spp. to thrive where *Intestinibacter* cannot, or by directly compromising the integrity of the microbe. Evidence for the latter can be found in a pharmaco-materials study whereby metformin attached to gold nanoparticles had the highest antibacterial and bactericidal activity against pathogens by compromising the cell wall when compared to other nonantibiotic drugs (*9*).

Interestingly, a cohort that was missing from the Forslund *et al.* study, and that would lend insight into the above observations, is the 30% of patients with type 2 diabetes who cannot tolerate metformin due to gastrointestinal distress. This common side effect often prevents patients from taking what is otherwise a relatively safe, effective, and inexpensive drug. Understanding whether these individuals have a distinct microbiome signature of their own that predisposes them to the unpleasant side effects creates an opportunity to alter the offending microbes through diet or other means, such that metformin becomes a viable treatment option. Within the existing metformin-treated cohort in the Forslund *et al.* study, the authors suggest that microbial genes encoding virulence factors and involved in gas production are enriched. However, the associations are unclear, as the implication for inclusion in this cohort is that these individuals are tolerant to the drug. Furthermore, metformin is often prescribed in combination with another antidiabetic drug, and it remains unclear whether the stratification also accounted for combination therapy.

Overall, Forslund *et al.* make a strong case for the importance of stratifying for any ubiquitously prescribed drug in a disease of interest when looking for microbial signatures (see the figure). For prospective studies, exclusion criteria aim to identify such confounding factors and exclude them in the first place. However, for most diseases, it is rare to find enough patients who have not undergone treatment to sufficiently power a study; nor is it ethical to take a patient off a drug that is controlling a disease for the purpose of a study. Therefore, we are left with the less than ideal option of keeping careful patient records and stratifying post hoc. However, what this study truly underscores is the need for more investigation into drug-microbiome interactions and the mechanisms therein. ■

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CANCER

Tracking the origins of tumorigenesis

A zebrafish model allows visualization of embryonic reprogramming during melanoma initiation

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Cancer arises through mutations that transform normal cells into cells that proliferate in an uncontrolled manner, form a tumor, invade the underlying tissue, and then metastasize to distant organs (*1*). Although the genetic events required to induce tumor formation are relatively well known (*2*), the additional early downstream molecular events that are required to reprogram normal cells into cancer cells are still poorly understood. On page 464 of this issue, Kaufman *et al.* report the development of an elegant transgenic reporter system that allows the early steps of tumor initiation to be tracked in situ. They find that oncogene-expressing melanocytes are reprogrammed into neural crest–like progenitors before progressing into invasive tumors (*3*).

Melanomas arise from the transformation of melanocytes, pigment-producing cells, which are derived from neural crest progenitors (NCPs) during embryonic development (*4*). Melanoma formation is associated with mutations in *BRAF*, N-*RAS*, and other oncogenes or tumor suppressor genes (*5*). In zebrafish, melanocytes are responsible for the pigmented stripes located on the scales of the fish. Transgenic overexpression in fish melanocytes of a mutated form of *BRAF* [with the mutation $Val^{600} \rightarrow$ Glu (V600E), the most frequent driver mutation in human melanoma] induced the formation of benign nevi, mole-like features; the concomitant deletion of *p53* promoted the progression of these nevi into malignant melanomas (*6*). Even though all melanocytes expressed the $BRAF^{V600E}$ oncogene and were deficient for *p53*, very few eventually formed melanomas, indicating that other mechanisms besides $BRAF^{V600E}$ and *p53* loss of function are needed for tumor initiation.

To better elucidate these mechanisms, Kaufman *et al.* generated transgenic zebrafish to visualize and characterize the early steps of melanoma formation in situ. They engineered fish expressing a crestin-GFP (green fluorescent protein) reporter gene, which faithfully recapitulates crestin expression during embryogenesis and in melanomas (*7*). Crestin-GFP is invariably expressed, prior to the malignant transition, in all lesions that will eventually progress into invasive tumors; this suggests that crestin-GFP marks a point of no return during tumorigenesis and represents one of the earliest molecular states associated with tumor initiation. The survival and propagation of crestin-GFP–expressing cells after transplantation in the scales of $BRAF^{V600E}/p53^{-/-}$ fish further supports the idea that these early crestin-GFP^{+} patches are already tumorigenic. The reexpression of markers of embryonic NCP cells during melanoma initiation supports the notion that oncogene-expressing cells progressing into invasive tumors are reprogrammed into a state that resembles their embryonic progenitor counterpart. The embryonic reprogramming of adult stem cells during tumor initiation was previously reported during initiation of basal cell carcinoma, the most frequent cancer in humans (*8*).

> ***"...crestin-GFP marks a point of no return during tumorigenesis..."***

The authors identified a 296–base pair minimal promoter/enhancer element that regulates crestin-GFP transgene expression during embryonic development and melanoma formation. This element contains binding sites for multiple transcription factors, including Sox10, Pax3, Mitf, and Tfap2, that regulate NCP specification and differentiation (*9*). Mutations in these transcription factor binding sites decreased the specificity of crestin-GFP transgene expression during embryogenesis, supporting the notion that Sox10 together with Mitf and Tfap2 control crestin-GFP expression during melanocyte development. It will be important to assess

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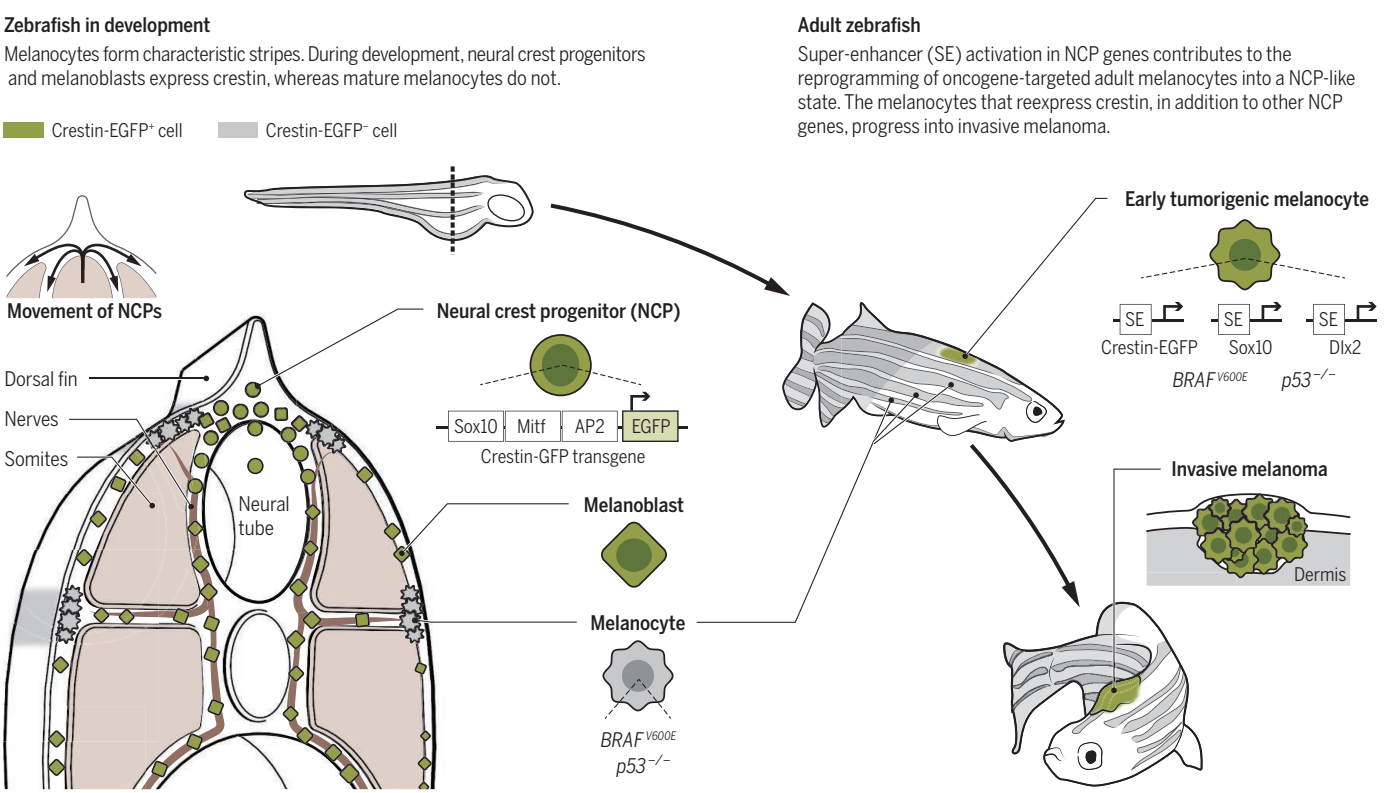

Reprogrammed to be transformed. During zebrafish embryonic development, NCPs express crestin, marked by crestin-GFP (green), which is regulated by specific transcription factors; NCPs then migrate and give rise to melanocytes (gray), which down-regulate crestin expression. In the *BRAF*^{V600E}*/p53* zebrafish, among the oncogene-expressing melanocytes, only a few reexpress crestin-GFP and will invariably progress to invasive melanoma. Super-enhancers (SE) associated with key neural crest genes are activated during the reprogramming of oncogene-targeted melanocytes into a NCP-like state.

whether the same set of transcription factors and enhancer regions controls crestin-GFP during melanoma initiation. Microarray analysis of early crestin-GFP$^+$ patches, compared to adjacent crestin-GFP$^-$ scales, showed an enrichment for NCPs (Mitf, Sox10) and melanoma-expressed genes. Bioinformatic analysis of the genes overexpressed in crestin-GFP$^+$ cells during melanoma initiation revealed a positive correlation with zebrafish and human neural crest gene signatures, strengthening the notion that key elements of the NCP state reemerge at the time of melanoma initiation.

Sox10 is a transcription factor essential for melanocyte development (*4*) and a key regulator of melanoma formation (*10*, *11*). Kaufman *et al.* showed that Sox10 overexpression in melanocytes increased crestin-GFP reporter expression and accelerated melanoma onset, suggesting that Sox10 overexpression promotes the establishment of a NCP state and melanoma formation. In contrast, mutating Sox10 by means of CRISPR/Cas9 gene editing resulted in a delay in tumor onset. Moreover, in the tumors that developed, Sox10-targeted alleles were strongly enriched for noninactivating mutations, suggesting that Sox10 activity is necessary for melanoma initiation. By combining transcriptional, chromatin, and epigenetic profiling, the authors propose that super-enhancer elements associated with neural crest genes, including crestin and Sox10, are active in crestin-GFP–marked preneoplastic lesions and are likely to contribute to the reprogramming of oncogene-targeted melanocytes into a NCP fate during melanoma formation.

Defining more precisely the early steps of tumor initiation and why only some oncogene-targeted cells eventually progress into invasive cancer, whereas others do not, is critical for our basic understanding of tumorigenesis and for cancer prevention and treatment. The reprogramming of oncogene-targeted cells into embryonic-like progenitors is potentially amenable to drug targeting. For example, blocking Wnt signaling during basal cell carcinoma initiation completely prevents embryonic reprogramming and skin tumorigenesis (*8*). Genetic, transcriptional, and epigenetic profiling of tumor cells isolated by fluorescence-activated cell sorting at different stages of tumor initiation will be important to define more precisely how oncogene-targeted melanocytes develop into invasive tumors. For example, tumor-initiating cells may need to accumulate further somatic mutations or other chromosomal abnormalities to progress. Because Sox10 is already expressed in melanocytes and benign nevi, although at lower levels (*12*), it will be important to define the other transcription factors, epigenetic regulators, or signaling pathways that are essential for tumor initiation. The competence of melanocytes to be reprogrammed and to initiate tumorigenesis could either be a stochastic process or be predefined by the fate of the cells initially targeted. Indeed, stem cells might be more easily reprogrammed than progenitors or differentiated cells. We also need to know whether the underlying microenvironment of the oncogene-targeted cells influences tumor initiation. Crestin is unique to zebrafish, raising the question of whether there is an equivalent marker of NCP and early melanoma-initiating cells in mice and humans. ■

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