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Original contribution

# Autocrine activation of platelet-derived growth factor receptor α in metastatic papillary thyroid cancer☆

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**Summary** Metastatic dissemination of papillary thyroid cancer has been reported to be strongly associated with expression of platelet-derived growth factor (PDGFR) α and altered TTF1 function. However, the status of PDGF ligands in papillary thyroid cancer and the potential role of these ligands in metastatic disease are obscure. We assessed the prevalence of PDGF ligands in benign and malignant thyroid tumors to determine if ligand upregulation is associated with α-isoform (PDGF-AA or PDGF-BB) or the β-isoform (PDGF-BB or PDGF-DD) of PDGFR in individual tumors. The immunohistochemical expression of PDGFRα, PDGF-AA, PDGF-BB, and PDGF-DD was surveyed in follicular adenomas (n = 55), papillary thyroid carcinomas (103 with and 59 without nodal metastases), and lymph node metastasis (n = 12). There is an augmented tendency for PDGF-AA expression in node-positive papillary thyroid cancer metastases ($P < .0001$). Although PDGF-BB and -DD were commonly identified, there was no relationship between the presence of these cytokines and malignant disease or metastases. Logistic regression demonstrated that PDGF-AA expression was significantly associated with the presence of PDGFRα (odds ratio = 4.6, $P = .004$) and recurrent disease. When either PDGFRα or PDGF-AA was used to predict the presence of metastases, the sensitivity achieved was 86% and 88%, respectively, whereas specificities were lower at 71% and 61%, respectively. The augmented coexpression of PDGF-AA and PDGFRα in metastatic papillary thyroid cancers suggests that an autocrine signaling loop may contribute to nodal infiltration. Combined testing for the expression of PDGF-AA and PDGFRα may identify those patients with papillary thyroid cancer at risk of metastatic disease and resistance to therapy.


## 1. Introduction

The incidence of thyroid cancer has steadily risen over the past 4 decades, making it the most prevalent type of endocrine cancer globally [1]. In North America, it is expected to become the fourth most commonly diagnosed cancer by 2030 [2]. This increase is mainly due to a tripling in the incidence of the papillary histology subtype, which accounts for nearly 90%

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of thyroid cancers [3,4]. Papillary thyroid carcinomas are highly inclined to lymph node metastases, with a likelihood of 60% in anatomic studies of adult patients [5-7]. Nodal infiltration is associated with increased risk of recurrence, and in many cases, repeated surgery is necessitated when disease recurs because of inherent resistance to routine radioactive iodine treatment. For approximately 10%-15% of all thyroid cancer patients, repeated operations may result in significant complications such as recurrent laryngeal nerve injury and permanent hypoparathyroidism, both of which can negatively impact quality of life and survival [6,8-10].

Traditional prognostic factors such as age or histological features provide an incomplete understanding of the natural history of disease to predict malignancy in indeterminate nodules or metastases in proven cases of cancer [11]. Tumor size and patient age are well accepted, but imperfect, prognostic surrogates that are used to stratify patients for surgery as well as for the subsequent application of radioactive iodine. Unfortunately, many patients are over- or undertreated given our inability to predict the natural history of thyroid cancer. Serial biochemical and ultrasound investigations remain the workhorses for follow-up as patients are assessed for the presence of metastases, which can occur decades after initial diagnosis [11,12]. Genetic tests designed to discriminate between benign and malignant nodules have been attempted and are used in select settings for indeterminate nodules. However, tests designed to predict metastatic disease or poor outcomes based on resistance to radioactive iodine therapy are not available [11,13]. Molecular indicators capable of predicting the risk of nodal infiltration are needed for the effective management of metastatic thyroid carcinomas.

Recently, Zhang et al reported a strong positive association between nodal metastasis and platelet-derived growth factor receptor (PDGFR) α expression in papillary thyroid cancer tumors [14]. Biopsies from primary tumors with metastases exhibited high levels of PDGFRα, whereas adenomas and primary tumors without metastases showed low or minimal PDGFRα staining. PDGFRα was also shown to drive thyroid follicular cell dedifferentiation and the epithelial-mesenchymal transition through a number of signaling moieties including the MAPK, STAT3, and PI3K/Akt pathways [15,16]. In these same studies, PDGFRβ was found to be a more ubiquitous component of thyroid neoplasms and did not appear to vary qualitatively between malignant disease with or without metastases. The role of PDGFRα in driving malignancy is not limited to thyroid neoplasms. In a human lung carcinoma model, PDGFRα signaling promoted cell proliferation and migration [17]. PDGFRα signaling is also associated with tumor progression and metastasis in colorectal cancer [18]. Other cancers reported to express PDGFRα with prognostic implications include renal, breast, ovarian, prostate, melanoma, and bone cancer [19].

In the current study, we examine the ligand stimulatory environment for the α and β receptors of PDGF by assessing the relative distribution of PDGF-AA, -BB, and -DD in benign and malignant thyroid neoplasms. Through the immunohistochemical staining of a large cohort of patient samples that include metastatic disease, we aim to identify if individual, or multiple, ligands are selectively expressed in metastatic disease specimens. PDGFRα staining was also performed to identify a correlation between PDGF ligand and α-receptor expression in individual tumors, potentially generating an autocrine feedback loop. Outlining the distribution of PDGF ligands across patient samples may result in the development of adjunct immunohistochemical tests to complement existing methods used to identify patients at risk of nodal metastases in papillary thyroid cancer.

## 2. Materials and methods

### 2.1. Patients and tissues

Ethics approval was obtained through the University of Alberta Heath Research Ethics Board (Pro00178), and informed consent was obtained from all patients. The tissue microarrays (TMAs) were constructed using 229 formalin-fixed, paraffin-embedded archived samples including 55 follicular adenomas and 162 papillary thyroid carcinomas (103 with and 59 without nodal metastases) and 12 lymph node metastases. Within the carcinoma subgroup, about 70% (n = 161) represent the classic variants, with nearly all of the remaining cases comprising follicular variants (nonencapsulated) and with a small number of cases (n = 7) described with significant elements of columnar, tall cell, and/or diffuse sclerosing components. Two pathologists separately confirmed histological diagnoses of the primary tumors and the presence of metastases in the nodes sectioned. The classification of thyroid tumors was based on the World Health Organization criteria (2004). Clinical data were obtained from a database maintained at the Cross Cancer Institute. *Recurrence* is defined as an increase in unstimulated thyroglobulin levels of greater than 0.4 ng/mL or stimulated thyroglobulin levels greater than 2 ng/mL, and/or pathologic evidence of recurrence based on surgical or fine needle aspiration biopsy.

### 2.2. Immunohistochemistry and scoring

Immunohistochemical (IHC) staining was performed using standard techniques. Briefly, TMAs were freed of paraffin in xylene and rehydrated through graded alcohols. Heat-induced antigen retrieval was performed by pressure-cooking slides in the microwave with citrate buffer (pH 6.0) for 20 minutes. Endogenous peroxidase activity was quenched by incubating slides in 3% $H_2O_2$ for 10 minutes followed by treatment with avidin/biotin blocking reagent (Dako Burlington, Ontario, Canada) for 15 minutes at room temperature. Immunoreactivity was then assessed by overnight incubation with antibodies at 4°C in a humidified chamber. Antibodies for PDGFRα were purchased from Cell Signaling Technology (Danvers, MA), and those for PDGF-AA, PDGF-BB, and PDGF-DD were from Santa Cruz Biotechnology (Santa Cruz, CA). The antibody dilutions used are shown