学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士（生命科学）　氏 名　李 雪

学 位 論 文 題 名

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）

Cancer invasion and metastasis are landmark events that transform a locally growing tumor into a systemic, metastatic, and life-threatening disease. Radiotherapy, as an important treatment option for cancer, is the medical use of localized ionizing radiation (IR). However, radiotherapy may also lead to the progression to a more aggressive and malignant post-radiation tumor, due to the development of resistant cancer cell sub-populations with enhanced proliferative ability as well as invasive and angiogenic properties, which results in the failure of radiotherapy and low survival rate of patients. And the mechanism of IR-induced cancer cell malignancy is still poorly understood.

We previously showed that non-small cell lung cancer (NSCLC) A549 cells survived IR (IR cells) acquired higher invasiveness in a three-dimensional (3D) collagen matrix compared to the wild type counterpart (P cells), and the essential role of integrin β1 subunit in the IR-induced invasiveness. The purpose of this research is to further clarify that how IR cells increase their invasiveness by examining altered gene expression and signaling pathways in IR cells compared with those in P cells.

First, we surveyed integrin expression pattern, and found that expression levels of the integrin α2 and β1 subunits were specifically elevated in IR cells. Knockdown of α2 expression or functional blockade of integrin α2β1 resulted in a round morphology of IR cells, and abrogated their invasiveness in the collagen matrix (Figure 1), suggesting the molecule's essential role in cell spreading and invasion in 3D collagen matrix.

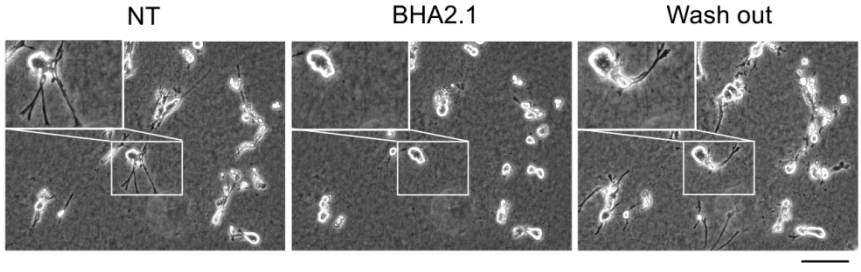

Figure 1 Functional blockade of integrin α2β1 in IR cells by BHA2.1 antibody inhibited the elongation of IR cells in 3D collagen matrix. NT: Non-treated. BHA2.1: BHA2.1 treated. Wash out: wash out BHA2.1 by fresh medium. Bar=100 μm.

In addition, we studied epidermal growth factor receptor (EGFR); a tyrosine kinase receptor that is found frequently overexpressed or harbors constitutively active mutations in NSCLC. Quantitative RT-PCR and western blotting showed that EGFR was also overexpressed and activated in IR cells. Treatment with specific EGFR tyrosine kinase inhibitor, PD168393, decreased the ratio of elongated cells and cell invasiveness (Figure 2).

Then several important downstream signaling molecules, those have been reported under regulation of integrin α2β1 and/or EGFR, including Akt, extracellular signal-regulated kinase (Erk1/2), p38 mitogen-activated protein kinase (p38 MAPK) and signal transducer and activator of transcription 3 (Stat3) were studied. Among them, Erk1/2 and Akt were found highly activated in IR cells (Figure 3). And only inhibition of Akt activation by treating with phosphoinositide 3-kinase (PI3K) inhibitor LY294002 decreased IR cell invasion. The activation of PI3K/Akt was found sustained by integrin α2β1 and EGFR. In conclusion, we demonstrated that integrin α2β1 and EGFR cooperatively promote higher invasiveness of IR-survived lung cancer cells, partially through the PI3K/Akt signaling pathway, and might serve as alternative targets in combination with radiotherapy.

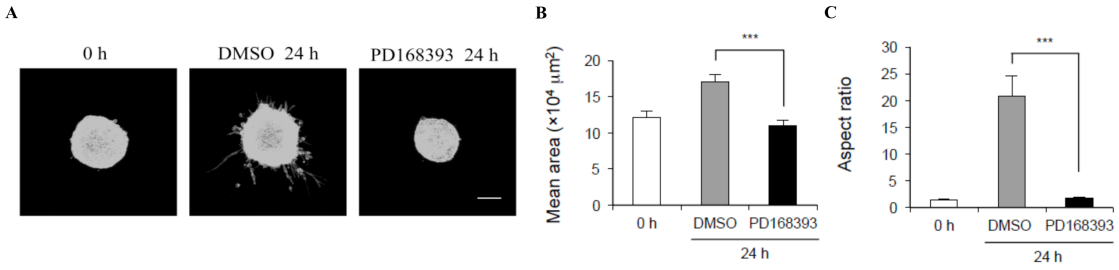

Figure 2. Three-dimensional (3D) spheroid invasion assay showed that inhibition of EGFR by PD168393 decreased IR cell invasiveness. (A) Confocal images of representative MFP488 phalloidin-stained IR spheroids in collagen gel for 0 h and IR spheroids treated with DMSO or PD168393 for 24 h. Scale bar, 200 μm. (B) Quantification of the area of spheroids by ImageJ software. (C) The aspect ratio of spheroids was calculated from perimeter2/[4π(area)]. Results are represented as mean values ± S.D (***p < 0.001) from three independent experiments in triplicate.

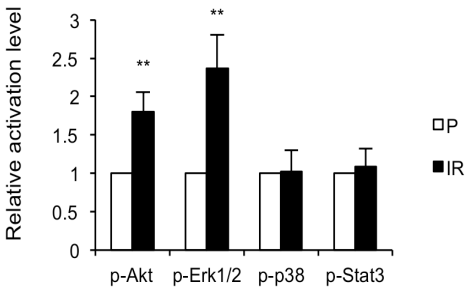

Figure 3. Expression analysis of the total and phosphorylated forms of Akt (Ser473), Erk1/2 (Thr202/Tyr204), p38 (Thr180/Tyr182) and Stat3 (Ser727) by western blotting. Intensity of signals was quantified by densitometry and normalized with GAPDH. Results are represented as mean values ± S.D of relative protein level (**p<0.01) from 3 independent experiments, indicated as fold change relative to P cells.