

Some recent works on diagnosis and treatment of gastric cancer

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Aiming at earlier diagnosis and better result of treatment of gastric cancer, we endeavored to do some laboratory and clinical studies in recent years. The following is a brief sketch of these works.

PREPARATION AND USES OF MONOCLONAL ANTIBODIES

By means of cell fusion technic we established several hybridoma cell lines capable of producing antigastric cancer monoclonal antibodies^[1]. These antibodies were named MG series among which MGa-7, MGa-9, MGa-11 and MGb-2 were used more widely^[2]. The corresponding antigens of these antibodies were different from the known cancer related antigens such as CEA, AFP, X-hap ten, Tn antigen, etc. Their chemical nature was proved to be lipoprotein, glycoprotein, or glycolipid. Immunoelectron microscopy demonstrated that these antigens were chiefly distributed on the cell membranes and/ or in the cytoplasm of the gastric cancer cells, being especially abundant in the microvilli, the fossate surface of the cell membrane, the coarse endoplasmic reticulum and the microcysts.

Diagnostic uses

Immunohistological and cytological diagnosis Owing to the high specificity of these McAbs, they were used to determine the source of some metastatic tumors by immunohistological examination when ordinary histological staining gave ambiguous results^[3].

Dysplasia of gastric mucosa is quite common in atrophic gastritis. Its outcome is highly variable. It may remain stationary, regress or undergo

malignant degeneration after a certain period. Hence it is deemed as a precancerous lesion, causing anxiety to the patients and their relatives and leading to repeated gastroscopy with increased economical burden and physical sufferings. So far there has been no reliable method to predict the relative risk of malignant degeneration. We found that immunohistochemical staining of the gastric mucosa with MGa-7 may serve as an important parameter for judging the relative risk of malignant degeneration. In 156 patients with dysplasia of gastric mucosa, 84 gave positive reaction and 72 gave negative reaction. Patients of each group were followed up for 2 - 4 years by repeated gastroscopies. Among the positive reactors, gastric cancer was detected in 17 cases, 7 of which were in the early stage. Among the 72 negative reactors, no one developed gastric cancer.

Serological diagnosis Detection of the MG series McAb corresponding antigens (MG-Ags) in blood either by ELISA^[4] or by radioimmunoassay gave positive result in 58.7% to 72.8% of gastric cancer patients^[5]. A follow-up study in a group of patients undergoing gastrectomy showed that after the excision of the tumor, MG-Ags in serum decreased in titre or became undetectable, while in those with unresectable gastric cancer or metastasis to remote sites, the serum titre did not change significantly. Hence it could serve as a preliminary test in the diagnosis for gastric cancer and could be used for the surveillance of relapse after excision and for the appraisal of treatment efficacy. Owing to the simplicity and low cost of these serological tests, they were used as a screening measure in the mass survey for gastric cancer, many symptomless cases being discovered and properly treated.

More recently a new method for determination of serum MG-Ags was established through the construction of mAb-pXJ19 chimera^[6]. The mAb possesses specific affinity for gastric cancer related antigen, the pXJ19 is a recombinant template DNA and can be amplified by a pair of designed primers. This method was much more sensitive for detection of gastric cancer related antigen, yielding positive reaction in 85.2% of gastric cancer patients.

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Targeting therapy

Immunoconjugates Since MG series monoclonal antibodies possess specific affinity to gastric cancer tissue, they may carry anticancer agents to the targeted tumor cells. Various immunoconjugates were produced by linking these McAbs ricin, ricin A chain, methotrexate, daunorubicin, adriamycin, epirubicin, mitomycin C, bleomycin, cisplatin and radioactive iodine^[7]. After conjugation, both the immunological affinity of the antibodies for the tumor cells and the pharmacological activity of the cytotoxic agents were well preserved. These immunoconjugates exhibited selective cytotoxicity to gastric cancer cells. In vitro studies demonstrated that they could inhibit the growth rate of cancer cells. In vivo experiments showed that they could inhibit the growth of transplanted gastric cancer in nude mice much more effectively than unconjugated McAb or anticancer agents^[8]. In order to demonstrate the process of internalization of these immunoconjugates, we carried out observations by double dynamic electron microscopy labeling technic, using streptavidin-gold and sheep antimouse IgG gold probes^[9]. It was found that the main entry fashion of the conjugates was through non-coated microinvagination, followed by coated pits and interiorization of microvilli. Intracellularly, the endocytosed conjugates were transported from tubovesicular structures to multivesicular bodies and finally to lysosomes, where they were degraded. In the presence of verapamil, they stayed longer in tubovesicular structures, therefore, increased amount of them would remain in the cytosol. Thus cytotoxic effect could be augmented.

Immunoliposomes Immunoliposomes are spherical capsules with double-layered phospholipid coats, 40 nm - 120 nm in diameter, incorporating with antigastric cancer monoclonal antibodies. It could entrap a large number (6000 - 10000) of molecules of anticancer agents. By virtue of the specific affinity of the mAb to the gastric cancer related antigen, the immunoliposomes carrying the anti-tumor agents could be concentrated in the tumor cells manifesting a selective cytotoxic effect on the gastric cancer cells.

Boron neutron capture therapy Boron neutron capture therapy (BNCT) is based on the nuclear reaction ($^{10}\text{B}_5 + {}^1_0\text{n} + {}^7\text{Li}_3 \rightarrow {}^4\text{He}_2$), yielding lithium atom and alpha particles with high LET when ^{10}B is irradiated with thermal neutron^[10]. The essential factors for a successful BNCT are a large number of ^{10}B atoms concentrated in tumor cell and an adequate fluence rate of thermal neutrons. We

prepared immunoliposomes, 40nm in diameter, conjugated with mAb MGb-2 and entrapping a ^{10}B rich compound $(\text{Et-}_4\text{N})_2\text{-}^{10}\text{B}_{10}\text{H}_{10}$. There were 1.4×10^4 atoms of ^{10}B encapsulated and 20 molecules of MGb-2 incorporated per liposome. The immunoliposomes showed specific affinity to the gastric cancer cells and could deliver sufficient amount of ^{10}B to them. Thus when irradiated with thermal neutrons, the gastric cancer cells were selectively killed.

EXPERIMENTAL GENE THERAPY

The development of malignant tumors is believed to be related with the overactivation of the oncogenes and the inactivation of the tumor suppressor genes. So it is a logical approach to treat malignant tumors by inhibiting the oncogenes. The oncogenes related with gastric cancer include *c-myc*, *ras*, *c-erbB-2*, *K-sam*, *hst*, *n-myc*, *met*, *p53* (mutant form), etc. and telomerase, cyclin D1, PCNA, etc. are also oncogenic for gastric cancer. In recent years we used experimental gene therapy in the following ways.

Gene transfection of specific ribozyme of c-erb B2

It was found that the amplification and overexpression of c-erb B2 bear a close relationship with the occurrence, development and metastasis of malignant tumors. A specific ribozyme RZ1 for c-erb B2 mRNA, which can be splitted and inactivated by it, was constructed and transplanted into the gastric cancer cell line SGC 7901. The transfected cell line SGC/RZ1 manifested remarkable changes in growth rate, cell cycle, morphology and tumorigenicity. In comparison with the original SGC-7901 cells, the growth rate was inhibited by 55%. Under flow cytometry, there was a 44% decrease of cells in S phase. Electron microscopy demonstrated vacuole degeneration, karyopyknosis and apoptosis in many cells. In nude mice, the transplanted SGC/RZ1 cells showed a delayed tumor-formation time. The size of the tumor was much smaller than that of the control.

Transfection of antisense RNA of PCNA

Proliferating cell nuclear antigen (PCNA) is a cofactor for DNA polymerase, playing a very important role in the replication of DNA, proliferation of cells and regulation of cell cycle. Inhibition of PCNA expression would bring about changes of malignant behavior of tumor cells. To testify this, we transfected antisense RNA of PCNA into SGC-7901 cells. The transfected cells manifested retardation of growth rate,

degeneration, necrosis and apoptosis. The tumorigenic power in nude mice was inhibited.

Transfection of wild type p53 gene

Wild type *p53* (*wtp53*) gene is a tumor suppressor gene. When *wtp53* cDNA was transplanted into SGC-7901 cells, the transfected cells (SGC7901/*wtp53*) manifested decreased growth rate and prolonged doubling time. Transmission electron microscopy revealed shrinkage of the cells and characteristic morphological features of apoptosis. By flow cytometry, the percentage of cells in G1 phase increased while that in S phase decreased in comparison with the parental SGC 7901 cells.

Transfection of herpes simplex virus thymidine kinase (HSV-TK) gene

HSV-TK can turn the nontoxic ganciclovir (GCV) into phosphorylated GCV, which is a potent inhibitor of DNA synthesis. When HSV-TK-mRNA was transfected into gastric cancer cells and the transfected cells were exposed to GCV in the culture medium, they were killed in a dose-dependent fashion.

Transfection of cyclin D1 antisense RNA

Cyclin D1 gene, located on the chromosome 11q13 region, has been found in many cancers, including gastric cancer, and is regarded to be related to carcinogenesis. To testify the effect of inhibiting cyclin D1 in gastric cancer cell, cyclin D1 antisense RNA was first constructed and then was transfected into SGC-7901 cells. The transfected cells displayed a much longer doubling time, an increased percentage of cells in G1-G0 phase, and marked inhibition of tumorigenicity in nude mice.

Transfection of telomerase antisense RNA

Recent studies indicated that the activation of telomerase is a very important factor for the uncontrolled proliferation of tumor cells. We found that telomerase was detected in 84.2% of 38 cases of gastric cancer, and only 5.2% of normal gastric mucosa. After SGC-7901 cell was transfected with human telomerase anti-sense RNA, its growth rate slowed down in culture. Morphological changes of necrosis and apoptosis occurred, and oncogenic property was reduced.

The above-mentioned preliminary experiments indicate that gene therapy might be a promising approach to the treatment of gastric cancer.

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