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Exploring the dynamic behavior of leukocytes with zebrafish

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Abstract

Cell migration is a complex and intricate network of physical, chemical, and molecular events that ultimately leads to cell motility. This phenomenon is involved in both physiological and pathological processes, such as proper immune and inflammatory responses. Dysregulation of cell migration machinery in immune cells can have a tremendous impact in the trajectory of inflammation, infection, and resolution. The small vertebrate, the Zebrafish, has a remarkable capacity for genetic and pharmacological manipulation aligned to transparency that enables modulation and visualization of cell migration *in vivo* non-invasively. Such characteristics revolutionized the field of leukocyte biology, particularly neutrophils. In this review we will focus on leukocyte migration and highlight findings made in the Zebrafish that demonstrate how this small vertebrate system is a unique model to perform *in vivo* imaging and study mechanisms that regulate the dynamic behavior of immune cells in their native environment under homeostasis or upon challenge.

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Keywords

Cell Migration; Zebrafish; Non-invasive Imaging

Zebrafish model – an unparallel system to study *in vivo* cell migration.

Zebrafish, *danio rerio*, are a small vertebrate model that first gained traction in the 1980s for their fast external development and genetic tractability [1]. Zebrafish has over 70% of gene homology with humans and 84% of disease-causing genes have at least one zebrafish orthologue [2]. The community has developed a wide number of disease models, and transgenic lines readily available (ZFIN; zfin.org) [3] to study cell dynamics in real time in their environemnt. Moreover, the zebrafish optical transparency and small size have proven to be an ideal model to follow cells in real time in a whole-animal context. The development of transgenic lines with fluorescently tagged neutrophils [4–6] macrophages [7,8] and T cells [9–12] provided access to visualize immune cell migration behaviors and dynamics *in vivo* under homeostasis or upon challenges (e.g., wounding, infection, or malignancy). The use of this system has revolutionized the study cell morphology, migration, and dynamics both in health and disease [5,9,13–15].

Revolutionizing the study of neutrophil migration

Directed cell migration, towards or away from diverse environmental cues, relies on four events which consist of generating, sensing, transmitting, and executing the signal, recently termed the “four pillars of directed migration (reviewed in [16]).” Leukocyte motility is often classified as an ameboid type of migration, which is characterized as an adhesion-independent movement observed for example in neutrophils [17]. However, macrophages can also display a mesenchymal migration pattern, which is protease- and podosome-dependent [17] (Figure 1).

The use of the zebrafish has drastically changed the way biologists study cell migration through interstitial tissues, particularly neutrophil migration(reviewed in [18]). Through the generation of the first transgenic lines that enabled the visualization and tracking of neutrophils in their native microenvironment, the dogma that all neutrophils were doomed to die at injury sites was challenged. In 2006, *Huttenlocher et al* reported for the first time that neutrophils have the capacity to migrate away from the inflammatory site back into the vasculature [4]. With this discovery, and later support in a mammalian model more than a decade later [19], a new area of research emerged with exceptional potential for therapeutical target to promote resolution [20]. The specific mechanism that neutrophils use to undergo reverse migration is still unclear (reviewed in [21–23]). Up to now some of the main findings in the zebrafish suggest that macrophages control neutrophil reverse migration through redox and Src family kinase signals, via macrophage-neutrophil contact [24]. Zebrafish Cxcl8a and Cxcr2 are required for neutrophil reverse migration and resolution *in vivo* [25], as well as lipid mediators such as, prostaglandin E2 (PGE2) and lipoxin A4 (LXA4) [26]. In addition to signals that drive reverse migration, there are also signals that retain neutrophils at sites of injury such as the activation of the oxygen-sensing

transcription factor hypoxia-inducible factor-1α (HIF-1α) or CXCL12 / CXCR4 signal axis, inhibition of these signals promotes reverse migration of neutrophils and accelerates resolution [27,28] (Figure 2). Interestingly, neutrophils that undergo reverse migration have a unique phenotype, compared to those in circulation or tissue resident neutrophils, which seems to be associated with dissipating inflammation throughout the body [29]. However, zebrafish studies from *Ellet et al*, showed that neutrophils' response and antibacterial effect after reverse migration upon secondary injury were not significantly different from those at the recruitment stage [30]. Not surprisingly, the zebrafish are being used as a main high throughput screening tool to identify new drugs to modulate this phenomenon, as a way to promote resolution [20,31,32].

Myeloid cells migrate towards signal gradients, called chemoattractants that trigger actin polymerization at one end and depolymerization at the other [3]. Actin formation and assembly are important features for leukocyte motility. As actin is polymerized and protrusions form, global inhibition will generate contractile force, providing the cell polarity and movement towards the chemoattractant. In neutrophils, contractile tension is more important than protrusions when interpreting chemoattractant gradients [33]. Neutrophil motility (random and upon challenge) relies on the persistent polarization of stable F-actin via Actin-related protein 2/3 complex (Arp2/3). For such PI(3)K activation at the leading edge through the modulation of both Rac-mediated protrusion and F-actin polarity is needed. PI(3)K activation resulted in accumulation of dynamics of its products PI(3,4,5)P3-PI(3,4)P2 during neutrophil migration, as visualized in living zebrafish using radiometric imaging [34]. *In vivo* visualization of microtubule dynamics during neutrophil migration in intact tissues showed a strong polarity toward the rear of the cell, with microtubule-organizing center (MTOC) localized in front of the nucleus[17,35]. Furthermore the microtubule cytoskeleton suppresses neutrophil polarity and motility through negative regulation of both Rho and Rac in a PI(3)K-independent manner [36]. In response to wounding, the heterotrimeric G protein subunit Gβ1 signaling controls neutrophil migration by activating PI(3)K and modulates actin dynamics [37]. Recently, lattice lightsheet microscopy (LLSM) has been used to visualize neutrophils *in vivo*, revealing that migrating neutrophils commonly carry thin trailing extensions at the uropod, with surprising length that are left behind as cell moves away [38]. Uropod extension dynamics and detachment are regulated by myosin II [38]. Additionally, intravascular behaviors like leukocyte rolling and transmigration were visualized using non-invasive intravital microscopy, allowing detailed visualization of rolling neutrophils extending cytoplasmic tethers or lengthier slings that attached and detached as neutrophils moved along the inner endothelium [38].

Temporally and spatially coordinated gradients of chemoattractants and chemorepellents are crucial in mediating neutrophil directional motility in interstitial tissues, characterized by distinct phases including scouting, amplification, and stabilization (reviewed in [21,39]). Under normal conditions most neutrophils reside in the hematopoietic tissue and upon stimulus of the promigratory pathway and the CXCR2 receptor, neutrophils are released into circulation. The primary ligand of CXCR2, CXCL8, is not found in the mouse genome. However, zebrafish have two homologs of Cxcl8 (Cxcl8a and Cxcl8b), which make it a unique and suitable vertebrate model to study migration and the circulation of neutrophils into the bloodstream [40]. Upon mild and severe tailfin wounding in zebrafish, Cxcl8b

controls the release of neutrophils into the bloodstream and Cxcl8a recruits neutrophils in circulation to the wound [41]. In response to injury, neutrophils (and macrophages) are recruited to injury from distal sites and also mobilize into peripheral blood, using abluminal endothelial surfaces of lymphatic and blood vessels as migration highways [42]. After leaving the blood vessels neutrophils are guided by chemotactic signals, through G-coupled protein receptors (GPCRs) such as BLT1, CXCR1, and CXCR2 [43]. CXCR2 activation causes intracellular calcium to rise [44]. This influx in calcium concentration comes from cytosolic storage and permeable ion channels like transient receptor potential channels (TRP) [44]. Using an extended mathematical model to study the trajectory of neutrophil migration *in vivo* and *in vitro* towards chemotactic signals, it was found that chemotaxis results in anomalous migration, a Brownian motion, moving towards the signal [44]. Interestingly, CXCR2 inhibition and a TRPC6 knockout (KO), resulted in tempered migration, also defined as a lapse in the memory of migration [44].

One of the most complex phenomena that involves neutrophil coordinated motility is swarming behavior. Upon tissue damage neutrophil migration changes from passive surveillance to coordinated swarming in response to chemoattractant signals, such as leukotriene B4 (LTB4) that is sensed by BLT1 [43]. While external cues drive neutrophil activation, recruitment is largely sustained by local and self-organized cues [45]. Zebrafish have emerged as a unique system capable of studying swarming behavior, at a single cell level. Combining new biosensors and live imaging in zebrafish, it has been shown that pioneer neutrophils sense the damage signal ATP, which leads to LTB4 synthesis that is triggered by calcium flux upon neutrophil contact with the necrotic tissue [43]. The subsequent neutrophil coordinated motion and swarming is contact-dependent via connexin-43 (Cx43) hemichannels, which enhance chemoattractant biosynthesis and sustain cluster growth [43]. Inhibition of neutrophil Cx43 impairs clearance of wound-colonizing bacteria, supporting a crucial role in the sensing and propagation of alarm signals in the formation of the dense antimicrobial cell masses that block opportunistic pathogens to breach tissue barriers [43]. In addition, pioneer neutrophils adopt distinct phenotypes from other migrating neutrophils, developing a rounded morphology which is coupled with a reduction in speed and displacement [46]. The release of extracellular traps (NETs) that contain chromatin and myeloperoxidase from pioneering neutrophils promotes neutrophil swarming [46] supporting that pioneer neutrophil and the mechanisms that are triggered upon sensing alarm signals in damaged tissues, are crucial in regulating swarming formation [43].

Neutrophils are recruited to sites of injury and infection through the detection of endogenous signals called damage associated molecule patterns (DAMPs) or by exogenous molecules, pathogen associated molecular patterns (PAMPs) [21]. DAMPs that drive early neutrophil recruitment, such as ATP, Calcium and hydrogen peroxide [47,48] increase chemokine production, specifically Cxcl8 [49], which further activate neutrophil recruitment and neutrophil release into circulation [21]. While neutrophils respond to both tissue injury and infection, it remains unclear what signals differentiate these two responses. Comparing transcriptomic profiles from zebrafish neutrophils the myeloid derived growth factor (MYDGF) emerged as a damage signal that regulates neutrophil interstitial motility and inflammation through a HIF-1α pathway towards damaged tissues, but not infected [50].

New regulators of neutrophil migration are emerging. Interestingly, mitochondrial outer membrane protein Mitofusin 2 (MFN2) regulates cell migration and the actin cytoskeleton *in vivo* [51]. In addition, micro-RNAs (miRs), which are known as powerful regulators of neutrophil biology, have emerged also as key modulators of neutrophil chemotaxia *in vivo* [52]. miR-99 for example, directly targets the transcriptional factor RAR-related orphan receptor alpha (*roraa*), which has important functions in other immune cells, but is necessary for neutrophil recruitment and receptor activation [53]. The therapeutical potential of selectively targeted miRs to manipulate neutrophil migration, at different inflammatory and disease contexts, is a largely unexplored field that zebrafish can serve as a fast large-scale screening resource.

Cell migratory plasticity of macrophages.

Neutrophils and macrophages display distinct modes of motility [17]. While neutrophils display ameboid cell migration characteristics, *in vivo* live imaging of macrophages in a sterile wound context revealed that macrophages display behavioral plasticity during inflammation and change their shape from amoeboid to elongated during healing [54]. Macrophages are therefore reprogramed at injury sites based on environmental cues that regulate their polarization and behavior switching, such as 15-lipoxygenase (LOX) [54]. Additionally, morphometric analysis identified four different cell shapes that macrophages adopt, consisting of amoeboid, star-like, elongated, and rounded shapes [55].

Interestingly the dynamic morphological changes of macrophages during migration regulate their speed and motility [55]. Mesenchymal migration of macrophages depends on proteases to degrade the extracellular matrix (ECM) [17]. Inhibition of macrophage proteolysis, using an MMP-9 inhibitor, causes macrophages to adopt an ameboid like shape impacting their migration patterns [55]. Mesenchymal migration is also a Rac dependent process, as Rac inhibition dampens macrophage migration [56] and degradation of the ECM [55]. Macrophage's ability to degrade and remodel the ECM is extremely important during development, cancer metastasis, and cell intravasation [55]. Consequently, Rac inhibition of macrophages in the aorta-gonad-mesonephros (AGM) leads to ineffective hematopoietic stem cell colonization and organization in the caudal hematopoietic tissue (CHT) [55]. Additionally, the ADP ribosylation factor (ARF) GTPase activation protein ASAP1 that regulates cytoskeletal dynamics, small GTP-binding protein receptor recycling, and intracellular vesicle trafficking has been shown to regulate macrophage migration. Zebrafish larvae deficient in Asap1 displayed impaired macrophage migration to tail injury or upon infection with *M. marinum*, impacting susceptibility to infection [57].

Focal adhesions are fundamental molecular structures that link the cell cytoskeleton and ECM and are responsible for proper cell migration [58]. They are dynamically assembled and disassembled at the leading and trailing edge of the cell, generating mechanical forces causing the cell cytoskeleton to contract, facilitating cell movement [58]. Paxillin a core protein in focal adhesions that signals downstream to activate cell migration, is regulated by several phosphorylation events that recruit adaptor proteins, such as CRKII that activate guanine exchange factors (GEFs) [58]. Neutrophils and macrophages rely differently on paxillin during migration through interstitial tissues *in vivo* [17]. Indeed,

although neutrophils express the cytoskeletal proteins vinculin and paxillin, they do not form mature focal adhesions contrary to macrophages [59]. Using zebrafish, the visualization of focal adhesions became possible by overexpressing fluorescently tagged paxillin both in macrophages and neutrophils. Elongated ameboid macrophages form paxillin-containing puncta display reduced speed and increased directional persistence, while the ameboid morphology of migrating neutrophils lacks defined paxillin puncta allowing these cells to migrate fast [17].

Macrophage random migration is also dependent on Arp2/3; however, upon the activation of directional migration this mechanism is less reliant on Arp2/3, suggesting other actin nucleators are involved [17]. Additionally, as in neutrophils radiometric live imaging of PIP3 using PHAKT-GFP biosensor in macrophages allowed visualization of PIP3 localization at the leading edge, however projections at the rearward side of the cell during both protrusion elongation and retraction also localize PIP3 [17]. Microtubules network, which are essential in promoting turnover of adhesion complexes and regulating the actin cytoskeleton, also present a distinct localization in macrophages and neutrophils. *In vivo* visualization of microtubules with ensconsin microtubule-binding domain fluorescent probe (EMTB-3X GFP), showed that microtubules localize both at the rear and the front in macrophages, while neutrophil microtubules localize toward the rear of the polarized cell [17].

Dendritic Cell and Lymphocyte migration – un unexplored field in zebrafish model.

As in mammal, dendritic cells (DCs) form a link between the adaptive and innate immune systems through their ability to stimulate T-cell activation and proliferation serving as antigen presenting cells (APCs) [60,61]. A population of dendritic like cells have been identified in the kidney marrow of adult zebrafish using differential lectin binding assays [60]. More recently, using cross-organ single cell transcriptomic analysis Zhou *et. al.*, described two major classes of DCs, conventional DCs (cDCs) and plasmacytoid DCS (pDCs), found widely distributed in the non-lymphoid organs of zebrafish and emerging at larval stages. Functional studies on DCs and their migration patterns and behavior are now possible due to the generation of specific reporter lines that allow visualization and tracking of diferent subsets of DCs and distinction from macrophage subsets[62] (Table 1). Like the innate immune system, the adaptive immune system between fish and mammals is remarkably conserved, reviewed [63]. Zebrafish have B-cells that express Ig proteins, as well as T-cells with receptor components that are mediated by Rag1 and Rag2 protein rearrangements [9]. Additionally, although zebrafish have many lymphocyte receptor homologs, they lack antibodies for these populations, relying heavily on transgenic lines to visualize cell dynamics, including lymphocyte migration [9]. For many years lymphocyte migration has not been properly explored in the zebrafish model, even though specific transgenic reporter lines for B and T cells have been developed in the last two decades (Table 1). Recent advancements and important contributions from the Huttenlocher lab might change this trend [64]. In mammals naïve T-cells migrate from the lymph nodes, located throughout the body, to scan for antigens. With a combination of scRNA-seq and microscopy, a lymphoid network, devoid of lymph nodes, that performs whole-body antigen

surveillance was identified in zebrafish [64]. This tessellated lymphoid network (TLN) maintains many characteristics of motile lymphocytes, including morphology and directed and coordinated movement [64]. Using scRNA-seq revealed three major APC populations and naïve like T-cells that express *ccr7*, both indicative hallmarks of lymph nodes [64]. T-cells within this network collectively and directionally migrate, but in the context of infection transition to single cell Brownian walk, allowing these cells to interact with APCs, activate, and differentiate [64].This groundbreaking study from Robertson *et. al.* [64] will for sure push the field to make use of the tremendous characteristics of the zebrafish that associated with the increasing knowledge on fish immunology and evolution of the immune system, will open opportunities for new research programs be established focused on lymphocyte cell dynamics and migration.

Boosting research in rare genetic diseases and discovery of novel therapeutics.

Zebrafish has emerged as a powerful in vivo model to investigate “untouched” human rare diseases that present as severe, chronic, progressive, and mostly with a genetic cause. Zebrafish has been pivotal in offering an affordable system to increase knowledge about the pathophysiological mechanism of disease in rare conditions including blood, skeletal and neurological rare diseases (reviewed in [65–68]). The zebrafish is currently a main choice to confirm gene discovery, to perform genetic analysis of human variants, or to study orthologs to mouse lethal allele. A group of rare blood diseases named primary immunodeficiencies (PIDs) represent a heterogeneous group of over 200 genetic disorders characterized by the partial or complete absence of the immune system or its improper activity (i.e., function and migration) [67]. Patients with PIDs present increased vulnerability to germs such as bacteria, viruses, fungi, and protozoa. There are over 230 identified PID-causing genes, but many more for sure to be discovered. In the last two decades, zebrafish have been used to model different PIDs and determine how genes associated with different diseases impact production, function and migration of immune cells. Some of the available zebrafish PIDs models are: Wiskott-Aldrich syndrome (WAS) [69] warts, hypo-gammaglobulinemia, infections, and myelokathexis (WHIM) syndrome [70], chronic granulomatous disease (CGD) [71], leukocyte adhesion deficiency (LAD) [72,73] REF, activated PI3K delta syndrome (APDS)[74]. Most of these models were used to explore mechanisms that impact myeloid cells but the impact in dendritic cells, T and B cells is still to be explored. Additionally, the use of PID models to design therapeutics to restore proper immune response in patients with PIDs is still vastly unexplored and deserves further attention since it could provide valuable approaches for patients. Access to next-generation sequencing techniques that quickly identify gene defects in small populations (or single patients) with inherited diseases aligned to the latest genome-editing techniques such as CRIPR/Cas9, and the numerous unique features of the zebrafish model, are offering an unprecedent opportunity to model and understand the physiopathology of PIDs and other rare genetic human diseases. Interestingly, even though many human disease models have been developed and fully characterized in zebrafish, their use in designing and developing new therapeutics is far from reaching its full potential. In the years to come private sector

investment needs to be stimulated and encouraged so that they can transition from in vitro to in vivo drug screening pipelines with zebrafish models.

Future areas to explore leukocyte dynamics.

Evaluating leukocyte migration within their *in vivo* environment is crucial to better understand their physiological behavior in the context of homeostasis and disease. As the scientific community becomes more elucidated and open to non-mammalian animal systems, they will transition from conventional 2D models to more complex and physiological scenarios, such as the zebrafish. The combination of an optimized *in vitro* system with *in vivo* approaches where cells are being exposed to multiple migration-inducing cues, simultaneously, is key to understanding leukocyte migration in the context of disease. A big question that zebrafish will continue to assess is how cells switch their migration direction, particularly neutrophils. Are neutrophils reprogrammed at the injury site and disseminate inflammation upon reverse migration, or is this a specific subset of neutrophils? Our most recent work shows that the presence of metainflammation drastically impacts neutrophil recruitment. We found that neutrophils are activated at the nonalcoholic fatty liver and able to reverse migrate displaying a fast and exacerbated migratory behavior towards an injury. In vivo time lapse microscopy showed that neutrophil exposed to metainflammation move faster than control larvae and seem to be unable to slow down at the wound suggesting defects in migration machinery [75]. So, how is neutrophil polarization and subsequent forward and reverse migration impacted by the presence of systemic inflammation (e.g., metainflammation or inflammaging) Clarification in this area is key to properly develop and use therapies that target neutrophil migration in scenarios of neutrophilic inflammation that are sustaining chronic or hyperactive inflammation (autoimmune diseases, cytokine storm, chronic lung disease, cancer early progression, etc.) and contributing to poor outcomes. Another important topic to expand our knowledge is how leukocytes use different migration modes (amoeboid versus mesenchymal) to migrate in response to various environmental cues that are present in different disease states. Are macrophages the only leukocytes that display morphological plasticity *in vivo*? Do dendritic cells behavior similarly? And B and T cells, what are the cell dynamics and the mechanisms these cells use to migrate in tissues and organs? New emergent techniques such as spatial-transcriptomics [76], single cell proteomics [77], or behavioral immune landscapes of inflammation [78] will play important roles in answering this and many other questions. Finally, studying immune cell trafficking through non-invasive intravital imaging, and establishing consistency of analysis across different laboratories is crucial for proper advancement of the field of cell migration [79].

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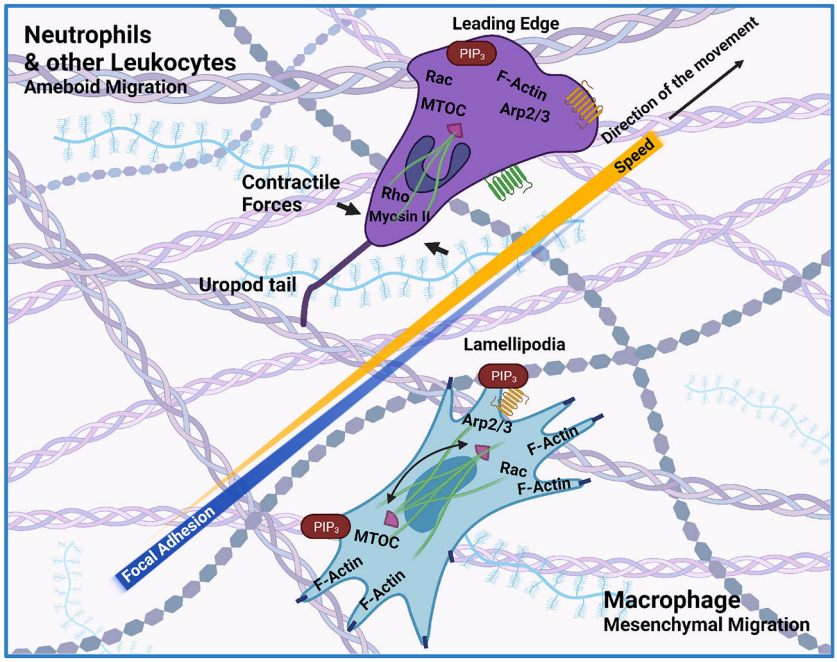

	Ameboid (Propulsion)	Mesenchymal (Traction)
Migration Speed	Fast	Slow
Polarity	Front and rear	Competing lamellipodia
Adhesion	Relatively week (mostly intracellular)	Strong (with ECM), well defined adhesion complexes
Proteolysis	Proteolysis independent	ECM proteolysis dependent
Migration in vivo	Squeezing through ECM pores	Traction via adhesion to ECM
Actin	Actomyosin contractability rear and F-actin at leading edge	F-actin in lamellipodia, actomyosin minifilaments attached to focal adhesions
MTOC localization	In front of the nucleus	Alternate from front and rear of the nucleus

Figure 1: Amoeboid and mesenchymal migration - Similarities and differences.

Overall, all leukocytes, including neutrophils use an ameboid migration pattern. This type of migration does not rely on proteolytic degradation of the ECM nor strong focal adhesion therefore this type of migration is fast. Establishment of a clear cell polarity is crucial for proper migration with F-actin localizing at the leading edge together with PIP3 gradient. Rho activation and Myosin II generate contractile forces that are responsible for the propulsion of neutrophils. Recently, using LLSM it has been shown that neutrophils display a long Uropod trail that eventually loses while migrating. Interestingly MTOC localizes in

the front of the nucleus. Macrophages are known by their morphological plasticity and can also acquire mesenchymal phenotypes with a slow migration speed. This type of migration uses lamellipodia that competes to guide cells through the interstitial tissue. F-actin is localized in lamellipodia, and small myosin filaments attach to focal adhesion allowing cell to interact strongly with ECM. Mesenchymal migration relies heavily on traction. Interestingly, contrary to ameboid cells MTOC is located both at the rear and front of macrophage nucleus.

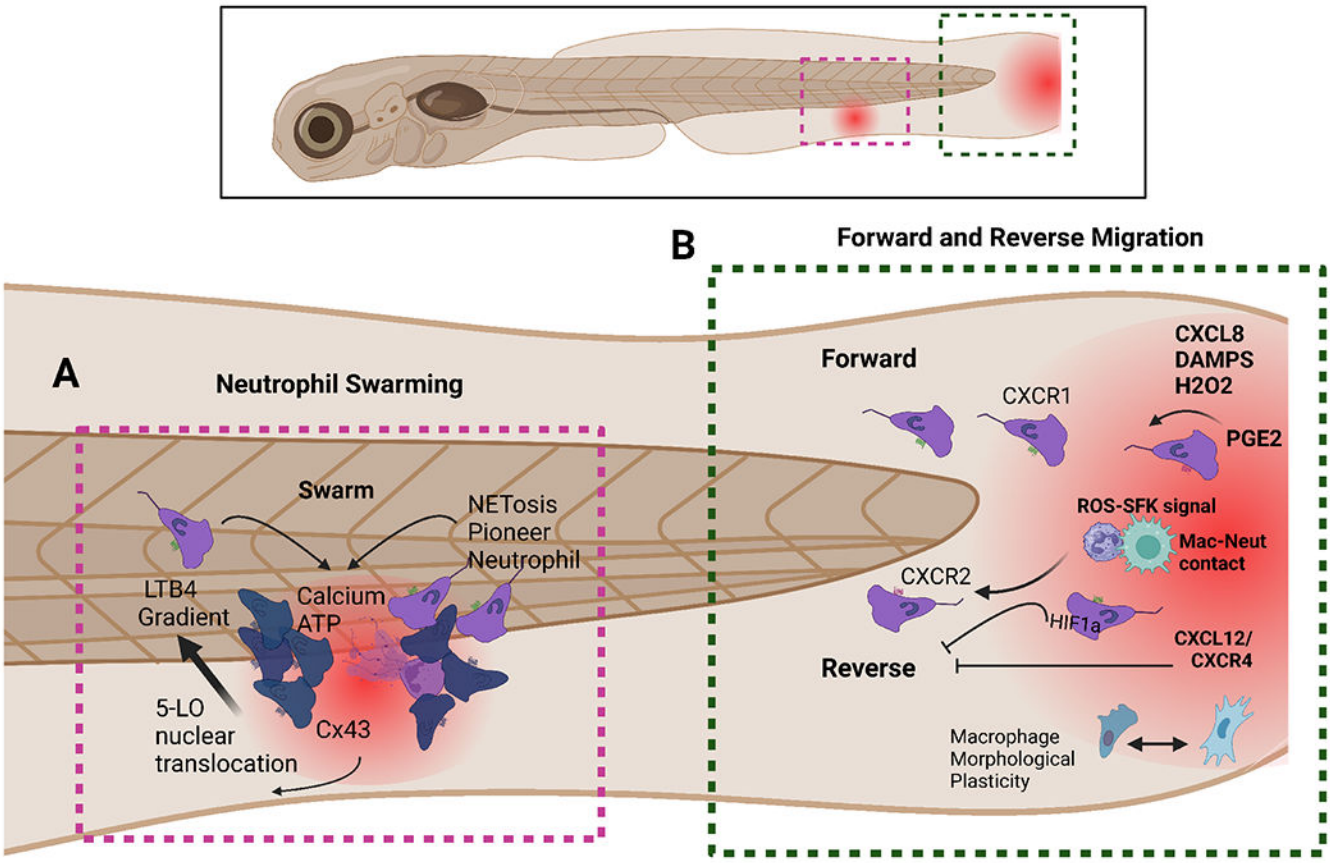

Figure 2: Myeloid migration becomes clearer with zebrafish.

In the last decade and a half, the neutrophil community has been engaged in unravelling the mechanism and pathological role of reverse migration. **A.** Calcium alarm signals in neutrophil clusters locally promote attractant synthesis and are dependent on ATP sensing and contact with necrotic tissue. Clustering neutrophils initiate and propagate calcium alarm signals via Cx43 channels Neutrophil swarms and Cx43 restrict wound colonization by opportunistic bacteria. **B.** CXCL8 and other chemoattractants regulate neutrophil migration to injuries via CXCR1. CXCR2 on the other hand is needed for forward migration. Additionally, several mediators coordinate neutrophil reverse migration such as PGE2. Some other molecules such as ROS-SFK signal in macrophages and cell-cell contact are also vital for proper neutrophil reverse migration. Cxcl12/CXCR4 signal has been identified as a druggable retention signal for neutrophils at tissue damage that can be blocked using AMD3100.

Table 1:

Transgenic reporter zebrafish lines targeting Lymphocytes and Dendritic cells.

Transgenic line	Immune cell	Allele (Ref.)
Tg(rag1:GFP)	Lymphocytes	la5Tg [10]
Tg(rag2:GFP)	Lymphocytes	zdf8Tg & 1a6Tg [11,80]
Tg2(rag2:mCherry)	Lymphocytes	bu3Tg [81]
Tg(lck:lck-EGFP)	T Cells	cz1Tg [9]
TgBAC(foxp3a:TagRFP,cryaa:EGFP)	Treg	vcc3Tg [82]
TgBAC(cd4-1:mCherry)	CD4 T Cells	umc13Tg [83]
Tg(-14cd79a:EGFP)	B Cells	fcc97Tg [84]
Tg(-5cd79b:EGFP)	B Cells	fcc90Tg [84]
Tg(Cau.Ighm:EGFP)	B Cells	sd19Tg [85]
TgBAC(ccl35.1:EGFP)	Dendritic cells	n.a. [62]