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Original Article

Role of topoisomerase I and thymidylate synthase expression in sporadic colorectal cancer: Associations with clinicopathological and molecular features

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ABSTRACT

Topoisomerase I (Topo I) and thymidylate synthase (TS) are essential enzymes for the replication, transcription and repair of DNA, and are potential biomarkers in colorectal cancer (CRC). The aim of the study was to correlate the tissue expression of Topo I and TS in sporadic CRCs with relevant pathological and molecular features and patients' outcome.

Topo I and TS expression was assessed by immunostaining in 112 consecutive primary CRCs. Increased expression of Topo I was found in 36% of tumors, preferentially rectal (50%) and with not otherwise specified (NOS) histology (44%). Topo I expression was associated with 18q allelic loss (LOH), ($p = 0.013$), microsatellite stable phenotype ($p = 0.002$) and normal expression of mismatch proteins hMLH1 and hMSH2 ($p = 0.0012$ and $p = 0.02$, respectively). High TS expression was found in 60% of tumors, more frequently in distal sites (62%) and with NOS histology (66%); no association with microsatellite instability was observed.

Topo I seems to be involved in the chromosomal instability pathway of sporadic CRCs. Conversely, high TS expression is unlikely to affect the clinical behavior of microsatellite unstable CRCs.

Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second in women worldwide. According to WHO, there were 1.2 million new diagnoses of CRC in 2008, and more than 608,000 deaths [1,2]. CRC development follows two major pathways of genetic instability: chromosomal instability (CIN), observed in 85% of sporadic CRCs, and microsatellite instability (MSI), accounting for approximately 15% of cases [3]. CIN-related CRCs are characterized by gross chromosomal rearrangements, aneuploidy, defects in checkpoints for G1/S entry, and loss of heterozygosity (LOH) in particular at chromosomal arm 18q [3–5]. Conversely, MSI-related CRCs have diploid or nearly diploid karyotypes and show defects of the DNA mismatch repair system (MMR) genes [3]. CRCs referred to CIN or MSI pathway display relevant clinic-pathological differences as to tumor site, histology and response to adjuvant therapy [6]. Several studies have also demonstrated that the molecular phenotype may affect CRC outcome [7–10]. In particular, CRCs with MSI have been associated with improved survival [11], whereas 18qLOH is a molecular marker of adverse prognosis [7,8,12–21].

Several biological markers have been investigated for a prognostic role in CRC [22], among which are topoisomerase I (Topo I) and thymidylate synthase (TS), whose expression levels correlate with survival although this evidence is controversial among different studies [23–28].

Topo I is an essential enzyme in regulating the topology of supercoiled DNA by transiently cleaving of one of the two strands [29]. Antineoplastic drugs targeting Topo I, such as irinotecan and camptothecins, form stable Topo I-DNA cleavage complexes and inhibit Topo I activity, thus preventing DNA religation [30,31]. Topo I is expressed in primary CRCs and metastases, but it is debated whether its expression can predict the response to anti-Topo I treatments [32–35].

TS catalyzes the conversion of dUMP to dTMP and is essential for 'de novo' DNA synthesis [36,37]. The expression of TS may affect tumor sensitivity to fluoropyrimidines, such as 5-fluorouracil

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Table 1
Main clinicopathological features of patients.

	N (%)
Gender	
Male	58 (52)
Female	54 (48)
Age (years)	
Average	69.2
Range	27–94
AJCC stage	
I	12 (11)
II	60 (53)
III	37 (33)
IV	3 (3)
Tumor site	
Proximal	42 (37)
Distal	50 (45)
Rectum	20 (18)
Tumor grade	
G1–G2	43 (38)
G3	69 (62)
Histological type	
Adenocarcinoma, NOS	74 (66)
Mucinous adenocarcinoma	38 (34)
Adjuvant therapy	
Yes	12 (11)
No	99 (89)

(5-FU) [38]. 5-FU-based treatment is the standard of care for adjuvant therapy of CRC in combination with oxaliplatin [39] and for the treatment of metastatic disease in association with oxaliplatin or irinotecan [40–43].

The aim of the present study was to assess the expression of Topo I and TS in tumor tissue by immunostaining and to correlate it with pathological and clinical variables, patients' outcomes and molecular characteristics, such as MSI status, LOH at different loci and other markers implicated in CRC carcinogenesis.

Materials and methods

Patients

The study is a retrospective evaluation of 112 unselected, consecutive, primary CRCs that underwent curative resection between January 1997 and April 1999 at our institution (Table 1). There were 58 males (age range 27–94 yr, mean 69) and 54 females (age range 45–91 yr, mean 69). Tumors were located in the proximal colon in 42 patients (37%), in the distal colon in 50 patients (45%), in the rectum in 20 patients (18%), and they were predominantly poorly differentiated (G3) adenocarcinomas (62%) with not otherwise specified (NOS) histology (66%). The tumor stage was determined according to the American Joint Committee on Carcinoma (AJCC) system [44]; most cases were stage II (53%).

All cases were deemed sporadic, based on the absence of relevant family history as recorded prospectively at the initial patient interview.

During the study period, a uniform surgical management protocol was adopted. Data on clinical and pathological characteristics and chemotherapy were obtained from surgical, pathological and oncological records. Recurrence and survival data were followed-up to September 2007 (censoring date) using databases on hospital admission and the National Central Registry on death recording. Overall recurrence rate was 16% (18 patients); 12 patients (11%) had local recurrence without metastases, 6 patients (5%) had local recurrence with metastases.

Patients with AJCC stage III colon cancer under 75 years old were eligible for adjuvant chemotherapy [45]; however, this treatment option was exerted at discretion of the patient and the oncologist. The standard drug regimen was 375 mg/m^2/d 5-FU and 20 mg/m^2/d levamisole, 5 days/week every 4 weeks for 6 months. Patients with rectal cancer received irradiation therapy administered in a dosage of 40 Gy, divided into 16 daily doses of 2.5 Gy each (4 doses/week for 4 weeks) before surgery [46].

Patients were observed at 3-month intervals for 24 months after the completion of therapy, every 6 months for 3 years, and then yearly. History and physical examination, complete blood cell and platelet count, liver chemistries, ultrasound and carcinoembryonic antigen measurement were performed at each visit; chest X-ray, colonoscopy and CT were performed once a year.

All CRC specimens underwent histopathological analysis by the same gastrointestinal pathologists (B.C. and S.E.M.), who were unaware of the interim results of molecular genetic and immunohistochemical analysis.

The study protocol was approved by the Human Ethics Committee of the University of Parma.

Immunohistochemistry

For immunohistochemical analysis, tumors were routinely fixed in buffered 10% formalin immediately after surgery and embedded in paraffin. To avoid loss of immunoreactivity due to prolonged storage, the sections were freshly cut, and all slides were processed simultaneously with antibodies from the same batch, including positive and negative controls.

The following primary antibodies were used: anti-Topo I (clone 1D6, Sanbio, The Netherlands, working dilution: 1:100), anti-TS (clone TS106, Neomarkers, Fremont, CA, USA, working dilution: 1:200).

For antigen retrieval, sections were treated with 10 mM citrate buffer at pH 6.0, in a 750 W microwave oven for three 5-min cycles. The sections were developed with the streptavidin–biotin kit (LSAB2, Dako) in accordance with the manufacturer's specifications and counterstained with hematoxylin. Positive controls were CRCs previously assayed with strong positivity; negative controls consisted of substituting normal serum for the primary antibodies.

The immunostains were concurrently evaluated by two observers blinded to the clinical and pathological data. The scores of the two observers were averaged for every sample. In the vast majority of cases, the observers were in agreement.

The expected/shown expression of Topo I or TS was very faint in normal colorectal epithelial cells that were used as internal reference to evaluate the intensity of expression in tumor cells.

For Topo I, a score for intensity and distribution of nuclear staining was assigned according to a 4-tier system. Intensity ranged from 0 to 3 (0 = no staining, 1 = weakly positive, 2 = moderately positive, and 3 = strongly positive staining). The staining distribution considered the percentage of positive tumor cells and ranged from 0 to 3 (0 = 0 to 5%, 1 = 6% to 25%, 2 = 26% to 50%, 3 = 51% to 100%). An overall Topo I expression score was calculated as the sum of the intensity and distribution scores in each case. Cases with a total score of at least 4 were considered high expression tumors (with altered pattern), whereas cases with a total score of 0–3 were considered negative or low expression tumors (with normal pattern) [32].

TS expression levels were estimated semiquantitatively based on the intensity of cytoplasmic staining that has been previously validated as an accurate measure of protein levels [47]. In particular, TS staining considered both positive tumor cell percentage and staining intensity as follows: distribution score 0: <1% positive cells, score 1: 1–20%, score 2: 21–50%, score 3: 51–80%, score 4: >80%; intensity: score 1 (weak), score 2 (moderate), and score 3 (strong).