Thesis abstract

Age Dependent Epigenetic Regulation of TET3
(TET3 の年齢依存性エピジェネティック制御)

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Background:

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 1-3 proteins. 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.

Purpose:

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

Methods:

[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 form spleen of 10 6-10-week-old and 10 1.5-2 year-old C57BL/6 mice.

[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.

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

[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 was performed by hMeDIP kit and Methylminer Methylated DNA Enrichment Kit, respectively, followed by real-time PCR.

Results:

Genomic hmC levels and expression levels of TET family genes.

We 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, we 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.

We then examined mRNA expression levels of mouse T cells. As we 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

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

We checked the hmC level in regions which are located on CpG island and on the upstream or gene body of mouse *TET1* and *TET3*. We 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, we 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.

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 we 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).

We 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.

Experimental conversion of mC to hmC in mouse ADHR by TET3

In 293T cells co-transfected with the methylated-ADHR-pGL3basic and mouseTET3-pcDNA4, we 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.

Discussion:

We 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.

We discovered an age-dependent hmC region (ADHR), in which hmC level was naturally decreased in aging process. There are 2 transcription start sites for *TET3* gene,

one starts from exon 0, the other from exon 1. The usage of these alternative transcription start sites may be important. In young T cells, because of the high hmC level in ADHR, *TET3* transcription may begin with exon 0. This could maintain the *TET3* mRNA level and facilitate the mC to hmC conversion in ADHR, providing the positive feedback system. This auto-regulation might be decreased in aged T cells because of the decreased hmC level in ADHR.

Conclusion:

We demonstrated a potential mechanism of age-dependent epigenetic regulation through positive feedback of *TET3* expression and mC to hmC conversion in its own gene body. This might represent an important physiologic system for alteration of T-cell function, as well as susceptibility of T cells to tumorigenesis, after aging.