The Role of B7-H3 in Tumorigenesis and Tumor Cell Growth in Head and Neck Squamous Cell Carcinoma

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Introduction: Head and Neck Squamous Cell Carcinoma (HNSCC) represents the 6th most common cancer worldwide. Despite advancements in combined-modality therapies, about 50% of patients will experience a recurrence of disease. Locoregional recurrences of HNSCC have a poor 5-year survival of 15-20% and recurrent HNSCC is a major cause of morbidity among head and neck cancer patients. The combination of poor outcomes and high rates of disease recurrence in HNSCC patients underscores the need for targeted therapeutic options, which in turn necessitates a deep mechanistic understanding of the molecular processes driving tumor pathogenesis. In a recent Phase II clinical trial conducted at the University of Cincinnati, it was found that HNSCC patients who were unresponsive to pembrolizumab had significantly higher levels of B7-H3 (CD276) expression. This seems to suggest that B7-H3 plays a prominent role in diminishing patient response to anti-PD1 therapy in the setting of HNSCC.

Methods: Utilizing a lentiviral transduction model, we transduced three different HNSCC cell lines (HN5, Cal27, FADU) with a lentiviral vector expressing Puro^r, GFP and B7-H3 targeting shRNA. We transduced each cell line with two different B7-H3 shRNA constructs and one scrambled shRNA construct. Transduced cell lines were selected using 1:2000 puromycin and GFP and then assessed for changes in B7-H3 expression using Western blotting and Flow Cytometry. Next, we generated single cell clones from each of our transduced cell lines. For each of our transduced cell lines, we quantified changes in cell proliferation and apoptosis using an MTS Proliferation assay and BrdU Flow assay. We also wanted to assess downstream changes in gene expression following B7-H3 knock-down and this was achieved using Western blotting to look at changes in STAT3, ERK, and Akt signaling pathways.

Results: Knock-down of B7-H3 produced a concomitant reduction in pERK 1/2 and pAkt levels without affecting levels of ERK 1/2 and AKT suggesting that B7-H3 plays a prominent intracellular role in maintaining tumor promoting signaling pathways. Similar changes were not seen in the levels of pSTAT3 seemingly indicating that B7-H3 does not play a direct role in this pathway. Interestingly, decreases in B7-H3 protein levels did not seem to affect cell proliferation or produce an increase in cancer cell apoptosis.

Conclusion: The results of our experiments seem to suggest that in addition to its role as an extracellular signaling molecule, B7-H3 also plays a prominent intracellular role in tumorigenesis. However, knock-down of B7-H3 alone is not sufficient to halt cell growth or induce cell death in HNSCC. Inhibition of B7-H3 using antibodies in colorectal carcinoma has been shown to reverse radioresistance through actions on the ERK 1/2 signaling pathway. Given the similar effect of B7-H3 knock-down on ERK 1/2 signaling in HNSCC, our next aim will be to assess whether or not endogenous B7-H3 knock-down can produce a similar reversal of radioresistance in HNSCC.

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