum cancer	Well differentiated adenocarcinoma	–	ND

[1] Relative expression of EGF-R was determined by comparing with that in A431 cells (+++) and with that in NIH3T3 cells (–). Relative expression of p21 was compared with that in PH1-3 cells (++) and that in NIH3T3 cells (+).

[2] Not done.

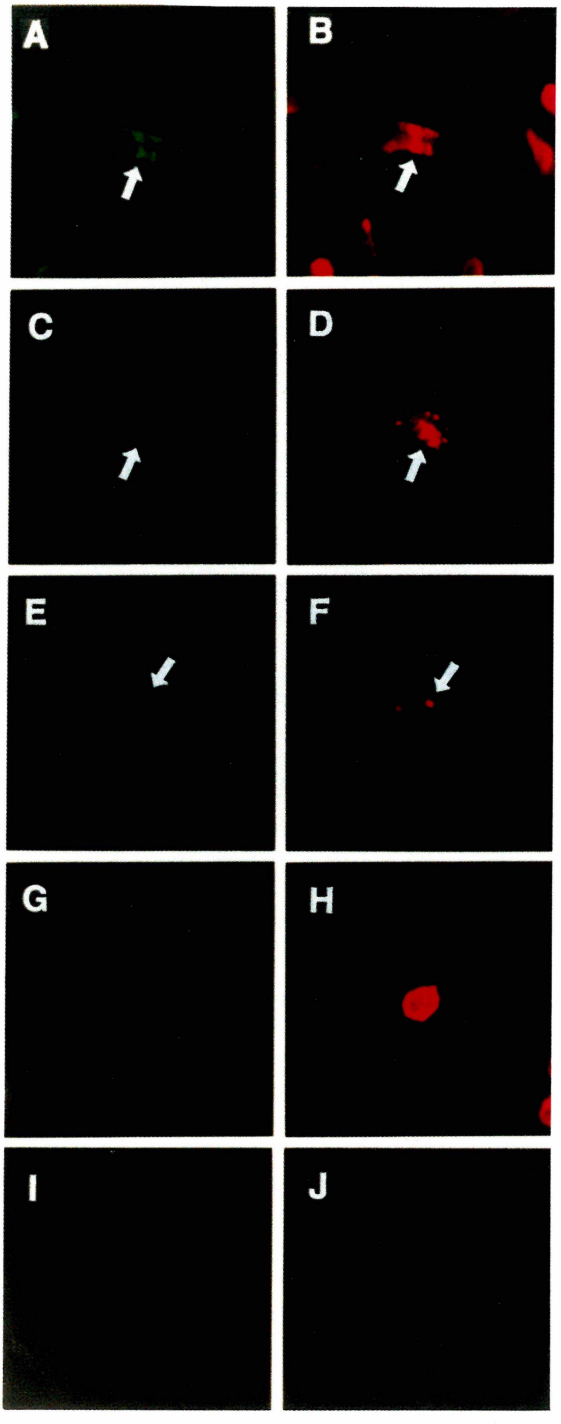

Fig. 1. — Double indirect immunofluorescent analysis of gynecologic tumor cells. Primary cultures of tumor cells from patients numbered 21 (A-D, I and J), 23 (E and F) and 24 (G and H) were simultaneously reacted for 1 h with mouse anti-EGF-R monoclonal antibody (1 : 20 dilution) and rabbit anti-p21 HAS 2 polyclonal antibody (1 : 60 dilution) (A - H), or with preimmune rabbit serum (1 : 60 dilution) (J), and then treated for 1 h with fluorescein-conjugated anti-mouse IgG (1 : 100 dilution) and rhodamine-conugated anti-rabbit IgG (1 : 100 dilution). Cells indicated in I was treated with only second antibodies. Fluorescence of fluorescein (EGF-R) (A,C,E,G, and I) and rhodamine (p21) (B, D, F, H and J) was shown in left and right figures, respectively. Each pair of A to H shows the same cells. (Original magnification: × 400).

were determined by comparing the fluorescence with that of NIH3T3 mouse fibroblasts as a negative control and with that of A431 human epidermoid carcinoma cells as a positive control [14]. The expression of p21 was compared with that of NIH3T3 cells and v-Ha-*ras*-transformed NIH3T3 cells, PH1-3 [15]. High expression of EGF-R was observed in 21 out of 26 cases (81%) and that of p21 was in 14 out of 18 cases (78%), suggesting that these oncogene products might have a role in most but not all of gynecologic carcinogenesis. There was no definitive correlation between the expression of EGF-R or p21 and the tissue type or the histological type of tumors (Table 1).

Some results of double indirect immunofluorescent analysis are shown in Fig. 1. Green-colored and red-colored photographs indicate the localization of EGF-R and p21, respectively.

Neither EGF-R nor p21 was distributed uniformly throughout cells but there were some concentrated areas in the cytoplasm (Fig. 1). Both EGF-R and p21 showed punctate distribution (Fig. 1B, D and F) and they were frequently co-localized. Some co-localized areas are indicated by arrows (compare each pair of Fig. 1A to F). It has previously been suggested that p21 is localized in plasma membrane [16], however, we occasionally observed such membrane localization of p21 (Fig. 1H). Neither omission of the first antibodies nor the use of preimmune rabbit serum produced any significant fluorescence (Fig. 1I and J, respectively).

DISCUSSION

High frequencies of elevated expression of EGF-R (81%) and p21 (78%) indicate that these two oncogene products are involved in carcinogenesis of gynecologic tumors. These high percentages are comparable to the frequent expression of p21 in stomach cancer cells (68%) [17] and in colon adenocarcinomas (77.1%) [18].

It is generally argued that p21 is localized in plasma membrane [16] whereas many results of immunohistochemical staining of cancer tissues have indicated that p21 is found in cytoplasm [17, 19]. Most primary cultures of gynecologic tumors showed punctate distribution of p21 (Fig. 1, data not shown). Similar granular pattern of p21 was also observed in *ras*-transformed NIH3T3 cells using polyclonal antibody HAS 2 [20] or NCC-RAS-001 monoclonal antibody [21] and in normal human colon, colon polyps and carcinomas using monoclonal antibody mES 13 [22]. Thus p21 may be localized not only in plasma membrane but also in cytoplasmic vesicles.

The co-localization of EGF-R and p21 suggests that these two proteins can interact with each other. We have recently found that p21 inhibits lysosomal cysteine proteinases [23], which can preferentially degrade EGF-R *in vitro* [24]. If p21 is associated with EGF-R *in vivo,* p21 could protect EGF-R against those proteases [25].

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