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RESEARCH

Different Characteristics of Mitochondrial Microsatellite Instability Between Uterine Leiomyomas and Leiomyosarcomas

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Abstract Uterine leiomyomas are benign tumors of the uterus that arise clonally from smooth muscle cells of the myometrium and are the most common reason for hysterectomies. The aim of this study was to evaluate mitochondrial microsatellite instability (mtMSI) in uterine leiomyomas and leiomyosarcomas to clarify the molecular pathogenetic distinction between these tumors. DNA was isolated from paired normal and tumoral tissues in 50 patients with uterine leiomyomas and 14 patients with leiomyosarcomas. mtMSI was analyzed by using eight microsatellite markers. Our result showed that mitochondrial microsatellite instability was not found in all uterine leiomyomas. However, 3 (21.4%) of 14 patients with leiomyosarcomas had mtMSI and the frequencies of mtMSI in these tumors were significantly different ($p<0.01$). Distinctive characteristics of mitochondrial genetic instability in uterine leiomyomas and leiomyosarcomas suggested the potential of mtMSI as a marker for differential diagnosis between them.

Keywords Genetic instability · Mitochondrial microsatellite instability · Uterine leiomyoma · Uterine leiomyosarcomas

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Introduction

Uterine leiomyomas are benign monoclonal tumors of the smooth muscle cells of the myometrium. Their prevalence among all women has been estimated to be as high as 77% [1]. Despite their prevalence, leiomyomas have remained enigmatic, with the incidence, natural history, and progression incompletely understood [2]. Uterine leiomyosarcomas are rare gynecologic malignancy and account for only 1% of all uterine malignancies. They are generally considered to be highly malignant with poor prognosis due to high recurrence rate. Histological similarity of leiomyomas and leiomyosarcomas can lead clinicians to misdiagnose early-stage leiomyosarcomas as leiomyomas [3]. Some authors described leiomyosarcomas as malignant degenerative change of leiomyomas, however, this argument is still in debate [3–5].

Various experiments have been carried out previously to clarify the relation between uterine leiomyomas and leiomyosarcomas. In current years, increased attention has been focused on genetic instability in cancer, and microsatellite instability (MSI or nuclear MSI, nMSI) has been studied in various cancers [6–9]. Previous research has studied nMSI and loss of heterozygosity (LOH) in uterine leiomyomas and leiomyosarcomas. LOH was frequently seen in leiomyosarcomas, whereas it was absent in leiomyomas [10]. However, nMSI presented non-distinguishable patterns. [10–12].

Mitochondrial DNA (mtDNA) is made up of 16,569 bp circular, double-stranded DNA molecules and has its own independent genome. The mutation rate of mitochondrial DNA is 10- to 100-fold higher than that of nuclear DNA because of the high concentration of reactive oxygen species (ROS) in the mitochondrial inner membrane, few repair mechanisms, and absence of mtDNA-coating proteins like the histones in the nucleus [13–15]. Previous research also has analyzed mitochondrial MSI (mtMSI) in various cancers, including breast, gastric, ovarian, endometrial, and colorectal cancers [16–19]. According to these studies, mitochondrial and nuclear MSI showed no significant associations, suggesting that different systems are responsible for mitochondrial and nuclear genetic instabilities in tumor cells. Though nMSI in leiomyomas and leiomyosarcomas has been studied by many authors, there is no study about mtMSI in these tumors.

In the present article, we have analyzed mtMSI, one of the markers of genetic instability, in uterine leiomyomas and leiomyosarcomas. Based on previous studies [20–22], mtMSI was investigated in these tumors by using eight microsatellite markers.

Materials and Methods

Samples and DNA Extract

Our study groups were comprised of fifty patients with uterine leiomyomas (26–72 years old) and fourteen patients with uterine leiomyosarcomas (33–85 years old). Since 2003, we have acquired both paired normal and tumor samples from hysterectomy specimens from patients being treated for suspected uterine tumors by the Division of Gynecologic Oncology at Dongsan Medical Center. Gynecological pathologist reviewed these samples to confirm the diagnosis of uterine leiomyomas and leiomyosarcomas. Diagnosis of leiomyosarcoma was based on coagulative tumor cell necrosis, atypia, and mitotic index according to the Stanford criteria [23]. Non-tumorous or non-inflammatory tissues were used as a control. All samples were fixed in formalin and embedded in paraffin. The institutional regional review board (IRB) approved the research proposal, and informed consent was obtained from all individuals involved in the study.

All of the tumors and paired normal tissues obtained were microdissected under a light microscope using a 30-gauge needle and transferred to 100 μL of extraction buffer (10 mM Tris-HCl, 1% Tween, 0.1 mg/mL proteinase K, 1 mM EDTA, pH 8.0), respectively. The mixture was incubated overnight at 37°C and boiled for 10 min to inactivate the proteinase K. The DNA solution was purified using the QIAquick Gel Extraction Kit protocol (Qiagen, Chatsworth, CA, USA). The purified DNA was eluted in 50 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Mitochondrial Microsatellite Instability Analysis

Mitochondrial microsatellite instability (mtMSI) was analyzed by using eight microsatellite markers as previously described [20–22]. We performed the PCR using a thermal cycler (model 2400, Applied Biosystems, Foster City, CA, USA) as follows: 35 cycles of 40 s at 94°C for denaturation, 40 s at 56°C for annealing, and 40 s at 72°C for extension. Final extension was performed at 72°C for 10 min. The PCR products were electrophoresed on an agarose gel and stained with ethidium bromide to confirm the size of the bands. The PCR products were also denatured in formamide loading buffer (95% formamide, 20 mmol EDTA, 10 mmol NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol) and electrophoresed through 7.5% and 10% polyacrylamide gels. The bands were visualized by silver staining [21, 24]. mtMSI was defined as either a band shift or an appearance of a novel band in DNA from tumoral tissue with more than one marker. We repeated all experiments at least twice to rule out any artifacts.

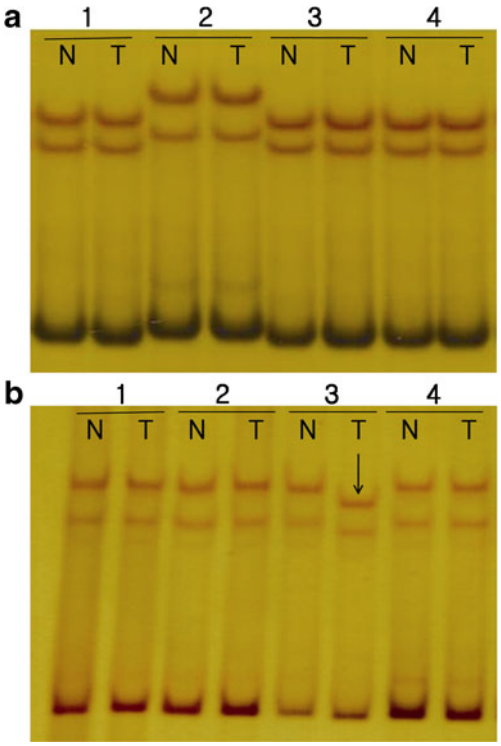

Fig. 1 Representative examples of mitochondrial microsatellite instability at the (CA)n of the D-loop in uterine leiomyomas (**a**) and leiomyosarcomas (**b**). **a** In all patients with leiomyomas, tumor tissues (*lanes T*) did not show any band shift compared to the paired normal tissues (*lanes N*). **b** In the case 3, tumor tissue (*lane T*) showed mobility-shift band (*arrow*) compared to the paired normal tissue (*lane N*)

Mitochondrial DNA Sequencing

Direct DNA sequencing was performed on those PCR products that showed altered band mobility in mtMSI analysis. Variations in mtDNA sequences between tumor and matched normal tissue were analyzed for nucleotide sequencing by Macrogen Inc, Korea.

Statistical Analysis

The statistical analysis of experimental values was performed by one-way analysis of variance (ANOVA) and, subsequently, by Fisher's exact probability and Chi-square tests using the SPSS 15.0 for Windows Program. A statistically significant difference was accepted at a *P*-value of <0.05 similar to other medical studies.

Results

MtMSI was examined in 50 uterine leiomyomas and 14 leiomyosarcomas with eight mitochondrial microsatellite markers. No mtMSI was detected in any of the leiomyomas, however, mtMSI was found in 3 (21.4%) of 14 patients with leiomyosarcomas (Fig. 1a and b). The locations of mtMSI in leiomyosarcomas were the (C)n (np303–np309) and the (CA)n (np514–np523) of the D-loop and the (A)7 (np4605–np4611) of *ND2,* respectively. The frequencies of mtMSI in leiomyomas and leiomyosarcomas were significantly different ($p < 0.01$). Repeated test results demonstrated consistent results and other markers did not reveal any instability. The outcomes of direct DNA sequencing showed significant differences in mtDNA sequences between tumor and matched normal tissues (Fig. 2). C insertion at np303, (CA) deletion at np514, and A deletion at np4605 were found in leiomyosarcomas tissue compared to paired normal tissue. The result of direct DNA sequencing indicated that mitochondrial DNA which we focused in these tumors was appropriately investigated.

Discussion

To the best of our knowledge, this article evaluates mitochondrial microsatellite instability (mtMSI) in uterine leiomyomas and leiomyosarcomas for the first time. Recent studies have focused on basic mitochondrial genetics because of the high frequency of mitochondrial mutation and of the association of mitochondria with various diseases. Instability of mtDNA is associated not only with several types of cancer, but also with neuromuscular diseases; additionally, large deletions of mtDNA are

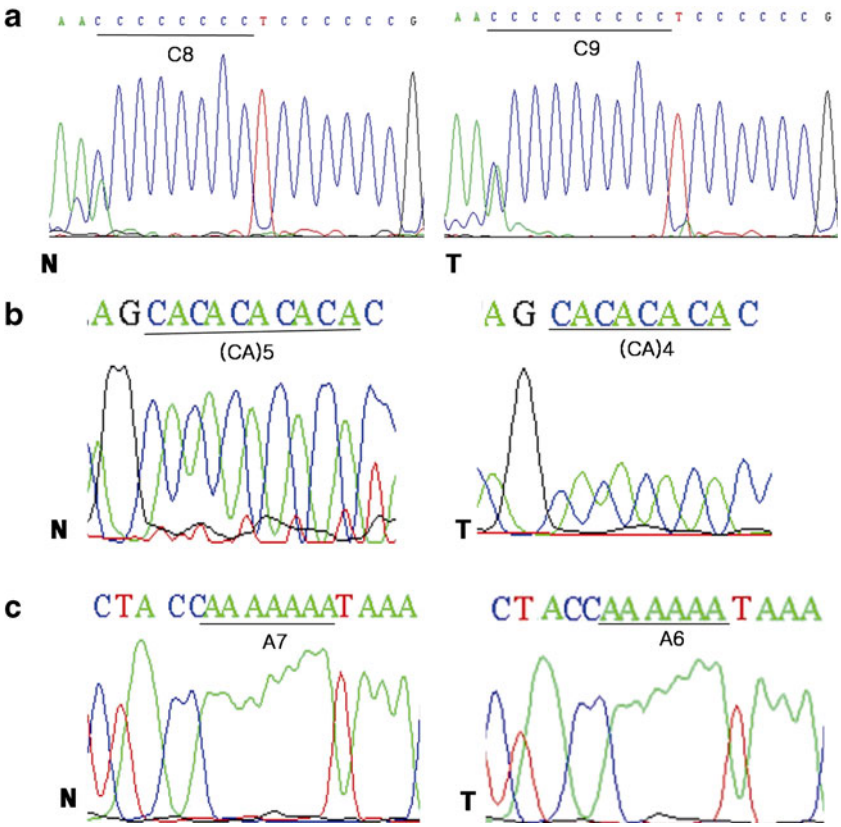

Fig. 2 The results of direct sequencing of mtDNA in leiomyosarcomas. **a** Sequencing analysis of D-loop region showed an insertion of C nucleotide (np303–np309) in tumor tissue (T) as compared with normal tissue (N). **b** Sequencing analysis of D-loop region showed a deletion of CA nucleotides (np514–np523) in tumor tissue (T) as compared with normal tissue (N). **c** Sequencing analysis of *ND2* showed a deletion of A nucleotide (np4605–np4611) in tumor tissue (T) as compared with normal tissue (N)

associated with the aging process and age-related disorders such as diabetes, deafness, and many others [15]. We examined the suggested link between uterine tumors and mtMSI, considering the high incidence and broad distribution of mtMSI in human cancers [17, 18].

Various experiments have been carried out previously to clarify the relation between uterine leiomyomas and leiomyosarcomas. Cytogenetic studies showed that leiomyosarcomas usually had both numerical and structural aberrations [25–27]. However, approximately 40% of leiomyomas only had simple cytogenetic abnormalities [4, 28–30]. According to their studies, the main aberrations in leiomyomas included a translocation between chromosomes 12 and 14, deletions and rearrangements on chromosome 1, trisomy 12, and deletions of the long arm of chromosome 7. Comparative study of the genomic hybridization (CGH) and loss of heterozygosity (LOH) have found no specific aberrations shared by leiomyomas and leiomyosarcomas [5, 10]. However, a recent study using molecular and immunohistochemical methods showed that some leiomyosarcomas may arise from a specific subset of leiomyomas [31].

To clarify the role of genetic instability in uterine leiomyomas and leiomyosarcomas, researches on nMSI have been carried out. Ethnic differences in nMSI were found in leiomyomas. nMSI was found frequently in white patients with leiomyomas, however, no leiomyomas in black patients had nMSI [12, 32]. Quade et al. [10] reported that no leiomyomas and 3 (19%) of 16 leiomyosarcomas had nMSI, however, they did not provide detailed data. Previous study reported that MSI-H (more than 30–40% of the markers examined or 2 of the five Bethesda panel markers) was not found in leiomyosarcomas except the study by Risinger et al. (20%, 1/5) [10, 12, 33].

Previous articles described that nuclear and mitochondrial genetic instabilities had different mechanisms in various cancers [16–18]. To clarify the characteristics of these tumors, we investigated mtMSI in leiomyomas and leiomyosarcomas. MtMSI was not observed in all of 50 leiomyomas. Previous studies on nMSI [10, 12] and our result on mtMSI suggest that neither nuclear nor mitochondrial genetic instabilities are relevant to tumorigenesis of uterine leiomyomas. On the other hand, mtMSI was found in 21.4% (3/14) of uterine leiomyosarcomas and their differences of mtMSI were statically significant ($p < 0.01$). It suggested a possibility of mtMSI as a marker for differential diagnosis between uterine leiomyomas and leiomyosarcomas.

The leiomyosarcomas had mtMSI in the (C)n and the (CA)n of the D-loop and the (A)7 of *ND2*. The D-loop region of mtDNA is highly polymorphic and contains hotspots for genetic instability in various types of tumor [34, 35]. Instability in this region may decrease in the copy number and alter the gene expression in the mitochondrial genome, because the D-loop is involved in the control of replication and transcription of mtDNA [36]. Our previous and present studies showed that instability of *ND2* was found in gastric cancer and uterine cancer, respectively [22]. These data indicated that genetic instability not only in the D-loop region but also in *ND2* was associated with these cancers. However, the implication of the D-loop region and *ND2* remains to be confirmed.

In the present study, we confirmed mitochondrial genetic instability of uterine leiomyomas and leiomyosarcomas for the first time. Different characteristics of mtMSI between leiomyomas and leiomyosarcomas contribute to current understanding about their relationship and mtMSI may be a useful marker for differential diagnosis. To clarify the role of mtMSI in uterine leiomyomas and uterine leiomyosarcomas, further studies with larger series of uterine leiomyomas and leiomyosarcomas are expected.

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Conflict of interest statement The authors declare that there are no conflicts of interest

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