Inhibition Of Retinoblastoma Tumor Suppressor Activity By RNA Interference In Lung Cancer Lines Deregulates Growth

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Background: Inactivation of retinoblastoma (RB) tumor suppressor function occurs frequently in lung cancer, enabling unregulated cell cycle progression. Short hairpin RNA (shRNA) can be constructed to target specific sequences and efficiently knockdown protein _expression. We hypothesized that RB activity in lung cancer cells can be precisely targeted for inhibition using shRNA, disrupting cell cycle checkpoint control.

Methods: A small interfering RNA was delivered by the plasmid pMSCVpuro containing an insert for a human RB short hairpin targeted sequence (RB3C) in the A/B pocket. NCI-H520 human non-small cell lung cancer cells, which carry wild-type Rb, were transfected with pMSCVpuro-RB3C, or the empty vector control. Transfectants harboring the construct were selected with puromycin. Protein _expression was determined by immunoblotting. Exponentially expanding cells were counted to establish growth curves. Tumors were grown as xenografts in nude mice by subcutaneous flank injection of 5 million cells in 50ìl mixed with 50ìl Matrigel. Tumor volume was calculated as v=ð (width² x length)/6. About eight hours before euthanizing the animals, they were injected with BrdU. Slides were prepared from the tumors for immunohistochemical determination of the BrdU incorporation. Immunohistochemical analysis was done for RB _expression, as well as for targets of RB signaling pathways, including cyclin A, topoisomerase II alpha, and thymidylate synthase.

Results: Transfection resulted in a dramatically diminished RB _expression. As a result, there was an increase in the levels of certain targets of RB-mediated control, including topoisomerase II and thymidylate synthase. The growth of cells harboring pMSCVpuro-RB3C was increased compared to empty vector controls: 10.2±2.0 vs 4.6±0.2 at 4 days, 82.6±9.9 vs 23.6±5.6 at 8 days, and 480.6±37.7 vs 159.4±36.2 at 12 days (fold increase in cell count). The tumor growth in nude mice was also increased with RB knockdown compared to control: 8.3±1.0 vs 4.6±2.4 (fold increase in tumor volume at euthanization). Immunohistochemistry staining knockdown of RB activity demonstrated an increased _expression of targets of RB.

Conclusions: Downregulation of RB is efficiently achieved in lung cancer cell lines by RNA interference. Targets of RB control are deregulated with RB knockdown. Depletion of RB increases growth in vitro and in a murine xenograft model. This will serve as an ideal system to further evaluate the role of RB activity on responses to chemotherapeutic agents.