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Mini review

Mutations leading to constitutive active gp130/JAK1/STAT3 pathway

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ABSTRACT

Constitutive activation of STAT (Signal Transducer and Activator of Transcription) transcription factors is a common feature identified in numerous tumors. Inflammatory hepatocellular adenomas (IHCA) are benign liver tumors characterized by an inflammatory phenotype and an overexpression of STAT3 target genes. Recurrent somatic mutations in major actors belonging to the IL6/JAK/STAT3 pathway have been identified in these tumors. (1) 60% of IHCA show IL-6 signal transducer (*IL6ST*; gp130) mutations; (2) 10% harbor mutations of the Fyn-related kinase FRK; (3) 5% harbor mutations in STAT3; (4) 5% harbor somatic mutations in the GNAS complex locus; and (5) 1% of IHCA harbor mutations in the Janus kinase 1 (JAK1). All these IHCA-associated mutations promote the constitutive activation of STAT3. In this review, we discuss the role of these mutated genes in IHCA and other tumors.

1. Introduction

Constitutive activation of STAT (Signal Transducer and Activator of Transcription) proteins, and particularly of STAT3 and STAT5, is a common feature identified in numerous solid and hematological tumors [1,2].

STAT transcription factors are directly phosphorylated and activated by the Janus kinase (JAK1–JAK3) family of tyrosine kinases, which plays pivotal roles in the development of both solid and hematopoietic tumors. In interleukin-6 (IL-6) signaling, the ligand binds its specific α-receptor, IL6R (also known as gp80), and generates hetero-dimeric IL-6/IL6R complexes that associate with two molecules of the signaling component of the interleukin-6 receptor, gp130, resulting in a hexameric structure that activates the signaling functions of gp130. Gp130 activation then induces the phosphorylation of JAK kinase 1 (JAK1) and JAK2, which in turn phosphorylate STAT3 on tyrosine-705, triggering STAT3 homodimerization, nuclear translocation, DNA binding and the activation of inflammatory STAT3 transcription targets genes [3,4].

Hepatocellular adenomas (HCA) are rare benign liver tumors that usually develop in young females following the use of contraceptives [5]. HCA are a heterogeneous entity and 4 major molecular subgroups have been defined so far, based on both genotypic and phenotypic features [6,7]. HCA are defined by (1) mutations inactivating HNF1A (H-HCA, 35% of the HCA) [8,9], (2) activation of the β-catenin by mutations in exons 3, 7 and 8 (bHCA, 20%) [10,11], (3) inflammatory phenotype with STAT3 activation (IHCA), and (4) the remaining unclassified tumors (UHCA, 10%). Inflammatory hepatocellular adenomas (IHCA) account for 45–60% of HCA, hence represent the most common subtype of this disease. This subtype of adenomas exhibits strong pathological hallmarks: inflammatory infiltrates, dystrophic arteries, and sinusoidal dilatation [12]. Inflammatory HCA show a cytoplasmic overexpression of SAA and CRP, two proteins of the acute phase of inflammation, in the tumor hepatocytes. This particular subtype of HCA occurs most frequently in women on oral contraceptives, but has also been associated with obesity and alcohol abuse [12].

We described the oncogenes that explain the hepatocytes proliferation and the inflammatory phenotype (“oncogene-induced inflammation”) in these tumors. (1) 60% of IHCA harbor activating somatic mutations in the interleukin (IL)-6 signal

Abbreviations: HCA, hepatocellular adenoma; IHCA, inflammatory HCA; HCC, hepatocellular carcinoma; STATs, ignal transducer and activator of transcription; JAK, Janus kinase; LGL, large granular lymphocytic leukemia; ALCL, anaplastic large cell lymphoma; TCL, T-cell lymphoma; ALL, acute myeloid leukemia.

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transducer (*IL6ST*) locus, gene coding for gp130, the co-receptor and signal transducer of the IL-6 receptor (IL6R or gp80) [13,14]; (2) 10% harbor mutations of the Fyn-related kinase FRK [11]; (3) 5% harbor somatic mutations in signal transducer and activator of transcription 3 (STAT3) [15]; (4) 5% harbor somatic mutations in the GNAS complex locus (GNAS) encoding, among other proteins, the G-protein α subunit [16]; and (5) 1% of IHCA harbor mutations in the Janus kinase 1 (JAK1) [11]. Notably, all these IHCA-associated mutations promote the constitutive activation of STAT3 (Fig. 1).

2. Mutations identified in IHCA

2.1. Gp130 mutations

The most preeminent oncogene identified was gp130 (coded by the *IL6ST* gene) [13,14]. Gp130 is a cell surface signaling receptor shared by all IL-6 family cytokines (IL-6, IL-11, IL27, ciliary neurotrophic factor [CNTF], cardiotrophin-1 [CT-1], cardiotrophin-like cytokines [CLC], leukaemia inhibitory factor [LIF], oncostatin M [OSM] and neuropoitin [NPN]) [17–19].

Somatic activating mutations of gp130 have been identified in 60% of inflammatory HCA. Gp130 mutations are mutually exclusive with recurrent mutations in HNF1A, STAT3, FRK, GNAS and JAK1 that are also recurrently found mutated in HCA [9,11,15,16]. The spectrum of *IL6ST* mutations included 20 distinct in-frame deletions, one missense substitution and three in-frame insertion–deletions [14] (Fig. 2a). Almost all of these mutations modify 1 to 26 amino acids neighbouring the IL-6/IL-6R binding site (also known as cytokine-receptor homology region, CHR) located in D2 domain of gp130. All of these mutations are predicted to disrupt key residues involved in the gp130-IL-6 binding interface. Specifically, the most frequent alterations target residues 186–191, which are essential for the interaction between gp130 and IL-6. A rare in-frame deletion targeting four residues (A418–F421) was identified at the D4–D5 junction, a region important for flexible dimerization of the gp130 extracellular domains.

All the gp130 mutations identified in IHCA resulted in a ligand independent constitutive activation of gp130. When transfected into hepatocellular cell lines, gp130 mutants induced constitutive STAT3 activation and subsequent upregulation of acute-phase proteins, as SOCS3 and CRP, both known targets of STAT3-induced transcriptional regulation [13].

Recently, it has been shown that gp130 activation is regulated by the connectivity of two gp130 extracellular domains involved in contacting the cytokine IL-6. The mutations identified in IHCA are thought to impair an interdomain hydrophobic interaction inducing conformational changes within this region of the protein, leading to constitutive activation [20].

Schmidt-Arras and colleagues showed that oncogenic gp130 mutants are predominantly localized to intracellular compartments, to the endoplasmic reticulum (ER) and early endosomes. ER retention of gp130 mutant was linked to prolonged association with the ER quality control component calnexin. Signals emitted by mutant gp130 from both intracellular pools contribute to ligand-independent cell proliferation and induction of downstream signals [21].

Constitutively active gp130 mutants expression has also been shown to trigger activation in the intestine of the transcriptional regulators YAP and Notch, that control tissue growth and regeneration [22]. This activation was independent of STAT3 and was mediated by tyrosine kinases Src and Yes, which phosphorylate YAP and induce its stabilization and nuclear translocation. Through YAP and Notch, intestinal gp130 signaling stimulates epithelial cell proliferation, causes aberrant differentiation and confers resistance to mucosal erosion. Interestingly Src, but not JAK inhibitors, were shown to be able to block IL-6- or gp130 mutants-induced YAP activation and stabilization in human colorectal cells and mouse small intestinal organoids [22].

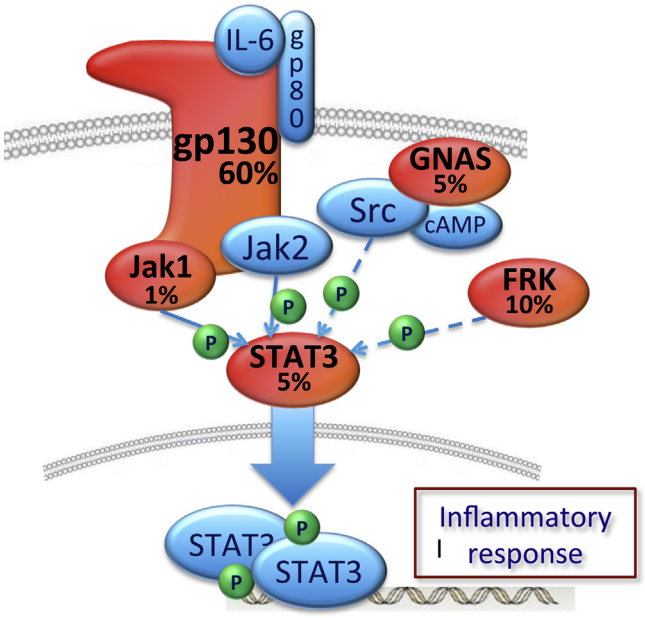

Fig. 1. *Different genetic alterations identified in inflammatory adenoma (IHCA) among the gp130/JAK/STAT3 pathway*. Mutations activating gp130, FRK, STAT3, GNAS, or JAK1 are identified in 60%, 10%, 5%, 5% and 1% of IHCA, respectively. All these mutants (in red) promote STAT3 activation in tumor hepatocytes.

2.2. FRK mutations

FRK is a tyrosine kinase related to the Src kinase family [23]. Src family consists in 9 members: Src, Fyn and Yes that are ubiquitously expressed, and Blk, Fgr, Hck, Lck, Lyn and Yrk, expressed in specific tissues. Three proteins, Brk, Srm and FRK, have similar structures and compose the Src-like family. All these proteins share a Src homologue 2 (SH2) and a SH3 domain, that play a role in protein–protein interactions, and a catalytic kinase domain [24].

FRK somatic mutations have been identified in 10% of IHCA [11]. Two hot-spots were identified: one missense substitution at position E346 and in-frame deletions or in-frame deletions-insertions located at codons V378-F380 (Fig. 2b). The E346 codon is very well conserved across species and among all the Src kinases. A mutation of the Src kinase protein corresponding to the FRK E346G substitution has been shown to be able to activate Src *in vitro*, conferring oncogenic proprieties and enhancing its kinase activity [25].

FRK mutants identified in IHCA exhibited an increased kinase activity compared with the wild-type FRK. When expressed in hepatocellular cell lines, FRK mutants are able to induce a persistent STAT3 activation leading to a strong acute inflammatory response, with the overexpression of inflammatory target genes, independently from ligand induction.

Ba/F3 cells, an interleukin-3 (IL-3)-dependent murine pro-B cell line, were able to grow upon IL-3 withdrawal when expressing the FRK mutants. Ba/F3 cells with stable expression of the VK FRK mutant resulted in tumor growth after subcutaneous injection in nude mice, in contrast to Ba/F3 cells expressing wild-type FRK, and allografted tumors showed nuclear and Y705-phosphorylated STAT3 [11].

2.3. STAT3 mutations

Mutations of STAT3 itself were identified in 5% of IHCA [15]. All mutations identified were somatic and monoallelic, and led to

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