Journal of Cancer Research and Practice xxx (2017) 1–5

Contents lists available at ScienceDirect

Journal of Cancer Research and Practice

journal homepage: http://www.journals.elsevier.com/journal-of-cancer-research-and-practice

Characteristics of lymphocyte-infiltrating papillary thyroid cancer

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ARTICLE INFO

Article history:
Received 26 January 2017
Received in revised form
10 March 2017
Accepted 17 March 2017
Available online xxx

Keywords:
Lymphocyte
Papillary thyroid cancer
Survival
Immune
Differentiation

ABSTRACT

Background: The tumor-promoting or tumor-suppressing role of tumor-associated lymphocytes remains a subject of debate. We examined thyroid cancer data from The Cancer Genome Atlas in an attempt to define the relationship between lymphocyte infiltrates and clinical and molecular presentations.
Methods: Patient characteristics and transcriptome profiling were compared between groups dichotomized by the percentage of tumor-infiltrating lymphocytes of the primary tumor. Differentially expressed genes were subjected to functional enrichment analyses.
Results: In 52% of the tumors, there was no lymphocyte infiltration. Papillary thyroid cancer with infiltrating lymphocytes was associated with classical histologic features, multifocality, and lymph node metastasis. Patients with lymphocyte-infiltrating cancer had a longer overall survival duration (log-rank $P = 0.018$). A total of 3151 differentially expressed genes were identified. Pathways related to immune response were upregulated, where the expression of several thyroid-related genes was downregulated.
Conclusion: Papillary thyroid cancer with tumor-infiltrating lymphocytes is associated with an upregulation of immune response and cytokine production, along with a trend which suggests an overall survival benefit.

1. Introduction

Since Rudolf Virchow identified leukocytes in tumor tissues in 1863, inflammation has been intimately implicated in cancer development and progression. The presence of an inflammatory infiltrate in tumor tissue may represent inflammatory conditions preceding the development of malignancy. Studies have shown that an inflammatory milieu could contribute to proliferation and survival of malignant cells, sabotage adaptive immunity, and stimulate angiogenesis and metastasis.[1] On the other hand, inflammatory infiltrates may represent the host response to the tumor. Heterogeneous immune infiltrates have been shown in diverse tissue types and may portend an improved prognosis.[2]

Different types of tumor-associated inflammatory cells have been identified in thyroid cancer.[3] These consist of cells of the innate immune system (mainly macrophages, mast cells, and neutrophils) as well as cells associated with an adaptive immune response (T and B cells). In general, innate immune cells are more likely to have tumor-promoting properties.[4] Previously, we have found that thyroid cancer patients in the higher tertile of neutrophil-to-lymphocyte ratio had a significantly larger tumor size and a high recurrence risk.[5] In contrast, the role of adaptive immune cells is still a matter of debate. Several studies have shown that patients whose tumors are not infiltrated by lymphocytes present a high recurrence rate, suggesting that the presence of lymphocytes in the thyroid tumor microenvironment indicates a favorable prognosis.[6] On the contrary, other researchers reported that thyroid cancer with tumor-associated lymphocytes exhibited higher disease stage and increased incidence of invasion and lymph node metastasis.[7] Furthermore, the enrichment of $CD8^+$ lymphocytes has been associated with disease recurrence.[8]

Lymphocytic infiltrates in thyroid cancer may suggest a background of chronic lymphocytic thyroiditis. Thyroiditis is characterized by a cellular immune response with dense lymphocytic infiltration of the thyroid gland, as well as by a humoral immune response leading to the production of autoantibodies against thyroid antigens. Recently, a meta-analysis investigated the correlation between papillary thyroid cancer and histologically proven Hashimoto's thyroiditis.[9] The results revealed that thyroiditis was more

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Peer review under responsibility of The Chinese Oncology Society.

http://dx.doi.org/10.1016/j.jcrpr.2017.03.003

Please cite this article in press as: Kuo C-Y, et al., Characteristics of lymphocyte-infiltrating papillary thyroid cancer, Journal of Cancer Research and Practice (2017), http://dx.doi.org/10.1016/j.jcrpr.2017.03.003

frequently observed in papillary thyroid cancer than in benign thyroid diseases, and cancer patients with thyroiditis had a longer duration of recurrence-free survival. This seemingly paradoxical phenomenon deserves further investigation, because it may provide insight into the immune dysregulation involved in both disorders. In the present study, we examined the data of The Cancer Genome Atlas (TCGA) project in an attempt to define the relationship between lymphocyte infiltrates and clinical and molecular presentations.

2. Materials and methods

Patient characteristics and preprocessed gene expression data of patients with papillary thyroid cancer were downloaded from Genomic Data Commons (https://gdc.cancer.gov/) in August 2016.[10] The mRNA expression profile was obtained by Illumina HiSeq 2000 RNA sequencing version 2 level 3 data, and expressed as RNA-Seq by Expectation Maximization (RSEM) values.[11,12] The information on new tumor event, disease status, and patient mortality was updated to incorporate the latest follow-up. Patients who had missing transcriptome profiling or who lacked tumor-infiltrating lymphocyte data were excluded from the analysis. Finally, a total of 497 patients were included in the study.

The percentage of tumor-infiltrating lymphocytes of the primary tumor was significantly skewed, ranging from 0 to 30% (mean 1.5%, median 0%). There were no tumor-infiltrating lymphocytes in 257 (52%) of the cases. We used the X-tile software (Yale University, New Haven, CT, USA) to determine an optimal cut-off point.[13,14] For categorical variables, the chi-square test, Fisher's exact test, or the Cochran-Armitage trend test was used to determine the difference between groups.[15] For continuous variables, unpaired two-tailed Student's t-test was performed.[16] Overall survival and recurrence-free survival were compared using Kaplan-Meier curves and log-rank tests. The level of statistical significance was chosen as $P < 0.05$.

To identify transcriptome changes in association with lymphocyte infiltration, the gene expression profile of the tumors with tumor-infiltrating lymphocytes less or more frequent than the cut-off were thoroughly compared. Median RSEM values of all gene expression data obtained from RNA sequencing were calculated[10]; then, genes with a median value of less than 4.1374 (the first quartile) were excluded to avoid any variation caused by low-level expressions. The expression levels of remaining genes were compared one by one between the two groups using Wilcoxon rank sum tests. Genes with a P value of less than 0.01 (differentially expressed genes) were subjected to functional enrichment analyses. Spearman's rank correlation test was used for correlation studies.[17]

The Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation of differentially expressed genes was analyzed using Web-based Gene Set Analysis Toolkit (WebGestalt, http://www.webgestalt.org/) and the Database for Annotation, Visualization and Integration Discovery (DAVID, https://david.ncifcrf.gov/).[18] In WebGestalt, the minimum number of genes for enrichment was set at 5, and the significance analysis was performed using the hypergeometric test with the significance level set at $P = 0.01$. In DAVID, the parameters were set to their default values.

3. Results

In the present study, nearly one-half of the tumors were absent for lymphocyte infiltration. The cut-off of lymphocyte infiltration was determined at 1%. In total, 258 (52%) patients had tumor-infiltrating lymphocytes less than 1%, while 239 (48%) patients had tumor-infiltrating lymphocytes greater than or equal to 1%. The characteristics of the two groups are listed in Table 1. Papillary thyroid cancer with infiltrating lymphocytes ≥1% was associated with classical histologic features, multifocality, and lymph node metastasis. However, there was no difference in TNM stage or the frequency of *BRAF* mutation between the two groups. As shown in Fig. 1, papillary thyroid cancer with infiltrating lymphocytes ≥1% was associated with a better overall survival (log-rank $P = 0.018$), but there was no difference in recurrence-free survival (log-rank $P = 0.851$). Histological subtype did not influence overall survival (log-rank $P = 0.419$) or recurrence-free survival (log-rank $P = 0.274$).

There were 3151 differentially expressed genes identified from the transcriptome profiling data of 497 patients (Fig. 2). Among

Table 1
Clinicopathological features of 497 patients with papillary thyroid cancer grouped by the percentage of tumor-infiltrating lymphocytes.

	Lymphocyte <1% (n = 258)	Lymphocyte ≥ 1% (n = 239)	P value
Histology, n (%)			**0.001**
Classical	178 (69%)	176 (74%)	
Follicular variant	64 (25%)	34 (14%)	
Tall cell	15 (6%)	21 (9%)	
Others	1 (0%)	8 (3%)	
Female, n (%)	191 (74%)	172 (72%)	0.604
Family history, n (%)	15 (6%)	10 (4%)	0.406
Age (years)	48 ± 16	47 ± 16	0.577
Tumor size (cm)	2.9 ± 1.7	2.9 ± 1.7	0.868
Extrathyroidal extension, n (%)			0.339
None	179 (70%)	149 (66%)	
Minimal	67 (26%)	66 (29%)	
Advanced	9 (4%)	10 (4%)	
Multifocality, n (%)	105 (42%)	118 (50%)	**0.048**
Lymph node metastasis, n (%)	101 (39%)	121 (51%)	**0.010**
Distant metastasis, n (%)	7 (3%)	1 (0%)	0.070
TNM stage, n (%)			0.933
Stage I	141 (55%)	140 (59%)	
Stage II	38 (15%)	14 (6%)	
Stage III	53 (21%)	56 (24%)	
Stage IV	25 (10%)	28 (12%)	
BRAF mutation, n (%)	133 (52%)	119 (50%)	0.695

Data are expressed as number (percentage) or mean ± SD.
Bold-fond numbers indicate statistical significance ($P < 0.05$).

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