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Absence of JH2 domain mutation of the tyrosine kinase JAK2 in renal cell carcinomas

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To the Editor

Signaling pathways initiated by a variety of cytokines and growth factors are essential for cell differentiation, growth and survival. Their dysregulation can contribute to human malignancies. Janus kinases (JAK), which are cytoplasmic tyrosine kinases, play a crucial role in signaling transduction cascades triggered by cytokines whose cognate receptors lack intrinsic tyrosine kinase activity. The JAK family of kinases comprises four known mammalian members, i.e., TYK2, JAK1, JAK2 and JAK3, which associate with the intracellular domains of a large number of cytokine and growth factor receptors. Receptor dimerization after ligand binding leads to activation of JAKs and the subsequent phosphorylation of receptor tyrosine sites that provide docking sites for various SH2-containing signaling molecules, including signal transducer and activator of transcription (STAT). Thus, JAKs constitute important links between cytokine receptors and downstream molecules in signaling pathways.

All members of the JAK family have very similar structures. Each member possesses seven highly conserved domains (JHl–JH7). The JAK C-terminal region consists of tandem kinase and kinase-like (pseudokinase) modules, termed JH1 and JH2, respectively. The JH1 domain contains the typical conserved residues identified in tyrosine specific kinases. Phosphorylation of JH1 is essential for full kinase activity. The JH2 domain appears to have a complex regulatory function on the JH1 catalytic domain. The remaining regions (JH3–JH7) of JAKs may be essential for interacting with their cognate cellular receptors and for coordinating JAK functions.

The *JAK2* gene (mapped to 9p24) contains 23 exons encoding an 1132-amino acid protein. An acquired *JAK2* gene mutation (V617F) has been recently associated with myeloproliferative disorders [1]. The JAK2 V617F mutation, which causes the substitution of phenylalanine for valine at codon 617 in the JH2 domain, predicts to disrupt the autoinhibition of the JH1 catalytic domain by the JH2 domain. This gain-of-function mutation is detectable in the majority of patients with polycythemia vera and in many with essential thrombocythemia or idiopathic myelofibrosis [2]. More recently, several other *JAK2* mutations, including K539L (a replacement of lysine at position 539 with leucine), F537-K539delinsL (a replacement of phenylalanine at position 537 through lysine at position 539 by a single leucine), H538QK539L (a substitution of glutamine for histidine at position 538 and leucine for lysine at position 539), N542-E543del (a deletion of asparagine at position 542 and glutamic acid at position 543) and L611S (a substitute of serine for leucine at position 611), have also been detected in

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(Received 25 May 2007; accepted 6 June 2007)

ISSN 0284-186X print/ISSN 1651-226X online
DOI: 10.1080/02841860701499390

Table I. Primers used for DGGE analysis.

Exon	Primer* (5′→3′)	Product size (bp)
10	Forward: TGGAGCAATTCATACTTTCAGTG Reverse: CAATGTCACATGAATGTAAATCAAGA	323
11	Forward: TCTTCCTCATTGAATGTATTTTCTT Reverse: TGAGAGCACATCTTTAAACAGCA	325
12	Forward: TGCTGAAAGTAGGAGAAAGTGC Reverse: CTGACACCTAGCTGTGATCCTG	339
13	Forward: TGAGTTTTGCCAATTTAATTTCTT Reverse: TTGTTTCCAGGTAAAGATAATTTGT	381
14	Forward: TTTTGGGGGCTTGAACATAC Reverse: CCTTTACACCACTGCCCAAG	330
15	Forward: AAAATAGGCCTGATTATTCAAATG Reverse: TGTGAAAGAAATATGAAAGTTCAGAGA	371
16	Forward: AAGGTTGGTGTGGCATTACA Reverse: GAAAGCTGGAAAATCAATAAGATCA	294

*a 40-nucleotide CG-clamp was attached to the beginning of the forward primer of each primer pairs. To reach optimal DGGE conditions, the primers were modified by addition of a stretch of G/C or A/T nucleotides (two to ten) to the primer beginning, which was determined by computer simulation of DNA melting using the software WinMelt Version 2 (BioRad, Hercules, CA).

V617F-negative patients with myeloproliferative disorders [3,4]. These mutations are also located in the JH2 pseudokinase domain of JAK2 and all result in a constitutive tyrosine phosphorylation activity of this tyrosine kinase.

Clear cell renal cell cancer (RCC) is the most frequent renal cancer subtype that derives from proximal renal tubular cells. Proximal tubular cells of the kidney produce erythropoietin (Epo), a cytokine that stimulates the proliferation of hemopoietic progenitors. Polycythemia vera due to elevated erythropoetin levels is a well known paraneoplastic syndrome in RCC patients. JAK2 is directly linked to erythropoietin receptor (EpoR), therefore mediating EpoR signaling. It is known that mutational activation of JAK2 induces Epo hypersensitivity. An increased expression of both Epo and EpoR at the levels of transcript and/or protein has been described in human primary RCC and in established renal cancer cell lines, indicating the presence of a constitutively activated EpoR-JAK2 signaling in RCC. Previous experiments have shown that activation of EpoR can stimulate proliferation of renal carcinoma cells. These lead to the speculation that aberrant EpoR-JAK2 signaling activation in RCC may be induced by JAK2 mutation. In this context, we employed denaturing gradient gel electrophoresis (DGGE) assay to screen for *JAK2* gene mutations in primary clear cell RCCs obtained from 35 patients who underwent partial or radical nephrectomy between 1993 and 2003 at the University Hospital of Zurich, Switzerland. This study obtained approval from local ethics committee and patients gave consent for tissue collection and analyses. Sixteen tumors were in pT1 or 2 stages, while the remaining 19 ones in pT3 stage. The isolation of DNA was conducted as described previously [5]. DGGE-based mutation analysis was performed to screen for mutation within exons 10–16 of *JAK2* gene, which encode the JH2 regulatory domain. These exons and their exon-intron junctions were PCR-amplified from tumor DNA using primers given in Table I. PCR amplification and DGGE were carried out as described elsewhere [5]. Samples showing aberrant bands were sequenced using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). However, no mutation in the JH2 region of *JAK2* gene was detected in these RCC patients.

Accumulating evidence suggests that JAK kinases contribute to the pathogenesis of various human neoplastic diseases. In addition to the well-described role of JAK2 in myeloproliferative disorders, direct downstream molecules of JAK kinases, i.e., STATs, are constitutively activated in a variety of hematological malignancies and solid tumors. JAK activities are generally required for the activation of STATs. However, a constitutive activation of JAKs could result from either JAK gain-of-function mutation or an increased activity of their upstream molecules. Our data demonstrate absence of JH2 domain mutation of JAK2 in human clear cell RCC, suggesting that the activation of EpoR-JAK-STAT signaling may not be related to JAK2 mutation in RCC.

Acknowledgements

This work was supported by the Cancer League of Canton of Zurich, Switzerland.

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The role of surgery in jaw bone necrosis associated with long-term use of bisphosphonates

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In last years, several cases of osteonecrosis of the jaw (ONJ) associated with the use of bisphosphonates have been reported [1–7]. In our experience, the development of this complication in breast cancer patients seems to be higher than in other studies (frequency of about 6%) [8]. The pathogenesis of ONJ is unclear. An analogy with 'phossy jaw' has been reported, suggesting a potential alteration in the normal phosphorus and calcium metabolism in these patients [9,10]. Persistence of hypercalcemia and secondary hyperparathiroidism may eventually predispose to the development of this condition [11]. Pamidronate and zoledronic acid exert direct effects on osteoclastic activity through different mechanisms, and may induce osteoclast apoptosis [12]. The antiangiogenic effect attributed to bisphosphonates might play a role, together with microtrauma, inflammation and chronic infection [13–16].

ONJ can severely affect quality of life, and even becoming the major concern of cancer patients because of severe pain, difficulties in performing oral hygiene and in eating.

Its clinical management is controversial and no satisfactory therapy is currently available [17]. The use of hyperbaric oxygen seems to be ineffective [18]. Antibiotic therapy may be temporarily useful but does not induce clinical resolution. Temporary discontinuation of bisphosphonates may be advocated, although clinical benefit has yet not been proven. Non-surgical treatments have always been preferred: minimal debridement, cover exposed bone, protective stint [19]. In some cases of progressive disease a major surgical approach may be considered [20]. Recurrent ONJ should be taken into account when considering risks and benefits resulting from the surgical procedure [17]. Recently it has been observed that users of intravenous bisphosphonates had an increased risk of inflammatory conditions, osteomyelitis, and surgical procedures of the jaw and facial bones. The increased risk may reflect an increased risk for osteonecrosis of the jaw, even in misdiagnosed cases [21].

In this short report we describe our experience of surgery in patients with jaw bone necrosis.

The first patient is a 64 year old man affected from prostate cancer with bone, pulmonary and nodal metastasis. He received treatment with zoledronic acid from November 2004 to April 2005. In July 2005 he started to complain paresthesia in left emi-mandibular area, with no pain. Symptoms gradually worsened, with recurrent dental abscesses, treated with antibiotic therapy, and progressive pain localized

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(Received 18 June 2007; accepted 15 July 2007)

ISSN 0284-186X print/ISSN 1651-226X online
DOI: 10.1080/02841860701592418