学 位 論 文 審 査 の 要 旨
Doctoral Dissertation Evaluation Summary

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（大学院生命科学院）

学 位 論 文 題 名
Title of Doctoral Dissertation

Investigation of irradiation-induced increased invasiveness of lung cancer cells in three-dimensional collagen matrix and the involvement of integrin α2β1 and epidermal growth factor receptor

（放射線照射が誘引する肺がん細胞の 3 次元コラーゲン基質中における浸潤性の亢進とインテグリン α2β1 および上皮成長因子受容体の関与に関する研究）

<Doctoral Dissertation title>
（<Doctoral Dissertation title in Japanese>）

博士学位論文審査等の結果について（報告）
Results of Evaluation of the Doctoral Dissertation (Report)

Radiotherapy is the medical use of ionizing radiation (IR), and is considered a non-invasive local treatment, affecting mainly the cells and tissues that are situated inside the beam of IR. More than half of all cancer patients receive radiation therapy during their course of treatment. However, increasing evidences suggested that IR could cause increased malignancy in the repopulated cancer cells, which is emerging as a contributor to the limited benefit of radiotherapy. Therefore, the identification of molecules and the mechanism of IR-induced metastatic cancer progression are required for improving the efficacy of radiotherapy and patient survival rate.

This dissertation is a summary of research achievement of how IR-promoted the invasiveness of lung cancer cells *in vitro*. IR-survived cells (IR cells) were compared with parent cells (P cells) in three-dimensional (3D) collagen type I matrix, the main component of extracellular matrix. This 3D culture revealed the difference of morphology between P and IR cells, and provided a better platform than two-dimensional stiff substrate culture used in other studies. And the invasiveness of cancer cells was investigated by two kinds of invasion assay. One is to quantify the invasion speed of single cells in 3D collagen matrix by time-lapse observation, which showed alteration of cell morphology and　invasive behavior in real-time. Another one is to quantify the aspect ratio of cell

spheroid after culturing in 3D collagen matrix for 1 day. The spheroids exhibit several relevant physiological traits including similar morphology, formation of cell-cell bonds, decreased proliferation rates, increased cell survival, tumor dormancy and a hypoxic core. Applying this model in a 3D culture invasion assay provides a more physiological approach for assessing tumor invasion and providing a visual component that can be quantitated through image analysis.

This study investigated the mechanism by which IR cells increase their invasiveness by examining altered gene expression and signaling pathways in IR cells compared with those in P cells. By using many standard biotechnologies such as qRT-PCR, siRNA and western blotting, two key molecules have been found to be responsible for IR-induced invasiveness of lung cancer cells including integrin α2β1 and epidermal growth factor receptor (EGFR). The downstream signaling pathways and the relationship among the integrin α2β1, EGFR and downstreams were also studied to further reveal the signaling transduction in IR cells. Akt and extracellular signal-regulated kinase (Erk1/2), two important signaling molecules known to be essential for radioresistance, were demonstrated more active in IR cells; whereas, phosphoinositide 3-kinase (PI3K)/Akt was shown to be involved in the signal transduction of IR cell invasion, and was regulated by both integrin α2β1 and EGFR.

In conclusion, the author has new findings on the importance of integrin α2β1 and the epidermal growth factor receptor (EGFR) in the invasive ability of lung cancer cells that survive ionizing radiation. Recent work has underlined the implication of integrin α2β1 in cancer cell invasion and metastasis. While this dissertation first time showed a pivotal role of integrin α2β1 in the elongated morphology and increased invasiveness of lung cancer cells those survived IR. Moreover, although it has been demonstrated that advantage of EGFR inhibition on radiosensitization of cancer cells is mainly due to a reduction in cell proliferation and clonogenic survival, the results of this dissertation provided new evidence for the importance of EGFR inhibition on control of cancer invasion.

Considering these results, integrin α2β1 and EGFR were suggested as potential therapeutic targets for non-small cell lung cancer and these will contribute to the prevention of the recurrence of secondary tumors after radiotherapy. Therefore, we acknowledge that the author is qualified to be granted the Doctorate of Life Science from Hokkaido University.