Confocal imaging studies cast doubt on nuclear localization of JAK2 V617F

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To the editor:

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The most common mutation in myeloproliferative neoplasm (MPN)
substitutes a valine to phenylalanine at position 617 of JAK2
(JAK2V617F). Recently, Rinaldi et al reported both a nuclear and
cytoplasmic localization of JAK2 in K562 cells. A previous report
also indicated that JAK2V617F translocates to the nucleus, where
it is proposed to phosphorylate histone H3. These findings remain
controversial, in part because of nonspecific binding of the
commercial anti-JAK2 antibodies used in most studies. To avoid reliance on antibodies, we also studied the cellular localization of
JAK2 wild-type (WT) and V617F expressed as chimeric proteins
fused to visible fluorescent proteins (VFP; derivatives of green
fluorescent protein). Transfections were performed using an Amazka
electroporation method (Lonza AG) with at least a 60% rate of
transfection efficiency in each cell line.

Confocal images of Baf3/EpoR transiently transfected with
JAK2WT-VFP or JAK2V617F-VFP constructs showed both fluores-
cent proteins localized predominantly on the plasma membrane,
with a faint signal in the cytosol and no fluorescent signal in the
nucleus (Figure 1A-B). Addition of erythropoietin did not induce
JAK2 nuclear translocation (not shown). The nuclear dye Hoechst
was used as a positive control to stain nuclei in these experiments.
Similar results were obtained with different plasmids (pcDNA3.1
or pcEF), regardless of the VFP derivative used (mCitrine or YFP).

Z-stack analysis ruled out true nuclear localization of occasional
spots over the nucleus. We also detected no significant staining for
JAK2 in the nuclei of BaF3 cells labeled with primary antibodies
specific for JAK2 (Cell Signaling Technology, #3230), and secondary
anti–rabbit Cy5-conjugated secondary antibodies (Jackson
Immunoresearch Laboratories). These antibodies were also used
in previous studies.

We repeated these experiments in JAK2V617F-positive HEL
cells and in JAK2V617F-negative K-562 cells. Again, there was no
nuclear localization of endogenous JAK2 in either cell type.
Further, JAK2 has a strong plasma membrane localization after transient transfection (HEL), or stable transfection (K562), with the
JAK2WT-VFP or JAK2V617F-VFP expression plasmid. Plots
below Figure 1C and D provide quantitative analysis of the
2 fluorescence patterns, with no overlap between JAK2 and the
nuclear stain. Western blotting experiments following cell fraction-
ation of untransfected HEL cells showed JAK2V617F as a single
band, exclusively in the cytosol/membrane fraction, as was tubulin
used as a positive control. In contrast, phospho-Histone 3 was
found exclusively in the nuclear extract (Figure 1E). These results,
which report the distribution of endogenous JAK2V617F, suggest
that the addition of VFPs does not alter the cellular location of
JAK2 protein.

In summary, our results do not support the conclusions of
Rinaldi et al2 and Dawson et al,3 who reported a significant fraction
of JAK2V617F to be localized to nuclei of cells. Like Behrmann et
al,4 we found JAK2WT, and also JAK2V617F, to be targeted
primarily to the plasma membrane and excluded from the nucleus.
We note that Rinaldi et al2 also evaluated the cellular localization of
JAK2V617F in a group of 10 MPN patients. Nuclear JAK2 was
seen in only 3%-5% of the cells from these patients, which
corresponded to the fraction of CD34+ cells. We conclude that the
bulk of neoplastic cells in MPN are therefore unlikely to rely on
nuclear JAK2 for proliferative signals. It remains possible that
certain JAK2 variants may uniquely target the nuclei of hematopoietic
progenitors, where the functional significance is presently
unknown. We suggest that whenever JAK2 or JAK2V617F are
detected in the nucleus, the JAK2 gene or/and cDNA be fully
sequenced, looking for novel mutations or deletions.

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Acknowledgments: We thank members of the Wilson and Hermouet
laboratories for technical assistance, Unm Cancer Center Microscopy facility,
Radek Skoda (University Hospital Basel) for the gift of BaF3/EpoR cells, and
Serge Haan (University of Luxembourg) for the gift of the pEi-JAK2-WT-VFP
construct. This work was funded by a Leukemia & Lymphoma Society

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Specialized Center of Research (B.W., project PI). F.G., C.C., and S.H. are members of the MPN&MPNr-EuroNet program.

Contribution: F.G., S.H., and B.W. designed research and wrote the paper; F.G., M.S., and C.C. performed research; and F.G., M.S., C.C., and B.W. analyzed data.

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