Abnormal Fibroblasts Drive Pigmented Skin Lesions in a Mouse Model of Carney Complex

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Carney complex (CNC) is a rare autosomal dominant tumor predisposition syndrome that features focal skin pigmentation, myxomas, endocrine overactivity, and pigmented schwannomas (Carney et al., 1985). Abnormal skin pigmentation is seen in >80% of patients with the syndrome. Although lentigiosis in the central face, lips, and other areas (e.g., inside the mouth, canthus of the eyes, external genitalia) is the most common finding (Figure 1), individuals may also have Spitz, blue, or other nevi (Saggini and Brandi, 2011). Approximately 70% of patients with CNC carry deleterious germline sequence variants in PRKAR1A, which encodes the type 1a regulatory subunit of the cAMP-dependent protein kinase (protein kinase A [PKA]) (Kirschner et al., 2000). As a result of mutation/loss of the regulatory subunit, tumors of patients with CNC have elevated PKA activity. This has been modeled in a variety of mouse tissues, including in the thyroid and adrenal glands (Pringle et al., 2012; Sahut-Barnola et al., 2010). However, there is a paucity of knowledge regarding the origin of pigmented skin lesions and significant ambiguity about myxoma formation, especially because mouse models have not recapitulated these phenotypes.
Clinical Implications

- Cutaneous nevi in Carney complex are caused by non-cell autonomous Prkar1a-null dermal fibroblasts instructing pigmentary melanocytes through paracrine signaling.
- Relevant fibroblasts that express the steroidogenic transcription factor SF1 (NR5A1) are present in the dermis. These cells appear to lose SF1 expression in postnatal mice, whereas SF1 expression persists in fibroblasts in human skin.
- An analogous mechanism may also cause myxomas in Carney complex.

Pigmented skin lesions are not cell autonomous

In their recent article in the journal, Sahut Barnola et al. (2022) report that mice carrying a conditional null allele of Prkar1a developed pigmented skin lesions when gene knockout was driven by the promoter for Sf1 (known formally as Nr5a1). SF1-cre is expressed from embryonic days 10.5–11.5 onward in the pituitary, hypothalamus, and steroidogenic tissues, and it is easily visualized in the gonads and the adrenal glands. Previous characterization of Sf1-cre;Prkar1a<sup>fl/fl</sup> mice, also designated as AdKO<sub>2.0</sub> (Amaya et al., 2022), showed that the mice exhibit adrenal-based hypercortisolism. The authors discount the role of glucocorticoids in the skin phenotype observed in this study because two other similar models generated by this group using other adrenal-specific cre lines (Akr1b7-cre and Aldosterone synthase-cre) also exhibit glucocorticoid excess but lack skin pigmentation.

To explore this observation, the authors perform lineage-tracing experiments using a lineage-marking cre reporter line that causes cells to express an enhanced GFP (EGFP) permanently once cre is expressed. This provides a straightforward way of identifying cells that have undergone Prkar1a excision. Using this approach, the authors identified a population of EGFP+ cells in the dermis of mice during embryogenesis producing cre-marked cells that persisted into adulthood. Staining for SF1 itself in postnatal skin did not reveal the protein, suggesting that SF1 expression is transient and limited to embryogenesis in mice. This observation contrasts with a study reporting that SF1+ cells can be detected in the dermis of adult human skin (Patel et al., 2001). Assuming that these human data are correct, the lack of SF1+ cells in the mouse dermis may reflect interspecies differences in skin structure (as discussed by the authors), or it may be related to technical issues that are associated with detecting low levels of this transcription factor.

The striking finding made by the authors is that cells marked by deletion of Prkar1a in the pigmented lesions are not those that are melanogenic, as judged by a lack of colocalization of EGFP as a lineage marker and Melan-A as a marker for melanocytes. Instead, the SF1 lineage marker colocalized to a subset of Vimentin-positive cells in the dermis. Vimentin is a mesenchymal marker that identifies fibroblasts in most tissue types, including the skin, and approximately 35% of Vimentin-positive cells were marked with EGFP. The authors conclude that pigmented lesions occur in the mice because of crosstalk between Prkar1a-null dermal fibroblasts and genetically normal dermal melanocytes.

Fibroblasts promote melanogenesis through hepatocyte GF and likely through endothelin-3

Fibroblasts play an important role in regulating melanocytes by secreting a number of paracrine factors that bind to their receptors and modulate intracellular signaling cascades linked to melanocyte functions (Wang et al., 2017). The authors focused on three ligands secreted by fibroblasts—hepatocyte GF (HGF), endothelin-3 (EDN3), and Kit ligand (KITLG)—and their corresponding receptors (MET, EDNRB, and C-kit, respectively) that are expressed on melanocytes. Quantitative RT-PCR revealed that expression of the Hgf–Met and Edn3–Ednrb dyads were upregulated in AdKO<sub>2.0</sub> compared with that in wild-type (WT) skin, whereas the levels of KITLG–C-kit mRNA were unchanged. To explore these effects, fibroblasts were isolated from WT and AdKO<sub>2.0</sub> skin and grown in vitro. The authors determined that Prkar1a expression was decreased by about one third in cultured AdKO<sub>2.0</sub> fibroblasts, consistent with the observation that about one third of Vimentin-positive cells expressed EGFP. The authors also showed that expression of Hgf and Edn3 was markedly increased in AdKO<sub>2.0</sub> cells and that HGF levels in the medium were ~16-fold elevated. The levels of EDN3 were not examined. Conditioned medium from the AdKO<sub>2.0</sub> fibroblasts was able to increase melanocyte proliferation and melanosome density in primary melanocytes. These data indicate that AdKO<sub>2.0</sub> fibroblasts produce factors (HGF and possibly EDN3) that affect paracrine signaling from fibroblasts to dermal melanocytes. In this way, closely approximated fibroblasts upregulate gene expression and secrete factors that educate dermal melanocytes and stimulate melanogenesis in lentigines-prone areas.

To confirm the relevance of this observation to humans, the authors obtained pigmented lesions from individuals with CNC. In blue nevi from these patients, elevated HGF staining

Figure 1. Typical pigmentation in patients with the CNC. This photograph shows typical centrofacial pigmentation in a patient aged 25 years with CNC. Note the presence of lentigines (denoted as L) in the periorbital area as well as a darker nevus (denoted as N) on the cheek. This patient also exhibits a small myxoma (denoted as M) on the eyelid, which may be related to pigmentary lesions, as discussed in this article. The patient has graciously provided consent for the use of this photograph. CNC, Carney complex.
was observed in a subset of Vimentin-positive cells in the dermis, although these HGF-bright cells were not observed in typical lentigines. Immunofluorescence suggested that cells that exhibited enhanced HGF levels also lacked staining for PRKAR1A, consistent with the observations in mouse skin.

Cell autonomy, tumors, and PRKAR1A
The other significant implication of this paper is that myxomas, which are also common in patients with CNC, may arise from crosstalk between dermal fibroblasts and melanocytes or other epithelial cell lineages. In mouse myxomas, Prkar1a-null cells comprised about 10% of the cell nuclei, consistent with a noncell-autonomous mechanism and possibly explaining why the bulk of cells from human myxomas may stain for PRKAR1A protein. In contrast, the authors conclude in this paper that adrenal nodules in patients with CNC exhibit cell-autonomous tumorigenesis because secretory adrenal nodules that formed in primary pigmented nodular adrenocortical disease do not stain for PRKAR1A. The same observations have been made in mouse tumor models involving other cAMP-responsive tissues such as the thyroid, where ablation of PRKAR1A from the target tissues was sufficient to produce tumors (Pringle et al., 2012). Cardiac-specific knockout (Yin et al., 2008) of Prkar1a also produced myxomatous changes in muscle, although it was not determined whether this was cell autonomous or nonautonomous. The contribution of cell-autonomous versus that of nonautonomous effects may be tissue- and/or tumor-type specific.

Conclusion
In this study, it has been shown that ablation of Prkar1a in a subset of dermal fibroblasts induces the expression of the promelanogenic factors, HGF and EDN3, which in turn, increases the number of dermal melanocytes and/or stimulates melanogenesis in lentigines-prone areas. AdKO2.0 mice develop cutaneous tumors that are hallmarks of the skin lesions found in patients with CNC. The occurrence of myxomas in sites where blue nevi initially develop suggests a relationship between these pigmented lesions and myxoma formation.

The cell lineage-tracing experiments that are described in this manuscript suggest that the pigmented skin lesions typical of CNC form through the PRKAR1A-dependent activation of a specific subpopulation of dermal fibroblast progenitors that express the steroidogenic transcription factor SF1 during embryogenesis. In mice, SF1 expression is lost postnatally, although this may not be the case in humans. From a therapeutic perspective, it may be worth considering whether antagonists of HGF/MET and/or EDN3/EDNRB may be useful treatments for aggressive skin lesions and/or myxomas, especially whether these arise in the setting of mutations that increase PKA signaling in the fibroblasts.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES