

A Preliminary Study on 3D In Vitro Tumor Models of Non-small Cell Lung Cancer (NSCLC) under Hypo-fractionated Stereotactic Body Radiotherapy (SBRT)

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Abstract

Objectives: To grow the 3D in vitro tumor models with A549 and H1229 cell lines of NSCLC. To irradiate 3D In Vitro tumors with hypo-fractionated SBRT similar to RTOG0813 and RTOG0915 protocols to investigate the treatment response.

Methods: Two human cell lines of NSCLC - A549 and H1229 were obtained from tissue culture core facility at our local university. The tumor cells were grown in Roswell Park Memorial Institute (RPMI) 1640 media supplemented with 10% fetal bovine serum (FBS), 0.5 mM sodium pyruvate, 10 mM HEPES, and 1% penicillin / streptomycin in a humidified incubator containing 5% CO₂ at 37 °C. The soft agar colony was formed according to the co-author's previous publication [1-2], where the top layer of the mixture was made up of a lighter concentration of agar (0.3 mL of 0.36% agar medium) and 3x10⁴ tumor cells of either A549 or H1229 in a 24 well-plate. The bottom layer was formed with a denser concentration of agar (0.5 mL of 1% agar medium). On top of the heterogeneous agar gels, RPMI-1640 media supplemented with 10% fetal bovine serum (FBS), 0.5 mM sodium pyruvate, 10 mM HEPES, and 1% penicillin / streptomycin were added to agar gels one day after the plating. Subsequently, cell media was replaced weekly. After 21 days, 3D tumor models were formed and irradiated with SBRT protocols similar to RTOG0815 and RTOG0915, i.e., 40Gy in 5 fx, 50Gy in 5 fx, 60Gy in 5 fx, 34Gy in single fx, and 48Gy in 4 fx were delivered on a clinically commissioned TrueBeam linac. Each treatment was triplicated and compared to the control condition with no radiation. The cells were allowed to grow for another 5 days before termination. Five pictures of colonies were taken with microscope magnifications of 10X for each well. The number of colonies was counted with counting tools in Photoshop software. The minimum 50 cells (i.e., larger than 100 micrometers) was counted as a 3D tumor colony from three independent wells under the same radiation dose and fraction conditions.

Results: The survival fraction was calculated as the number of 3D tumor colonies with radiation divided by that without radiation. For A549 tumors, a decreased trend was observed with dose escalation of RTOG0813. The survival fraction was 1.000+/-0.023, 0.915+/-0.161, 0.648+/-0.235, and 0.398+/- 0.043 for 0, 40Gy in 5fx, 50Gy in 5 fx, and 60Gy in 5 fx, respectively. In contrast, H1229's 3D tumor models had survival fraction of 1.000+/-0.197, 0.993+/-0.141, 0.784+/-0.197, and 0.811+/-0.111 for the RTOG0813 dose escalation respectively. For RTOG0915 arms, the single fractional radiotherapy 34Gy had much smaller survival fraction than the 48Gy in 4 fx arm. For A549's 3D tumors, the survival fraction was 0.607+/-0.252 and 0.955+/-0.265 for the

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single fx dose of 34Gy arm and 48Gy in 4fx arm, respectively. For H1229, the survival fraction was 0.940 ± 0.173 and 0.947 ± 0.176 , respectively. The detailed analysis of in vitro MRI diffusion study and the cell-free DNA in the cell media will be conducted in the future.

Conclusions: The 3D tumor models of NSCLC cell lines - A549 and H1229 - were developed in the heterogeneous agar gel. Subsequently, fractionated radiation similar to SBRT of RTOG0813 and RTOG0915 was conducted. Decreased 3D tumor colony number was observed, especially for the single fractionated 34Gy. Future studies will be conducted to further analyze the size and number change in the 3D tumor models to correlate with the radiation dose and fractionation.