The growth properties and CEA localization of cultured signet ring cell carcinoma transplanted into athymic nude mice

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The signet ring cell carcinoma is one type of gastric carcinoma. The growth properties of this particular type are not fully understood. This study has been undertaken to elucidate the features of the tumor growth of the cells transplanted into athymic nude mice which correlated with the cell features in vitro.

In vitro, the signet ring cell carcinoma cell line, MA IV, was established from a pleural effusion of a 36-year-old male patient. These cells were cultured with a Ham's F-10 Medium containing 20% fetal bovine serum for two years in vitro with the following characteristics: The doubling time was 33.6 hours. The model chromosomal analysis showed 46, XY, +8, mar in 74% cells. These cells ranged microscopically from 7 to 20 μm in size and round in shape, having a pleomorphic nucleus, and containing PAS and alcian blue positive granules and vesicles in cytoplasm. Immunohistochemically carcinoembryonic antigen (CEA) was localized on the cell membrane and CEA production correlated with cell growth property by 3H thymidine and BrdU uptake. Signet ring cells were classified as mature cells and immature cells with a morphological appearance. The nuclei of mature cells were compressed by abundant mucous granules and seen in the periphery of the cytoplasm. However, immature cells had round nuclei which were seen in the center of the cells, and the mucous granules were scarcely distributed. The morphological features changed corresponding to the maturing process and subsequent CEA production.

To transplant the carcinoma cells, athymic nude mice (nu/nu) having BALB/c genetic background were used. Five to eight-week-old mice were fed under germ free conditions. After 6 months, 1 × 10⁸ signet ring cells were injected into the subcutaneous tissue on the back of seven athymic mice with 500 rad radiation. Two weeks after cell transplantation, the mice were sacrificed. The tumor-bearing tissues were extirpated and evaluated histologically with HE, PAS and alcian blue stains in addition to the observations of the immunohistochemical localization of CEA. The CEA antibody (DAKO Co.) absorbed human spleen extract in order to avoid the localization of non specific cross reacting antigen (NCA).

In this experiment, tumor growth was not observed in seven nude mice without radiation, however, in two of seven mice with radiation, tumor growth was observed (Fig. 1a). Histologically, the tumor revealed the invasion of immature cells into the surrounding tissue (Fig. 1b). Mature cells with ghost cells were noted in the central zone of the tumor. The cytoplasms of these cells were stained with alcian blue and PAS stains. CEA localizations were observed in the cytoplasm of
invasive immature cells into the surrounding tissue (Fig. 2), while mature cells showed a weakly positive reaction on the plasma membrane. The fibroblasts in the stroma of this tumor were growing around cancer cells, and the morphological appearance of growing stroma was observed as scirrhous carcinoma, which suggested the interaction of cancer cells and stromal cells. The morphological and immunohistochemical findings of cancer cells in vivo were similar to those of cells in vitro.

We concluded that the signet ring cells in vivo had been morphologically changed to the invasive pattern which correlated with excessive CEA production.

**Key words:** carcinoembryonic antigen (CEA), gastric carcinoma, signet ring cell carcinoma, athymic nude mouse, characterization

**References**


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