

論文 / 著書情報
Article / Book Information

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Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

(博士課程)
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論文要旨

THESIS SUMMARY

系・コース : Life Science and
Department of, Graduate major in Technology 系
コース

申請学位 (専攻分野) : 博士
Academic Degree Requested Doctor of ()

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要旨 (英文 800 語程度)

Thesis Summary (approx.800 English Words)

Thesis title is “ROR2 regulates survival of murine osteosarcoma cells in lung capillary” and consist of five chapters.

Chapter 1 “General introduction” describes the research background and purpose of this study. Lung metastasis is a major cause of mortality in patients with osteosarcoma (OS). Surgical and chemotherapeutic treatments have significantly improved survival in patients with non-metastatic osteosarcoma. However, metastatic disease interferes with long-term cure and can decrease 5-year survival rate to 20%. A better understanding of the molecular mechanism of OS lung metastasis may facilitate development of new therapeutic strategies to prevent the metastasis. Wnt signaling consists of two main groups: the canonical or β -catenin-dependent Wnt signaling pathway, and noncanonical or β -catenin-independent Wnt signaling pathway. Although the Wnt signaling pathway is involved in the progression of OS, the involvement of noncanonical Wnt signaling in OS progression has not been studied as much as canonical Wnt signaling. Therefore, my research focused on noncanonical (β -catenin-independent) Wnt signaling pathway. I aimed to identify pro-metastatic regulators associated with noncanonical Wnt signaling pathway in the sublines of murine OS cell line LM8. I believed that the data presented would provide new therapeutic targets useful for developing treatments of patients with metastatic OS.

Chapter 2 “ROR2 function is required for lung metastasis of LM8” describes the identification and analysis of ROR2 as a key factor associated with lung metastasis in LM8. Based on analysis of gene expression profiles between sublines of LM8 with high (LM8-H) and low (LM8-L) metastatic ability, I have identified receptor tyrosine kinase-like orphan receptor 2 (ROR2), a receptor of the non-canonical Wnt signaling pathway, as a gene that plays an important role in lung metastasis of OS. LM8-H knocked out of *Ror2* (H/*Ror2*-KO) significantly reduced lung metastasis.

Chapter 3 “ROR2 is an upstream regulator of cytoglobin” describes ROR2 as an alternative regulator of cytoglobin (CYGB) in LM8. Previous study has shown that lymphoid enhancer-binding factor 1 (LEF1), a compensate transcription factor of canonical Wnt signaling, promotes the extravasation ability of LM8 during lung metastasis via regulating CYGB expression. In vitro studies revealed that CYGB plays a crucial role in the transmigration ability of LM8. In chapter 3, I have found that loss of ROR2 expression in LM8-H reduced *Cygb* mRNA level while ROR2 expression did not correlate with the expression of LEF1. Moreover, knockout or overexpression of *Ror2* significantly reduced or enhanced transmigration ability of LM8-H or LM8-L cells, respectively. These data suggested that ROR2 is an upstream regulator of *Cygb*.

Chapter 4 “ROR2-AKT axis regulates anoikis resistance of LM8 cells” describes the function of ROR2 in cell survival in lung capillaries and its underlying molecular mechanism. In an experiment in which fluorescently labeled LM8 sublines were intravenously administered, the ratio of fluorescence intensity day 2 to day 0 in the lung was correlated with the expression level of ROR2: the fluorescence intensity on day 2 was significantly reduced in the lungs administered with cells having low ROR2 expression level. In vitro studies revealed that ROR2 function is involved in the resistance of LM8 to anoikis, a type of cell death induced by loss of proper extracellular matrix attachment. Furthermore, I showed that ROR2 contributes to LM8 anoikis resistance via AKT activation that the anoikis resistance is associated with *Itga6* and *Itgb5*

expression, which was altered by AKT inhibition by MK2206 under low adhesion conditions: Under low adhesion conditions, MK2206 significantly reduced *Itgb5* expression but increase *Itga6* expression. These results suggest that the ROR2-AKT-ITGB5 axis may play an important role in lung metastasis of LM8 through anoikis resistance in lung capillaries.

Chapter 5 “Discussion and prospect” summarizes the results of my research and describes the experiments that need to be performed in the future. Several of the noncanonical Wnt signaling-related receptor genes were extracted by analysis using Kyoto Encyclopedia of Genes and Genomes (KEGG). Among them, I selected ROR2 and found that ROR2 has a crucial role in lung metastatic ability of LM8: Knockdown of *Ror2* significantly reduced lung metastasis of LM8 (Chapter 2) due to reduced cell survival in the lung capillaries (Chapter 4). Then, functions of ROR2 in LM8 transmigration ability (Chapter 3) and anoikis resistance (Chapter 4) were assessed *in vitro*. ROR2 has been reported in human OS cell as a regulator of migration and invasion, key steps in the initiation of metastasis. I successfully elucidated the novel function of ROR2 involved in cell survival in lung capillaries through AKT activation and transmigration ability through CYGB function which are the post-circulatory lung metastasis process of murine OS cells. To my knowledge, this is the first report describing the involvement of ROR2-AKT signaling in OS anoikis resistance. To explore anoikis regulatory mechanisms under low adhesion conditions in relation to ROR2-AKT-ITGB5 axis, further analysis of integrin protein levels in LM8 and of LM8 cells with engineered integrin genes is required.

These results of ROR2 function in anoikis resistance, invasion and transmigration, and MK2206 function for LM8 cell survival under low adhesion conditions suggest novel therapeutic strategies: the combination of MK2206 and blocking ROR2 signaling may contribute to therapeutic treatments for OS patients with lung metastasis.

備考：論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。

Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).

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