Evaluation Of Type 1 Growth Factor Receptor Family Expression In 205 Thyroid Lesions Reveals Diagnostic Utility And Targeted Therapeutic Potential For HER1, HER3, and HER4

Sam Wiseman[A,B,C,D], Griffith O[B,D], Melck A[A,B], Masoudi H[A,B], Rajput A[C], Gilks B[B,C,D], Jones S[B,D]

Department of Surgery St. Paul's Hospital[A], University of British Columbia[B], Genetic Pathology Evaluation Center at the Prostate Research Center of Vancouver General Hospital[C], & British Columbia Cancer Agency[D], Vancouver, British Columbia, Canada

STUDY HYPOTHESIS

Type 1 Growth Factor Receptor Family Members (HER1, HER2, HER3, and HER4) will have diagnostic and prognostic clinical utility.

STUDY ABSTRACT

Background: The accurate preoperative diagnosis and prognostication of thyroid cancer in individuals who present with nodular thyroid disease has remained a major clinical challenge.

Objectives: The aim of this study was to evaluate the diagnostic and prognostic utility of the type 1 growth factor receptor (T1GFR) family (HER1, HER2, HER3, HER4) in the management of DTC. A secondary objective was to evaluate the proportion of DTC expressing T1GFR family members.

Methods: Tissue microarrays (TMAs) consisting of 100 benign thyroid lesions, 105 malignant thyroid lesions were stained for HER1, HER2, HER3, and HER4. Correlation of clinicopathologic characteristics with expression of each of the markers was assessed with contingency table tests ($\chi 2$ or Fisher's Exact where appropriate) for categorical variables and the Mann-Whitney U-test for continuous variables. A *p* value of less than 0.05 was considered statistically significant. The Benjamini and Hochberg (BH) procedure was used to correct p-values for multiple testing. To test whether this combination of markers would be useful for diagnostic discrimination of DTC from benign samples, all four markers were submitted as potential variables to a Random Forests classifier.

Results: HER1, HER2, HER3, and HER4 were expressed in 76%, 2%, 57%, and 73% of DTC cases respectively. HER1 and HER3 showed significantly increased expression in DTC compared to benign thyroid lesions (76.3% vs. 59.6%, p=0.022 and 56.5% vs. 34.3%, p=0.013, respectively). HER4 showed significantly decreased expression in DTCs compared to benign thyroid lesions (72.7% vs. 85.9%, p=0.032). For HER2 there was no significant difference in expression between benign and DTC lesions. Before multiple testing correction, the expression of HER3 correlated with the presence of lymph node metastasis (p=0.012), tumor type (only follicular carcinoma stained negative) (p=0.013), and higher N stage (p=0.022); the expression of HER4 correlated with lower T stage (p=0.037). However, none of these associations were still significant after multiple testing correction by the BH procedure. A classifier targeting benign versus malignant status with all four markers as potential predictors displayed an accuracy, sensitivity and specificity of 67.2%, 60.4%, and 74.0%, respectively.

Conclusions: Expression of the T1GFR family helps distinguish DTCs from benign thyroid lesions and the high proportion of cancers which expressed HER1, HER3, and HER4 suggests that investigation of currently utilized anticancer agents, which target one or more of these family members, warrants further clinical study in individuals diagnosed with DTC.

INTRODUCTION

Thyroid nodules are extremely common, being palpable in 4% to 7% of adults in North America, with new nodules detected at a yearly rate of 0.1%.1 Each year approximately 19,500 new cases of thyroid cancer are diagnosed in the United States and 1,300 individuals die from this disease. The majority of individuals will be diagnosed with papillary carcinoma (80%), while the remainder of malignant thyroid neoplasms diagnosed are: follicular, medullary, and anaplastic tumors. Fine needle aspiration biopsy (FNAB) is currently considered the best diagnostic tool in the evaluation of thyroid nodules. The result of the FNAB cytology can be classified as: benign (70% of cases), malignant (5-10% of cases), indeterminate or suspicious (10-15% of cases), and nondiagnostic (10-15% of cases). While nondiagnostic FNAB can be repeated, it is the indeterminate or suspicious group that poses a dilemma for the clinician. Many individuals with indeterminate cytology will undergo surgery. Unfortunately, intraoperative pathologic assessment of thyroid nodules is of limited utility and has been discouraged by many investigators.On final pathology up to 50% of individuals with indeterminate cytology who undergo thyroidectomy are found to harbor benign disease. Recently, there has been much interest in the prognostic and treatment implications of the expression by multiple tumor types, especially breast and lung cancers, members of the type 1 growth factor receptor (T1GFR) family.The T1GFR family consists of 4 known transmembrane receptors: HER1 (epidermal growth factor receptor or EGFR), HER2 (c-erbB-2), HER3 (c-erbB-3), and HER4 (c-erbB-4). Study of the pattern of expression by the entire T1GFR family, their diagnostic utility, and their relationship to each other and to known thyroid cancer prognosticators, would be of considerable clinical importance.

METHODS

Patients and Methods

Two hundred and five subjects were selected from a Thyroid Surgery Patient Database which is prospectively maintained by one of the authors (SW). All patients had undergone thyroid surgery for benign or malignant disease between January 2001 and May 2005. The medical records of these patients were retrospectively reviewed and the following data were recorded: patient demographics, surgical procedure, pathology, extrathyroidal extension, vascular invasion, multifocality, completeness of resection, lymph node and distant metastases, adjuvant therapy in the form of radioactive iodine ablation (RAI) and external beam radiation therapy (EBRT), thyroglobulin levels, AJCC stage, AMES scores, length of follow-up, and disease status. We then obtained archival pathology specimens for these patients to construct TMAs and carry out subsequent immunohistochemical analysis of the four molecular markers, as described below. This study was carried out with the approval of the Research Ethics Boards.

Tissue Microarray Construction

The archival pathology specimens from these patients, including lymph node specimens where relevant, were reviewed by two pathologists to confirm the diagnosis and select the optimal area of the specimen to be included in the TMA. Paraffin-embedded tissue blocks were then created from these samples. TMA construction was performed utilizing a tissue-arraying instrument (Beecher Instruments, Silver Springs, MD) to remove two 0.6-mm core biopsies from the pre-selected regions of each primary block. They were then transferred side-by-side to defined array coordinates in one of two recipient TMA blocks; one recipient array contained 100 benign lesions and the other contained 105 malignant lesions.

Immunohistochemistry

A Leica microtome was used to cut serial 4-μm sections from the TMA blocks and transferred onto adhesive-coated glass slides. TMA slides were then processed following standard immunohistochemistry protocols using monoclonal antibodies against each of the markers. The antibodies used, dilutions, and antigen retrieval methods are shown in Table 1. Briefly, sections were deparaffinized and antigen retrieval performed as indicated. Subsequently, slides were incubated with primary antibody at a pre-determined optimal concentration for 32 minutes at 37.5°C. Secondary detection was done using a DAKO LSAB® detection system (DAKO, Carpenteria, CA) according to the manufacturer's instructions. Appropriate positive and negative control slides were used for each antibody.

Scoring

Two pathologists blinded to the clinical data, examined the sections at high power to determine the proportion of cells expressing the markers. These grading scales were based on previously published reports of immunohistochemistry studies involving these four markers, and are listed in Table 2. If the scores for the two samples from each specimen differed, the higher of the two was chosen for analysis. The scores were recorded in a standardized score sheet matching each TMA section (Microsoft Excel; Microsoft, Redmond, WA) by one of the investigators. The deconvoluted data was then transferred into the clinicopathologic database for statistical analysis.

Statistical Analysis

Correlation of clinicopathologic characteristics with expression of each of the markers and the significance of marker expression in malignant versus benign tissue was assessed with the $\chi 2$ test or Fisher's exact test where appropriate, using the SPSS statistical software package (Version 13.0; SPSS, Chicago, IL). Nine of the cancers in the malignant TMA were medullary or Hurthle cell carcinomas, and were thus excluded from the statistical analysis as we wanted a pure differentiated thyroid cancer cohort. A *p* value of less than 0.05 was considered statistically significant.

RESULTS

HER1, HER2, HER3, and HER4 were expressed in 76%, 2%, 57%, and 73% of DTC cases respectively. HER1 and HER3 showed significantly increased expression in DTC compared to benign thyroid lesions (76.3% vs. 59.6%, p=0.022 and 56.5% vs. 34.3%, p=0.013, respectively). HER4 showed significantly decreased expression in DTCs compared to benign thyroid lesions (72.7% vs. 85.9%, p=0.032). For HER2 there was no significant difference in expression between benign and DTC lesions.

Before multiple testing correction, the expression of HER3 correlated with the presence of lymph node metastasis (p=0.012), tumor type (only follicular carcinoma stained negative) (p=0.013), and higher N stage (p=0.022); the expression of HER4 correlated with lower T stage (p=0.037). However, none of these associations were still significant after multiple testing correction by the BH procedure.

A classifier targeting benign versus malignant status with all four markers as potential predictors displayed an accuracy, sensitivity and specificity of 67.2%, 60.4%, and 74.0%, respectively.

Table 1. Characteristics of molecular markers evaluated

Antibody	Isotype	Clone	Company	Catalog #	AgR	Conc	Localization	Scoring
EGFR	mouse monoclonal	2-18C9	DAKO	K1492	Proteinase K	ready	cytoplasmic +/or membranous	A
HER2	rabbit polyclonal	x	DAKO	A485	Steam 20min, TRS	1:500	cytoplasmic +/or membranous	B
HER3	rabbit polyclonal	x	NeoMarkers	RB-066-PO	None	1:200	cytoplasmic +/or membranous	A
HER4	mouse monoclonal	x	NeoMarkers	MS-637-PO	x	1:160	cytoplasmic +/or membranous	C

Table 2. Scoring system types for markers

Scoring Type	Scoring System
A	3+ = >75% of cells positive 2+ = 26-75% of cells positive 1+ = 5-25% of cells positive 0 = <5% of cells positive
B	1+ = Herceptest +ve 0 = Herceptest -ve
C	2+ = strong 1+ = weak 0 = negative

CONCLUSIONS

- Expression of the T1GFR family helps distinguish DTCs from benign thyroid lesions
- The high proportion of cancers which expressed HER1, HER3, and HER4 suggests that investigation of currently utilized anticancer agents, which target one or more of these family members, warrants further clinical study in individuals diagnosed with DTC