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Advances in the Studies of NK Cell Migrations Using Microfluidic Devices

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Introduction

Proper cell migrations are tightly regulated in unicellular and multi-cellular organisms [1]. For example, cell migration is a critical process in our immune system that will determine the development of different specific immune cell types, promote immune surveillance and the proper mounting of host defence against invading microbes [2,3].

Natural killer cells (NK) are granulocytic lymphocytes derived from bone marrow hematopoietic stem cells [4]. The molecular mechanism(s) that govern NK differentiation remains unclear. Soluble factors (such as IL-15, SCF, Flt3L and IL-7), transcription factors (e.g. Ikaros, E4BP4), and microenvironment of bone marrow are involved in this coordinated NK differentiation process [5-8]. Previous study has found DNA methylation is essential for the maintenance of clonal restricted expression of highly homologous KIR genes and alleles in NK cells [9]. However, in the NK cells, the enzyme that required for the establishment of DNA methylation at KIR gene loci remains unknown. A basic leucine zipper transcription factor E4BP4 plays a key role during development of NK cells [10].

Since recent study has found E4BP4 interacts with histone methyltransferase G9a [11] and G9a is able to maintain DNA methylation loci specifically in mammalian cells [12], NK cells may therefore maintain their clonal specificity by employment of E4BP4/G9a/DNMT complex in mammals. NK cells were first identified because of their "natural" ability to recognize and eliminate "abnormal" transformed cells without prior sensitization [4]. The outcome of such target recognition mechanism is determined by the balance of the signals generated by simultaneous engagement of activation and/or inhibitory NK receptors on a NK cell surface [13]. Upon activation, NK cells can mediate direct cytotoxic killing of target cells, and/or production of soluble chemokines and cytokines that are essential in inducing innate immune responses, shaping adaptive immunity and recruitment of other immune cells to the site of inflammation/infection [14]. Migratory properties of NK cells upon different stimulations are less studied *in vitro*, as compared to their other effector functions (cytotoxicity and cytokine responses).

NK cells trafficking to peripheral organs and/or inflamed lymph nodes to facilitate immunosurveillance directly or indirectly through their interactions with dendritic cell (DC), a critical cell type that regulates adaptive B and T cell functions [15,16]. DC produces a number of chemokines, such as chemokine (C-X-C motif) ligand 8/ IL-8 (CXCL8/IL-8), chemokine (C-X-C motif) ligand 9 (CXCL9), C-X-C Motif Chemokine Ligand 10/ IFN-γ-inducible protein 10 (CXCL10/IP-10) and C-X-C motif chemokine 11 (CXCL11), to induce NK-cell migrations [17,18]. NK cells acquire several chemokine surface receptors (ranging from CXC, CC, C and CX3C motif chemokine receptors) during development and maturation [16,19]. Preferential migration and localization of NK cells into the lymph nodes are dependent on chemokine receptors such as C-C chemokine receptor type 7 (CCR7), C-C chemokine receptor type 5 (CCR5) and C-X-C Motif Chemokine Receptor 3 (CXCR3) [20-24]. Whereas NK cells reside in blood, liver, spleen and inflammatory sites exhibit higher chemokine (C-X-C motif) receptor 1/ IL-8 receptor (CXCR1) and C-X3-C Motif Chemokine Receptor 1 (CX3CR1) surface expressions [22,25,26]. In addition, several non-chemokine family proteins such as chemerin and sphingosine 1-phosphate (S1P) 5 were also found important in orchestrating NK cell trafficking [27,28].

A Simple Microfluidic System for Imaging of NK Cell Trafficking

The conventional methods used in cell migration assays (such as the trans-well method) are useful but lack the ability to provide a stable gradient environment for single cell analysis (Table 1). Microfluidic systems offer its advantage to study the micro environment events in micrometer scale. It is easier to control and manipulate the fluidic behaviors using microfluidic devices. The microfluidic device based cell-migration studies have been rapidly growing over the past decade [29-32]. Particularly relevant to our work in NK cell migration, Lin et al. developed a simple "Y" shape microfluidic device for cell migration studies (Figure 1) [33]. In this device, a migration medium and a chemokine solution of interest are continuously infused into the device by syringe pumps through tubing and the inlets of the device, at a total flow rate of 0.2 μL/min to define a stable chemokine gradient. The cells were imaged at a few millimeters downstream of the "Y" junction.

Through close collaboration between the Kung lab and the Lin lab at the University of Manitoba, we recently established the first application of this Y-shaped device in the imaging and analyses of the abilities of the immature and mature DC to regulate murine IL-2 activated NK cell migrations *in vitro* [34]. We generated immature and lipopolysaccharide (LPS)-matured bone marrow derived DC (BMDC) using the established Granulocyte macrophage colony stimulating factor (GM-CSF) culture condition. Conditioned media from these DC cultures were collected and assayed for its ability to regulate NK cell migration in the microfluidic system. We reported that soluble factors released by immature and LPS-matured BMDC contained chemotactic signals which induced a modest and a high level of chemotactic movements of IL-2 activated NK cells *in vitro* respectively. We identified that CXCR3 is a key receptor on NK cells that mediated the migration. More interestingly, a surprising function of GM-CSF in repulsing murine NK cell migratory response was revealed in this microfluidic assay [34]. This study highlighted also key feature and difference of the two migration assays (trans-well and microfluidic assays): cell migration parameters (speed and chemotactic index) were

analyzed in the microfluidic assay, at the single-cell level on a 2D plane under a stable chemical gradient; in contrast, chemotaxis was measured as a net downward movement of a population of the migratory cells in the trans-well assay.

	Microfluidic devices	**Trans-well assay**
Ease of uses	Relative easy, but specific devices are needed	Easy, simple to set up and use
Cells analyzed	Migrating cells analyzed at a single-cell level	Migrating cells analyzed as a bulk population
Chemotaxic responses	Yes	Yes
Chemo-repulsion	Yes	No
Migratory responses under stable chemical gradient	Yes	No
Ability to mimic multiple, complex chemical gradients	Possible	No

Table 1: Side-by-side comparison of the microfluidic devices and conventional trans-well assay in the studies of NK-cell migrations *in vitro*

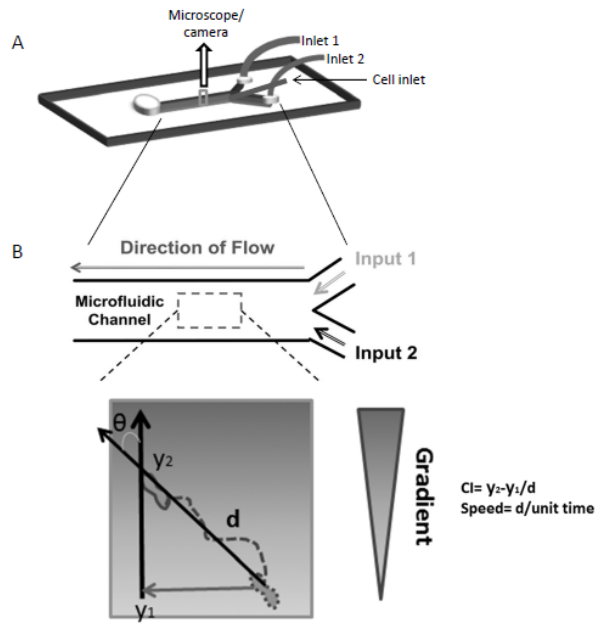

Figure 1: A Y-shaped microfluidic device used in cell migration studies. A. Schematic illustration of the Y-shaped microfluidic device for cell migration [33]. B. Illustration of the quantitative cell migration data analysis for each single cell videotaped under the microscopic view. CI, chemotactic index; y1, y2, displacement along each axis; d. distance travelled.

In addition to the application of this simple Y-shaped microfluidic device in the studies of NK-DC crosstalk, we have also used this platform to study NK cell migrations in the conditioned medium from tumor cells. Recent work in breast cancer patients and mouse breast cancer models suggests an important role of NK cells in controlling tumor growth and metastasis [35]. High level of CX3CL1 expression by breast cancer cells supported recruitment of tumor-infiltrating CD8+ T cells, NK and DC cells was correlated with a good prognosis for disease-free and overall survival [36]. Activated NK and NKT cells rendered adoptively transferred T cells resistant to myeloid-derived suppressor cells and effective in adoptive cellular therapy against breast cancer [37]. In addition, abilities to augment NK cells functions, such as depletion of regulatory T cells [38] and forced expression of CKb-11 chemokine in breast cancer cell line recruited NK cells to the tumor site [39], promoted tumor rejections. The mechanism behind the low infiltration of NK cells into the malignant breast cancer, as well as other tumor tissues [40] is not yet clear. As migrating immune cells at tumor sites are critical in mounting effective anti-tumor activity, it is possible that cancer cells produce factor(s) that prevent the infiltration of NK cells into the tumor environment. Studying NK cell migrations in tumor microenvironments in microfluidic based systems will provide important insights into our understanding of NK cell immunosurveillance of cancer and in the development of NK cell based immunotherapy.

Future Perspectives on Studying NK Cell Migration Using Microfluidic Devices

Our study demonstrated the effective use of a simple microfluidic device in experimental studies of directed NK cell migration in controlled gradient environments and for quantitative cell migration data analysis at the single cell level [34]. The study revealed interesting insights regarding migratory interactions between NK cells and dendritc cells. The main features of this Y shape device is its simple design, control of stable simple or co-existing chemical gradients and the ability to trace single cell migration in real-time. Indeed, the useful features of this device have also been applied to study chemotactic and chemo repulsive migration of other immune cells such as primary neutrophils, T lymphocytes, stem cells and various cell lines [33,41-46]. On the other hand, this simple microfluidic device has a number of limitations including varying gradient profiles along the gradient channel, requirement of external syringe pumps for chemical flow infusion, fluctuations of flows due to the step-pumping, low throughput and being as a simplified 2D system. The migration data analysis is further complicated by the uncontrolled cells initial positions in the gradient channel and it is difficult to configure multiple co-cultures in such a device. Therefore, further development of novel microfluidic devices to address these limitations will be critical to advance microfluidics-based NK cell migration research. Here we discuss our recent and envisioned future development in this direction.

First, to remove the requirement of external pumps, we have developed a microfluidic device, which creates the chemical flows by the pressure difference between the inlet and outlet reservoirs, and a balance zone design to equilibrate the pressure difference between flows from different inlets [46]. A chemical gradient is generated in the downstream channel and can be maintained over sufficiently long time for immune cell migration experiments. This device has been successfully demonstrated to study neutrophil migration and chemotaxis for both basic research and clinical application [46]. This device inherits the useful features of the simple Y shape device while frees the experiment from external pumps and avoid the gradient fluctuation due to step-pumping. We expect that this new device can improve the operation of NK cell migration experiments. On the other hand, more complicated microfluidic channel design [31] together with accurate low pressure pumps can be employed to manipulate gradient profiles and test its effect on NK cell migration.

Second, when cells are randomly seeded in the gradient channel, individual cells start to migrate from different initial positions in the chemical gradient and their subsequent gradient exposure throughout the migration experiment is different. We have recently shown that neutrophils' migratory response is influenced by their previous chemical gradient exposure [41], and we expect such memory effect applies to NK cell migration as well. Therefore, the current averaged migration parameters (e.g. chemotactic index and cell speed) of all the cells tracked in the gradient channel is inaccurate to reflect cell migratory responses. To overcome this limitation, we have recently developed a novel microfluidic device that combined the standalone gradient generator and a cell docking structure, which can pre-align the cells along a thin barrier microgroove adjacent to the gradient channel before the chemical gradient is applied. Therefore, the cells starting positions are identical [47]. Furthermore, the cell pre-alignment feature of this device permits instant cell migration quantification by measuring the cell migration distance into the gradient channel without requiring single cell tracking analysis.

Third, we have already demonstrated that microfluidic device can be used to study NK cell migration to complex physiological fluidic samples (e.g. conditioned medium from cell cultures) [34]. In a similar way, we have studied chemotactic migration of human neutrophils as well as stem cells to diseased tissue samples such as sputum samples from patients with chronic pulmonary disease or tissue extract from injured rat heart respectively [46,47]. Incorporating this approach to the new microfluidic devices will further strengthen the physiological and medical relevance of NK cell migration studies. With the new cell docking design, we have the ability to align NK cells and different relevant cell types of interest (e.g. cancer cells or dendritic cells) or even tissue samples in a defined pattern in the microfluidic device. This will allow us to directly study the migratory interactions between NK cells and other cell types in a controlled manner. Flow-based or flow-free-based microfluidic gradient generators can be used separately or in combination to control exchange of cell derived products in the cell co-cultures and the effect of flow-induced shear stress on cells. Further incorporation and development of microfluidic devices with controlled 3D extracellular matrices (ECM) will more closely mimic the in-vivo physiological environments.

Finally, NK cells are contained in blood leukocytes at a low percentage (i.e. 1-6% of leukocytes). Therefore, a large volume of blood sample is often required to isolate enough NK cells for cell migration experiments even with the microfluidic device. The ability to expand NK cells *in vitro* is slow and limited. Therefore, it is highly desirable to directly isolate and culture NK cells from while blood followed by microfluidic cell migration experiments on a single chip. Very recently, we have developed an all-on-chip method to integrate negative magnetic cell isolation directly from a small drop of whole blood (a few microliters) using the reagents from a new cell isolation kits (STEMCELL) and then perform chemotaxis experiments on the same device following cell isolation [47]. The whole assay is done within 25 minutes. We successfully applied this method to study chemotaxis of untouched neutrophils from human blood induced by both purified chemoattractants and sputum samples from chronic pulmonary disease patients [47]. Relevantly, a similar cell isolation kit for NK cells is available and our developed all-on-chip method can be readily applied to study NK cell migration.

Concluding Remarks

Cell migration is a highly ordered cell behavior enabled by the complex regulations and interactions of various molecules at multiple levels. Microfluidics-based migration assay supported measurements of cell migration in precisely defined chemical guiding fields. Highly diverse approaches have been explored for microfluidic gradient generation and chemotaxis studies [29-32], thus supporting further feasibility of creating novel microfluidic devices that will better mimic the complex compartmentalization and physiology of chemotactic factors *in vitro*. Understanding NK cell trafficking mechanism, particularly in inflamed peripheral sites, lymphoid tissues or tumor microenvironments will provide new insights into the development of NK cell-based therapeutic approaches against tumor and infections.

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References

1. Horwitz R, Webb D (2003) Cell migration. Curr Biol 13: R756-R759.
2. Friedl P, Weigelin B (2008) Interstitial leukocyte migration and immune function. Nat Immunol 9: 960-969.
3. Luster AD, Alon R, Von Andrian UH (2005) Immune cell migration in inflammation: present and future therapeutic targets. Nat Immunol 6: 1182-1190.
4. Campbell KS, Hasegawa J (2013) Natural killer cell biology: an update and future directions. J Allergy Clin Immunol 132: 536-544.
5. Williams NS, Kubota A, Bennett M, Kumar V, Takei F, et al. (2000) Clonal analysis of NK cell development from bone marrow progenitors in vitro: orderly acquisition of receptor gene expression. Eur J Immunol 30: 2074-2082.
6. Raulet DH, Vance RE (2006) Self-tolerance of natural killer cells. Nat Rev Immunol 6: 520-531.
7. Huntington ND, Vosshenrich CA, Di Santo JP (2007) Developmental pathways that generate natural-killer-cell diversity in mice and humans. Nat Rev Immunol 7: 703-714.
8. Vivier E, Van de Pavert SA, Cooper MD, Belz GT (2016) The evolution of innate lymphoid cells. Nat Immunol 17: 790-794.

9. Chan HW, Kurago ZB, Stewart CA, Wilson MJ, Martin MP, et al. (2003) DNA methylation maintains allele-specific KIR gene expression in human natural killer cells. J Exp Med 197: 245-255.
10. Gascoyne DM, Long E, Veiga-Fernandes H, de BJ, Williams O, et al. (2009)The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. Nat Immunol 10: 1118-1124.
11. Tong X, Zhang D, Buelow K, Guha A, Arthurs B, et al. (2013) Recruitment of histone methyltransferase G9a mediates transcriptional repression of Fgf21 gene by E4BP4 protein. J Biol Chem 288: 5417-5425.
12. Zhang T, Termanis A, Ozkan B, Bao XX, Culley J, et al. (2016) G9a/GLP Complex Maintains Imprinted DNA Methylation in Embryonic Stem Cells. Cell Rep 15: 77-85.
13. Lanier LL (2005) NK cell recognition. Annu Rev Immunol 23: 225-274.
14. Yokoyama W M (2005) Natural killer cell immune responses. Immunol Res 32: 317-326.
15. Fernandez NC, Flament C, Crepineau F, Angevin E, Vivier E, et al. (2002) Dendritic cells (DC) promote natural killer (NK) cell functions: dynamics of the human DC/NK cell cross talk. EurCytokine Netw 13: 17-27.
16. Walzer T, Vivier E (2011) G-protein-coupled receptors in control of natural killer cell migration. Trends Immunol 32: 486-492.
17. McColl S R (2002) Chemokines and dendritic cells: a crucial alliance. Immunol Cell Biol 80: 489-496.
18. Vujanovic L, Ballard W, Thorne SH, Vujanovic NL, Butterfield LH, et al. (2012) Adenovirus-engineered human dendritic cells induce natural killer cell chemotaxis via CXCL8/IL-8 and CXCL10/IP-10. Oncoimmunology.. 1: 448-457.
19. Murdoch C, Finn A (2000) Chemokine receptors and their role in inflammation and infectious diseases. Blood 95: 3032-3043.
20. Gustafsson K, Ingelsten M, Bergqvist L, Nystrom J, Andersson B, et al. (2008) Recruitment and activation of natural killer cells in vitro by a human dendritic cell vaccine. Cancer Res 68: 5965-5971.
21. Khan IA, Thomas SY, Moretto MM, Lee FS, Islam SA, et al. (2006) CCR5 is essential for NK cell trafficking and host survival following Toxoplasma gondii infection. PLoS Pathog 2: e49.
22. Maghazachi AA (2010) Role of chemokines in the biology of natural killer cells. Curr Top Microbiol Immunol 341: 37-58.
23. Martin-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, et al. (2004) Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. Nat Immunol 5: 1260-1265.
24. Somanchi SS, Somanchi A, Cooper LJ, and Lee DA (2012) Engineering lymph node homing of ex vivo-expanded human natural killer cells via trogocytosis of the chemokine receptor CCR7. Blood 119: 5164-5172.
25. Bernardini G, Sciume G, Santoni A (2013) Differential chemotactic receptor requirements for NK cell subset trafficking into bone marrow. Front Immunol 4: 12.
26. Shi FD, Ljunggren HG, La CA, Van KL (2011) Organ-specific features of natural killer cells. Nat Rev Immunol 11: 658-671.
27. Pachynski RK, Zabel BA, Kohrt HE, Tejeda NM, Monnier J, et al. (2012) The chemoattractant chemerin suppresses melanoma by recruiting natural killer cell antitumor defenses. J Exp Med 209: 1427-1435.
28. Walzer T, Chiossone L, Chaix J, Calver A, Carozzo C, et al. (2007) Natural killer cell trafficking in vivo requires a dedicated sphingosine 1-phosphate receptor. Nat Immunol 8: 1337-1344.
29. Irimia D, Ellett F (2016) Big insights from small volumes: deciphering complex leukocyte behaviors using microfluidics. J Leukoc Biol.
30. Kim S, Kim HJ, Jeon NL (2010) Biological applications of microfluidic gradient devices. Integr Biol (Camb) 2: 584-603.
31. Li JN, Baskaran H, Dertinger SK, Whitesides GM, Van de Water L, et al. (2002) Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device. Nat Biotechnol 20: 826-830.
32. Wu J, Wu X, Lin F (2013) Recent developments in microfluidics-based chemotaxis studies. Lab Chip 13: 2484-2499.
33. Lin F, Butcher EC (2006) T cell chemotaxis in a simple microfluidic device. Lab Chip 6: 1462-1469.
34. Mahmood S, Nandagopal S, Sow I, Lin F, Kung SK (2014) Microfluidic-based, live-cell analysis allows assessment of NK-cell migration in response to crosstalk with dendritic cells. Eur J Immunol 44: 2737-2748.
35. Mamessier E, Sylvain A, Thibult ML, Houvenaeghel G, Jacquemier J, et al. (2011) Human breast cancer cells enhance self-tolerance by promoting evasion from NK cell antitumor immunity. The Journal of Clinical Investigation 121: 3609–3622.
36. Park MH , Lee JS, Yoon JH (2012) High expression of CX3CL1 by tumor cells correlates with a good prognosis and increased tumor-infiltrating CD8+ T cells, natural killer cells, and dendritic cells in breast carcinoma. J Surg Oncol 106: 386-392.
37. Kmieciak M, Basu D, Payne KK, Toor A, Yacoub A, et al. (2011) Activated NKT cells and NK cells render T cells resistant to myeloid-derived suppressor cells and result in an effective adoptive cellular therapy against breast cancer in the FVBN202 transgenic mouse. J Immunol 187: 708-717.
38. Olkhanud PB, Baatar D, Bodogai M, Hakim F, Gress R, et al. (2009) Breast cancer lung metastasis requires expression of chemokine receptor CCR4 and regulatory T cells. Cancer Res 69: 5996-6004.
39. Braun SE, Chen K, Foster RG, Kim C H, Hromas R, et al. (2000) The CC chemokine CK beta-11/MIP-3 beta/ELC/Exodus 3 mediates tumor rejection of murine breast cancer cells through NK cells. J Immunol 164: 4025-4031.
40. Gulubova M, Manolova I, Kyurkchiev D, Julianov A, Altunkova I, et al. (2009) Decrease in intrahepatic CD56+ lymphocytes in gastric and colorectal cancer patients with liver metastases. APMIS. 117:870-879.
41. Halilovic I, Wu J, Alexander M, Lin F (2015) Neutrophil migration under spatially-varying chemoattractant gradient profiles. Biomed Microdevices 17: 9963.
42. Li H, Hou S, Wu X, Nandagopal S, Lin F, Kung S, et al. (2013) The tandem PH domain-containing protein 2 (TAPP2) regulates chemokine-induced cytoskeletal reorganization and malignant B cell migration. PLoS One 8: e57809.
43. Li H, Wu X, Hou S, Malek M, Kielkowska A, et al. (2016) Phosphatidylinositol-3,4-Bisphosphate and Its Binding Protein Lamellipodin Regulate Chemotaxis of Malignant B Lymphocytes. J Immunol 196: 586-595.
44. Nandagopal S, Wu D, Lin F (2011) Combinatorial guidance by CCR7 ligands for T lymphocytes migration in co-existing chemokine fields. PLoS One 6: e18183.
45. Wadhawan N, Kalkat H, Natarajan K, Ma X, Gajjeraman S, et al. (2012) Growth and positioning of adipose-derived stem cells in microfluidic devices. Lab Chip 12: 4829-4834.
46. Wu J, Hillier C, Komenda P, Lobato de FR, Levin D, et al. (2015) A Microfluidic Platform for Evaluating Neutrophil Chemotaxis Induced by Sputum from COPD Patients. PLoS One 10: e0126523.
47. Wu J, Hillier C, Komenda P, Lobato de FR, Santos S, et al. (2016) An all-on chip method for testing neutrophil chemotaxis induced by fMLP and COPD patient's sputum. Technology.