Innate Immunity

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Goals and Projects:

We are interested in the epithelial innate defense system, and how dysregulation of these defense mechanisms can lead to diseases. Epithelial tissue forms a protective lining of our body, including the outer surfaces that are exposed to the environment, as well as the inner body cavities, glands and ducts. It serves as an essential defense barrier stopping pathogens, both physically and chemically, right at the points of entry before they can cause diseases. Recently, we discovered a novel family of antimicrobial peptides in human corneal epithelial cells that kill various bacterial pathogens of both Gram types. These novel antimicrobial peptides are glycine-rich C-terminal fragments of cytokeratin 6 (K6). It is an unexpected role for keratin proteins since they have long been viewed as building blocks of cytoskeleton that primarily maintain structural integrity and resilience of epithelial cells. In general, keratins K8/K18 and K7/K19 constitute the primary and secondary cytokeratins in simple epithelial cells (e.g. gut, lung, retinal pigment epithelia); K5/K14/K15 and K6/K16 are found in stratified epithelial cells of skin, oral, esophageal, genital, conjunctival and corneal mucosa, as well as luminal cells of sweat, mammary and prostate glands. Given the wide distribution of keratin 6 among different epithelial tissues, as well as the structural and functional similarities shared among different keratins, our studies aim to advance understanding of epithelial innate defenses of the cornea and other sites of the body, and to contribute to new biocompatible anti-infective development in the post-antibiotic era we are fast approaching.

Research and Innovations:

Using corneal epithelial cell culture system and rodent disease models, coupled with an array of modern biochemical, molecular and cell biological, proteomic, and fluorescence microscopic tools, our ongoing research attempts to answer the following questions:

1. What are the mechanisms involved in fragmentation of cytokeratin 6 that produces its antimicrobial derivatives?
2. What signals the fragmentation?
3. What are the mechanisms for regulating level of keratin fragmentation, as well as production, secretion and activity of keratin-derived antimicrobial peptides?
4. How do keratin-derived antimicrobial peptides kill bacteria?
5. Do keratin-derived antimicrobial peptides have other protective roles against infection and other diseases?
6. Is keratin-based host defense widely used in other sites of the body? If this defense is dysregulated, could it lead to increased disease susceptibility?
7. Are keratin-derived antimicrobial peptides effective prophylactic and/or therapeutic agents against infection?

Endogenous proteolytic processes that produce antimicrobial keratin fragments. As keratin-derived antimicrobial peptides existing in the cytosol are C-terminal fragments of cytokeratin 6, they are likely cleaved from their full-length form by endogenous proteases. We are investigating whether this fragmentation is mediated by specific intracellular protease(s) and/or homeostatic degradation via proteasomes, as well as the signaling pathways that regulate the activity of these proteolytic processes.

Regulatory mechanisms for generation and activity of keratin-derived antimicrobial peptides. As epithelial barriers must remain structurally resilient under various environmental challenges, the level of keratin expression and the dynamics of keratin filament turnover are both highly regulated. One of the mechanisms

involves phosphorylation of keratin subunits (e.g. via MAPK kinases or protein kinase C), which in turn increases keratin solubilization (filament disassembly). Triggers for phosphorylation include various stress paradigms, apoptosis and mitosis. We are investigating the regulatory mechanisms, i.e. keratin phosphorylation, involved in production/secretion and activity of keratin-derived antimicrobial peptides in response to external triggers, i.e. bacteria and bacterial antigens that are relevant to corneal innate defense.

Bacterial killing mechanisms of keratin-derived antimicrobial peptides. We found that keratin-derived antimicrobial peptides (10-36 amino acids) have broad efficacy against both Gram-positive and Gram-negative human pathogens such as *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Escherichia coli*. Interestingly, the activity spectrum of individual peptide varies slightly. In addition, the peptides are salt-tolerant and their killing action appears to be independent of peptide/bacterial membrane electrostatic attraction. We are interested in elucidating the structure-activity relationships of these peptides to better understand how they work.

Roles of endogenous and secreted keratin-derived antimicrobial peptides in protecting the cornea and other sites against diseases. In addition to being antimicrobial, we are interested in determining if these keratin peptides have additional roles in host defense, e.g. their working relationships with other cellular factors during intracellular killing of bacteria, or with immune cells/factors which facilitate extracellular killing. We are also interested in determining if the antimicrobial keratin fragments play a role in certain keratin diseases, such as cirrhosis and inflammatory bowel disease, which are found to link to dysfunctional keratin turnover (decreased solubilization of keratin filaments).

Prophylactic and treatment use of keratin-derived antimicrobial peptides. As essential components and first-line defense factors of innate immunity, it is very common that antimicrobial peptides have broad-spectrum activity. Several human antimicrobial peptides have been shown to possess antibacterial, antifungal and antiviral activities. Some are even effective against multi-drug resistance bacteria with low tendency to induce bacterial resistance. Infectious and inflammatory corneal disease remains a major cause of visual impairment and blindness, and a significant public health burden globally. We are interested in examining the activities of keratin-derived antimicrobial peptides against major ocular pathogens, including fungi, viruses, Acanthamoeba and additional bacterial pathogens of both Gram types (i.e. the antibiotic-resistant strains). We are also interested in examining synergistic or additive effects of keratin-derived antimicrobial peptides with each other or with currently used antibiotics. By using suboptimal dosage and administration frequency of 19mer peptide in mouse models of keratitis (corneal infection), we found that keratin-derived antimicrobial eye drops applied to partially healed but disease susceptible mouse corneas can confer protection against infection caused by *P. aeruginosa*. The eye drops also helped reduce disease severity of ongoing infection in mice. While direct bactericidal activity is undoubtedly an important means to prevent infection, shorten healing time can also reduce tissue susceptibility to infection after wounding. It has been shown that antimicrobial peptides can promote wound healing, and that cytokeratin 6 plays a role in wound healing and tissue repair. Thus, we are interested in determining the wound healing effects of keratin-derived antimicrobial eye drops on non-infected, injured mouse corneas, and improving their protective/therapeutic capability against infection by optimization of dosage, administration frequency and dosage form.

Lab Staff Members:

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