En1 fibroblasts and melanoma

“...embryonic expression of *En1* defines a tissue resident lineage of fibroblasts in the dorsal skin of mice that appears to contribute significantly to the pathogenesis of cutaneous melanoma.”

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Melanoma results from a tumorigenic change in melanocytes of the skin. However, a melanoma is ultimately comprised of multiple cell types, including endothelial cells, fibroblasts, and inflammatory cells. The dynamic interactions between these cells maintain and support the progression of melanoma. Fibroblasts, in particular, play a prominent role in both promoting and maintaining tumorigenesis by laying down a supportive stroma [1]. In normal skin, melanocytes are regulated through E-cadherin mediated interactions. Malignant transformation leads to downregulation of E-cadherin and upregulation of N-cadherin, permitting direct heterotypic interactions between melanocytes and fibroblasts [2]. The transformed melanocytes secrete growth factors such as PDGF, TGF- β , IL-8 and bFGF that exert paracrine control over stroma formation by inducing the activation and proliferation of fibroblasts.

Until recently, the behavior of dermal fibroblasts *in vivo* could only be inferred indirectly. However, lineage tracing in transgenic mice is proving to be a powerful approach for tracking fibroblast function and fate *in vivo*. Traditionally used in developmental biology, lineage tracing has become an essential tool in fibroblast biology because it provides information about

how separate fibroblast lineages behave in the context of organ homeostasis and pathology. In particular, the use of lineage tracing has provided unprecedented levels of information about clonal dynamics and the organization of distinct fibroblast lineages in postnatal skin across both normal physiology and various fibrotic states [3,4].

In 1986, Harold Dvorak posited that tumors are wounds that do not heal, citing similarities between tumor stroma generation and wound healing [5]. We observed that a distinct lineage of dermal fibroblasts, defined by embryonic expression of *En1*, is responsible for the bulk of connective tissue deposition and fibrosis during cutaneous wound healing in mice, and in keeping with Dvorak's thinking, we found that this fibroblast lineage was also primarily responsible for producing the reactive stroma of cutaneous melanoma [4]. This finding may be explained by enhanced responsiveness of *En1* fibroblasts to signals secreted by tumor cells. In support of this notion, we observed that *En1* fibroblasts transplanted intradermally upregulated collagens and markers of fibroblast activation to a significantly greater degree than non *En1* fibroblasts [4].

The stromal response of *En1* fibroblasts in melanoma may also be heavily influenced by direct cell-to-cell contact. Existing literature

KEYWORDS

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- tumor

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demonstrates that melanoma and stromal fibroblasts interact through direct cell-to-cell contacts mediated by both connexins and cadherins [6,7]. The elevated expression of these proteins on the surface of *En1* fibroblasts may provide a possible mechanism by which tissue resident *En1* fibroblasts are preferentially recruited, activated, and maintained at the tumor site. Such a line of inquiry warrants further investigation.

Studies investigating the origin of tumor-associated fibroblasts have pointed to both resident cells and circulating bone marrow-derived precursors. While our results in mice do not unequivocally refute the possibility of circulating cells contributing to stroma formation in cutaneous melanoma, they do establish that such a contribution is minimal at best. We observed that the bulk of connective tissue deposition in melanoma is derived from *En1* fibroblasts and confirmed that *En1* lineage-derived cells were not present in the bone marrow of these mice. We feel that these results provide a substantial answer to this much-debated question. On a related note, many studies have suggested that a circulating fibrocyte population contributes significantly to connective tissue deposition in healing wounds; however, this was also incongruent with our findings.

Efforts to target the tumor stroma have been a subject of recent focus. In the case of melanoma, four broad categories are under investigation: protease inhibitors, receptor tyrosine kinase inhibitors, anti-integrins and anti-fibrosis drugs [8]. Results have been mixed, with some failures attributed to impairment in normal tissue function and host-tumor defense. A more targeted approach requires the identification and characterization of the fibroblast populations primarily involved in stroma formation. In this manner, culprit cells could be effectively eliminated while maintaining a microenvironment permissive to normal tissue

function and immune surveillance. In our mouse study, diphtheria toxin based ablation of *En1* fibroblasts during the early stages of melanoma tumorigenesis led to decreased total tumor size. Although this result is certainly encouraging and suggestive of the supportive role of *En1* fibroblasts in cutaneous melanoma, a more nuanced investigation of the effect of targeted stromal ablation on mortality and metastasis must be performed.

In summary, embryonic expression of *En1* defines a tissue resident lineage of fibroblasts in the dorsal skin of mice that appears to contribute significantly to the pathogenesis of cutaneous melanoma. Early work targeting this lineage through cell-based ablation has shown promise, but clinically viable strategies will require exploiting highly expressed markers on the surface of these fibroblasts. Although we have identified CD26 as a marker for *En1* fibroblasts in mice, this may not be the case in humans. Additionally, mouse studies often do not translate to humans. Therefore, further studies must be performed to identify a human marker for the mouse *En1* fibroblast equivalent and study whether inhibition of this lineage has comparable results. Moving forward, the characterization of additional tissue resident fibroblast lineages in other anatomic locations will also be critical for therapeutic exploitation of the tumor–stroma interaction in other cancers and tissues.

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