Discussion

Here, we report establishment and characterization of a novel Ph+ chronic myeloid leukemia cell line, TCC-S which was derived from a patient with Ph+ CML in BC. It grew well with the doubling time of 27.96 ± 0.97 hours, a similar time to other CML cell lines[10),11)].

More than 40 Ph+ CML cell lines have been established so far. It is known that the majority of P210 *BCR/ABL*-expressing Ph+ CML cell lines simultaneously express P190 *BCR/ABL* transcript, although the expression level of P190 *BCR/ABL* is low[12)]. The mechanism of this co-expression is considered to be due to the alternative splicing from the same pre-mRNA of *BCR/ABL*, not due to the existence of two clones, one which has the breakpoint within the major *BCR* and the other within the minor *BCR* in the *BCR* gene. In TCC-S cells, co-expression of P210 and P190 *BCR/ABL* transcripts was also observed, although the expression level of P190 *BCR/ABL* was much lower than that of P210 *BCR/ABL* transcript. Since we did not observe the

Table 3: Immuno-phenotype of the patient's bone marrow cells and TCC-S cells

Markers	Patient's bone marrow cells (%)	TCC-S cells (%)		
	Oct 2, 1991	Nov 29, 1991	Feb 27, 1992	Feb 13, 2004
CD2	1.9	1.5	8.3	1.7
CD3	1.1	1.1	9.5	2.1
CD4	16.9	44.4	21.2	77.5
CD5	2.6	2.6	7.1	2.0
CD7	7.0	3.8	15.5	1.7
CD8	3.2	3.2	10.4	1.6
CD10	0.1	0.1	10.4	1.5
CD13	16.7	55.2	35.3	63.8
CD14	6.2	6.2	10.3	2.0
CD19	0.1	0.1	11.2	2.8
CD20	0.2	0.2	10.2	1.5
CD33	51.6	57.4	73.6	99.7
CD34			14.4	1.3
HLA-DR	39.7	0.4	11.1	1.2
Control (%)	1.4	1.4	9.3	1.3

existence of 2 clones with a different breakpoint with FISH study, co-expression mechanism should be also due to the alternative splicing.

When the patient's BM cells were obtained for establishment of a cell line, the majority of the cells expressed myeloid antigens (CD13 and CD33), CD4 and HLA-DR. However, TCC-S cells showed a drastic increase of CD13 and CD33 expression and loss of HLA-DR expression, while they still retained CD4 expression (Table 3). CD4 is expressed in T-cells, but also in monocytes, and CD4 expression is usually observed in myeloid BC-derived CML cell lines. In the process of sub-culture, a lineage switch to myeloid direction must have occurred in TCC-S cells.

We defined TCC-S cells as triploid cells according to the ISCN (International System for Human Cytogenetic Nomenclature), because the chromosome number was 67 to 82 with 76 chromosomes as a mode number. However, the majority of the cells retained two der(9)del(9)(p12)t(9;22)(q34;q11)s, two del(9)(q21)s, two der(22)t(9;22)(q34;q11)s and two normal chromosome 22. Moreover, 5 of 28 cells showed XYY sex chromosome pattern with two Y chromosomes, nevertheless usually triploid karyotype shows XXY. Thus, it is likely that TCC-S was derived from a tetraploid cell.

Missing of normal chromosome 9 is occasionally seen among Ph+ CML cells lines[10), 11), 13), 14)] or patients, which gives rise to missing of a huge amount of genes. However, a partial loss of the long arm of normal chromosome 9 has been seldom seen among them[10), 11), 13), 14)], which results in missing of a restricted region including normal *ABL* gene at 9q34. TCC-S cells have del(9)(q21) with no existence of *ABL* genes which is also confirmed by FISH study.

Recently, submicroscopic deletions on the derivative chromosome 9 called "der(9) deletions" are identified in 10-15% of patients with CML[15)]. The deletions are usually large, spanning several megabases. They are located in the region flanking the *BCR/ABL* breakpoint on the der(9), involving the loss of sequences from chromosome 9, chromosome 22 or both, although deletions of sequence only from chromosome 22 represent only 5-10% of all deletions. CML patients carrying such deletions are known to have significantly an unfavorable prognosis than those without them if they are treated with interferon-alpha and cytosine arabinoside, or bone marrow transplantation, probably due to the loss of several tumor suppressor genes (TSGs) involved in the deleted region, although more recently, it has been reported that imatinib mesylate can overcome this disadvantage[16)]. However, the TSGs responsible for a poorer prognosis in CML patients with der(9) deletions have not yet been determined. Thus, if a candidate TSG in these deleted regions is transfected to TCC-S cells to investigate the therapeutic effect, TCC-S cells may provide a good tool to determine such TSGs.

ABL protein is ubiquitously expressed, and is considered to play a complex and important role as a cellular module that integrates signals from various extra- and intra-cellular sources[3)]. This protein influences decisions in regard to cell cycle and apoptosis, although this function still remains not fully understood due to lack of an adequate model system to investigate. TCC-S cells will be a useful tool also for studying the biological properties of ABL protein, if *ABL* gene is transfected and expressed in them.

In conclusion, we have established a novel triploid CML cell line which harbors only *BCR/ABL* gene and no normal *ABL* gene. This cell line will provide a useful tool for functional study of *ABL* in Ph+ CML.

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Corresponding Author: Yuko Sato, M.D., Ph.D., Division of Ultrafine Structure, Department of Pathology, Research Institute, International Medical Center of Japan, Toyama 1-21-1, Shinjuku-Ku, Tokyo, 162-0052, JAPAN. Direct TEL: 81(Japan)-3-5273-8602, FAX: 81(Japan)-3-5273-8603 e-mail: ysato@ri.imcj.go.jp