



Title	Impairment of double-strand breaks repair and aberrant splicing of ATM and MRE11 in leukemia-lymphoma cell lines with microsatellite instability
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学 位 論 文 名	Impairment of double-strand breaks repair and aberrant splicing of <i>ATM</i> and <i>MRE11</i> in leukemia-lymphoma cell lines with microsatellite instability (マイクロサテライト不安定性を有する白血病/リンパ腫細胞株は <i>ATM</i> 、 <i>MAE11</i> のスプライシング異常、DNA 二重鎖切断修復異常がみられる)
論 文 審 査 委 員	(主査) 教 授 青 笹 克 之 (副査) 教 授 仲 野 徹 教 授 金 倉 譲

論 文 内 容 の 要 旨

[Objective]

To ensure genomic integrity, cells have evolved several mechanisms to repair DNA damages. The DNA mismatch repair (MMR) system corrects errors that might occur during DNA replication, whereas homologous recombination (HR) and non-homologous end joining (NHEJ) are involved in the repair of DNA double-strand breaks (DSB). Disruption of the MMR system, as revealed by microsatellite instability (MSI), is characterized by the accelerated accumulation of single nucleotide mutations and resultant alterations in the microsatellite DNA sequences. Ataxia telangiectasia mutated (ATM) is a nuclear protein kinase, regarded as the primary activator of DSB repair pathway. MRE11-RAD50-NBS1 (MRN) complex plays a role in DSB repair, particularly in the HR pathways. In the present study, mutations of DSB repair genes together with the presence of microsatellite instability (MSI) were examined in 50 leukemia-lymphoma cell lines.

[Methods and Results]

Cell lines. A total of 50 leukemia-lymphoma cell lines derived from Burkitt lymphoma, diffuse large B-cell lymphoma, acute lymphoblastic leukemia, adult T-cell leukemia, NK/NKT-cell lymphoma, Hodgkin lymphoma, pyothorax-associated lymphoma (PAL), acute myeloid leukemia, and chronic myeloid leukemia were used in this study. Two colorectal carcinoma cell lines known to harbor MSI were used as positive controls, whereas two lymphoblastoid cell lines were used as negative controls, together with five normal peripheral blood samples.

Detection of MSI and mutations of *ATM*, *MRE11*, *RAD50*, *NBS1* and *ATR*. Genomic DNA was extracted from the cell lines and MSI was examined by the presence of mutations in both *BAT-25* and *BAT-26*. For each cell line, fragments containing mononucleotide repeats in intervening sequence (IVS) 7 of *ATM*, IVS 4 of *MRE11*, open reading frame (ORF) of *RAD50*, ORF of *NBS1*, and ORF of *ATR* were amplified by PCR, and analyzed with GeneScan analysis software. MSI was observed in 13 of 50 leukemia-lymphoma cell lines, including 11

lymphoid and two myeloid lines. No MSI was found in five normal blood samples. Mutations of the mononucleotide repeats in IVS 7 of *ATM* were found in 9 lines, in 8 MSI-positive lines and one MSI-negative cell line. Mutations of mononucleotide repeats in IVS 4 of *MRE11* were found in 6 lines : in 5 MSI-positive lines and one MSI-negative line. No mutations were observed in five normal blood samples. The frequency of *ATM* and *MRE11* mutation was significantly higher in MSI-positive than MSI-negative lines ($P < 0.01$). Mutation of mononucleotide repeat in the ORF of *RAD50* was found in only one cell line. No mutation was found in the mononucleotide repeats in the ORF of *NBS1* or in the ORF of *ATR*.

Detection of aberrant splicing in *ATM* (497del22) and *MRE11* (315del88). RNA was extracted and reverse transcribed from the cell lines. PCR amplification of the fragments of exon 8 of *ATM*, and exon 5 of *MRE11* were performed and the relative intensity (RI) of the wild-type and aberrant transcripts were measured. Sequencing was carried out by using a DNA sequencing kit. The intronic mononucleotide repeat mutations generated aberrant splicing in the next exon of *ATM* (497del22) and *MRE11* (315del88). The RI was stronger in 5 MSI-positive lines harboring *ATM* intronic mononucleotide mutations of 2 bp or more and in 4 MSI-positive lines with 1-bp *MRE11* intronic mononucleotide mutations, as compared to MSI-positive lines without the mutation.

Real-time PCR for assessment of expression levels of DSB repair genes. Expression levels of *ATM*, *MRE11*, *RAD50*, *NBS1* and *ATR* were analyzed using the TaqMan Gene Expression Assays™. The expression levels of *ATM* and *MRE11* in 13 MSI-positive lines were significantly higher than those in 37 MSI-negative lines ($P < 0.05$). No significant differences were found in the expression levels of *RAD50*, *NBS1* and *ATR* between MSI-positive and MSI-negative lines.

DNA DSB repair assays. To evaluate whether defects in MMR function may correlate with impairment of the DSB repair ability, DSB repair in each cell line were examined by using pulsed-field gel electrophoresis. DSB were quantified as the fraction of DNA in the compression zones relative to that in the wells. More than 80% of the DSB in IB-4 were repaired within 2 h of ionizing radiation exposure. Abrogation of DSB repair, as shown by unrepaired DSB in 8 MSI-positive lines was significantly higher than those in 14 MSI-negative lines ($P < 0.05$).

[Conclusion]

In the present study, we provide evidence that impairment of the MMR system generates aberrant transcripts in DSB repair genes, especially *ATM* and *MRE11* but not *RAD50*, *NBS1* or *ATR*, in hematolymphoid malignancies. This might result in inactivation of the DSB repair system, thus inducing a harmful chain reaction such as an acceleration of genome instability and accumulation of genetic damage.

論文審査の結果の要旨

DNA 修復遺伝子に異常がある場合、腫瘍発症の危険性が高まる。マイクロサテライト不安定性 (MSI) を有する大腸がんや他の上皮性悪性腫瘍には DNA 二重鎖切断修復遺伝子の変異が報告されているが、血液系の腫瘍についてはまだわかっていない。本研究は 50 種類のリンパ腫－白血病細胞株を用いて、DNA 二重鎖切断修復遺伝子変異と MSI を検討したものである。その結果、1. 50 種類のリンパ腫－白血病細胞株の中で、マイクロサテライト不安定性は 13 に検出された。*ATM* と *MAE11* の intronic mononucleotide repeat 変異はマイクロサテライト不安定性を示す細胞株で頻度が高い。2. この遺伝子変異により、スプライシング異常が起きる。3. *ATM* と *MRE11* の発現レベルはマイクロサテライト不安定性を有する細胞では高い。4. マイクロサテライト不安定を有する細胞株では DNA 修復が遅れる。

以上の研究は DNA ミスマッチ修復の異常により *ATM*、*MAE11*、の intronic mononucleotide repeat の短縮が起き、その結果スプライシング異常、相同組み換え異常を生じる。この異常の蓄積によりリンパ腫が発症することを示唆している。DNA ミスマッチ修復と相同組換え修復の異常がリンパ腫－白血病の発症に関与することを示唆した本研究は博士 (医学) の学位授与に値するものと認める。