論文の内容の要旨

論文題目　Investigation of a causal gene of familial myelodysplastic syndromes
（家族性骨髄異形成症候群の原因遺伝子の探索）

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MDS is mostly a sporadic diseases, however, familial cases of MDS/AML have been discovered recently. Although genomic mutational spectrum in sporadic MDS is gradually uncovered, little is known about the causal genes and the epidemiology of familial MDS/AML due to its rarity. In this study, I conducted a nationwide survey of familial MDS/AML, and a next-generation sequencing analysis using the samples obtained from the familial MDS/AML patients to elucidate the full picture including the epidemiology, the genomic mutational landscape, and the molecular pathogenesis.

A pedigree with four MDS patients was analyzed. Firstly, to investigate a causal gene of this pedigree, Sanger sequence was performed using the peripheral blood mononuclear cells obtained from the patient of the pedigree. Although thirty-six genes previously identified in sporadic and familial MDS patients were analyzed, no genomic mutations were observed in their hot-spots and the coding regions. Thus, it was hypothesized that this pedigree might have an unknown causal gene of familial MDS/AML. In order to identify the patient specific germ-line genomic mutations of the pedigree, whole exome sequence was performed with the samples obtained from two patients and two non-MDS healthy family members as control. As a result, twelve candidate genomic mutations were identified after eliminating reported and in-house SNPs, and the mutations shared with control samples. To investigate the recurrence of the candidate genomic mutations in familial MDS/AML, I performed a Japanese nationwide survey of familial MDS/AML. I obtained the clinical information of 24 patients in 16 pedigrees, and the clinical samples of seven patients from six pedigrees. In the collected 24 patients, the mean and the median age of the initial diagnosis were 49.6 and 58 years, respectively. WHO classification revealed that RAEB-2 was the largest group (25%), followed by AML (17%), RA (17%), and RCMD (17%). Chromosomal analysis showed that half of the patients had normal karyotypes. Subsequently, twelve single base mutations were analyzed by Sanger sequencing, however, these mutations were not identified in all the obtained samples of familial MDS/AML. Of the twelve candidate genes, I searched a gene with a similar function of the driver genes of

sporadic MDS by a literature survey. Among the candidates, I selected geneX for further functional assays. Subsequently, I checked the *geneX* genomic mutation status in sporadic MDS samples by Sanger sequence. Whole *geneX* coding regions of 40 samples were sequenced and a novel *geneX* mutation was identified in one sample (1/40 = 2.5%). Importantly, a public dataset (GSE9476) which included the expression profile of normal hematopoietic cells from 38 healthy participants and leukemic blast cells from 26 AML patients demonstrated that *geneX* expression was significantly lower in AML samples ($p<0.01$). Subsequently, I evaluated in vitro colony-formation capacity using *geneX*-knocked down 5-fluorouracil (5FU)-primed C57BL/6 mouse bone marrow (BM) cells. Strikingly, *geneX*-silenced BM cells showed enhanced in vitro colony-replating capacity. I also found c-kit, a hematopoietic stem/ progenitor cell marker, positive cells were increased in *geneX* shRNA-expressing colonies after the first round of colony-forming cell assay.

Although a previous report showed that *geneX* expression level was very low in one leukemia cell line, little is known about the relationships between X and hematological malignancies. X is a transcription factor, an E3-ubiquitin ligase, and X also regulates the expressions of the cohesin complex-associated genes that frequently mutated in sporadic MDS/AML. In this study, 5-FU primed murine *geneX*-knocked down cells survived longer than control cells. Considering that *geneX* expression level was low in leukemia cell line and leukemia samples, and loss-of-*geneX* increased replating capacity, X might behave as a tumor suppressor rather than an oncogene in hematological malignancies. In conclusion, I performed the first nationwide survey of familial MDS/AML, and elucidated the characteristics of familial MDS/AML patients in Japan. I also identified twelve candidate genes of familial MDS/AML by whole exome sequencing analysis. Of these, loss-of-*geneX* enhanced in vitro colony replating capacity. In addition, a novel *geneX* mutation was detected in sporadic MDS sample. This study could be an important first step for the understanding of familial MDS/AML.