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14. ABSTRACT: The purpose of this proposal was to elaborate on the viability of NIS-mediated genetically targeted radiotherapy as a possible novel therapeutic intervention in human breast carcinoma. Problems encountered with SK-Br-3 forced other cell lines to be utilized for tumor growth and imaging. A stable NIS expressing clone was derived from SK-Br-3 cell line. The ability of the clone to accumulate radioactivity was lost after several passages, which may be due to epigenetically silencing. The NIS expressing clone was unable to accumulate radioactivity in vitro. The acquisition of a pin-hole collimator enables mice bearing Ad-NIS treated tumors to be non-invasively imaged following radioactive administration. The imaging enables dosimetric calculation to be performed to determine the absorbed dose to the tumor. Correlations between the absorbed dose and therapeutic outcome can provide a possible prediction of tumor response. Real-time RT-PCR experiments were used to detect increased NIS expression after treatment with several histone deactylase inhibitors (HDACi), including sodium butyrate (SB), trichostatin A (TSA), in conjunction with the DNA methyltransferase inhibitor 5-aza-2'deoxycytidine.

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Human sodium iodide symporter

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Introduction

Breast cancer is one of the leading causes of cancer in women in the United States. Over 180,000 women are diagnosed every year, and more than 44,000 cancer-related deaths occur annually. Conventional treatments for breast cancer are surgery, radiotherapy, systemic hormone therapy and chemotherapy. Early detection has increased in recent years decreased mortality rates by 25% (1). However reoccurrence and metastatic disease continue to be problematic. Therefore a better treatment for reoccurrence and metastatic disease is necessary. Several tumors have detectable levels of sodium iodide symporter (NIS), which can be used in detection and therapy of the tumor (1, 2, 3). hNIS is responsible for the physiologic accumulation of iodide in the human thyroid. In addition to iodide, NIS has an affinity for several halides and pseudohalides, enabling imaging and potential therapies. Cells treated with retinoic acid, and different histone deacetyalse inhibitors were shown to increase endogenous levels of NIS (4, 5, 6). Directly targeting NIS will enable imaging of tumors and subsequent treatment with radioiodide. The tumor response then can be monitored over time.

In addition to forcing the reexpression of NIS in breast cancer cells, intra-tumoral delivery of adenoviral hNIS will enable gene transfer and direct targeting of malignant tissue. NIS can confer the ability to accumulate radionuclides in transduced cells. This results in an increase in radioactivity in the NIS expressing cells and surrounding tumor tissue.The accumulation and retention of radioiodide in NIS expressing tumors enable non-invasive imaging of the transgene, as well as therapy. The imaging can provide real time assessments. From the radioactivity determined, estimated absorbed doses can be calculated. The absorbed doses could be used as a prognostic indictor to predict therapeutic outcome in patients following NIS gene transfer radioiodide administration.

This report will examine the current data gathered from each experiment. Current problems encountered and how new technology is being utilized to analyze new data.

Body

The aim of task 2 was to create stable NIS expressing SK-Br-3 clones as well as empty vector control clones. The clones were generated with modest increase in radioiodide was observed. The clones however quickly lost expression of the sodium iodide symporter (NIS) as seen in another breast cancer stable cell line MDA-MB-435. The SK-Br-3 cells were then injected into nude mice but problems were encountered when the tumors did not develop in the mice. Another estrogen positive cell line was chosen to perform non-invasive imaging as well as follow therapeutic outcome of Ad-NIS treated tumors compared to Ad-Bgl II control tumors and address task 4.

T47D cells suspended in 0.1 PBS were injected subcutaneously into both flanks of 3 female athymic nude mice (Harlen Sprague-Dawley, Indianapolis IN). When the xenograft tumors were approximately 5 mm diameter, 2 x 10^9 pfu of Ad-NIS was injected into the right tumor and 2 x 10^9 pfu of Ad-Bgl II was injected into the left tumor. The mice received 1 mCi of I-131 by intraperitneal (i.p.) injection. The mice were imaged 1, 6, 12, and 24 hrs post I-131 injection using a gamma camera fitted with a pin-hole collimator. A strong signal was detected from the Ad-NIS tumors compared to the Ad-Bgl II control tumors (figure 1).

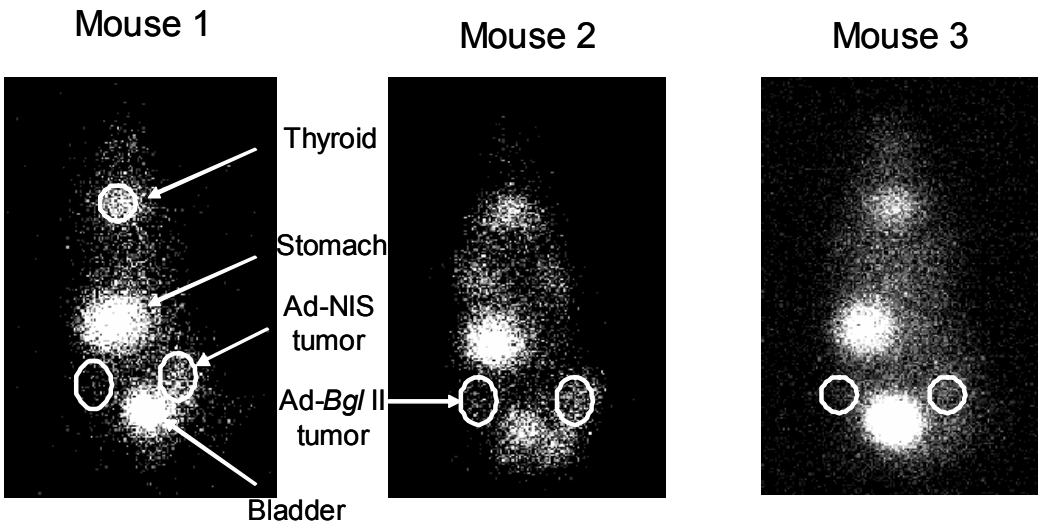

Figure 1. Non-invasive imaging of Ad-NIS and Ad-Bgl II injected tumors utilizing gamma camera scintigraphy 1 hr following I-131 administration. The mice were imaged 1, 6, 12, and 24 hrs following I-131 injections. The 3 mice shown here display a signal from the Ad-NIS injected tumor on the right flank compared to the Ad-Bgl II control tumor on the contralateral flank. The stomach, thyroid, and bladder were also visible in the images.

Region of interests were drawn over the tumors, thyroid and background. Time activity curves were generated for the mice. The average counts per minute (CPM) were graphed for Ad-NIS injected tumors compared to the Ad-Bgl II infected tumors (figure 1). The Ad-NIS tumors accumulated significantly higher levels of radioiodide compared to the control tumors and background. The Ad-Bgl II tumor displayed similar levels to background. The counts were "time" corrected.

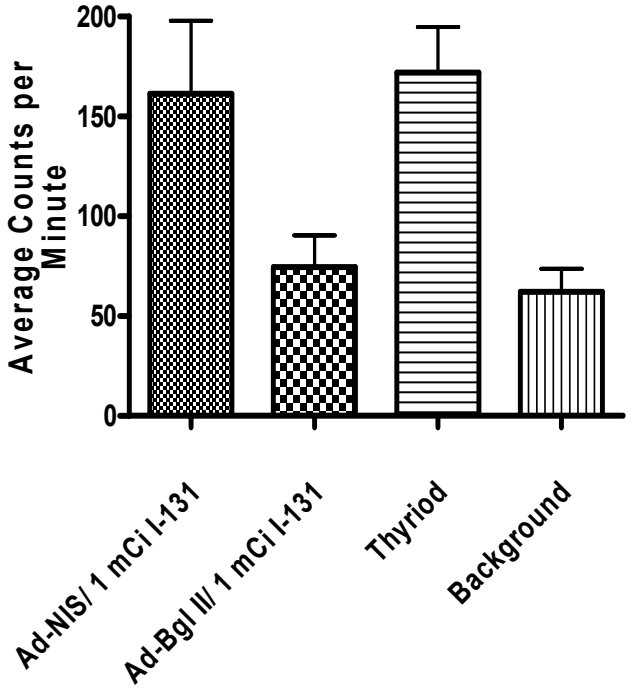

Figure 2. Average counts per minute acquired 1 hr following I-131 administration. The Ad-NIS injected tumors displayed a higher CPM than both Ad-Bgl II control tumors and background but equal to the thyroid. N=3

The activity was determined by comparing it to a known amount of radioactivity. Based on the counts received above time activity curves were generated for both Ad-NIS and Ad-Bgl II tumors. The averages were calculated for all three mice (figure 3). The Ad-NIS tumors accumulated radioactivity at a rapid rate compared to the controls. The radioactivity was slightly higher in the Ad-NIS tumors than the Ad-Bgl II tumors throughout the 24 hour imaging. Variation observed in the individual Ad-NIS time activity curves could be attributed to the adenovirus delivery.

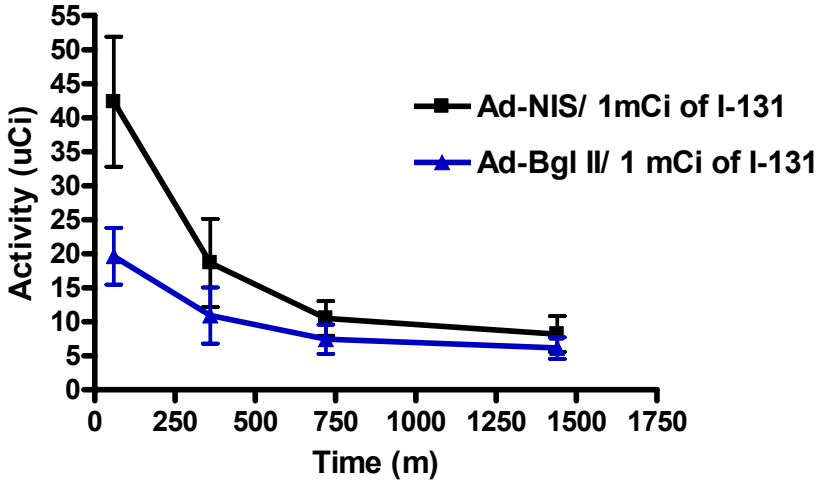

Figure 3. Time activity curves. The accumulation of radionuclide was quantified for the Ad-NIS and Ad-Bgl II tumors. The time activity curves were generated based on the counts received for Ad-NIS or Ad-Bgl II tumors. A significant difference was seen in the first time point $p<0.001$.

The estimated absorbed dose was calculated based on the counts detected from the tumors and the volumes of the tumors (radionuclide). The calculations were decay corrected. The Ad-NIS tumors received almost twice the dose as the control tumors (figure 4).

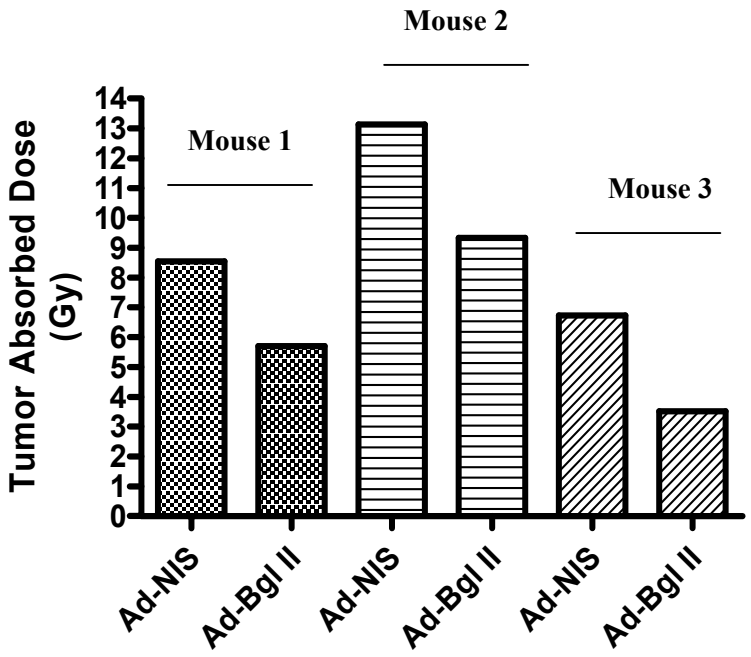

Figure 4. Absorbed dose delivered to the Ad-NIS or Ad-Bgl II tumor. The Ad-NIS tumor received a higher absorbed dose than its control tumor.

The absorbed doses delivered to the Ad-NIS tumors were 8.5, 13.2, and 6.8 Gy respectively. A slight variation was observed between the mice this could be due to radioactivity excreted in the urine during imaging as well as heterogeneity observed in the adenovirus injections. The average absorbed dose for Ad-NIS was approximately 1.5 times that of Ad-Bgl II tumors.

The tumors were measured throughout the experiment and therapeutic curves were generated for tumors injected with Ad-NIS or Ad-Bgl II followed by I-131. The tumors that received Ad-NIS + 1 mCi of I-131 displayed a delay in growth over time however Ad-Bgl II control tumors continued to grow after I-131 treatment (figure 5). This shows with just a modest increase in radioiodide accumulation a tumor response is demonstrated.

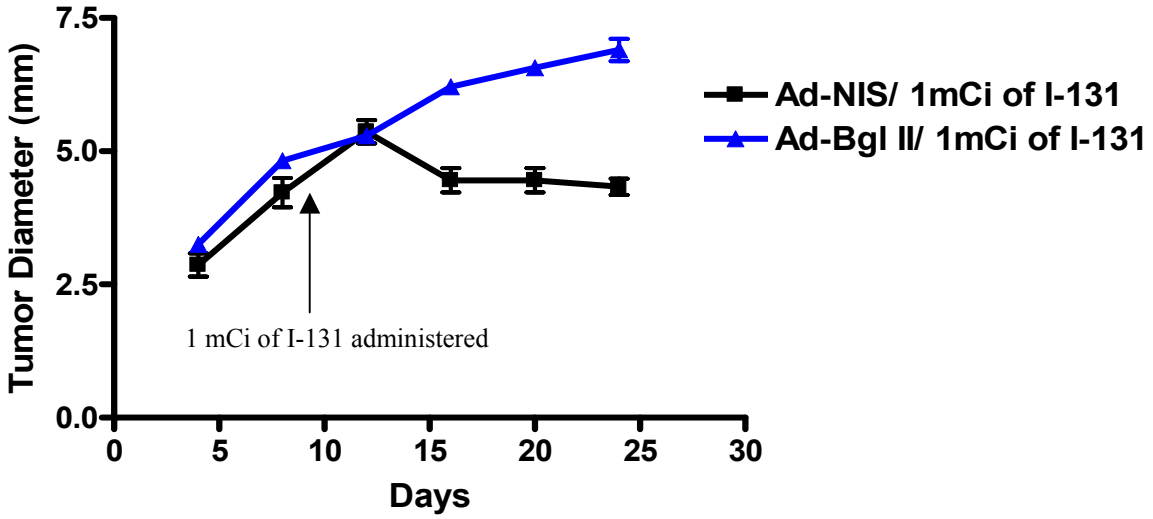

Figure 5. Tumor therapy curves comparing Ad-NIS/ I-131 and Ad-Bgl II/ I-131. The tumor diameter was measured every 4 days and followed throughout the experiment. The Ad-NIS displayed a slight regression in growth following I-131 injections. Then a delay in growth was observed compared to the control tumors. This experiment is ongoing. The difference between the two groups was significant. $P < 0.001$

In an effort to increase NIS expression in breast cancer cell lines and increase the tumor response, several histone deactylase inhibitors (HDACi), were tested. The HDACi including sodium butyrate (SB), trichostatin A (TSA), were tested in conjunction with the DNA methyltransferase inhibitor 5-aza-2'deoxycytidine. The RNA expression levels were analyzed using SYBR green real-time RT-PCR (ABI 7000 sequence detector, Applied Bioscience, Foster City CA). The primers were designed to span the 8^{th} and 9^{th} exon junction of human NIS (hNIS). T47D, SK-Br-3, MCF-7, and MDA-MB-231 were treated for 5 days with 1 μM 5-aza-2'deoxycytidine followed by 24 hrs with either 3 mM of SB, 150 ng of TSA or 1 μM of retinoic acid. No significant increase in relative NIS mRNA was detected with the addition of 5-aza-2'deoxycytidine. The activity did not increase in T47D, SK-Br-3, and MDA-MB-231 with the different treatments. Increase radioiodide was observed with RA treatment only (data not shown).

Key Research Accomplishments

Non-invasive imaging with Ad-NIS injected tumors compared to Ad-Bgl II tumors using the gamma camera fitted with a pin-hole collimator. Ad-NIS tumors were shown to accumulate more radioactivity than the control or background. The tumor was visualized for the 24 hr imaging time. This also enables the gene expression to be tracked and determine if it is in a part of the body which could be toxic.

Based on counts acquired from images estimated absorbed doses were calculated. The Ad-NIS tumors displayed higher absorbed doses in all 3 mice compared to the control. The dose could indicate if a high enough dose was deliveried to the tumor

Therapeutic curves were generated. The curves can be correlated to the absorbed dose to determine the therapeutic outcome following treatments. This could be clinically relevant in that it could indicate the tumor response based on the dose.

Reportable outcomes

1. Krager KJ, Gaut A, Madsen M*, et al.* Genetically Targeted Radiotherapy Using Sodium Iodide Symporter In Breast Cancer Cells. The 4th Era of Hope Department of Defense Breast Cancer Research Program Meeting. Philadelphia, PA; 2005.

2. Niu G, Krager K, Graham M, Hichwa R and Domann F. (2005) Noninvasive radiological imaging of pulmonary gene transfer and expression using the human sodium iodide symporter. *Eur J Nucl Med Mol Imaging*. May 32(5):534-40.

3. Krager KJ., *et al*. Quantitative Dosimetry and Biodistribution of Radionuclide Concentrator Therapy in Ad-NIS Transduced Head and Neck Carcinoma. 52nd Annual Meeting of the Radiation Research Society, Denver, CO 2005.

4. Krager KJ., *et al* Absorbed Dose Calculations Predict Therapeutic Response in Sodium Iodide Symporter (NIS) Expressing Tumors. The 13th International Congress of Radiation Research, San Francisco, CA 2007.

5. Matthew J. Provenzano, Matthew P. Fitzgerald, Kimberly Krager, Frederick E. Domann. Increased Iodine Uptake in Thyroid Carcinoma after Treatment with Sodium Butyrate and Decitabine (5-Aza-dC). In review

Conclusion

Mice were imaged using gamma camera scintigraphy to non-invasively measure the amount of radioactivity in each tumor. A strong signal was detected from the tumors injected with Ad-NIS following I-131 treatments. This allows tracking of gene expression to occur as well. The images were taken over a 24 hr time course, the activities generated from the images were used to derive estimated absorbed doses for each tumor. The Ad-NIS tumors received 8.5, 13.2, and 6.8 Gy respectively, which was 1.5 times more than the Ad-Bgl II control. The tumor diameter was measured to correlate the absorbed dose with any tumor response that was observed. The Ad-NIS tumors all displayed a slight regression in size followed by a delay in growth after I-131 administration. This suggested that the increase in radioiodide in the tumor was enough to elicit a tumor response in the Ad-NIS tumors only.

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