Zebrafish Integumentary System

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Introduction

The zebrafish, like all animals, is covered with sophisticated skin that acts as both a wall and a window to the external environment. The skin is traditionally divided into three layers. A superficial epidermis—comprising a stratified epithelium—provides barrier and sensory function. The underlying dermis is dominated by bony elasmoid scales and a deep collagenous stroma that together armor and support the muscles and organs. The relatively thin hypodermis lies deeper, at the boundary between skin and muscle, and harbors adipocytes and pigment cells. Below, we give a brief primer on the development and anatomy of zebrafish skin.

Epidermal Development and Anatomy

The epidermis represents the ultimate boundary between the fish and its environment. It forms a semiimpermeable barrier while also allowing sensory functions. The primary epidermis, known as the enveloping layer, first develops before gastrulation (Kimmel, Warga, & Schilling, 1990). Following gastrulation, the enveloping layer becomes the outermost skin cell layer, the periderm, and basal cells arise from nonneural ectoderm, thereby generating a bilayered epidermis (Bakkers, Hild, Kramer, Furutani-Seiki, & Hammerschmidt, 2002; Lee & Kimelman, 2002). Periderm cells are connected by tight junctions forming a solute barrier and are decorated with elaborate, wavy microridges that increase epidermal surface area and likely aid in mucous retention (Hawkes, 1974; Kiener, Selpsova-Friedrich, & Hunziker, 2008; Lam, Mangos, Green, Reiser, & Huttenlocher, 2015; Whetar 1970). This bilayered state persists through the early larval period until 6–7 standardized standard length [SSL (Parichy et al. 2009); ~6–7 mm standard length], when descendants of basal cells generate the intermediate suprabasal cell layers (Guzman, Ramos-Balderas, Carrillo-Rosas, & Maldonado, 2013; Lee, Asharani, & Carney, 2014).

In adult skin, there are two to eight layers of suprabasal cells over the surface of the fish, whereas superficial periderm and deep basal cells remain as monolayers (Fig. 8.1). Similar to mammalian epidermis, basal cells serve as epidermal stem cells, and their proliferation as well as suprabasal cell proliferation is regulated by ΔN-p63. Intermediate suprabasal cells are the most proliferative cells in the epidermis and most appear undifferentiated. It is possible they serve as a transient amplifying population, though precise lineages and stem cell kinetics of the epidermis remain unresolved (Guzman et al. 2013; Le Guellec, Morvan-Dubois, & Sire, 2004; Lee & Kimelman, 2002; Quilhac and Sire 1999; Richardson et al. 2013).

The epidermis harbors several additional cell types. Goblet cells secrete mucous and club cells produce alarm substances (Jevtov, Samuelsson, Yao, Amsterdam, & Ribbeck, 2014). Ionocytes and chemosensory cells aid in maintaining chemical homeostasis (Coccimiglio & Jonz, 2012; Cruz, Chao, & Hwang, 2013; Hwang & Chou, 2013). Somatosensory cells send their nerve endings into the epidermis enabling a sense of touch (Rasmussen, Vo, Sagasti, 2018). Moreover, the mechanosensory neuromasts of the lateral line are hosted by the epidermis and enable detection of water motions against the surface of the fish (Lee, Asharani, Carney, 2014; Metcalfe, 1985).
Development and Anatomy of the Hypodermis and Collagenous Dermal Stroma

The dermis contains a dense collagenous stroma—the stratum compactum—that imbues the skin with mechanical strength. Basal epidermal cells begin producing a primary collagenous stroma by 24 h postfertilization (hpf). Once initiated, the stroma thickens throughout the life of the fish. By 72 hpf, thin layer of dermal endothelial cells begins to accumulate along the surface of the muscles to constitute the hypodermis. These cells also contribute to the growth of the primary stroma. Basal epidermal and hypodermal cells continue to produce collagen through the early larval period, building an ever thicker collagenous stroma. Hypodermal cells remain a sparse layer in adult fish, where they likely provide trophic and other support to pigment cells comprising the stripe pattern and to dermal adipocytes (Fig. 8.1) (Hirata, Nakamura, & Kondo, 2005; Lang, Patterson, Gordon, Johnson, & Parichy, 2009; Le Guellec, Morvan-Dubois, Sire 2004; Minchin and Rawls 2017).

Coincident with stratification of the epidermis, the primary collagenous stroma of the dermis becomes organized into a plywood-like arrangement of orthogonally aligned collagen fibers. This stroma remains devoid of cells until ~8 mm SSL (Parichy, Elizondo, Mills, Gordon, Engeszer, 2009) when it is populated by dermal fibroblasts to generate the stratum compactum, a tough yet transparent structural component of the skin that resists tearing and may provide elastic recoil to the swim stroke (Fig. 8.1) (Szewciw and Barthelat 2017).

Development and Anatomy of Elasmoid Scales

Hundreds of arrowhead-shaped, calcified scales are embedded in the dermis of adult zebrafish (Fig. 8.2A). These overlap precisely like roof tiles so that every position along the body is covered by at least two scales, providing flexible armor that likely protects against puncture injuries that might be inflicted by cooccurring...
predators or other types of trauma (Figs. 8.1, 8.2A,B) (Engeszer, Patterson, Rao, & Parichy, 2007; Sire, Allizard, Babiar, Bourguignon, Quilhac, 1997; Zhu, Szewciw, Vernerey, Barthelat, 2013).

After the stratum compactum forms, in 8–10 SSL larvae, dermal cells accumulate at the superficial limit of the dermis, in close proximity to the epidermis. These superficial cells aggregate to form scale papillae (Le Guellec et al. 2004; Mongera and Nüsslein-Volhard 2013; Shimada et al. 2013; Sire et al. 1997). Dermal papillae form first in the caudal peduncle and above the ribs. Additional papillae are added in rows and columns to generate a half-offset hexagonal grid of scales (Fig. 8.2A,B) (Aman, Fulbright, & Parichy, 2018; Sire et al. 1997).

Initiation of scale papillae relies on interactions between epidermis and dermis involving ectodysplasin, Wnt/β-catenin, and fibroblast growth factor (Fgf) signaling pathways (Aman et al. 2018; Daane, Rohner, Konstantinidis, Djuranovic, & Harris, 2015; Harris et al. 2008; Rohner et al. 2009). Remarkably, interactions between signaling pathways and the cell behaviors they govern during zebrafish scale development resemble those governing formation and patterning of skin appendage like feathers and hair in terrestrial vertebrates. This implies that, despite profound differences in matrix composition—dermal calcified matrix of zebrafish scales and epidermal keratin of terrestrial appendages—all skin appendages likely share a common ancestry, and mechanisms that govern their early development are similar across vertebrates.

Following aggregation, dermal papillae produce the initial calcified matrix of the scale plate (Aman, Fulbright, Parichy, 2018; Sire et al. 1997). Developing scales are oriented toward the posterior by the planar cell polarity machinery, and their growth is driven by Sonic hedgehog (Shh) and Fgf-dependent proliferation and growth of osteoblast-like scale-forming cells accompanied by expansion of the calcified matrix (Aman et al. 2018; Cox et al. 2018; Iwasaki, Kuroda, Kawakami, & Wada, 2018; Rasmussen et al. 2018; Sire et al. 1997).

The structure of the scale plate consists of a basal layer of weakly ossified collagen, called isopedine, capped by the more strongly ossified external layer. At the posterior margin of the scale, a highly calcified, collagen-poor limiting layer is present (Fig. 8.2C). The scale-forming cells line the deep aspect of the scale and loop around the limiting layer at the posterior scale margin (Fig. 8.1). Gene expression in these posterior margin scale-forming cells is distinct from other scale-forming cells. It is this population that proliferates to add scale-forming cells during growth (Aman et al. 2018; Cox et al. 2018; Iwasaki et al. 2018). As growth proceeds, limiting layer matrix is deposited in waves, leading to concentric arcs of more heavily calcified circuli in the mature scale (Fig. 8.2C) (Sire et al. 1997). The scale...
plate is also punctuated by matrix-free channels, the radii, that organize and house neuronal processes and vasculature of the skin (Rasmussen et al. 2018; Sire et al. 1997). During scale outgrowth, epidermal Shh triggers accumulation of a thin layer of scale pocket cells underneath the scale-forming cells (Fig. 8.1). Epidermal cells invaginate along these cells yielding the final partially protruding organization of the adult scale (Aman et al. 2018; Sire et al. 1997).

The evolutionary relationships between elasmoid scale extracellular matrix and calcified matrices in mammals—enamel, dentin, cartilage, and bone—remain uncertain. The precise homologies of cells secreting these matrices are similarly ambiguous. Paleontological and other evidence indicate that thin, flexible scales of zebrafish and other teleosts descended from heavy, enameled rhomboid scales of ancient vertebrates. Such rhomboid scales contain clear examples of matrix resembling enamel, dentine, and bone, a state of zebrafish-calcified matrix-producing cells, and to make zebrafish an attractive system for understanding cellular and molecular basis of skin disease, wound healing, and regeneration. Indeed, zebrafish with mutations in genes associated with human cutaneous disease often recapitulate aspects of these diseases and can provide insights into pathology (Feitosa, Richardson, Bloch, & Hammerschmidt, 2011; Hatzold et al. 2016; Li et al. 2011a, 2011b). Zebrafish skin is also highly regenerative and is proving to be an excellent system for uncovering mechanisms that underlie injury response, wound healing, and tissue regeneration (Armstrong, Henner, Stewart, & Stankunas, 2017; Chen et al. 2016; Cox et al. 2018; Gault, Enyedi, & Niethammer, 2014; Kang, Nachtrab, & Poss, 2013; Richardson et al. 2013, 2016).

**Outlook**

Zebrafish skin affords the biomedical research community a powerful system to address fundamental questions of stem cell biology, patterning, morphogenesis, regeneration, and disease. Emerging genetic reagents, as well as imaging and analytical methods, will further enable the study of zebrafish skin and will likely contribute novel insights into these important areas of biology and pathology.

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**References**


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