**Abstract figure title: Morphological, histochemical, and immunohistochemical characteristics of alarm cells and their precursors in ruby-red-fin Shark, (rainbow Shark), Epalzeorhynchos frenatum (Teleostei: Cyprinidae).**

\***Diagram showing the** Precursor alarm cells (1) were shown to be resting on the basement membrane of the epidermis. Subsequently, they enlarged and became large oval-shaped cells (2). The superficial alarm cells gradually collapsed(3).

**\* Using semi-thin sections stained by toluidine blue**, the basal alarm cell appeared deeply stained by toluidine blue (1). While the Mid-epithelial alarm cell was faintly stained (2). The superficial alarm cells gradually acquired deep staining affinity toward the surface (3).

**A and B:** By general H&E staining the basal alarm precursor cells (1) were identified by a high cytoplasmic/nuclear ratio. They were columnar in shape, basally located, and had strongly eosinophilic cytoplasm. The mid-epithelial alarm cells gradually enlarged (2) and became round to oval-shaped. The superficial alarm cells were collapsed (3).

**C, D, E, F, G, H:** **Alarm cells were found to have an affinity for different histochemical stains(bromophenol blue,** **iron hematoxylin,**  **Sudan black B,** **Safranin O, and** **Wiegert stain )**The basal alarm precursor cells (1) had weak staining affinity and increased in mid-epithelial alarm cells (2), while superficial alarm cells (3) exhibited extensive staining affinity.

**J and I:** **trichrome stains were used to detect keratin in the epithelial cells.** Alarm precursors, and mid-epithelial alarm cells had affinity for fibrous-specific stains including methyl blue (I). Instead, superficial alarm cells had staining affinity for orange G using Mallory triple trichrome stain (J).

**K and L:** **Alarm cells had endocrine properties that were identified by** silver staining (K) and (L) Synaptophysin immunostaining.

**M:** **The basal alarm precursor cells (1) had extensive immunoaffinity for PCNA**. Some alarm cells retained PCNA immunoaffinity and others lost it (2 and 3).

**N and O:** **The proteolytic activity of alarm cells was investigated using MMP-9.** Both the basal (1) and some of the mid-epithelial alarm cells strongly expressed MMP-9 (2a). Other mid-epithelial alarm cells (2b), located more superficially, as well as the superficial alarm cells (3), had less immunoaffinity for MMP-9.

**P, Q and R:** **Alarm precursor cells were CD117-positive.** The basal alarm precursor cells (1) had weak staining affinity. The mid-epithelial alarm cells (2) were CD117-positive and exhibited CD117-positive fine granules. The superficial alarm cells (3) had strong CD117 immunoaffinity and had large CD117-positive granules.

**S and T:** **The affinity of alarm cells for enzymatic reactivity, lipases, and alkaline phosphatase was studied**. The mid-epithelial alarm cells had strong staining affinity for lipase (2). The basal alarm cells (1) had weak staining affinity for alkaline phosphatase. The mid-epithelial (2) and superficial alarm cells (3) had strong staining affinity for alkaline phosphatase.

**U and V:** **Staining affinity of alarm cells and their precursors for Acridine orange.** The basal alarm cells appeared deep orange (1), while the mid-epithelial alarm cells (2) had orange granules and the superficial alarm cells (3) had either yellow or orange reactivity for Acridine orange.

**In conclusion, alarm cells are unique epidermal cells with multiple functions, playing immunological, endocrine, and angiogenic roles. They also retain stemness and proliferative properties.**