Airineme-mediated intercellular communication

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Abstract

Intercellular communication is indispensable across multicellular organisms, and any aberration in this process can give rise to significant anomalies in developmental and homeostatic processes. Thus, a comprehensive understanding of its mechanisms is imperative for addressing human health-related concerns. Recent advances have expanded our understanding of intercellular communication by elucidating additional signaling modalities alongside established mechanisms. Notably, cellular protrusion-mediated long-range communication, characterized by physical contact through thin and elongated cellular protrusions between cells involved in signal transmission and reception, has emerged as a significant intercellular signaling paradigm. This chapter delves into the exploration of a signaling cellular protrusion termed 'airinemes,' discovered in the zebrafish skin. It covers their identified signaling roles and the cellular and molecular mechanisms that underpin their functionality.

Keywords: Intercellular communication, airinemes, macrophages, metaphocytes, zebrafish, pigment cells, cytonemes, Notch, xanthophores, melanophores, cellular protrusions

1. Introduction

Cell-to-cell signaling is essential in all multicellular organisms. Even single-celled organisms also communicate with each other for their better survival or adapting to environmental changes. Thus, communication between cells is an essential part of living organisms, and understanding the mechanisms of intercellular communication is critical in fundamental Biology and Medicine. It is well known that there are a handful of intercellular communication mechanisms documented. Those include juxtacrine, paracrine, endocrine, and neuronal signaling mechanisms. In addition, in recent years, studies have identified that cells can communicate by long, thin cellular protrusions that physically contact between signal sending and receiving cells at a distance [1-6].

These cellular protrusions look like typical filopodia but serve a distinct role in transmitting major morphogenetic signals. This mode of cellular protrusion-mediated communication has now been observed across various species and tissues *in vivo,* supported by functional validations [7-11]. In contrast to typical filopodia, many of these signal-conveying protrusions exhibit lengths several orders of magnitude longer and demonstrate remarkable dynamic extension and retraction [7, 10, 12]. Despite variations in morphology, exact signaling mechanisms, cytoskeletal composition, and the involvement of other cellular components, all these protrusions share a common function in mediating long-range intercellular communication.

Various types of cellular protrusions have been identified so far, broadly falling into two categories: closed-ended structures like cytonemes or open-ended formations such as tunneling nanotubes [13, 14]. Nevertheless, recent evidence indicates the existence of cellular protrusions with distinct features and morphologies in diverse species and contexts. For instance, during Kupffer's vesicle formation in gastrulating zebrafish, it has been proposed that large vesicle-like structures, known as migrasomes, located at the tips of retraction fibers from the rear of migrating cells, facilitate long-distance cell-cell communication [15, 16]. These findings suggest the possibility of numerous yet unidentified forms of cellular protrusions in nature.

Recent studies have identified a distinctive type of signaling cellular protrusions originating from zebrafish pigment cells, termed ‘airinemes’ [1, 17, 18]. Airinemes share both similarities and differences with cytonemes and other cellular protrusions. However, one of the notable differences between airinemes and others is that airineme-mediated signaling relies on the skin-resident macrophage and they have vesicle-like structures at the tips of their protrusions. Macrophages, recognized as immune phagocytes responsible for clearing dead cells and foreign pathogens [19], play a novel role in airineme-mediated signaling. This discovery unveils a previously unknown function of macrophages in facilitating cellular protrusion-mediated signaling between non-immune cells [20].

2. Airineme-mediated signaling in pigment pattern formation

Airinemes are long, thin cellular protrusions initially identified from pigment cells in zebrafish skin that play a crucial role in mediating long-distance signaling between different pigment cell types during pigment pattern formation. Like other signaling cellular protrusions, airinemes can be visualized using membrane-targeted fluorescent tags [1, 3, 5, 6]. Given the thin and elongated nature of airinemes, cytosolic fluorescent protein expression is not effective to label them. Instead, fluorophores such as EGFP or mCherry, fused with plasma membrane targeting signals like

Palmitoylation, Lyn, or CAXX motif, can be employed under the control of the *aox5*, a lineage specific promoter in yellow pigment cell called xanthophore to effectively label airinemes [1]. Although their ultrastructure has not been explored with electron microscopy, analysis of fluorescently labelled confocal images indicates that the airinemes filaments are less than a micron in diameter, extend up to two to three hundred micrometers, and demonstrate dynamic extension and retraction, with extension occurring at a faster rate than retraction. Unlike other cellular protrusions such as cytonemes or tunneling nanotubes, airinemes exhibit highly curved trajectories and possess a membranous vesicle at their tip (Fig.1) [1]. The adult zebrafish exhibits a horizontal zebra pattern characterized by alternating dark stripes and yellow/orange interstripes. This pattern is created by two types of pigment cells: dark melanophores, forming the stripes, and yellow/orange xanthophores, constituting the interstripes. In addition, silvery iridophores are present throughout the zebrafish flank. The formation of this stripe pattern occurs during metamorphic stages where various tissue remodeling takes place including pigment pattern development. Notably, intercellular communication between all three pigment cell types is essential for the proper pigment pattern formation [21, 22]. In the early stages of metamorphosis, postembryonic melanophores begin to differentiate across the zebrafish trunk. As a result, pre-positioned xanthophores within the interstripe become intermingled with these newly differentiating melanophores [22], and the stripe pattern is not yet fully formed. Subsequently, these postembryonic melanophores migrate out of the interstripe, coalescing into stripes and establishing a clear boundary between the stripes and interstripe [22].
Laser ablation experiments and genetic cell ablation experiments have indicated that intercellular communication between melanophores and xanthophores is critical for pigment pattern formation. The failure of pigment pattern formation occurs if either of these cell types is ablated. Based on previous studies, it is assumed that xanthophores repel postembryonic melanophores in the interstripe [23, 24]. However, findings revealed that undifferentiated xanthophores, referred to as xanthoblasts or ‘cryptic xanthophores’ intermingled with differentiated melanophores in the stripes. These xanthoblasts extend airinemes, targeting differentiating melanophores in the interstripe rather than those already differentiated melanophores in the stripes [25]. Airinemes deliver Delta C-carrying airineme vesicles specifically to these target melanophores, activating Notch signaling. It has been suggested that the Notch pathway, in turn, activates Kit signaling, which is crucial for melanophore migration and survival [26, 27]. Thus, the study suggests that melanophores located in the interstripe receive signals from xanthoblasts in the stripes via airinemes, prompting their migration out of the interstripe [1].

3. Macrophage requirement in airineme-mediated signaling

One distinctive feature of airinemes distinguishing them from other cellular protrusions, such as cytonemes tunneling nanotubes, is their dependence on tissue-resident macrophages for signaling. Airinemes also exhibit unique meandering trajectories. These highly curved airineme filaments result from macrophages pulling and dragging airinemes vesicles, with the filaments trailing back as the macrophage migrates (Fig. 1). Studies have shown a significant decrease in airineme extension in *csf1r* mutant zebrafish, which lacks macrophages. Genetic ablation of skin resident macrophages also led to a substantial reduction in airineme extension frequency, resulting in a pigment pattern defect. It also revealed that the speed of airineme extension depends on the migration speed of macrophages. These findings strongly indicate the critical role of macrophages in airineme-mediated intercellular signaling [20]. However, this raises a question about whether macrophages play dual roles in immune response and signaling or if specific macrophage subpopulations are dedicated to airineme signaling.
Subsequent investigations have unveiled two morphologically distinct macrophage populations in zebrafish skin. One is characterized by a relatively larger size and dendritic shape, while the other

exhibits a smaller amoeboid morphology. Interestingly, it turns out that the smaller macrophage subpopulation actively pulls airineme vesicles and drags airinemes as they migrate. The migration speed of this population aligns with the airineme extension speed, suggesting a preferential interaction between these amoeboid macrophages and airinemes [28].
Further analysis indicates that these airineme-pulling cells overlap with a macrophage subpopulation termed 'metaphocyte,' known to be derived from the ectoderm or endoderm in zebrafish skin [19, 29]. Specific markers for metaphocytes, such as *grn2* and *cldnh*, are highly expressed in the small amoeboid population but not in the larger dendritic macrophages. Notably, metaphocyte-specific ablation resulted in the abolition of airineme extension, highlighting the essential role of metaphocytes in airineme-mediated intercellular communication during metamorphosis in zebrafish skin [28]. Despite metaphocytes expressing genes associated with phagocytosis, these cells appear co-opted for signaling in this specific context [28, 29]. Additionally, the study identified that metaphocyte-mediated airineme signaling is dependent on the activity of Matrix Metalloproteinase-9 (MMP-9). It was suggested that MMP-9 expression in metaphocytes allows them to migrate deeper into the hypodermis, where xanthoblasts located, exhibiting a faster migration speed compared to the MMP-9 low dendritic macrophage subpopulation [28].

4. Airineme vesicles

Airinemes exhibit a distinctive trait characterized by the presence of vesicle-like membranous structures at their tips, housing DeltaC (and potentially other Delta ligands). Dynamic live imaging reveals the origin of airineme vesicles from the surface of xanthoblasts, the undifferentiated/unpigmented yellow pigment cells in zebrafish [1]. These vesicles are then conveyed from signal-sending cells to target cells via metaphocytes. While a more detailed exploration is imperative, there is a presumption that airineme vesicle precursors manifest as outwardly protruded, bleb-like structures (referred to as airineme blebs) formed at the plasma membrane before airineme extension. Abundant in phosphatidylserine (PtdSer), a well-characterized 'eat-me' signal for macrophages [19], these precursors are predominantly observed in airineme-producing xanthoblasts rather than in differentiated xanthophores (Figure 1). This pattern aligns with the observed high airineme extension frequency in xanthoblasts compared to the low frequency in xanthophores [1].
The regulatory molecular mechanisms governing these structures remain elusive. Comparable outward plasma membrane extrusion is well-known in microvesicles, also called 'ectosomes,' which prominently expose phosphatidylserine (PtdSer) to the outer membrane. Although further investigation is necessary, this observation suggests potential shared molecular pathways between airineme vesicle precursors and ectosomes in their formation [30].
An intriguing question arises concerning the packaging of DeltaC into airineme blebs. Evidence suggests that DeltaC is packaged in the blebs before macrophage uptake, excluding the period during or after airineme extension. This is substantiated by the apparent expression of DeltaC in airineme vesicle precursors on the surface of xanthoblasts. Notably, not all airineme vesicle precursors exhibit DeltaC positivity, hinting at a presumed packaging during the maturation phase of these precursors [1].

5. Target-Specific Airineme Signaling

As previously mentioned, airineme vesicles exhibit precise delivery to target melanophores situated in the interstripe, intermingled with xanthophores during metamorphic stages. When airinemes encounter non-target pigment cells, such as xanthophores or iridophores, airineme

vesicles are detached from the filament, engulfed by dragging metaphocytes, and subsequently eliminated. Therefore, a mechanism must be in place to ensure an efficient target search [20].
In contrast to cytonemes or tunneling nanotubes, which are straight protrusions, airinemes display highly curved protrusions. While straight protrusions may take less time to reach their targets, they also increase the risk of missing the target due to their ballistic nature. Conversely, curved protrusions allow for a larger spatial exploration, offering a better chance of finding targets, albeit with a longer travel time (diffusive). Depending on the context, one strategy may be more effective than the other.
In the zebrafish skin, where various cell types are densely packed into the epidermis and hypodermis alongside metaphocytes and pigment cells, airinemes must locate their target cells among a mixture of non-target cells. Therefore, a diffusive search mechanism is conceivable to suit this context better. Through a combination of mathematical modeling and biological assessments of airineme curvatures, it has been revealed that the degree of curvature in airineme filaments is optimized for efficient target search. Also, the study discovered that there is a trade-off between directional sensing and target cell contact [31].

5. Conclusion

The discoveries mentioned above highlight the complexity of signal propagation mechanisms, challenging our previous understanding. While the evidence supporting the functional significance of cellular protrusion-mediated signaling, including airinemes, cytonemes, and others, is rapidly expanding, it remains incompletely understood, with its potential applications for human health-related issues largely untapped. Nevertheless, considering that signaling mechanisms mediated by cellular protrusions play a pivotal role in conveying major morphogenetic signals, any misregulation of these processes undoubtedly has the potential to contribute to numerous human diseases [32]. Therefore, further comprehensive studies are essential to unravel the intricacies of these potentially crucial signaling mechanisms.
In this context, essential questions that demand attention include: (1) unraveling the underlying molecular mechanisms governing airineme target specificity, (2) exploring the ultrastructure of airineme filaments and vesicles to gain valuable insights into their formation and regulation, (3) enhancing our understanding of the nature of metaphocyte-airineme interactions, (4) identifying airineme-specific molecular regulators, and (5) investigating the existence of airineme-mediated intercellular signaling in mammalian contexts. Importantly, given their role in transmitting key signaling molecules, the potential existence of airinemes in humans raises the prospect that their malfunction could be linked to certain human diseases, even though this is not currently recognized.
For technical advancements, it is imperative to develop unbiased large-scale analysis tools for time-lapse movies. Given the temporal nature of airinemes, long-term live imaging currently stands as the most effective method for their detection. Analyzing extensive time-lapse data demands highly trained individuals for consistent analyses. Therefore, recent AI-based unbiased image analysis tools have the potential to propel the field forward and unveil unnoticed cellular behaviors that may elude human eyes.

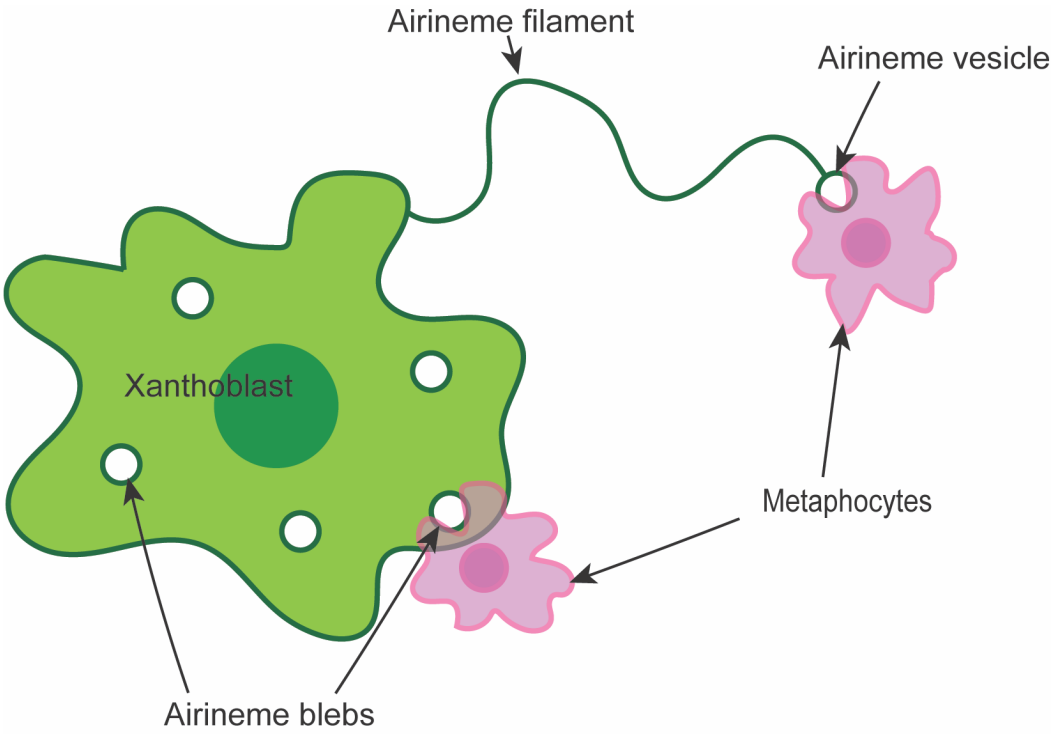

Figure 1. Airinemes and airineme-pulling metaphocytes.
A metaphocyte (bottom left) recognizing a phosphatidylserine (PtdSer)-rich airineme bleb engages in a 'nibbling' interaction. Another metaphocyte (upper right) is depicted dragging an airineme bleb, referred to as an 'airineme vesicle' after metaphocyte pull, during migration, with a trailing filament.

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