Chapter 1

Introductory Chapter: Keratins - What to Do with Too Much? What to Do with Too Little?

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Additional information is available at the end of the chapter

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1. Introduction

Keratin, from the Greek word for horn, **κέρατο**, denotes the proteinaceous covering layers and structures produced by chordates, including mammals, birds, fish, reptiles, and amphibians. The dead outermost layer of the epidermis, hair and wool, horns, claws, hooves, feathers, and scales is composed of keratin. Keratin is completely insoluble in water and is resistant to proteases that degrade other proteins—1000-year-old Egyptian and other ancient mummies often have full head of hair, virtually undamaged keratin. Keratin proteins can be either alpha-helical in structure, in the skin, hair, and wool of mammals, or parallel sheets of beta-pleated polypeptide chains found in the feathers of birds and scales of reptiles. Rich in amino acid cysteine, keratins become covalently crosslinked via disulfide bonds, which confers a great chemical and biochemical stability to keratin. Thus, keratin serves as important resilient structural and protective functions for the organism.

Importantly, *keratin* is also the resilient structural intracellular protein that protects living epithelial cells from mechanical damage or stress. In cytoplasm, keratin constitutes a filamentous cytoskeletal protein network, extending from the nucleus to the cell periphery, the intermediate filaments, thicker than the actin filaments but thinner than microtubules [1]. Two large families of keratin genes encode multiple proteins with both common and cell-type-specific functions [2].

The indispensable fundamental intracellular keratin functions are revealed in congenital human skin diseases caused by mutations in keratin genes, for example, Epidermolysis bullosa simplex and Epidermolytic hyperkeratosis or in Meesmann's Corneal Dystrophy, the disease caused by a mutation in the gene specifically encoding a corneal keratin [3]. Most

keratin gene mutations have a dominant-negative effect, disrupting the filamentous structure formation even from the natural allele and leaving the cell with a deficient cytoskeleton.

2. What to do with too little?

Several chapters in this volume address the diseases associated with keratin deficiencies (see manuscripts by Komine et al., Zhang et al.). Corrective gene therapy approaches attempt to specifically target the mutant keratin gene allele, thus allowing the normal keratin protein to decrease cell fragility [4]. Short inhibitory RNA (siRNA) technology was effectively used to downregulate mutant K6a and K14 allele expressions in cultured PC and EBS cells, respectively [5–7]. This mutation-specific siRNA therapy has been used in a human clinical trial, resulting in effective siRNA treatment of a skin disorder [8]. The functional redundancy of keratins in tissues affected by keratin mutation allows for a possibility to use gene-specific silencing, rather than allele-specific siRNA. Spliceosome-mediated RNA trans-splicing uses the endogenous spliceosome machinery to excise mutant exons and was used to replace the first seven exons of the KRT14 gene in an EBS cell line [9].

Induced pluripotent stem cells and even patient-specific-induced pluripotent stem cells have been generated for use in treatment of inherited keratinopathies [10–12]. Such cell-based therapies have been proposed in conjunction with CRISPR/Cas9- and TALEN-based gene-editing techniques for targeting mutations in the keratin genes [13–15].

A naturally occurring phenomenon, whereby a subpopulation of mutant cells spontaneously reverts to the wild-type phenotype, "revertant mosaicism," has been observed in several patients with EB [16–20]. Revertant mosaicism keratinopathies have two major advantages: (1) the revertant skin is visible and easily accessible and (2) the revertant keratinocytes often have a growth advantage over their mutant progenitors, and so may outgrow and correct the patient's ichthyotic phenotype [21]. Harvesting, expanding, and autologous re-grafting the revertant tissue therefore may be feasible in a clinical setting.

3. What to do with too much?

The increased importance of ecological dangers and ways to alleviate them focused attention on the very large volume of keratin industrial waste. Several chapters in this volume address the incipient remediation efforts (see manuscripts by Ningthoujam et al., Sharma et al., and Nugroho et al.). The mechanical and chemical methodology, cumbersome and inadequate, seems to be giving in to the new biological technology. We can expect deeper understanding of the microbiome and its efficient biodegradation capabilities to play ever more important role. Especially promising are the studies of complex microbiomes, and we can expect in the not-so-distant future that combinations, communities of specific microbes, will be able to convert the obnoxious keratin waste into delightful new materials [22, 23].

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