Janus kinases (JAK) play important roles in proliferation, differentiation, and apoptosis of both normal and neoplastic cells (1). The JAKs receive signaling from cytokine and growth factor receptors, and subsequently activate downstream proteins, including signal transducers and activators of transcription (2). The JAKs consists of JAK1, JAK2, JAK3, and TYK2 that share structural and functional homologies with each other (1).

Activation of JAKs is implicated in tumorigenesis, and genetic alterations of genes encoding JAKs are partly responsible for activations of JAK signaling (3). A chromosomal translocation results in fusion of JAK2 and TEL to generate TEL-JAK2 kinase, which is detected in some patients with leukemias (4).

Recently, a missense somatic mutation in JAK homology 2 pseudokinase domain of JAK2 gene (JAK2 p.V617F) has been reported in chronic myeloproliferative disorders (5, 6). The mutation was observed in most polycythemia vera as well as in about one third of both essential thrombocytemia and myeloid metaplasia with myelofibrosis. Functionally, this mutation constitutively activates JAK2 signaling, suggesting its role in the neoplastic transformation (5, 6). In addition to the JAK2 mutations, JAK3 mutations were also detected in acute megakaryoblastic leukemia (p.A572V, p.V722I, and p.P132T), and these mutations were activating mutations that confer growth factor-independent growth on cells (7). These data indicate that not only JAK2 but also other JAK genes are mutated in human cancers, and suggest a possibility that JAK genes might be targets for somatic mutations in human cancers. Because activation of JAK signaling frequently occurs in human cancers (3, 8), it is also necessary to identify whether the activation is originated from somatic mutations of JAK genes.

Recently, research groups in the United States and the United Kingdom screened all of the kinase genes for the detection of somatic mutations in several cancer types and found that somatic mutations of JAK1, JAK3, and TYK2 genes are rare in the cancers (9, 10). However, following questions about JAK gene mutations in cancers remain unanswered: (a) whether other types of human cancers besides the ones analyzed in the previous studies have JAK mutations; and (b) whether there is any ethnic difference of the mutations. In this study, we investigated presence of JAK1, JAK3, and TYK2 mutations in acute adulthood leukemias and common solid cancers (breast,
colorectal, lung, gastric, and hepatocellular carcinomas) by a PCR-single strand conformation polymorphism (SSCP) assay.

**Materials and Methods**

**Tissue samples.** Methacarn-fixed cancer tissues from Korean patients with 90 breast ductal carcinomas, 47 colorectal carcinomas, 47 gastric carcinomas, 47 hepatocellular carcinomas, and 47 non-small cell lung cancers, and fresh bone marrow aspirates of 186 acute adulthood leukemias and 30 multiple myelomas (age range 20-80) were used for this study. The acute leukemias consisted of 105 acute myelogenous leukemias (AML), 80 acute lymphoblastic leukemias (ALL; 69 B-ALL and 11 T-ALL), and 1 undifferentiated acute leukemia. The AML samples consisted of 8 AMLs minimally differentiated, 15 AMLs without maturation, 21 AMLs with maturation, 7 acute myelomonocytic leukemias, 7 acute monoblastic and monocytic leukemias, 2 acute monocytic leukemias, 15 AML with t(8;21)(q22;q22), 6 AMLs with t(15;17)(q22;q12), 11 AMLs with t(16;16)(p13;q22), 12 acute erythroid leukemias, 15 AML with t(1;19)(q22;q13), 20 AMLs with abnormal bone marrow eosinophils Inv(16)(p13q22), 12 acute promyelocytic leukemias [AML with t(15;17)(q22;q12)] 11 AMLs with multilineage dysplasia, and 1 AML and myelodysplastic syndrome, therapy-related according to WHO classification. The colorectal carcinomas originated from cecum (n = 1), ascending colon (n = 9), transverse colon (n = 2), descending colon (n = 2), sigmoid colon (n = 13), and rectum (n = 20). The gastric carcinomas consisted of 18 diffuse-type, 15 intestinal-type, and 14 mixed-type gastric adenocarcinomas by Lauren’s classification, and 7 early and 40 advanced gastric carcinomas according to the depth of invasion. The breast carcinomas consisted of 14 ductal carcinomas in situ and 76 invasive ductal carcinomas. The hepatocellular carcinomas consisted of 5 grade I, 22 grade II, and 20 grade III cancers by Edmondson’s classification (11). The non-small cell lung cancer samples consisted of 25 adenocarcinomas and 22 squamous cell carcinomas. We analyzed the primary tumors but not the metastatic lesions. Approval for this study was obtained from the Catholic University of Korea, College of Medicine’s institutional review board.

**SSCP analysis and DNA sequencing.** For the solid cancers, malignant cells and normal cells were selectively procured from H&E-stained slides using a 301/2 gauge hypodermic needle affixed to a micromanipulator, as described previously (12). In this study, primary lesions, but not the metastatic lesions, were analyzed for the mutation detection. DNA extraction was done by a modified single-step DNA extraction method (12). For the leukemias, we extracted DNA from nonfixed fresh bone marrows of the patients. To date, JAK2 p.V617F (exon 12), JAK3 p.P132T (exon 4), AKT2 p.A572V (exon 13), and JAK3 p.V722I (exon 16) mutations have been detected in human cancers (7, 9, and 10). Thus, we focused our mutational analyses on the corresponding exons of JAK1, JAK2, and TYK2 genes (JAK1 exons 5, 14, and 17; JAK2 exons 4, 13, and 17; and TYK2 exons 14 and 17). Genomic DNA each from cancer cells and normal cells were amplified by PCR with primer pairs (Table 1). Radiosotope was incorporated into the PCR products for detection by autoradiogram. After SSCP, DNAs showing mobility shifts were cut out from the dried gel and

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cancer</th>
<th>Cancer subtype</th>
<th>Nucleotide change (predicted amino acid change)</th>
<th>Notch1 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>JAK1</strong></td>
<td>Acute leukemia #104</td>
<td>T-ALL</td>
<td>c.1972G&gt;T (p.V657F)</td>
<td>—</td>
</tr>
<tr>
<td><strong>JAK1</strong></td>
<td>Acute leukemia #136</td>
<td>T-ALL</td>
<td>c.1972G&gt;T (p.V657F)</td>
<td>p.F1593S</td>
</tr>
<tr>
<td><strong>JAK1</strong></td>
<td>Acute leukemia #182</td>
<td>AML with t(15;17)(q22;q12)</td>
<td>c.1972G&gt;T (p.V657F)</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>JAK1</strong></td>
<td>Acute leukemia #120</td>
<td>T-ALL</td>
<td>c.2347C&gt;T (p.L783F)</td>
<td>p.F1593delInsPEH</td>
</tr>
<tr>
<td><strong>JAK1</strong></td>
<td>NSCLC #196</td>
<td>Adenocarcinoma (without EGFR mutation)</td>
<td>c.2345C&gt;T (p.T782M)</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>JAK1</strong></td>
<td>Breast cancer #72</td>
<td>Invasive ductal carcinoma</td>
<td>c.1939C&gt;T (p.H647Y)</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>JAK3</strong></td>
<td>Breast cancer #113</td>
<td>Invasive ductal carcinoma</td>
<td>c.2143G&gt;A (p.V715I)</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>JAK3</strong></td>
<td>Breast cancer #50</td>
<td>Invasive ductal carcinoma</td>
<td>c.2143G&gt;A (p.V715I)</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>JAK3</strong></td>
<td>Gastric cancer #247</td>
<td>AGC, diffuse type</td>
<td>c.420+28G&gt;A (unknown)</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Abbreviations: N.D., not determined; NSCLC, non–small cell lung cancer; AGC, advanced gastric carcinoma; EGFR, epidermal growth factor receptor.

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**Table 1. Primer sequences of JAK1, JAK3, and TYK2 genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Sequences</th>
<th>Size of PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>JAK1</strong></td>
<td>Exon 5</td>
<td>F: 5'-ACAATTGGCATCCATTTTCTCTG-3' R: 5'-CCTGGGCGCAACTTCTCTCTA-3'</td>
<td>204</td>
</tr>
<tr>
<td><strong>JAK1</strong></td>
<td>Exon 14</td>
<td>F: 5'-CTGGCCCTGAGAAAGAGACACACT-3' R: 5'-GAAGACTGCTGAAACGACACC-3'</td>
<td>203</td>
</tr>
<tr>
<td><strong>JAK1</strong></td>
<td>Exon 4</td>
<td>F: 5'-GGTGCTTCCCTCCTCCTCACAGG-3' R: 5'-TCTTATGTTGCCCCCTTTTG-3'</td>
<td>191</td>
</tr>
<tr>
<td><strong>JAK3</strong></td>
<td>Exon 11</td>
<td>F: 5'-AACCCACGGATCTCCTCCTCC-3' R: 5'-GGGGCTCTCACTGTCTCCAG-3'</td>
<td>133</td>
</tr>
<tr>
<td><strong>JAK3</strong></td>
<td>Exon 16</td>
<td>F: 5'-CTCAGTCTCAGCAGAAGAG-3' R: 5'-CAAAGTTGGGTTGCGAGAC-3'</td>
<td>201</td>
</tr>
<tr>
<td><strong>TYK2</strong></td>
<td>Exon 14</td>
<td>F: 5'-GGAGGGGTTGCGGTTGTCG-3' R: 5'-GGCGGAGCACCTACACTTT-3'</td>
<td>150</td>
</tr>
<tr>
<td><strong>TYK2</strong></td>
<td>Exon 17</td>
<td>F: 5'-CTGACCATGGCCTCCCAAGG-3' R: 5'-GAGGGCCAAGGGTCTCTCA-3'</td>
<td>200</td>
</tr>
</tbody>
</table>
reamplified for 30 cycles using the same primer sets. Sequencing of the PCR products was carried out using the cyclic sequencing kit (Perkin-Elmer). The procedures of PCR and SSCP analysis were done as described previously (12). We previously analyzed JAK2 V617F mutation in the same cancer tissues, and the data were compared in this study (13).

For the 11 T-ALLs, we analyzed Notch1 mutation in exons 26, 27, and 34 by a direct sequencing method. We focused the analysis on these areas because with rare exceptions, all of the Notch1 mutations have been detected in these areas (14).

Reverse transcription-PCR. To see whether mutated JAKs are expressed in the cancers, we analyzed mRNA expression of the mutated JAKs by reverse transcription-PCR. Total RNA was extracted from fresh bone marrow aspirates using TRIzol LS reagent following the manufacturer’s protocol (Life Technology). Reverse transcription and subsequent PCR were done using a polymerase in a single step without changing reaction mixtures (SuperScript III One-Step RT-PCR system; Invitrogen). To detect the JAK1 c.1972G>T (p.V658F) mutation, a forward primer (5'-GTCTTAGACCCCAGCCACAG-3') and a reverse primer (5'-CCCATTACGGTGCTGTC-3') were used for the RT-PCR. To detect the JAK1 c.2347C>T (p.L783F) mutation, a forward primer (5'-CCCATTACGGTGCTGTC-3') and a reverse primer (5'-CCGGCTTTTCATAGAATCTCT-3') were used. The PCR products were run on agarose gels, and the corresponding bands were cut out from the gel. Sequencing of the PCR products was carried out using a capillary automatic sequencer (ABI Prism Genetic Analyzer; Applied Biosystems) according to the manufacturer’s recommendation.

Results

Overall, this study detected six JAK1, three JAK3, and no TYK2 mutations (Table 2). The PCR-SSCP analysis of the JAK1 gene in the 438 cancers identified aberrantly migrating bands in 6 cancers (Fig. 1). DNA sequence analysis of the aberrant bands led to identification of JAK1 mutations in 4 acute leukemias (4 of 186; 2.2%), 1 breast carcinoma (1 of 90; 1.1%), and 1 non–small cell lung cancer (1 of 47; 2.1%; Fig. 1; Table 2). Among the acute leukemias, T-ALL harbored 3 JAK1 mutations (3 of 11; 27.3%). None of the normal samples from the patients with the JAK1 mutations showed evidence of mutations by the SSCP (Fig. 1), indicating the mutations had
risen somatically. We also confirm the mutation by a direct DNA sequencing (data not shown). We repeated the experiments twice, including PCR, SSCP, and DNA sequencing analysis to ensure the specificity of the results, and found that the data were consistent (data not shown). The JAK1 mutations consisted of three c.1972G>T (p.V658F), one c.2347C>T (p.L783F), one c.2345C>T (p.T782M), and one c.1939C>T (p.K647Y; Fig. 1; Table 2). The JAK1 p.V658F mutation was observed in two T-ALLs and an AML with t(15;17)(q22;q12). The JAK1 p.L783F, the JAK1 p.T782M, and the JAK1 p.K647Y were found in a T-ALL, a lung adenocarcinoma, and an invasive ductal carcinoma of breast, respectively. Among the 11 T-ALLs, 3 had Notch1 mutations. In the three T-ALLs with JAK1 mutations, two (case #120 and 136) were found to have Notch1 mutations. The case #120 and the case #136 had p.F1593delinsPEH mutation and p.F1593S mutation (Table 2). However, the association between Notch1 and JAK1 mutation in the T-ALL was not significant (P = 0.072, χ² test).

For JAK3 gene, three somatic mutations were found in the cancers [2 in 90 breast carcinomas (2.2%) and 1 in 47 gastric carcinomas (2.1%)]. The JAK3 mutations consist of one missense mutation [c.2143G>A (p.V715I)] and two mutations in intron sequences (Fig. 2; Table 2).

From our previous data on the JAK2 p.V617F mutation in the same cancer tissues (13), we identified none of the cancers with the JAK1 mutations nor the JAK3 mutations harbored JAK2 p.V617F mutation. With respect to allelic status of the mutations, all of the SSCP of the cancers either with JAK1 or JAK3 mutations at the mutation sites showed both wild-type and aberrant bands (Figs. 1 and 2), and direct sequencing analysis also revealed both mutant and wild-type sequences (data not shown), indicating the mutations were heterozygous.

To see whether the sequence variants detected in this study are indeed somatic mutations confined to the cancers or polymorphisms in the population, we analyzed the altered DNA sequences in normal tissues from 100 healthy persons. However, we did not find the same sequence variants in the samples. Also, the SSCP from the cancer DNA showed mobility shifts, whereas the SSCP from the corresponding normal DNA from the same patients did not show any mobility shift (Figs. 1 and 2). Because polymorphisms should be detected in both normal and cancer cells, we concluded that
the detected sequence variations in this study are somatic mutations, not polymorphisms.

To see the expression of the mutant JAK1, we analyzed mRNA expression of JAK1 in four leukemias with the JAK1 mutations (acute leukemia #104, #120, #136, and #182). For the c.1972G>T (p.V658F) mutant, two (#136 and #182) of the three leukemias strongly expressed JAK1 mRNA (Fig. 3A). For the c.2347C>T (p.L783F) mutant, the leukemia with the mutation (#120) strongly expressed JAK1 mRNA (Fig. 3A). By DNA sequencing, we found the RT-PCR products (#120, #136, and #182) represented mutated JAK1 as well as wild-type JAK1 (Fig. 3B and C). As for the gastric, breast, and lung cancers with JAK1 or JAK3 mutations, we could not analyze mRNA expression, because the tissues for RT-PCR were not available.

Discussion

A dominant role of the JAK2 p.V617F mutation in the development of chronic myeloproliferative disorder (5, 6) led us to analyze mutations of other JAK genes in common human cancers. In this study, we found somatic mutations of either JAK1 or JAK3 in T-ALL, AML, gastric carcinoma, breast carcinoma, and non–small cell lung cancer but neither in colorectal nor hepatocellular carcinomas. Interestingly, whereas JAK1 mutation in T-ALL was frequent (27.3%), the mutations of JAK1 and JAK3 in other cancers were infrequent (up to 2.1%). Furthermore, we observed no significant difference of JAK gene mutations in gastric, colorectal, breast, and lung cancers between our study (Korean patients) and the previous studies done in Western countries (Fisher’s exact test, P > 0.05), indicating there is no ethnic difference of the mutations. Our data indicate that JAK1 and JAK3 mutations occur rarely in common human cancers, but their incidences may vary depending on cancer types.

The JAK1 p.V658F mutation is homologous to the JAK2 p.V617F mutation (National Center for Biotechnology Information database), which constitutively activates JAK2 signaling (5). Recently, Staerk et al. (15) showed that JAK1 p.V658F mutation led to constitutive activation of JAK1. The JAK2 p.V617F mutant may activate JAK2 by relieving the inhibition of JAK homology 1 domain by the JAK homology 2 domain where p.V618F resides. We hypothesize that JAK1 p.V658F mutant may activate JAK1 signaling similarly and contribute to tumorigenesis in vivo. We found JAK1 mutations p.T782M and p.L783F. Interestingly, the corresponding residue of JAK1 leucine at position 783 is JAK3 valine at 715, a replacement of which was observed in this study (JAK3 p.V715I). A close proximity of these three mutations (JAK1 p.T782M, JAK1 p.L783F, and JAK3 p.V715I) suggests that these residues may be important in JAK function, and that these mutations might be functional in tumorigenesis. Of note, we found that most of the mutated JAK1 were actively expressed in the acute leukemias at mRNA level. However, one of the leukemias with JAK1 p.V658F mutation did not strongly express the mutated JAK1. Epigenetic modifications such as hypermethylation in CpG island contribute to the loss of protein expression. Recent studies showed that up to 5% of human genes can be hypermethylated in their CpG islands in the promoters (16). Whether the expression of JAK1 p.V658F mutation is inactivated by hypermethylation should be further studied. Mutations at consensus donor and acceptor sequences of an intron have been shown to cause cryptic splice site use and exon skipping in various human disease genes (6). The intron +5G of the donor sequences is a conserved sequence (6). Thus, although functional studies have not been done, it could be conceived that the splice-site mutation (JAK3 c.420+5G>A) found in this study might result in abnormal splicing of JAK3 mRNA. In contrast to JAK1 p.V658F mutation, however, other JAK
mutations have not been tested for functional consequences. Therefore, whether they are "functional" or "passenger" mutations remains unknown at this stage.

T-ALL is an aggressive malignant disease of T-cell precursors (17). For survival and proliferation of both normal and neoplastic hematopoietic cells, cytokines play crucial roles (3). Interleukin-7 (IL-7) that is implicated in normal T-cell survival and proliferation activates both JAK1 and JAK3 (3). Interleukin-7 also promotes proliferation of T-ALL (18), suggesting its role in pathophysiology of T-ALL. In the present study, we detected two types of JAK1 mutation in the T-ALL. One of them was the JAK1 p.V658F mutation that had been proven to be an activating mutation (15). The other one was JAK1 p.L527P, the function of which has not been known. As in the case with JAK2 p.V617F mutation of polycythemia vera, the T-ALL cells with the JAK1 mutations might possibly proliferate without cytokines such as interleukin-7. Notch 1 mutation is the best-known mutation in T-ALL, which leads to aberrant Notch signaling (14). Our data showed that three of the T-ALLs with JAK1 mutations harbored Notch1 mutation as well. However, their association was statistically insignificant.

Twice and used primers around 200 bp (150-204 bps), it can be found that the amplified fragments are 200 bps or less in size (19). It is possible that the sensitivity of PCR-SSCP is generally believed to be high if the amplified fragments are 200 bps or less in size. However, because we have analyzed the samples by SSCP twice and used primers around 200 bp (150-204 bps), it can be thought that the missing of JAK1 mutations, if any, would be very rare in this study. Also, we analyzed the JAK1 mutation in 50 of the 186 acute leukemias used in this study by direct sequencing as well as by SSCP, and no additional mutation was detected by the direct sequencing (data not shown), suggesting that the SSCP is a sensitive method in detecting mutations in this study.

There have been large scale mutational studies on kinase-encoding genes in colorectal, lung, breast, gastric, ovarian and renal carcinomas, melanomas, and gliomas (9, 10). They detected somatic mutations of JAK genes in some cancers, including JAK1 p.E886K in a colorectal carcinoma, JAK1 p.N707Is*7 in a gastric carcinoma, JAK2 p.K191Q in an ovarian carcinoma, JAK3 p.L527P in a gastric carcinoma, and TYK2 p.H732R in a colorectal carcinoma. However, the incidences of the JAK mutations were very low (below 5%), and there was no recurrent mutation in them (9, 10). Moreover, these mutations are not overlapped with the JAK mutations detected in our study, indicating somatic mutations of JAK genes might be passenger mutations and rare in common solid cancers. JAK mutations may be specific to rare hematopoietic disorders such as polycythemia vera (JAK2) and T-ALL (JAK1).

In summary, the present study identified novel mutations of JAK1 and JAK3 in T-ALL, AML, gastric cancer, breast cancer, and lung cancer. Although T-ALL harbored frequent JAK1 mutation, other cancers did not harbor frequent mutation of any JAK gene. The most impressive examples of recent cancer therapies target activated kinases by genetic alterations such epidermal growth factor receptor mutation, ERBB2 amplification, and BCR-ABL translocation (20–22). Thus, research will further focus on evaluating kinases as promising molecular targets for cancer treatment. Because most of the JAK1 mutations detected in this study had been proven to be an activating mutation (V658F), this mutation might have a potential utility for the development of antineoplastic drug targeting T-ALL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

Somatic Mutations of JAK1 and JAK3 in Acute Leukemias and Solid Cancers

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