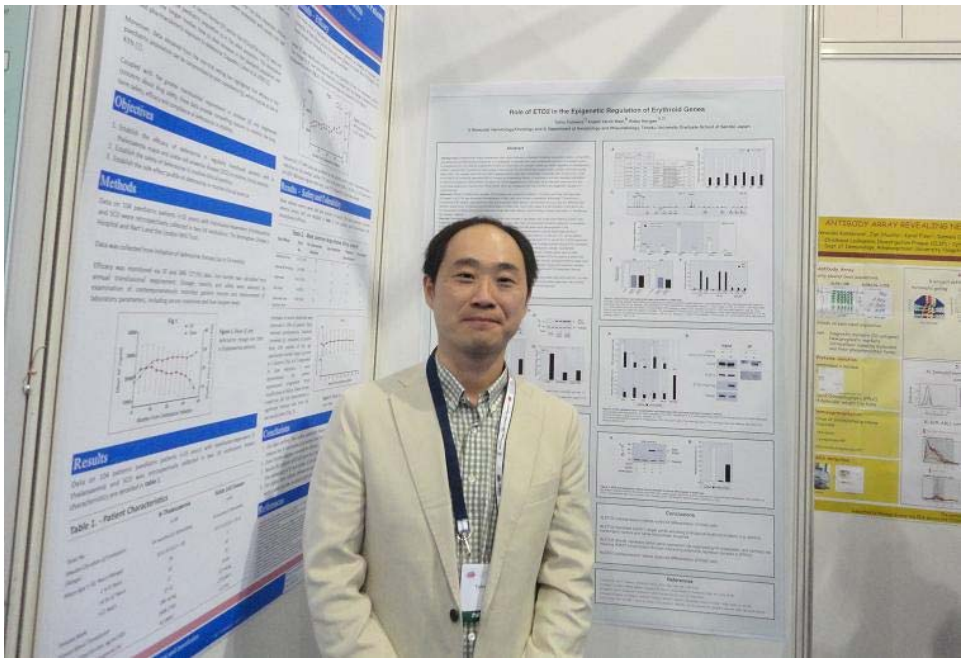
会/受賞報告書

16th EHA Congress Travel Award
for JSH Young Hematologists

血液分子治療学寄附講座　助教
藤原 亨



今回、日本血液学会より「16th EHA Congress Travel Award for JSH Young Hematologists」を頂き、大変光栄に思います。ご指導・ご推薦を頂きました張替秀郎先生に心から感謝申し上げます。

今回、私は転写抑制因子として知られているETO2を介した、赤芽球系遺伝子の発現制御機構に関する研究成果を、2011年6月にロンドンにて開催された第16回欧州血液学会で発表いたしました。まだ本テーマに取り組みはじめて日は浅いのですが、今回の発表を通じて足りないものは何かを確認する良い機会であったと思います。これからも本賞に恥じぬよう、一層の努力をしなければならないと感じております。

受賞研究：

Role of ETO2 in the epigenetic regulation of erythroid genes

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抄録：

(Background) Developmental control mechanisms often utilize multimeric complexes containing transcription factors, coregulators, and additional non-DNA binding components. It is challenging to ascertain how such components contribute to complex function at endogenous loci. We recently analyzed the function of components of a complex containing master regulators of hematopoiesis (GATA-1 and Scl/TAL1) and the non-DNA binding components ETO2, the LIM domain protein LMO2, and the chromatin looping factor LDB1. We revealed that ETO2 and LMO2 regulate distinct target gene ensembles in erythroid cells. Furthermore, it was found that ETO2 commonly represses GATA-1 function via suppressing histone H3 acetylation, and also regulates methylation of histone H3 at lysine 27 (H3-trimeK27) at select loci, which suggested that ETO2 might be an important determinant of the erythroblast epigenome (Fujiwara et al. PNAS. 2010). Here, we investigated the role of ETO2 in the epigenetic regulation of erythroid genes.

(Methods) *CBFA2T3* mRNA (which encodes ETO2 protein) was cloned into pcDNA3.1 (Clontech) and Flexi HaloTag vector (Promega), and ETO2 was transiently overexpressed in K562 cells using Amaxa nucleofection technology™ (Amaxa Inc.). Quantitative ChIP analysis was performed using anti-acetylated H3K9 (abcam), anti-trimethyl H3 (Lys 9 and 27) (Millipore) and anti-Myc (Santa Cruz). To induce erythroid differentiation of K562 cells, hemin was treated at a concentration of 30 uM for 24h. For transcription profiling, human whole expression array was used (Agilent). Gene Ontology analysis was based on DAVID software (http://david.abcc.ncifcrf.gov/home.jsp).

(Results) Overexpression of ETO2 in hemin-treated K562 cells resulted in decreased ratio of benzidine-staining positive cells, suggesting that ETO2 retards the erythroid differentiation of K562 cells. Next, we conducted microarray analysis to characterize ETO2 target gene ensemble in K562 cells. The analysis demonstrated that 598 genes were downregulated in the ETO2-overexpressed cells (> 2 fold). To test what percentages of ETO2-repressed genes could be direct target genes of GATA-1 or GATA-2 in K562 cells, we merged the microarray results with ChIP-seq profile (n= 5,749 and n=21,167 for GATA-1 and GATA-2 ChIP-seq, respectively) (Fujiwara et al. Mol Cell. 2009), and demonstrated that 23.1% and 40.5% of ETO2-repressed genes contained significant GATA-1 and GATA-2 peaks in their loci, respectively. Gene Ontology analysis among ETO2-repressed genes revealed significant enrichment of genes related to “oxygen transporter” and “hemoglobin complex” (p=0.00128). Overexpression of ETO2 in K562 cells resulted in the significant decrease in the expression of globin genes such as *HBG*, *HBB*, *HBE1*, *HBA*, *HBM* and *HBZ* by quantitative RT-PCR. Quantitative ChIP analysis revealed ETO2 occupancy at globin HS2. Furthermore, the overexpression significantly increased H3-trimeK27 and reduced acetylated H3K9 at γ-globin promoter. Co-immunoprecipitation analysis revealed the interaction between ETO2 and EZH2, a member of polycomb repressor complex. We are currently analyzing the mechanism of ETO2-dependent transcriptional repression and how ETO2-dependent histone marks are established in erythroid cells.

(Conclusion) In conjunction with the evidence that ETO2 binds histone deacetylases and associates with GATA-Scl/TAL1 complex that binds epigenetic modifiers, ETO2 appears to have important roles in establishing the erythroblast epigenome. We consider this is important from the perspective of elucidating mechanisms of hematopoiesis and leukemogenesis.