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Critical role of FANCC in JAK2 V617F mutant-induced resistance to DNA cross-linking drugs

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ABSTRACT

A point mutation (V617F) of tyrosine kinase Janus kinase 2 (JAK2) is found in the majority of patients with myeloproliferative neoplasms (MPNs) and an aberrant signaling pathway induced by constitutively active JAK2 V617F mutant is a hallmark of MPNs. Cells transformed by JAK2 V617F mutant exhibited resistance to anti-cancer drugs such as cisplatin (CDDP), mitomycin C (MMC) and bleomycin (BLM). We first found that the expression of FANCC, a member of the Fanconi anemia (FA) proteins, was significantly induced by JAK2 V617F mutant through activation of signal transducers and activators of transcription 5 (STAT5). In addition, monoubiqitination and foci formation of FANCD2, which are critical for activation of the FA pathway, were increased in cells transformed by JAK2 V617F mutant, compared to cells expressing wild-type JAK2. Interestingly, knockdown of FANCC in cells expressing JAK2 V617F mutant induced not only the reduction of monoubiqitination and foci formation of FANCD2 but also the enhancement of sensitivity to DNA damage induced by CDDP and MMC but not BLM. Taken together, FANCC is most likely to be critical for resistance to DNA cross-linking drug-induced DNA damage in cells transformed by JAK2 V617F mutant.

1. Introduction

The Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway plays an important role in hematopoiesis, and its deregulation promotes cell growth and prevents apoptosis in a variety of hematological malignancies such as acute lymphoid leukemia and chronic myeloid leukemia [1–3]. Previously, a somatic mutation of JAK2, V617F, was identified in myeloproliferative neoplasm (MPNs) patients including 95% of polycythemia vera (PV) patients and ~50% of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF) [4,5]. In the presence of erythropoietin receptor (EpoR), JAK2 V617F mutant is constitutively active and confers growth-factor independence on Ba/F3 cells, suggesting that JAK2 V617F mutant functions as a potent oncogene product [6]. Importantly, apoptotic cell death caused by IL-3 deprivation was prevented by the expression of JAK2 V617F mutant [7]. In addition, transformed cells by JAK2 V617F mutant and EpoR exhibited resistance to a DNA cross-linking drug, cisplatin (CDDP) [8], suggesting that JAK2 V617F mutant activates survival signals against apoptosis induced by not only cytokine removal but also DNA damage.

In erythropoietin (Epo) signaling, JAK2 is activated and then phosphorylates a number of tyrosine residues in the intracellular domain of EpoR. Phosphorylated erythropoietin receptor (EpoR) provides a platform for the recruitment and activation of signaling mediators through Src homology 2 (SH2) domain-mediated interactions, including STAT5, phosphoinositide 3-kinase (PI3K)–Akt pathway and Ras–ERK pathway, which are known to be involved in Epo-induced cell proliferation and survival [9–11]. When EpoR was coexpressed, JAK2 V617F mutant induced aberrant activation of several transcription factors, such as STAT3, STAT5, AP-1 and c-Myc, which are involved in the proliferation, survival and tolerance of DNA damage, and this is independent of Epo stimulation [8,12–15]. It is simply speculated that JAK2 V617F mutant induces the expression of target genes of these transcription factors, and it is also possible that some of these contribute to JAK2 V617F mutant-induced transformation activity; however, it is still unclear which target genes harbor the central roles in transforming activity induced by JAK2 V617F mutant.

Fanconi anemia (FA) is a rare genetic cancer-susceptibility syndrome that is characterized by congenital abnormalities, bone-marrow failure and cellular sensitivity to DNA cross-linking agents such as cisplatin (CDDP) and mitomycin C (MMC) [16–18]. FA is caused by a deficiency in any of at least 15 genes that encode FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN,

Abbreviations: ATM, ataxia-telangiectasia mutated; Epo, erythropoietin; EpoR, erythropoietin receptor; ET, essential thrombocythemia; FA, Fanconi anemia; JAK2, Janus kinase 2; MPNs, myeloproliferative neoplasms; PMF, primary myelofibrosis; PV, polycythemia vera; RT-PCR, reverse transcriptase-polymerase chain reaction; STAT5, signal transducers and activators of transcription 5; TAE, Tris–acetic acid–EDTA; TBE, Tris–boric acid–EDTA.

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FANCO and FANCP proteins. Although the underlying molecular mechanisms of FA are incompletely understood, it has been reported that eight of the FA proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM) form a nuclear core complex that functions as an E3 ubiquitin ligase. FANCD2 is the substrate of the E3 ligase activity of FANCI in the FA core complex when cells are treated with DNA-damaging agents or during replication stress. Monoubiquitinated FANCD2 translocates to chromatin and accumulates in subnuclear foci, which are considered to be the sites of DNA damage and DNA repair. Thus, it is thought that DNA repair occurs through activation of this FA pathway [19–21].

In this study, we searched for the gene cluster induced by JAK2 V617F mutant, using DNA array screening. Here, we found that expression of FANCC was induced through STAT5 activation downstream of JAK2 V617F mutant. Interestingly, we demonstrated that FANCC expression significantly contributed to JAK2 V617F mutant-induced tolerance to CDDP and MMC.

2. Materials and methods

2.1. Reagents

Recombinant human erythropoietin (Epo) (ESPO 3000) and mitomycin C (MMC) were purchased from Kirin Brewery Co. (Tokyo, Japan). Recombinant murine IL-3 was purchased from PEPROTECH (Rocky Hill, NJ, USA). Cisplatin (CDDP) and bleomycin (BLM) were purchased from Nihon-Kayaku (Hyogo, Japan). Anti-phospho-JAK2 (Y1007/1008) antibody, anti-phospho-STAT5 antibody (Y694), anti-STAT5 antibody and anti-phospho-gamma Histone H2AX (S139) antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-HA antibody and anti-Flag antibody were purchased from Roche (Basel, Switzerland) and Sigma (St. Louis, MO), respectively. Anti-FANCC antibody, anti-FANCD2 antibody and anti-β-actin antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Peroxidase-conjugated rabbit anti-mouse and goat anti-rabbit secondary antibodies

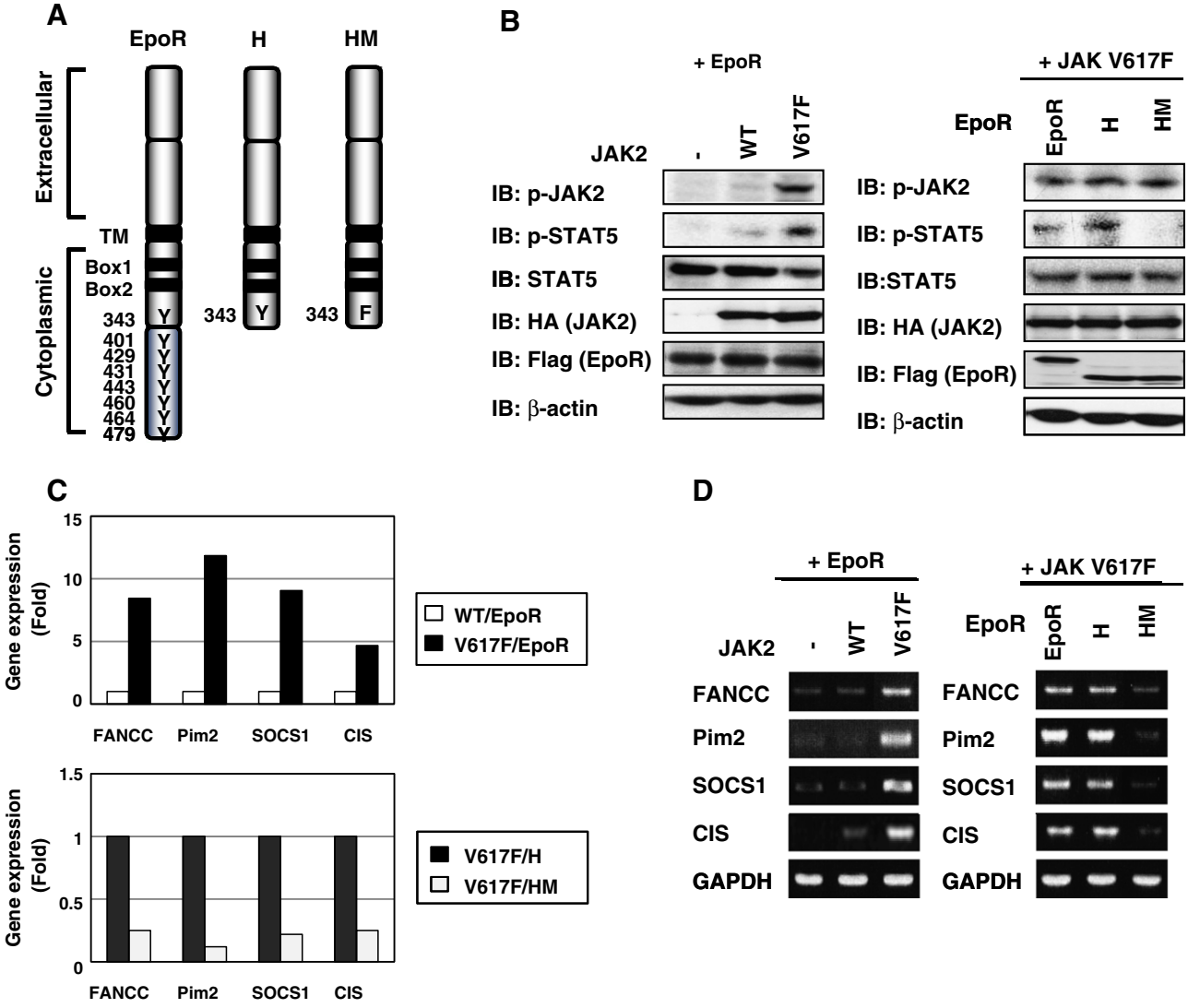

Fig. 1. JAK2 V617F mutant induces expression of FANCC as well as Pim2, SOCS1 and CIS. (A) Structure of EpoR and its deletion mutants, EpoR-H and EpoR-HM. (B–D) Ba/F3 cells were infected with empty virus (-), retroviruses encoding wild-type JAK2 c-HA, JAK2 V617F mutant c-HA, EpoR c-Flag, deletion mutant of EpoR c-Flag (H) or deletion mutant of EpoR c-Flag with a point mutation of Y343F (HM). (B) The transduced Ba/F3 cells were cultured without Epo for 12 h. Whole cell lysates were immunoblotted (IB) with anti-phospho-JAK2 (Y1007/1008) antibody, anti-phospho-STAT5 antibody (Y694), anti-STAT5 antibody, anti-HA antibody, anti-Flag antibody or anti-β-actin antibody. (C) Total RNAs were prepared from WT/EpoR cells, V617F/EpoR cells, V617F/H cells and V617F/HM cells that were cultured without Epo for 12 h. The change of the gene expression between WT/EpoR cells and V617F/EpoR cells and between V617F/H cells and V617F/HM cells was determined by DNA array. The fold expression of FANCC, Pim2, SOCS1 and CIS was graphed. (D) Total RNAs were prepared from transduced Ba/F3 cells that were cultured without Epo for 12 h. mRNA expression of FANCC, Pim2, SOCS1, CIS and GAPDH was determined by RT-PCR analysis.