Lung Cancer 125 (2018) 128–135

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

# Detection of abundant megakaryocytes in pulmonary artery blood in lung cancer patients using a microfluidic platform

Hitoshi Dejima[a,b], Hayao Nakanishi[a,c,*], Hiroaki Kuroda[b], Mayumi Yoshimura[a], Noriaki Sakakura[b], Nanae Ueda[a], Yuko Ohta[a], Rie Tanaka[c], Sayomi Mori[c], Tatsuya Yoshida[d], Toyoaki Hida[d], Noriyoshi Sawabata[e], Yasushi Yatabe[a], Yukinori Sakao[b]

[a] Department of Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya, 464-8681, Japan
[b] Department of Thoracic Surgery, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya, 464-8681, Japan
[c] Laboratory of Pathology and Clinical Research, Aichi Cancer Center Aichi Hospital, 18 Kuriyada Kakemachi, Okazaki, Aichi, 444-0011, Japan
[d] Department of Thoracic Oncology, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya, 464-8681, Japan
[e] Department of Thoracic and Cardiovascular Surgery, Nara Medical University School of Medicine, 840, Shijo-cho, Kashihara, Nara, 634-8521, Japan

ARTICLE INFO

*Keywords:*
Circulating megakaryocytes
Lung cancer patients
Microfluidic device
Pulmonary arterial blood
Thrombopoiesis

ABSTRACT

*Objectives:* The lung was recently re-discovered as a hematopoietic organ for platelet production in mice. However, evidence for the role of the lung in thrombopoiesis in humans is still limited. In this study, we examined megakaryocytes in the pulmonary and systemic circulation, specifically in pulmonary arterial blood (PAB), venous blood (PVB) and peripheral blood using a newly developed microfluidic platform for rare cell isolation.

*Materials and methods:* We analyzed 23 lung cancer patients who underwent surgery in our institute. PAB and PVB were obtained from the resected lung immediately after surgery. Blood samples were size-selected using a filtration-based microfluidic device and enriched rare cells on glass slide specimens were stained with Papanicolaou (Pap), immunocytochemistry (ICC), and immunofluorescence (IF). Lung tissues were also analyzed by immunohistochemistry.

*Results:* Pap/ICC/IF showed the presence of abundant CD61 + /cytokeratin- giant cells with a megakaryocyte lineage in PAB, but only a few in PVB. These megakaryocytes were found to consist of CD61 + /CD41 + immature megakaryocytes and CD61 + /CD41- mature megakaryocytes with the potential to produce platelets. These findings were confirmed by the conventional hematological analysis of blood smears stained with Giemsa. In analysis of lung cancer, CD61 + megakaryocytes were observed exclusively in the capillaries of non-cancerous tissue, whereas platelets were selectively observed in the tumor blood vessels of cancerous tissue.

*Conclusions:* These results indicate that numerous megakaryocytes migrate from systemic bone marrows to accumulate in PAs and arrest of mature megakaryocytes in the capillaries of normal lung, suggesting the possibility that the lung plays a physiological role in the systemic thrombopoiesis in lung cancer patients.

## 1. Introduction

Megakaryocytes are multinucleated giant cells that produce platelets and release them into the systemic circulation. Megakaryocytes develop from bipotent megakaryocytic-erythroid progenitor (MEPs) following stimulation with primary signals such as thrombopoietin (TPO), differentiating through the following sequence; MEPs–promegakaryocytes–megakaryocytes–proplatelets–platelets. Many signaling molecules are involved in this process including GM-CSF, IL-3, IL-6, IL-11 and some chemokines [1–4]. It has long been

*Abbreviations:* PAB, pulmonary arterial blood; PVB, pulmonary venous blood; PB, peripheral blood; Pap, Papanicolaou; ICC, immunocytochemistry; IHC, immunohistochemistry; IF, immunofluorescence; 3D, 3-dimensional; TPO, thrombopoietin; CK, cytokeratin; CTC, circulating tumor cells

* Corresponding author at: Laboratory of Pathology and Clinical Research, Aichi Cancer Center Aichi Hospital, 18 Kuriyada Kakemachi, Okazaki, Aichi, 444-0011, Japan.

*E-mail addresses:* Hdejima@mdanderson.org (H. Dejima), hnakanis@aichi-cc.jp (H. Nakanishi), h-kuroda@aichi-cc.jp (H. Kuroda), m_yoshimura@aichi-cc.jp (M. Yoshimura), nsakakura@aichi-cc.jp (N. Sakakura), nueda@aichi-cc.jp (N. Ueda), hdejima@aichi-cc.jp (Y. Ohta), hdejim@aichi-cc.jp (R. Tanaka), kentaikensa@acc-aichi.com (S. Mori), t.yoshida@aichi-cc.jp (T. Yoshida), 107974@aichi-cc.jp (T. Hida), nsawabata@naramed-u.ac.jp (N. Sawabata), yyatabe@aichi-cc.jp (Y. Yatabe), ysakao@med.teikyo-u.ac.jp (Y. Sakao).

https://doi.org/10.1016/j.lungcan.2018.09.011
Received 8 August 2018; Accepted 14 September 2018
0169-5002/ © 2018 Elsevier B.V. All rights reserved.

believed that the majority of such thrombopoiesis events occur in the bone marrow [5,6]. In the past, several investigators reported the presence of megakaryocytes in lung capillaries and that platelets are released from these megakaryocytes in mice. However, this concept has not been widely accepted because most evidences were indirect and based on the histological and ultrastructural observation mostly in rodents [7–9]. Recently, however, Lefrancais et al directly and quantitatively demonstrated that the lung plays a crucial role in platelet production in mice. Using a direct imaging method to detect lung microcirculation in real-time in transgenic mice expressing megakaryocyte-specific PF4-Cre reporter gene, they observed megakaryocytes trapped in lung capillaries of lung parenchyma that had migrated from bone marrows. They showed that the lung contributes to the platelet biogenesis and is responsible for approximately 50% of total platelet production in mice [10,11].

During the past several decades, evidences of circulating megakaryocytes in the blood of various vessels such as central veins have accumulated in clinical settings based on morphological analysis of the specimens derived from patients with cardiovascular disorders [12–17]. In addition, the megakaryocytes reportedly were found to be trapped in the capillaries of the lung tissues in autopsy cases [18]. From these observations, it has been suggested that megakaryocytes observed in the lung play some role in thrombopoiesis in clinical settings. Recently, Ouzegdouh et al [19] reported the evidence to support pulmonary platelet production in human condition. However, there is no systematic studies on the relationship between pulmonary circulating megakaryocytes and platelet production in the human lung. The dynamics of megakaryocytes and the role of megakaryocytes in thrombopoiesis in humans remain to be elucidated. One reason for this is the lack of a device that can efficiently and conveniently enrich and subsequently detect rare megakaryocytes in the blood.

We recently developed a filtration-based microfluidic platform for rare cell isolation such as circulating tumor cells (CTCs) [20]. This device contains a 3 dimensional (3D) metal filter that can size-dependently enrich CTC and cytologically detect CTCs using glass slide specimens stained by Pap and ICC under light microscopy [21]. During investigation of CTCs in pulmonary blood using this microfluidic device, we unexpectedly found a large number of multinucleated giant cells that were morphologically different from CTCs in pulmonary arterial blood (PAB). This observation prompted us to examine the characteristics of these giant cells in the pulmonary blood in more detail. In the present study, we report that these giant cells are megakaryocytes with both mature and immature phenotypes and that they are also located in the capillaries vessels of the lung. We discuss the potential role of these megakaryocytes in thrombopoiesis and tumor development in patients with lung cancer.

## 2. Material and methods

### 2.1. Reagents

Mouse monoclonal antibody against human CD41 (TP-80) was purchased from Nichirei (Tokyo, Japan). Mouse monoclonal antibodies against human CD61(Y2/51), CD34 (QBEnd10) and CD68 (KP1) was purchased from DAKO (Carpinteria, CA, USA). Mouse monoclonal antibody against wide-spectrum human cytokeratin (Oscar) was purchased from Merck Millipore (Darmstadt, Germany). Mouse monoclonal antibody to human α-tubulin was obtained from Sigma Aldrich (St Louis, MO, USA). For direct labeling of the above antibodies, Zenon Alexa Fluor 488 and Alexa Fluor 567 mouse labeling kit was obtained from Invitrogen (Eugene, OR, USA). Hoechst 33,342 was obtained from Dojindo Lab. (Kumamoto, Japan).

### 2.2. Patients and ethics statement

Primary lung cancer patients (n = 23) with Stage I-III disease who underwent surgery at the Aichi Cancer Center Central Hospital (2017–2018) were enrolled in this study. Average age was 52 years old (range, 34–81 years). Patients' characteristics are summarized in Table 1. Patient blood samples (1–5 ml) were collected in EDTA-2Na tubes from pulmonary arteries (PAs) and pulmonary veins (PVs), immediately (~3 min) after lobectomy with a 26 G needle and kept at room temperature until use within 3–4 h. Peripheral blood (PB) samples (5–10 ml) were obtained from a cubital vein before surgery. This study was approved by the institutional review board of Aichi Cancer Center (ACC approved number: 2016-1-074) and written informed consent was obtained from individual patients prior to sample collection. The tumor stage and histology of lung cancer was estimated by a pathologist based on the 8th UICC criteria.

**Table 1**
Patients Characteristics.

| Parameter | Patients N = 23 | Megakaryocytes (/ml) | | P-value |
|---|---|---|---|---|
| | | < 200 | ≥ 200 | |
| Sex | | | | |
| Male | 13 | 6 | 7 | 0.76 |
| Female | 10 | 4 | 6 | |
| Smoking | | | | |
| Current/Former | 13 | 7 | 6 | 0.25 |
| Never | 10 | 3 | 7 | |
| CEA (ng/ml) | | | | |
| < 5.0 | 15 | 5 | 10 | 0.18 |
| ≥ 5.0 | 8 | 5 | 3 | |
| Histology | | | | |
| Adeno Ca. | 15 | 5 | 10 | 0.18 |
| Non-adeno Ca. | 8 | 5 | 3 | |
| pStage | | | | |
| I | 12 | 4 | 8 | 0.31 |
| II-III | 11 | 6 | 5 | |
| Vascular invasion | | | | |
| Presence | 7 | 3 | 4 | 0.97 |
| Absecnce | 16 | 7 | 9 | |
| LN metastasis | | | | |
| Presence | 15 | 8 | 7 | 0.19 |
| Absence | 8 | 2 | 6 | |

Ca = Carcinoma, pStage = Pathological stage, LN = lymph node.

### 2.3. Fabrication of filtration-based microfluidic device

A microfluidic device with a 3D metal filter for rare cell enrichment was produced by injection molding technology. A 3D metal (nickel or nickel alloy) filter that consists of an 8 μm pore in the lower layer and a 30 μm-sized cell capture hole in the upper layer was produced by microfabrication technology in combination with electroforming processes (Optnics Precision Co. Ltd. Tochigi, Japan) as described previously [20,21].

### 2.4. Enrichment of rare cells and transfer to a glass slide

The rare cell enrichment device consisted of a blood reservoir, filter unit, waste outlet and a syringe pump. The process of enrichment of rare cells using this microfluidic device, transfer from the filter to a glass slide and subsequent cytological staining of rare cells on a glass slide specimen was performed as described previously [21]. Briefly, whole blood obtained from PAs and PVs blood in patients with lung cancer were diluted 10-fold with PBS/5 mM EDTA and applied to the reservoir directly by aspiration using a syringe pump at a flow rate of 2.5 ml/min. After filtration and washing of blood, cells on a filter in the device was fixed with 10% buffered formalin in PBS for 10–15 min, followed by washing with PBS/5 mM EDTA. The filter was detached from the device and placed upside down onto a glass slide (MAS coat, Matsunami, Osaka, Japan) and then immersed with PBS under a glass cover slip. Rare cells with a diameter more than 10 μm trapped in the

Download English Version:

https://daneshyari.com/en/article/11029319

Download Persian Version:

https://daneshyari.com/article/11029319

Daneshyari.com