THE DIFFERENTIATION OF HUMAN GASTRIC ADENOCARCINOMA CELL LINE MGc80-3 INDUCED BY DIBUTYRYL cAMP IN VITRO

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For providing some experimental basis in establishing malignant phenotypic reversed indexes of gastric carcinoma cells, human gastric adenocarcinoma cell line MGc80-3 was induced by dBcAMP in vitro to appraise the effect of gastric carcinoma cell differentiation by chemical inducers.

Under light microscope, MGc80-3 cells, after treated with 1 mM dBcAMP, tended to be flat and disperse, and their volume gradually enlarged, with their nucleus relatively smaller and their shape rather regular. Morphological changes, like norma differentiated epithelial cells, were observed. The cells attached firmly, grew slowly, their growth curve showed inhibitory rate amounted to 52.87%, and cellular division exponent displayed their peak value 1.5 times less than that of MGc80-3 cells. It was clear that dBcAMP could effectively inhibit the multiplication activity of MGc80-3 cells. After dBcAMP treatment, remarkable changes of cell subface charges was indicated by cell electrophoresis, the ratio dropped to 3.043 from 3.968, and their retardant ratio reached up to 31.2%. cAMP content in cells after this treatment, detected by cAMP and cGMP radioimmunoassay, was enhanced by 2.42 times, and cAMP/cGMP ratio, by 1.73 times. Thus, cAMP level within MGc80-3 cells was raised obviously by dBcAMP. Heterotransplantation experiments showed that tumorigenic rate of MGc80-3 cells (transplanted subcutaneously to BALB/c mice) amounted to 100%, and that of the cells after this treatment was only 5.6%. Their tumorigenic ability was extremely reduced.

These results confirmed that dBcAMP was able to change malignant phenotypic characteristics of MGc80-3 cells and produce a reversed alteration: Thus, it has a remarkable inductive effect in differentiating gastric carcinoma cells. All these characteristics were also considered as the reference indexes in appraising reversed effect for the homologous cancer cells.

In recent years, a number of research showed that malignant tumor cells can be differentiated by cellular differentiating inducers. In order to explore the method and means of regulating the growth of cancer cells and controlling their malignant phenotypes artificially, O2-Dibutyryl adenosine-3', 5' cyclic monophosphate (dBcAMP) has been used as a differentiating inducer. It can inhibit the multiplicative activity of many animal and human transformed cells and tumor cell lines effectively, impel the cellular morphological, behaviour and biochemical features to produce a reversional alternation and restore their normal phenotype. In inducing differentiation research, it is an important basis of judging the malignant phenotypical reversion of cancer cells to inspect changes of the main malignant characteristics. The main differences between cancer and normal cells in vitro are displayed by the strengthening of cellular multiplicative capability, the change of cell surface features, the descending of endogenous cAMP level, havin the heterotransplanting tumorigenic character, and so on. MGc80-3 cell is a human gastric low differentiated mucous adenocarcinoma cell line, having the features of lower pathological differentiation degree, faster multiplication and higher malignant degree. It is an idea model for unfolding the study of gastric carcinoma cell in vitro. We appraised the differentiating effect of MGc80-3 cell induced by dBcAMP, according to changes of the characteristics above-mentioned, with the purpose of providing some data for establishing the malignant phenotypic reversional indexes of gastric carcinoma cell.

MATERIALS AND METHODS

Cell Culture and Induced Treatment

MGc80-3 cells were cultured in RPMI-1640 medium supplemented with 20% fetal calf serum,
and penicillin, streptomycin and kanamycin in just the right amount. The cells in logarithmic growth phase were harvested and seeded in 25 ml culture flask at a density of $12.5 \times 10^4$ cells per ml. Each flask contained 2 ml of cellular suspension. Cells were incubated at 37°C in an atmosphere of air: 5% CO$_2$. Induced treatment was performed after seeding for 24 hours, the culture medium was aspirated and replaced with the culture medium containing 1 mmol/L dBcAMP (purchased from Sigma Chemical Co.), and the culture medium in control group was replaced with new medium without dBcAMP, then incubated consecutively for experiment.

**Cell Growth Curve and Cell Mitotic Index Determination**

MGc80-3 cells seeded for 3 days were harvested in cellular suspension at a density of $5 \times 10^4$ cells per ml and subcultured in a lot of small culture flask and little penicillin bottles with cover slip strip, the small culture flask contained 2 ml of cellular suspension and the little penicillin bottles 1 ml. Induced treatment was performed after subculturing for 24 hours and culture medium was replaced once every three days during the incubation. From the first to seventh day after adding inducer, each three flasks of cells from experiment and control groups were harvested every day, the viable cells were counted by trypan blue dye exclusion, and their average values were adopted. The cover slip strips in each three little penicillin bottles were taken at the same time, fixed in Bouin-Hollonde fixation fluid and stained with HE. The mitotic cells in 1,000 cells at each cover slip strip were counted every day, and their average values were adopted. The experiments were repeated three times, their results were unanimous basically, and took one experiment among them as the criterion.

**Cell Electrophoresis**

MGc80-3 cells and the cells after 1 mmol/L dBcAMP treatment for 10 days were harvested, washed with D-Hank's solution twice and resuspended at the density $5 \times 10^7$ cells per ml. Both the experiment and control cells in the amount of $5 \times 10^6$ cells were inoculated at a same mouse. The experimental cells were subcutaneously inoculated at left foreleg armpit and the control cells were at the same position of right foreleg. Then each mouse was injected with 2.5 ml cortisone twice at that day and the next day. After inoculating for 14 days, tumor tissues were taken, measured their size and weighted, then compared the tumorigenic ratio.

**RESULTS**

**Effect of dBcAMP on the Morphology and Multiplication of MGc80-3 Cell**

There were manifold shapes in MGc80-3