Our long-term goal is to address two important clinical problems related to patients with thyroid tumors. The first one is the incidental discovery of impalpable thyroid nodules, in an estimated 20-67% of general population. Fine-needle aspiration (FNA) is considered the best initial diagnostic tool in the evaluation of thyroid nodules. However, inconclusive diagnosis (suspicious) occurs in nearly 5-40% of all FNA. Guidelines for evaluation and management of patients with thyroid nodules suggest that all patients with a cytological report of suspicious should be referred to surgery for a more accurate diagnosis. Only 5-20% of these suspicious nodules, when removed, are indeed malignant on histology. Molecular markers could help to avoid a large number of surgeries. We previously performed SAGE in a follicular thyroid adenoma and follicular thyroid carcinoma. This analysis and subsequent validation revealed that four novel markers (Ddit3, Arg2, C1orf24 and Itm1) differed in the two types of tumor. A linear combination of expression levels distinguished FTC from FTA with an estimated predictive accuracy of 0.83. In this project we propose to improve the sensitivity and specificity of our test and perform function analysis for Itm1 and C1orf24. The second clinical problem is that recurrence of thyroid tumors is high, ranging from 20-40% of patients with differentiated thyroid tumors. Lymph nodes account for 60-75% of all neck recurrences. This project proposes to identify genes that are involved in lymph node metastases. To this end, SAGE will be performed in matched normal thyroid, primary tumor and lymph node metastasis. We believe that, besides understanding the mechanism of metastases, the molecular markers identified from gene expression profile must offer an alternative in the follow-up of patients with thyroid carcinoma and ultimately may reveal a target gene for therapy.
Biomarkers of papillary thyroid carcinoma (PTC) metastasis can accurately identify metastatic cells and aggressive tumor behavior. Serial Analysis of Gene Expression (SAGE) was performed on matched-normal thyroid tissue, primary PTC and a PTC lymph node metastasis. The genome-wide expression analysis and further validation in a larger set of matched-samples identified Limd2 and Ptprc as consistently different between the tumor and metastatic samples (P < 0.0045). Ltb had borderline significance. The PTC SAGE library was compared to the previous generated follicular thyroid carcinoma library (FTC) and normal thyroid, to identify papillary thyroid carcinoma (PTC)-associated transcripts. We identified three genes (Cst6, Cxcl14, Dhrs3) strongly associated with PTC. Additionally, Cst6 and Cxcl14 were positively correlated with the presence of metastasis and brafV600E mutational status. Noteworthy, braf mutation was previously associated with the presence of metastasis. Our findings suggested that these genes may be induced subsequently to Braf activation and, therefore, may be downstream in the Braf/Mek/Erk signaling pathway. To improve the accuracy of our test, custom antibody for C1ORF24 and ITM1 and commercially available DDIT3 and ARG2 were tested in 127 benign and malignant thyroid tissue sections (which are a source for diagnosis errors by immunohistochemistry – IHC).

We improved this diagnostic test by adding C1ORF24 and ITM1 custom antibodies and demonstrating its use on a wider variety of thyroid pathologies. We recommend that testing of all four cancer biomarkers now be advanced to larger trials. Use of one or more of these antibodies should improve diagnostic accuracy of suspicious thyroid nodules from both tissue sections and FNA samples.

Representative results of immunohistochemical analysis. Strong brown staining for PTPRC was observed in the surrounding immune cells in metastatic lymph node. LTB was positive for tumor cells within a lymph node, as revealed by the brown immunostaining.