Splice variant forms of the TCRζ mRNA in SLE patients

The Second Department of Internal Medicine, Saitama Medical Center, Saitama Medical School, Kawagoe, Saitama, JAPAN
Research center for Genomic Medicine, Saitama Medical School, Hidaka, Saitama, JAPAN
Kensei TSUZAKA

It has been reported that the reduction in the tyrosine phosphorylation and decreased expression of TCRζ (ζ) protein play crucial roles in the pathogenesis of systemic lupus erythematosus (SLE). We have previously reported some SLE patients exhibited mutations of ζmRNA open reading frame including a splice variant of the ζmRNA lacking the 36-bp exon7 (ζmRNA/exon7−), accompanied with the down-regulation of ζ protein.1-2 We have also reported predominant expression of the ζmRNA with alternatively spliced 3′-untranslated region (3′ UTR) (ζmRNA/as–3′ UTR) and decreased expression of ζmRNA with the wild form 3′ UTR (ζmRNA/w–3′ UTR) was related to the decreased expression of ζ protein.3 By using a retroviral expression system, AS3′ UTR mutants (MA5.8 cells deficient in ζ protein that have been transfected with ζmRNA/as–3′ UTR) and EX7− mutants (MA5.8 cells transfected with ζmRNA/exon7−) exhibited a reduction in the expression of TCR/CD3 complex including ζ on their cell surface as well as the decreased production of IL−2 after the stimulation with anti-CD3 antibody, compared with that in WT3′ UTR mutants (MA5.8 cells transfected with ζmRNA/w–3′ UTR).4,5 Furthermore, the real-time PCR analyses showed that the half-lives of ζmRNA/as–3′ UTR in AS3′ UTR mutants and ζmRNA/exon7− in EX7− mutants were much shorter than that of ζmRNA/w–3′ UTR in WT3′ UTR mutants, demonstrating the lower stability of these two forms of the splice variants of ζmRNA.4,5 To investigate whether SLE T cells accompanied with diminished expression of TCR/CD3 complex including ζ exhibit differential transcription patterns that are indicative of SLE, we compared the gene expression profile by the DNA microarray analysis. Total RNA was collected 24 hours after stimulating the MA5.8 mutants with anti-CD3 antibody and reverse transcribed. Compared was differential gene expression between AS3′ UTR and WT, or EX7− and WT mutants. Expression of the identified genes was confirmed by the real-time PCR. Compared with the WT mutants, eight and ten of 8,747 genes were up-regulated, and 53 and 34 genes were down-regulated in AS3′ UTR and EX7− mutants, respectively, by DNA microarray analysis. Real-time PCR revealed that the relative mRNA expression of some adhesion molecules as well as granzyme A, selenium binding protein 2, solute carrier family 4, and lipocalin2 were up-regulated (defined as more than 3-folds) commonly both in AS3′ UTR and EX7− compared with WT. On the other hand, those of several cytokines including IL−2, IL−15, IL−18, and TGF−β2 were commonly decreased (defined as less than 1/5). From these observations, down-regulation of the TCR/CD3 complex including ζ and the accompanying up-regulated those gene expression including adhesion molecules may help to better understand the mechanism underlying T-cell dysfunction and pathogenesis of SLE.

Reference: