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# Mutational analysis of JAK1 gene in human hepatocellular carcinoma

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The *Janus kinase 1* (JAK1) gene encodes a cytoplasmic tyrosine kinase that is noncovalently associated with a variety of cytokine receptors and plays a nonredundant role in cell proliferation, survival, and differentiation. The mutated forms of JAK1 often altered the activation of JAK1 and then changed the activation of JAK1/STAT pathways, and this may contribute to cancer development and progression. Thus, to investigate whether genetic mutations of JAK1 gene are associated in hepatocellular carcinoma (HCC) progression, we analyzed genetic alterations of JAK1 gene in 84 human HCCs by single-strand conformational polymorphism (SSCP) and direct sequencing. Of 24 exons of JAK1 gene, 12 exons were previously reported to have mutations, we searched genetic alteration of JAK1 in these exons. Overall, one missense mutation (1.2%) was found. In addition, 12 cases (14%) were found to have single nucleotide polymorphism (14%) in exon 14. Taken together, we found one novel missense mutation of JAK1 gene in hepatocellular carcinomas with some polymorphisms. Although the functional assessment of this novel mutant remains to be completed, JAK1 mutation may contribute to the tumor development in liver cancer.

*Key words: JAK1 gene, hepatocellular carcinoma, mutation*

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and its mortality is third among all cancers and one of main causes of cancer death especially in Asia and Africa [1, 2] . Recent studies have found that genetic alterations of tumor suppressor genes or oncogenes such as p53, β-catenin, and AXIN1 are involved in hepatocarcinogenesis [3–5]. but the frequency of mutation of these genes appears to be very low in HCC patients. Furthermore, it is unclear how these genetic changes precisely reflect the clinical characteristics of individual HCC patients. Therefore, the molecular events underlying HCC in most patients still remain unknown.

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway plays a significant role in various physiological processes, including immune function, cell growth, differentiation, and hematopoiesis [6–11]. Recently, accumulating evidence indicates that abnormalities in the JAK/STAT pathway are involved in the oncogenesis of several cancers [12–14]. JAK is a small family consisting of JAK1, JAK2, JAK3 and Tyk2 [15]. Among them, JAK1 plays an essential and nonredundant role in mediating biological responses induced a specific subgroup of cytokines controlling cell differentiation and proliferation.

The family members are widely expressed in a variety of different cell types, with the exception of JAK3, which is selectively expressed in cells of hematopoietic origin [8]. There are two kinase domains in the structure of JAKs, one is carboxyterminal kinase domain named JH1 and the other is a pseudokinase domain named JH2, like the twofaced god Janus of Roman, so the JAK were also named as Janus kinases of family [10], but only the JH1 domain has functional kinase activity [6, 7]. The pseudokinase domain, as its name implies, lacks catalytic activity but has essential regulatory functions [16–19]. JAKs also have an SH2-like domain, but the ability of this region to bind phosphotyrosine has not been established. The amino terminus of JAKs comprises a band-four-point-one, ezrin, radixin, moesin (FERM) domain, which is critical for binding cytokine receptors [17, 20, 21].

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For the unique structure and important functions of the JAKs family in signaling pathway, more and more researchers focus on JAKs with pathogenesis of tumor. For example, overexpression of JAK is suggested that the one of the oncogenic events leading to the constitutive activation of the JAK/STAT pathway [22]. The suppressor of cytokine signaling-3 (SOCS-3) and interferon-alpha (IFN-α) were found negatively regulate cell growth and cell mobility by inhibiting JAK/STAT pathway in HCC cells [23, 24]. Previous studies reported that the activation of JAK/STAT pathways were ubiquitous in human HCC, 49.4% of human HCC patients could be seen the constitutive activation of JAK/STAT3 pathway [25, 26].

JAK2 and JAK3 mutations have recently been identified in several hematological malignancies, and the resulting mutant JAK proteins induce the constitutive activation of STAT5 and STAT3. Somatic gain-of function JAK1 mutations have been reported in 18% of adult T-cell acute lymphoblastic leukemia (T-ALL) patients [27]. Among the newly identified JAK1 mutants, a point mutation in the pseudokinase domain (A634D) induced the strongest level of signaling. However, few studies have been made in solid tumors in regarding JAK1 mutation. Thus, we investigated somatic mutation of the JAK1 gene in 84 human hepatocellular carcinomas by using single strand conformational polymorphism (SSCP) and sequencing in order to investigate whether genetic alterations of JAK1 gene are involved with tumor development or progression of hepatocellular carcinoma.

## Materials and methods

*Tissue samples.* Eighty-four frozen HCCs and their corresponding background liver tissue samples in 84 patients after resection of HCC were evaluated. Approval was obtained from the institutional review board of the Catholic University of Korea, College of Medicine. Informed consent was provided according to the Declaration of Helsinki. There was no evidence of familial cancer in any of the patients. The average age of the patients was 52 years (range 26–89 years); 69 were men and 15 were women. The background liver showed cirrhosis in 59 (70.2%) patients, chronic active hepatitis in 10 (11.9%), chronic persistent hepatitis in four (4.8%), and nonspecific change in 11 (13.1%). HBV was detected in 76 (90.5%) and HCV in four (4.8%). Clinically, there were 13 samples of histological grade I, 60 of grade II and 11 of grade III. To confirm diagnosis, one 6-μm section stained with hematoxylin and eosin was independently reviewed by three pathologists.

*DNA extraction.* Frozen tumor and normal tissue samples were ground to very fine powder in liquid nitrogen, suspended in lysis buffer and treated with proteinase K. DNA extraction was performed by a modified single step DNA extraction method, as described previously [28].

*Mutational analysis.* For screening the mutations of JAK1 gene in HCC, we designed 12 primer sets of 24 exons, covering all the reported mutational exons of JAK1. Each polymerase chain reaction (PCR) was performed under stan-

**Table 1. Primer sequences of JAK1 gene used in this study**

| | Primer | Nucleotide sequence | Product size (bp) |
|---|---|---|---|
| Exon 4 | Forward | **5' GGT CCC CTT TGC CAC AAT 3'** | 216 |
| | Reverse | **5' CTG GGC CCA AAC TTC CTA C 3'** | |
| Exon 5 | Forward | **5' GGC TCT CAT GGT TTC TCC 3'** | 243 |
| | Reverse | **5' AAC ACC ACC ATC CTC ACA 3'** | |
| Exon 7 | Forward | **5' AAC CCT GTC CCT TTT ATG TAT C 3'** | 239 |
| | Reverse | **5' TAA GAG CTT CTG GGA CAA ACT 3'** | |
| Exon 8 | Forward | **5' CCG TGG GAA TTT CTT CTC 3'** | 221 |
| | Reverse | **5' CAC ACC AAA GGC AAC TGA 3'** | |
| Exon 9 | Forward | **5' TCT TTC TCC CTT TGC CTA CT 3'** | 201 |
| | Reverse | **5' CAC CTC ATG GCT GTA TGG 3'** | |
| Exon 10 | Forward | **5' TCT GGT CAT TTC CCG AAT AGC A 3'** | 230 |
| | Reverse | **5' TCT GGC ACA GGG AGA CGA AC 3'** | |
| Exon 13 | Forward | **5' CTG GCC TGA GAC ATT CCT ATG 3'** | 150 |
| | Reverse | **5' CCC CTT TGA AAG AGA ACA CAC T 3'** | |
| Exon 14 | Forward | **5' CAG ACC AGG TTC CAG ACA TGG CTA T 3'** | 200 |
| | Reverse | **5' GTG GGA CCA TTA TGG ACA TCA GGA C3'** | |
| Exon 15 | Forward | **5' AAT AGA ATG CGG GAA GGA 3'** | 211 |
| | Reverse | **5' AGG GAA GAG AGG GGA GAC 3'** | |
| Exon 16 | Forward | **5' GAG ATC CCA GAA ACT GCT CCA TC 3'** | 239 |
| | Reverse | **5' GAA AGC CCT CAC TTG CCT CAC 3'** | |
| Exon 17 | Forward | **5' TGG GGC TGA GAA GTT TG 3'** | 232 |
| | Reverse | **5' AGC ACA TGG CAG GTC TTA 3'** | |
| Exon 18 | Forward | **5' CTT GGG GAG AAA CAG GAG 3'** | 196 |
| | Reverse | **5' AGC AGC ACG GGT GTA AC 3'** | |
| Exon 21 | Forward | **5' ATG TGC TTG ACT TTT ACT TCT C 3'** | 225 |
| | Reverse | **5' CCT AGT GGT TTG ATT CAG TTA C 3'** | |

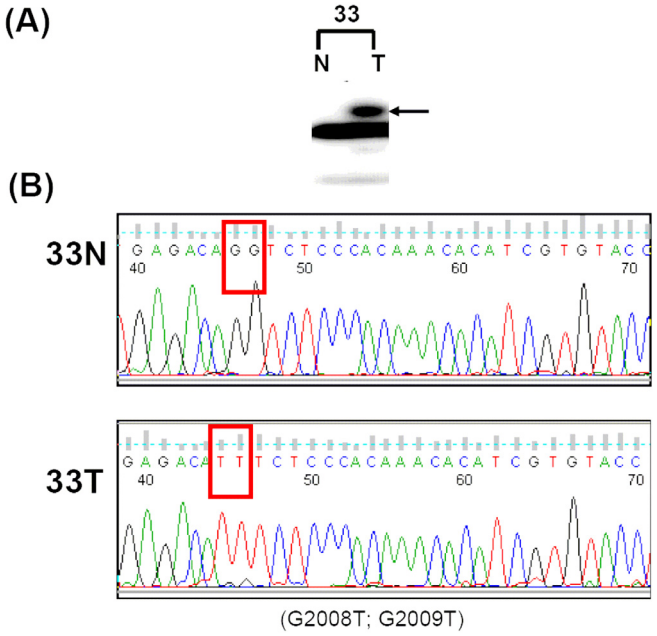


**Figure 1. Representative SSCP and DNA sequencing of the JAK1 gene in human hepatocellular carcinoma tissues. SSCP (A) and DNA sequencing analysis (B) of the JAK1 gene from tumor tissue (T) and normal tissue (N).** A **Aberrant bands marked by arrow (lane T) compared with SSCP from normal tissue.** B **Nucleotide changes at 2008(2008 G>T; Q646V) and 2009 (2009 G>T; H 647 F) in tumor tissue compared to normal tissue.**

dard conditions in a 10μl reaction mixture containing 1μl (20ng) of template DNA, 0.5 μM of each primer 0.2 μM deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 0.4 unit of Ampli Taq gold polymerase(Perkin-Elmer, Foster City, CA, USA), 0.5 μCi of $^{32}$P-dCTP(Amersham, Buckinghamshire, UK), and 1μl of 10×buffer. The reaction mixture was denatured for 12 min at 95°C and incubated for 35 cycles (denaturing for 30s at 95°C annealing for 30 s at 56–67°C, and extension for 30 s at 72°C) After amplification, PCR products were denatured for 5 min at 95°C at 1:1 dilution of sample buffer containing 95% formamide/20 mmol/L EDTA/0.05% bromophenol blue/0.05% xylene cyanol and were loaded onto a single strand conformation polymorphism (SSCP) gel (FMC Mutation Detection Enhancement system; Intermountain Scientific, Kaysville, UT, USA) with 10% glycerol. Samples were electrophoresed at 8 W at room temperature overnight. After electrophoresis, the gels were transferred to 3 MM Whatman paper and dried, and autoradiography was performed with FUJI MEDICAL X-RAY FILM(FUJIFILM Corporation, JAPAN ). For the detection of mutations, all the aberrant mobility bands of the SSCP experiments were cut out from the SSCP gel. DNA samples were extracted from the gel with 50μL distiled water incubating at 50°C for 24h and amplified for 39 cycles using the same primer sets. The amplified samples were loaded onto agarose gel and purified with QIAquick Gel Extraction Kit. The purified PCR products were sent to cosmo4 company (Seoul of Korea) for sequencing.

## Results

*Mutational analysis* We analyzed all the previously reported mutational exons (Table 1.) of JAK1 by PCR-SSCP. From this, we found 19 genetic alterations in exon 5,9,10,13,14 of JAK1 gene (Table 2). Direct sequencing of aberrant migrating bands on SSCP gel resulted one missense mutation: (2008 G>T; Q646V), (2009 G>T; H 647 F) and rest of them were silent or mutation in intron (Fig.1, Table 2). We also found some SNPs in other exons. Interestingly, the samples of No.2 and No.148 have SNPs both in exon 5 and exon 10. The SNPs are A to G transiton at nucleotide 616 (616A>G; G182G) in exon 5 and a C to T transition at nucleotide 1654 (1654C>T; I530I) in exon 10. Most interestingly, there are 12 samples (14%) have the same SNP situation at nucleotide 2225 (2225C>T; S685S) in exon 14.

## Discussion

The Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway mediates signaling by cytokines, which control survival, proliferation and differentiation of several cell types. Constitutive JAK activation leads to persistent activation of STAT transcription factors, and several cancers exhibit constitutive STAT activation, in the absence of JAK or STAT activating mutations.

Mutational studies have showed that the mutation in exon 4 and exon 8 of the JAK1 gene could result in complete loss of the protein in several different prostate cancer cell lines [29]. Analysis of the homologous mutation in JAK1 (V658F) also showed that the homologous mutation induced autonomous growth of cytokine-dependent cells and constitutive

**Table 2. Summary of JAK1 mutations in HCC**

| Exons | No. of Tissues | Nucleotide | Amino acid | |
|---|---|---|---|---|
| Exon 5 | 2T, 148T | A616G | G182G | Silent |
| Exon 9 | 78T, 143T | C(Intron)T | | |
| Exon 10 | 2T, 148T | C1654T | I530I | Silent |
| Exon 13 | 33T | G2008T;G2009T | Q646H;V647F | Missense |
| Exon 14 | 3T;9T;15T;29T;33T,40T;47T; 60T,68T;73T;80T;105T | C2225T | S685S | Silent |

activation of STAT5, STAT3, mitogen-activated protein kinase, and Akt signaling in Ba/F3 cells [30]. Although this homologous mutation locate in the pseudokinase domain of JAK1 and this region lacks catalytic activity, we may speculate that it has essential regulatory functions that might regulate the activity of JAK1 and then alter the activity of JAK/STAT pathways. In liver cancer, it has repored that constitutive activation of JAK/STAT pathways was ubiquitous in human HCC [25, 26]. In this study, we found two missense mutations (Q646V; H647F) in the pseudokinase domain of JAK1 in human hepatocellular carcinoma. This implies that it may change the activation of JAK1 and contribute to the development of human hepatocellular carcinoma.

In conclusion, we report here one mutation in exon 13 of JAK1 gene in 84 human hepatocellular carcinomas. In spite of low frequency of mutation, we suggest that mutation of JAK1 gene may contribute to the development and progression of hepatocellular carcinoma through altering activation of JAK-STAT signaling. Further functional analysis of the mutation identified in this study will broaden our understanding of the pathogenesis of hepatocellular carcinoma.

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