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*Letter to the Editor*

# Augmentation of Colony Formation of Cultured Cell Lines by Pleural Effusion from Patients with Lung Cancer

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THE EFFECTS of malignant pleural effusions obtained from patients with lung cancer on the colony formation by several cultured cell lines was examined by the soft agar clonogenic assay. The cell lines used were PC-7 and PC-9 (pulmonary adenocarcinoma), N231 and N857 (pulmonary small cell carcinoma), Raji (Burkitt lymphoma), K562 (chronic myelogenous leukemia) and MKN45 (gastric carcinoma).

Pleural effusions, confirmed by cytology to be of malignant origin, were obtained from seven patients without prior chemotherapy. There were five adenocarcinomas (Nos. 1, 2, 4, 5 and 7), one small cell carcinoma (No. 3) and one large cell carcinoma (No. 6). The pleural effusions were centrifuged for 10 min at 1500 rpm and the supernatant filtered through a 0.22 μm millipore filter. Protein concentrations as determined with protein assay kit (Bio-Rad Company) in the cell-free fluid ranged from 2.2 to 3.5 g/dl.

The soft agar clonogenic assay, previously described by Hamburger and Salmon [1], with some modification was employed. Briefly, 1 ml of tumor cell suspension ($1 \times 10^4 - 1 \times 10^5$/ml) of 0.3% agar in CMRL 1066 medium (GIBCO) supplemented with 10% horse serum, penicillin (100 units/ml), streptomycin (100 μg/ml), glutamine (2 mM), $CaCl_2$ (4 mM), insulin (3 units/ml), asparagine (0.6 mg/ml), DEAE-dextran (0.5 mg/ml) and freshly prepared 2-mercaptoethanol (0.5 mM) was plated onto 1 ml of bottom layer of 0.5% agar in McCoy's 5A medium supplemented with 15% heat-inactivated fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/m), Na pyruvate (0.22 mg/ml), glutamine (1 mM), serine (42 μg/ml), 10 ml of 3% tryptic soy broth, asparagine (0.6 mg/ml) and DEAE-dextran (0.5 mg/ml). The effect of pleural effusion on the colony forming ability was examined by adding cell-free pleural effusion to the top layer at the final concentration of 50%.

Relative plating efficiency was expressed as:

$$\frac{\text{No. of colonies/No. of cells plated (with pleural effusion)}}{\text{No. of colonies/No. of cells plated (without pleural effusion)}}$$

Table 1 shows the relation between the relative plating efficiency and the protein concentration in the pleural effusion. These effusions were listed in the order of increasing protein content. The effect of the pleural effusion on colony formation depended on the protein concentration and tumor cell line. The pleural effusions including a protein concentration >3.0 g/dl increased significantly the

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*Table 1. Relative plating efficiency and protein concentration in pleural effusion*

| Pleural effusion | | | Relative plating efficiency | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | Histology* | Protein concentration (g/dl) | PC-7 | PC-9 | N231 | N857 | Raji | K562 | MKN45 | Mean ± S.D. | *P* value† |
| 1 | Adeno. | 2.2 | 0.04 | 1.19 | 1.55 | 1.48 | 0.66 | 0.73 | 1.21 | 0.98 ± 0.50 | N.S. |
| 2 | Adeno. | 2.8 | 0.54 | 1.16 | 0.86 | 1.32 | 1.65 | 1.09 | 1.23 | 1.12 ± 0.39 | N.S. |
| 3 | Small | 3.2 | 1.09 | 1.49 | 1.53 | 2.30 | 2.30 | 1.04 | 1.27 | 1.53 ± 0.46 | <0.01 |
| 4 | Adeno. | 3.3 | 0.75 | 1.43 | 1.76 | 2.37 | 2.18 | 0.99 | 1.07 | 1.50 ± 0.60 | =0.05 |
| 5 | Adeno. | 3.3 | 1.51 | 1.48 | 1.99 | 3.25 | 1.87 | 1.01 | 1.52 | 1.80 ± 0.69 | <0.01 |
| 6 | Large | 3.4 | 1.67 | 1.21 | 1.28 | 1.83 | 0.94 | 1.19 | 1.32 | 1.35 ± 0.36 | <0.01 |
| 7 | Adeno. | 3.5 | 1.22 | 1.28 | 1.05 | 2.18 | 2.50 | 1.22 | 1.12 | 1.48 ± 0.61 | <0.01 |
| Mean number of colonies formed in control plate§ | | | 1032 | 1136 | 530 | 285 | 898 | 929 | 1642 | | |

*Adeno. = adenocarcinoma, Small = small cell carcinoma, Large = large cell carcinoma.
†All data are analyzed for significance by the paired *t*-test. *P* values are calculated by comparing the experimental groups with control.
‡N.S.: not significant.
§Mean of duplicate or triplicate experiments.

relative plating efficiency (Nos. 3, 4, 5, 6 and 7) ($P<0.01$–$0.05$). In addition, plating efficiency was augmented in majority of tumor cell lines especially in N231, N857 and Raji.

There are some reports demonstrating that cell-free malignant effusion augmented the plating efficiency of fresh human tumor cells [2, 3] and that epidermal growth factor (EGF) and platelet derived growth factor (PDGF) stimulated colony formation by freshly isolated as well as serially cultured tumor cells [4, 5]. This is the first report on the growth stimulation of cultured cell lines by malignant pleural effusion. On the other hand, neither EGF nor PDGF showed increases in plating efficiency (data not presented). This experiment indicated that malignant pleural effusions caused by lung cancer augmented the plating efficiency of majority of cultured cell lines. The mechanisms of stimulation are not clear.

These preliminary results warrant further investigation to identify the active substance in pleural effusions.

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