A Novel Mouse Model of Metastatic Thyroid Carcinoma Using Human Adipose Tissue-Derived Stromal/Stem Cells

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Abstract. Background/Aim: Scientists have been in quest for the best in vivo model to evaluate chemotherapies for radioiodine-resistant metastatic thyroid carcinomas. Human adipose tissue-derived stromal/stem cells (ASCs) have been found to promote in vitro growth and in vivo tumorigenesis. In the present study, we describe a novel model of metastatic human thyroid carcinoma by combining ASCs with the papillary thyroid cancer, K1 cell line. Materials and Methods: Three groups of severe combined immunodeficient mice were investigated. The first group was injected subcutaneously with K1 cells plus ASCs, the second group with K1 cell only, and the last group with ASCs only. Mean tumor volumes and standard deviations were calculated and compared. Results: Concomitant injection of ASCs with the K1 cell line led to the development of significantly larger tumors compared to the other groups (p<0.05). In addition, the lungs of this group demonstrated gross tumor metastasis and pathological features of high-grade neoplasms. Conclusion: In the present study we describe a novel mouse model using ASCs with the potential to be used for assessment of new treatments for the management of metastatic thyroid carcinomas.

More than 56,000 cases of thyroid cancer are diagnosed each year in the U.S., and its incidence is rising the fastest among all major types of cancer (1). Surgery followed by radioactive iodine (RAI) therapy is the treatment of choice for well-differentiated thyroid neoplasms (papillary and follicular thyroid carcinoma) (2-4). However, the 10-year recurrence rate is 20-30% among patients who are older, have larger tumors, have extra-thyroidal disease, or develop extensive lymph-node metastases (4-6). Additionally, there is no currently effective treatment for radioiodine-resistant metastatic disease, with a 10-year survival rate of less than 15% (7).

An appropriate pre-clinical in vivo model that can mimic human cancer progression and response to treatment is a primary goal of many researchers. The advancement of human tumor xenograft models, which use human malignant cells from different cell lines transplanted into immune-deficient mice, provide clinically significant targets as well as metastatic sites, thus laying some groundwork for anticancer drug development (8-12).

Human adipose tissue-derived stromal/stem cells (ASCs) are a vital source of growth factors and have potent angiogenic and regenerative properties that make them capable of treating many medical and surgical problems (13). ASCs are increasingly being used for regenerative purposes such as soft tissue reconstruction following mastectomy. There is some concern, however, that these same characteristics that make ASCs so great for regenerative purposes make them potentially mitogenic for cancer cells. Recurrence of cancer, and metastases are all feared outcomes from the use of ASCs (14). In addition, subcutaneous and orthotopic xenograft models of human thyroid cancer have been created using different cell lines including follicular, papillary and anaplastic tumor types (15). However, the main challenge has been to produce an established metastatic human thyroid carcinoma model.
ASCs have been shown to increase tumor volume and invasion/metastasis when co-injected with cancer cells in xenografts tumors models (16-23). Furthermore, evaluation of ASCs presence in xenograft thyroid tumor models is currently lacking.

In the present study, ASCs co-injected with the papillary thyroid tumor cell line K1 (harboring heterozygous mutation in \textit{BRAF} (V600E T>A missense mutation at nucleotide 1796)) were used to develop a reproducible xenograft tumor model in nude mice that can be exploited to evaluate and build novel therapeutics for metastatic thyroid tumors. \textit{BRAF}, a serine-threonine kinase, is translocated to the cell membrane following activation by RAS. \textit{BRAF} phosphorylates mitogen activated protein kinase (MAPK) and activates it and its downstream signaling pathways. \textit{BRAFV600E} gene mutations result in enhanced/constitutive kinase activity, and have been reported in many types of malignancies, including melanomas, colorectal cancers, serous ovarian cancers, and non-small cell lung cancers (24-26). The \textit{BRAFV600E} mutation is also found in up to 83% of thyroid cancers (27-29).

In the present study we describe a novel subcutaneous model of metastatic human thyroid carcinoma using human adipose tissue-derived stromal/stem cells in addition to a K1 cell line.

Materials and Methods

\textit{Cell culture and preparation.} The K1 cell line, derived from a primary papillary thyroid carcinoma, was obtained from Sigma (St. Louis, MO, USA). The cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 0.1 mM non-essential amino acids, 1 mM sodium-pyruvate, and 100 U/100 μg/ml of penicillin/streptomycin. ASCs were isolated as previously described (30, 31). Briefly, the subcutaneous abdominal adipose tissues from a healthy female patient during elective surgery were collected with the patient’s informed consent. Written consent was obtained by the plastic surgeons performing the surgery under a protocol approved by the Institutional Review Board of the Pennington Biomedical Research Center Institution. Tissues were provided to the investigators with all identifying information removed. The percentage of ASCs that were positive for individual surface markers were as follows: CD29, 99.03; CD105, 98.82; CD45, 11.87; CD34, 93.94; CD44, 12.7; CD73, 88.48; CD90, 93.46. ASCs were cultured in ASC growth medium [DMEM/F-12 Ham’s, 10% FBS (Hyclone, Logan, UT, USA), 1% penicillin-streptomycin/0.25 g fungizone] on polystyrene tissue culture.

\textit{Subcutaneous mouse models of thyroid cancer.} All animal work was performed at the Tulane University School of Medicine, in accordance with federal, local, and institutional guidelines and with approved IACUC protocol (#2941R2) from Tulane University. For the subcutaneous injection, 0.5×10⁶ K1 cell pellets were dissolved alone or combined with 0.5×10⁶ ASCs (1:1 ratio) in 150 μl phosphate-buffered saline (PBS) and matrigel basement membrane matrix (BD Bioscience, San Jose, CA, USA). Six-week-old inbred homozygous athymic BALB/C nude (nu/nu) male mice (Charles River, Wilmington, MA, USA) were housed in a pathogen-free barrier facility. Twelve mice were randomized into three experimental groups and inoculated subcutaneously in the flank. Five mice were injected with 0.5×10⁶ K1 cells and 0.5×10⁶ ASCs, another five mice were injected with 0.5×10⁶ K1 cells only, and the last two mice were injected with 0.5×10⁶ ASCs only. One bolus was injected in the right flank region of each mouse depending on its group. Palpable tumors were allowed to develop for 4 weeks. No surgical or anesthetic complications were noticed in any of the mice. All 12 mice underwent necropsy 42 days after tumor cell injection. At the end of the experiment, mice were euthanized by exposure to CO₂ and tumors and mouse lungs, spleen, and liver were removed for further evaluation. Tumor volume was calculated under the assumption that tumors were ellipsoid in shape, using the formula \((π×a²×b/6)\), where \(a\) is the short axis, \(b\) is the long axis of the tumor, and \(π\approx3.14159\).

\textit{Hematoxylin and eosin staining of tumors and mouse tissues.} At the end of the experiment, tumors and mouse organs were removed from the animals, photographed and then tissues were stored in 10% neutral buffered formalin for paraffin embedding/sectioning and hematoxylin and eosin (H&E) staining. Paraffin-embedded tumor and mouse tissues were sectioned (5 μm) and stained with H&E.

\textit{Statistical analysis.} Student’s \(t\)-tests were performed using the Microsoft Excel Software to compare the calculated mean tumor volume and standard error of the mean (SEM) between groups. \(p\)-Values less than 0.05 were considered significant.

Results

Following injection of cells, tumor growth was monitored for 42 days. The mean tumor volume was (862±33.6 mm³) in the K1-cells group and (1982±216 mm³) in the K1-cells-plus-ASCs group. The subcutaneous implantation of K1 cells-plus-ASCs produced significantly larger tumors by
volume, than the implantation of K1 cells-alone (p-value <0.05) (Figure 1). No palpable tumor growth was observed in mice injected with ASCs alone.

Local invasion into adjacent structures was visualized in all mice from the K1 cells-plus-ASCs group (Figure 2A and B). In addition, multiple micrometastases to the lungs were observed in all five (100%) lung samples of this group. Detailed histological analyses of these metastases demonstrated features of high-grade malignant neoplasms, characterized by nuclear atypia, marked cellular pleomorphism, and necrosis (Figure 3A and B). Some micrometastases were visualized in the group injected with K1 cells (40%), but not in ASCs alone.

**Discussion**

Follicular and papillary thyroid cancers tend to be less aggressive, well-differentiated tumors that often exhibit a good response to standard therapy regimens. On the other end of the spectrum, undifferentiated or anaplastic thyroid cancer has a poor prognosis with a tendency to metastasize to other organs, primarily the lungs. Due to the fact that anaplastic thyroid cancer is often resistant to current standard radioactive iodine or chemotherapy regimens, new treatment alternatives are desperately needed. On this path, considerable effort has been devoted to developing an animal model.
model for aggressive thyroid cancer. Such a model may serve as the cornerstone to creating novel treatment strategies.

Nucera et al. describe a novel orthotopic thyroid cancer model using distinctive 8505c thyroid cancer cells that demonstrate features of aggressive tumors (32). What distinguishes our model, however, is the use of a thyroid tumor cell line with the addition of human ASCs implanted subcutaneously, which demonstrate on impressive ability to increase tumor size, invasion and metastasis. The mean tumor size in our model was 1982±216 mm³ when both K1 cells and ASCs were injected subcutaneously, compared to 18.4±3.5 mm³ when 8505c thyroid cells were injected subcutaneously and 246.9±68.8 mm³ when the same cell line was orthotopically-transplanted as shown in the model of Nucera et al. In both models the same number of cancer cells were used (0.5x10⁶).

To our knowledge, this is the first model in which ASCs are used to produce metastatic thyroid cancer. When ASCs along with papillary thyroid cancer, K1 cells were injected subcutaneously, the tumor size 42 days following the injection was significantly larger than using K1 cells-alone. In addition to promoting dramatic tumor growth, ASCs were found at distant metastatic sites in all of the implanted mice. This indicates that these cells have the capability to extravasate into the circulation from a primary tumor site and then seed within metastatic organs. Mice injected with K1 cells-alone demonstrated significantly less micrometastases and ASCs-alone did not demonstrate any metastases. Although the model described by Nucera et al. did demonstrate lung metastases in orthotopically-implanted mice using the distinctive 8505c thyroid cell line, metastases to other organs were not visualized.

Histologically, lung samples of all five mice (100%) injected subcutaneously with ASCs plus K1 cells showed a high nuclear-to-cytoplasmic ratio, nuclear pleomorphisms, necrosis, and atypia, all being features of a high-grade carcinoma. However, mice injected with K1 cells-alone demonstrated micrometastases in only two out of five mice (40%) and ASCs-alone did not show any metastases to the lungs. In the study by Nucera et al. only orthotopic placement of the tumors led to lung metastases while subcutaneous placement led to relatively small tumors. This difference in overall growth and metastasis suggests the importance of an appropriate microenvironment and organ-specific angiogenesis necessary for tumors to take on an aggressive pattern. Our experiment demonstrates, however, that with the addition of ASCs, which possess their own array of growth factors and angiogenic properties, orthotopic placement becomes much less critical in the development of an aggressive tumor type.

The main challenge of subcutaneous models has been that they do not closely-reproduce the primary site or the sites of metastases of common human cancers. Although orthotopic tumor placement appears to better-mimic the microenvironment and metastatic patterns of human cancer, the subcutaneous model is technically easier to generate (12). There is, however, widespread use of subcutaneous tumor models, especially in therapeutic screening studies suggesting that correlations between subcutaneous tumor model data and clinical activity are good.

We conclude that the implantation of K1 cells combined with human ASCs can be used to produce an aggressive thyroid cancer model with metastatic lesions. This novel model of advanced human thyroid cancer is theoretically possible, easily reproducible, and is a potential tool for studying tumor growth, aggressiveness, and development as well as the therapeutic options to combat these characteristics.

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Conflicts of Interest

None.

References

Kandil et al.: A Metastatic Thyroid Cancer Model


