CIGARETTE SMOKE EXTRACT TREATMENT IMPROVES THE INVASION ABILITY OF LUNG CANCER CELLS CO-CULTURED WITH FIBROBLAST IN 3D CELLULAR SPHEROIDS

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Conventional two-dimensional (2D) monolayer cell cultures lack the three-dimensional (3D) architectures as those of real tissues *in vivo*. In cancer research, comparing 2D cell cultures with 3D cellular spheroids, the latter have features closer to those of a real tumor, such as cell-cell interaction, molecule responses [1]. Cigarette smoke is a main risk factor for lung cancers because it may participate in lung tumor invasion and metastasis [2]. However, the effect of cigarette smoke in 3D microenvironment of a lung tumor is still unclear. In the present study, we treated 3D cellular spheroids of co-cultured lung cancer cells and fibroblasts with cigarette smoke extract (CSE) to observe the variations of cell viability and invasion ability with light-sheet fluorescence microscopy (LSFM). LSFM is suitable for the observations on cellular spheroids because of its low phototoxicity and 3D imaging capability [3]. Figure 1 shows the LSFM systems used in this work.

We employed a microfluidic culture device to form co-culture cellular spheroids of lung fibroblast MRC-5 and lung cancer cell CL1-0 [4]. The cancer cells and fibroblasts were labelled with different dyes, such that we could employ LSFM to measure the intensity variation of individual types of cells. Figure 2 is the LSFM images of the co-culture spheroids without and with the treatments of CSE. Our experimental data show that CSE reduced the signal of fibroblasts, while that of the cancer cells sustained. It seems that the cancer cells have a higher resistance to the toxicity of CSE. We also verified the invasion ability of co-culture spheroid under the CSE treatment. Figure 3 shows that CSE could enhance the invasion ability of both fibroblasts and lung cancer cells as the spheroid was placed in Matrigel®.

Previous studies indicated CSE could improve the growth of tumor by reverse Warburg effect through the enhancement of autophagy in the surrounding fibroblasts [5]. We are now analyzing the expressions of specific proteins related to autophagy in the fibroblasts under the treatment of CSE. The preliminary results showed that the CSE treatment increased the expression of LC3B and decreased p62 in fibroblast. These changes in protein expressions indicated the promotion of autophagy. Therefore, we hypothesized that the autophagy of fibroblast was involved in the viability and invasion ability of cancer cells under the CSE treatment. More data will be presented in the Conference.

Word Count: 386

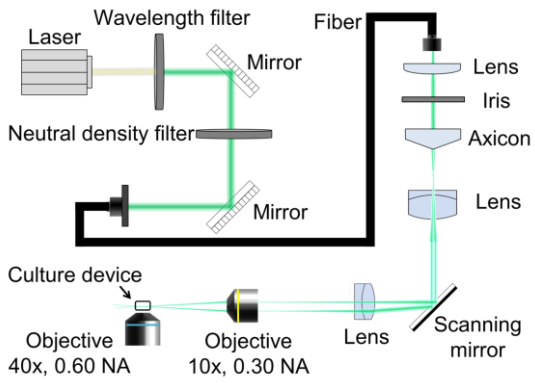

Fig. 1 Scheme of the LSFM system used in this work. The light sheet is generated by scanning a Bessel beam produced with an axicon transversely to the imaging direction. NA, numerical aperture.

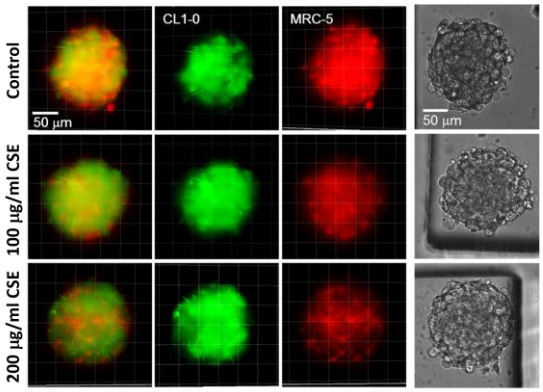

Fig. 2 Bright-field and LSFM images of the co-culture spheroids without and with the treatment of CSE.

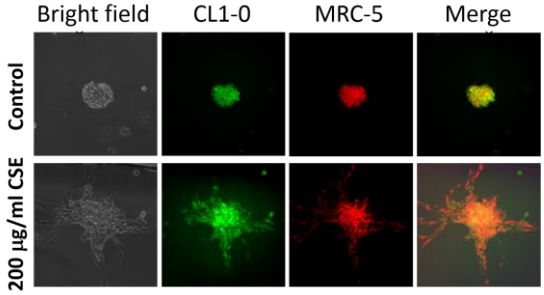

Fig. 3 3D invasion assay of CSE treatment on CL1-0 lung cancer cell and MRC-5 fibroblast co-culture spheroids in Matrigel®. All the images were collected after 48 hours of CSE treatment.

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