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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Table 1  Conditions for measuring β-glucuronidase in culture medium

| Medium (mL) | Water (mL) | Acetate buffer (mL) | Substrate final | Molarity (mL) | Incubation time at 37 °C (h) | Alkalizing reagent (mL) | Final pH | Coloremeter wavelength (nm) |
|------------|-----------|---------------------|-----------------|--------------|------------------------------|------------------------|---------|--------------------------|
| 0.2        | 0.4       | 0.2                 | 4.5             | 0.2          | 0.006                         | 18                     | 10.2    | 540                      |

*Glycine-Duponal reagent: 15.01 g of glycine dissolved in 900 mL of H2O 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.

\[^{1}\text{P < 0.001 vs CX1, CCL187.}\]

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

| Cell line | Differentiation degree\(^{(2)}\) | Invasiveness\(^{(3)}\) | \(n\) | β-glucuronidase activity, \(\mu g/10^6\) cells·h \(t\) |
|-----------|----------------------------------|---------------------|-----|----------------------------------|
| CX1       | Good                             | Low                 | 6   | 1.29 ± 0.17                      |
| CCL187    | Good                             | Low                 | 6   | 1.96 ± 0.28                      |
| CCL229    | Good                             | High                | 6   | 3.37 ± 0.34\(^{(4)}\)            |
| CCL227    | Poor                             | High                | 6   | 2.46 ± 0.18\(^{(5)}\)            |
| CCL228    | Poor                             | High                | 6   | 2.73 ± 0.19\(^{(6)}\)            |
| Clone A   | Poor                             | High                | 6   | 3.22 ± 0.38                      |

\[^{(1)}\]CX1, CCL187, CCL229, CCL227, CCL228, Clone A

\[^{(2)}\]Glycogenosis

\[^{(3)}\]Invasion

\[^{(4)}\]Collagenase

\[^{(5)}\]Glycogen

\[^{(6)}\]β-glucuronidase

\[^{(7)}\]P < 0.001 vs CX1, CCL187.

Rena 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 °C for future use. β-glucuronidase activity levels in the collected medium was determined by Fischman’s method\[^{(1)}\]. Phenolphthalein standard curve was set up in a range of 0 mg/L to 40 mg/L. The substrate was phenolphthalein mono-β-D glucurononic 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\[^{(2)}\]\(^{(3)}\), 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) collagenses on tumor invasion and metastasis\[^{(4)}\]\(^{(5)}\), 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\[^{(6)}\] 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\[^{(7)}\] reported that the β-glucuronidase activity level in stomach cancer was higher than that in non-cancerous tissues. Nicolson et al\[^{(8)}\] 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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