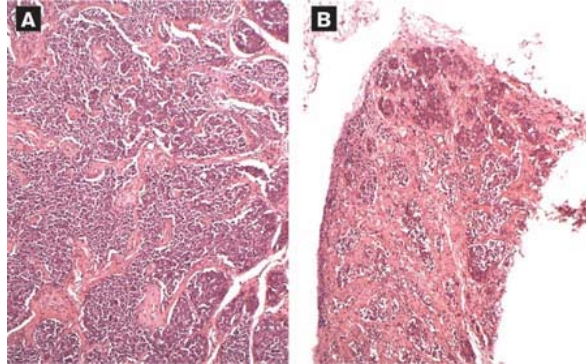


was injected into the control mouse (our previous experiments have consistently demonstrated no fluorescence in any control mice injected [N = 15];¹³⁻¹⁶). Some 24 to 48 hours after injection, the mice were sacrificed, examined, and photographed with an Olympus small-animal imaging system (470-nm light source) at AntiCancer, Inc., a biotechnology firm in San Diego. The mice were also illuminated with the blue LED flashlight and viewed through the filtered goggles. The tumor masses subsequently underwent hematoxylin and eosin (H&E) staining to confirm the histopathology of the cell line in the control and study mice (**figure 1**).

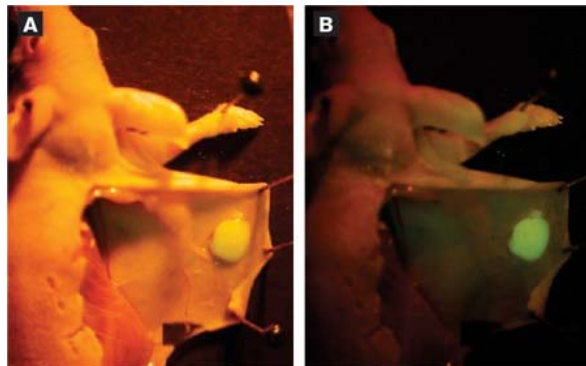
Slides contain biopsies from study mouse B (A) and the control mouse (B) (H&E, original magnification $\times 10$).



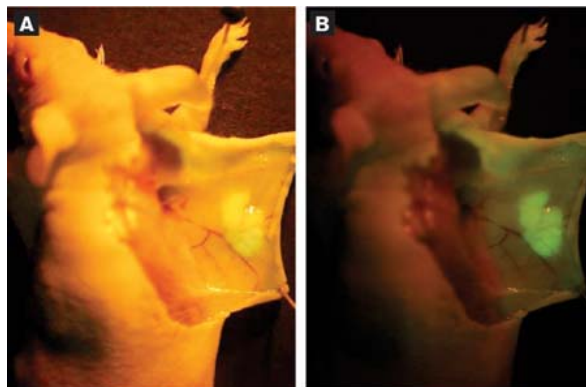
Results

Under white light, the tumor nodules in this experiment were grossly visible (**figure 2, A; figure 3, A; and figure 4, A**). When the tumors were illuminated with the Olympus imaging system, they were just as clearly visible with bright green fluorescence in the study mice (**figure 2, B, and figure 3, B**) but not in the control mouse (**figure 4, B**). In the study mice, the fluoresced tumor tissue was easily distinguishable from the surrounding normal tissue, and the margins were extremely distinct. In the control mouse, fluorescence was undetectable even when the background lighting was low.

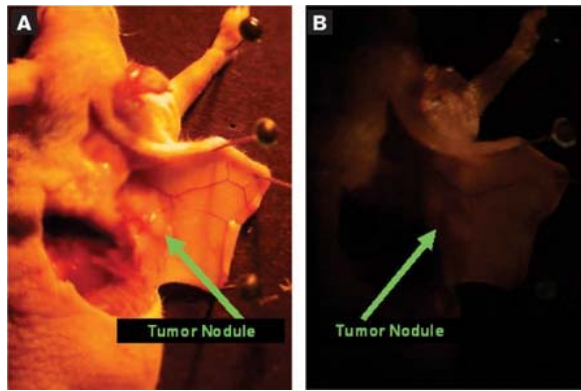
The nodule in study mouse A is seen under white light (A) and under the 470-nm light source (B).



The nodule in study mouse B is seen under white light (A) and 470-nm light (B)



In the control mouse, the nodule is seen under white light (A), but it does not fluoresce under 470-nm light (B)



The bright green fluorescence was also easily visible to the naked eye with the use of the blue LED flashlight and filtered goggles to simulate the surgical setting. Our previous experiments with breast and colon cancer cell lines have also shown bright green fluorescence with the blue LED flashlight and filtered goggles.^{13–16} We have also previously shown that this fluorescence remains bright from 24 hours to as long as 120 hours.^{13–16}

Discussion

As is the case with most primary cancers, effective treatment of medullary thyroid cancer requires a complete excision with no residual tumor. However, aggressive surgery in all areas at risk can be difficult to complete successfully. If all malignant tissue could be made fluorescent, surgeons might feel more confident in their ability to adequately remove it because fluorescence would highlight occult nodal metastases and possibly establish true tumor margins. Additionally, tumor fluorescence might allow for tumor restaging and a more aggressive resection if fluorescence were to be found outside the planned area of resection. Hence our development of FAAST.

If future studies in humans were to validate our findings with FAAST, at some point surgeons would theoretically be able to resect only what is fluorescent in the operative field. More accurate, specific, and complete resections would result in more limited tissue removal, fewer complications, less operating room time, and possibly better (or at least equivalent) local control and survival rates.

By using a human medullary thyroid cancer cell line for this in vivo mouse experiment, we have shown that tumor fluorescence can be easily induced by using fluorophore-tagged antitumor antigen antibodies. For this technology to become useful in more general applications, the antigenic expression of the primary tumor must be known and fluorophore-tagged antibodies directed against that antigen must be available. Additionally, antibody binding needs to be specific enough so that most of the antibody is not sequestered in other sites of the body. These obstacles notwithstanding, it is not difficult to conceive that if a battery of antibodies (e.g., anti-CEA, CA15-3, CA19-9, CA125, antiprostata-specific membrane, etc.) were available, multiple antigens on the same tumor could be bound at the same time to increase fluorescence. Additionally, a wide variety of different primary tumors could be marked with fluorophore-tagged antibodies simultaneously. We have demonstrated in previous work that this technology works equally well for antigen-expressing breast and colon cancer cell lines^{13–16} and for pancreatic cancer cell lines, as well. If the tumor burden is sufficient, it could potentially be used to identify metastases to locoregional lymph nodes.

Based on our results, we believe there is the encouraging possibility that FAAST could someday help surgeons through the difficult process of localizing recurrent medullary carcinoma of the thyroid in previously dissected areas of the head and neck. In the animal model, FAAST is simple to use and requires no complex or expensive technical equipment. Although the need to dim the overhead lights intermittently during surgery might limit some clinical applications, we do not anticipate that this should be a major obstacle because many procedures, such as endoscopic surgery, are already routinely performed in low lighting conditions. We believe that FAAST technology offers significant promise for surgeons involved in the diagnosis and treatment of patients with medullary carcinoma of the thyroid, as well as for those who treat many other primary cancers. Based on our initial experiments in thyroid, breast, and colon cancer, we hope to expand our in vivo labeling technology to other types of primary cancers, including other head and neck cancers.

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