RESEARCH HIGHLIGHT

Treatment for giant congenital nevi moves a step closer

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Giant congenital melanocytic nevus, defined as a pigmented melanocytic lesion present at birth that will reach a diameter of ≥ 20 cm in adulthood, is associated with severe complications including malignant transformation into cutaneous melanoma, affects the central nervous system (neurocutaneous melanosis), and has major psychosocial impact due to its unsightly appearance. In a study recently published in *Cell*, Choi et al. used three mouse preclinical models to search for new non-invasive treatments, describing a promising strategy that promotes the clearing of these pigmented lesions by the innate immune system.

Nevus (or mole) is a common and benign skin lesion composed of a cluster of melanocytes (cells that make a substance called melanin, which gives color to skin and eyes). These lesions are usually triggered by somatic mutations in the *BRAF* gene that are acquired after birth. They remain relatively small in size and only rarely become malignant. In contrast, giant congenital melanocytic nevi (GCMN) occur in only ~0.2% of births[1] and are often driven by activating mutations in *NRAS* (Q61K or Q61R).[2] This particular subtype can become extremely large in size and, in up to 12% of the cases,[1] may lead to development of melanoma, the most aggressive forms of skin cancer. The surgical excision/removal of these lesions, one of the most effective current treatment options, can only be applied to small lesions and cause extensive scarring.

In a recent study, Choi and colleagues searched for locally applied pharmacologic agents that can be used to avoid surgery.[3]

The authors used three different and complementary preclinical models (Fig. 1a). In one model, they crossed a lox-stop-lox (LSL) $Nras^{Q61R}$ knock-in mutant allele[4] with a transgenic allele expressing the Cre recombinase under the control of the dopachrome tautomerase (*Dct*) promoter. This promoter drives expression of mutant $Nras^{Q61R}$ in embryonic melanoblasts and formation of congenital nevi that exhibit histopathological features of human GCMN. As previously reported in human patients,[5] the mutant melanocytic cells were proliferative (Mki67$^+$) in neonatal nevi before entering a growth arrest phase, as illustrated by lack of expression of Mki67 and upregulation of the senescence marker $p16^{Ink4a}$, in adult mice.

The authors also crossed the LSL $Nras^{Q61R}$ knock-in allele with the tamoxifen-inducible tyrosinase (*Tyr*)-$CreER^{T2}$ allele.[6] In this second model, melanocytic lesions can be induced after birth in a spatiotemporally-controlled manner. Both inducible and constitutive $Nras^{Q61R}$ models exhibited increased melanoma susceptibility, especially on a $Nras^{Q61R}$ homozygous background. Finally, the authors used xenograft models in which human resected congenital melanocytic nevus (CMN) tissue was grafted into immunodeficient SHO mice lacking adaptive immunity, but retaining a partly functional innate immune system.

Having established the suitability of these models, Choi and colleagues demonstrated, as previously shown,[7] that significant depigmentation of the melanocytic lesions can be induced by intralesional injection treatment with several MEK or PI3K inhibitors. Similar effects could even be observed upon topical application of these agents when used in combination.

Importantly, several lines of evidence indicated that nevus regression was accompanied by an activation of the immune system. This observation incentivized the authors to test a method that directly stimulates the immune system, known as hapten-based immunotherapy (Fig. 1b). It is based on the local administration of low molecular weight chemicals such as squaric acid dibutylester (SADBE), which become antigenic when bound to carrier molecules by eliciting "contact hypersensitivity-like" responses.[8] Noteworthily, SADBE is already used by dermatologists as a topical agent for alopecia areata and warts, and is known to induce depigmentation of the skin or vitiligo.[9]

Remarkably, they show that repeated topical application of SADBE successfully reduced the number of congenital nevi's melanocytes and thus both proliferative and growth-arrested lesions. Evidence of local inflammation and recruitment of (M1-polarized) macrophages accompanied nevus cell destruction. Choi et al. demonstrated that the SADBE-dependent clearing of congenital nevi could be prevented upon depletion of (F4/80) macrophages, but not CD4$^+$ or CD8$^+$ T-cells, D19$^+$ B-cells or NK1.1$^+$ NK cells. Accordingly, SADBE treatment of human CMN tissue grafted into SHO mice led to a striking melanocyte decrease in the giant nevus xenografts.

Last but not least, long-term monitoring of a large cohort of *Dct*-Cre;$Nras^{Q61R/+}$ mice exposed to SADBE showed that these mice were fully protected from melanoma development (for more than one year) within the treated body regions. Melanomas only developed in the untreated control cohort (as expected) and in regions of the body that were not exposed to the chemical in the SADBE/experimental cohort.

In summary, this study presents a set of GCMN preclinical mouse models that complement other *NRAS*-driven mouse models[7,10,11] and can be used to study GCMN biology and develop/test new treatment options for children with this lifelong challenging condition. Using such models, the authors identify a promising hapten-based immunotherapy approach. As the authors point out, the future clinical implementation of this strategy remains, however, to be further validated for two main reasons. (1) The study is so far limited to preclinical mouse models and mouse skin differs

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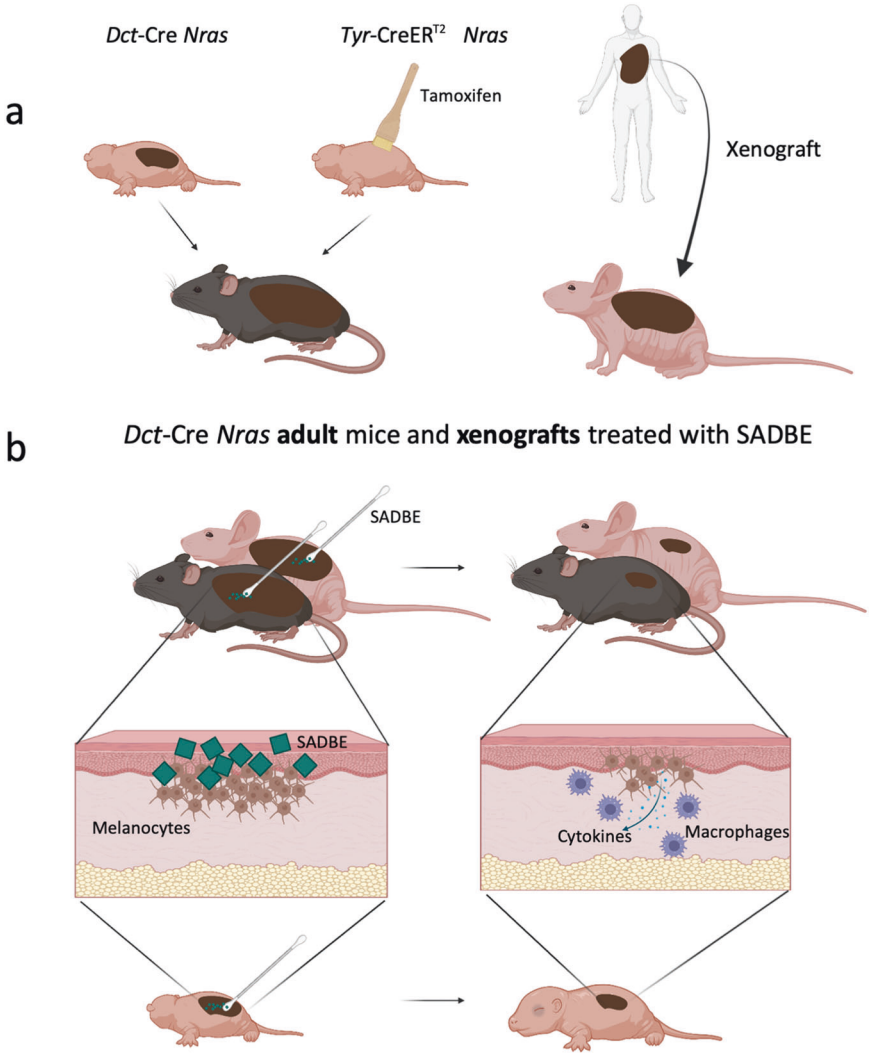

Fig. 1 Modeling and targeting giant congenital nevi in mice. a Representation of the *Tyr*-CreER^{T2} *Nras, Dct*-Cre *Nras*, and xenograft mouse models that recapitulate the giant congenital nevi. **b** Treatment of the adult and neonatal *Dct*-Cre *Nras* mice and the GCMN xenografts in an adult mouse with SADBE significantly diminishes pigmentation. Created with BioRender.com.

structurally from human skin. Importantly, topical exposure to SADBE causes drastic depigmentation in both mice and humans. (2) It remains unclear whether doses of SADBE capable of minimizing toxicity while maintaining efficacy at regressing CMN cells can be found in patients. Irrespectively, this elegant study clearly opens a new window of therapeutic opportunities for GCMN patients that should (and will) be further explored.

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ADDITIONAL INFORMATION

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