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Harnessing mismatch repair to model sporadic cancers

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Two mouse models that initiate sporadic tumors seek to recapitulate spontaneously arising human cancers

Human tumors are initiated and progress via mutation and selection of oncogenes, tumor suppressor genes and other facilitating genes^{1,2}. Such tumor evolution is mediated by the interactions of the cancer cells with their immediate tissue microenvironment and by the complex systems biology of the whole organism. Modeling human cancers in animals has been a long-term research goal and has been most successful in the mouse, where innovative technologies have been added to the long-established genetic flexibility of the organism³. However, in many cases mouse models may not fully reproduce the corresponding human cancers as it has been difficult to pinpoint the exact targets and/or pathways that lead to sporadic human tumors; and mouse gene–targeted strategies typically do not replicate the prolonged nature of sporadic mutation and selection. Two studies published in this issue of *Nature Methods* now present mouse systems that model sporadic tumor initiation^{4,5}. However, it is important to note that these models only begin to recapitulate the first steps in the cascade of mutation and selection process(es) that drive cancer progression (Fig. 1).

Both studies use an out-of-frame run of mononucleotides, or microsatellite sequences, within the coding sequence of the Cre recombinase. Sporadic activation of the Cre recombinase occurs by a frameshift reversion mutation (also known as microsatellite instability or MSI)⁶ that is normally suppressed by mismatch repair. The activated Cre may then activate or inactivate genes containing proximal *loxP* recombination sites. The strategies of these two groups then diverge, and their utility as global models for sporadic tumorigenesis may be interrogated.

Akyol *et al.*⁴ placed the frameshifted *Cre* (22GCre) under the control of the human *CDX2P9.5* to homeobox gene promoter and generated transgenic mice (Fig. 1). Expression of *CDX2P9.5* is confined to the caudal region of the developing mouse embryo and is tightly restricted to the distal small intestine, cecum and colon in late-gestation and adult tissues. Such tissue specificity restricts activated Cre to the intestinal tract for subsequent analysis. Because MSI is rare with a normal mismatch repair system in place, eight germline transgenic lines were required to identify one that contained an allele (or alleles) with enough 22GCre transgene copies (~80) to detect measurable sporadic activation. As most human colon tumors are characterized by loss of the adenomatous polyposis coli (*APC*) (gene, the researchers bred the transgenic 22GCre line with mice heterozygous homozygous for an *Apc^{flax}* allele containing *loxP* sites flanking exon 14. Sporadic frameshift activation of 22GCre resulted in pre-neoplastic cells containing an inactive APC protein that developed into adenomatous polyps; ultimately the mice succumbed to florid polyposis by 10–27 d.

Miller *et al.*⁵ engineered a frameshifted *12ACre* that was targeted to disrupt exon 2 of the mismatch repair gene *Pms2* (*Pms2^{cre}*; Fig. 1). The resulting mice carry *12ACre* under the control of *Pms2* promoter. Homozygous *Pms2^{cre/cre}* display a *de facto* mismatch-repair deficiency evidenced by substantially elevated MSI. Breeding *Pms2^{cre/cre}* mice with the *Cre*-

inducible *Gt(rosa)26^{tm1Sor} (Rosa26) lacZ* reporter mouse and visualization with X-gal identified activated Cre-induced blue spots or ribbons of intestinal staining from the crypt base to the villus tips. Thus, the elevated MSI in the mismatch repair-deficient mouse appeared sufficient to activate one or both copies of *12ACre* in these intestinal cells. Breeding the *Pms2^{cre/cre}* mouse with targeted constitutively active *K-Ras^{G12V}* gene containing an upstream transcription stop site bound by *loxP* sites, resulted in sporadic activation of K-Ras^{G12V} and ultimately the formation of lung tumors. Although the authors observed sporadic activation of *12ACre* in intestine, pancreas, liver and muscle, the occurrence of lung tumors most likely reflects the strong penetrance of activated Ras in the lung epithelium. Notably, the number of intestinal blue 'spots' did not change in the mice carrying the *loxP* site-flanked stop-K-Ras^{G12V}, although villi involvement per patch increased and included some irregularly sized villi. Taken as a whole, these observations tend to support a role for mutant Ras in mouse intestinal cancer progression rather than initiation

Although another group had cleverly used a frame-shifted gene to detect and examine sporadic MSI in mouse tissues^{7,8}, the use of the frameshift in Cre to model sporadic tumor initiation is novel. Yet all such models have inherent problems when considering their global use as sporadic initiators tumorigenesis. For example, how many copies of the *22GCre* transgene are necessary to observe significant sporadic frameshift activation of Cre in other tissues that may have different rates of frameshift mutations⁸? By contrast, the *Pms2^{cre/cre}* model suffers from the fact that it maintains an inherent mismatch repair defect, which itself results in tumors (mostly lymphoma) and a 50% survival of ~10 months. If one were to use this largely non-tissue-specific model, the studies would *a priori* be confined to the months before the time that *Pms2^{-/-}* tumors arise. Moreover, one might argue that the inherent mismatch repair defect and associated MSI could become a tumor promotor that encourages an alternate pathway for tumorigenesis, which would not normally depend on MSI. Miller *et al.*⁵ suggest that the largely mismatch repair-proficient heterozygous *Pms2^{cre/+}* mouse may be sufficient to induce sporadic activation of *12ACre* and reduce or eliminate the *Pms2*-dependent tumorigenesis. This supposition is based on the fact that spontaneous Cre activation is still observed, albeit at a 100–1,000-fold lower frequency, in the small intestines at least. However, this idea has not yet been tested.

What might constitute other cassette designs for both controlled and tissue-specific sporadic activation of tumorigenesis? One would wish for the tissue specificity of Akyol *et al.*⁴, as well as the elevated MSI of Miller *et al.*⁵, without the associated mismatch repair-dependent tumorigenesis. Could one transiently suppress mismatch repair long enough to activate the frameshift in Cre? One might imagine the development of a nondisruptive targeted delivery vector with a multiple cloning site upstream of a frameshifted Cre. This would allow easy introduction of a tissue-specific promoter. A *U6* promoter containing an intervening tet-repression site upstream of a mismatch repair gene-specific small interfering RNA or microRNA might be desirable to induce transient inactivation of mismatch repair by providing dietary tetracycline; reducing or eliminating mismatch repair-dependent tumorigenesis and providing timed or stage-specific sporadic activation of Cre.

Harnessing mismatch repair to induce sporadic tissue-specific mutation of oncogenes and tumor suppressor genes may indeed develop into a globally useful model. However, even with a controlled sporadic initiation mechanism, modeling mouse tumors to mimic human cancer progression pathways will still require some expansive genetic manipulations. Regardless, the idea that understanding the sporadic nature of tumor initiation and progression in different tissues will lead to an improved understanding of therapeutic targets is exciting and clearly seems closer on the horizon.

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Figure 1.

Two mouse models designed to recapitulate the initial sporadic mutation step(s) of tumorigenesis. **(a)** Akyol *et al.*⁴ constructed a transgenic mouse containing ~80 copies of a 22GCre driven by a tissue-specific promoter. **(b)** Miller *et al.*⁵ targeted the mouse *Pms2* gene by replacing exon2 with a frameshift 12ACre. In both models, sporadic frameshift reversion to 21GCre or 11ACre result in mosaic activation of the Cre recombinase, which in turn may act on oncogene or tumor suppressor genes containing proximal *loxP* sites. IRES, internal ribosome entry site.