LETTER TO THE EDITOR

Clonal evolution in UKE-1 cell line leading to an increase in JAK2 copy number

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The JAK2 V617F mutation is present in the vast majority of polycythemia vera patients, and in about half of patients with primary myelofibrosis and essential thrombocythemia. A correlation between MPN disease phenotype and the proportion of JAK2 V617F mutant alleles has lead to the need for sensitive and reproducible molecular techniques to assess the so-called JAK2 V617F allele burden in patient samples. A 2009 multicenter study conducted by Lippert et al. assessed the concordance of JAK2 V617F allele burden quantitation from various diagnostic laboratories. The study demonstrated the importance of using well-defined and accurate standards to calibrate JAK2 V617F quantitative assays. To that end, the authors suggested the use of plasmid DNA dilutions along with a known genomic DNA sample as an internal control to allow for the most precise quantification of JAK2 V617F allele burden. As an alternative to plasmids, the diploid UKE-1 cell line, having two copies of mutated JAK2 per cell, was suggested as a useful genomic DNA standard.

There are several JAK2 V617F-positive cell lines derived from patients with history of myeloproliferative or myelodysplastic disorders (HEL, MB-02, MUTZ-8, SET-2 and UKE-1) that differ in number of JAK2 mutant alleles. These cell lines are good research tools for elucidating the pathobiology of MPN, and are potential standard material as well. However, HEL cells have more than two copies of JAK2 and should not be used as a standard for quantification of JAK2 V617F allele burden. The UKE-1 cell line has recently been shown to be homozygous for the JAK2 V617F mutation and may therefore be a useful as a genomic DNA standard. UKE-1 was derived from an essential thrombocythemia patient transformed into acute leukemia in 1979. Phenotypic analysis of leukemic cells revealed coexpression of myeloid and endothelial antigens. Cytogenetic analysis of cells showed bclonality with the following karyotype: 48,XX,+8,+14[14]/45,XX,-7,del(11)(p14)[8]. UKE-1 cells were cultured in Iscove modified Dulbecco medium supplemented with 10% horse serum and 10% horse serum, 10% fetal calf serum, 10% horse serum and 10% fetal calf serum, 10% horse serum and 10% fetal calf serum.

Here, we show that UKE-1 cells in our hands underwent clonal evolution during in vitro culture, leading to an increase of JAK2 gene copy. Use of these cells as a standard would cause an underestimation of the JAK2 V617F allele burden.

The UKE-1 cells used in this study had been cultured for 5 months, frozen for 16 months, then thawed. We then cultivated the UKE-1 cells and a human myeloid cell line, HL60, for 3 weeks before making serial dilutions of UKE-1 in HL60 (100% UKE-1 through 0% UKE-1). This widely used human myeloid cell line derives from leukocytes of a patient with acute promyelocytic leukemia in 1979. Initial cytogenetic analysis revealed 75% of aneuploid cells (44 chromosomes) with structural anomalies on chromosomes 7, 9, 10 and number anomalies on chromosomes 5, 8, 18, X. The cell mixtures were washed twice in 1× phosphate-buffered saline and pelleted. Genomic DNA was extracted with the FlexiGene DNA kit (Qiagen, Courtaboeuf, France), and JAK2 V617F quantification was performed using the plasmid based JAK2 Mutantaq kit (Ipsogen SA, Marseille, France) according to manufacturer recommendations.

Throughout the range of dilutions, we found that the measured JAK2 V617F allele burden in the UKE-1/HL60 mixtures was consistently higher than expected (Figure 1). Similar findings were found in another lab. It was found that the JAK2 V617F allele burden was consistently overestimated when Mutantaq kit was used on a range of dilution of the line UKE-1: 2.4% for 1%, 67% for 50% in mean (Cassinat, personal data). One possible explanation for this discrepancy is an abnormal JAK2 copy number in one of the cell lines. We therefore performed conventional and molecular cytogenetic analysis of the HL60 and UKE-1 cells. Karyotypes were determined using standard cytogenetic techniques and described according to the International System for Human Cytogenetic Nomenclature (ISCN 2009). In order to assess JAK2 copy number, fluorescence in situ hybridization with bacterial artificial chromosomes (FISH-BAC) was performed as previously described. A BAC-targeting JAK2 (RP11-982A21 on 9p24.1 band) labeled in SpectrumGreen (Abbott, Rungis, France) and a reference BAC (RP11-115G2 located on 9p21.2) labeled in SpectrumOrange (Abbott) were applied to metaphase preparations and analyzed.

The karyotype of the HL60 cell line was 45,X,del(3)(p27),add(5)(q21.2),der(8;14)(q24;q11),+18,add(9)(q34),add(11)(q23),i(13)(q10),add(17)(p11),+5,add(8)(q24),del(9)(p21),del(10)(p12),del(11)(q22),der(8)t(4;8)(q24;q21),del(12)(p13),+18,add(21)(q11),del(2)(p13),-17,add(5)(q35),+15,del(11)(p11.1),+19,add(11)(p15),add(13)(q14),del(15)(q13),+17,add(12)(q24),der(18)(q22),-20,add(11)(q23),-21,der(9)(q34),+22 (data not shown). FISH-BAC with JAK2 probe found an interstitial deletion of chromosome 9 without JAK2 deletion (data not shown) in HL60 lineage.

Cytogenetic analysis of UKE-1 revealed two clones: a pseudodiploid clone (previously observed in 2008 in our lab) (Figure 2a) and a tetraploid clone (observed for the first time) (Figure 2b).

Figure 1. Measured versus expected JAK2 V617F percentage. Serial dilutions of UKE-1 cells (supposedly homozygous for mutant JAK2 V617F) in HL60 cells (homozygous for wild-type JAK2) were made and the percentage of JAK2 V617F in each dilution was quantified using the JAK2 Mutantaq Ipsogen kit. For each dilution, the measured JAK2 V617F percentage was significantly greater than the theoretical ratio shown by the dotted line (P = 0.002).
JAK2 on abnormal chromosome 9 (Figure 2c). As a consequence, there is an unbalanced ratio of wild-type JAK2 and JAK2 V617F within the quantification standard, leading to an underestimation of the JAK2 mutational load when using this UKE-1 cell line as the standard in the analysis of patient samples.

These results highlight the possibility of clonal evolution of the UKE-1 cell line with an increase in JAK2 gene copy number. The cell line has deviated following culture in our hands because the tetraploid clone was not present when supplying in 2008 and only two copies of JAK2 where detectable at this time. Mechanisms of this genetic instability may be related to an excessive reactive oxygen species production that promotes DNA double-strand breaks and altered repair, as this has been reported in several myeloid diseases and cell lines.10 In MPN, it has been shown that the JAK2 V617F mutation induces an increase in spontaneous homologous recombination leading to a hyperrecombination state.11 JAK2 V617F mutation may also affect p53 response to DNA damage.12

Several clinical studies show that measuring the JAK2 V617F allele burden may be important to assess prognosis and adjust treatment in MPN patients.2,3,13,14 Accurate quantification of allele burden requires a well-defined genetic standard for calibration. Besides, cooperative works are underway to standardize and harmonize the q-PCR techniques. This study shows that using the UKE-1 cell line as a standard for JAK2 V617F quantification must be avoided, as cells in culture can acquire multiple copies of the JAK2 gene.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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