approach to quantify overdiagnosis is a randomized controlled trial of screening; however, it is unlikely that such a trial will ever be conducted owing to the sample size and long follow-up needed to detect mortality benefits (most patients with melanoma can already expect excellent long-term survival); a screening trial powered to detect an improvement in long-term survival would need to be massive in scale. New statistical methods that allow analyses of prospective cohort studies to mimic the characteristics of randomized controlled trials may be able to fill the gap.

There is no doubt that the detection of melanoma is undergoing a frameshift, in part due to new technologies, including dermoscopy, total-body photography, and artificial intelligence. Further studies are needed to understand the effects of opportunistic screening so that clinical practice is predicated on evidence of the benefits and harms of different screening strategies. We need better tools to distinguish between indolent lesions and those likely to progress; here also lies the challenge of reconciling the outwardly different perspectives of clinicians (striving for sensitivity and patient benefits) and epidemiologists (striving for specificity and population benefits).

The findings of Kurtansky et al. (2021) highlight the increasing burden of melanoma in the United States and globally. Early detection is only one approach for reducing disease burden, and we must not forget that cutaneous melanoma is a largely preventable disease. Indeed, one might argue that these findings should remind us to focus efforts on prevention as the most cost-effective control measure and as one that has the potential to simultaneously mitigate the problems of rising incidence and overdiagnosis.

CONFLICT OF INTEREST
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REFERENCES

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States and Fates of Skin Fibroblasts Revealed through Chromatin Accessibility
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Adult mammalian skin harbors a myriad of stromal populations, but it is only recently that we have been able to untangle their developmental origins, lineage-specific functions, and fluctuations in influence during skin development, aging, and injury/repair. Thompson et al. (2022) now unveil the epigenetic status underlying the heterogeneity of skin stromal cells. They also show that neonatal skin stromal cells exhibit chromatin landscapes that shape two epigenetically distinct lineages: dermal papilla and adipocyte fates.

Stromal cells of adult skin reside in specialized niches and exhibit distinctive functions. For example, papillary fibroblasts are positioned directly beneath basal keratinocytes (KCs) at the border between the dermis and epidermis. This close interaction of papillary fibroblasts with KCs is particularly important for the generation of hair follicles during development and later for the progression of hair growth cycles. Papillary stromal cells, particularly in perinatal stages,
have the capacity to differentiate into follicle-associated dermal papilla, dermal sheath, arrector pili muscle, and papillary fibroblasts (Driskell et al., 2013). On the contrary, reticular stromal cells reside within the deeper layers of the dermis and, throughout skin maturation, may differentiate into intradermal and hypodermal adipocytes. This stromal population also serves as a source of reticular fibroblasts that may differentiate into myofibroblasts during skin injury (Driskell et al., 2013). Within the adult hypodermis is a specialized connective tissue layer termed fascia, wherein a specialized population of fascia fibroblasts resides. Fascia fibroblasts also serve as an important source of myofibroblasts and uniquely mobilize loose connective tissue from the fascia microenvironment to sites of injury, supporting the healing of open wounds (Correa-Gallegos et al., 2019).

“Understanding the transcriptome and chromatin architecture profiles of the cell types in neonatal skin will provide a platform for understanding how to transform aging adult skin to be regenerative.”

The specialties of each of these stromal populations reflect their gene expression signatures. Taking full advantage of single-cell RNA sequencing (scRNAseq), recent studies have described transcriptionally defined populations that mirror the classical stromal cell types in the skin (Joost et al., 2020; Philippeos et al., 2018). These new technologies have also faithfully predicted the differentiation trajectories taken by the papillary fibroblasts observed in vivo (Phan et al., 2020).

**Early differentiation of skin stromal cells**

Gene expression is intimately linked to open chromatin landscapes, in which chromatin accessibility of gene promoters translates into an active gene expression. Detection of open chromatin loci using a single-cell assay for transposase-accessible chromatin sequencing (scATACseq) coupled with scRNAseq has shown that chromatin accessibility is a major driver of cell differentiation (Ma et al., 2020). Thompson et al. (2022) now report a study implementing parallel chromatin accessibility and RNA single-cell omics methodologies in mouse neonatal skin as a means to better understand the functional diversity of stromal cells that develop during skin maturation. Thomson et al. (2022) show that when it comes to neonatal skin stages, the gene expression signatures currently used to discriminate between functionally mature fibroblasts are not supported by chromatin states at this immature stage. Instead, they identify two early branches of cellular commitment that include dermal papilla and adipocytes, whereas all other fibroblastic fates remain uncommitted.

Supporting their claims, Thompson et al. (2022) investigated several markers that are currently used to distinguish between adult papillary and reticular fibroblasts such as Dpp4/CD26, Ly6a/Sca1, and Dlk1. First, Thompson et al. (2022) used computational clustering of scRNAseq data to show that distinct populations express the mRNA-associated markers as expected. Intriguingly, this was not always the case for open chromatin as analyzed through the scATACseq data. The authors observed that despite the markers’ restricted expression at the RNA level, chromatin accessibility for these specific markers was homogeneous for all fibroblast populations. This general accessibility, in what are deemed to be restricted markers, was suggested by the authors as an explanation behind the discrepancies in the reported expression of some of these markers in adulthood. Particularly, Dpp4/CD26 has been associated with fibroblasts of deep dermal layers during adulthood (Rinkevich et al., 2015), whereas it is expressed in upper papillary fibroblasts at neonatal stages. The authors point out that correlating the scRNAseq and scATACseq datasets revealed a more conservative separation between papillary and reticular lineages.

In silico correlational analysis indicated that chromatin landscapes in neonatal skin stromal cells funnel their potential fates into either dermal papilla or adipocyte differentiation paths (Figure 1). This is consistent with previous in vivo findings with chamber transplantation assays that use sorted papillary or reticular/hypodermal fractions, where the
capacity to generate hair follicles was restricted to the papillary lineage (Driskell et al., 2013). Remarkably, the chamber assay successfully reconstitutes new hair follicles only when using neonatal but not adult papillary fractions. The findings by Thompson et al. (2022) of an early bimodal only when using neonatal but not adult papillary fractions. The findings by Thompson et al. (2022) of an early bimodal lineage-chromatin separation into dermal papilla and adipocytes provide an explanation for the requirement of neonatal populations in the chamber assay because neonatal papillary fibroblasts possess the superior capacity to differentiate into hair follicle-sustaining populations owing to their still accessible chromatin. Intriguingly, reports indicate that dermal papilla cells promoting hair follicle neogenesis occurring in large wounds originate from nonpapillary sources (Abbasi et al., 2020). Although these are rare occurrences (Phan et al., 2021), these experimental findings indicate that lineage-specific chromatin landscapes might not be anatomically restricted in the adult skin.

Chromatin accessibility and regenerative versus scar-type repair

“Understanding the transcriptome and chromatin architecture profiles of the cell types in neonatal skin will provide a platform for understanding how to transform aging adult skin to be regenerative.”

When the authors analyzed myofibroblast-related genes, they noticed that despite showing a lack of gene expression in the scRNAseq data, scATACseq profiles indicate open chromatin accessibility. This discrepancy between chromatin accessibility and gene expression is explained as resulting from an immature state of fibroblasts in the neonatal skin.

Although this study was performed on newborn skin, recent reports from adult skin indicate that the chromatin accessibility for myofibroblast markers is restricted to myofibroblasts and is not universal to other fibroblasts (Foster et al., 2021). The authors argued that these differences in chromatin accessibility might result from maturation that is still undergoing in the chromatin landscape of neonatal skin. The findings by Thompson et al. (2022) suggest that conversion into myofibroblasts is not restricted at this stage of development and that all fibroblast populations have the capacity to transit into wound myofibroblast at neonatal stages, contrary to those at adult stages where only fibroblasts from deeper layers contribute to functional myofibroblasts in wounds (Correa-Gallegos et al., 2019; Driskell et al., 2013). The open chromatin profiles of markers of myofibroblasts and mature fibroblasts suggest a plastic state in stromal cells during neonatal stages.

Alternatively, it is plausible that functionally committed populations do already exist at newborn stages that have not yet been segregated anatomically to upper versus lower dermal compartments. Indeed, we and others have shown that En1-lineage-positive fibroblasts (EPFs) are the primary scar-producing fibroblasts in adult skin (Rinkevich et al., 2015). Embryonic lineage tracing of EPFs into adulthood shows that they are present within both papillary, reticular, and fascia compartments and are not anatomically segregated. It could well be that chromatin accessibility studies in combination with lineage tracing methods rather than the use of classical adult markers would reveal separate chromatic landscapes that mimic adult fibroblast functions at these early stages.

Injuries in neonatal stages result in more regeneration than those in adults. Previous reports also showed that newborn skin wounds are populated prominently by papillary fibroblasts, whereas adult skin wounds are populated by fibroblasts from deeper skin compartments such as reticular and fascia compartments (Phan et al., 2021). The plasticity facilitated by open chromatin in neonatal fibroblasts might be in part responsible for the prevalence of a more regenerative and scarless phenotype seen in neonatal skin wounds. Conversely, the suggested chromatin maturation occurring during development would limit the capacity of stromal populations to transit into regenerative states, resulting in a scar-type repair seen in reticular and fascia compartments. Understanding and clinically exploiting the precise chromatin configurations in skin stromal cells might become a powerful approach to treat and prevent pathological scarring and chronic nonhealing wounds in the clinic.

Thompson et al. (2022)’s report applying parallel single-cell multomics reveals unprecedented intimate details of fibroblast plasticity that shapes fibroblast heterogeneity. This represents an in-depth look into the early commitment steps of skin stromal cells. Additional studies using multomics complementing approaches such as this are needed to fully reveal the biological foundations of skin fibroblast heterogeneity as the skin matures into adulthood.

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REFERENCES

In their article in the *Journal of Investigative Dermatology*, Conway et al. (2021) describes the CpG island methylator phenotype (CIMP) subclass occurring in 16% of invasive primary melanomas (Figure 1). Cutaneous melanoma is one of the deadliest forms of skin cancer in North America, Europe, and Australia. Early diagnosis and prognostic classification of primary melanoma are of critical importance to reduce morbidity and healthcare costs and improve survival (Siegel et al., 2021). The utilization of molecular omics in the study of primary melanomas is rapidly growing, and the molecular factors involved in the progression of primary melanoma are being delineated. Understanding these molecular alterations may improve the early identification of patients who will eventually develop aggressive metastatic disease. This would inform clinical decisions on diagnosis, prognosis, and treatment strategies. To date, the major emphasis of molecular omics analyses of primary melanomas has been related to specific sequence variations. However, only a single nucleotide variation, the *BRAF* V600E single nucleotide variation, occurs with an average frequency >50%, and other single nucleotide variation's are much less frequent in primary melanomas. There is a need for a more useful molecular omics assessment that has more generalized clinical utility for predicting poor outcomes of invasive primary melanomas.

Understanding CIMP subtype in early-stage melanoma lesions

Conway et al. (2021) performed genome-wide methylation analysis using the HM450K BeadChip Illumina array (Illumina, Carlsbad, CA) and classified 89 invasive primary melanomas into three methylation subclasses integrated with clinical data: low methylation, intermediate methylation, and CIMP. Briefly, CIMP is a pattern of extensive DNA hypermethylation on cytosine in CpG islands of the human genome (Greenberg et al., 2014). The CIMP epigenetic phenomenon has been reported in the past as a universal pancancer epigenetic event that becomes more prominent in advanced stages of various cancers (Miller et al., 2016). Conway et al. (2021) showed that patients with the CIMP subclass of invasive primary melanomas had a significantly worse melanoma-specific survival when considering known prognostic factors (hazard ratio = 11.84; confidence interval = 4.65–30.20). A prominent feature of the CIMP subclass of primary melanomas is a poor tumor-infiltrating lymphocyte response associated with limited antitumor immunity. This study shows the clinical utility of CIMP in a subtype of early-stage invasive primary melanomas.

CIMP involves the enhancement of methylation in variable CpG islands of gene promoter regions as well as other genomic regions. The hypermethylation of CpG (island) sites is not uniform. This epigenetic phenomenon may be due to a higher activity of DNA methyltransferase 1, which is involved in specific methylation of CpG sites during mitosis of cells (Zhang et al., 2021). Other epigenetic factors may also promote CIMP phenotypes.

It is important to determine whether methylation of specific CpG sites in CIMP is responsible for regulation of selective gene expression. Using The Cancer Genome Atlas (TCGA) skin database, the authors found an association between CIMP and mRNA expression related to specific genes. However, a direct paired one-to-one correlation of specimens is required for validation of these findings. Although gene promoter regions were predominantly assessed for CpG island methylation, noncoding regions that include methylated-in-tumor loci as well as other noncoding loci (Greenberg et al., 2014; Tanemura et al., 2009) can be indicators of CIMP. The HM450K BeadChip Illumina array has more in-depth coverage of the whole genome that includes more CpG island sites at gene promoter and noncoding regions. A more comprehensive epigenomic platform that involves new approaches with in-depth whole-genome methylation sequencing with further validation using targeted quantitative