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Non-invasive visualization of mast cell recruitment and its effects in lung cancer by optical reporter gene imaging and glucose metabolism monitoring

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ABSTRACT

The inability to monitor the *in vivo* dynamics of mast cells (MCs) limits the better understanding of its role in cancer progression. Here, we report on noninvasive imaging of MC migration to tumor lesions in mice and evaluation of the effects of migrated MCs on tumor progression through reporter gene-based *in vivo* optical imaging and glucose metabolism monitoring in cancer with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) *in vitro* and *in vivo*. Murine MCs (MC-9) and Lewis lung cancer cells (LLC) expressing an enhanced firefly luciferase (effluc) gene were established, termed MC-9/effluc and LLC/effluc, respectively. MC-9/effluc cell migration to LLC tumor lesions was initially detected within 1 h post-transfer and distinct bioluminescence imaging signals emitted from MC-9/effluc cells were observed at tumor sites until 96 h. *In vivo* optical imaging as well as a biodistribution study with ^{18}F-FDG demonstrated more rapid tumor growth and upregulated glucose uptake potentially associated with MC migration to tumor lesions. These results suggest that the combination of a reporter gene-based optical imaging approach and glucose metabolism status monitoring with ^{18}F-FDG represents a promising tool to better understand the biological role of MCs in tumor microenvironments and to develop new therapeutic drugs to regulate their involvement in enhanced tumor growth.

1. Introduction

Mast cells (MCs) are immune cells derived from hematopoietic precursors that are a central player in immediate-allergic reactions [1–3]. Aggregation of the high-affinity IgE receptor (FcεRI) on MCs as well as its stimulation by neuropeptides such as substance P (SP)

Abbreviations: MCs, mast cells; BLI, bioluminescence imaging; ^{18}F-FDG, ^{18}F-fluorodeoxyglucose; effluc, enhanced firefly luciferase; PGD_2, prostaglandin D_2; LTC_4, leukotriene C_4; c-kit, CD117; LLC, Lewis lung cancer; CCK, cell counting kit; DNP, dinitrophenyl; β-Hex, beta-hexosaminidase; CM, conditioned media; SFM, serum free media; HBSS, Hank's balanced salt solution; %ID/g, percentage of injected dose per gram of tissue.

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triggers the release of preformed mediators such as histamine, protease, and newly synthesized inflammatory mediators (cf. prostaglandin D_2 (PGD_2), leukotriene C_4 (LTC_4), and various cytokines) [4–7]. MCs are the only cell type that stores preformed tumor necrosis factor [8], which is thereby rapidly released and affects the recruitment as well as activation of T-cells [9,10]. The ability to release multiple mediators allows MCs to actively interact with other cell types in their surrounding environment and participate in the onset of mast-associated diseases including anaphylaxis, asthma, rhinitis, atopic dermatitis, and cancer [3,11–15].

Increasing evidence from preclinical and clinical studies has revealed that MCs are able to directly affect the proliferation and invasion of tumor cell through the modulation of inflammation and angiogenesis [16–20]. Furthermore, MCs can aid tumors to not only organize their microenvironments but also to modulate the host immune response to tumor cells in an indirect manner. For these reasons, many efforts have been attempted to not only investigate the biological role of MCs in cancer progression but also to identify promising therapeutic drugs to modulate their involvement in tumor progression. However, many limitations exist toward understanding the complex biological, pathological, and immunological factors involved in the link between MCs and tumor-bearing hosts owing to the limited ability to monitor the dynamics of MCs *in vivo*. Accordingly, straightforward non-invasive and quantitative approaches are urgently required to facilitate the evaluation of the dynamic functions of MCs in tumor microenvironments.

Molecular-genetic imaging can be conducted via the introduction of several types of reporter genes and their specific substrates, thereby allowing the *in vivo* imaging of proliferation, localization, and migration of reporter-tagged cells with specialized imaging instruments. Among reporter gene-based imaging strategies, *in vivo* optical imaging using bioluminescent reporter genes including firefly luciferase, Renilla luciferase, NanoLuc luciferase, and Gaussia luciferase has been extensively applied for cell tracking of e.g. immune and stem cells owing to the sensitivity of detection, simple imaging procedures, and lack of requirement for complex imaging facilities including positron emission tomography and magnetic resonance imaging instruments.

In this study, we demonstrate the serial monitoring of MC migration to tumor lesions in a mouse model of lung cancer using reporter gene-based optical imaging (Fig. 1). For visualization of the *in vivo* dynamics of MCs, we selected the murine MC line MC-9, which is derived from mouse fetal liver and exhibits IL-3 dependency, because it shows unique characteristic of MCs such as IgE/Ag-stimulated degranulation and eicosanoid synthesis (LTC_4 and PGD_2), in addition to high expression of FcεRI and c-Kit as phenotypic markers for fully differentiated MCs. For the *in vivo* cancer model, we chose Lewis lung cancer cells as a lung cancer model, because it represents one of the most aggressive and lethal cancers known to affect humans and has been shown to exhibit a high density of infiltrated MCs to the tumor lesion and a good correlation between MCs and microvessel densities, which is associated with prediction of poor clinical outcome [21–24]. In addition, for the sensitive and quantitative tracking of MCs and monitoring of cancer cell proliferation both *in vitro* and *in vivo*, the highly sensitive optical reporter gene enhanced firefly luciferase (effluc) was introduced. As the reporter retrovirus expressing the *effluc* gene also included the *Thy1.1* gene as a co-reporter, we were able to easily sort out effluc-positive cells through the use of a microbead-conjugated Thy1.1-specific antibody and a magnetic separation system. Furthermore, ^{18}F-fluorodeoxyglucose (^{18}F-FDG) was used to evaluate the status of glucose metabolism in the lung cancer model in response to MCs both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Mice

Specific pathogen-free, six-week-old female C57B6 mice were purchased from SLC, Inc. (Shizuoka, Japan). Mice were acclimatized to the vivarium for at least two weeks and then utilized for the *in vivo* study. All described procedures were reviewed and approved by the Kyungpook National University Animal Care and Use Committee and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals.

2.2. Cells

The murine MC line MC-9 was derived from mouse fetal liver and is dependent on IL-3. MC-9 cells were grown in DMEM media with high glucose (Hyclone, Logan, UT) containing 10% fetal bovine serum (Hyclone), 100 U/mL penicillin (Gibco, Grand Island, NY), and 5% poke-weed mitogen-spleen cell conditioned medium as a source of IL-3 and cultured at 37 °C in a 5% CO_2 atmosphere. MC-9 and Lewis lung cancer cells (LLCs) were each transduced with retrovirus expressing both effluc and *Thy1.1* genes [25]. Thy1.1-positive cells were enriched using CD90.1 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). The established stable cells co-expressing effluc and Thy1.1 were termed MC-9/effluc or LLC/effluc cells.

2.3. Fluorescence activated cell sorting (FACS) analysis

To determine the level of *Thy1.1* gene expression in MC-9/effluc cells, cells were labeled with APC-Cy7 conjugated anti mouse CD90.1 antibodies (Biolegend, San Diego, CA) for 30 min at 4 °C. To detect the expression of c-kit and FcεRIa in MC-9 cells or MC-9/effluc cells, cells were stained with APC-Cy7 conjugated anti-mouse CD117 (c-kit) and FITC conjugated anti mouse FcεRIa antibodies (Biolegend). Stained cells were washed twice in phosphate buffered saline (PBS) containing 1% bovine serum albumin. Flow cytometric analysis was performed using a BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA) and data were analyzed using FlowJo software (Tree Star, Ashland, OR).

2.4. In vitro luciferase assay

To examine the luciferase activity of either MC-9/effluc or LLC/effluc, a specified number of cells were plated in 96-well black plates with clear bottoms. After 24 h incubation, each well was supplemented with 3 μL D-luciferin (30 mg/mL, PerkinElmer, Waltham, MA) and then the bioluminescence signals were measured using an IVIS Lumina III *in vivo* imaging system (PerkinElmer).

2.5. Conditioned media (CM) preparation

For the preparation of CM from LLCs, LLCs were grown to 70–80% confluence in complete culture media. The medium was replaced with serum-free RPMI-1640 and cells were cultured for an additional 48 h. Medium was collected and filtered using 0.22-μm filters (Millipore, Billerica, MA).

For preparation of CM from MCs, MC-9/effluc cells were re-suspended in serum-free RPMI-1640 and cultured for 48 h. Medium was collected and filtered using 0.22-μm filters (Millipore).

2.6. Degranulation and eicosanoids assay

For cell stimulation, either parental MC-9 or MC-9/effluc cells