

Aging-associated genes *TNFRSF12A* and *CHI3L1* contribute to thyroid cancer: An evidence for the involvement of hypoxia as a driver

MENG LIAN^{1*}, HONGBAO CAO^{2-4*}, ANCHA BARANOVA^{4,5}, KAMIL CAN KURAL⁴,
LIZHEN HOU¹, SHIZHI HE¹, QING SHAO⁶ and JUGAO FANG¹

¹Department of Otorhinolaryngology Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing 100730; ²Department of Psychiatry, First Hospital/First Clinical Medical College of Shanxi Medical University, Taiyuan, Shanxi 030001, P.R. China; ³Department of Genomics Research, R&D Solutions, Elsevier Inc., Rockville, MD 20852; ⁴School of Systems Biology, George Mason University, Fairfax, VA 22030, USA; ⁵Research Centre for Medical Genetics, Moscow 115478, Russia; ⁶Department of Breast and Thyroid Surgery, Jiangyin People's Hospital, Jiangyin, Jiangsu 214400, P.R. China

Received March 26, 2019; Accepted August 16, 2019

DOI: 10.3892/ol.2020.11530

Abstract. The prevalence of thyroid cancer (TC) is high in the elderly. The present study was based on the hypothesis that genes, which have increased activity with aging, may play a role in the development of TC. A large-scale literature-based data analysis was conducted to explore the genes that are implicated in both TC and aging. Subsequently, a mega-analysis of 16 RNA expression datasets (1,222 samples: 439 healthy controls, and 783 patients with TC) was conducted to test a set of genes associated with aging but not TC. To uncover a possible link between these genes and TC, a functional pathway analysis was conducted, and the results were validated by analysis of gene co-expression. A multiple linear regression (MLR) model was employed to study the possible influence of sample size, population region and study age on the gene expression levels in TC. A total of 262 and 816 genes were identified to have increased activity with aging and TC, respectively; with a significant overlap of 63 genes ($P < 3.82 \times 10^{-35}$). The mega-analysis revealed two aging-associated genes (*CHI3L1* and *TNFRSF12A*) to be significantly associated with TC ($P < 2.05 \times 10^{-8}$), and identified the association with multiple hypoxia-driven pathways through functional pathway analysis, also confirmed by the co-expression analysis. The MLR analysis identified population region as a significant factor contributing to the

expression levels of *CHI3L1* and *TNFRSF12A* in TC samples ($P < 3.24 \times 10^{-4}$). The determination of genes that promote aging was warranted due to their possible involvement in TC. The present study suggests *CHI3L1* and *TNFRSF12A* as novel common risk genes associated with both aging and TC.

Introduction

Aging is a fundamental biological process accompanied by alterations in the regulatory activities performed by the endocrine system, including the thyroid gland (1). On the other hand, with aging, the prevalence of hypo- and hyperthyroidism increases (2). Both hypo- and hyperpituitarism are believed to affect longevity (3). Defining a physiological norm for the thyroid hormones is complicated in the elderly by the gradual resetting of the hypothalamic-pituitary-thyroid axis, which leads to increased levels of thyroid stimulating hormone (4).

The prevalence of thyroid neoplasms is increased in the elderly; these tumors are more aggressive in men than in women (5). The mortality rate of thyroid cancer (TC) gradually increases with age, from the ages of 40-45 (6). Notably, young survivors of TC have increased risks for other aging-related diseases, including diabetes, disorders of lipid metabolism, eye disorders, ear conditions and diseases of the musculoskeletal system and connective tissue (7). A majority of TC arise from the epithelial elements of the gland, including thyrocytes and follicular cells. The classification of thyroid tumors into two major groups, differentiated (including papillary, follicular, and medullary) or undifferentiated (anaplastic) carcinoma, based on clinical features and morphology was supported by advances in molecular studies (8).

Importantly, a recent study on DNA methylation and histone modification patterns in >2,000 tumors collected from patients of various ages revealed that most tumor types did not demonstrate age-associated changes in DNA methylation (9), which is in agreement with the theory that the epigenetic clock in cancer cells is reprogrammed (9). Remarkably, this

Correspondence to: Dr Qing Shao, Department of Breast and Thyroid Surgery, Jiangyin People's Hospital, 163 Shoushan Road, Jiangyin, Jiangsu 214400, P.R. China
E-mail: q.shao@gousinfo.com

*Contributed equally

Key words: thyroid cancer, aging, mega-analysis, multiple linear regression model, pathway analysis

theory does not hold for thyroid tumors, which displayed age-associated differential methylation of CpGs (9). In fact, age at diagnosis serves as a strong indicator of prognosis in TC, particularly in well-differentiated tumors (10,11), with an order of magnitude difference in hazard of death from cancer between the youngest and oldest cohorts. It is also of note that thyroid tissue displays the lowest level of DNA methylation changes in cancer and one of the lowest with aging (9).

Overall, previous studies indicate the presence of an association between thyroid carcinogenesis and aging. The present study explored whether genes that promote aging may also play a role in the development of TC. Therefore, besides the identification of previously identified common genes, those that are known to be associated with aging but not with TC are worthy of further study. A mega-analysis of thyroid cancer datasets obtained from Gene Expression Omnibus (GEO) identified two genes, *TNFRSF12A* and *CHI3LI*, as likely contributors to both the process of aging and the thyroid carcinogenesis.

Materials and methods

Study workflow. The workflow was organized as follows. Firstly, the large-scale literature-based mining effort for thyroid cancer (TC)- and aging-associated gene sets was undertaken; these gene sets were compared. For each gene from the list implicated in aging alone, a mega-analysis was conducted in 16 publicly available expression datasets retrieved from GEO. For genes that showed significant change in expression across analyzed datasets, functional pathway analysis and protein-protein interaction (PPI) by co-expression analysis were conducted, then conclusions on their pathogenic significance in TC were made.

Literature-based relation data. Relation data for both aging and TC were extracted and analyzed using Pathway Studio (www.pathwaystudio.com), and the results were downloaded into a genetic database Aging_TC, hosted at <http://database.gousinfo.com>. The downloadable format of the database was available at http://gousinfo.com/database/Data_Genetic/Aging_TC.xlsx, with different section of the results presented in different worksheets (e.g., age-specific genes were presented in worksheet 'Aging_alone genes'). In this study, we referred to these worksheets in the form of Aging_TC→'worksheet name'. Beside the list of analyzed genes (Aging_TC→Aging_alone genes, Aging_TC→TC_alone genes, and Aging_TC→Common genes), supporting references for each disease-gene relation are presented (Aging_TC→Ref for Aging_alone genes, Aging_TC→Ref for TC_alone genes, and Aging_TC→Ref for Common genes), including titles of the references and the sentences describing identified disease-gene relationships. The information could be used to locate a detailed description of an association of a candidate gene with aging and/or TC. Please refer to Aging_TC→DataNote for the decryption of each worksheet.

Data selection for mega-analysis. The relevant expression datasets available at GEO (<https://www.ncbi.nlm.nih.gov/geo/>) were retrieved with the keyword 'thyroid cancer' (n=91). The following criteria were applied: i) Organism, *Homo sapiens*; ii) data type, RNA expression; iii) sample

size, ≥10; and iv) the studies were performed according to case-control design. A total of 16 datasets remained available for the mega-analysis.

Mega-analysis models. To discern the effect sizes of the selected genes in a case vs. control expression comparison, both fixed-effect and random-effects models were employed. The expression log fold change (LFC) was used as the effect size. The results derived from both models were compared. In order to assess the variance within and between different studies, the heterogeneity of the mega-analysis was analyzed. In the case that total variance Q was equal to or smaller than the expected between-study variance df, the statistic $ISq=100\% \times (Q-df)/Q$ was set as 0, and a fixed-effect model was selected for the mega-analysis. Otherwise, a random-effects model was selected. The genes with significant effects were identified according to the following criteria: $P < 1 \times 10^{-7}$ and effect size (LFC) >1 or <-1.

Multiple linear regression analysis. A multiple linear regression (MLR) model was employed to study the possible influence of three factors on the changes in gene expression in TC: Sample size, population region, and study age. P-values and 95% confidence interval (CI) were reported for each of the factors.

Pathway analysis. For the set of genes identified through expression mega-analysis described above, a functional pathway analysis was conducted with an aim to identify potential biological associations between the selected genes and the TC. The analysis was performed using the 'Shortest Path' module of Pathway Studio (www.pathwaystudio.com).

Co-expression analysis. For each pair of the genes/proteins identified in the pathway analysis, a study of their co-expression was executed. The purpose of this analysis was to identify possible protein-protein interaction (PPI) of the proteins involved. The Fisher's Z was employed as the effect size for the mega-analysis, as shown in the equation below:

$$\text{Fisher Z} = 0.5 \times \log \left(\frac{1 + \text{Correlation}}{1 - \text{Correlation}} \right) \quad (1)$$

All co-expression associations with P-value $< 1 \times 10^{-4}$ and Fisher's Z-value ≥ 0.3 or ≤ -0.3 were identified as significant, and then presented as a Cytoscape-imaged PPI network.

Results

Genes commonly affected by the process of aging and by the carcinogenesis in the thyroid gland. The curated Aging_TC database identified 262 genes with substantially increased expression or activity with aging (supported by 1,495 scientific references) and 816 genes associated with the pathogenesis of TC (supported by 4,169 references). A total of 63 genes were identified to be involved in both aging and TC (right tail Fisher's exact test, $P=3.82 \times 10^{-35}$; Fig. S1), which accounts for about a quarter of the genes associated with aging (24.05%). The descriptions of these 63 genes are presented in Table I. Further information on these genes was also provided in

Table I. Common genes associated with aging and thyroid cancer.

| Name | Entrez Gene ID | Human chromosome position |
|-----------------|---|---------------------------|
| <i>SERPINC1</i> | 462;304917;11905 | 1q25.1 |
| <i>IL17A</i> | 16171;301289;3605 | 6p12.2;6p12 |
| <i>EDN1</i> | 1906;24323;13614 | 6p24.1 |
| <i>LCN2</i> | 16819;170496;3934 | 9q34.11;9q34 |
| <i>PTGS2</i> | 19225;29527;5743 | 1q31.1;1q25.2-q25.3 |
| <i>HLA-G</i> | 24747;14991;3135 | 6p21.3;6p22.1 |
| <i>CSF1</i> | 1435;12977;78965 | 1p13.3 |
| <i>MIF</i> | 4282;17319;81683 | 22q11.23 |
| <i>PTH</i> | 19226;24694;5741 | 11p15.3;11p15.3-p15.1 |
| <i>CYP27B1</i> | 1594;13115;114700 | 12q14.1 |
| <i>CYP24A1</i> | 25279;1591;13081 | 20q13;20q13.2 |
| <i>HUWE1</i> | 59026;10075 | Xp11.22 |
| <i>EIF2AK2</i> | 5610;54287;19106 | 2p22-p21;2p22.2 |
| <i>PDGFRB</i> | 5159;24629;18596 | 5q32;5q33.1 |
| <i>APP</i> | 54226;351;11820 | 21q21.3 |
| <i>HYOU1</i> | 10525;12282;192235 | 11q23.1-q23.3;11q23.3 |
| <i>EGF</i> | 25313;1950;13645 | 4q25 |
| <i>TNF</i> | 24835;21926;7124 | 6p21.3;6p21.33 |
| <i>HMOX1</i> | 15368;3162;24451 | 22q12.3;22q13.1 |
| <i>MIR21</i> | 406991 | 17q23.1 |
| <i>MMP9</i> | 81687;17395;4318 | 20q11.2-q13.1;20q13.12 |
| <i>FAS</i> | 246097;14102;355 | 10q23.31;10q24.1 |
| <i>DPP4</i> | 25253;1803;13482 | 2q24.3;2q24.2 |
| <i>KLK3</i> | 18048;18050;354;13648;16619;16618;16613;16624;16612;16623;16622; 13646;16617;16616;16615 | 19q13.33;19q13.41 |
| <i>PROS1</i> | 19128;81750;5627 | 3q11.2;3q11.1 |
| <i>FASLG</i> | 14103;356;25385 | 1q23;1q24.3 |
| <i>GPX3</i> | 2878;64317;14778 | 5q23;5q33.1 |
| <i>ALB</i> | 11657;213;24186 | 4q13.3 |
| <i>DCN</i> | 1634;13179;29139 | 12q21.33 |
| <i>B3GAT1</i> | 27087;76898;117108;964 | 11q25 |
| <i>ADIPOQ</i> | 9370;246253;11450 | 3q27;3q27.3 |
| <i>ARG2</i> | 11847;29215;384 | 14q24.1 |
| <i>RUNX3</i> | 12399;156726;864 | 1p36.11;1p36 |
| <i>PRDX1</i> | 5052;18477;100363379;117254 | 1p34.1 |
| <i>HTRA1</i> | 5654;65164;56213 | 10q26.13;10q26.3 |
| <i>RCAN1</i> | 54720;1827 | 21q22.12 |
| <i>CTNNB1</i> | 84353;12387;1499 | 3p21;3p22.1 |
| <i>BAG3</i> | 9531;29810;293524 | 10q25.2-q26.2;10q26.11 |
| <i>TGFB1</i> | 21803;7040;59086 | 19q13.1;19q13.2 |
| <i>TGFA</i> | 24827;21802;7039 | 2p13;2p13.3 |
| <i>CTSD</i> | 1509;171293;13033 | 11p15.5 |
| <i>MUC1</i> | 4169;4582 | 1q21;1q22 |
| <i>TRAP1</i> | 68015;10131;287069 | 16p13.3 |
| <i>CDKN1A</i> | 12575;114851;1026 | 6p21.2 |
| <i>CCNA2</i> | 12428;114494;890 | 4q27 |
| <i>CCR2</i> | 1231;12772;729230 | 3p21.31 |
| <i>IL21</i> | 59067;365769;60505 | 4q26-q27;4q27 |
| <i>BAX</i> | 12028;24887;581 | 19q13.33;19q13.3-q13.4 |
| <i>IL4</i> | 287287;3565;16189 | 5q31.1 |
| <i>ENO1</i> | 2023;24333;433182;13806 | 1p36.23;1p36.2 |
| <i>HGF</i> | 3082;15234;24446 | 7q21.11;7q21.1 |

Table I. Continued.

| Name | Entrez Gene ID | Human chromosome position |
|-----------------|--------------------|---------------------------|
| <i>TNFRSF10</i> | 246775;22035;8743 | 3q26.31;3q26 |
| <i>IL6</i> | 24498;3569;16193 | 7p15.3;7p21 |
| <i>THRA</i> | 7067;81812;21833 | 17q11.2;17q21.1 |
| <i>ANGPT2</i> | 89805;11601;285 | 8p23.1 |
| <i>EP300</i> | 170915;2033;328572 | 22q13.2 |
| <i>GSTM1</i> | 2944;14863;24424 | 1p13.3 |
| <i>CCL2</i> | 287562;6347;20293 | 17q12;17q11.2-q12 |
| <i>TXNIP</i> | 117514;56338;10628 | 1q21.1 |
| <i>GSTT1</i> | 2952 | 22q11.23 |
| <i>POSTN</i> | 50706;361945;10631 | 13q13.3 |
| <i>NQO1</i> | 18104;1728;24314 | 16q22.1 |
| <i>IL10</i> | 16153;25325;3586 | 1q31-q32;1q32.1 |

Aging_TC→Common genes and Aging_TC→Ref for common genes.

In order to assess the functional profile of the 63 genes associated with both aging and TC, a gene set enrichment analysis (GSEA) against the GO and Pathway Studio Ontology was conducted. The GSEA showed that these common genes were mainly involved in the protein kinase domain, cell proliferation, tissue development, gland development and response to hormone processes. Specifically, this analysis uncovered a total of three pathways/gene sets associated with cell apoptosis (26 unique genes) and 2 pathways/gene sets associated with cell growth proliferation (25 unique genes). A bar plot of the 39 pathways and enriched genes out of the 63 common genes are presented in Fig. S2.

Gene expression analysis result. Although there was a significant overlap between aging- and TC-associated gene sets ($n=63$; $P=3.82 \times 10^{-35}$), a majority of the aging-associated genes ($n=199$ or 75.95%) have not been yet implicated in the pathogenesis of TC. Therefore, the association between each of these 199 genes with TC was examined, using 16 gene expression datasets shown in Table II (12-26). The detailed description of the results is presented in Aging_TC→Mega-analysis. The significance of association criteria ($P < 1 \times 10^{-7}$ and absolute LFC > 1) was met by two genes and presented in Table III.

For each gene, a LFC was estimated from the majority of the 16 studies (a total of 16 and 14 studies for *CHI3L1* and *TNFRSF12A*, respectively). As shown in Fig. 1, the mRNA expression levels of *TNFRSF12A* demonstrated strong between-study variances (ISq=54.07% and Q test $P=8.2 \times 10^{-3}$); therefore, the random-effects model was selected for the mega-analysis. In contrast, no significant between-study variance was observed for the gene *CHI3L1* (ISq=14.73% and Q test $P > 0.28$); therefore, the fixed-effect model was selected for analysis of its mRNA expression levels. This identified the sample population region (country) as a significant factor that influences the LFC of both genes in the case of TC ($P < 3.24 \times 10^{-4}$; Table III).

Functional pathway analysis. According to the *de novo* approach selected for the identification of novel TC-associated

genes, no prior direct link to the pathogenesis of TC were known. However, Pathway Studio-guided 'shortest path analysis' revealed plausible associations of *CHI3L1* and *TNFRSF12A* genes with TC, with a set of common interactions (Fig. 2A).

Using Pathway Studio, the 'shortest path' analysis was conducted to identify associated genes that link *CHI3L1* and *TNFRSF12A*-encoded molecules to the pathogenesis of TC in a unidirectional way. For example, the association between *CHI3L1* and transforming growth factor $\beta 1$ (TGF $\beta 1$) in TC was identified. YKL-40, also known as chitinase-3-like protein 1 (*CHI3L1*), is a secreted glycoprotein that binds to interleukin-13 receptor $\alpha 2$ (IL-13R $\alpha 2$) and subsequently stimulates the production of TGF- $\beta 1$ (27), a key molecule in thyroid carcinogenesis and considered as a new prognostic and therapeutic target for TC (28,29). The details for all other associations are presented in Fig. 2A, and are described in Aging_TC→TC-2Genes_potential pathways. This reference information included the type of association, the amount of underlying supporting references, and the relevant sentences where these relationships were identified and described. The shortest pathway analysis was conducted using the Pathway Studio (www.pathwaystudio.com). All TC-associated genes reported in the shortest pathway analysis were employed in the identification of the TC-associated genes.

In order to confirm the associations depicted in Fig. 2A, another mega-analysis was conducted using 16 datasets, with the purpose to evaluate the co-expression pattern of mRNAs encoded by *CHI3L1* and *TNFRSF12A*, and 31 genes that link the two genes with TC. The association between *CHI3L1* and *TNFRSF12A* and 13 of the 31 genes presented in Fig. 2A was validated ($P < 0.005$; Fig. 2B). Please refer to Aging_TC→MetaResults_ShortestPath for the detailed description of the associations presented in Fig. 2B. In addition, a PPI network connecting the products of *CHI3L1* and *TNFRSF12A* and the 'bridge' genes ($n=31$) were generated using Cytoscape (Fig. 3). The detailed information of the mega-analysis for the co-expression was presented in Aging-TC→PPI, including a list of the genes (nodes) of the PPI network, and the mega-analysis results for each pair of gene-gene correlation.

Table II. Datasets used for thyroid cancer-aging relation mega-analysis.

| Study | Dataset GEO ID | Control, n | Case, n | Country | Refs. |
|-----------------------------------|----------------|------------|---------|-----------|---------|
| Jarzab <i>et al</i> , 2015 | GSE35570 | 51 | 65 | Poland | (12) |
| Rusinek <i>et al</i> , 2015 | GSE58545 | 18 | 27 | Poland | (13) |
| Swierniak <i>et al</i> , 2015 | GSE58689 | 18 | 27 | Poland | (13) |
| Tarabichi <i>et al</i> , 2015 | GSE60542 | 34 | 33 | Belgium | (14) |
| von Roemeling <i>et al</i> , 2015 | GSE65144 | 13 | 12 | USA | (15) |
| Versteyhe <i>et al</i> , 2013 | GSE39156 | 16 | 48 | Belgium | (16) |
| Pita <i>et al</i> , 2013 | GSE53157 | 3 | 24 | Portugal | (17) |
| Tomas <i>et al</i> , 2012 | GSE29265 | 20 | 29 | Belgium | N/A |
| Tomas <i>et al</i> , 2012 | GSE33630 | 45 | 60 | Belgium | (18,19) |
| Giordano <i>et al</i> , 2011 | GSE27155 | 4 | 95 | USA | (20,21) |
| Yu <i>et al</i> , 2008 | GSE5364 | 58 | 270 | Singapore | (22) |
| Fontaine <i>et al</i> , 2007 | GSE6339 | 135 | 48 | France | (23) |
| Salvatore <i>et al</i> , 2007 | GSE9115 | 4 | 15 | USA | (24) |
| Reyes <i>et al</i> , 2006 | GSE3678 | 7 | 7 | USA | N/A |
| Vasko <i>et al</i> , 2006 | GSE6004 | 4 | 14 | USA | (25) |
| Liyanarachchi <i>et al</i> , 2005 | GSE3467 | 9 | 9 | USA | (26) |

N/A, not applicable.

Table III. Significant genes from mega-analysis [$P < 1 \times 10^{-7}$ and $\text{abs(LFC)} > 1$] involved in aging and thyroid cancer.

| Gene name | Mega-analysis results | | | | | Multiple linear regression analysis for three factors | | |
|------------------|-----------------------|-------------------|------|------------|------------------------|---|-----------------------|---------------|
| | Random effects model | Datasets included | LFC | STD of LFC | P-value | Sample size | Population region | Year of study |
| <i>CHI3L1</i> | 0 | 16 | 2.88 | 0.42 | 5.10×10^{-12} | 0.24 | 2.77×10^{-4} | 0.15 |
| <i>TNFRSF12A</i> | 1 | 14 | 1.79 | 0.33 | 2.05×10^{-8} | 0.35 | 3.24×10^{-4} | 0.56 |

LFC, log fold change. STD, standard deviation.

Discussion

The present study aimed to identify novel molecular pathways, which connect the process of aging and the development of TC. By removing all known associations between curated sets of genes involved in aging and TC, uncovered aging-associated contributors to TC were identified. According to the pre-selected significance of association criteria ($P < 1 \times 10^{-7}$ and $\text{abs(LFC)} > 1$), two aging-associated genes, *TNFRSF12A* and *CHI3L1*, were found to be involved in the development of TC.

The role of *TNFRSF12A* and *CHI3L1* in the aging-associated disease is well described, whereas no apparent connections to the malignant processes in the thyroid were reported thus far. *TNFRSF12A* encodes for an exclusive receptor for tumor necrosis factor-related weak inducer of apoptosis (*TWEAK*), an interacting pair of molecules involved

in age-associated pathological changes in skeletal muscle and other organs (30-32). *CHI3L1* encodes for YKL-40, a glycoprotein upregulated in a variety of inflammatory conditions commonly found in the elderly, including chronic obstructive pulmonary disease and neurodegenerative diseases (33,34).

Employing a Pathway Studio-guided 'shortest path analysis' revealed plausible associations of *TNFRSF12A* and *CHI3L1* with TC, simultaneously highlighting a set of common interactors that included the signaling molecules *AKT1*, *RAC1*, *MAPK1* and the soluble proteins *TNF- α* , albumin, *TIMPI* and *MMP9*.

Among these, the association of *TNFRSF12A* and *CHI3L1* with *AKT1* was notable, as this molecule was both overexpressed and over-activated in TC (35). Moreover, increased Akt signaling led to increased Bcl-2 promoter activity and cell survival (36). The interaction between *TNFRSF12A* and *TWEAK* also positively regulated pro-survival molecules

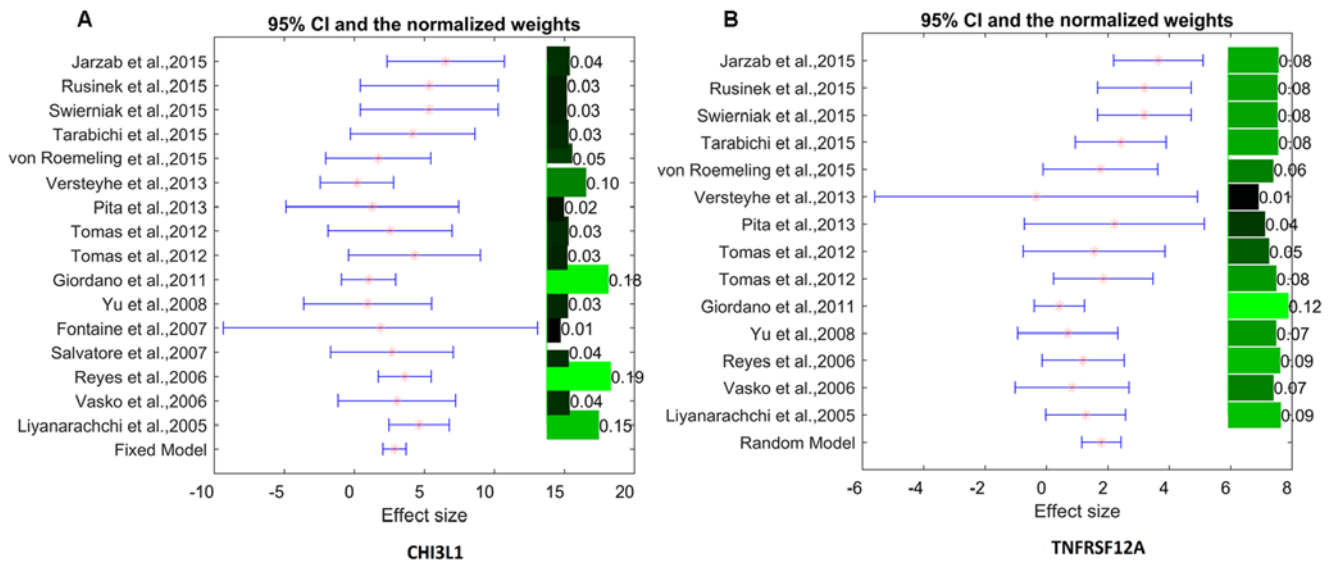


Figure 1. Effect size, 95% CI and weights of the expression levels of *TNFRSF12A* and *CHI3L1*. (A) Mega-analysis results for *CHI3L1*; (B) Mega-analysis results for *TNFRSF12A*. Fixed-effect model and random-effects model were employed for *CHI3L1* and *TNFRSF12A*, respectively. CI, confidence interval.

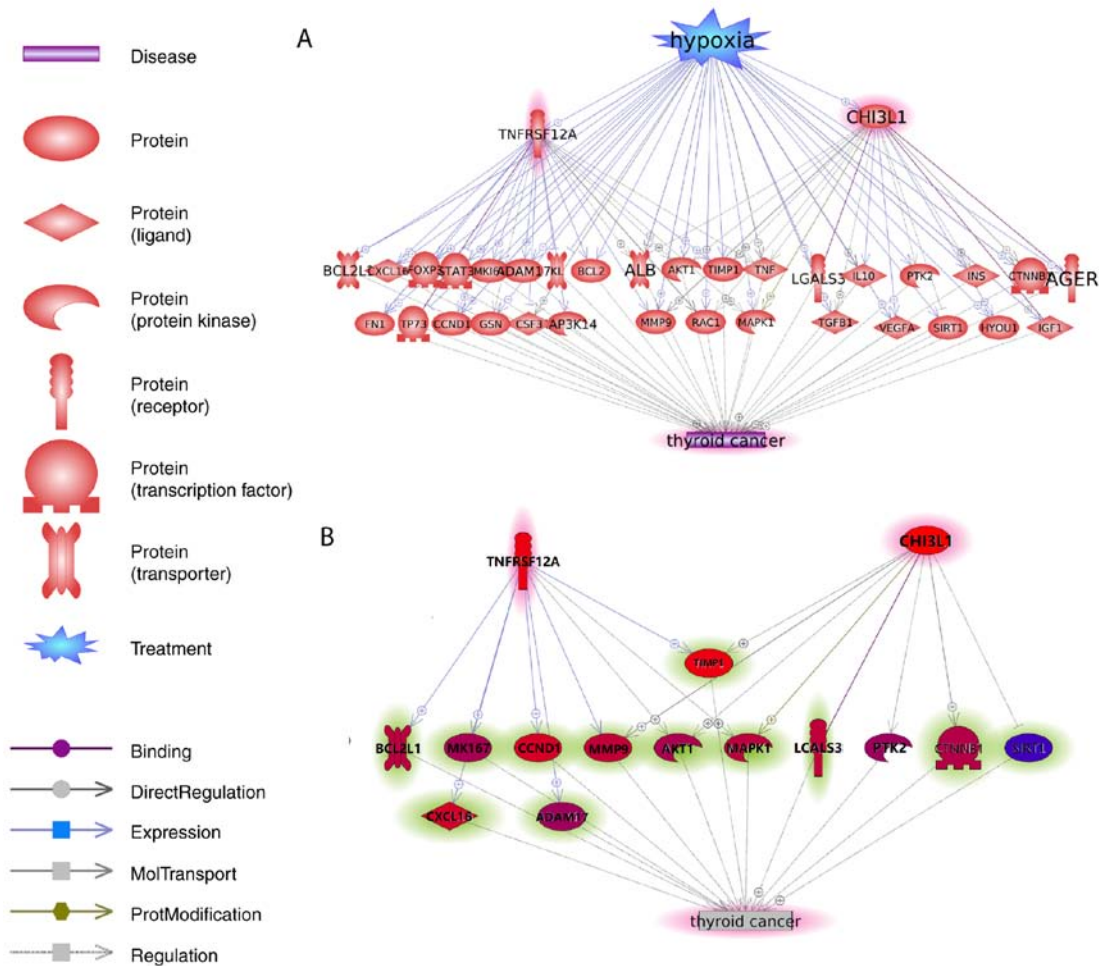


Figure 2. Hypoxia driven interaction network that links *CHI3L1* and *TNFRSF12A* to thyroid cancer. (A) The network generated in Pathway Studio environment (www.pathwaystudio.com). Each relation (edge) in the figure has one or more supporting references. (B) Network containing only protein-protein interactions validated by 14 thyroid cancer expression datasets.

of the *Bcl2* family (37,38), and, therefore augmented the pro-survival signal of *AKT1* (39).

Among the soluble molecules, angiogenic matrix metalloproteinase (*MMP*)9 was found to be associated with

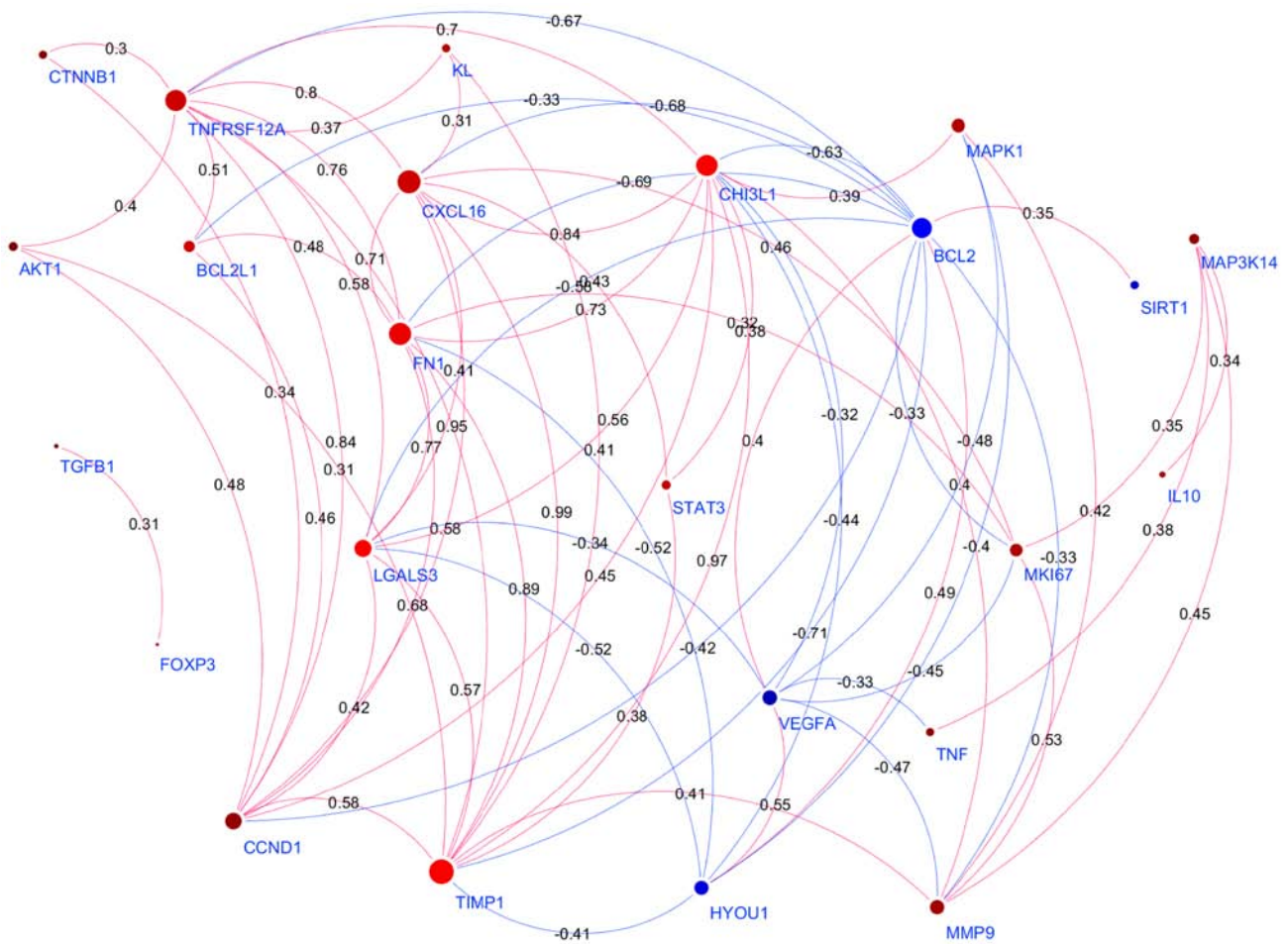


Figure 3. Protein-protein interaction network generated in Cytoscape for *CHI3L1* and *TNFRSF12A* and the 'bridge' genes. Each edge represents a significant association ($P < 1 \times 10^{-4}$) between the mRNA expression levels encoded by *CHI3L1* and *TNFRSF12A*. Positive associations are highlighted in red; negative associations are highlighted in blue. A red node represents an overall positive association within the network, whereas a blue node represents an overall negative association. The size of the node represents the centrality of the respective protein. The larger the size, the higher the centrality of the protein. The labels on the edge represent the correction coefficients in terms of Fisher's Z-value as derived from the mega-analysis.

TNFRSF12A and *CHI3L1*, which participates in follicular TC cell invasion (40). *TIMP-1*, acts as an inhibitor for *MMP9* expression that is often co-expressed with this molecule in thyroid tumors and serves as a reliable surrogate marker for BRAF-mutated status and likely aggressiveness (41). Notably, *TIMP-1* serves as a prominent PPI hub gene in the Cytoscape network built upon co-expression of *TNFRSF12A* and *CHI3L1*, along with *LGALS3*, a specific biomarker of well-differentiated thyroid carcinomas (42), *CXCL16*, which mediated the involvement of macrophages in the invasion of papillary TCs (43) and fibronectin, an EMT biomarker which promoted migration and invasion of papillary thyroid cancers (44).

Importantly, many of the molecules discussed above were upregulated in response to hypoxia (45), or stimulated the expression of key mediators of the antihypoxic response (46), or both (47,48). Both *HIF-1a* and *HIF-2a* were reported to be expressed in TC (49). Moreover, a growing evidence points out that hypoxia plays a significant role in the maintenance of thyroid cancer stem cells (CSC) (50). General hypoxia due to diminished vascularization is a well-known characteristic of aging tissues, possibly due to endothelial dysfunction as well decreased function of endothelial progenitor cell function (51,52). For more information regarding the hypoxia-gene

regulation presented in Fig. 2A, please refer to Aging_TC→TC_2Genes_potential pathway. Therefore, it can be speculated that the age-dependent increase in hypoxia may also contribute to the increased aggressiveness of TC observed in the elderly (10,11).

The results could be influenced by multiple factors, including sample size and sample source. Considering the fact that the pathology of disease could change with time, the publication date/age was checked in the present study. The MLR analysis showed that country region was a significant factor that could influence the gene expression levels of TC genes, but not the other two factors. Other influencing factors of TC gene activities could include mortality or epigenetics. However, due to be the limitation of the metadata, these factors were not amiable for analysis in the datasets employed in the present study, which would be valuable for future studies.

In conclusion, the present study conducted a functional pathway and co-expression analysis, and mined a set of genes associated with aging. *TNFRSF12A* and *CHI3L1* were identified as previously unrecognized contributors to the development of thyroid tumors, which are known for unusual increase in aggressiveness in the elderly. An analysis of the

Cytoscape network built upon co-expression of *TNFRSF12A* and *CHI3L1* points towards tissue hypoxia as a bridging factor, which is common for the pathophysiology of aging and the development of TC.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Authors' contributions

ML, HC, AB and QS developed the study design, analyzed the data, and wrote the original manuscript. KCK, LH, SH and JF contributed to data analysis and manuscript writing and revision. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Da Costa VM and Rosenthal D: Effects of aging on thyroidal function and proliferation. *Curr Aging Sci* 1: 101-104, 2008.
- Cooper DS: Thyroid disease in the oldest old: The exception to the rule. *JAMA* 292: 2651-2654, 2004.
- Jones CM and Boelaert K: The endocrinology of aging: A mini-review. *Gerontology* 61: 291-300, 2015.
- Pasqualetti G, Caraccio N, Dell'Agnetto U and Monzani F: Cognitive function and the aging process: The peculiar role of mild thyroid failure. *Recent Pat Endocr Metab Immune Drug Discov* 10: 4-10, 2016.
- Rukhman N and Silverberg A: Thyroid cancer in older men. *Aging Male* 14: 91-98, 2011.
- Mazzaferrri EL and Kloos RT: Current approaches to primary therapy for papillary and follicular thyroid cancer. *J Clin Endocrinol Metab* 86: 1447-1463, 2001.
- Blackburn BE, Ganz PA, Rowe K, Snyder J, Wan Y, Deshmukh V, Newman M, Fraser A, Smith K, Herget K, *et al*: Aging-related disease risks among young thyroid cancer survivors. *Cancer Epidemiol Biomarkers Prev* 26: 1695-1704, 2017.
- De Lellis RA, Lloyd RV, Heitz PU and Eng C: World Health Organization classification of tumours. Pathology and genetics of tumours of endocrine organs. IARC Press, Lyon, France, 2004.
- Pérez RF, Tejedor JR, Bayón GF, Fernández AF and Fraga MF: Distinct chromatin signatures of DNA hypomethylation in aging and cancer. *Aging Cell* 17: e12744, 2018.
- Krook KA, Fedewa SA and Chen AY: Prognostic indicators in well-differentiated thyroid carcinoma when controlling for stage and treatment. *Laryngoscope* 125: 1021-1027, 2015.
- Ganly I, Nixon IJ, Wang LY, Palmer FL, Migliacci JC, Aniss A, Sywak M, Eskander AE, Freeman JL, Campbell MJ, *et al*: Survival from differentiated thyroid cancer: What has age got to do with it? *Thyroid* 25: 1106-1114, 2015.
- Handkiewicz-Junak D, Swierniak M, Rusinek D, Oczko-Wojciechowska M, Dom G, Maenhaut C, Unger K, Detours V, Bogdanova T, Thomas G, *et al*: Gene signature of the post-Chernobyl papillary thyroid cancer. *Eur J Nucl Med Mol Imaging* 43: 1267-1277, 2016.
- Rusinek D, Swierniak M, Chmielik E, Kowal M, Kowalska M, Cyplinska R, Czarniecka A, Piglowski W, Korfanty J, Chekan M, *et al*: BRAFV600E-associated gene expression profile: Early changes in the transcriptome, based on a transgenic mouse model of papillary thyroid carcinoma. *PLoS One* 10: e0143688, 2015.
- Tarabichi M, Saiselet M, Trésallet C, Hoang C, Larsimont D, Andry G, Maenhaut C and Detours V: Revisiting the transcriptional analysis of primary tumours and associated nodal metastases with enhanced biological and statistical controls: application to thyroid cancer. *Br J Cancer* 112: 1665-1674, 2015.
- von Roemeling CA, Marlow LA, Pinkerton AB, Crist A, Miller J, Tun HW, Smallridge RC and Copland JA: Aberrant lipid metabolism in anaplastic thyroid carcinoma reveals stearoyl CoA desaturase 1 as a novel therapeutic target. *J Clin Endocrinol Metab* 100: E697-E709, 2015.
- Versteijhe S, Driessens N, Ghaddab C, Tarabichi M, Hoste C, Dumont JE, Miot F, Corvilain B and Detours V: Comparative analysis of the thyrocytes and T cells: responses to H₂O₂ and radiation reveals an H₂O₂-induced antioxidant transcriptional program in thyrocytes. *J Clin Endocrinol Metab* 98: E1645-E1654, 2013.
- Pita JM, Banito A, Cavaco BM and Leite V: Gene expression profiling associated with the progression to poorly differentiated thyroid carcinomas. *Br J Cancer* 101: 1782-1791, 2009.
- Dom G, Tarabichi M, Unger K, Thomas G, Oczko-Wojciechowska M, Bogdanova T, Jarzab B, Dumont JE, Detours V and Maenhaut C: A gene expression signature distinguishes normal tissues of sporadic and radiation-induced papillary thyroid carcinomas. *Br J Cancer* 107: 994-1000, 2012.
- Tomás G, Tarabichi M, Gacquer D, Hébrant A, Dom G, Dumont JE, Keutgen X, Fahey TJ III, Maenhaut C and Detours V: A general method to derive robust organ-specific gene expression-based differentiation indices: application to thyroid cancer diagnostic. *Oncogene* 31: 4490-4498, 2012.
- Giordano TJ, Au AY, Kuick R, Thomas DG, Rhodes DR, Wilhelm KG Jr, Vinco M, Misek DE, Sanders D, Zhu Z, *et al*: Delineation, functional validation, and bioinformatic evaluation of gene expression in thyroid follicular carcinomas with the PAX8-PPARG translocation. *Clin Cancer Res* 12: 1983-1993, 2006.
- Giordano TJ, Kuick R, Thomas DG, Misek DE, Vinco M, Sanders D, Zhu Z, Ciampi R, Roh M, Shedden K, *et al*: Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. *Oncogene* 24: 6646-6656, 2005.
- Yu K, Ganesan K, Tan LK, Laban M, Wu J, Zhao XD, Li H, Leung CH, Zhu Y, Wei CL, *et al*: A precisely regulated gene expression cassette potentially modulates metastasis and survival in multiple solid cancers. *PLoS Genet* 4: e1000129, 2008.
- Fontaine JF, Mirebeau-Prunier D, Franc B, Triau S, Rodien P, Houlgatte R, Malthièry Y and Savagner F: Microarray analysis refines classification of non-medullary thyroid tumours of uncertain malignancy. *Oncogene* 27: 2228-2236, 2008.
- Salvatore G, Nappi TC, Salerno P, Jiang Y, Garbi C, Ugolini C, Miccoli P, Basolo F, Castellone MD, Cirafici AM, *et al*: A cell proliferation and chromosomal instability signature in anaplastic thyroid carcinoma. *Cancer Res* 67: 10148-10158, 2007.
- Vasko V, Espinosa AV, Scouten W, He H, Auer H, Liyanarachchi S, Larin A, Savchenko V, Francis GL, de la Chapelle A, *et al*: Gene expression and functional evidence of epithelial-to-mesenchymal transition in papillary thyroid carcinoma invasion. *Proc Natl Acad Sci USA* 104: 2803-2808, 2007.
- He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, *et al*: The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci USA* 102: 19075-19080, 2005.

27. He CH, Lee CG, Dela Cruz CS, Lee CM, Zhou Y, Ahangari F, Ma B, Herzog EL, Rosenberg SA, Li Y, *et al*: Chitinase 3-like 1 regulates cellular and tissue responses via IL-13 receptor $\alpha 2$. *Cell Rep* 4: 830-841, 2013.
28. Pisarev MA, Thomasz L and Juvenal GJ: Role of transforming growth factor beta in the regulation of thyroid function and growth. *Thyroid* 19: 881-892, 2009.
29. Zhong J, Liu C, Zhang QH, Chen L, Shen YY, Chen YJ, Zeng X, Zu XY and Cao RX: TGF- $\beta 1$ induces HMGA1 expression: The role of HMGA1 in thyroid cancer proliferation and invasion. *Int J Oncol* 50: 1567-1578, 2017.
30. Tajrishi MM, Sato S, Shin J, Zheng TS, Burkly LC and Kumar A: The TWEAK-Fn14 dyad is involved in age-associated pathological changes in skeletal muscle. *Biochem Biophys Res Commun* 446: 1219-1224, 2014.
31. Van Kirk CA, VanGuilder HD, Young M, Farley JA, Sonntag WE and Freeman WM. Age-related alterations in retinal neurovascular and inflammatory transcripts. *Mol Vis* 17: 1261-1274, 2011.
32. Hénaut L, Sanz AB, Martin-Sanchez D, Carrasco S, Villa-Bellosta R, Aldamiz-Echevarria G, Massy ZA, Sanchez-Nino MD and Ortiz A: TWEAK favors phosphate-induced calcification of vascular smooth muscle cells through canonical and non-canonical activation of NF κ B. *Cell Death Dis* 7: e2305, 2016.
33. Tong X, Wang D, Liu S, Ma Y, Li Z, Tian P and Fan H: The YKL-40 protein is a potential biomarker for COPD: A meta-analysis and systematic review. *Int J Chron Obstruct Pulmon Dis* 13: 409-418, 2018.
34. Baldacci F, Lista S, Cavedo E, Bonuccelli U and Hampel H: Diagnostic function of the neuroinflammatory biomarker YKL-40 in Alzheimer's disease and other neurodegenerative diseases. *Expert Rev Proteomics* 14: 285-299, 2017.
35. Ringel MD, Hayre N, Saito J, Saunier B, Schuppert F, Burch H, Bernet V, Burman KD, Kohn LD and Saji M: Overexpression and overactivation of Akt in thyroid carcinoma. *Cancer Res* 61: 6105-6111, 2001.
36. Sen P, Mukherjee S, Ray D and Raha S: Involvement of the Akt/PKB signaling pathway with disease processes. *Mol Cell Biochem* 253: 241-246, 2003.
37. Tran NL, McDonough WS, Savitch BA, Sawyer TF, Winkles JA and Berens ME: The tumor necrosis factor-like weak inducer of apoptosis (TWEAK)-fibroblast growth factor-inducible 14 (Fn14) signaling system regulates glioma cell survival via NF κ B pathway activation and BCL-XL/BCL-W expression. *J Biol Chem* 280: 3483-3492, 2005.
38. Ameri H, Liu H, Liu R, Ha Y, Paulucci-Holthausen AA, Hu S, Motamedi M, Godley BF, Tilton RG and Zhang W: TWEAK/Fn14 pathway is a novel mediator of retinal neovascularization. *Invest Ophthalmol Vis Sci* 55: 801-813, 2014.
39. Whitsett TG, Cheng E, Inge L, Asrani K, Jameson NM, Hostetter G, Weiss GJ, Kingsley CB, Loftus JC, Bremner R, *et al*: Elevated expression of Fn14 in non-small cell lung cancer correlates with activated EGFR and promotes tumor cell migration and invasion. *Am J Pathol* 181: 111-120, 2012.
40. Kalhori V and Törnquist K: MMP2 and MMP9 participate in SIP-induced invasion of follicular ML-1 thyroid cancer cells. *Mol Cell Endocrinol* 404: 113-122, 2015.
41. Maeta H, Ohgi S and Terada T: Protein expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinase 1 and 2 in papillary thyroid carcinomas. *Virchows Arch* 438: 121-128, 2001.
42. Trimboli P, Virili C, Romanelli F, Crescenzi A and Giovannella L: Galectin-3 performance in histologic a cytologic assessment of thyroid nodules: A systematic review and meta-analysis. *Int J Mol Sci* 18: E1756, 2017.
43. Cho SW, Kim YA, Sun HJ, Kim YA, Oh BC, Yi KH, Park DJ and Park YJ: CXCL16 signaling mediated macrophage effects on tumor invasion of papillary thyroid carcinoma. *Endocr Relat Cancer* 23: 113-124, 2016.
44. Xia S, Wang C, Postma EL, Yang Y, Ni X and Zhan W: Fibronectin 1 promotes migration and invasion of papillary thyroid cancer and predicts papillary thyroid cancer lymph node metastasis. *Onco Targets Ther* 10: 1743-1755, 2017.
45. Semenza GL: The hypoxic tumor microenvironment: A driving force for breast cancer progression. *Biochim Biophys Acta* 1863: 382-391, 2016.
46. Yu X, Zhao R, Lin S, Bai X, Zhang L, Yuan S and Sun L: CXCL16 induces angiogenesis in autocrine signaling pathway involving hypoxia-inducible factor 1 α in human umbilical vein endothelial cells. *Oncol Rep* 35: 1557-1565, 2016.
47. Zheng J, Lu W, Wang C, Xing Y, Chen X and Ai Z: Galectin-3 induced by hypoxia promotes cell migration in thyroid cancer cells. *Oncotarget* 8: 101475-101488, 2017.
48. Cui H, Grosso S, Schelter F, Mari B and Krüger A: On the pro-metastatic stress response to cancer therapies: Evidence for a positive co-operation between TIMP-1, HIF-1 α , and miR-210. *Front Pharmacol* 3: 134, 2012.
49. Burrows N, Resch J, Cowen RL, von Wasielewski R, Hoang-Vu C, West CM, Williams KJ and Brabant G: Expression of hypoxia-inducible factor 1 alpha in thyroid carcinomas. *Endocr Relat Cancer* 17: 61-72, 2010.
50. Mahkamova K, Latar N, Aspinall S and Meeson A: Hypoxia increases thyroid cancer stem cell-enriched side population. *World J Surg* 42: 350-357, 2018.
51. Hoeng MR, Bianchi C, Rosenzweig A and Sellke FW: Decreased vascular repair and neovascularization with aging: Mechanisms and clinical relevance with an emphasis on hypoxia-inducible factor-1. *Curr Mol Med* 8: 754-767, 2008.
52. Valli A, Harris AL and Kessler BM: Hypoxia metabolism in aging. *Aging (Albany NY)* 7: 465-466, 2015.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.