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Original article

Inhibition of hTERT expression by MAP kinase inhibitor induces cell death in renal cell carcinoma

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Abstract

Background: Human telomerase reverse transcriptase (hTERT) is one of the components of telomerase enzyme and its activity is associated with cell proliferation and differentiation. Extracellular signal regulated kinase (ERK)-mitogen activated protein kinase signaling pathway play an important role in hTERT expression. The present study was conducted to ascertain hTERT messenger RNA (mRNA) expression in renal cell carcinoma (RCC) and its association with clinicopathological characteristics. Further, we also explored hTERT targeting as possible tumor therapeutic.

Methods: A total of 96 histopathologically confirmed RCC cases and corresponding normal tissues were subjected to hTERT gene expression using real-time PCR. Two RCC cell lines viz. ACHN and A498 were treated with MEK inhibitor (U0126) and hTERT mRNA expression, telomerase activity, cell viability, migration, and apoptosis were evaluated.

Results: The hTERT mRNA levels were found to be significantly higher in RCC as compared with corresponding normal renal tissues ($P = 0.040$) as well as in high grades to that of low grades clear cell RCC ($P = 0.008$). Significantly diminished ERK phosphorylation and hTERT mRNA expression concomitantly reduced telomerase activity that was observed after U0126 treatment. Subsequently, cell viability and migration were significantly inhibited after treatment with U0126 in both the cell lines to that of control ($P = 0.001$). In addition, U0126-treated cells showed significantly increased apoptosis ($P = 0.001$) to that of controls.

Conclusion: Henceforth, this study infer that, hTERT expression can be used as a possible diagnostic marker for RCC, and inhibition of hTERT expression by hampering ERK-mitogen activated protein kinase cascade may be used as a promising anticancer target in RCC.

Keywords: Renal cell carcinoma; hTERT; U0126; ERK-MAP kinase; Anticancer

1. Introduction

Renal cell carcinoma (RCC) is one of the most lethal and common renal tumor, which constitutes 3% of all adult malignancies. Moreover, 90% of all primary renal cancers are associated with RCC [1]. Clinical outcome of RCC is worst among cancers of urinary system. Intriguingly, RCC does not responded to conventional chemotherapy, radiation therapy, and immunotherapy [2,3]. Current diagnosis and prognosis of RCC as well as the effective treatment are confined owing to indigent molecular understanding of disease process. Henceforth, there is an urgent need of comprehensive understanding of RCC pathogenesis as well as early diagnosis and to establish an effective therapeutics. In view of these facts, it is utmost important to understand molecular pathogenesis of RCC.

Telomeres are specialized structures at the ends of human chromosomes that get shortened with each cell division. Telomerase is a ribonucleoprotein reverse transcriptase enzyme that adds telomeric repeats $(TTAGGG)_n$ at the ends of chromosomes thus compensates loss of repeats, that are otherwise lost with successive cell divisions [4].

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Telomerase is expressed in the most types of tumors but not in most of the somatic cells. This observation supports that telomerase activity is necessary for the proliferation of cancer cells and it can be used as a powerful strategy for anticancer therapy [5]. Augmented telomerase activity is directly correlated with uncontrolled division of cells, which is a hallmark of cancer [6]. Human telomerase reverse transcriptase (hTERT) is an active component of telomerase and is responsible for its catalytic activity. hTERT is found to be up-regulated in 85%–90% of cancers [7]. The dependence of telomerase enzyme on hTERT for its activity makes hTERT a potential anticancer target.

Present study demonstrated an elevated level of expression of hTERT gene expression in RCC. Therefore, we conjectured that hTERT would be a good target from therapeutic aspect in RCC. It is noteworthy here that an association between mitogen activated protein (MAP) kinase and hTERT gene promoter activity has been demonstrated previously [8]. Therefore, inhibition of MAP kinase signaling mechanism may have a subsequent effect on hTERT gene expression along with telomerase activity. Therefore, it may indulge in regulation of tumor cell immortality through hTERT gene expression.

In view of these facts, the present study was designed to investigate the effects of extracellular signal regulated kinase (ERK)-MAP kinase inhibition on hTERT expression and telomerase activity. Further, we investigated the effect of hTERT inhibition on proliferation, migration, and cell cycle progression in RCC cell lines (ACHN and A498).

2. Materials and methods

2.1. Patients

The present study was approved by the institute ethics committee and an informed consent was obtained from patients. A total 96 cases of histopathologically proven RCC as well as corresponding normal renal tissues were included in this study. None of the patients received radiotherapy and preoperative chemotherapy. Tumor staging and grading of clear cell RCC type were performed according to TNM staging [9] and Fuhrman grading [10], respectively. Detailed clinical characteristics of patients are given in Table 1. Following nephrectomy, tissue samples were taken from the tumor and grossly normal renal parenchyma separately. The samples were snap frozen in liquid nitrogen and stored at −80°C till further use.

2.2. Real-time PCR

Total RNA was isolated from normal and tumor tissue using PureLink RNA Mini Kit (Invitrogen, CA) as per manufacturer's instruction. Reverse transcription of 1 μg of RNA was performed with first-strand c-DNA synthesis using SuperScript^{III} using polyA primer (Invitrogen, CA). Real-time PCR analysis was performed on 7300 RT-PCR system (Roche, IN) using the light cycler RNA Master SYBR Green kit (Roche, IN). The primer used for hTERT mRNA were as follows: sense, 5′-GCCTGAGCTGTACTT TGTCAA-3′; antisense, 5′-CGCAAACAGGCTTGTTCTC-CATGTC-3′. For internal control, levels of β-actin mRNA were measured using the following primers: sense, 5′-CGA GCGCGGCTACAGCTT-3′; antisense, 5′-TCCTTAATGT-CACGCACGATTT-3′. The fold change in hTERT mRNA expression in each tumor sample was calculated relative to normal renal tissue (control) by the ΔΔCT method using the following equation [11]:

Fold change $= 2^{-(\text{Tumor } \Delta Ct \_ \text{ Control } \Delta Ct)}$, where ΔCt = Ct (hTERT)−Ct (β-actin).

Table 1
Clinical characteristic of the patients.

Patients, *n*	96
Sex, *n*	
Male	63
Female	33
Age (y), mean ± SD	53 ± 12.4
BMI, mean ± SD	23.47 ± 0.146
Commonest presenting complaints, *n*	
Hematuria	52
Flank pain	31
Both	24
High fever	25
Incidental radiological examination	15
Histologic subtype, *n*	
Clear cell RCC	69
Papillary	13
Sarcomatoid	07
Chromophobe	06
Collecting duct	01
TNM stage (clear cell), *n* (%)	
Low stages (stage I and II)	46 (66.7)
High stages (stage III and IV)	23 (33.3)
Furhman grade (clear cell), *n* (%)	
Low grades (I and II)	40 (58)
High grades (III and IV)	29 (42)

SD = standard deviation.

2.3. Cell culture

RCC cell lines (ACHN and A498) were purchased from National Centre for Cell Sciences, Pune, India. These cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were allowed to 60%–80% confluent at 37°C in 5% CO_2 atmosphere. For experiments, cells were divided into 4 groups and they are untreated cells, epidermal growth factor (EGF) (80 ng/ml) treated cells, U0126 (20 μM) treated cells, and cells treated with both EGF (80 ng/ml) and U0126 (20 μM).

2.4. ELISA for ERK1/2 phosphorylation

The phosphorylation status of ERK1/2 was checked using RayBio cell-based ERK1/2 (Thr202/Tyr204) enzyme-linked