Available online at www.sciencedirect.com

SCIENCE DIRECT

Gynecologic Oncology 98 (2005) 427 – 433

Gynecologic Oncology

www.elsevier.com/locate/ygyno

Aberrant expression and mutations of TGF-β receptor type II gene in endometrial cancer

Junko Sakaguchi[a], Satoru Kyo[a,*], Taro Kanaya[a], Yoshiko Maida[a], Manabu Hashimoto[a], Mitsuhiro Nakamura[a], Kiyofumi Yamada[b], Masaki Inoue[a]

[a]*Department of Obstetrics and Gynecology, Kanazawa University Graduate School of Medical Science, 13-1, Takaramachi, Kanazawa, Ishikawa 920-8641, Japan*

[b]*Laboratory of neuropsychopharmacology, Department of Clinical Pharmacy, Faculty of Pharmaceutical Science, Kanazawa University, 13-1, Takaramachi, Kanazawa, Ishikawa 920-8641, Japan*

Received 20 January 2005
Available online 1 July 2005

Abstract

Objective. Transforming growth factor β (TGF-β) is a multifunctional cytokine that strongly inhibits epithelial cell growth. Disabling of TGF-β signaling is thought to be involved in development of a variety of tumors in which abnormal expression or function of TGF-β receptor plays critical roles. In the present study, we examined aberrant expression and mutation of the gene *TGF-β receptor type II* (*TβRII*) in endometrial cancers of endometrioid subtype.

Methods and results. Real-time PCR analysis using surgical tissue specimens of 27 endometrial cancers and 24 normal endometria revealed that endometrial cancers had significantly decreased levels of TβRII mRNA expression (mean level 2.44 ± 2.65), compared to normal endometria (mean level 7.23 ± 6.07) ($P < 0.001$). Methylation status of *TβRII* promoter containing 30 CpGs was examined by bisulfite sequencing analysis, and 98% (51/52) of the patients were found to have unmethylated *TβRII* promoter, indicating that promoter hypermethylation is not the major cause of decreased expression of *TβRII* in endometrial cancers. Mutational analysis revealed that 15.1% (8/53) of endometrial cancers had frameshift mutations at polyadenine repeats in exon 3 of the *TβRII* gene. Notably, these mutations were preferentially accumulated in patients with MSI-H phenotype (7/19:37%) ($P < 0.001$) or with those with methylated *MLH1* promoters (6/16:38%) ($P < 0.01$). Thus, it appears that the *TβRII* gene is a target of mismatch repair deficiency.

Conclusion. Taken together, we found that the decreased expression of *TβRII* as well as frameshift mutation of *TβRII* via mismatch repair deficiency frequently occurs in this tumor type, possibly causing loss of receptor function and unresponsiveness of TGF-β signaling that may lead to endometrial carcinogenesis.

Keywords: TGF-β-receptor type II; Mutation; Methylation; Endometrial cancer

Introduction

Transforming growth factor β (TGF-β) is a multifunctional cytokine that strongly inhibits epithelial cell growth [1,2]. TGF-β initiates signaling across the plasma membrane to the nucleus by binding to the specific transmembrane receptor TGF-β receptor type II (TβRII), which is a constitutively active serine/threonine kinase that recruits and phosphorylates type I receptor (TβRI). Activated TβRI directly phosphorylates intracellular transcription factors known as R-Smads (receptor-specific substrates), specifically Smad2 or Smad3, which combines with co-Smad4; the resulting complex translocates to the nucleus to activate TGF-β-regulated genes [3,4].

In normal epithelial cells, TGF-β acts as a tumor suppressor by inhibiting cellular proliferation or by promoting cellular differentiation or apoptosis [5]. In the initial stages of tumorigenesis, a cell loses its TGF-β-mediated

* Corresponding author. Fax: +81 76 234 4266.
E-mail address: satoruky@med.kanazawa-u.ac.jp (S. Kyo).

0090-8258/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2005.04.031

growth inhibition as a result of mutation or loss of expression of the genes for one or more components of the TGF-β signaling pathway [6]. The first mutations found in the TGF-β signaling pathway were detected in *TβRII*; several cancers were found to have mutations in or loss of expression of this receptor gene. The coding region of this gene has a sequence of 10 consecutive adenine nucleotides; the addition or deletion of an adenine within this region results in the production of a truncated, functionally inactive receptor. By the analysis of hereditary nonpoliposis colorectal cancers (HNPCC), a familial cancer syndrome, characterized by a high incidence of colon, endometrial and gastric cancers, these mutations were reported to be tightly associated with microsatellite instability (MSI) [7]. Although MSI+ phenotype is characteristic of HNPCC-related cancers, it is also present in many sporadic colon, endometrial and gastric cancers [8]. Subsequent studies demonstrated that such mutations were also common in sporadic colorectal and gastric cancers, associated with MSI [9–11]. These findings proposed a concept that *TβRII* is a target of mismatch repair (MMR) deficiency. In regard to endometrial cancer, only one report demonstrated that mutation of *TβRII* is relatively rare even in MSI+ tumors [10], and it remains unclear whether the above concept can be applied to endometrial cancer.

Another known dysfunction of *TβRII* is promoter hypermethylation, which has been detected in some lung cancer cell lines [12]. However, the roles of hypermethylation of *TβRII* in human carcinogenesis are still largely unknown.

TGF-β seems to play an important role as an autocrine, paracrine and endocrine factor in human endometrium, a unique tissue that undergoes cyclic regeneration in a menstrual cycle-dependent manner [13]. Different expression patterns of the TGF-β isoforms and their specific receptors have been demonstrated not only in cycling endometrium but also in the process of endometrial carcinogenesis [14–16]. There have been reports of disabling of TGF-β signaling at different steps early in endometrial carcinogenesis, in which TGF-β receptor abnormality plays critical roles [17]. In the present study, we sought to investigate how TGF-β signaling is disabled in endometrial carcinogenesis by analyzing expression and mutation of the *TβRII* gene in relation to MMR deficiency in sporadic endometrial cancers.

Materials and methods

Tissue sample

Surgical tissue specimens were obtained from patients who underwent hysterectomy to treat primary endometrial cancers or benign tumors such as uterine myomas at the Department of Obstetrics and Gynecology, Kanazawa University Hospital. All cancers and normal endometria were histologically confirmed and diagnosed by 2 independent pathologists. All samples were collected after obtaining written informed consent from the patients. Half of each tissue sample was histologically examined, and the remaining half of each sample was frozen at −80°C until DNA extraction.

Real-time PCR

Total RNA was extracted from surgical tissue specimens using NucleoSpin RNA II (Machery-Nagel, Duren, Germany) and was reverse transcribed using SuperScript II Reverse Transcriptase and oligo (dT) primers (SuperScript First-strand Synthesis System for RT-PCR; Invitrogen, Carlsbad, CA, USA). The mRNA expression of the *TβRII* gene was measured by fluorescence-based, TaqMan real-time RT-PCR, using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed with a 40-μl reaction mixture containing 2× TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM forward and reverse primers, 200 nM probe (Table 1). After 2 min at 50°C and 10 min at 95°C, amplification was performed in 40 cycles of 95°C for 15 s and 60°C for 60 s [18]. As the control, GAPDH mRNA expression was examined. To detect GAPDH mRNA expression, assays on demand gene expression products (20× primer and probe mix) for GAPDH (Applied Biosystems) were used. The amplification plot, the graph of the increment of fluorescence reporter signal versus cycle number during PCR, is examined early in the reaction, at a point that represents the logarithmic phase of product accumulation.

Bisulfite sequencing

Genomic DNA was extracted from surgical tissue specimens using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) and was subjected to bisulfite sequencing and mutation analyses. DNA was modified with sodium bisulfite using a CpGenome DNA Modification Kit (Intergen Co., Purchase, NY, USA). After conversion, the methylation status of the *TGFβRII* promoter region was examined. Modified DNA was amplified with PCR consisting of initial denature at 95°C for 10 min followed by 40 cycles of denature at 94°C for 45 s, 52°C for 30 s and 72°C for 30 s using the primers TGFβRII-p5F (or p6F) and TGFβRII-p4R (Table 1). The PCR products were purified using a Qiagen Gel Extraction Kit (Qiagen), cloned into the TA vector pGEM-T Easy (Promega, Madison, WI, USA) and sequenced. At least 2 independent clones were picked up and examined.

Analysis of microsatellite instability (MSI)

Samples of DNA from endometrial cancers and normal areas of the endometrium were analyzed using a panel of 5