Lara Crow/NPG

TUMORIGENESIS

Order matters

> gene expression patterns depended on the order in which the mutations were acquired

In tumour cells containing many mutations, it is unclear whether mutation order influences phenotypes. Although early mutations might dictate which later mutations can confer a selective advantage, some mutations can occur either early or late in tumorigenesis, suggesting that order might not be important. Ortmann, Kent *et al.* present intriguing evidence that the order of acquisition of two mutations (that can occur either before or after the other) in myeloproliferative neoplasms can affect tumour cell biology and clinical phenotypes.

Approximately 10% of patients with myeloproliferative neoplasms — such as polycythemia vera or essential thrombocythemia — have mutations in Janus kinase 2 (*JAK2*) and TET methylcytosine dioxygenase 2 (*TET2*). The authors sequenced *TET2* in 246 patients carrying the *JAK2*^{V617F} mutation and identified 24 patients who had both mutations. Subclone analyses were carried out to determine the order in which mutations were acquired; these experiments also established that most patients had clonal stability. Within the cohort, 12 patients had first acquired *JAK2* mutations (*JAK2*-first), and 12 had initial *TET2* mutations (*TET2*-first). *TET2*-first patients were significantly older at disease diagnosis than *JAK2*-first patients (this was also confirmed in a second cohort of 24 more patients). Furthermore, *JAK2*-first patients were more likely to have a diagnosis of polycythemia vera than essential thrombocythemia, and had a greater risk of thrombosis.

The two patient groups had different ratios of immature haematopoietic progenitors, so the authors analysed individual haematopoietic stem and progenitor cells (HSPCs) isolated from patients and cultured *in vitro*, and found that proliferation was increased in *JAK2*^{V617F} single-mutant cells, but not in double-mutant cells from either *TET2*-first or *JAK2*-first patients, suggesting that the *TET2* background affects the ability of *JAK2*^{V617F} to promote proliferation. In addition, progenitor formation was decreased by *JAK2* mutations in *TET2*-first patients, but increased by *TET2* mutations in *JAK2*-first patients. Genotyping was consistent with this result, showing that the HSPC compartment contained primarily single-mutant cells in *TET2*-first patients and double-mutant cells in *JAK2*-first patients. Interestingly, *JAK2*-first patients had similar distributions of genotypes throughout the haematopoietic hierarchy, but the proportion of double-mutant cells in *TET2*-first patients increased in later stage erythroid and megakaryocytic progenitors. These data suggest that *TET2* mutations increase the fitness of progenitors, and that *JAK2* mutations promote differentiation into more committed progenitors.

Transcriptional profiling of colonies from four *TET2*-first and three *JAK2*-first patients indicated that gene expression patterns depended on the order in which the mutations were acquired. This analysis identified 10 genes that were upregulated by *JAK2*^{V617F} in *JAK2*-first cells but downregulated by *JAK2*^{V617F} in *TET2*-first cells. Six of these were implicated in proliferation, which is consistent with the observed differences in proliferation in patient-derived HSPCs.

In vitro experiments suggested that the JAK inhibitor ruxolitinib reduced the proportion of both single-mutant and double-mutant colonies in *JAK2*-first patients, but there was no effect on colonies of either genotype from *TET2*-first patients, suggesting differences in sensitivity to JAK inhibition.

It will be interesting to determine whether mutation order affects phenotypes in other tumour types, and the mechanisms by which mutation order influences biology and therapeutic sensitivity.

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