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DIFFERENTIAL EXPRESSION AND RESPONSE OF GROWTH FACTORS IN DIFFERENT METASTATIC VARIANTS OF HUMAN PULMONARY GIANT CELL CARCINOMA

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The cell lines PGbE1 and PGLH7, which have high and low metastatic potential respectively, were two different variants isolated from human pulmonary giant cell carcinoma cell line PG. This study compared the expression and response of several growth factors TGFα, TGFβ, bFGF, IL-6, IL-8 and ANG in the two cell lines. By using RT-PCR analysis and [^{3}H] thymidine incorporation assay, it was found that IL-6, TGFα and their receptors *IL-6R and EGFR were expressed at higher level* in PGbE1 cells than in PGLH7 cells, while no significant differences were found in the expression of ANG, bFGF, IL-8, IL-8R and TGFβ. Recombinant IL-6 and TGFα stimulated the proliferation of both cells, while TGFβ had dual effects. These results suggest that ANG, bFGF IL-6, IL-8 and TGFα, β may be involved in the proliferation of pulmonary giant cell carcinoma via autocrine mechanism, and IL-6 and TGFα may play an important role in the metastasis of tumor cells.

Key words: Growth factors, Metastasis, RT-PCR, Human pulmonary giant cell carcinoma.

It has been reported that bFGF, IL-6, IL-8, TGFα, β, bombesin and other growth factors regulated the proliferation of several kinds of tumor cells, including human lung cancers, via autocrine as well as paracrine mechanism.[1-4] It also has been shown that TGFα, IL-8, bFGF and angiogenesin (ANG) are involved in the tumor angiogenesis.[5,6] Since tumor proliferation and angiogenesis are essential process of metastasis, these data suggest that these growth factors may also play an important role in the tumor metastasis.

PG is a stably, highly metastatic cell line of human pulmonary giant cell carcinoma, from which two variants, PGbE1 and PGLH7, were isolated with *different metastatic potentials*.[7,8] Being of the same source, their difference in metastatic potential could be a consequence of differential expression of some metastasis related genes. A comparative study of the expression of growth factors in the two different metastatic variants may provide clues about the role (s) of growth factors in the metastatic process. In this study we examined the expression and response of several growth factors in PGbE1 and PGLH7 cells.

MATERIALS AND METHODS

Cells

PGbE1 and PGLH7 cells were isolated as described.[7,8] Cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) and appropriate amount of antibiotics. Cultures were kept at 37 ℃ in 5% CO_2 saturated incubator.

RNA Isolation, Reverse Transcription and PCR

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Amplification

Total cellular RNA was isolated by Chomczynski rapid one-step method,[9] reversely transcribed and amplified as described previously.[10]

Growth Assay

Growth assay was carried out by using [^{3}H] thymidine incorporation as described else where.[11] PGbE1 and PGLH7 cells were seeded in 96-well microtiter plates at the density of 5×10^3/well and grown to 70% confluency in RPMI-1640 containing 10% FCS. The cells were then cultured with serum free medium for 48 h. After serum starvation, cells were incubated with different concentration (0.25–20 ng/ml) of TGFα, TGFβ and IL-6 for 16 h, and then were pulse labeled with 0.5 uCi [^{3}H] thymidine per well for 4–6 h before being harvested. All experiments were conducted at least twice and by triplicate determinations. Control without added growth factors were considered as 100%.

RESULTS

Expression of Growth Factors in PGbE1 and PGLH7 Cells

The expression of growth factors was examined with RT-PCR technique. To assess the linearity between the PCR products and cDNA templates, lug of total RNA were reverse transcribed, and 50 ng cDNA was used to be amplified for 30 cyclesly for each factor detection. As a control on the quality of the RNA preparation and on the efficiency of the reverse transcription, primers of β-acin were included. As shown in Figure 1, TGFα, EGFR, IL-6 and IL-6R were expressed at higher levels in PGbE1 cells than in PGLH7 cells, while on difference were observed in the expression of bFGF, IL-8, IL-8R, TGFβ and ANG between the two cell lines.

Effects of TGFα, TGFβ and IL-6 on the Proliferation of PGbE1 and PGLH7 Cells

We next examined the effects of exogenous recombinant TGFα, TGFβ and IL-6 on the proliferation of the two cells by using [^{3}H] thymidine incorporation, the two cell lines exhibited different response to different growth factors. For example, though TGFα generally stimulated the proliferation of both cells, the effect was stronger on PGLH7 cells than on PGbE1 cells at higher concentration, while the effect was stronger on PGbE1 cells than on PGLH7 cells when the concentration was lower than 0.05 ng/ml (Figure 2). IL-6 showed a dose-dependent stimulatory effect on both cells, and the effect peaked at 2 ng/ml and 10 ng/ml for PGbE1 and PGLH7, respectively (Figure 3). TGFβ appeared to exert dual effects. At low concentration (<0.5 ng/ml), it stimulated cell proliferation with a stronger effect on PGLH7 cell than on PGbE1 cell, and at high concentration (>0.5 ng/ml), it inhibited cell growth with a weaker effect on PGLH7 cell than on PGbE1 cell (Figure 4).

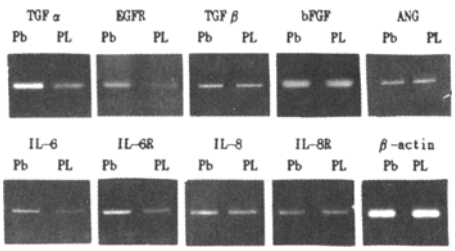

Fig. 1. Expression of growth factors and receptors in PGbE1 and PGLH7 cells, detected by RT-PCR. 50 ng RNA were used for growth factors and receptors, and 5 ng RNA for β-actin.

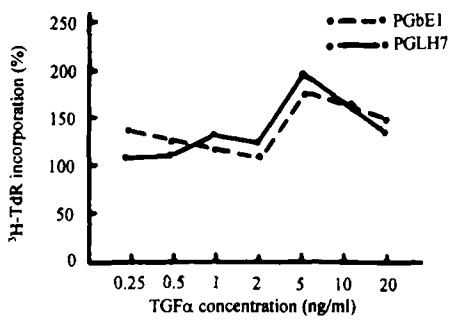

Fig. 2. Effect of recombinant TGFα on the proliferation of PGbE1 cells and PGLH7 cells.

DISCUSSION

The results described above demonstrate that

both PGbE1 and PGLH7 cells expressed TGFα, TGFβ, IL-6, IL-8, bFGF and ANG and receptors EGFR, IL-6R, IL-8R, suggesting that these factors may act as autocrine growth factors in the proliferation of lung cancer cells *in vitro*.

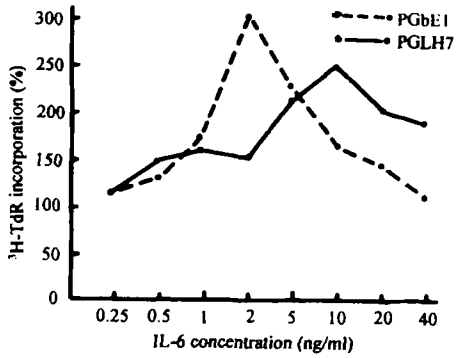

Fig. 3. Effect of recombinant IL-6 on the proliferation of PGbE1 cells and PGLH7 cells.

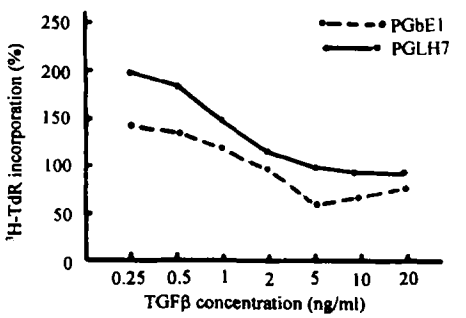

Fig. 4. Effect of recombinant TGFβ on the proliferation of PGbE1 cells and PGLH7 cells.

TGFα function as an autocrine growth factor in a variety of transformed and malignant cells *in vivo* and *in vitro*.[1, 12] In the present study, we observed a marked increase in TGFα and EGFR expressions in PGbE1 cell compared to PGLH7 cell. Furthermore, an autocrine level (pg/ml) of exogenous TGFα exerted higher stimulatory effect on PGbE1 cells than that on PGLH7 cells. In summary, the high metastatic cells exhibited elevated expression of and response to TGFα. These data suggest that TGFα may increase the metastatic potential of lung cancer via an increased autocrine production.

IL-6 is generally viewed as a member of the hemopoietic growth factor/cytokine family, many studies have emphasized its possible role in the proliferation of hematologic malignancies. Evidences are accumulating that IL-6 may act as autocrine or paracrine growth factor for nonhematologic malignacies.[3] In this study, IL-6 and its receptor coexpressed in PGbE1 and PGLH7 cells, with the expression level higher in PGbE1 cell than in the latter. Exogenous IL-6 exerted stimulatory effect on the proliferation of both cells in a dose dependent manner, with the highest effect dosage of 2 ng/ml and 10 ng/ml for PGbE1 and PGLH7, respectively. The high sensitivity of PGbE1 cell to IL-6 was consistent with its elevated expression of the receptor. These results suggest that IL-6 function not only as an autocrine also as a paracrine growth stimulating factor for lung cancer cells, and that IL-6 may enhance the metastatic potential of human lung cancer by increasing its autocrine production or increasing the amount of cell surface receptors with increased sensitivity to paracrine IL-6.

TGFβ was expressed at the same level in the two cells, low concentration of TGFβ stimulate the proliferation of both cells. It was concluded that TGFβ may act as an autocrine growth stimulator for lung cancer, but does not affect its metastatic potential. bFGF, ANG and IL-8, which are members of the angiogensis growth factor family,[6] exhibited no differences in their expression between the high and low metastatic cells, suggesting that angiogenesis has little to do with metastatic potentials of tumor cells, though it is necessary for tumor metastasis.

In summary. The growth factors TGFα, TGFβ, bFGF, ANG, IL-6 and IL-8 may be involved in the proliferation of lung cancer cells and/or in the tumor angiogenesis via an autocrine mechanism. The elevated expression of TGFα and IL-6 may enhance the metastatic potential of the expressing cells by increasing their selective growth predomination.

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