The Molecular Revolution in Cutaneous Biology: Emerging Landscape in Genomic Dermatology: New Mechanistic Ideas, Gene Editing, and Therapeutic Breakthroughs

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Stunning technological advances in genomics have led to spectacular breakthroughs in the understanding of the underlying defects, biological pathways and therapeutic targets of skin diseases leading to new therapeutic interventions. Next-generation sequencing has revolutionized the identification of disease-causing genes and has a profound impact in deciphering gene and protein signatures in rare and frequent skin diseases. Gene addition strategies have shown efficacy in junctional EB and in recessive dystrophic EB (RDEB). TALENs and Cripsr/Cas9 have emerged as highly efficient new tools to edit genomic sequences to creat new models and to correct or disrupt mutated genes to treat human diseases. Therapeutic approaches have not been limited to DNA modification and strategies at the mRNA, protein and cellular levels have also emerged, some of which have already proven clinical efficacy in RDEB. Improved understanding of the pathogenesis of skin disorders has led to the development of specific drugs or repurposing of existing medicines as in basal cell nevus syndrome, alopecia areata, melanoma and EB simplex. These discoveries pave the way for improved targeted personalized medicine for rare and frequent diseases. It is likely that a growing number of orphan skin diseases will benefit from combinatory new therapies in a near future.

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Abbreviations: AA, alopecia areata; CRISPR/Cas9, clustered, regularly interspaced, short palindromic repeat/cas 9; HR, homologous recombination; iPSC, induced pluripotent stem cell; MSC, mesenchymal stromal cell; NHEJ, nonhomologous end-joining; RDEB, recessive dystrophic epidermolysis bullosa; TALEN, transcription activator-like effector nuclease

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NEW MECHANISTIC IDEAS

Over the last decade, technological advances in genomics have generated an unprecedented wealth of new information on underlying defects, mechanisms, and therapeutic targets of skin diseases. They have led to the development of biological systems to profile and understand gene, RNA, and protein networks and biological cascades in disease models and in patients. Functional genomics has shown complex webs of interactions, and integrative biology has become essential to capturing, comparing, and understanding these networks. Transcriptomics allows deciphering of disease signatures and signaling networks to discover mechanisms for disease variability (Odorisio et al., 2014) and to define groups of skin conditions with shared functional profiles, which may help redefine a new nosology of skin diseases (Inkeles et al., 2015). Technological advances in mass spectrometry-based proteomics now allow measurement of changes in protein abundance, posttranslational modifications, and protein-protein interactions at the scale of the proteome, resulting in the identification of mechanisms of skin diseases and targets for therapeutic intervention (Altelaar et al., 2013; Küttner et al., 2013). Epigenomics, epistasis, metabolome, microbiome, and phenomics are also being developed to account for the complexity of biosystems and to set up the bases for integrative genomics (Hawkins et al., 2010; Mitra et al., 2013; Topol, 2014).

Genomic medicine could lead to a more comprehensive understanding of skin diseases, to personalized medicine, and to improved knowledge of genotype-phenotype interactions and is essential for making new medicines. In fact, spectacular progress has been made in targeted therapies derived from improved understanding of the disease pathogenesis. Some of the best examples have led to the development of specific and highly effective small-molecule drugs blocking the disease biological cascade, and others have used gene therapy or cell therapy to replace the function of a defective gene, to provide cells with high potential to reverse the phenotype, or to repurpose existing drugs.

Next-generation sequencing (Metzker, 2010) has been instrumental in identifying new disease-causing genes and unfolding the genetic landscape of a number of genetic skin diseases. Next-generation sequencing brings a new paradigm for the geneticist, allowing for the identification of Mendelian genes from very rare diseases and/or when no large families are available. However, the rapid identification in an

individual of hundreds of new variants with little or no known biological significance complicates the work, and it may be difficult to identify the causative sequence variation if no other families can be enrolled. The growing body of data generated by next-generation sequencing and biological databases calls for the development of new tools that can help biologists and physicians get access to and understand that information (Cui et al., 2015).

New tools have emerged allowing for easier functional validation of sequence variants in vivo. Because of its unique features (transparency allowing easy visualization of internal organs, fast extrauterine development, large offspring), the zebrafish model, which was for a long time used for developmental studies, has now become an attractive model for genetic research. The possibility of efficiently knocking down specific genes in vivo using morpholino oligonucleotides (Nasevicius and Ekker, 2000), and subsequently the rescue by transfer of mRNA encoding a wild-type or mutated version of a human cDNA, allows for the rapid confirmation of the damaging nature of a specific sequence variant. The zebrafish model has proved useful in studying not only heritable skin disorders (Kim et al., 2010; Li et al., 2011; Li and Uitto, 2014; Postel et al., 2013) but also wound healing (Richardson et al., 2013) and melanoma (Lister et al., 2014).

GENE EDITING

Gene editing is based on the capacity of tailored nucleases to induce a DNA double-strand break at a chosen chromosome site. The double-strand break will further be repaired imperfectly by the nonhomologous end-joining (NHEJ) mechanism, leading to gene disruption or perfectly by homologous recombination (HR) if a donor template is provided. The rapid evolution of gene-editing tools using engineered nucleases, from meganucleases and zinc finger nucleases 15 years ago to transcription activator-like effector nucleases (TALENS) (Gupta and Musunuru, 2014) and clustered, regularly interspaced, short palindromic repeat/cas 9 (CRISPR/Cas9) (Chen et al., 2015) today, permits gene inactivation genes through NHEJ repair and introduction of mutations or correction of disease mutations through HR repair in cells, zygotes, and embryos in various animal models (mainly limited to mice and fruit flies before) much more easily (Sander and Joung, 2014). Indeed, zinc finger nuclease-mediated targeted gene disruption was first achieved in vertebrates to generate a model of albinism and vitiligo in *Xenopus laevis* (Nakajima et al., 2012), followed by the use of TALENs in mice (Wang et al., 2013a) and, later, CRISPR/Cas9 in zebrafish (Hwang et al., 2013), mice (Wang et al., 2013b) and rats (Ma et al., 2014). The versatility of these new tools also makes them candidates for more efficient and safer molecular therapeutics in the future.

Significant achievements in the area of gene editing for genetic skin disorders have been obtained for junctional, and recessive (RDEB), and dominant dystrophic epidermolysis bullosa over the last 5 years (Webber and Tolar, 2015). First, nuclease-mediated gene editing for *COL7A1* was achieved in RDEB patient-derived fibroblasts using TALEN-mediated HR with minimal off-target activity (Osborn et al., 2013). The researchers used a donor template harboring a floxed-phosphoglycerate kinase—puromycin cassette in addition to homologous arms to correct a mutation in exon 14 through HR and to select for edited cells. These results showed the ability of TALENs to induce site-specific editing, resulting in gene correction in this region of *COL7A1*. Also, TALEN-mediated correction of a recurrent RDEB mutation was recently achieved by Chamorro et al. (2016). In our laboratory, we could correct two disease-causing *COL7A1* mutations through meganuclease-mediated HR in an RDEB patient's derived keratinocyte cell line and in primary RDEB keratinocytes and fibroblasts (Izmiryan et al., 2016). HR has a lower efficiency compared with NHEJ, and competition between the two mechanisms of double-strand break repair may impair efficient gene correction or gene addition. To favor HR over NHEJ, two recent studies used specific inhibitors of key molecules of the NHEJ pathway, leading to increased precise gene editing frequency, which is required for clinical applications (Chu et al., 2015; Maruyama et al., 2015).

The feasibility of site-specific genome editing through NHEJ has also been investigated in dominant negative disorders such as dominant dystrophic epidermolysis bullosa. In a proof-of-concept study, Shinkuma et al. (2016) successfully used TALENs and CRISPRs to inactivate dominant negative mutations in induced pluripotent stem cells iPSCs derived from dominant dystrophic epidermolysis bullosa fibroblasts. Later, these gene-edited iPSCs were differentiated into keratinocytes and fibroblasts, and restoration of type VII collagen expression could be shown by reverse transcriptase-PCR and Western blot analyses.

Future studies will aim at improving the efficacy and specificity of Cas9 nuclease to limit and prevent CRISPR/Cas9-mediated off-target activity and to optimize this system for in vivo applications (Kuscu et al., 2014; Wang et al., 2015). For this purpose, recently developed new Cas9 variants and Cas9 nickase have shown improved specificity to the target site (Kleinstiver et al., 2015; Shen et al., 2014).

THERAPEUTIC BREAKTHROUGHS

Gene-, protein-, and cell-based therapeutic approaches have mainly been applied to genetic skin diseases, more specifically to inherited epidermolysis bullosa and to pachyonychia congenita. Pharmacological approaches have been successfully used for Gorlin syndrome and for frequent skin conditions, including basal cell carcinoma, alopecia areata (AA), and melanoma, as a result of an improved understanding of their pathogenesis that lead to specific drug development or repurposing of existing drugs.

Gene-, cell-, and protein-based therapeutic approaches for genetic skin diseases

Gene therapy. Ex vivo gene therapy for inherited skin disorders has been developed in several preclinical studies, with a focus on junctional epidermolysis bullosa and RDEB (reviewed by Abdul-Wahab et al., 2014; Uitto et al., 2016; Vanden Oever and Tolar, 2014). In 2006, a patient suffering from generalized nonlethal junctional epidermolysis bullosa underwent successful transplantation with genetically modified epithelial sheets made from autologous keratinocytes corrected with a classical retroviral vector encoding the β3