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Review

The telomere proteins in tumorigenesis and clinical outcomes of oral squamous cell carcinoma

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SUMMARY

The "Hallmarks of Cancer" describe the ways by which cancer cells bypass homeostasis. Escape from replicative senescence is one of the earliest features of cancer cells. Maintenance of the telomeres through reactivation of telomerase was initially associated with replicative immortality in various cancers. The shelterin complex, a telomeric hexaprotein association, plays a key role in telomere maintenance and in the hallmarks of cancer. Some shelterin proteins are overexpressed in diverse cancers and can promote tumorigenesis in animal models. Shelterin can also have an impact on tumor size, tumor growth and resistance to treatment. Studies into the expression level of shelterin in oral squamous cell carcinoma (OSCC) report contradictory results. Moreover, the exact role of these proteins in OSCC tumorigenesis remains uncertain. In this review, we examined the data linking telomeres and hallmarks of OSCC. Furthermore, we examined the literature concerning telomeres and the clinical outcome of OSCC. Finally, we propose a model encompassing the role of shelterin proteins in oral tumorigenesis and treatment outcome.

Introduction

Telomeres are the distal ends of linear eukaryotic chromosomes and are composed of tandemly repeated DNA elements 5′-TTAGGG-3′. These terminal sequences are capped by specific telomeric proteins and adopt a particular three-dimensional conformation called the T-loop. The formation of this loop is made possible by the existence of a telomeric 3′ single-stranded overhang that invades the double-stranded part of the telomeric DNA. These features prevent the telomeres from being recognized as double-strand breaks of DNA. Among the telomeric proteins, the shelterin complex promotes the folding of linear telomeres into a T-loop and protects them from being processed by DNA repair mechanisms (ATM, HDR, NHEJ, BER, and NER) [1–6]. In humans, the shelterin complex consists of six proteins associated with telomeres: the telomeric repeat-binding factors 1 and 2 (TRF1 and TRF2), the TRF2 and TRF1 interacting protein (TIN2), the repressor activator protein 1 (RAP1), the protection of telomeres 1 (POT1) and the POT1 interacting protein (TPP1) [7].

In normal human somatic cells, homeostasis is guaranteed by telomere attrition during each replication cycle. This phenomenon is responsible for aging and the replicative senescence-based limited life span of the cells. Cancer is characterized by uncontrolled

Abbreviations: APC, antigen presenting cell; ALT, alternative lenghtening telomeres; ATM, ataxia telangiectasia mutated; AAH, atypical adenomatous hyperplasia; AML, acute myelogenous leukemia; BAC, bronchioloalveolar adenocarcinoma; BER, base excision repair; CSC, cancer stem cells; CTL, cytotoxic T lymphocytes; DNA, deoxyribonucleic acid; DN, dysplastic nodule; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinases; GC, gastric cancer; GSC, glioblastoma stem cells; HCC, hepatocellular carcinoma; HDR, homology directed repair; hnRNP A1, heterogenous nuclear ribonucleoprotein A1; hTERT, human telomerase reverse transcriptase; hTR, human telomerase RNA component; IFNγ, interferon gamma; IHC, immunohistochemistry; MAPK, mitogen activated protein kinases; mRNA, messenger ribonucleic acid; NER, nucleotide excision repair; NHEJ, non-homologous end joining; NHEK, normal human epidermal keratinocytes; NK, natural killers; NME2, metastasis suppressor non-metastatic 2; OSCC, oral squamous cell carcinoma; PDGFRβ, platelet derived growth factor receptor beta; PI3K, phosphoinositide 3-kinase; POT1, protection of telomeres 1; RAP1, repressor activator protein 1; RCC, renal cell carcinoma; REST, repressor element 1 silencing transcription factor; RT-PCR, reverse transcription polymerase chain reaction; shRNA, short hairpin ribonucleic acid; siRNA, small interfering ribonucleic acid; TGFα, transforming growth factor alpha; TIN2, TRF2 and TRF1 Interacting protein; TNFα, tumor necrosis factor alpha; TPP1, POT1 interacting protein; TRF1, telomeric repeat-binding factor 1; TRF2, telomeric repeat-binding factor 2; TUNEL, TdT-mediated dUTP nick end labeling; XPF, xeroderma pigmentosum group F-complementing protein; ZSCAN4, zinc finger and SCAN domain containing 4 gene.

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proliferation and spread of abnormal cells. Cancer cells evolve from normal cells that successively acquire new capabilities enabling them to proliferate, invade and produce metastases. In the very first steps of carcinogenesis cancer cells trigger replicative immortality to keep ahead of the senescence and apoptosis induced by enhanced division. Most cancer cells bypass aging through reactivation of telomerase activity, which maintains telomere length and enables replicative immortality, one of the "Hallmarks of Cancer" [8]. These hallmarks were initially described as six essential alterations by which cells evade control and become neoplastic: sustaining growth signaling, evading growth suppressors, enabling replicative immortality, evading apoptosis, sustaining angiogenesis and activating invasion and metastases [9]. Recently, avoidance of immune destruction or deregulation of cellular energetics have been added to these hallmarks [10]. To achieve these objectives, cancer cells must first undergo mutations in oncogenes and/or tumor suppressor genes that free them from the checkpoints that prevent proliferation of genomically damaged cells [11,12].

Oral squamous cell carcinoma (OSCC), a malignancy arising from epithelial cells of the oral mucosa, is the eighth most frequent cancer in France [13]. Late diagnosis and the side effects of anticancer treatments (mucitis, osteonecrosis and loss of secretion of saliva) highlight the need for novel diagnostic and therapeutic biomarkers. Although many authors have studied telomerase in cancer, very few studies have evaluated the involvement and the expression of proteins of the shelterin complex in OSCC. Moreover, the results of these studies were in diametrically opposition in some cases. These discrepant results point to the need for further investigation into the understanding of the role of the telomeric proteins in OSCC tumorigenesis and the associated clinical outcome.

In this review, we will summarize the results of studies investigating the role of shelterin in the induction and propagation of cancer. We will also discuss the potential use of shelterin components as prognostic markers in OSCC and/or predictive markers of success/failure of treatment. Finally we will evaluate the opportunity to consider components of the shelterin complex as relevant targets for anticancer therapies.

Shelterin expression in various cancers and OSCC

Shelterin proteins are up-regulated in various cancers. Pal et al. examined TRF1 and TRF2 expression in 92 samples of renal cell carcinoma (RCC) tissue and in the A-498 RCC cell line by RT-PCR and immunohistochemistry (IHC) [14]. They found significant overexpression of the TRF1 and TRF2 mRNA in tumor tissues compared to normal kidney (respectively $P = 0.005$ and $P = 0.0048$), which was confirmed by IHC. Likewise, expression of TRF1 was significantly increased in high-grade dysplastic nodules of the liver and further increased in hepatocellular carcinoma (HCC) [15].

Dong et al. reported TRF2 overexpression in SW480 colon carcinoma cells and in 19 out of 39 tumor tissues compared to normal cells and tissues [16]. This result was also confirmed by RT-PCR in colorectal carcinoma samples compared to normal tissues ($P < 0.05$). Valls-Bautista et al. assessed normal and tumor samples of 83 patients who underwent surgery for colorectal cancer. High levels of TRF1 were observed in 68.7% of tumor samples, while the majority of normal samples (59%) were negative or weak [17]. In another study, 20 gastric carcinomas (GC) were examined by RT-PCR. Among these, 10 (50%) and 12 (60%), respectively expressed TRF1 and TRF2 at higher levels than did non-neoplastic mucosa [18]. A more recent study confirmed these results; the expression of the TRF1, TRF2, and TIN2 proteins was significantly higher in precancerous lesions, GC and GC with lymph node metastasis compared with normal gastric mucosa tissues ($P < 0.01$) [19].

De Divitiis and La Torre reported variable levels of TRF1and TRF2 expression in meningioma samples depending of the stage of the disease [20]. Up-regulation of TRF1 and TRF2 was found in low grade astrocytomas (LGA), while expression of these proteins was down-regulated in anaplastic astrocytomas and glioblastoma multiform [21].

Increased expression of POT1, TPP1, TIN2 and RAP1 was reported in multiple myeloma compared to monoclonal gammopathy with significant differences for POT1 gene ($P = 0.002$) [22]

Inversely, some studies showed that shelterin proteins were down-regulated in various cancers. TRF1 mRNAs were significantly down-regulated in cancers compared to non-cancerous mucosa to maintain telomeres in gastric cancer [23]. Expression of TRF1 and TRF2 mRNA was higher in normal cells than in human malignant hematopoietic cell lines and in 16 samples of patients with acute leukemia [24]. This result was partially confirmed by another study in which the expression level of the TRF1 protein was significantly higher ($P < 0.01$) in normal bone marrow compared to bone marrow of acute leukemia patients [25]. Studies into the expression of shelterin have obtained discrepant results for the head and neck location. In 74 specimens of esophageal SCC, the expression rate of TRF1 ($P = 0.002$) in the tumor tissues was higher than in the normal paired tissues [26]. Sainger et al. assessed 75 OSCC protein samples from Indian patients for TRF1 and TRF2 expression by immunoblotting with monoclonal antibodies. They found that the expression of TRF-2 but not of the TRF-1 protein was significantly higher ($P < 0.001$) in malignant tissues compared to the adjacent normal tissue in OSCC [27]. Finally, Chuang et al. analyzed 256 OSCC samples from Chinese patients. They observed a lower expression of TRF1 and TRF2 proteins by immunoblotting and IHC, in OSCC compared to normal paired tissues in 5 out of 256 specimens [28].

The apparent discrepancies between these studies can be explained. First, the elevated male–female sex-ratio (239 of 256 cases) in the latter study was not representative of that described in other studies of Asian patients [29,30]. Second, the polyclonal antibody used in this study (Santa Cruz Biotechnologies Inc.) was not the same as the monoclonal one used by other authors. In addition, no information concerning validation of the antibody by a knock-down approach can be found.

Although TRF1 down-regulation and TRF2 overexpression seem to be common features of a large variety of cancer cells, the discrepant results obtained in OSCC emphasize the difficulty of concluding whether shelterin component expression plays a role in tumorigenesis and prognosis. In particular, the lack of homogeneity in the technical approaches may account for discrepancies in the results obtained by the different teams. Furthermore, the other components of shelterin (RAP1, TIN2, TPP1, POT1) are not documented enough in cancer.

Telomere proteins in OSCC tumorigenesis

All the clinical parameters of a cancer such as tumor growth (size and speed), tumor grade, tumor invasion, metastases and treatment response depend on biological phenotypes called the hallmarks of cancer. As previously described, telomeres are key components of cancer cells. It thus appears interesting to highlight the influence of telomere components in OSCC tumorigenesis to better understand their impact on clinical outcome.

Genomic instability

Oral carcinogenesis is a multistep process that begins with multiple mutations in genes transforming normal tissue first into dysplastic and then into tumor tissue [12].