GENETICS

Myeloproliferative neoplasms —order of mutations counts!

Myeloproliferative neoplasms are chronic myeloid diseases that can reflect an early stage of tumorigenesis. Patients with these neoplasms are identified early in the disease trajectory because of the overproduction of mature cells types. *JAK2* is mutated in the vast majority of patients with myeloproliferative neoplasms and *TET2* is mutated in ~10% of patients. These neoplasms offer a unique opportunity to assess disease course over a long period of time, because differences in patient variables (such as age, sex, therapy and genetic background) can be controlled for, thus enabling direct comparison of genetically distinct subclones that develop within a patient.

Despite the enormous progress in our understanding of the accumulation of somatic mutations and the associated genetic complexity of cancer, it is not known whether the order in which mutations in a tumour are acquired have any relevance to disease characteristics. David Kent and coauthors addressed this question by studying mutation order in a set of myeloproliferative neoplasms in which approximately 10% of patients progress to more-severe disease—acute leukaemia. They provide compelling evidence that mutation order influences the biology and outcome of the disease.

The researchers screened the exons of 246 patients with myeloproliferative disorders (consisting of polycythemia vera, essential thrombocythemia, and myelofibrosis disorders) harbouring the *JAK2* V617F mutation, and identified a group of 24 patients in whom mutation order could be established. As Kent explains: “We analysed mutation order by growing individual red cell colonies from patient blood samples (each colony was derived from a single cell) and we established whether no genes, one gene or two genes were mutated. Each patient is a composite of a non-mutant, single mutant

(either the *JAK2* or *TET2* mutation alone), or double mutant (both *JAK2* and *TET2* mutations).” The researchers then surveyed the clinical characteristics of these patients and the molecular and cellular features of the stem and progenitor cells present in patient samples to look for differences between patients who acquired a ‘*JAK2*-first’ mutation and those that acquired a ‘*TET2*-first’ mutation.

Patients in whom the *JAK2* mutation was acquired first had a greater likelihood of having polycythemia vera than essential thrombocythemia, an increased risk of thrombosis and greater sensitivity to the *JAK2*-inhibitor ruxolitinib on colony formation *in vitro*, compared with patients showing the *TET2*-first mutational profile. Furthermore, the order of the mutations influenced cell proliferative response, as progenitor expansion of individual double-mutation haematopoietic stem cells and progenitor cells was considerably different in *TET2*-first patients and *JAK2*-first patients. The proliferation of progenitor cells was enhanced by acquisition of a *JAK2* mutation on a *TET2* normal background, but not on a *TET2*-mutant background.

“This study shows for the first time that the order in which mutations are acquired impacts on the clinical and biological features of disease. Patients who acquire a *JAK2* mutation prior to a *TET2* mutation show signs of disease approximately 10 years earlier, have an increased risk of thrombotic events, and are more responsive to *JAK* inhibitor therapy in cell culture experiments,” explains Kent. The researchers subsequently showed that individual stem cells and progenitor cells with the same genetic mutations (for example, double mutant for *TET2* and *JAK2*) had completely distinct molecular and cellular characteristics that were dependent on the order in which the mutations were acquired.

Kent highlights the true significance of these striking research findings:

“This work establishes a paradigm that mutation order matters in cancer.” Importantly, the approach from this study could help to improve our understanding of how and when cancer cells develop mutations. Crucially, the order in which genetic faults appear can affect how patients respond to different therapies.

The results of this important research illustrate that the order in which mutations are acquired are likely to be relevant in many cancers and could provide useful insights into the origins of cancer. By knowing how patients might respond differently to drugs as a result of the mutational order, we might be able to personalize treatment more efficiently and cost-effectively for patients.

In the future, Kent and his colleagues plan to further define the following: “First, we would like to extend the search to other mutations in the myeloproliferative neoplasms, then to other myeloid malignancies, and ideally other researchers would also search in other cancer types. Second, we hope that a prospective clinical trial could be established to determine whether order impacts on progression to more-severe disease or dictates response to *JAK*-inhibitor therapy.”

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