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081 Modulation of the aryl hydrocarbon receptor by adipose tissue: Implications for skin carcinogenesis

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Identifying signaling networks in melanoma tumors that promote the uncontrolled growth of BRAF mutant melanocytes

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Melanocytic nevi (AKA moles) are benign proliferations of melanocytes induced by the same BRAF mutation that initiates melanoma. BRAF mutant melanocytes in nevi eventually undergo growth arrest whereas melanomas do not, raising the question that how do melanoma tumors escape growth arrest and continue to progress uncontrolledly? In answering this question, we recently determined that simply crossing the BRAF mutation from black mice into an isogenic albino background was sufficient to induce melanoma formation. BRAF mutant albino tumors did not have any new mutations, indicating that factors that influence tumor formation in these models must be epigenetic. We next sought to define signaling networks present in BRAF mutant albino melanomas that are absent in BRAF mutant black nevi. Single cell RNA sequencing analysis of melanoma tumors identified discrete macrophage and fibroblast populations that were observed in BRAF induced melanomas but not in BRAF induced nevi. Specifically, we identified a distinct population of melanocytes with a Schwann cell-like gene expression signature (termed S cells) that have a similar gene expression pattern to the neural crest stem cells that are thought to regulate BRAF inhibitor resistance. These S cells were observed in both BRAF and BRAF PTEN melanoma tumors as well as human melanomas. Intriguingly, the S cells present in nevi had a different gene expression pattern than the S cells observed adjacent to melanomas. Taken together, these results indicates that discrete cells in the melanocytic lineage promote tumorigenesis by secreting growth promoting signals that allow BRAF mutant melanocytes to overcome growth arrest.

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Current treatment options for patients with unresectable cutaneous melanoma

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Cutaneous Melanoma (CM) remains the leading cause of skin-related mortality in the U.S. The U.S. Surveillance, Epidemiology and End Results database reports 5.6% of all new cancer cases in 2021 attributed to CM, with incidences rising annually since 2009. Several subtypes of CM are thought to arise from early driver mutations in BRAF, RAS, and NF1 genes resulting in unopposed activation of MAPK and PI3K signaling pathways, unregulated growth and malignant transformation of melanocytes. Due to the metastatic potential of CM, emphasis on systemic treatment has been an area of interest when surgical resection is insufficient for curative prognosis. Our case involves a 65 year old patient who presents for management of a 8.5mm tumor with ulceration and multiple associated subcutaneous satellite lesions on his left scapula. At his most recent follow up, he has experienced a 5kg weight loss, fatigue, shortness of breath, and non-tender axillary lymphadenopathy. Excisional biopsy revealed nodular-variant malignant melanoma, and immunoperoxidase studies were positive for SOX10 and Melan-A, confirming the diagnosis. CT of the chest showed enlarged bilateral axillary lymph nodes with areas of necrosis, as well as punctate bilateral pulmonary nodules, consistent with metastasis. Based on the American Joint Committee of Cancer classification, our patient has T4N2M1b consistent with stage IV melanoma and a 53% one year prognostic survival. Due to his unresectable tumor, systemic treatment using immune checkpoint inhibitors or targeted therapy is indicated. As of 2011, the FDA approved immunomodulator therapy, such as CTLA4 inhibitors and PD1 inhibitors, which promote CD8+ T cell death. Of these, nivolumab, a PD1 inhibitor, has been shown to have increased response rates (43% vs 19%), and 3 year survival (52% vs 37%) than CTLA-4 inhibitors, such as ipilimumab. Due to these benefits and lower side effect profile, we chose nivolumab as the drug of choice for our patient.

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Modulation of the aryl hydrocarbon receptor by adipose tissue: Implications for skin carcinogenesis

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The aryl hydrocarbon receptor(AhR) is a ligand-activated transcription factor that responds to chemical carcinogens. An endogenous AhR agonist, 6-Formylindolo[3,2-b]carbazole, is produced following ultraviolet light(UV) absorption by tryptophan. Epidemiological studies show a correlation between exposure to AhR agonists and non melanoma skin cancer(NMSC). As an example, AhR activation promotes metabolism of pro-carcinogens to carcinogens such as the metabolism of benzo(a)pyrene to benzo[a]pyrene diol epoxide(BPDE). There are differing epidemiological studies which explain the relationship between obesity and NMSC—some demonstrate a positive association while others demonstrate a negative association. However, evidence supporting an inverse correlation is relatively weak and heavily confounded by UV exposure among body mass index groups. Our objective was to determine the role of AhR in adipose tissue-stimulated malignant transformation. RNA-seq analysis demonstrated that secretions from adipocytes induced AhR-regulated phase I metabolizing enzymes in a non-tumorigenic, mouse epidermal cell line, JB6 P^+, including CYP1A1 which metabolizes B[a]P to BPDE. Phase II detoxifying enzymes remained unchanged or reduced suggesting xenobiotic metabolism by adipocyte secretions. Together, B[a]P and adipocyte secretions induce malignant transformation, assessed by the JB6 P^+ soft agar clonogenic assay. Primary human keratinocytes, cultured with adipocyte-conditioned medium for 24 hours, demonstrated elevated AhR protein levels and induction in CYP1A1 and CYP1B1 mRNA(3.6 and 10.2-fold)compared to the media control cells. Understanding how adipocytes modulate AhR activity in skin with or without environmental AhR ligands will lead to new therapeutic strategies to prevent epidermal cell transformation.

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***In vivo* tracking of clonal dynamics shows three phases of UV-induced skin carcinogenesis**

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We hypothesize that skin cancer emerges from a combination of mutations and tissue disruption. We characterize clonal dynamics and transcriptional signatures during skin carcinogenesis using lineage tracing. For 3 months, we UV-irradiated fluorophore expressing K14Cre-ERT2 Confetti mice. Clone volumes were computed from 3-D digitized confocal z-stacks. scRNAseq was used to compare UV-exposed (EXP) epidermis, non-exposed (NON), and tumors. Over 6 months from the UV initiation, we generated 914 serial images of the EXP/NON skin. We analyzed 16,135 clones from the EXP and 21,506 clones from the NON skin. Median clone size did not differ between UV treatments and did not change with time. However, the mean size of EXP clones were 50% larger than NON ones, with a >6-fold increase in variance. The number of large EXP clones increased dramatically by months 3-4, plateauing at months 5-6. scRNAseq of EXP/NON epidermis and tumors revealed 16 clusters corresponding to different differentiation states. We observed dynamic changes to the clusters when progressing from normal to chronically exposed skin, and then to tumors. Development of large clones preceded high penetrance mutations. EXP clusters were associated with expression of cystatins (*Scfa 3*, *BC100530*), and alarmins/proliferative keratins (*Krt16*, *Krt6a*). Flr-expressing keratinocytes from large EXP clones exhibited altered keratinocyte differentiation, inflammation, and upregulation of metabolic regulators. These results show an initial disruption of tissue (2-3 months) permitting the emergence of unusually large clones (phase 1), followed by inter-clonal competition and mutational buildup (phase 2) and the emergence of lesions (phase 3).

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Defining the immune response in basal cell carcinoma spontaneous regression

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In the US, more people are diagnosed with basal cell carcinoma (BCC) than any other cancer, but treatment of advanced tumors is frequently complicated by drug resistance. Interestingly, 20-29% of human BCC tumors are reported to shrink spontaneously in the absence of treatment through an unknown mechanism. With case studies suggesting a role for immune activation in this tumor regression, we hypothesize that T cells are recruited to growing BCCs to promote spontaneous tumor shrinkage. The inducible Gli1CreERT2; Ptch fl/fl transgenic BCC mouse model shows microtumor growth in the skin followed by significant reduction ($p<0.001$) in tumor area after 10 weeks. Using this model of regression in the absence of treatment, we assess the role of immune system interactions in promoting this mechanism and as a potential therapeutic target. Analysis of immune infiltration, bulk and single-cell RNA-sequencing of tumor-bearing skin shows evidence of an inflammatory neutrophil response during tumor growth followed by a transient cytotoxic T cell response. By assessing T cell necessity and the sufficiency of upregulated cytokines in inducing anti-tumor responses, we aim to define the immune response against BCC tumors and determine the role of effector T cells in regulating regression. This study will identify the cell populations, pathways, and interactions critical to the tumor regression mechanism and BCC pathogenesis. This will provide candidate therapeutic targets to enhance tumor regression, thus facilitating the development of alternative therapies to treat advanced BCCs.

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mRNA methylation in skin tumorigenesis and therapeutic resistance

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Analogous to DNA and histone modifications, RNA molecules are chemically modified, the study of which gives rise to the field called epitranscriptomics. Among these modifications, N^6-methyladenosine (m^6A) RNA methylation is the most abundant internal modification in messenger RNA (mRNA) and non-coding RNA in eukaryotic cells, which regulates RNA metabolism, including RNA decay, translation and nuclear processing. However, the regulatory and functional role of m^6A RNA methylation in skin cancer remain poorly understood. Recently we demonstrated that the m^6A mRNA demethylase FTO promotes tumorigenesis and resistance to immunotherapy in melanoma. In addition, using skin-specific conditional knockout mouse models, in combination with in vitro cellular models, recently we have demonstrated a pivotal role of m^6A RNA methylation in skin cancer development. FTO as an N^6-methyladenosine (m^6A) RNA demethylase is degraded by selective autophagy, which is impaired by low-level arsenic exposure to promote tumorigenesis. We found that in arsenic-associated human skin lesions, FTO is up-regulated, while m^6A RNA methylation is down-regulated. In keratinocytes, chronic relevant low-level arsenic exposure up-regulated FTO, down-regulated m^6A RNA methylation, and induced malignant transformation and tumorigenesis. Moreover, in mice, epidermis-specific *FTO* deletion prevented skin tumorigenesis induced by arsenic and UVB irradiation. Targeting FTO genetically or pharmacologically inhibits the tumorigenicity of arsenic-transformed tumor cells. We identified *NEDD4L* as the m^6A-modified gene target of FTO. Finally, arsenic stabilizes FTO protein through inhibiting p62-mediated selective autophagy. Our study reveals FTO-mediated dysregulation of mRNA m^6A methylation as an epitranscriptomic mechanism to promote arsenic tumorigenicity and may open up new opportunities to reduce skin cancer burden.