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# In vivo fluorescence of medullary carcinoma of the thyroid: A technology with potential to improve visualization of malignant tissue at surgical resection

**Abstract**

Medullary carcinoma of the thyroid requires aggressive treatment because of its potential to metastasize and because of the current limitations of preoperative localization and systemic therapy. If these tumors could be made to fluoresce in vivo with tagged fluorophore antibodies against tumor antigens, surgeons would be able obtain additional information in the operating room to facilitate a more complete resection. Based on the success of our previous work in breast and colon cancer models, we conducted an animal study of in vivo tumor fluorescence of a human medullary thyroid cell line in which bright tumor fluorescence is visible during dissection. To accomplish this, we used an inexpensive and commercially available handheld, blue (470 nm), light-emitting diode flashlight and filtered goggles (520 nm). This procedure, which we call the *fluorescent antibody-assisted surgical technique* (FAAST), is easy to perform, requires no complex or expensive technical equipment, and has the potential to be applied to a wide variety of tumors. To the best of our knowledge, this is the first experiment of its kind to be reported in the literature.

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## Introduction

Thyroid surgery carries a low risk of complications when it is performed by competent, well-trained, and meticulous surgeons. The pioneering work of Kocher, Billroth, Mayo, Crile, and Lahey laid the foundation for modern-day techniques for safe and adequate resections of the thyroid.[1] Nevertheless, even when surgery is in the best of hands, complications such as postoperative wound infections, delayed healing, edema, hematoma, seroma, and nerve injury can occur, regardless of whether surgery is performed for a simple goiter or for a thyroid malignancy. Surgery for thyroid cancer can be made more difficult by local invasion, which also increases the risk of complications. Additionally, in cases of recurrence, scarring from a previous surgery may add to the difficulty of localizing the disease and achieving adequate resection with clear margins.

Medullary carcinoma of the thyroid is a rare malignancy of neuroendocrine origin.[2] It can occur independently or as a component of multiple endocrine neoplasia syndrome 2A or 2B. Medullary carcinoma of the thyroid arises from parafollicular cells (C cells) derived from ultimobranchial bodies. Tumors are typically solid and well circumscribed but not encapsulated. The cells are round to polygonal, and they contain calcitonin, carcinoembryonic antigen (CEA), and chromogranin; they also express other markers. Cervical adenopathy is common.

Medullary carcinoma of the thyroid requires particularly aggressive treatment because of its potential to metastasize and because the effectiveness of preoperative localization with imaging and current systemic therapy is limited.[2–12] Treatment requires complete surgical excision of the primary tumor and all locoregional disease. Typically, this involves a central neck dissection, as well as a unilateral or bilateral lateral dissection. Multifocality is common, so careful attention to removal of all malignant tissue is crucial to optimizing long-term disease-free survival. Any advantage that the surgeon might have in this respect would be welcome. One such advantage would be the ability to make thyroid cancers fluorescent in vivo. Fluorescent labeling of a tumor in vivo might provide a clear identification of all malignant tissue and allow for restaging of a tumor intraoperatively, with the result being a more accurate and complete resection of all malignant tissue.

Using the nude mouse model, we have previously demonstrated that human breast and colon cancers, including locoregional nodal metastases, can be made fluorescent in vivo with fluorophore-tagged antitumor antigen antibodies.[13–16] We call this procedure the *fluorescent antibody-assisted surgical technique* (FAAST). In this article, we present the findings of our study of tumor fluorescence of a human medullary carcinoma of the thyroid cell line in mice. To the best of our knowledge, this is the first report of in vivo tumor fluorescence of a human thyroid cell line in which bright tumor fluorescence was made visible by a simple, inexpensive, commercially available, handheld, blue light-emitting diode (LED) flashlight (fitted with a 470-nm band-pass filter) and easily viewed through filtered (520 nm) goggles.

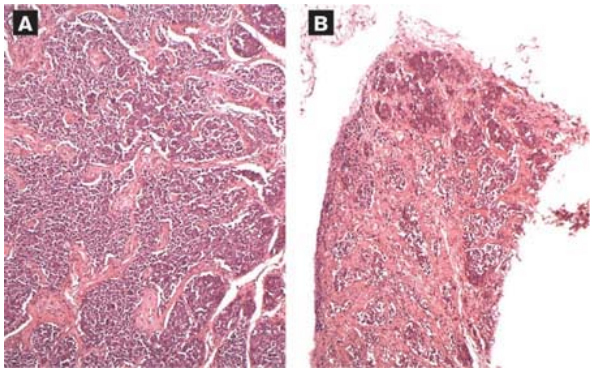
## Materials and methods

Cells from a human medullary carcinoma of the thyroid cell line were injected subcutaneously into 4 nu/nu mice (3 study mice and 1 control). Approximately 3 weeks later, 3- to 5-mm subcutaneous nodules were visible and palpable. The carcinoma cells were known to express CEA and CA15-3 tumor antigens.

Anti–CA15-3 (mouse anti–human CA15-3) monoclonal antibodies were tagged with a green fluorophore from a commercially available labeling kit. Fluorophore-tagged anti–CA15-3 (100 µl of 1 mg/ml antibody solution) was injected into the tail vein of the 3 study mice. Fluorophore-tagged mouse IgG

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**).

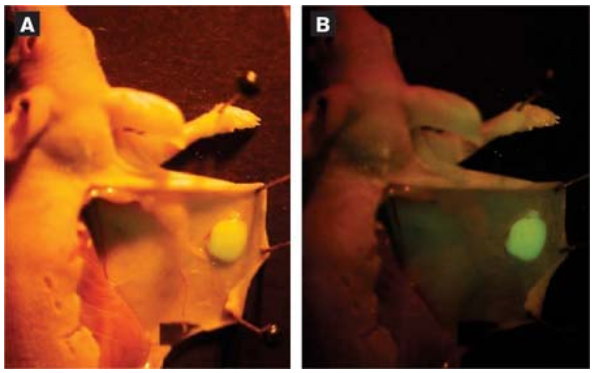
**Slides contain biopsies from study mouse B (A) and the control mouse (B) (H&E, original magnification ×10).**



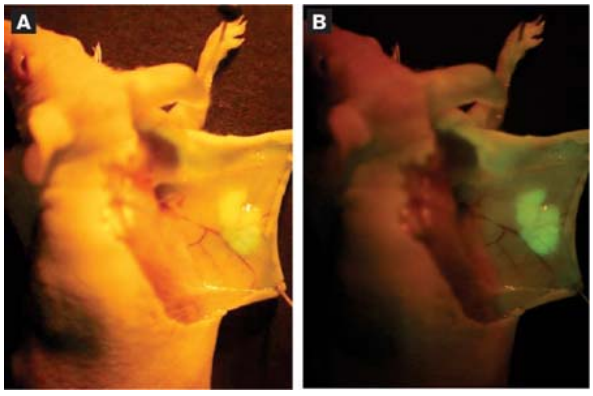

## Results

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.

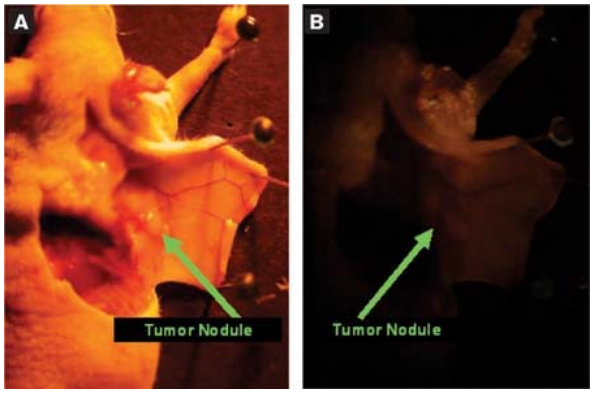
**The nodule in study mouse A is seen under white light (A) and under the 470-nm light source (B).**




**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, antiprostate-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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