Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779-90.

Supplementary Appendix

Detection of LOH on chromosome 9p

LOH was determined by fluorescent microsatellite PCR using the following primer sequences (asterisks indicate the primers that were labeled with a fluorescent dye at the 5' end):

D9S1779	sense	*CCCTGCCAGGTGTGCATCTG
	antisense	GTTTCTTCAGGCTCCCTCGTGGCTCT
D9S288	sense	GTTTCTTAGCAACCTCAACAGGG
	antisense	*AATCATCCAGAAAGGCCA
D9S1810	sense	GTTTCTTCTGACAGCAGAGCATCC
	antisense	*CAAGCAAAACTTTTTATTGTGA
D9S1681	sense	GTTTCTTCAGATTCAGCCATGTTC
	antisense	*AGGCAGTTGCACAGATAG
D9S1852	sense	GTTTCTTGAATCACAACATACACCCAC
	antisense	*GAAACATTCTTTTACAAGTAACATT
D9S235	sense	*CTGTATGGAGAGAGAATACG
	antisense	GTTTCTTGGTCTCTCCGGTATACTCA
D9S157	sense	GTTTCTTAGCAAGGCAAGCCACATTTC
	antisense	*TGGGGATGCCCAGATAACTATATC
D9S925	sense	*TGTGAGCCAAGGCCTTATAG
	antisense	GTCTGGGTTCTCCAAAGAAA
D9S162	sense	*GCAATGACCAGTTAAGGTTC
	antisense	AATTCCCACAACAAATCTCC
D9S161	sense	*TGCTGCATAACAAATTACCAC
	antisense	GTTTCTTCATGCCTAGACTCCTGATCC

Gene Copy Number

The following primers were used: 1429 TCCTCAGAACGTTGATGGCA and 1430 GTGCTTTGGTCCCTTTATATGGA for *JAK2*, and 1283 CCATGGACGACGGGT-TTCT and 1284 TGTACAGGACGTAGGAGGGTGA for *LOC221154*. The SYBR Green PCR Master Mix (Applied Biosystems) was used and reactions were analyzed by the 7000 Sequence Detection System (Applied Biosystems). The threshold cycles for *JAK2* and *LOC221154* were simultaneously measured for each sample in duplicates. The relative quantification was performed with the $\Delta\Delta C_T$ method (Applied Biosystems, manufacturer's recommendations) using a calibrator DNA sample from granulocytes of a healthy person.

JAK2 Sequencing

The *JAK2* cDNA was sequenced using the following primers:

Jak2-1	sense	GCGACGGCAAATGTTCTGAA
	antisense	GCCACTGAGCAAAGAGGTAA
Jak2-2	sense	GTGCTGAAGCTCCTCTTCTT
	antisense	CCCTCTTGGTTTGCTTGCTT
Jak2-3	sense	CAGTGGTCAAGAGGGAAACA
	antisense	TGTCTGAGCGAACAGTTTCC
Jak2-4	sense	CAACCTCAGTGGGACAAAGA
	antisense	TTCTCGTCTCCACAGACACA
Jak2-5	sense	GAGCAAGCTTTCTCACAAGC
	antisense	AATTCTGCCCACTTTGGTGC
Jak2-6	sense	TGCAGTGGAGGAGATAAACC
	antisense	TTTAGATTACGCCGACCAGC
Jak2-7	sense	CCTGAAATCCCTACAGCATG

antisense TCATCCAGCCATGTTATCCC

Genomic sequencing of exon 14 of *JAK2* was carried out with primers 1391 GGACAACAGTCAAACAACAATTCTTT and 1392 ACTGACACCTAGCTGTGATC. The DNA fragments were sequenced using the BigDye Terminator v3.1 chemistry (Applied Biosystems).

DNA constructs

The mutant *JAK2* cDNA was amplified by RT-PCR using granulocyte RNA isolated from a patient homozygous for the mutation using the primers 1395 GCGACGGCAAATGTTCTGAA and 1404 AATTCTGCCCACTTTGGTGC. The resulting cDNA fragment was cloned into the pCR 2.1-TOPO vector (Invitrogen). An 1.4 kb *Xho*I-*Nco*I fragment containing the V617F mutation, was introduced to the wild type human *JAK2* cDNA in the expression vector pMSCVpuro (kindly provided by Dr. J. Cools, Flanders Interuniversity Institute for Biotechnology, Leuven, Belgium). The *JAK2* cDNA in the resulting expression construct was verified by sequencing.

Cell Proliferation Assays

Stably transfected BaF3 cells were selected with puromycin (2.5 μg/ml). Transiently transfected UT-7/TPO cells were selected using the MACSelect 4.1 transient expression system (Miltenyi Biotech). The proliferation of the cells grown for 3-4 days in presence or absence of cytokines was assessed using the Cell proliferation kit II XTT (Roche Molecular Biochemicals). To determine the survival of the transfected cells without IL-3, we plated cells that were washed four times in PBS at a density of 10^5 cells/ml in RPMI medium

containing 10% fetal calf serum (FCS). The viability of the cells was determined using the trypan blue exclusion assay (Sigma).