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Effect of preoperative cancer treatment on epidermal growth factor receptor (EGFR) receptor expression level in ABY-029 guided sarcoma surgery

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Abstract

Surgical excision via wide local excision (WLE) of the primary sarcoma tumor is a mainstay of treatment due to the limited effectiveness of chemotherapy and radiation. Even with attempts at WLE, 22–34% of the patient will be diagnosed with a positive margin by the pathologist, necessitating additional radiation or surgery. Recent studies have demonstrated reduced local recurrence when using fluorescence-guided surgery (FGS) to detect residual sarcoma following attempted WLE.

ABY-029 is an anti-EGFR Affibody® molecule labeled with IRDye800CW that is currently under Phase 0 human trial for FGS. To date, several studies have been performed to evaluate ABY-029 signal intensity in untreated human sarcoma xenografts; however, many patients undergoing cancer surgery have received pre-operative radiation and/or chemotherapy, which can affect tissue properties and tumor molecule expression level. Determining the effects of radiation and chemotherapy exposure on fluorophore binding in sarcomas may influence best practices in implementing FGS for sarcoma.

In this project, fluorophore signal intensities in tumor and surrounding tissue were measured and compared to the receptor concentration determined by immunohistochemistry. Here, we report the result for one EGFR positive synovial sarcoma cell lines, SW982. Four groups of human dose equivalent therapies – control, radiation, chemotherapy (Doxorubicin) and radiation followed by chemotherapy – were given to the tumor-bearing mice. The difference between groups can be used to determine the effects of preoperative sarcoma therapies on EGFR expression, ABY-029 uptake, and optical properties of tissues.

Keywords

Chemotherapy; radiation; fluorescence-guided surgery; sarcoma; immunohistochemistry; drug delivery and tissue characterization

1. INTRODUCTION

Sarcomas are a heterogeneous group of connective tissue cancers with survival rates that are highly dependent on subtype and grade [1]. Because chemotherapy and radiation have

limited effectiveness for sarcomas, surgical excision via wide local excision (WLE) of the primary tumor is a mainstay of treatment. WLE requires the tumor to be removed with a layer of normal, non-cancerous tissue surrounding the tumor. Even with attempts at WLE, 22–34% of the patient will be diagnosed with a positive margin by the pathologist, necessitating additional radiation or surgery [2]. As a result, there is growing interest in fluorescence-guided surgery (FGS), to help identify tumor tissue and intraoperatively guide tumor removal. Some recent studies have demonstrated reduced local recurrence when using FGS to detect residual sarcoma following attempted WLE [3–4].

In soft-tissue sarcoma surgery, epidermal growth factor receptor (EGFR) can be a good target molecule, overexpressed in 43–78% of tumors [5]. ABY-029 is an anti-EGFR Affibody® molecule labeled with IRDye800CW that is currently under Phase 0 human trial for FGS. ABY-029 has features preferable to anti-EGFR antibodies due to its 1) smaller size and faster diffusion while retaining high EGFR specificity; 2) shorter plasma half-life, permitting same-day administration; 3) low immunogenicity; and 4) low toxicity [6].

To date, several studies have been performed to evaluate ABY-029 signal intensity in untreated human sarcoma xenografts; however, many patients undergoing cancer surgery have received pre-operative radiation and/or chemotherapy, which can affect tissue properties and tumor molecule expression level. Determining the effects of radiation and chemotherapy exposure on fluorophore binding in sarcomas may influence best practice in implementing FGS for sarcoma.

In this project, fluorophore signal intensities in tumor and surrounding tissue were evaluated after radiation and chemotherapy exposures typical for sarcoma. An EGFR positive cell line, SW982, was implanted subcutaneously into NSG mice. Once the tumors reached a size of 200 mm³, four groups of human dose equivalent therapies – control, radiation, chemotherapy (Doxorubicin) and radiation followed by chemotherapy – were given to the tumor-bearing mice. At the end of the treatment, the tumor and adjoining relevant connective tissues were removed for fluorescence imaging and further pathological analysis. The difference between groups can be used to determine the effects of preoperative sarcoma therapies on EGFR expression, ABY-029 uptake, optical properties of human sarcomas and connective tissues, and the accuracy of subsurface fluorescence-guided margin estimation. In this conference proceeding, the result from one of the EGFR positive cell lines, SW982, was shown. Statistically significant differences were observed between the control and groups that contain chemotherapy. However, the EGFR expression level was not increased in the chemotherapy group indicating a nonspecific accumulation of the target agent in the tumor area for the SW982 tumor cell line.

2. METHODS

2.1 Animal experiments

All animal experiments were performed in accordance with guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at the Dartmouth-Hitchcock Medical Center. Twenty female NSG mice (The Jackson Laboratory, Bar Harbor, ME) were used to develop tumor xenografts. One million SW982 cells were implanted subcutaneously

at the flank area. In order to maintain the cell at the location and promote cell growth, tumor cells were mixed with an equal volume of Matrigel (Corning, Corning, NY) before injection. Animal health, weight and tumor size were measured twice every week.

The whole experiment design is shown in Fig. 1. After 8–9 weeks, when the tumors reached a size of 200 mm³, the mice were separated into 4 groups with different treatment courses. After each treatment, animals were given a week to recover from the treatment similar to human patients. The control group was not given any treatment. Animals in the chemotherapy group were given at 0.75 mg/kg of Doxorubicin (Mylan, Canonsburg, PA) in 200 µL solution weekly for 4 weeks. For the radiation therapy, human patients with soft-tissue sarcoma typically receive 50 Gy radiation prior to surgical excision. Mice were given 10 doses of 3 Gy irradiation using LINAC system (Varian, UK) in a course over 2 weeks to establish a biological equivalent dose without causing animal morbidity. For the combined group, animals irradiated for 2 weeks followed by one week of recovery and then given 4 weeks of chemotherapy.

Once the whole course of treatments were finished, animals were given six-times the human equivalent dose of ABY-029, 4 hours prior to the sacrifice. Animals were sacrificed by cervical dislocation. Four types of tissues, tumor, fat tissue, leg muscle, and sciatic nerve, were harvested from the animal. The tumor tissue was cut in half to show the distribution of the dye in the entire tissue. All tissues were placed on a glass slide and then put in the Odyssey system (LI-COR Biosciences, Lincoln, NE) for imaging. The tumor tissues were then placed in a cuvette for paraformaldehyde fixation and sent for pathology analysis. Immunohistochemistry (IHC) stain was performed to visualize the expression of epidermal growth factor receptor (EGFR) in brown color. The slides were digitized at 20X magnification for further analysis.

2.2 Image analysis

Two sets of image data were analyzed, the tissue fluorescence and IHC images. Region of interest (ROI) was selected engulfing each piece of tissue, and then the averaged values were calculated over the ROI. In order to precisely compare the tumor fluorescence versus the IHC measured EGFR expression level, the tumor ROI was selected based on the IHC image. For the IHC image, two data sets, the mean signal intensity and the percent area of the EGFR signal in the whole tumor region were measured and recorded. An ROI was selected circulating the whole tumor region based on the presence of EGFR signal. The mean value was calculated over the ROI. Another threshold based on the signal intensity was used in the ROI, the selected pixels were divided by the total pixel numbers in the ROI to calculate the percent area. These two sets of data were then compared with the fluorescence signal intensity in the tumor region and evaluate the change of the signal based on the therapy group.

2.3 Statistics

Origin 2019 (OriginLab Corp., Northampton, MA) was used for all statistical analyses. The animal survival curve was drawn using the Kaplan-Meier Estimator. The overall differences in fluorescence for all tissues were measured by one-way ANOVA. The two-sample t-test

was used to determine the difference between tumor signals in each treatment group. Statistical significance was based on $p < 0.05$. All data are presented as mean SD.

3. RESULTS AND DISCUSSION

3.1 Animal survival

The tumor growth timespan for SW982 is two to three times longer than other soft-tissue sarcoma cell lines we have experience with, including the EGFR negative cell line, MES-SA. Inward growth toward the muscle and bone region of the tumor was noticed when removing the tumor, indicating an invasive growth pattern. Unlike other subcutaneously implanted tumors, this cell line tends to grow invasively without a noticeable change in tumor size. This also prolonged the growing period measured by the subcutaneous tumor size.

All treatments were initiated at about 7-week after tumor implantation when the tumor size reached 200 mm³. The mice were sacrificed if their weight fell below 15% of their original body weight. Due to the criteria, all animals in the combined group failed to finish the 4-week of chemotherapy and were dropped from the study around 2-weeks in the course of treatment. On the other hand, most of the mice in the chemotherapy and radiation group finished the whole course of the therapy and were sacrificed after a week of the recovery break.

3.2 Fluorescence signal intensity in tissue

The effects of radiation and chemotherapy in molecular-guided sarcoma surgery were evaluated in this study using imaging probe ABY-029. The fluorescence signals in tumor, sciatic nerve, muscle, and adipose tissue were shown in Figure 3 after four different treatments. Each group contained 5 mice. Looking at the tumor signal alone, there were statistically significant differences between the groups treated with chemotherapy, both chemotherapy group and combined group, and the non-chemotherapy treated group, control, and radiation group. Elevated tumor-to-background contrast should be expected after chemotherapy treatment, which can be beneficial for fluorescence-guided surgery. Since the combined group only went through 2-week of chemotherapy compared to 4-week of chemotherapy for the chemo-group, the slightly lower mean value indicates a time-related effect from the chemotherapy treatment.

On the other hand, radiation therapy did not show a statistically significant effect on the fluorescence signal compared to the control group. Both sciatic nerve and muscle tissue worked well as a background correcting tissue showing fewer changes among all groups. The adipose tissue, likely due to the weight and overall health of the animal, demonstrates a large inter- and intra-group variance. The variance in signal intensity is related to the weight loss, density of the adipose tissue, and the blood spill in the process of the tissue harvesting. All these possible reasons made the fat tissue an unideal standard to compare with the signal from tumor tissue.

In order to demonstrate the effect of change in EGFR expression in the fluorescence signal, the immunohistochemistry (IHC) stain of EGFR is compared with the fluorescence signal,

Fig. 4. Unexpectedly, the EGFR expression level does not change much in the SW982 cell line based on the IHC staining. For the mean IHC value, only the combined therapy group has a statistically significant difference to the control group. All other groups did not show statistically significant difference with each other. Similarly, in the analysis of the percent area of brown within the whole tumor tissue, only the combined group stood out from the other groups. In contrary, the fluorescence signal can help to distinguish the chemotherapy group. This result indicates that the EGFR expression level was increased only after the combined treatment of radiation and chemotherapy for the SW982 tumor. One of the possible reasons for the nonspecific retention of the EGFR targeted imaging agent can be the induced angiogenesis after chemotherapy instead of increasing the expression level of EGFR. The effect of angiogenesis induced by Doxorubicin has been reported [7, 8]. No clear pattern can be drawn from the radiation group using both the IHC staining and fluorescence delivery.

In conclusion, this project indicates a potential change in the current preclinical fluorescence-guided surgery research under a clinical setting with patients under the treatment of different therapies. Further investigation will be performed to verify the effect of therapies on different tumor lines in this study. To verify the effect of angiogenesis in the fluorescence signal, staining of the vasculature was planned for future studies.

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Figure 1. Schematic diagram of the workflow to evaluate the effect of therapies, including: tumor implantation, therapy application, fluorescent agent administration, tissue harvest, fluorescence imaging, pathology analysis and final image analysis.

Figure 2. Kaplan-Meier survival curve for SW982 tumor cell line in NSG mice (n=5 for each group). The chemotherapy started on day 63. The radiation therapy for both radiation and combined groups started on day 64. Time duration was shown in days.

Figure 3. Comparing the effect of control, chemotherapy, radiation and radiation followed by chemotherapy on the ABY-029 fluorescence signal intensity in tumor (blue, n = 8–10), sciatic nerve (orange, n = 5), muscle (gray, n = 5) and adipose tissue (yellow, n = 5). * indicates $p < 0.05$. Statistical results shown for tumor groups only.

Figure 4. Plot of the ABY-029 fluorescence signal intensity against the percent area of EGFR staining in immunohistochemistry for control (yellow, n = 5), chemotherapy (green, n = 4), radiation (blue, n = 4) and combined (brown, n = 3).