Mechanisms of Chemical Cooperative Carcinogenesis by Epidermal Langerhans Cells

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Cutaneous squamous cell carcinoma (SCC) is the most prevalent invasive malignancy with metastatic potential. The epidermis is exposed to a variety of environmental DNA-damaging chemicals, principal among which are polyaromatic hydrocarbons (PAHs) ubiquitous in the environment, tobacco smoke, and broiled meats. Langerhans cells (LCs) comprise a network of dendritic cells situated adjacent to basal, suprabasal, and follicular infundibular keratinocytes that when mutated can give rise to SCC, and LC-intact mice are markedly more susceptible than LC-deficient mice to chemical carcinogenesis provoked by initiation with the model PAH, 7,12-dimethylbenz[*a*]anthracene (DMBA). LCs rapidly internalize and accumulate DMBA as numerous membrane-independent cytoplasmic foci. Repopulation of LC-deficient mice using fetal liver LC-precursors restores DMBA-induced tumor susceptibility. LC expression of p450 enzyme CYP1B1 is required for maximal rapid induction of DNA-damage within adjacent keratinocytes and their efficient neoplastic transformation; however, effects of tumor progression also attributable to the presence of LC were revealed as CYP1B1 independent. Thus, LCs make multifaceted contributions to cutaneous carcinogenesis, including via the handling and metabolism of chemical mutagens. Such findings suggest a cooperative carcinogenesis role for myeloid-derived cells resident within cancer susceptible epithelial tissues principally by influencing early events in malignant transformation.

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INTRODUCTION

The skin and other epithelial tissues are continually exposed to environmental chemicals and toxins, including those with carcinogenic potential. Mutagenic polyaromatic hydrocarbons (PAHs), generated largely as a result of industrial combustion exhaust and wastewater treatment discharges, have become prevalent in the atmosphere, soil, and ground water. Automobile emissions, processed asphalt, coal burning, grilled or charred meats, and tobacco smoke also all contribute substantially to human PAH exposure. Furthermore, a variety of cosmetics and shampoos are made with coal tar and therefore may contain PAHs. As lipophilic compounds, PAHs are readily absorbed by cells; thus, PAH levels within plants and animals may be much higher than the PAH levels detected in their environments (ATSDR/CDC, 2011).

Skin exposure to PAHs may occur by direct contact, as well as by distribution to the skin after systemic absorption. People who smoke tobacco, for example, are exposed to high levels of the PAH benz[*a*]anthracene (Werley *et al.*, 2008) and show a >50% increased risk of developing SCC (Leonardi-Bee *et al.*, 2012). Mutagenic PAHs form stable, bulky covalent DNA-PAH adducts that lead to double-stranded DNA breaks and nucleic base mismatch reads. The cellular handling and metabolism of PAHs are therefore fundamental to understanding early events in chemical carcinogenesis. Most prior studies, however, have focused on the target epithelial cells of transformation, largely ignoring the other locally resident cells that may have substantial influence on these events.

Within the epidermis, a network of dendritic Langerhans cells (LCs) extend to contact the vast majority of basal, suprabasal, and infundibular keratinocytes, in which tumor suppressor gene (e.g., p53) and oncogene (e.g., Ras) mutations may precipitate squamous cell carcinomas (SCCs). Recently, we demonstrated that LC-deficient (huLangerin-DTA transgenic; DTA (Kaplan *et al.*, 2005)) mice are strikingly resistant to two-stage chemical carcinogenesis, and to *Hras* mutagenesis by dimethylbenz[*a*]anthracene (DMBA) (Modi *et al.*, 2012), despite the fact that the keratinocytes of such mice contain all the requisite enzymes for metabolism of DMBA. The abrogation of the requirement of LC for keratinocyte genotoxicity and tumor induction after topical application of the 3,4-diol DMBA metabolite implicated preferential metabolism of DMBA by LC to reactive intermediates, over detoxification. Consistent with this, murine LCs were found to express cytochrome p450 enzyme CYP1B1, but not CYP1A1,

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Abbreviations: DMBA, dimethylbenz[a]anthracene; DTA, huLangerin-DTA transgenic Langerhans cell deficient mouse; E, embryonic day; LC, Langerhans cell; PAH, polyaromatic hydrocarbon; SCC, squamous cell carcinoma; TPA, 12-O-tetradecanoylphorbol-13-acetate

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and human LCs express markedly higher levels of CYP1B1 than of CYP1A1. Thus, we hypothesized that the high ratio of CYP1B1:CYP1A1 within LC biased metabolism of DMBA to mutagenic metabolites, inducing DNA damage and provoking carcinogenesis in adjacent keratinocytes that themselves expressed high levels of both p450 enzymes, but in the inverse ratio (CYP1A1 > CYP1B1).

To further investigate the mechanisms by which LCs facilitate chemical carcinogenesis, we herein studied the kinetics of LC handling of DMBA and demonstrate that LCs rapidly internalize and accumulate DMBA as numerous membrane-independent cytoplasmic foci that are excluded from LC nuclei. We developed a strategy for the repopulation of LC-deficient mice using fetal liver LC-precursors, and reestablished DMBA-induced tumor susceptibility, proportionate to levels of LC reconstitution. By neonatal repopulation of LC-deficient mice with fetal liver LC-precursors isolated from CYP1B1-deficient donors, we reveal that LC expression of CYP1B1 is required for maximal rapid induction of DNA damage within adjacent keratinocytes, and as well as for their efficient neoplastic transformation. Nonetheless, LCs also show the capacity to mediate pro-carcinogenic effects independently of CYP1B1. For example, consistent with a role for LC in increasing the bioavailability of mutagen independently of metabolism, mice repopulated with LC from CYP1B1 −/− donors are more susceptible to DMBA-induced DNA damage compared with LC-deficient mice. In addition, effects on tumor progression attributable to the presence of LC were revealed as CYP1B1 independent. Thus, LCs make multifaceted contributions to cutaneous carcinogenesis via their handling and metabolism of chemical mutagens, as well as their potentiation of tumor progression.

RESULTS AND DISCUSSION

LCs internalize DMBA and facilitate cutaneous carcinogenesis

Keratinocytes possess all the enzymatic requirements to metabolize DMBA to mutagenic forms; yet, application of DMBA followed by weekly promotion with the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) much more readily facilitates tumor outgrowth in the presence of LC (Figure 1a). These data are consistent with mechanisms where LCs enhance DMBA mutagenesis within adjacent epidermal stem cells. Moreover, 24 hours after topical cutaneous exposure to DMBA, we observed LC mRNA expression, surface protein, and morphologic changes indicative of aryl hydrocarbon receptor–mediated activation. In addition to our previous observation of increased CYP1B1 expression (Modi *et al.*, 2012), the LC cell line XS106 also displayed mRNA levels increased for CD80 and CD207 and decreased for TLR4 (Figure 1b). As well, 24 hours after percutaneous application of DMBA, isolated LC displayed increased CD86 surface protein (Figure 1c) and, *in vivo*, converted from a dendritic to a more rounded morphology (Figure 1d and e). These changes are all consistent with that previously reported for aryl hydrocarbon receptor xenobiotic response element activation in myeloid-derived cells (Simones and Shepherd, 2011; Ilchmann *et al.*, 2012). Thus, we sought to better understand the characteristics of LC internalization of DMBA, and

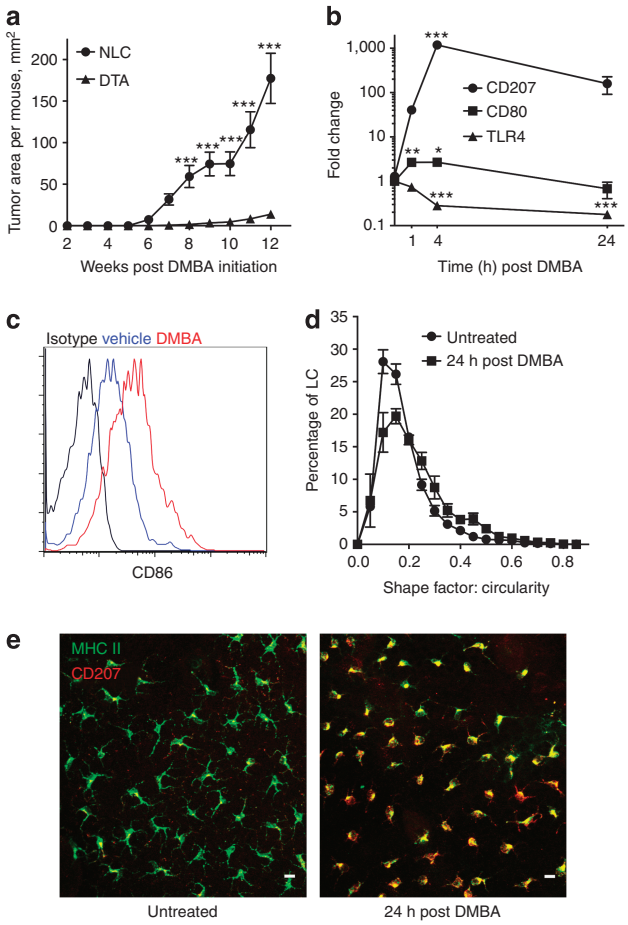

Figure 1. Langerhans cells (LCs) facilitate dimethylbenz[*a*]anthracene (DMBA)-initiated cutaneous carcinogenesis and display phenotypic and morphologic changes in response to DMBA exposure. (**a**) Carcinogenesis initiated in LC-intact (NLC) and LC-deficient (DTA) mice by application of DMBA (400 nmole) followed by twice weekly 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (20 nmole, $n = 13$–15 mice per group, $^{***}P \leqslant 0.001$). (**b**) XS106 was treated with 64 μM DMBA and changes in gene expression monitored by quantitative real-time PCR, $^{*}P < 0.05$, $^{**}P \leqslant 0.01$, $^{***}P \leqslant 0.001$. (**c**) FVB/N dorsal bodywall was treated with vehicle (acetone) or 400 nmole DMBA, and epidermal cell suspensions were prepared 24 hours later and stained for flow cytometric analysis. Population shown is gated on CD45+ CD207+ cells. LCs from untreated (not shown) and vehicle-treated mice had similar CD86 expression (ΔMFI = 20.4 vs 17.3; P = n.s.). LC CD86 expression increased following DMBA treatment (ΔMFI = 54.4, $P = 0.0013$ vs vehicle, $n = 3$ mice/group). (**d**, **e**) FVB/N dorsal ears were treated with 35 nmoles DMBA, and epidermal sheets were prepared 24 hours later and stained with CD207 (red) + MHC II (green). LCs were identified and their shape factor (circularity ranging from 0 to 1, circle = 1. Mean ± SE untreated 0.1825 ± 0.0019, DMBA treated 0.2254 ± 0.0028, $P < 0.0001$) measured using Volocity 6.2, $n = 3$ mice per group. Scale bar = 10 μm.

monitored XS106, as well as isolated murine epidermal LC, by time-lapse two-photon microscopy after *in vitro* exposure to DMBA. The multi-ringed structures of PAHs provide predictable spectral fluorescence properties, and DMBA fluoresces in the 440 nm wavelength range upon excitation with UV light. Two-photon microscopy systems using pulsed femtosecond laser exposures provide high sensitivity with less