[課程―２]

審査の結果の要旨

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This thesis sought to detect causative mutations in patients with Ellis-van Creveld (EVC) syndrome and to evaluate functional role of mutated *EFCAB7* on regulation in EVC protein-complex and Hedgehog (Hh) signaling.

Seven unrelated EVC families and 100 healthy Vietnamese controls were recruited at Children's Hospital 2, Ho Chi Minh City, Vietnam from September 2015. Genetic analysis was conducted by analyzing entire coding region, exon-intron boundaries of *EVC, EVC2, IQCE, EFCAB7* and *WDR35* by Sanger sequencing. For patients and their relatives with novel mutations, quantitative RT-PCR was done to evaluate the mRNA expression of *EVC* or *EVC2*. To detect heterozygous deletion of the causative gene in a possible carrier, who had no mutations in Sanger sequencing, single nucleotide polymorphism (SNP) array analysis was performed. SNP array result was confirmed by long range PCR and direct sequencing.

To assess the functional role of novel mutated EFCAB7, ECH1 391H (one of the domains in EFCAB7 protein) was tagged with FLAG. The binding partner of EFCAB7, WEYER, which consists of 87 amino acids located in C-terminal of EVC2, was fused with GST. The binding capacity of WEYER-ECH1 was assessed after pull-down assay. Next, full-length *EFCAB7* 1171C/wild-type was transfected into HEK 293T cells. *EVC, EVC2* and *IQCE* mRNA expression levels in *EFCAB7* 1171C and wild-type transfected cells were examined. Finally, co-transfection of *EFCAB7* 1171C and shRNA *EVC* plasmids into HEK 293T cells was established, GLI3R/GLI3FL ratio after stimulation was calculated by Western Blotting.

This study found the followings:

1. Compound heterozygous mutations of *EVC* (c.1715C>G-p.S572X, 16.4kb partial deletion-del IVS8-11) and *EVC2* (c.769G>T-p.E177X, c.2476C>T-p.R826X) genes were detected in Vietnamese families diagnosed with EVC. In addition, heterozygous *EFCAB7* c.1171T>C (p.Y391H) was detected in one positive-*EVC* family. A proband and his father showed atypical cardiac defect, short chordae of atrioventricular valves, whereas the sibling in the same family with only *EVC* mutations had common phenotypes.
2. Motivated by these results, functional role of mutated *EFCAB7* in EVC complex was examined. The binding amount of FLAG-EFCAB7 domain 391H to GST-WEYER of EVC2 was three times higher than that of wild-type ($P < 0.001$). The relative *EVC* mRNA expression level in *EFCAB7 1171C* transfected cells was significantly higher than in wild-type cells ($P=0.004$). Transfection of shRNA-*EVC* together with wild-type *EFCAB7* severely perturbed the expression of *EVC2*. Whereas *EVC2* expression was not altered in mutated

EFCAB7 cells, co-transfection of shRNA-*EVC* resulted in around 20% level of *EVC2* expression compared to control. It suggested that mutated *EFCAB7* 1171C might increase *EVC2* mRNA expression in *EVC* knockdown cells. Taken together, both *EFCAB7* and *EVC* may be required for normal *EVC2* transcription.

3. Functional role of mutated *EFCAB7* via GLI3 processing was accessed. The ratio of GLI3R/GLI3FL in co-transfected cells (shRNA-*EVC* and mutated *EFCAB7*) was significantly different from that in shRNA-*EVC* and wild-type *EFCAB7* also imply that mutated EFCAB7 modulate Hh signaling together with EVC depletion.

In conclusion, comprehensive analysis was conducted in this study to understand the genetic background for EVC. The study provided a detailed genetic landscape of EVC in Vietnam populations for the first time. The results from Sanger sequencing and SNP array in this study combined with previous findings indicate that *EFCAB7* gene is a genetic modifier of Ellis-van Creveld syndrome. *EFCAB7* c.1171T>C (p.Y391H) mutation was firstly co-detected in positive-*EVC* cases, which modified EFCAB7-EVC2 interaction, transcription levels of EVC-complex molecules and GLI3 activation in Hh signaling. The data generated in this study provide a solid basis for future in vivo study on *EFCAB7* c.1171T>C mutation. Considering the importance and the novelty of the work, it is worthy of the award of Doctor of Health Science to the candidate.