Metastasis

Major Finding: Whether metastases were seeded mono- or polyclonally depended on cancer site and treatment.

Concept: Metastasis typically occurred two to four years prior to diagnosis of the primary tumor.

Impact: Understanding the genetic underpinnings of metastasis could help develop and time treatments.

METASTASIS HAS MULTIPLE ORIGINS AND OCCURS EARLY IN TUMORIGENESIS

Genetic studies of metastasis, the primary cause of cancer death, have been hindered in part by a lack of paired primary tumors and metastases available for research. In a study aiming to assess the natural history and clonal evolution of metastasis along with the effects of treatment, Hu and colleagues used 457 tumor samples from 136 patients (39 with colorectal cancer, 30 with lung cancer, and 67 with breast cancer), including 99 untreated metastases and 100 treated metastases. In each cancer type, the majority of clonal driver mutations were shared between primary tumors and metastases, whereas subclonal driver mutations were less commonly shared. Private clonal driver mutations were less common across all three cancer types, and less common still were such mutations in untreated metastases, implying that these metastases most often arise from a primary tumor's dominant clone. In contrast, private clonal drivers were found much more often in treated metastases, supporting the notion that clonal evolution is promoted by treatment. Further analysis revealed that the site of metastasis and treatment status were predictive of the prevalence of polyclonal seeding. For example, axillary lymph node metastases were more likely to be of polyclonal origin than distant metastases, perhaps due to multiple dissemination events from primary tumors to these nearby sites, and untreated distant metastases more often arose from polyclonal seeds than treated metastases, possibly reflecting treatment-induced selection. Consistent with the fact that metastasis-private clonal mutations were relatively rare, suggesting that metastatic seeding occurred early, computational modeling predicted that, on average, metastases were seeded two to four years prior to diagnosis of the primary tumor. Collectively, these findings provide meticulously detailed insight into the genetics underlying the metastatic process and highlight the importance of understanding how and when metastasis occurs to better design and apply treatments. ■

Hu Z, Li Z, Ma Z, Curtis C. Multi-cancer analysis of clonality and the timing of systemic spread in paired primary tumors and metastases. Nat Genet 2020 May 18 [Epub ahead of print].

Genome Editing

Major Finding: p53 pathway activation, inactivating *TP53* mutations, and DNA damage were common with Cas9 expression.

Concept: Experiments showed that genetic and chemical perturbation screens may be confounded by this effect.

Impact: These unanticipated sequelae of Cas9 expression should be considered when using CRISPR–Cas9 systems.

CAS9 EXPRESSION CAUSES p53 PATHWAY ACTIVATION AND *TP53* MUTATIONS

CRISPR–Cas9-mediated genome editing is now ubiquitous in biological research and may have clinical applications, but not all of the potential consequences of introducing Cas9 into cells are known. Enache, Rendo, and colleagues investigated the effects of inducing Cas9 expression in 165 human cancer cell lines and found substantial transcriptional differences between Cas9-expressing and wild-type (WT) cells. Specifically, 25 of the Cas9-expressing cell lines exhibited marked activation of the p53 pathway, and this effect was more common in cell lines with WT *TP53* rather than *TP53* harboring inactivating mutations. This observation, along with the well-established role of p53 in the DNA-damage response, raised the possibility that Cas9 induction could be associated with DNA damage. Indeed, Cas9 expression increased the number of DNA double-strand breaks in cells. In line with this finding, inactivating *TP53* mutations emerged or expanded in cell lines expressing Cas9, as would be expected if p53 activity was a hindrance to stable Cas9 expression. In fact, *TP53* landed in the top 4% of genes mutated in response to Cas9 expression. Additionally, a cell-competition assay revealed that expansion of cell populations harboring *TP53*-inactivating mutations was accelerated by Cas9 expression, an effect not seen with other tumor-suppressor genes. Demonstrating the functional relevance of these findings, genetic perturbation screens using CRISPR–Cas9-based gene editing or RNAi methods revealed notable discrepancies in $TP53^{WT}$ cell lines, and Cas9-expressing breast cancer cells were more sensitive to the MDM2 inhibitor nutlin-3 than WT cells. Thus, Cas9 expression may interfere with the interpretation of both genetic and chemical perturbation assays. In summary, this work demonstrates a previously unknown effect of Cas9 expression on p53, potentially producing consequences that should be considered in experiments and future therapeutics that employ CRISPR–Cas9-based techniques. ■

Enache OM, Rendo V, Abdusamad M, Lam D, Davison D, Pal S, et al. Cas9 activates the p53 pathway and selects for p53-inactivating mutations. Nat Genet 2020 May 18 [Epub ahead of print].

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