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Topoisomerase I Protein Expression and Prognosis of Patients with Colorectal Cancer

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Topoisomerase I (Topo I) is known as a target for chemotherapy in advanced or recurrent colorectal cancer. In order to prolong the survival of patients with colorectal cancer or to prevent ineffective chemotherapy, we evaluated clinicopathological characteristics of Topo I protein in colorectal cancer. Also, we estimated whether Topo I protein expression of primary tumors could be a parameter for chemosensitivity of Topo I inhibitor in patients with cancer recurrence. Immunohistochemical detection of Topo I protein was performed in 104 surgically obtained specimens. Topo I protein was detected in 45 of 104 patients (43.2%). Topo I protein expression closely correlated with tumor progression, histpathological differentiation and poor prognosis of patients. Sixteen patients with recurrent cancer had been treated with Topo I inhibitor. Topo I inhibitor significantly prolonged the survival of 12 patients who had Topo I-positive primary tumors. Topo I protein expression in colorectal cancer may be a biological marker for chemosensitivity of tumors against Topo I inhibitors.

Key words: chemosensitivity; colorectal cancer; DNA topoisomerase I; immunohistochemistry; prognosis

DNA topoisomerase I (Topo I) belongs to the DNA topoisomerase multimember family, which is essential for DNA topology modulation. Topo I transiently cleaves one strand of DNA, allowing relaxation of the supercoiled DNA. This process is important in cell replication, translation, recombination and repair (Gupta et al., 1995). Through Western and Northern blotting, Topo I protein and mRNA level were found to be more abundant in several human tumors than in normal tissues (Husain et al., 1994; Rowinsky et al., 1994; van der Zee et al., 1994; Giaccone et al., 1995).

Topo I is also a target for anticancer drugs, camptothecin and its derivatives (O'Leary and Muggia, 1998). Topo I-inhibiting drugs interfere with Topo I function by binding to Topo I at its active site, and prevent re-ligation of the DNA strand (Goldwassew et al., 1995). Camptothecin inhibits Topo I by forming stable Topo I–DNA cleavage complexes, and is specifically cytotoxic for S-phase cells (Hsiang et al., 1989). In vitro, tumor cells with a high level of Topo I protein respond better to Topo I inhibitors (Staley et al., 1999).

CPT-11, a derivative of camptothecin, has been used as one of the key drugs for treating colorectal cancer (Paradiso et al., 2004; Vallböhmer et al., 2006). In the last few years, it has been shown that when used in combination with 5-fluorouracil and leucovorin, both CPT-11 and oxaliplatin

Abbreviation: Topo I, topoisomerase I

treatments demonstrated significant improvement in the clinical outcome of patients with advanced colorectal cancer (Doillard et al., 2000; de Gramont et al., 2000). However, response rates for these chemotherapeutic regimens still remain about 40% to 50%. In the present study, we investigated the clinicopathological characteristics of colorectal tumors with Topo I protein expression. Also, we analyzed Topo I protein expression in primary tumors could be a biomarker of chemosensitivity for recurrent patients or not.

Materials and Methods

Tumor samples

We obtained tumors and non-cancerous normal mucosa from 104 patients who underwent colorectal resection between 1992 and 2001. Samples were collected immediately after surgical resection of specimens. The tissues were fixed in 10% buffered formalin and embedded in paraffin. Four micrometer histological sections were destined to specific immunohistochemical determinations.

Immunohistochemistry

After paraffin-embedded sections on the slides were dewaxed and rehydrated gradually with graded alcohols, antigen retrieval was performed by autoclaving in 10 mol citrate buffer for 30 min. Endogenous peroxidase activity was blocked by methanol and 0.3% hydrogen peroxide. Slides were then incubated with primary anti Topo I monoclonal antibody (Clone 1D6, 1:50 dilution, Novocastra Laboratories, Newcastle, United Kingdom) for 1 h at room temperature. After incubation, the specimens were washed twice with phosphate-buffered saline solution (pH 7.6) and processed with the streptavidin-biotin peroxidase method according to the manufacturer's recommendations. The slides were then incubated in diaminobenzidine tetrahydrochloride and hydrogen-peroxide chromogen substrate for 10 min at room temperature, washed in running water for 2 to 3 min, counterstained in Mayer's haematoxylin. Normal tonsil tissue was used as a positive control. It is known that normal tonsil tissue is well stained by immunohistochemistry which uses Topo I antibody (Rasheed and Rubin, 2003).

Scoring system

Tumor cells expressing Topo I immunoreactivity were quantified by 2 independent observers who evaluated at least 1,000 neoplastic cells in consecutive areas of neoplastic tissues. If there was a Topo I-positive cell in the tumor, the sample was classified as "positive". If there were no Topo I-positive cells, the sample was classified as "negative" (Paradiso et al., 2004).

Patients

Clinicopathological findings of colorectal cancer were defined according to Dukes' classification (Dukes and Bussey, 1958). In all 104 patients, curative colorectal resection was performed between 1992 and 2001 at Tottori University Hospital. Patients agreed to the use of their tissues by informed consent. None of the patients received preoperative chemotherapy, and all were followed until December 2006. The types of cancer recurrence were established by computed tomography, performed at least twice a year.

Statistical analysis

The chi-square test was used to compare the differences between the 2 groups. The overall and disease-free survivals were estimated using Kaplan-Meier's method and compared using a 2-sided log rank test. Cox's proportional hazards regression model was used to estimate the predictive power of Topo I protein expression on clinical outcome. Two-sided tests were computed, and $P < 0.05$ was considered statistically significant.

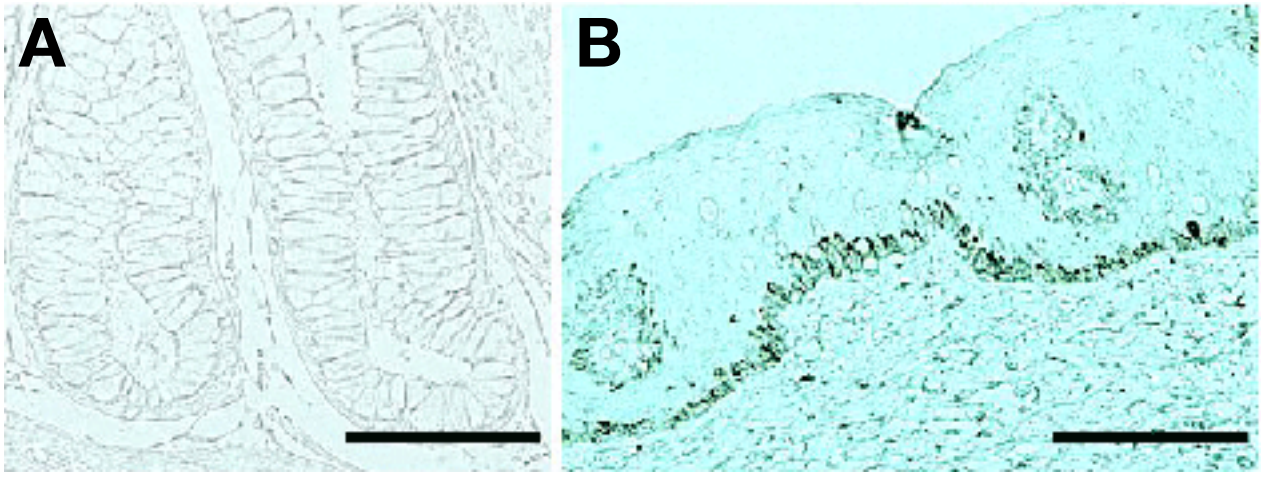

Fig. 1. Immunohistochemical topoisomerase I protein expression in normal colorectal mucosa adjacent to tumor (**A**) and in normal skin (**B**). Bar = 200 μm.

Results

Topo I expression in tumors and in non-cancerous tissues

Topo I protein expression was not detected in normal colorectal mucosa. But it was detected in normal cells in basal layer of the skin adjacent to rectal cancer of patiens who had undergone amputation of the rectum (Fig. 1). Topo I immunostaining was mainly located in the nucleus of cancer cells (Fig. 2).

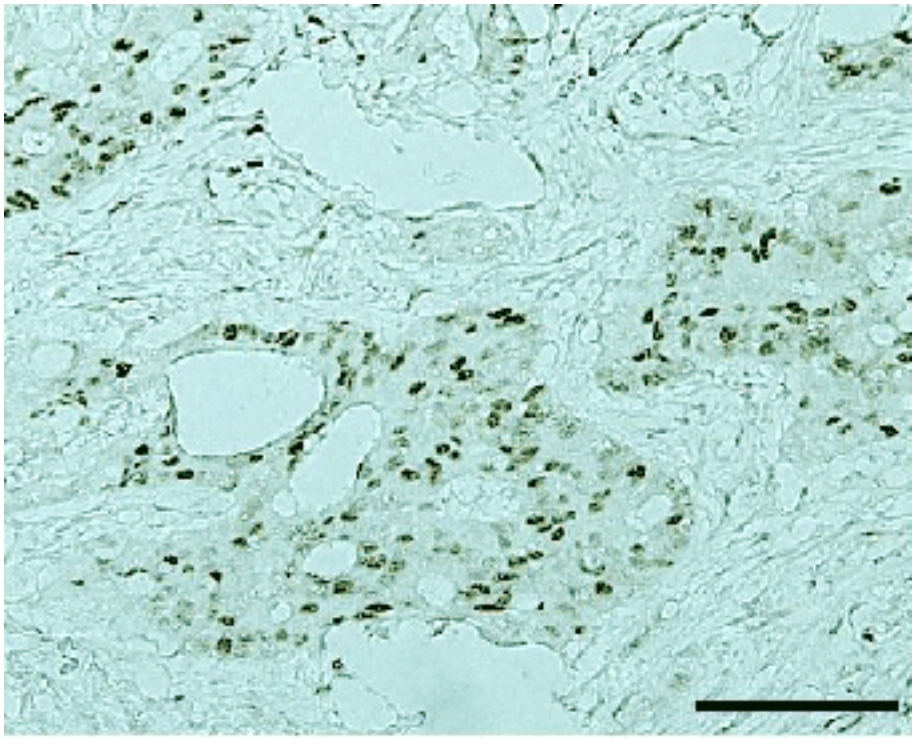

Fig. 2. Immunohistochemical topoisomerase I protein expression in advanced colorectal cancer. Strong nuclear expression of the protein is noted. Bar = 200 μm.