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学位論文題目	Age Dependent Epigenetic Regulation of TET3 (TET3 の年齢依存エピジェネティック制御)

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## 論文の内容の要旨

### (目的)

Over the past decade, epigenetic modification has been considered to play fundamental roles in cell regulatory processes and human diseases. Hydroxymethylcytosine (hmC) is a modified form of cytosine, which is enzymatically converted from methylcytosine (mC) by tet methylcytosine dioxygenase (TET) proteins (TET 1-3). While mC is a well-known epigenetic mark, little is known about its distribution and function. Although changes in methylation with aging have been variably described, very little information is available on the relationship between hmC and aging. We found that genomic hmC content was inversely correlated with age in T cells in human and mouse. The mechanism of hmC alteration with age, however, remains to be elucidated.

He conducted this study to clarify mechanisms of the age-dependent decrease of the hmC content and responsible TET proteins.

### (対象と方法)

1.T cell preparation: Human CD3-positive cells were obtained from peripheral blood of 53 healthy volunteers (20-83 years). Mouse CD3-positive cells were prepared from spleen of 10 6-10-week-old and 10 1.5-2 year-old C57BL/6 mice.

2.Analysis of hmC and mC: Genomic region containing hmC was enriched by the hydroxymethylated DNA immunoprecipitation (hMeDIP) method, and analyzed by real time PCR. Genomic region containing mC was

enriched by the Methylminer Methylated DNA Enrichment Kit, and analyzed by real time PCR.

3.Northern blot analysis: Northern blot analysis was used to detect the new exon and the various sizes of *TET3* mRNA in different cell types.

4.Analysis of the newly identified age-dependent hmC region: The newly identified age-dependent hmC region (ADHR) of mouse was amplified by PCR and inserted in the pGL3 basic vector (ADHR-pGL3basic). ADHR-pGL3basic was methylated by a CpG methyltransferase. 293T cells were co-transfected with methylated-ADHR-pGL3basic and mouse *TET3*-pcDNA4. hmC and mC analysis were performed by hMeDIP kit and Methylminer Methylated DNA Enrichment Kit, respectively, followed by real-time PCR.

(結果)

1.Genomic hmC levels and expression levels of TET family genes.

He found that the mRNA expression levels of *TET1* and *TET3* were significantly decreased with age in human T cells, while those of *TET2* were not changed with age. Because the preliminary results indicated that the genomic hmC content was decreased with age, he directly compared the genomic hmC content with the mRNA expression levels of *TET1*, *TET2*, and *TET3*, and found that the mRNA expression levels of *TET3* were significantly correlated with the hmC content, while those of *TET1* and *TET2* were not.

He then examined mRNA expression levels of mouse T cells. As he expected, the mRNA expression levels of *TET1* and *TET3* in aged mouse T cells were significantly lower than those in young mouse T cells, while those of *TET2* were not changed with age

2.Mechanism of age-dependent regulation of *TET3* expression.

He checked the hmC level in regions which are located on CpG island and at the upstream or gene body of mouse *TET1* and *TET3*. He found that a region spanning about 300 bp at the upstream of *TET3* showed higher hmC levels in young mouse T cells than those in aged mouse T cells. This region was located about 13 kb from the first coding exon of mouse *TET3*. In *TET1*, hmC levels were not different between young and aged mouse T cells.

In human, he also found a region with 79% homology with that of mouse located approximately 60 kb upstream of the first coding exon of human *TET3*. The hmC levels in this region were decreased with age. In contrast, the mC levels in this region were not different between young and aged human T cells.

3.Discovery of a new exon at the upstream of mouse and human *TET3*

The database of mRNA sequencing indicated that the human ADHR was overlapped with the 700 bp transcribed region, which he designated exon 0. By RT-PCR analysis using primers made from exon 0 to exon 1, the PCR product with the expected size, with the 13-kb deduced intron spliced out, was visualized, demonstrating that exon 0 was indeed expressed as a part of mRNA. The expression levels of exon 0-containing mRNA was higher in human T cells prepared from two volunteers at 22 years old and 29 years old than those prepared from volunteers at 62 years old and 69 years old, and than several human cell lines (Jurkat, 293T, HeLa).

He then examined the mRNA species by Northern blot for two mouse cell lines, NIH3T3 fibroblast cell line and EL4 lymphoblastic lymphoma cell line, using the probe corresponding to a part of the mouse version of exon 0 and exon 3. A single mRNA species corresponding to an expected size of 11.6 kb was detected with the exon 0 probe in both cell lines, showing a higher expression level in EL4 than in NIH3T3. When the exon 3 probe was used, the size of main mRNA species expressed in NIH3T3 appeared to be shorter than that expressed in EL4,

possibly corresponding to the mRNA species with (EL4) and without (NIH3T3) exon 0.

#### 4.Experimental conversion of mC to hmC in mouse ADHR by TET3

In 293T cells co-transfected with the methylated-ADHR-pGL3basic and mouseTET3-pcDNA4, he found that the hmC level in ADHR was increased compared to the controls, indicating that a part of mC in the ADHR was converted to hmC by exogenously expressed mouse TET3. In contrast, the change of mC level in ADHR was undetectable, indicating that only a small part of mC was converted to hmC.

(考察)

He demonstrated a dramatic age-dependent decrease in the genomic hmC content in both human and mouse T cells. This could be caused by the gradual decrease in the *TET3* expression levels during aging. The decrease in the genomic hmC content might play a role in alteration of the T cell function in elderly people.

### 審査の結果の要旨

(批評)

本研究では、ヒドロキシメチールシトシン (hmC) が加齢に伴い減少する分子機構を明らかにすることを目的とした。彼は、ヒトおよびマウスの T 細胞において、ゲノムの hmC が年齢依存的に減少することを見いだした。さらに、mC から hmC に変換する TET ファミリー蛋白 (TET1-3) の TET3mRNA 発現が hmC 量と関連していること、加齢とともに減少することなどを明らかにした。以上の結果から、加齢 T 細胞において、TET3 発現がエピジェネティックに hmC 発現を制御している可能性を明らかにした。本研究成果はすでに国際雑誌に掲載され、世界的に高い評価を得ている。

平成 27 年 1 月 7 日、学位論文審査委員会において、審査委員全員出席のもと論文について説明を求め、関連事項について質疑応答を行い、最終試験を行った。その結果、審査委員全員が合格と判定した。

よって、著者は博士 (医学) の学位を受けるのに十分な資格を有するものと認める。