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ORIGINAL RESEARCH

Determination of β-glucuronidase in human colorectal carcinoma cell lines

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Abstract

AIM: To study the relationship between β-glucuronidase and the invasiveness of human colorectal carcinoma cell lines.

METHODS: Six colorectal carcinoma cell lines, including three well-differentiated (CX1, CCL187, and CCL229) and three poorly differentiated ones (CCL227, CCL228, and Clone A), were analyzed by Fischman's method to determine the concentration of β-glucuronidase in the medium.

RESULTS: Low levels of β-glucuronidase (activity range: 1.29 to 1.96 μg/106 cells·h) were associated with poor invasiveness. This finding was in contrast to the elevated levels of the enzyme (2.46-3.37 μg/106·h) detected in the medium derived from the more aggressively invasive cells (CCL 227, CCL 228, Clone A, and CCL 229).

CONCLUSION: Highly invasive colorectal carcinoma cells secreted higher levels of β-glucuronidase than the poorly invasive cells. Determination of secreted β-glucuronidase might represent a useful in vitro measurement tool to assess the invasiveness of colorectal carcinoma.

Key words: Colorectal neoplasms; β-glucuronidase invasiveness; Cell lines

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INTRODUCTION

The major cause of death in patients with malignant neoplasms is metastasis. The spread of tumor cells from the primary site to other organs involves a sequence of complex biologic events. Improving our knowledge about the mechanisms of metastasis, at a biologic level, may enable us to detect the pre-metastatic state and inhibit tumor invasion. The following multistep cascade contributes to the initiation of tumor metastasis: (1) tumor cells attach to the extracellular matrix by means of cell-surface receptors; (2) tumor cells degrade the matrix by secreting specific degradative enzymes; and (3) tumor cells migrate through the degraded matrix, facilitating metastasis[1]. Matrix degradative enzymes, such as urokinase, type Ⅳ collagenase, β-glucuronidase and Cathepsin B, play important roles in this process.

β-glucuronidase, a lysosomal acid enzyme that acts to degrade proteoglycan, the major component of basement membrane, participates in the processes of tumor invasion and metastasis. In this study, an attempt has been made to establish a relationship between β-glucuronidase secretion and the primitive nature of *in vitro* colorectal carcinoma. Accordingly, the β-glucuronidase secretory capacities of six colorectal carcinoma cell lines, representing varying degrees of differentiation and invasiveness, were compared. The results indicated that the poorly and highly invasive cells could be separated on the basis of β-glucuronidase secretion. This biochemical marker may prove useful in assessing the primitive characteristics of cultured colorectal carcinoma cells.

MATERIALS AND METHODS

Materials

Human colorectal carcinoma cell lines that had originated from tumor tissues and were well established in serial subcultures were used in this study. The cell lines CX1, CCL187, CCL227, CCL228, CCL229, and Clone A were generous gifts from the Dana Farber Cancer Institute of Harvard Medical School, USA.

Methods

The cell lines were maintained in Dulbecco's modified Eagle's medium (GIBCO, BRL) supplemented with 10% calf serum and 2 U/mL gentamycin. Cell cultures were incubated at 37 ℃ in a humidified atmosphere of 95% air and 5% carbon dioxide.

The culture medium was collected as follows. Colorectal carci-

Table 1 Conditions for measuring β-glucuronidase in culture medium

Medium (mL)	Water (mL)	Acetate buffer (mL)	pH	Substrate final	Molarity (mL)	Incubation time at 37 ℃ (h)	Alkalinizing reagent (mL)	Final pH	Coloremeter wavelength (nm)
0.2	0.4	0.2	4.5	0.2	0.006	18	2[1] + 3 mL H_2O	10.2	540

[1]Glycine-Duponal reagent: 15.01 g of glycine dissolved in 900 mL of H_2O and brought to pH 11.7 by addition of 50% NaOH solution. Duponal (sodium lauryl sulfate) was added to produce a final concentration of 0.2% and water was added to achieve a final volume of 1 L.

Table 2 Activity of secreted β-glucuronidase in culture medium of six colorectal carcinoma cell lines

Cell line	Differentiation degree[3]	Invasiveness[3,4]	n	β-glucuronidase activity, $\bar{x} \pm s$
CX1	Good	Low	6	1.29 ± 0.17
CCL187	Good	Low	6	1.96 ± 0.28
CCL229	Good	High	6	3.37 ± 0.34[b]
CCL227	Poor	High	6	2.46 ± 0.18[b]
CCL228	Poor	High	6	2.73 ± 0.19[b]
Clone A	Poor	High	6	3.22 ± 0.38

[b]$P < 0.001$ *vs* CX1, CCL187.

noma cells were seeded in 100 mL flasks (2.5×10^5 cells/mL). After 3 d of culture, the medium was refreshed completely. After an additional day of culturing, the medium was harvested and the cells enumerated. The collected medium was condensed (mL/5×10^6 cells) and stored at 4 ℃ for future use. β-glucuronidase activity levels in the collected medium was determined by Fischman's method[2]. Phenolphthalein standard curve was set up in a range of 0 mg/L to 40 mg/L. The substrate was phenolphthalein mono-β-D glucosiduronic acid sodium salt. The conditions for measuring medium levels of β-glucuronidase are shown in Table 1. One enzyme activity unit equated to 1 μg of released phenolphalein/10^6 cells·h. The results were analyzed by Student's *t*-test.

RESULTS

The medium from each cell line was analyzed for activity of β-glucuronidase. The well differentiated and poorly invasive cell lines CX1 and CCL187 were found to be low secretors of the enzyme (activity range: 1.29-1.96 μg/10^6 cells·h). In contrast, the poorly differentiated and highly invasive cell lines CCL227, CCL228 and Clone A, as well as the well differentiated CCL229 with high invasiveness[3], were relatively more active in this respect, with β-glucuronidase activities ranging between 2.46 μg/10^6 cells·h and 3.37 μg/10^6 cells·h (Table 2).

DISCUSSION

Recent studies have highlighted the association of matrix degradative enzymes with malignant tumors, and have suggested that these enzymes may play a role in tumor invasion and metastasis. Although a lot of work has been done to investigate the effects of urokinase and type (WTBZ) Ⅳ (WTB1) collagenase on tumor invasion and metastasis[5,6], there are few reports about β-glucuronidase in this respect, especially in regards to colorectal carcinoma. β-glucuronidase, a lysosomal acid enzyme that can degrade proteoglycan, the major component of basement membrane, is known to participate in the process of tumor invasion and metastasis. Poole[7] reported that β-glucuronidase activity was high in experimental rat tumors and that the enzyme was present in the matrix ahead of the invading tumor. Dai *et al*[8] reported that the β-glucuronidase activity level in stomach cancer was higher than that in non-cancerous tissues. Nicolson *et al*[9] confirmed that highly metastatic melanoma cells secreted higher levels of β-glucuronidase and degraded subendothelial basement membrane at a higher rate than poorly metastatic melanoma cells. All these findings have supported the hypothesis that β-glucuronidase is closely related to tumor metastasis.

In order to illustrate the relationship between β-glucuronidase secretion and invasiveness of human colorectal carcinoma, we analyzed the culture medium from six cell lines to determine the activity of β-glucuronidase within. The results indicated that the highly invasive cell lines secreted higher levels of β-glucuronidase than the poorly invasive ones, supporting the notion that β-glucuronidase might contribute to colorectal carcinoma invasion and metastasis. Moreover, determination of secreted β-glucuronidase might represent a useful measurement tool for the invasiveness of *in vitro* colorectal carcinoma.

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