# A heightened radiosensitivity of stromal fibroblasts in 3D matrix

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Our recent study suggested that stromal fibroblasts can sensitize cocultured epithelial cells to radiation exposure. Since stromal fibroblasts exhibit highly elongated cytoplasmic extensions (pseudopodia), which as shown in our previous study are essential to guide neighboring epithelial cells to form branching ducts, we asked whether radiation could interfere with the formation of fibroblasts' pseudopodium, which would then impair their ability to structurally and functionally support the associated epithelial cells. For this, HMFs were seeded in 3D and were either mock-treated or irradiated 24 h later. The cultures were fixed at day 6 and the actin cytoskeleton was stained with Alexa Fluor-488-labled phalloidin and cell nuclei with DAPI dye to visualize cell shape. Significantly, the pseudopodia of fibroblasts were indeed significantly diminished by radiation exposure, leading to marked alteration of cell morphology from spindle-like to rounded appearance. In parallel experiments using the standard 2D culture, little, if any, difference in morphology was detected in IR-treated cells when compared with mock-treated cells. To examine whether the increased sensitivity of HMFs in 3D to IR-induced morphology alteration could reflect cellular radiosensitivity in general, we analyzed phosphorylation of histone H2AX, a commonly used marker for DNA damage. After an initial time course experiment, the 60 min time point was chosen to monitor IR-induced H2AX phosphorylation. Interestingly, when compared with the standard 2D culture, HMFs displayed markedly increased number of p-H2AX positive cells under the 3D condition following exposure of an acute dose of 0.1 Gy. We are currently investigating mechanisms underlying the heightened radiosensitivity of stromal fibroblasts in 3D matrix.