論文の内容の要旨

論文題目　Application of a murine stem cell system to analyzing impaired T cell development from BCR-ABL+ hematopoietic stem cell

（マウス幹細胞システムを用いたBCR-ABL陽性造血幹細胞のT細胞分化障害の解析）

氏名　チャンダ ビディシャ

Chanda Bidisha

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized by the presence of the Philadelphia (Ph) chromosome, formally termed as der(22)t(9;22)(q34;q11), in which the ABL gene at 9q34 is juxtaposed to the 5.8-kb limited region of the BCR gene at 22q11, resulting in the generation of 210 kD BCR-ABL oncoprotein, p210. In rare cases of CML, two other breakpoint regions of the BCR gene are fused to the ABL gene and produce 190 and 230 kD chimeric proteins, respectively. This active tyrosine kinase is responsible for the clonal amplification of leukemia cells by inhibition of apoptosis and stimulation of cell cycling.CML is believed to originate from a hematopoietic stem cell carrying the BCR-ABL fusion gene. The concept that leukemic cells in CML originate from a deregulated stem cell compartment was at first indicated by the demonstration of the Ph chromosome in erythroid and myeloid cells as well as megakaryocytes and later also in B-lymphoblastoid cells. Furthermore, evidence that some of the BCR-ABL+ cells can also differentiate into endothelial cells was reported, suggesting the

hemangioblast origin of the leukemic clone. In spite of the putative stem cell origin and the competence for differentiation toward mature B cells, there is a longstanding consensus that CML never involves the T cell lineage at least in chronic phase. One possible explanation is that the BCR-ABL oncoprotein may be harmful to differentiating T cell progenitors, as has been suggested to be the case in fibroblasts

To gain insight into the reason why the BCR-ABL+ leukemic clone has a very limited ability to differentiate toward mature T cells, I applied the conditionally-active BCR-ABL kinase to the *in vitro* model system of T cell differentiation from murine iPSCs and HSCs. We used induced pluripotent stem cell (iPSCs) in our in vitro study because the concept leukemic clones have been originated from hemangioblast, beyond the hematopoietic stem cell level.

C57BL/6 MEFs were reprogrammed using a polycistronic lenti viral Tet-On vector encoding human Oct4, Sox2 and Klf4, which were tandemly linked via porcine teschovirus-1 2A peptides, together with another lentiviral vector expressing rtTA driven by the EF-1α promoter. Almost all the vector sequences including the transgenes were deleted by adenovirus-mediated transduction of Cre-recombinase after derivation of iPSCs, and only remnant 600-bp LTRs containing a single loxP site remained in the genome. A clone of MEF-iPSCs were retrovirally transduced with p190ΔccER, a ligand-controllable p190-estrogen receptor fusion protein, whose tyrosine kinase activity absolutely depends on 4-hydroxytamoxyfen (4-HT).For T cell lineage differentiation, p190ΔccER-MEF-iPSCs were recovered from a feeder-free culture supplemented with LIF and plated onto a sub-confluent OP9-DL1 monolayer in the presence of 5ng/ml Flt3 ligand and 1ng/ml IL7 with or without 0.5 μM 4-HT.After 3 weeks of culture, iPSC-derived blood cells were collected and subjected to FACS analysis for their lineage confirmation. About 70% of lymphocyte-like cells from the 4-HT (-) culture expressed CD3, but only 20% of counterparts from the 4-HT(+) culture expressed CD3, suggesting impaired T cell

development by BCR-ABL.

To define the stages of impairment in T cell development more specifically we did the same study by using murine hematopoietic stem cell. Next C57BL/6 mouse bone marrow was collected after treatment intravenous of 5FU injection and bone marrow cells were sorted by lin-c-kit+Sca1+ mouse antibody KSL cells were transduced with same p190ΔccER retroviral vector or CS-CDF- tetO-BCR-ABL and CS-EF-rtTA2AEGFP lenti viral vector and induced them into T cells differentiation on sub-confluent OP9-DL1 monolayer in the presence of human Flt3 ligand and mouse IL7 with or without 0.5 µM 4-HT or doxycycline 4µg/ml. After 2 weeks of culture FACS analysis was done and it revealed that 95% 4-HT (-) cells were expressed CD3+TCRβ double positive but only 30% cells were double positive in case of 4HT (+) cultured cells. Next to specify the stages of T cells developmental arrest by BCR-ABL 4HT (+) and 4-HT (-) cultured cells were labeled by CD117, CD25 and CD44 anti mouse antibody and analyzed by FACS. Approximately 90% of 4-HT (-) cultured cells were in DN2 ($CD44^+CD25^+CD117^+$) stage where in 4HT (+) cultured cells were only 50% in DN2 ($CD44^+CD25^+CD117^+$) stage rest of 50% cells in DN1 ($CD44^+CD25^-CD117^+$) stage. BCR-ABL positive cells constituted the two populations according to the expression level of EGFP, and the $EGFP^{low}$ population arrested at the DN1 ($CD44^+CD25^-CD117^+$) stage, while $EGFP^{high}$ population. BCR-ABL impairs T cell development possibly through interfering of IL-7Receptor alpha down regulation.

It appears that BCR-ABL disturbs transition from the DN1 ($c\text{-}Kit^+CD44^+CD25^-$) to the DN2 ($c\text{-}Kit^+CD44^+CD25^+$) stage of T cell differentiation from iPSCs and HSCs. Intriguingly, BCR-ABL positive cells constituted the two populations according to the expression level of EGFP, and the $EGFP^{low}$ population arrested at the DN1 stage, while $EGFP^{high}$ population

proceeded to the DN2 stage. Assuming that the EGFP intensity reflects the expression level of BCR-ABL directly or inversely, absolute BCR-ABL kinase activity may determine the T cell fate. Although the precise mechanism of impaired T cell development by BCR-ABL is to be elucidated, it is likely that BCR-ABL down-regulates the Notch and/or IL7 signal, considering that the majority of BCR-ABL+ progenitor cells arrested at the DN1 stage. Especially, one possible speculation is that BCR-ABL may perturb the IL7 signaling pathway at any point, since IL7 signal is also transmitted through tyrosine kinase cascades including the Jak-Stats. Furthermore, it should be noted that DN1 thymocytes are still capable of transdifferentiation toward myeloid cells in murine hematopoietic system. Direct reprogramming of somatic cells to pluripotent stem cells is markedly valuable for pathophysiological investigation as well as therapeutic application in the near future. Widely used reprogramming vectors were retrovirus- or lentivirus-based vectors, which integrated into the host genome in multiple copies, resulting in the potential risk of insertional mutagenesis of endogenous genes. For the above investigation I developed and tested a single, polycistronic lentiviral vector that can be deleted after reprogramming to pluripotency. Additionally, I applied the Tet-on system to expression of reprogramming factors as another safe-guard, instead of constitutive promoters. Unexpected low efficiency of reprogramming is probably due to the low titer of viral vectors produced in small scale and unpurified.