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The 58-kDa microspherule protein (MSP58) represses human telomerase reverse transcriptase (hTERT) gene expression and cell proliferation by interacting with telomerase transcriptional element-interacting factor (TEIF)

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ABSTRACT

58-kDa microspherule protein (MSP58) plays an important role in a variety of cellular processes including transcriptional regulation, cell proliferation and oncogenic transformation. Currently, the mechanisms underlying the oncogenic effect of MSP58 are not fully understood. The human telomerase reverse transcriptase (*hTERT*) gene, which encodes an essential component for telomerase activity that is involved in cellular immortalization and transformation, is strictly regulated at the gene transcription level. Our previous study revealed a novel function of MSP58 in cellular senescence. Here we identify telomerase transcriptional element-interacting factor (TEIF) as a novel MSP58-interacting protein and determine the effect of MSP58 on *hTERT* transcription. This study thus provides evidence showing MSP58 to be a negative regulator of *hTERT* expression and telomerase activity. Luciferase reporter assays indicated that MSP58 could suppress the transcription of *hTERT* promoter. Additionally, stable overexpression of MSP58 protein in HT1080 and 293T cells decreased both endogenous *hTERT* expression and telomerase activity. Conversely, their upregulation was induced by MSP58 silencing. Chromatin immunoprecipitation assays showed that MSP58 binds to the *hTERT* proximal promoter. Furthermore, overexpression of MSP58 inhibited TEIF-mediated *hTERT* transactivation, telomerase activation, and cell proliferation promotion. The inhibitory effect of MSP58 occurred through inhibition of TEIF binding to DNA. Ultimately, the HT1080-implanted xenograft mouse model confirmed these cellular effects. Together, our findings provide new insights into both the biological function of MSP58 and the regulation of telomerase/hTERT expression.

Abbreviations: MSP58, 58-kDa microspherule protein; hTERT, human telomerase reverse transcriptase; TEIF, Telomerase transcriptional element-interacting factor; USF1, upstream stimulatory factor 1; TCF-4, T cell transcription factor 4; PI3K, phosphoinositide 3-kinase; NFAT, nuclear factor of activated T cell; HMGA2, high mobility group A2; Bmi-1, B-lymphoma Moloney murine leukemia virus insertion region-1; WT1, Wilms tumor 1; Mad1, max dimer protein 1; MZF-2, myeloid zinc-finger protein 2; NFX1, nuclear factor binds to the X1 box; Smad3, SMAD family member 3; menin (MEN1), multiple endocrine neoplasia type 1; IRF-4, interferon regulatory factor 4; IRF8, interferon regulatory factor 8; UBF, upstream binding factor; qPCR, quantitative PCR; MOF, males absent on the first; NuRD, nucleosome remodeling and deacetylase; Rb, retinoblastoma protein; Nde1, nudE nuclear distribution gene E homolog 1; STRA13, stimulated by retinoic acid 13; TOJ3, target of Jun 3

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1. Introduction

Telomerase is a ribonucleoprotein complex with reverse-transcriptase enzymatic activity which is responsible for adding hexameric repeats (TTAGGG) to telomeric ends of linear chromosomes [1]. The human core telomerase enzyme consists of a catalytic subunit (human telomerase reverse transcriptase; hTERT) which bears the enzymatic activity of telomerase and an RNA moiety (human telomerase RNA; hTR) that is used as a template for telomeric repeat synthesis [2]. Maintenance of telomere lengths by telomerase is required for cells to be able to proliferate indefinitely and to escape from replicative senescence. In adult humans, telomerase is present in human stem cells and germ cells but is barely detectable in the vast majority of differentiated somatic cells [3]. However, telomerase can be reactivated in most cancers and not only stabilize their telomere size but also directly regulate multiple cancer-promoting pathways [4]. Activation of telomerase is a critical step during cellular immortalization and malignant transformation [5]. Notably, telomerase or *hTERT* reactivation is detected in up to 90% of human cancers [6]. Moreover, it has been shown that ectopic expression of *hTERT* can restore telomerase activity in many telomerase-negative cell types, thereby facilitating immortalization [7,8]. Furthermore, introduction of a dominant negative form of *hTERT* or an antisense vector targeted against *hTERT* inhibits telomerase activity in human cancer cells and eventually induces apoptosis or differentiation [9]. Therefore, *hTERT* expression is defined as the rate-limiting factor in regulating telomerase activity and is a hallmark of cancer.

Numerous studies suggest that telomerase enzymatic activity and expressions of telomerase components are regulated at multiple levels, including transcription and post-transcription, and by chaperone-mediated folding and proper localization; however, the major control mechanism of telomerase regulation seems to be at the level of *hTERT* transcription [10]. Deletion analyses in reporter assays showed that the approximately 200-bp proximal region of the *hTERT* promoter is important for maintaining basal transcriptional activity [11,12]. The *hTERT* promoter contains several putative transcription factor-binding sites, including c-Myc [13–15], Sp1 [11,12], E2F [16], USF [17], hypoxia-inducible factor (HIF)-1 [18], and TCF4 [19]. Additional studies have demonstrated that PI3K [20], NFAT [21], HMGA2 [22], the Bmi-1 oncogene [23], and the papillomavirus E6 protein [24] can also stimulate *hTERT* promoter expression. In contrast, WT1 [25], Mad1 [26], MZF-2 [27], NFX1 [28], Smad3 [29], menin [30], and p53 [31,32] can negatively regulate *hTERT* promoter expression. Distinct repression and activation mechanisms likely operate in different cell contexts. For example, estrogen upregulates telomerase in both mammary and ovarian epithelial cells [33,34]; IRF-4 and IRF-8, two lymphoid cell-specific transcription factors, activate *hTERT* transcription in immune cells [35]; and E2F-1 has opposing actions toward *hTERT* gene expression in human tumors and normal somatic cells [16,36]. Epigenetic regulatory mechanisms, such as promoter DNA methylation [37] and histone acetylation [38], also modulate *hTERT* expression.

The 58-kDa microspherule protein (MSP58, also known as MCRS1) was originally identified as the interaction partner of the p120 nucleolar protein [39]. Further studies showed that in nuclei and nucleoli, MSP58 functions to regulate transcription through its interactions with the transcription factors Daxx, STRA13, and UBF [40–42]. Interestingly, microspherule protein 2 (MCRS2) was identified as an interacting partner of a potent inhibitor of telomerase, LPTS/PinX1, and hTERT [43]. MCRS2 inhibits telomerase activity in vitro and long-term overexpression of MCRS2 in cancer cell lines leads to telomere shortening. *Drosophila* MCRS2 is co-purified with RNA polymerase II complexes and is required for normal levels of cyclin gene expression [44]. Notably, MSP58 and MCRS2 have been shown to be associated with several chromatin-remodeling complexes including Mi-2β (a component of the NuRD complex), INO80, Brg1 (a subunit of the SWI/SNF complex), and histone acetyltransferase males absent on the first (MOF) [42,45–48]. Furthermore, p78, an isoform of MSP58, has been identified as a centrosomal protein that is required for maintaining centrosome homeostasis [49,50]. Importantly, a number of studies have indicated that MSP58 plays a key role in oncogenesis. For instance, MSP58 and TOJ3 (a quail homologue of MSP58) behave as oncogenes in fibroblast transformation assays, whereas the tumor suppressor, phosphatase and tensin homologue (PTEN), suppresses the transforming activity of MSP58 [51,52].

In a previous study, we demonstrated that MSP58 induces cellular senescence through the p53/p21 pathway [48]. Identifying potential novel binding partner(s) of MSP58 may help elucidate the role it plays in controlling cell proliferation; to do this, we performed yeast two-hybrid screening using MSP58 as bait. We identified a novel MSP58-interacting protein, telomerase transcriptional element-interacting factor (TEIF), that was found to bind to the *hTERT* promoter and activates its transcription and telomerase activities [53]. Notably, TEIF overexpression has been detected in tumor tissues and cell lines, parallel to *hTERT* [53,54]. In this study, we examined the potential contribution of MSP58 to the transcriptional regulation of *hTERT*. Our data revealed that overexpression of MSP58 repressed *hTERT* transcription and telomerase activity, in part, through interaction with TEIF. Our findings reveal MSP58 to be a negative regulator of *hTERT* expression and telomerase activity.

2. Materials and methods

2.1. Plasmids and antibodies

Yeast constructs expressing LexA-MSP58 and pGal-AD-TEIF, a mammalian vector expressing Flag-MSP58 and EGFP-MSP58, a pQCXIP-GFP-MSP58 retroviral vector, and a bacterial vector expressing GST-MSP58 were as described previously [40,48,53]. MSP58 deletion mutants for expressing LexA fusion in yeast and Flag-tagged fusion in mammalian cells were generated by inserting polymerase chain reaction (PCR) fragments respectively coding MSP58 amino acids 1–300 and 300–462 into the pBTM116 and pCMV-Tag2 (Stratagene, La Jolla, CA, USA) vectors. The full-length TEIF (amino acids 1–786) was cloned into the pcDNA3.1-HA expression vector (Invitrogen) to generate HA-TEIF. The TEIF and its deletion mutants for expressing Gal4 activation domain fusion in yeast and HA-tagged fusion in mammalian cells were generated by inserting PCR fragments respectively coding the full-length TEIF, and amino acids 1–395 and 396–786 into the pACT2 (BD Biosciences Clontech) and pcDNA3.1-HA vectors. Human Daxx complementary (c) DNA was purchased from GeneCopoeia (Germantown, MD, USA). Full-length Daxx was cloned into the pCDNA3.1-HA-expressing vector (Invitrogen) to generate HA-Daxx. hTERT retrovirus vector pLPC-hTERT (Clontech laboratories, CA, USA) was obtained from Dr. Yu-Ten Ju (National Taiwan University, Taipei, Taiwan). Full-length hTERT was cloned into the pCMV-Tag2 vector (Stratagene) to generate Flag-hTERT. The hTERT promoter deletion mutants p548 (−548 to +50), p212 (−212 to +50), p196 (−196 to +50), p177 (−177 to +50), and p95 (−95 to +50) were cloned upstream of the firefly luciferase reporter in the pGL3-Basic vector (Promega, Madison, WI, USA) following a previously described protocol [55]. The luciferase reporter plasmid, TERTLuc800, as previously described [15], was a gift from Dr. Kou-Juey Wu (National Yang-Ming University, Taipei, Taiwan). Short-hairpin (sh)RNA targeting MSP58 was expressed from the pLKO.1 hairpin vector, which harbors an expression cassette for a puromycin resistance gene. shMSP58 sequences used were shMSP58 1: 5′-CCTGCGATTCGTCTTCCTTAT-3′ and shMSP58 2: 5′-CAACAACTCTGTGGTGGAGAT-3′. pLKO.1-shLuc (TRCN0000072243; shLuc) was used as a control. These vectors were obtained from The RNAi Consortium (TRC) library (National RNAi Core Facility, Academia Sinica, Taipei, Taiwan). The pSUPER vector expressing small-interfering (si)RNA of human MSP58 was constructed according to instructions from OligoEngine (Seattle, WA, USA). The 19-nucleotide target sequences for siMSP58 were si-3 (5′-GAAGAAGAAGGTATCCAAA-3′) and si-5 (5′-CAAGGTGTCATCAAGC