A note on the functions of bc1-2 in human solid tumors in situ

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Summary

Dual functions of bc1-2 product, (1) inhibition of apoptosis and (2) inhibition of cell growth, were analyzed in human endometrial adenocarcinoma tissue using serial tissue sections and immunohistochemical and histochemical procedures. As conclusion, bc1-2 product was speculated to have at least two functions; (1) suppression of apoptosis of cells in G0 phase, and (2) blocking the entrance of G0 cells into cell division cycle.

Introduction

Bc1-2 has been known to protect cells from apoptosis in normal and malignant tissues [1, 2]. In addition, overexpression of bc1-2 inhibits growth of solid tumor cells in vitro [3]. Thus the function(s) of bc1-2 product seems to be dual when one considers prolongation of life and inhibition of growth of the cells. To demonstrate these two aspects of bc1-2 function(s) in human solid tumor in vivo, we performed serial section analysis of 10 cases of human endometrial adenocarcinomas [4-6]. All the cases were well-differentiated types and had not undergone preoperative chemotherapy.

Materials and Methods

Immunohistochemical or histochemical procedures were applied to formalin-fixed and paraffin-embedded tissue which had been serially cut 1 micrometer in thickness. The section series were stained with hematoxylin eosin, Ki-67 (immunostain) and bc1-2 (immunostain), and DNA-nick ends labeled by NEL, a modified method of TUNEL [7].

At first, bc1-2 staining pattern (normal lymphocytes were used as an internal control because of their constant strong signal) and NEL signal were compared and analyzed [4]. As in Fig. 1, bc1-2 positive carcinoma nests tended to be NEL negative, indicating that bc1-2 blocks in general apoptotic DNA fragmentation in human endometrial adenocarcinoma.

Then, the status of bc1-2 expression and cell proliferation was compared and analyzed [5]. Presence of Ki-67 antigen was used as a hallmark of proliferating cells, and Ki-67 negative cells were judged as those in the G0 phase [8, 9]. As shown in Fig. 2-1, an inverse correlation was found between the degree of bcl-2 expression and cell growth fraction (fraction of proliferating tumor cells of the total tumor cells); the quantitative data have been already described in reference no. 5.

Results

The results indicate that bcl-2 overexpression is found mainly in the G0 cell population. This fact indicates further that bc1-2 blocked G0 cells from entering the cell cycle, in other words possible "growth inhibition" by bc1-2 product.

From these two results, we suggest that bc1-2 inhibits apoptosis of cells mainly in the G0 phase. In cultured cells, a correlation between an apoptosis – suppressing effect of bc1-2 and cell cycle status has not yet been established [10, 11]. To conform this speculation, the correlation between Ki-67 stain, NEL and bc1-2 immunostain was analyzed [6]. As shown in Fig. 2-2, even when bc1-2 overexpression was absent, apoptotic DNA fragmentation occurred randomly irrespective of phases in the cell cycle (quantitative data were given in reference no. 6). The fact that a random distribution of apoptosis is shown throughout the cell cycle including G0 was alreally reported in the in vitro system [12]. Thus, apoptosis in G0 cells certainly exists among human endometrial adenocarcinomas, and this supports the above hypothesis that "bc1-2 suppresses apoptosis of cells mainly in G0".

Conclusions

In conclusion, we speculate that bc1-2 product has at least two functions: (1) suppression of apoptosis of cells in the G0 phase, and (2) blocking the entrance of G0 cells into the cell division cycle.

It should be necessary to clarify a correlation of the above-mentioned two functions. This problem could be a key point in understanding the correlation between prolongation of the life and growth potential of cells.

Acknowledgement

This communication was based on the results in our previous three articles (references no. 4-6), and the figures were taken from the articles.

The authors thank Drs. Moriaki Hayashi and Hiromichi Matsudaira for their expert advice.

Revised manuscript accepted for publication July 5, 1999

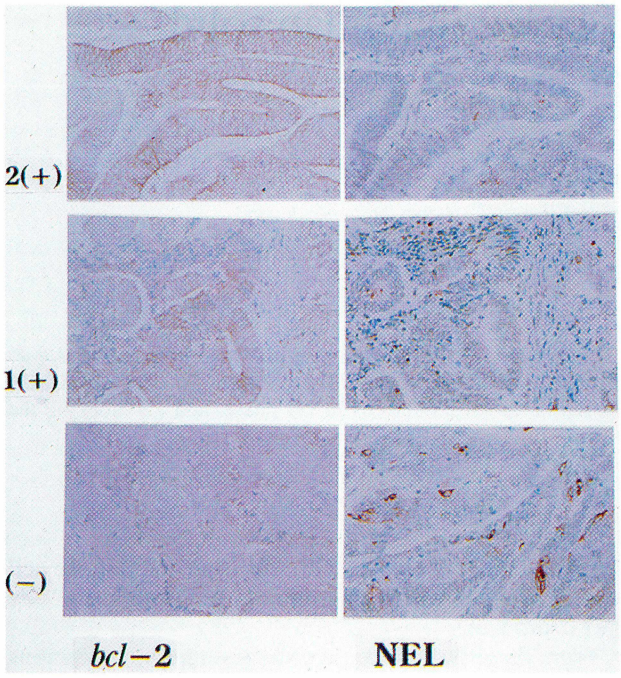

Figure 1. — Staining results for bc1-2 products (A, ++; C, +; E, –) and NEL (B, D, F). Bc1-2 positive cells are generally NEL negative. Staining intensity for bc1-2 was expressed as negative (–), weakly positive (+) or strongly positive (++), in comparison with that of normal lymphocytes used as a standard. (Taken from Kuwashima et al. 1996, ref. no. 4).

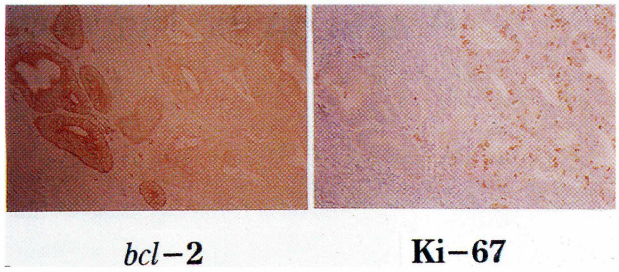

Figure 2.1. — Low magnification view of the carcinoma stained for bc1-2 (A) and Ki-67 (B). Bc1-2 positive nests (right) are almost Ki-67 negative, and bc1-2 negative nests (left) contained a large number of Ki-67 positive nuclei. (Taken from Kuwashima *et al.* 1997, ref. no. 5).

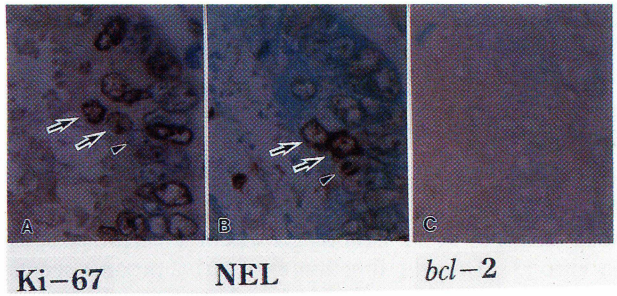

Figure 2.2. — Staining results for Ki-67 (A), NEL (B) and the bc1-2 product (C). Arrows indicate "proliferating apoptotic cells" and arrow heads "quiescent apoptotic cells". (Taken from Kuwashima *et al.* 1997, ref. no. 6).

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