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EDITORIAL

Telomere function in colorectal cancer

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Abstract

Colorectal cancer is the third most common form of cancer and the second leading cause of cancer-related death in the western world. Tumour cells acquire the hallmarks of cancer during the carcinogenic selection process. Cell immortality is one of the principal features acquired during this process which involves the stabilization of telomere length. It is achieved mainly, by telomerase activation. Thus, the discovery of telomeres and telomerase allowed an understanding of the mechanisms by which cells can become immortalized. Different studies have shown that tumour cells have shorter telomeres than nontumour cells and have detected telomerase activity in the majority of tumours. Survival studies have determined that telomere maintenance and telomerase activity are associated with poor prognosis. Taking into account all the results achieved by different groups, quantification and evaluation of telomerase activity and measurement of telomere length may be useful methods for additional biologic and prognostic staging of colorectal carcinoma.

Key words: Colorectal cancer; Telomeres; Immortality

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INTRODUCTION

Colorectal cancer (CRC) is the third most common form of cancer and the second leading cause of cancer-related death in the western world. More than 940 000 cases occur annually worldwide and nearly 500 000 die from it each year. It represents 9.5% of all cancers diagnosed and there is a high incidence in USA, Australia, Japan and Europe. Sporadic colorectal cancers account for 70%-80% of cases whereas familiar syndromes constitute up to 25% and are usually diagnosed at early ages. A major cause for sporadic CRC is a diet rich in fat, refined carbohydrates and animal protein, combined with low physical activity. Therapy is usually through surgery, which in many cases is followed by chemotherapy. Overall five-year survival is around 50% in Europe. It has been improving during the last decade and this tendency is expected to continue[1,2].

Colorectal cancer is a disease originating from the epithelial cells lining the gastrointestinal tract. Hereditary or somatic mutations in specific DNA sequences, among which are included DNA replication or DNA repair genes, and also the *APC*, *K-Ras*, *NOD2* and *p53* genes lead to unrestricted cell division[3,4].

Carcinogenesis is a multistep, multifocal process characterized by the accumulation of genetic and molecular abnormalities. It is well known that normal human somatic cells have a finite limit of cultivation when grown in vitro unless immortalization protocols are carried out. Current knowledge suggests that the progression of cancer from a premalignant to a malignant state is, consistent with a mechanistic model, based on of the principle of natural selection. Tumour cells acquire the hallmarks of cancer during this carcinogenic selection process[5]. Cell immortality is one of the principal features acquired during this process which involves the stabilization of telomere length. It is achieved, mainly, by telomerase activation. Thus, the discovery of telomeres and telomerase allowed an understanding of the mechanisms by which cells can become immortalized[6].

Telomeres are essential for chromosomal stability and integrity, protecting the ends of chromosomes from degradation and preventing chromosomal end fusions and recombination[7]. Indeed, loss of telomere function can be a major mechanism for the generation of chromosomal abnormalities[8,9]. Telomeres are the end cap on chromosomes and consist of repetitions of six nucleotides. In humans, the sequence is 5'-(TTAGGG)$_n$-3' and they vary in length from 5 to 15 Kb[10-12]. Telomeres end in an essential 3' single-stranded overhang of 50 to 400 nt[13-15]. Electron microscopy studies suggest this overhang can loop back and integrate into the duplex repeat tract, forming a "t-loop" [16]. Due to replication deficiencies (end replication problems) and telomere end processing, telomeres shorten progressively with replication in normal somatic cells and, eventually, trigger senescence. Cells that lose the ability to senesce because of mutations in p53 protein continue dividing, till they enter "crisis", where extensive telomere shortening results in chromosomal fusion and cell death. This telomere length-dependent growth inhibition which prevents critically short telomeres and, thereby, potentially unprotected chromosomes, is thought to be a barrier for unlimited cellular proliferation[17-22].

A group of proteins also play important roles in the regulation of telomere length maintenance and in the formation of a protective end-cap that prevents chromosome fusion. The mammalian telomeric core complex serves to form and protect the telomere, and has been termed shelterin. This complex includes proteins that bind directly to the telomeric DNA (TRF1, TRF2 and POT1) and telomere-associated proteins that are recruited to telomeres by the former (TIN2, TPP1 and Rap1)[23,24]. Other proteins, many of which are more commonly associated with DNA repair, are also found at telomeric ends. Examples include DNA-PK, the MRN complex, PARP1/2, Tankyrase 1/2, ATM, ERCC1/XPF, RAD51D, WRN and BLM[25].

It has been postulated that dysfunctional telomeres could play a causal role in carcinogenesis by instigating chromosomal instability, thus promoting neoplastic transformation[26-30]. Results from telomerase-knockout mouse models in which animals possessing critically short telomeres exhibit an increased cancer incidence support this concept[31,32]. In particular, Artandi *et al*[33] demonstrated that crossing telomerase- knockout mice with *p53* +/- mice resulted in a shift in the spectrum of tumours normally seen in the *p53*-defective background (primarily lymphomas and sarcomas) to one dominated by carcinomas displaying the types of karyotypic aberrations (e.g. nonreciprocal translocations) often observed in human epithelial cancers.

Human telomerase was discovered for the first time in cancer cells (HeLa) in 1989. The core enzyme consists of two subunits: the RNA component (hTR) which serves as a template for telomere synthesis and a catalytic protein, the telomerase reverse transcriptase (hTERT)[34-36]. Together, the telomerase ribonucleoprotein complex is responsible for adding telomere repeats to the very ends of chromosomes and thus compensates for replication- or damage-dependent loss of telomere sequences[37]. Over expression of telomerase has been found in more than 90% of human cancers, leading to the hypothesis that telomerase plays an important role in the pathogenesis of cancer[38-40]. Given the importance of telomerase to tumourigenesis, multiple studies have assessed the use of pharmacological, immunological and targeting technologies to diminish or abolish the expression of telomerase hTR and hTERT as a novel therapeutic strategy for cancer[40].

Some tumours, around 7%-10%, do not express telomerase and they maintain their telomeres through a mechanism termed alternative lengthening of telomeres or ALT[41]. ALT-positive cells are characterized by very long telomeres (> 40 Kb) as well as an extremely large variation in telomere length within the same nucleus. Another hallmark of the ALT mechanism is the presence of ALT-associated promyelocytic leukaemia protein (PML) nuclear bodies, the APBs, subnuclear structures containing PML protein, telomeric DNA, telomere-binding proteins and several proteins involved in DNA synthesis and recombination[42].

Telomere lengths are the result of the balance of proliferate telomere losses and *de novo* telomere synthesis. They serve as an indicator of the ability of each tumour to compensate for replicative telomere losses. When examined by Southern blotting analysis (Figure 1), the telomeres of invasive human cancers often appear shorter than their normal tissue counterparts[28]. The combined observations of short telomeres, plus the frequent activation of telomerase in human epithelial cancers, suggest that the majority of tumours undergo critical telomere shortening at some point during their development. This could simply be a consequence of the end-replication problem combined with extensive cell turnover occurring during tumour expansion. On

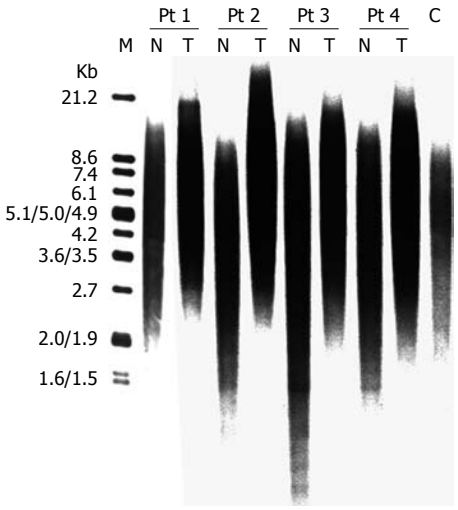

Figure 1 Representative X-ray film for telomere length analysis by Southern blotting. Pt1-Pt4: Patients with colorectal carcinoma; T: Tumour tissues; N: Their paired normal samples; C: Positive control; M: DNA molecular weight marker; kb: Kilobase pairs.

the other hand, if telomere shortening occurs early, it could be playing an important role during the initiation stage of tumourigenesis. Thus, the timing of the occurrence of telomere shortening during human cancer development is a critical question[5].

TELOMERES AS MOLECULAR PROGNOSTIC FACTORS IN COLORECTAL CANCER

Two studies carried out in 57 and 91 patients showed that median telomere lengths, measured by Southern blotting, in cancer tissue were significantly shorter than matched adjacent mucosa ($P < 0.001$ and $P = 0.020$, respectively)[43,44]. A significant positive correlation was observed between nontumour telomere length and tumour telomere length by Garcia-Aranda *et al*[44] and Nakamura *et al*[45]. Patient-by-patient comparison of matched tissue samples showed that 86% of samples (49 tumours) had telomere shortening[43]. Similar results were obtained by Engelhardt *et al*[46] and Nakamura *et al*[45] who found telomere shortening in 90% and 77% of colon tumours, respectively. Tatsumoto *et al*[47] arbitrarily defined TRFs as shortened when TRF length of tumour tissues was shorter than 80%. Among the primary cancers, Garcia-Aranda *et al*[44] and Tatsumoto *et al*[47] detected the shortened TRF lengths in 38% and 39.6% of tumours, respectively. The mean ratio of telomere lengths in cancer tissue to corresponding non cancerous mucosa was very similar in two different studies: 0.84 and 0.87[43,44].

The clinical significance of a correlation between telomere length in CRC tissue and stage is controversial. Some reports reveal a significant association between telomere length and stage in cancer tissue[43,45]. Thus, according to Gertler *et al*[43] telomeres in early-stage tumours (UICC stage Ⅰ) were significantly shorter than telomeres of advanced tumours (UICC stages Ⅱ through Ⅳ). The telomere length ratio of cancer to non-cancerous tissue increased within higher stage groups, approaching statistical significance ($P < 0.060$)[43]. Engelhardt *et al*[46] also reported on significantly longer telomeres in late-stage Dukes' C and D tumours compared with early stage Dukes' A and B tumours. Garcia-Aranda *et al*[44] failed to find this association. However, they defined a correlation between tumour telomere length and tumour location ($P = 0.005$) and tumour differentiation ($P = 0.046$). With this regard, tumours of the right colon displayed significantly shorter telomeres compared with tumours located in other sites. When considering tumour status (telomere shortening, maintainance or elongation), borderline-significant differences were observed for tumour differentiation ($P = 0.098$). Well differentiated and moderately differentiated tumours demonstrated telomere maintenance, whereas telomere dysfunction was detected in the majority of poorly differentiated tumours. Tatsumoto *et al*[47] assessed there was no significant relationship between altered TRF length and Dukes' classification nor other clinic-pathological parameters (tumour site, tumour size, lymph node metastasis, or histology), although mean telomere length in advanced stages tumours was slightly longer than that of early stage tumours (Table 1). Other available studies did not associate telomere length with tumour stage or depth of tumour invasion in patients with colorectal carcinoma[48-50].

O'Sullivan *et al*[51] have shown that telomere dysfunction is an early event in neoplastic progression in ulcerative colitis, and is related to chromosomal instability and anaphase bridge formation. This may facilitate the molecular evolution of tumourigenesis in cells by accelerating chromosomal instability. Recently, Raynaud *et al*[52] showed that telomere length depended on stage in CRC. They determined by FISH that the decrease in staining with the transition from normal tissue to LGD and with that from LGD to HGD was statistically significant ($P < 0.0001$ and $P = 0.012$, respectively). During later stages of the carcinogenic process, staining increased with invasiveness, reaching the levels observed in normal cells in invasive carcinoma. This increase in staining associated with the transition from HGD to IC was also statistically significant ($P = 0.006$). This attrition peaks in HGD, and it is only when the full invasive potential of the tumour has been reached that telomere length returns to levels close to those observed in normal tissue. Raynaud *et al*[52] hypothesized that this telomere attrition may have a protective effect against cancer because critically short telomeres are known to induce replicative senescence. Consequently, only cells that find a way to maintain their telomeres, thus allowing unlimited cell division and some degree of genomic stabilization, will be able to pass through this bottleneck and progress to give rise to an invasive tumour[5]. Meeker *et al*[5] support a model whereby telomere dysfunction induces chromosomal instability as an early initiating event in many, perhaps most, human epithelial cancers. Plentz *et al*[53] agree with this model. They showed for the first time that telomere

Table 1 Summary of the relationship between telomere length and clinic-pathological parameters in CRC described by different authors

Authors	No. of CRC patients	Telomeres and clinic-pathological parameters
Engelhardt *et al*[46], 1997	100	Telomeres in early stage Dukes' A and B tumours were significantly shorter than telomeres in late-stage Dukes' C and D tumours
Gertler *et al*[43], 2004	57	Telomeres of UICC stage I tumours were significantly shorter than telomeres of UICC stages Ⅱ-Ⅳ tumours. Telomere length ratio of cancer to no cancer tissue was shown to be an independent prognostic parameter for overall survival. Telomere length ratio > 0.90 implied a 3.3 times higher RR compared with patients who had telomere shortening
Garcia-Aranda *et al*[44], 2006	91	Telomeres of tumours of the right colon displayed significantly shorter telomeres compared with tumours located in other sites. A significantly poor clinical outcome in the group of patients who had tumours with longer telomeres was observed both in overall survival and disease-free survival. This parameter was found to be a significant prognostic factor independent of tumour stage. RR was 6.48 in patients who had tumours with longer telomeres

CRC: Colorectal cancer.

shortening characterizes the adenoma-carcinoma transition and that telomere shortening at this transition specifically affects epithelial cells, the cell type of origin of colorectal cancer. These authors suggest that carcinomas arise from chromosomally unstable epithelial cells with critical short telomeres which have lost DNA damage responses. Subsequently or simultaneously, a variety of cofactors are necessary for cancer progression, including activation of telomerase to stabilize telomeres and alleviate chromosomal instability to a level allowing further cell divisions and cell survival. It should also be considered that several proteins have been implicated in the formation of a protective higher-order capping structure at the telomeres, and experimental changes in the level of expression or function of several of these proteins have been shown to affect telomere length, both positively and negatively[45,54,55]. Taken together, telomeres may rapidly shorten as a result of inefficiently repaired DNA damage caused by oxidative stress[56].

Different results were presented by Engelhardt *et al*[46] who measured telomere length in premalignant lesions, CRCs and adjacent normal tissues by Southern blotting. Telomeres in colon cancer were considerably shorter than those in adjacent normal tissues ($P = 0.002$), whereas polyps and colitis had telomere lengths comparable to those of the normal adjacent tissues ($P = 0.312$ and $P = 0.830$, respectively).

Takagi *et al*[57] studied the relationship between microsatellite instability and telomere shortening in colorectal cancer. They assessed that microsatellite instability correlated significantly with frequency of telomere shortening ($P = 0.018$) and concluded that the relationship identified between microsatellite instability and telomere shortening might suggest some association between the DNA mismatch repair system and the telomere maintenance mechanism in colorectal cancers.

Patients with tumours that maintain telomere length showed an increased hazard rate in CRC. Gertler *et al*[43] described a 5-year survival of 25.6% ± 13.8% in patients with a TRF T/TRF N greater than 0.9, whereas those with a ratio ⩽ 0.9, showed a 5-year overall survival rate of 78.2% ± 6.9% ($P < 0.002$). In multivariate analysis (Cox regression), the telomere length ratio of cancer to non cancerous tissue was shown to be an independent prognostic parameter for overall survival ($P < 0.03$). The relative risk (RR) of death for patients with a telomere length ratio > 0.90 was 3.3 times higher compared with patients who had telomere shortening (95% CI: 1.2 to 9.0). The only other independent prognostic factor for overall survival was lymphatic vessel invasion, with a relative risk of 4.1 (95% CI: 1.5 to 11.6; $P < 0.01$).

Garcia-Aranda *et al*[44] considered two groups to analyze the impact of telomere length in patient prognosis. Group 1 included patients in the first and second quartiles and Group 2 included patients in the third and fourth quartiles. A significantly poor clinical outcome in the group of patients who had tumours with longer telomeres was observed both in overall survival and disease-free survival ($P = 0.020$ and $P = 0.060$, respectively). The differences found between these analyses derived from the fact that three patients who had recurrent tumours did not die during follow-up; on the other hand, one patient without tumour recurrence died because of further complications. Using the multivariate Cox proportional hazards model, this parameter was found to be a significant prognostic factor independent of tumour stage ($P = 0.040$). The relative risk was 6.48 in patients included in Group 2 (Table 1).

TELOMERASE AS A COLORECTAL CANCER MARKER

The most frequently used method to evaluate telomerase activity is the Telomere Repeat Amplification Protocol (TRAP assay), originally described by Kim *et al*[38] (Figure 2). Previous studies demonstrated increased telomerase activity in colorectal cancer tissue and even suggested a prognostic value for patients with colorectal carcinoma[45].

Chadeneau *et al*[58] described an association between telomerase activity and acquisition of malignancy in human colorectal cancer. Engelhardt *et al*[46] and Boldrini *et al*[59] found that dysplastic polyps and adenocarcinoma samples were telomerase positive, with higher levels in cancer tissues compared to dysplastic lesions. Engelhardt *et al*[46] postulated that some of the early-stage adenomas

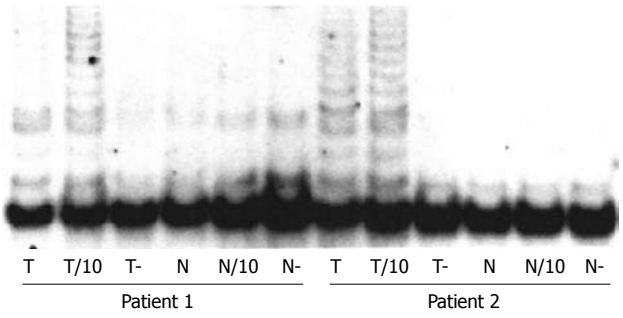

Figure 2 Telomerase activity in tissue extracts from two patients affected by colorectal cancer. In this representative experiment, telomerase activity was investigated in tumour (T) and non tumour (N) samples using the telomerase repeat amplification protocol (TRAP). As it is indicated in the figure, to avoid the effect of Taq DNA polymerase inhibitors, different extract dilutions were investigated.

may be telomerase negative, because they have not acquired all of the genetic abnormalities compared to the later-stage adenomas. Therefore, multistep genetic alterations in the preneoplastic development of adenomas may be involved in the selection of cell immortality and may lead to expression of telomerase activity in the more advanced adenomas. Boldrini *et al*[59] determined that high telomerase activity was associated with late-staged cancers and metastasis, what provided arguments supporting the role of telomerase not only in the development but also in the progression of colorectal carcinoma.

In studies carried out in a total of 17, 30, 41, 50, 91, 97, 100, 103 and 108 patients diagnosed with colon adenocarcinoma, positive telomerase activity was detected in 82.4% (14 tumours), 100% (30 tumours), 83% (34 tumours), 100% (50 tumours), 81.3% (74 tumours), 92.8% (90 tumours), 96% (96 tumours), 90% (93 tumours) and 81.5% (88 tumours) of the colon carcinoma samples, respectively[44,45,47,60-65].

Telomerase activity is related to the Dukes' stage: patients at stage A and B showed a lower percentage of positivity than the ones at stages C or D[45,47,61,62,64,66]. Tumours localized in colon showed a higher percentage of positivity than tumours localized in the rectum[61,64]. Maláska *et al*[65] and Okayasu *et al*[67] reported a correlation between positive telomerase activity and lymph node metastasis. Moreover, Maláska *et al*[65] found a tendency towards higher telomerase activity in patients with distant metastases, although this lacked statistical significance. Shoji *et al*[60] found that telomerase index (TI = log telomerase activity of cancer tissue - log telomerase activity of normal mucosa) increased significantly with depth of invasion ($P = 0.013$). Additionally, there was a significant difference in TI between tumours with and without venous invasion ($P < 0.001$): TI was higher in telomerase positive tumours. Garcia-Aranda *et al*[44] described tumour recurrence as a borderline-significant parameter, because all tumours which were positive for recurrence during follow-up showed telomerase activity ($P = 0.068$). These authors established an association between telomerase positive tumours and age and gender: higher rates of telomerase activity were detected in the group of patients older than 69 years-old ($P = 0.003$) and in females ($P = 0.057$) (Table 2). However, other authors disagree as they found no significant association between telomerase activity in tumour or normal mucosa related to clinical variables (gender and age) or to the anatomopathologic characteristics (histopathological grade, histology type, tumour localization, depth invasion (T), lymph node invasion (N), distance metastasis (M), and stage TNM classification)[63,68].

Kawanishi-Tabata *et al*[69] analysed 122 surgical specimens of human stage Ⅱ colorectal carcinoma. Telomerase activity was detected in 98 tumours (80%). The colon was the primary site of the tumour in 52 cases, whereas the rectum was the primary tumour site in 70 cases. Among the 52 colon tumour specimens evaluated, 47 (90%) were telomerase positive, whereas only 51 of the 70 rectal tumour specimens (73%) were positive for telomerase activity ($P = 0.020$). These results showed concordance with the ones presented by Lukman *et al*[61] and Sanz-Casla *et al*[64]: more patients with colon than with rectal carcinomas were telomerase positive in the series they analysed. Kawanishi-Tabata *et al*[69] also found that telomerase positive tumours presented more frequently in females ($P = 0.100$), as Garcia-Aranda *et al*[44], and were larger ($P = 0.040$) (Table 2).

Neither Garcia-Aranda *et al*[44] nor Tatsumoto *et al*[47] reported significant differences between TRF lengths in telomerase positive tumours versus telomerase negative tumours in CRC.

Tatsumoto *et al*[47] were the first to associate telomerase activity levels and patient prognosis. Kaplan-Meier overall survival curves of 100 patients showed that 5-year survival rate in the patients with high telomerase activity was 43%, whereas that in all remaining patients was 81%. The prognosis of patients with high telomerase activity was significantly worse than those for other patients ($P < 0.010$). Disease-free survival curves of 87 patients after curative surgery, showed significant difference between the tumour-free survival rates with and without high telomerase activity ($P < 0.010$). Among these patients who underwent curative surgery, 13 (38%) of 34 tumours with high telomerase activity recurred, whereas only 7 (13%) of 52 other tumours did ($P = 0.016$). Thus, curative tumours with high telomerase activity had significantly higher recurrence rates than other tumours. Considering that telomerase activity levels did not significantly correlate with stage of disease or Dukes' classification in the present study, the up-regulation of telomerase activity was considered an independent prognosis-associated factor in patients with colorectal cancer (Table 2).

Sanz-Casla *et al*[64] performed multivariate analysis to study the impact of telomerase activity in prognosis in 103 patients undergoing surgery for colorectal cancer. These data revealed that by adjusting for tumour stage, telomerase activity could be used to predict the risk of death or recurrence ($P < 0.001$) (Table 2).

Garcia-Aranda *et al*[44] identified telomerase activity as a marker of a trend toward a poor prognosis. Although no significant differences were found between patients

Table 2 Summary of the relationship between telomerase activity and clinic-pathological parameters in CRC described by different authors

Authors	No. of CRC patients	Telomerase and clinic-pathological parameters
Engelhardt *et al*[46], 1997	100	Lower rates of telomerase activity were detected in tumours at early stages
Okayasu *et al*[67], 1998	37[1]	There is a correlation between positive telomerase activity and lymph node metastasis
Lukman *et al*[61], 2000	17	Lower rates of telomerase activity were detected in tumours at early stages. Telomerase activity detection is more frequent in colon than in rectal tumours
Shoji *et al*[60], 2000	30	TI increased significantly with depth of invasion. TI was higher in telomerase positive tumours with venous invasion
Tatsumoto *et al*[47], 2000	100	Lower rates of telomerase activity were detected in tumours at early stages. The overall survival and the disease-free survival of patients showing high telomerase activity was significantly worse than those for other patients. Telomerase activity was an independent prognosis-associated factor
Ghori *et al*[66], 2002	30/20[2]	Lower rates of telomerase activity were detected in tumours at early stages
Kawanishi-Tabata *et al*[69], 2002	122[1]	Telomerase activity detection is more frequent in colon than in rectal tumours at stage Ⅱ. Telomerase positive tumours at stage II were larger than telomerase negative tumours. Disease-free survival for patients with telomerase negative tumours was shorter than for patients with telomerase positive tumours
Maláska *et al*[65], 2004	41	There is a correlation between positive telomerase activity and lymph node metastasis
Sanz-Casla *et al*[64], 2005	103	Lower rates of telomerase activity were detected in tumours at early stages. Telomerase activity detection is more frequent in colon than in rectal tumours. Telomerase activity could be used to predict the risk of death or recurrence
Garcia-Aranda *et al*[44], 2006	91	Higher rates of telomerase activity were detected in patients older than 69 years-old. Patients who had tumours with telomerase activity and high telomere length ratios had a significantly shorter disease-free survival compared with patients whose tumours showed lower telomere length ratios
Bautista *et al*[72], 2007	108	Patients with low TI rectal tumours showed a higher recurrence-free survival and overall survival probability. TI was an independent prognostic factor for predicting the recurrence in the first two years after surgery and for survival in rectal cancer patients
Vidaurreta *et al*[62], 2007	97	Lower rates of telomerase activity were detected in tumours at early stages. The overall survival of patients with telomerase activity was significantly worse than those for other patients

[1]Number of tumour samples; [2]30 tumour samples and 20 adjacent normal mucosa samples.

with telomerase positive tumours and patients with telomerase negative tumours, no recurrences were detected in the latter, during follow-up (P = 0.110). These differences translated into differences in overall survival. Therefore, this study revealed that no deaths were detected in the group of patients with telomerase negative tumours (P = 0.110). In addition, patients who had tumours with telomerase activity and high telomere length ratios had a significantly shorter disease-free survival compared with patients whose tumours showed lower telomere length ratios (P = 0.030) (Table 2).

Vidaurreta *et al*[62] determined overall survival and found that none of the patients with negative telomerase died during the follow-up period (P = 0.040). It should be considered that 53.6% of the population was subjected to adjuvant chemotherapy based on 5-fluorouracil (5-FU). These authors found no significant differences between the group of treated patients and the group of non-treated patients, with respect to the adjuvant chemotherapy treatment, when it was stratified by stages. These results are supported by previous ones which assessed that patients who underwent chemotherapy with 5-FU and their tumours had low telomerase activity, showed a tendency to chemo sensitivity (Table 2).

Recently, Ohnishi *et al*[63] evaluated telomerase activity from peripheral blood samples in 120 CRC patients who underwent curative surgical treatment. In univariate analysis, they found recurrence was significantly correlated with positive telomerase activity in the mesenteric vein (P = 0.002), positive telomerase activity in the peripheral vein (P = 0.003), histological type except well differentiated adenocarcinoma (P = 0.001), lymphatic infiltration (P = 0.044) and lymph node metastasis at surgery (P < 0.0001). In multivariate analysis, positive telomerase activity in peripheral vein was significantly associated with the existence of recurrence (HR: 3.13; P = 0.037). Ohnishi *et al*[63] assessed that measuring telomerase activity in peripheral blood samples seemed to be effective in predicting future recurrence to a degree greater than macroscopically examined tumour depth or other clinicopathological parameters.

Contrary to the results presented in this review, there is one study in which neither telomerase nor telomere length were predictive factors for overall survival[45]. However, patients with a very short survival (< 10 mo), all of whom had Dukes C (n = I) and Dukes D (n = 4) tumour stages, had high telomerase activity and short telomeres, whereas patients with extended survival (> 45 mo) had significantly lower telomerase activity and longer telomeres.

On the other hand, telomerase activity emerged as the only factor impacting disease-free survival (P = 0.050) in a series of patients with stage Ⅱ tumours[69] (Table 2). The authors of this study determined that the prognosis for patients with telomerase negative tumours was worse than that for patients with telomerase positive tumours. No association was found between telomerase activity and overall survival. They discussed that the poor prognosis observed in telomerase positive patients by other groups may, in some cases, represent an advanced stage of disease. In addition, tumours lacking both telomerase

and p53 could have a worse prognosis than telomerase positive tumours, as telomere shortening leads to mutations, chromosome rearrangements and translocations, which promote cellular transformations. Moreover, defects in mismatch repair genes, which have been reported in hereditary nonpolyposis colorectal carcinoma and spontaneous tumours, may facilitate cell proliferation and survival in the absence of telomerase due to activation of a recombination-dependent pathway for telomere maintenance and the accumulation of tumour-promoting mutations[69-71].

Bautista *et al*[72] determined TI to classify tumours in 2 groups: tumours with low telomerase index and tumours with high telomerase index. In 54 patients with colon cancer, they did not find a significant association between either recurrence-free survival nor overall survival and telomerase index. However, these associations were found when 41 rectal cancers were evaluated ($P = 0.020$ and $P = 0.010$, respectively): patients with tumours with low telomerase index showed a higher recurrence-free survival and overall survival probability. Moreover, when multivariate analysis was applied, Bautista *et al*[72] found that the telomerase index was an independent prognostic factor for predicting the recurrence in the first two years after surgery (95% CI: 1.09 to 10.8; $P = 0.030$) and for survival in rectal cancer patients (95% CI: 1.07 to 12.7; $P = 0.030$). This fact suggests a different behaviour for telomerase depending on its localization (Table 2). The findings of the present study are in accordance with other studies that identified differences in the etiologic, pathologic, and clinical behaviour of colon and rectal tumours: local recurrence occurs more frequently in patients with rectal cancer, and distant metastases occur more frequently in patients with colon cancer[73-75]. It is, therefore, reasonable to suggest that the etiologic factors and molecular bases may differ between colon and rectal cancers[72].

CONCLUSION

Gertler *et al*[43], Engelhardt *et al*[46] and Boldrini *et al*[59] support the hypothesis that sufficient (hTERT-mediated) telomere stabilization is achieved late in tumourigenesis after extensive cell proliferation and telomere shortening have already taken place. Telomere maintenance or even elongation seems to be essential for the tumour to maintain its (indefinite) proliferate capacity and to continue further tumour invasion and progression[76,77]. Effective (hTERT-mediated) telomere length stabilization might thus be a selection criterion for colorectal carcinoma to proceed from early to advanced tumour stages, illustrated by higher telomere length ratios in advanced tumours compared with early-stage tumours[45]. Moreover, telomerase evaluation may help to confirm the malignant transformation in polypoid colorectal lesions with different levels of dysplastic alterations[60].

Hahn *et al*[78] and Hahn *et al*[77,79] identified hTERT-mediated telomere maintenance as a key step in cell immortalization and neoplastic transformation of human cells and also stated that cells are selected for reactivated telomerase.

Taking into account all the results achieved by different groups, quantification and evaluation of telomerase activity and measurement of telomere length may be useful methods for additional biologic and prognostic staging of colorectal carcinoma. Moreover, the results presented by Garcia-Aranda *et al*[44] may help to identify a subgroup of patients with CRC who have a good clinical outcome among patients with telomerase positive tumours.

Gertler *et al*[43] consider that telomerase activity might be bypassed by alternative lengthening of telomeres or influenced by additional factors such as telomerase inhibitors, alternate splicing of hTERT transcripts[80], and changes of hTERT mRNA at the posttranscriptional level[81]. In agreement with that, Kawanishi-Tabata *et al*[69] propose the inclusion of other markers such as tumour suppressors or oncogenes to evaluate prognosis in CRC as clinical correlation studies based solely on telomerase activity may not be adequate. Therefore, it has been suggested that telomere length is the most reliable and most significant parameter of telomere regulation. It has the highest prognostic potential and it best defines possible candidates for new therapeutic protocols, when calculated as the ratio of cancer to non cancerous tissue[43,44,59].

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