HETEROGENEITY IN ANTIGENIC EXPRESSION AND RADIOSENSITIVITY IN HUMAN COLON CARCINOMA CELL LINES

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SUMMARY

A panel of human colon carcinoma cell lines were characterized regarding both antigenic heterogeneity and variations in radiosensitivity. Monoclonal antibodies were used to study the expression of carcinoembryonic antigen (CEA), gastrointestinal cancer antigen (GICA or CA 19-9) and carcinoma-associated antigen (CA-50). Radiosensitivity was studied with the clonogenic survival technique. Three cell lines, LS 174T, HCTC, and SW 1116 stained positive for all three antigens. HT-29 was positive for CA 19-9 and CA-50 whereas Caco-2 was positive for CEA and CA 19-9. The cell lines SW 620 and LIM 1215 only stained positive for one of the antigens, CA-50 and CEA, respectively. In nearly all positive cases the stainings were very heterogeneous with mixtures of positive and negative cells. One exception was the HCTC cells which stained homogeneously for the CA 19-9 and CA-50 antigens. The neuroendocrinelike COLO 320 cells were negative in all cases. The radiosensitivity varied strongly between the cell lines with $D_0$-values between 0.8 and 1.9, extrapolation numbers between 2.0 and 4.7, $D_0$-values between 1.1 and 2.8. The surviving fraction at 2 Gy varied between 0.3 and 0.7 with HCTC as the most radiosensitive and HT-29 as the most radioresistant cell line. Thus, there were differences in antigenic expression and intrinsic radiosensitivity between the cell lines and antigenic heterogeneities within each cell line. The analyzed panel of cell lines will be valuable in studies of dose-effect relations for monoclonal antibodies labeled with toxic radionuclides simulating both antigenic heterogeneity and variations in radiosensitivity.

Key words: carcinoembryonic antigen; CA 19-9; CA-50; CEA; cell lines; colorectal carcinoma; cultured cells; radiation effects; tumor markers.

INTRODUCTION

Colorectal cancer is one of the most common malignant tumors, with an increasing incidence in Western Europe and North America (35). The primary treatment is surgery. Radiotherapy is used complementary for different reasons, e.g., to decrease the risk of local recurrences, as preoperative treatment of a primarily non-resectable tumor, or as treatment of a local recurrence (22). Colorectal carcinoma has often been considered as an intermediately radiosensitive tumor (12,18), and a considerable heterogeneity in the response to radiation therapy was seen in clinical studies (7,13,34,38).

The tumor markers CEA (glycoprotein with a molecular weight of about 180 kDa), CA 19-9 and CA-50 (both carbohydrates with sialo-ganglioside determinants and with molecular weights of about 36 kDa) are found in fetal tissue and are often reexpressed in gastrointestinal tumors in adults (25). The expression of these antigens has been reported to be heterogeneous in human tumors (14,28) and in transplanted human colorectal carcinoma tumors grown on immunodeficient rodents (2,17).

Monoclonal antibodies are presently used for clinical determinations of serum levels of the CEA, CA-50, and CA 19-9 antigens (3,40,45,46,48,52) and scintigraphic visualization of the spatial antigen distribution has recently been tried (1,4-6,36). Antibodies to the antigens CEA and CA 19-9 are not specific or sensitive enough to detect colorectal cancer (32) but they are claimed to be useful in the "follow up" of patients (3,46). It has been proposed that analysis of two or more markers together (e.g., CA-50, CEA, and CA 19-9) might give improvements in the diagnostic procedures (40,45,52).

Human colorectal carcinoma cells transplanted in immunodeficient mice or rats have in some cases been used as a model for immunotherapy with $^{131}$I- or $^{90}$Y-labeled antibodies to CEA (9,17,26,42,43,50).

There is a potential for therapy with radiolabeled toxic antibodies when the diagnostic procedures show that the antigens are strongly expressed. Such therapy could, for disseminated colorectal carcinomas, be a useful complementary method to other types of therapy (16,21,32,44). However, it is unclear to what extent heterogeneity in the antigenic expression will decrease the therapeutic effects of treatments with monoclonal antibodies labeled with toxic radionuclides. Furthermore, the results of such therapy might vary from patient to patient if the intrinsic radiosensitivity of the tumor cells show large variations. Both heterogeneity in the antigenic expression and variations in intrinsic radiosensitivity make the results difficult to predict. It is therefore necessary to develop models of colorectal carcinomas where the possible heterogeneity in antigenic ex-
The aim of this study was to characterize colon carcinoma cell lines regarding both heterogeneity in antigenic expression and variations in intrinsic radiosensitivity. This is of interest for planned experimental therapy with monoclonal antibodies loaded with different types of toxic agents such as radionucleides, toxins, or cytostatic drugs. Variations in intrinsic radiosensitivity is also of interest in studies of combined treatments with chemotherapy and other therapeutic modalities.

**Materials and Methods**

**Cell lines.** All studied cell lines (Table 1), except SW 620, came from primary colonic adenocarcinoma tumors. SW 620 came from a lymph node metastasis of an adenocarcinoma growing primarily in the colon. COLO 320 came from a tumor classified as an adenocarcinoma, but the tumor cells showed hormonal production like carcinoids and are probably of neuroendocrine origin. The COLO 320 cells were included in the study for comparative purposes.

**Culture conditions.** Monolayer cultures were grown in Ham’s F10 medium supplemented with 10% fetal bovine serum, 2 mM glutamine and antibiotics, penicillin 100 IU/ml, and streptomycin 100 /µg/ml. All ingredients were from Flow Laboratories, Stockholm, Sweden.

The cells were normally grown in 50-ml culture flasks containing 10 ml medium, in an incubator at 37 °C and in humid air with 5% CO₂. The culture medium was replaced with fresh medium at 2- to 3-day intervals. Subculture was routinely made before confluency was reached (i.e., below 10⁶ cells/cm²), usually every 10th day. The cell cultures were then treated with a quick rinse of phosphate buffered saline and then incubated for 5- to 15 min in trypsin-EDTA (Flow Laboratories).

**Growth curves.** At each subculture, a sample of cells was counted using a Cell-Counter 134, Analyse Instrument AB, Sweden. The cells were always counted during the logarithmic growth phase before confluency was reached. The number of cells was plotted logarithmically vs. days in culture, and doubling times were calculated. The experiments were repeated 3 to 5 times for each cell line.

**Immunohistochemistry.** Cells were plated on Thermanox tissue culture cover slips with a diameter of 13 mm (Flow Laboratories), and cultured for about 1 wk. The cells were then aceton fixed for 15 to 20 s, followed by a fast rinse in distilled H₂O. The carcinoma-associated antigens CEA, CA 19-9, and CA-50 were identified by monoclonal antibodies secreted by mouse hybridomas obtained after immunization with colon carcinoma cells. Anti-CEA 38S1, which is of IgG type, identifies an epitope of the CEA glycoprotein antigen, and was a gift of Dr. A. Hedrin, Pharmacia AB, Uppsala, Sweden (23). C 19-9, an IgG antibody, identifies a sialo-ganglioside containing a sialylated Lewis A determinant CA 19-9 and was kindly provided by Dr. H. Koprowski, Wistar Institute of Anatomy and Biology, Philadelphia, PA, (27). C-50, an IgM antibody suitable for CA-50 staining, with a similar but somewhat broader specificity than C 19-9, was a gift of Dr. L. Lindholm, Dep. of Medical Microbiology, University of Gothenburg, Sweden (25, 31). The avidin-biotin-peroxidase method was used (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA) according to a protocol previously described (14). Detection of the antigens was not analyzed.

**Classification of antigenic heterogeneity.** The relative number of positively stained cells in a culture was classified in three steps: <25, 25-75, and >75%. The spatial distribution of positive cells in each culture was graded with letters, A, B, and C, where A means an isotropic distribution of single positive cells (single positive cells randomly distributed among negative cells) and C means mainly isolated areas (clusters) of positive cells separated from areas with only negative cells. M means a mixture of both single positive cells and clusters, and H means homogeneous positive staining. The strength of the staining was graded in three steps: +, ++, or ++++. One plus means weak staining, two pluses intermediate, and three pluses strong staining.

**Irradiation.** Survival after irradiation was estimated as the fraction of cells with a reproductive capacity giving rise to colonies with at least 50 cells, according to the clonogenic survival technique originally described by Puck and Marcus (37). The cell cultures were trypsinized and a specified number of cells were plated in 50-ml culture flasks with 10 ml medium. The number of plated cells per flask varied in the range of 200 to 50 000 depending on the dose.

**Radiosensitivity.** After irradiation, cells were incubated for about 3 h at 37 °C and then harvested. The irradiation was performed at room temperature with 16 MV x-rays (Philips SL75/20) with a dose rate of 2.0 Gy/min at dose maximum depth 3 cm in a polystyrene phantom. The absorbed dose was measured during each irradiation with silicon diodes (Scanditronix AB, Uppsala, Sweden). The diodes were calibrated against an ion chamber before each irradiation. The ion chamber was calibrated in relation to the Swedish Secondary Standard Laboratory, Stockholm. The radiation field was 15 x 15 cm, and the cells were plated at a source distance of 1 m. The dose rate varied less than ±3% over the radiation field. Different doses (0, 1, 2, 4, 6, 8, and 10 Gy) were given to duplicate tasks.

**Results**

**Growth rate.** The doubling times, calculated from the growth curves, varied for the studied cell lines between 1.8 days for the fastest growing cell line, SW 620, up to 5.4 days for the slowest, Caco-2 (Table 1).

**Immunologic stainings. CEA.** Five cell lines (LS 174T, HCTC, Caco-2, LIM 1215, and SW 1116) stained positively for CEA to a moderate extent and with a variable amount of positive cells and with a varying grade of heterogenous distribution (Table 2) and Fig. 1). HT-29, SW 620, and the neuroendocrine-like COLO 320 cells were negative.

**CA 19-9.** Five cell lines (HT-29, LS 174T, HCTC, Caco-2, and SW 1116) were positively stained for the antigen CA 19-9.