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The effects of activin A on the migration of human breast cancer cells and neutrophils and their migratory interaction

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ABSTRACT

Activin A belongs to the superfamily of transforming growth factor beta (TGFβ) and is a critical regulatory cytokine in breast cancer and inflammation. However, the role of activin A in migration of breast cancer cells and immune cells was not well characterized. Here, a microfluidic device was used to examine the effect of activin A on the migration of human breast cancer cell line MDA-MB-231 cells and human blood neutrophils as well as their migratory interaction. We found that activin A promoted the basal migration but impaired epidermal growth factor (EGF)-induced migration of breast cancer cells. By contrast, activin A reduced neutrophil chemotaxis and transendothelial migration to N-Formyl-Met-Leu-Phe (fMLP). Finally, activin A promoted neutrophil chemotaxis to the supernatant from breast cancer cell culture. Collectively, our study revealed the different roles of activin A in regulating the migration of breast cancer cells and neutrophils and their migratory interaction. These findings suggested the potential of activin A as a therapeutic target for inflammation and breast cancers.

1. Introduction

Breast cancer is one of the most common malignant tumors in women [2,4]. Cancer cell migration critically enables cancer metastasis resulting in patient deterioration and eventually death [15]. In the tumor microenvironment, a number of inflammatory factors such as chemokines attract immune cells to the proximity of the corresponding tumor [10]. Neutrophils are not only innate immune cells, but also play an important immune function in the tumor microenvironment [23]. It has been reported that tumor-associated neutrophils (TAN) may differentiate into a protumorigenic phenotype [12], which is unfavorable to the treatment and recovery of cancer patients. Research to better understand common factors that can affect the functions of both breast cancer cells and neutrophils and their interaction is a promising approach for developing new therapeutic strategies for treating cancers. In this direction, the present study focused on an interesting multifunctional regulatory protein, activin A.

As members of the transforming growth factor beta (TGFβ) superfamily, activins are disulfide-linked homodimers of inhibin β subunits, mainly consisting of three members including activin A (βAβA), activin AB (βAβB) and activin B (βBβB) [22,24]). Activins were initially isolated for their capacity to induce the release of follicle-stimulating hormone (FSH) from pituitary extracts [30,35]). Among activins, activin A is the most abundant and potent one in tissues [27]. Over-expression of activin A was found in many cancers such as human breast cancers [21,9], oral and head-neck squamous cell carcinomas [6,20] and esophageal adenocarcinoma [28]. Increasing evidence suggests that activin A has different effects on the development or progression of a variety of endocrine-related cancers [20,24]. For example, activin A can promote proliferation of ovarian carcinoma cells and migration of oral squamous cell carcinomas. By

Abbreviation: BSA, Bovine serum albumin; EGF, Epidermal growth factor; FBS, Fetal bovine serum; fMLP, N-Formyl-Met-Leu-Phe; FSH, Follicle-stimulating hormone; HUVEC, Human umbilical vein endothelial cells; PDMS, Polydimethylsiloxane; TAN, Tumor-associated neutrophils; TGFβ, Transforming growth factor beta

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contrast, activin A can suppress the invasion of prostate cancer cells and proliferation of pituitary adenoma cells. Similarly, activin A plays pro- or anti-inflammatory roles in many diseases [36]. However, the effect of activin A in the migration of human breast cancer cells and human blood neutrophils was not well characterized. Moreover, whether activin A can affect neutrophil recruitment to the tumor microenvironment is not clear. These interesting questions motivated the present study to investigate the effect of activin A on the migration of breast cancer cells and neutrophils and their migratory interaction.

Owing to advantages in reduced reagent and sample consumption, microenvironment configuration, and controlled fluidic sample manipulation and chemical reaction, microfluidic devices were broadly applied for life science research and medical diagnosis [17,18,25,29]. In particular, microfluidic devices were widely used for cell migration and chemotaxis studies [3,25,31]. Therefore, microfluidics technology offered a useful approach for investigating activin A mediated cell migration in controlled gradient environment. In this study, we used a recently developed triple docking microfluidic device featured with three independently controlled gradient generators and cell docking modules (i.e. D^3-Chip; [34]; Fig. 1). A human metastatic breast cancer cell line, MDA-MB-231 cells, and human blood neutrophils were used as the model cell systems. The important questions to be addressed by this study include 1) Does activin A affect the migration of breast cancer cells and neutrophils? 2) What are the common or different effects of activin A in regulating the migration of breast cancer cells and neutrophils? 3) Does activin A mediate migratory interaction between neutrophils and breast cancers?

2. Materials and methods

2.1. Reagents and chemicals

Activin A was purchased from R & D Systems (Minnesota, US). Trizol, bovine serum albumin (BSA), FITC-Dextran, N-Formyl-Met-Leu-Phe (fMLP), DME/F12, DME/high-glucose, IMDM and epidermal growth factor (EGF) were purchased from Sigma-Aldrich (Oakville, ON, Canada). EasySep Direct human neutrophil isolation kit was purchased from STEMCELL Technologies (Vancouver, BC, Canada). RPMI-1640, fetal bovine serum (FBS) and Fluo-4 were purchased from Fisher Scientific (Ottawa, ON, Canada). Rat tail collagen type I and fibronectin were purchased from VWR (Edmonton, AB, Canada).

2.2. Microfluidic device preparation

In this study, microfluidic devices were fabricated using the standard photolithography and soft-lithography technique. The photomasks of the devices were designed using Solidworks (ver. 2013, Dassault Systemes S.A.) and the design was printed onto a transparency film at 24,000 dpi resolution (Fineline Imaging, Colorado, US). The master mold of the D^3-Chip integrating the cell docking structure was fabricated by two-layer photolithography [34]. Briefly, the channel geometries in each layer were defined by patterning the SU-8 photoresist (MicroChem Corporation, Westborough, MA, US) through the photomask on a silicon wafer. The first layer was used to define the cell-docking structure (3 μm thick). The second layer was used to define the flow channels (70 μm thick). The SU-8 master was then molded by polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning) by soft-lithography to create the negative replica. Inlets and outlets were punched out of the PDMS replica. The PDMS replica was then bonded to a glass slide by air plasma treatment. The microfluidic channels were coated with 2 μg/mL rat tail collagen type I (for MDA-MB-231 cells) and 0.25 mg/mL fibronectin (for neutrophils) for 30 min at 37 °C followed by BSA blocking for another hour in room temperature before cell migration experiments. The D^3-Chip was used for each experiment (Fig. 1**A**). Three independently controlled cell migration test units were configured on a single chip allowing parallel tests of different conditions (Fig. 1**B**). Identical and stable chemical gradient could be rapidly generated in the three test channels without requiring external pumps for up to 40 min, which was sufficient for neutrophil migration experiments (Fig. 1**C**). For cancer cell experiments, chemoattractant solution and medium were topped up intermittently to the inlet wells to maintain the stable gradient for a longer period of time. The cell

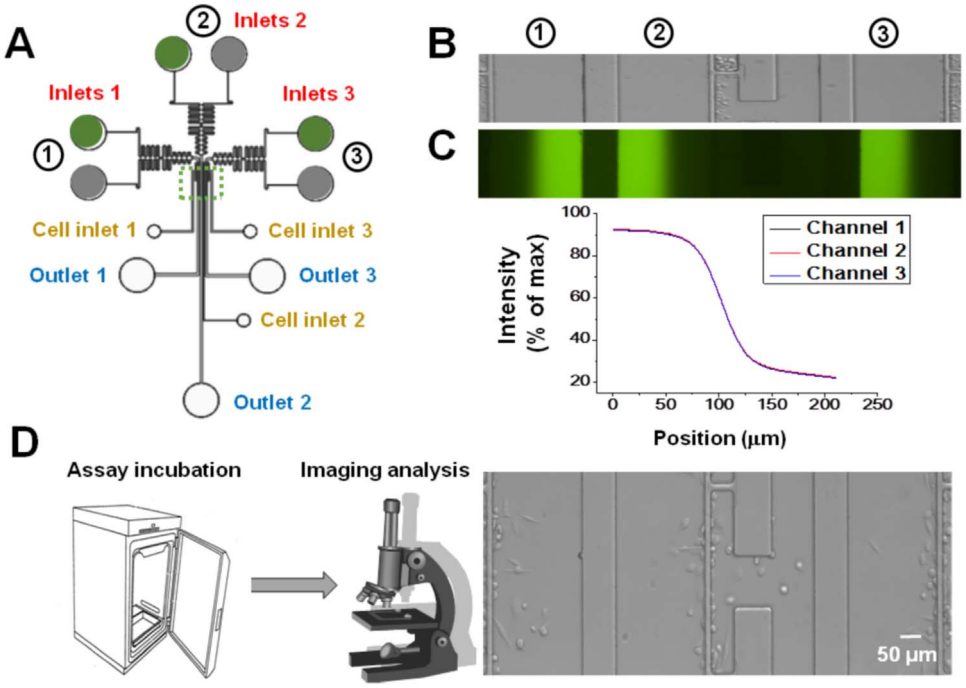

Fig. 1. Illustration of the microfluidic device. (A) Schematic illustration of the triple docking microfluidic device; (B) Image of the three microfluidic channels and docked cells; (C) The fluorescent image and profile plot of the FITC-Dextran gradient in the triple docking microfluidic device; (D) Illustration of the microfluidic cell migration assay that simply involves cell seeding and solution loading followed by incubation and final end-point imaging analysis. An image of MDA-MB-231 cells in the microfluidic device at the end of the assay was shown on the right panel.