COMMENTARY

A MASCOT for mosaic analysis

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Genetic mosaicism is a phenomenon that occurs naturally in multicellular organisms, by which genetically diverse cell populations arise through the process of somatic mutation within the organism. Somatic mutations that underlie genetic mosaicism result from intrinsic errors in DNA replication and/or exposure to exogenous genotoxic agents, such as radiation, ultraviolet light, or cigarette smoke. Genetic mosaicism is one of several mechanisms that promote cellular heterogeneity in tissues. Whereas most of the mosaicism that progressively emerges during life is inconsequential, i.e., unable to lead to pathological effects at the cellular and organismal level, a small proportion may produce deleterious effects and lead to pathologies such as cancer (1). In PNAS, Wang et al. (2) report an elegant mosaic system, MASCOT (mosaic analysis system with Cre or Tomato), enabling the temporally controlled introduction of mosaicism and fluorescent markers to a tissue of interest.

Cancer pathogenesis involves a succession of genetic alterations. A small fraction of randomly occurring genetic alterations that increase proliferative capacity, such as oncogenic mutations, confer a growth advantage, resulting in the expansion of the mutant cell clone within its cellular lineage (Fig. 1*A*). Subsequently, subclones within the pool of mutant cells can acquire additional advantageous alterations, resulting in subclonal expansion (3). As such, each round of clonal expansion generates a cell population with increasingly neoplastic and malignant phenotypes, eventually producing clinically manifest tumors that often harbor remarkable heterogeneity. This process is a textbook example of Darwinian natural selection, with each round of somatic mutation leading to the expansion of the fittest clones embedded in a pool of cells that are "wild type" (WT) with respect to the newly acquired mutation (Fig. 1*A*). Clonal hematopoiesis (CH) is a fascinating example of such genetic mosaicism, whereby hematopoietic stem cells acquire somatic mutations that confer the mutant cells a growth advantage, but that alone are insufficient to lead to the development of leukemia. However, patients with CH are significantly more likely to acquire subsequent cooperating mutations that produce acute myeloid leukemia, myelodysplastic syndrome, or myeloproliferative neoplasms (4, 5).

Geneticists have investigated gene function by genetically engineering mosaic animals—typically worms, fruit flies, and mice. Mosaic analysis has become a widely used tool to study the behavior of mutant clones in a WT cellular environment. Distinct from conventional gene knockout, where a gene of interest is inactivated at the beginning of life throughout the organism or in all cells of a particular cell lineage, mosaic models are based on conditional gene knockout techniques that circumvent the lethality caused by global loss of gene function. Furthermore, mosaic analysis provides the opportunity to directly compare the phenotypes of mutant cells with their neighboring WT cells in the same animal. To facilitate the analysis of genetic mosaic animals and examine the mutant clones at high anatomical resolution, many contemporary mosaic systems couple gene knockout to simultaneous labeling with a fluorescent marker that enables one to distinguish and track the mutant and WT cells. Several methods have been employed to create such mosaic animal models. In *Drosophila*, the mosaic analysis with repressible cell marker system allows selective labeling of homozygous mutant cells following Flp recombinase-induced targeted mitotic recombination to analyze phenotypic changes resulting from gene inactivation in high resolution during *Drosophila* development and neurogenesis (6). In mice, a similar approach was used to engineer the MADM (mosaic analysis with double markers) system, which makes use of Cre/loxP-directed interchromosomal recombination during mitosis to generate uniquely labeled homozygous mutant or WT cells in a heterozygous background. As the null allele resides on the same chromosome as the engineered fluorescent reporter, MADM allows extremely tight coupling of the two events—gene knockout and fluorescent labeling of mutant clones (7). However, the MADM system has a number of limitations. Given that gene knockout is

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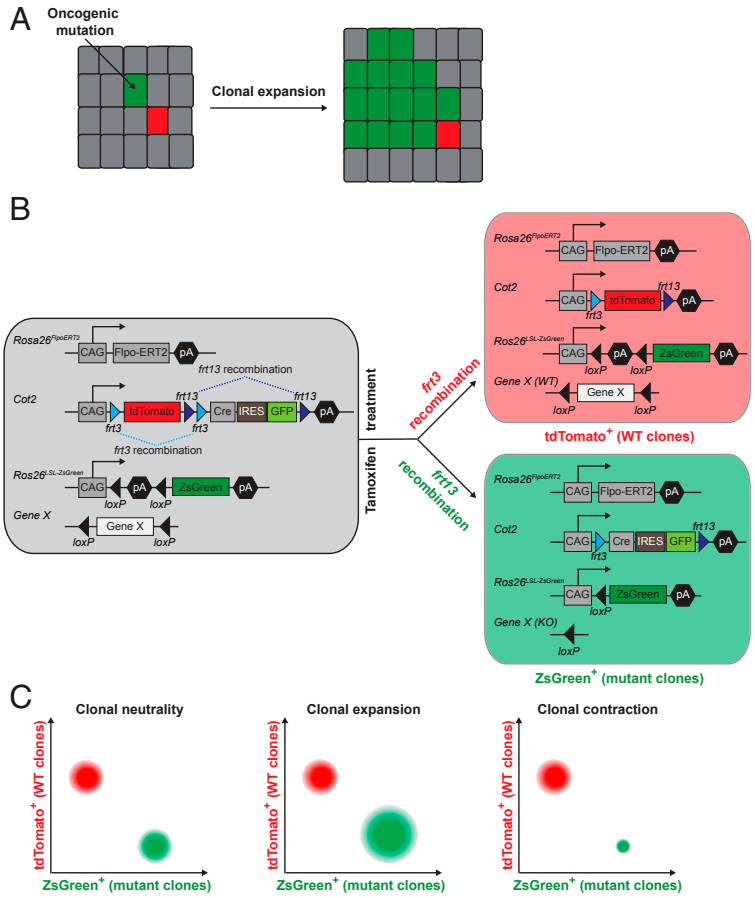

Fig. 1. (*A*) Mosaic tissue where cells with (green) or without (red) genetic alterations are surrounded by wild-type (WT) (gray) cells. Cells with a growth advantage (e.g., following an oncogenic mutation; green) display clonal expansion relative to the labeled (red) and unlabeled (gray) WT cells. (*B*) Components of the MASCOT system showing the two possible outcomes following tamoxifen-induced Flp/frt recombination. (*C*) Clonal growth monitored by quantification of the percentage of ZsGreen$^+$ cells/tdTomato$^+$ cells and displayed in schematized flow cytometry plots.

coupled to mitosis, gene deletion cannot be induced in postmitotic cells. Another limitation of the MADM system is that it requires the targeted introduction of MDAM cassettes at genes of interest, which is time-consuming and expensive. The MASTER (mosaic mutant analysis with spatial and temporal control of recombination) system overcomes these limitations by expression of a constitutive GFP-Cre fusion protein that can simultaneously delete floxed alleles and activate GFP within the same cells (8). The MASTER system thus has the advantage of being compatible with the large number of available mouse strains harboring floxed alleles. However, the system lacks labeling of an internal WT reference population with a fluorescent protein in the same tissue, which presents a challenge to the quantitative analysis of clonal dynamics.

MASCOT generates differentially labeled mutant and WT cells from the same animal using floxed alleles. The authors use MASCOT to investigate CH at an unprecedented resolution, providing insights into CH pathogenesis. The MASCOT system consists of three distinct alleles: 1) *Rosa26-FlpoER*, which initiates mosaicism (activation of Cre or tdTomato) in all cell types upon tamoxifen treatment; 2) MASCOT reporter that drives either the tdTomato marker or Cre in response to Flp-directed recombination; and 3) Cre-dependent *Rosa26-lox-stop-lox-ZsGreen* reporter for tracing Cre-expressing cells (Fig. 1*B*) (2). By combining these three different alleles with loxP-based conditional strains, one can delineate the cell-autonomous functions of any gene in different tissues at a desired time point in embryonic development or postnatal life, including in disease models. By quantifying the percentage of tdTomato$^+$ and ZsGreen$^+$ clones, one can easily determine whether the mutant clone marked by ZsGreen leads to clonal expansion, contraction, or neutrality (Fig. 1*C*).

To generate the MASCOT reporter, the authors placed tdTomato and Cre-IRES-GFP downstream of a ubiquitously active hybrid cytomegalovirus–chicken β-actin–rabbit β-globin (CAG) promoter, such that the coding sequence of each fluorescent protein is flanked by heterologous Flp recombinase target (frt) sites, leading to random excision of either tdTomato or Cre-GFP upon Flp activation. Using a piggyBac transposon, the authors introduced the MASCOT reporter into the mouse genome and selected progeny where the reporter was inserted into chromosome 2. This allele, named Cre or Tomato on chromosome 2 (*Cot2*), was selected for subsequent analysis. In mice harboring *Cot2*, the *Rosa26-FlpoER* allele, and the Cre-dependent *Rosa26-lox-stop-lox-ZsGreen* reporter, the authors observed up to 20 to 30% of labeling following tamoxifen treatment, and demonstrated that tdTomato$^+$ and ZsGreen$^+$ single-positive cells can be induced at similar frequency in a WT background. As a proof-of-principle application of the MASCOT system, the authors chose to target the well-characterized *Id3* and *Tet2* genes. In an elegant set of experiments, they show that the ZsGreen$^+$ cells indeed harbor the desired mutations and that the

phenotypes observed in, for example, *Tet2* knockout clones are consistent with what was observed in a gold standard bone marrow chimera competition experiment. Importantly, by using the MASCOT system, the authors uncovered several phenotypes that have not been previously reported, including a function for *Id3* in restricting the expansion of gut-resident macrophages and differential roles for *Tet2* in myeloid vs. lymphoid lineage development (2).

The MASCOT approach offers significant improvements over preexisting systems: It enables the labeling of both mitotically active and quiescent cells, as opposed to, for example, MADM where mosaicism is restricted to mitotic cells. The incorporation of tdTomato as an independent fluorescent marker for WT cells, as opposed to the labeling of solely the mutant clones with EGFP, provides an internal control to compare the phenotypes of mutant and WT clones in the same animal. There are multiple exciting applications for MASCOT beyond CH, including addressing how additional genetic or epigenetic alterations in subsets of established tumor cells impact tumor progression or drug resistance (2). To do this, one could cross the MASCOT alleles to a tumor model based on a germline cancer-causing mutation, such as the $Apc^{min/+}$ intestinal tumor model, or to chemical carcinogenesis models. Combining MASCOT and such models with floxed alleles of interest would enable the mosaic analysis of gene function in the context of tumor progression.

Although elegant, the MASCOT system is somewhat limited by the time and cost required to generate the desired crosses, as three to four generations of breeding are necessary to produce a sizable cohort of *MASCOT; Rosa26-lox-stop-lox-ZsGreen* mice harboring homozygous floxed alleles. Future efforts, such as the integration of multiple genetic elements into one locus, would greatly improve the utility of mosaic systems and significantly reduce the cost and duration of experiments. Another limitation of the MASCOT system is that mosaicism is induced in virtually all cell types, given the ubiquitous expression pattern of *Rosa26-FlpoER*. Although this issue could be addressed by combining MASCOT with lineage-specific *FlpoER* alleles in the future, very few such *FlpoER* strains are presently available. Finally, given that the ZsGreen reporter and the conditional gene knockout are independent of each other, it is not guaranteed that the knockout and the labeling of the mutant clones would be fully coupled (2).

The power of mosaic analysis models such as MASCOT is considerable, and future work should see the further development and application of such models. Recent additions to the armamentarium of available technologies, such as CRISPR, molecular barcoding, and single-cell omics, will undoubtedly add to the power and versatility of mosaic analysis in the coming years.

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