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Overexpression of EphB4, EphrinB2 and EGFR in Papillary Thyroid Carcinoma

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ABSTRACT

Objective:
- To examine the expression of EphB4, EphrinB2 and EGFR in tumor tissue and surrounding normal tissue in patients with papillary thyroid carcinoma (PTC)
- Evaluate the association between gene expression and tumor size, focality, extracapsular extension, neurovascular invasion, lymph node spread and clinical stage.

Methods:
Twenty one adult patients with newly diagnosed PTC underwent total thyroidectomy and level VI lymph node dissection. EphB4, EphrinB2 and EGFR expression in tumor and normal thyroid tissue was evaluated by complimentary DNA (cDNA) microarray, Western blot and immunohistochemistry (IHC).

Results:
cDNA microarray analysis (n=4)
- Increased average fold change in EphB4 (2.5, P<.001), EphrinB2 (2.4, P<.001) and EGFR (2.9, P<.001) expression in tumor vs. normal thyroid tissue.

IHC analysis (n=21)
- Higher mean expression of EphB4, EphrinB2 and EGFR in tumor versus normal thyroid tissue.
- Statistically significant association between PTC gene expression and lymph node spread (EphB4, EphrinB2), extracapsular extension (EphB4, EphrinB2), and clinical stage (EGFR).

Conclusion:
Overexpression of EphB4, EphrinB2 and EGFR is associated with PTC, thus providing potential targets for future PTC therapies.

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INTRODUCTION

- Papillary thyroid carcinoma (PTC) accounts for 70-80% of thyroid malignancies
- No method to predict or diagnose early lymph node involvement in order to guide targeted treatment.
- Molecular mechanisms involved in PTC invasion and lymph node spread: 1) Epidermal growth factor receptors (EGFR) 2) Eph receptors and their Ephrin ligands
- Molecular crosstalk between EGFR and Eph signaling noted in HNSCC may play a role in PTC tumorigenesis and spread as well.

Our objective is to identify key molecular constituents in the pathogenesis and spread of PTC, so that these biomarkers may be used as early predictors of lymph node involvement and potential therapeutic targets.

MATERIALS & METHODS

Twenty-one (21) adult patients with PTC underwent total thyroidectomy and level VI nodal dissection at a tertiary-level hospital setting

cDNA Microarray
Four samples (stage III) of matched tumor and normal tissue were randomly selected. mRNA was isolated from fresh tissue sections by standard methods [6]. Human universal RNA was used as a common reference for all experiments. The mRNA was then processed and analyzed via GeneChip U133 Plus 2.0 array (Affymetrix Inc.) against 47,500 genes. Raw data was imported to microArray database (mAdb) and analyzed by software tools provided by the Center for Information Technology, NIH.

Western blot
Western blot was performed on all 21 samples of matched tumor and normal tissue. Cell lysates were prepared as previously described [7]. β-actin was used as a loading control, and was measured for all genes.

Immunohistochemistry
Immunohistochemistry (IHC) analysis was conducted on all 21 samples of matched tumor and normal tissue

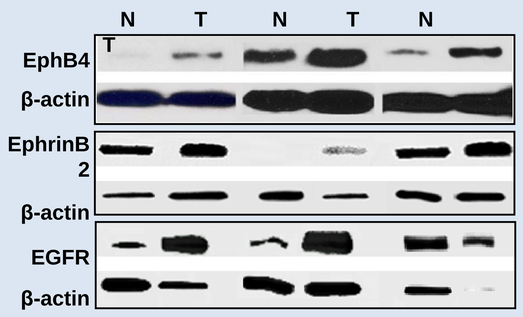

Figure 1. Western Blot of matched normal and tumor tissue (3 patients)

RESULTS

Patient Characteristics
- cDNA: differential expression of EphB4, EphrinB2, EGFR in tumor vs normal tissue (fold change ≥1.5)
- Western blot: EphrinB2, EphB4 and EGFR higher expression in tumor vs normal.
- IHC: EphB4 expression statistically significant association between tumor and LN invasion, ECS; EphrinB2 expression statistically significant association between tumor and LN invasion; EGFR expression statistically significant with clinical stage

Protein name	Gene Symbol	Fold change	*P*-value
Eph receptor B4	EPHB4	2.49	.004
Ephrin B2	EFNB2	2.42	.001
Epidermal growth factor receptor	EGFR	2.86	.003

Table 1. cDNA Microarray analysis. *P-value* from two-tailed *t*-test

Gene	Fold change (SE)	*P*-value
EphB4	-3.93 (0.14)	<.0001
EphrinB2	-3.66 (0.15)	<.0001
EGFR	-2.47 (0.07)	<.0001

Table 2. Immunohisto-chemistry analysis. log2 fold change of IOD data. *P-value* from Student's paired *t*-test (linear regression model adjusted for race).

Variable	EphB4		EphrinB2		EGFR	
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
Lymph node disease						
Yes	8436.96 (1558.66)	<.001	10429.02 (2429.49)	.001	3551.21 (1778.50)	.069
Extracapsular extension						
Yes	6591.78 (1232.23)	<.001	3784.57 (1943.52)	.071	1031.74 (1027.99)	.335
Neurovascular invasion						
Yes	-2032.74 (1853.41)	.294	-6201.15 (2587.12)	.033	66.26 (1583.73)	.967
Stage						
I-II	-2544.53 (1514.19)	.119	-868.21 (2383.10)	.722	-4113.91 (1054.0)	.001
III-IV	*Reference*					

Table 3. Multivariate linear regression analysis (IHC).

Character	Finding
Age, mean (range), y	46 (23-83)
Sex M F	 4 (19) 17 (81)
Tumor size, cm ≤1 1.1-2 2.1-4 >4	 5 (24) 8 (38) 6 (29) 2 (10)
Multifocal disease Yes No	 10 (48) 11 (52)
Lymph node involvement Yes No	 10 (48) 11 (52)
Extracapsular extension Yes No	 7(33) 14 (67)
Neurovascular invasion Yes No	 4 (19) 17 (81)
Clinical Stage I II III	 13 (62) 0 (0) 8 (38)

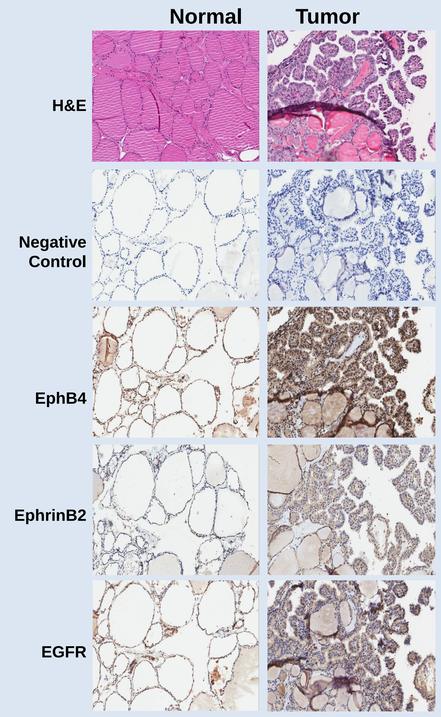

Figure 2. Immunohistochemistry analysis of PTC and matched normal thyroid tissue (x200 original magnification).

DISCUSSION

The precise mechanisms leading to PTC invasion and spread remain unknown.

EphB4, EphrinB2 and EGFR are involved in tumor angiogenesis

To our knowledge, this study is the first to demonstrate concurrent overexpression of EphB4, EphrinB2 and EGFR in PTC, and examine the relationship between differential expression of these biomarkers with PTC spread.

Activation of a multiplicity of downstream signaling cascades leading to nuclear gene transcription and, in the event of genetic mutation, uncontrolled cellular proliferation and invasion.
- EGFR-mediated upregulation of EphB4 signaling via activation of Akt in HNSCC [5].
- Akt pathway may play a role in EGF-mediated EphB4 upregulation in PTC.
- Molecular crosstalk and synergy that allows PTC cells to differentiate, and eventually migrate to regional lymph nodes.

Nodal involvement is an independent risk factor for tumor recurrence Prevention of recurrent disease in lymph nodes prevents revision surgery and morbidity in patients with PTC

CONCLUSION

- Elevated expression of EphB4, EphrinB2 and EGFR in PTC compared to normal tissue.
- Association between lymph node disease and EphB4 and EphrinB2 expression in tumor.
- Molecular markers involved in lymph node metastasis of PTC at a microscopic level; potential targets for directed therapy.

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