Differential gene expression profile of multinodular goiter

Wenberger Lanza Daniel de Figueiredo, Eraldo Ferreira Lopes, Deborah Laredo Jezini, Lorena Naciff Marçal, Enedina Nogueira de Assunção, Paulo Rodrigo Ribeiro Rodrigues, Adolfo José da Mota, Diego Monteiro de Carvalho, Spartaco Astolfi Filho, João Bosco Lopes Botelho

1 College of Medicine, Nilton Lins University, Manaus, Amazonas, Brazil, 2 Coari Institute of Health and Biotechnology, Federal University of Amazonas, Coari, Amazonas, Brazil, 3 Department of Internal Medicine, Federal University of Amazonas, Manaus, Amazonas, Brazil, 4 Institute of Biological Sciences, Federal University of Amazonas, Manaus, Amazonas, Brazil, 5 School of Health Sciences, State University of Amazonas, Manaus, Amazonas, Brazil

☯ These authors contributed equally to this work.

* dmdcarvalho@uea.edu.br

Abstract

Introduction
The goiter, a neglected heterogeneous molecular disease, remains a major indication for thyroidectomies in its endemic regions.

Objectives
This study analyzed differential gene expression in surgical specimens diagnosed with multinodular and compared the data to that of thyroid tissue without multinodular goiter from patients undergoing thyroidectomy in Manaus-AM, Brazil using RNA-seq technology.

Methodology
The transcriptome information of the surgical specimen fragments with and without multinodular goiter was accessed by Illumina HiSeq 2000 New Generation Sequencing (NGS) using the RNA-seq NEBNext Ultra™ RNA Library Prep Kit for Illumina®—#E7530L protocol and differential gene expression analysis.

Results
Differences were found between the gene expression profiles of the diseased tissues and those of the healthy control tissues; at least 70 genes were differentially expressed. The HOTS gene was expressed only in multinodular goiter tissues (p < 0.05).

Conclusion
These results demonstrate that the gene expression profile of multinodular goiter is protumoral and that HOTS can play a central role in multinodular goiter development.
Introduction

Partial or total removal of the thyroid gland affected by goiter is one of the most commonly performed surgeries in medical practice. The role of goiter as a risk factor for well-differentiated thyroid carcinoma is unclear; however, the prevalence of incidental carcinoma in patients operated for goiter in endemic areas is 10–12% [1, 2], which is greater than the overall prevalence of the disease (5.1%) [3].

Although goiter is the main indication for thyroidectomy in goitrogenic geographic areas, its molecular-genetic component has been scarcely studied compared with that of thyroid carcinoma. In addition, studies conducting massive sequencing for thyroid nodular diseases have focused on well-differentiated and undifferentiated thyroid carcinoma [4, 5].

Transcriptome analysis, which is gaining prevalence in studies on tumor diseases, allows a better understanding of gene expression profiles in tissues under different conditions, including the knowledge of non-coding RNAs (ncRNAs), monoallelic expression of imprinted genes, and several transcriptional phenomena, such as fluctuations in the expression of non-constitutive sequences [6].

The performance of ncRNAs, such as the products of the H19 gene, and their relationship with several types of cancer are well reported in the literature. This gene, which is never expressed beyond the embryonic period in normal conditions, has high expression in tumors related to tissue hypoxia and cancer. Aberrant expression patterns of this sequence occur in breast cancer [7] and melanoma [8].

In lung neoplasms, high expression of H19 is related to the epithelial-mesenchymal transition [9]. Its action on metabolic and cell cycle pathways is thought to be involved in the modulation of a pro-tumor state [10–12].

The need for a preoperative diagnosis due to gaps in the Bethesda cytological classification from fine needle aspiration (FNA) of thyroid nodules and the advent of molecular studies of these diseases have allowed the development of molecular tests for well-differentiated thyroid carcinoma, notably based on the identification of BRAF and RAS mutations as well as RET/PTC and PAX8/PPARy rearrangements [13, 14], among others such as Afirma GEC®, Thy-GenX TEST®, and ThyroSeq TEST®, all without relevant application for multinodular goiter.

This unprecedented study presents the occurrence of differentially expressed genes between tissues affected by multinodular goiter and disease-free tissues (hereafter referred to as controls) from specimens collected in a geographical area (Amazonas, Brazil) endemic for the disease.

Method

This study was approved by the Human Research Ethics Committee of the Adriano Jorge Hospital Foundation under CAAE 16463813.9.0000.0007 on June 1, 2013.

The study included transcriptome sequencing of two thyroid tissue fragments with multinodular goiter and one control tissue fragment from patients operated in a multinodular goiter endemic region (Manaus, AM, Brazil).

The thyroid fragments used in this study each measured 1 cm³ and were obtained directly from the surgical specimen after its removal from the cervical region by thyroidectomy. Tissue in the control group was obtained from thyroid tissue fragments from patients with thyroid adenoma, from a region of the thyroid gland 1.5 cm away from the nodule. Tissues were confirmed disease-free by pathology service analysis afterwards.

Immediately after collection, the fragments were stored in microcentrifuge tubes containing the preservative RNAlater™ Stabilization Solution (Thermo Fisher) in a -80 °C deep freezer.
until histological classification of the specimen by a pathologist as disease-free tissue or tissue with multinodular goiter.

The total RNA was prepared with TRIzol® Reagent (lifetechnologies™) protocol, following the manufacturer’s recommendations. All following steps to transcriptome sequencing was performed by GenOne Soluções em Biointecnoologia Facility (Rio de Janeiro, Brazil). RNA libraries were validated in an Agilent 2100 Bioanalyzer using the RNA 6000 nano Assay. The cDNA libraries were constructed by using NEBNext® Ultra™ RNA Library Prep Kit for Illumina®—#E7530L RNA-seq protocol with an expected output of 20 GB of data per sample and sequenced in the Illumina HiSeq 2000 platform.

Data analysis

The sequences exploratory analysis were carried out by the Bioinformatics group from the Central Laboratory of High Performance Technologies in Life Sciences (LaCTAD), State University of Campinas (UNICAMP, SP, Brazil). SRA data accession number: PRJNA810866. Reference genome mapping (Homo sapiens HG38) was performed using Bowtie2 [15], transcript quantification was performed using RSEM [16], and differentially expressed genes were analyzed using DESeq [17].

Differentially expressed genes were analyzed for biological function and protein class using the Panther tool [18] available on the Gene Ontology Consortium platform (http://www.geneontology.org/) [19], followed by protein-protein interaction analysis with the GeneMANIA tool (http://www.genemania.org/) [20–22].

Results

The differential expression analysis of two tissue fragments with multinodular goiter and a control tissue identified 65 differentially expressed genes and five pseudogenes, of which 61 were down-regulated and nine up-regulated in thyroid tissue with multinodular goiter compared with the control tissue (Fig 1).

The 70 differentially expressed gene sequences were classified into 62 protein-coding genes; five pseudogenes (SOR2D2P, PI4KAP1P, ZBTB45P1, TMSB4XP4, and PKD1P5); and three sequences related to the pre-mRNA of the transcription factor PRPF31 (RP11-1212a22.1, RP11-514P8.6, and RP11-958N24.2), indicating that one sequence was a product of the imprinted H19 locus encoding the HOTS nucleolar protein [Table 1].

A biological interaction analysis between functional genes identified 423 possible interactions using the Gene MANIA tool with co-expression greater than 50% Fig 2.

Discussion

Medical publications on thyroid surgical diseases are focused on the search for thyroid carcinoma biomarkers [23–26]. Initial immunohistochemistry and microarray studies comparing the expression profiles of normal, multinodular goiter, adenoma, and carcinoma tissue samples identified different patterns between the diseases but similarity between the groups of genes in the tissue with multinodular goiter and that with papillary carcinoma, which would explain the higher prevalence of incidental carcinoma (preoperatively unknown) in thyroids operated for multinodular goiter in goitrogenic areas and the existence of a common initial tumorigenesis factor [27, 28].

The literature is not clear about the molecular origin of multinodular goiter, which certainly involves epigenetic factors, heredity, and the classical iodine deficiency as well as iron and selenium deficiencies in the diet and exposure to foods rich in flavonoids and cyanogenic
substances, such as cassava. When chronic, these conditions would lead to mutations and the onset of nodules in the gland [29, 30].

In medical practice, when facing thyroid nodules, the presence of malignant lesions needs to be considered [31, 32] along with the preoperative FNA investigation and Bethesda’s cytological classification, which often need to be repeated, present variable sensitivity and agreement with histopathology, and are inconclusive in up to 30% of cases [33, 34].

The need to identify which patients with thyroid nodule should undergo surgery, new therapy strategies, or clinical follow-up justifies the investigation of the molecular characteristics of lesions to determine the risk of multinodular goiter malignancy.

In this study, 70 sequences were differentially expressed between multinodular goiter and disease-free tissue. The down-regulated genes in multinodular goiter were related to several molecular pathways, especially phospholipase C (PLCD4), apoptosis pathways (TNFRSF19), heat shock proteins (HSPA1A, HSPA6), growth factors (SHC3, NRG1), p53 proto-oncogene pathways (THBS1), and chaperone cell repair pathways (BAG3); on the other hand, the inflammatory (COL14A) and complement system (C4B) pathways were up-regulated in multinodular goiter tissue, in addition to the exclusive presence of an antisense transcription from the H19 locus, which encodes the nucleolar protein HOTS in multinodular goiter [35].

These findings are similar to the characteristics of tumor diseases with reduced apoptosis and cellular repair systems along with increased inflammatory activity in the presence of proto-tumor locus products, in this case the HOTS nucleolar protein, which, together with the IncRNA H19, would be possible inducers of cancerous breast, thyroid, liver, kidney, and lung lesions [36, 37].

The action of the H19 gene and its products in tumor onset and hyperplastic lesions is evident, with the antagonism of H19 IncRNA and p53 and the activity of one of its gene products, miR-675, in promoting cellular and chromosomal instability being well described, as well as its...
Table 1. List of genes differentially expressed in multinodular goiter and disease-free tissue when $p < 0.05$ (5%).

| Gene symbol | Gene description                                      | FC   | Interpretation       |
|-------------|--------------------------------------------------------|------|----------------------|
| HOTS        | H19 opposite tumor suppressor                          | $\text{Inf}^*$ | $\text{Up-regulated}$ |
| SORD2P      | Pseudogene                                             | 4.18 | $\text{Up-regulated}$ |
| C4B         | Complement C4-B                                        | 2.86 | $\text{Up-regulated}$ |
| C2CD4C      | C2 calcium-dependent domain-containing protein 4C       | 3.86 | $\text{Up-regulated}$ |
| C241377.2   | Protein LOC100996720                                    | 5.71 | $\text{up-regulated}$ |
| CPXM1       | Probable carboxypeptidase X1                           | 2.11 | $\text{up-regulated}$ |
| NAPRT       | Nicotinate phosphoribosyltransferase                    | 2.37 | $\text{up-regulated}$ |
| ST6GAL1     | Beta-galactoside alpha-2,6-sialyltransferase 1           | 1.86 | $\text{up-regulated}$ |
| COL14A1     | Collagen alpha-1(XIV) chain                            | 1.62 | $\text{up-regulated}$ |
| HSPA6       | Heat shock 70 kDa protein 6                             | -4.21| $\text{down-regulated}$ |
| C1QL4       | Complement C1q-like protein 4                           | -5.05| $\text{down-regulated}$ |
| PLCD4       | Phospholipase C                                         | -3.82| $\text{down-regulated}$ |
| ERRF1       | ERBB receptor feedback inhibitor 1                      | -2.84| $\text{down-regulated}$ |
| PCP4L1      | Purkinje cell protein 4-like protein 1                  | -5.27| $\text{down-regulated}$ |
| ATRNL1      | Attractin-like protein 1                                | -4.34| $\text{down-regulated}$ |
| MT1H        | Metallothionein-1H                                      | -2.71| $\text{down-regulated}$ |
| ABCA13      | ATP-binding cassette sub-family A member 13             | -3.32| $\text{down-regulated}$ |
| DNAJBI      | DnaJ homolog subfamily B member 1                       | -2.46| $\text{down-regulated}$ |
| CA12        | Carbonic anhydrase 12                                   | -2.89| $\text{down-regulated}$ |
| PKD1P5      | Pseudogene                                             | -2.89| $\text{down-regulated}$ |
| RP11-958N24.2| Uncharacterized                                       | -5.83| $\text{down-regulated}$ |
| ETV4        | ETS translocation variant 4                             | -3.48| $\text{down-regulated}$ |
| RP11-514P8.6| Uncharacterized                                       | 0    | $\text{down-regulated}$ |
| IGSF1       | Immunoglobulin superfamily member 1                    | -2.49| $\text{down-regulated}$ |
| RASD1       | Dexamethasone-induced Ras-related protein 1             | -2.51| $\text{down-regulated}$ |
| MRPL23      | 39S ribosomal protein L23mitochondrial                  | -3.47| $\text{down-regulated}$ |
| CPNE4       | Copine-4                                               | -7.62| $\text{down-regulated}$ |
| IL1RL1      | Interleukin-1 receptor-like 1                          | -4.71| $\text{down-regulated}$ |
| P4KAP1      | Pseudogene                                             | -2.95| $\text{down-regulated}$ |
| DLEC1       | Deleted in lung and esophageal cancer protein 1         | -3.45| $\text{down-regulated}$ |
| ZBTB45P1    | Pseudogene                                             | 0    | $\text{down-regulated}$ |
| LECT1       | Leukocyte cell-derived chemotaxin 1                     | -2.67| $\text{down-regulated}$ |
| SHC3        | SHC-transforming protein 3                              | -3.28| $\text{down-regulated}$ |
| SORCS1      | VPS10 domain-containing receptor SorCS1                | -2.95| $\text{down-regulated}$ |
| STARD9      | StAR-related lipid transfer protein 9                   | -2.34| $\text{down-regulated}$ |
| TMSB4XP4    | Pseudogene                                             | -4.16| $\text{down-regulated}$ |
| TMEM184A    | Transmembrane protein 184                              | -3.60| $\text{down-regulated}$ |
| PCSK2       | Neuroendocrine convertase 2                             | -2.22| $\text{down-regulated}$ |
| FOSB        | Protein fosB                                           | -1.72| $\text{down-regulated}$ |
| TRABD2A     | Metalloprotease TIK1                                    | -3.42| $\text{down-regulated}$ |
| GATM        | Glycine amidinotransferase, mitochondrial               | -1.82| $\text{down-regulated}$ |
| BAG3        | BAG family molecular chaperone regulator 3             | -1.88| $\text{down-regulated}$ |
| FAM105A     | Inactive ubiquitin thioesterase FAM105A                 | -2.32| $\text{down-regulated}$ |
| HSPA1B      | Heat shock 70 kDa protein 1B                            | -2.42| $\text{down-regulated}$ |
| MME         | Neprilysin                                              | -2.86| $\text{down-regulated}$ |
| PDE4C       | cAMP-specific 3',5'-cyclic phosphodiesterase 4C         | -3.47| $\text{down-regulated}$ |
| RTN4RL2     | Reticulon-4 receptor-like 2                             | -1.72| $\text{down-regulated}$ |

(Continued)
hypereexpression in the presence of external factors such as hypoxia [37]. A balance between the products of sense (lncRNA) and antisense (HOTS) H19 transcripts may be related to the regulation of cellular homeostasis.

There have been no studies on multinodular goiter using NGS and RNA-Seq with results similar to those described in the present study. In thyroid carcinoma, some lncRNA are discussed in the gene regulation of disease progression, such as PTCSC3 [38], with XLOC 051122 and XLOC 006074 [39] in local metastasis and PANDAR as a possible target in pro-apoptotic therapies for carcinoma [40].

The question of whether the presence of multinodular goiter can be considered a risk factor for thyroid carcinoma still raises discussion. Recent findings have shown that the same histopathologically diagnosed papillary lesion exhibits different protein expression behavior if the patient has a history of multinodular goiter prior to the diagnosis of neoplasia [40–42].

Other studies have shown the importance of membrane proteins in the development of hyperplastic and neoplastic thyroid diseases, especially connexins and aquaporins [43–45]. This study identified no differences between the expression profiles of connexins or aquaporins in different tissues, but STARD9 (apolipoprotein) and CPNE4 membrane proteins were down-regulated in multinodular goiter.

Thus, it was possible to identify molecular characteristics of multinodular goiter similar to those found in the genesis of neoplastic tumor lesions, including: 1) reduced cell repair activity;

Table 1. (Continued)

| Gene symbol | Gene description | FC | Interpretation |
|-------------|------------------|----|---------------|
| EDN3        | Endothelin-3     | -1.75 | down-regulated |
| NRK         | Nik-related protein kinase | -3.30 | down-regulated |
| EGR2        | E3 SUMO-protein ligase EGR2 | -1.63 | down-regulated |
| TNFRSF19    | Tumor necrosis factor receptor superfamily member 19 | -2.26 | down-regulated |
| ABCG3       | Canalicual multispecific organic anion transporter 2 | -2.23 | down-regulated |
| HSD17B6     | 17-beta-hydroxysteroid dehydrogenase type 6 | -1.72 | down-regulated |
| RP11-12I2A22.1 | Uncharacterized | -1.79 | down-regulated |
| CI5orf48    | Normal mucosa of esophagus-specific gene 1 protein;NMES1 | -2.57 | down-regulated |
| GATA5       | Transcription factor GATA-5 | -4.48 | down-regulated |
| FRAS1       | Extracellular matrix protein FRAS1 | -1.49 | down-regulated |
| PCDH8       | Protocadherin-8  | -3.16 | down-regulated |
| YJEFN3      | YjeF N-terminal domain-containing protein 3 | -2.36 | down-regulated |
| SPHKAP      | A-kinase anchor protein SPHKAP | -6.23 | down-regulated |
| TBX2        | T-box transcription factor TBX2 | -1.60 | down-regulated |
| KIAA1324    | UPF0577 protein  | -1.48 | down-regulated |
| KIF5A       | Kinesin heavy chain isoform 5A | -2.10 | down-regulated |
| ARHGPAP28   | Rho GTPase-activating protein 28 | -2.01 | down-regulated |
| CIorf233    | Fibronectin type-III domain-containing transmembrane protein | -1.94 | down-regulated |
| NRG1        | Pro-neuregulin-1, membrane-bound isoform | -1.83 | down-regulated |
| HSPA1A      | Heat shock 70 kDa protein 1A | -2.19 | down-regulated |
| THBS1       | Thrombospondin-1 | -1.67 | down-regulated |
| PLIN1       | Perilipin-1      | -2.51 | down-regulated |
| XKRX        | XK-related protein 2 | -4.19 | down-regulated |

*FC: log2FoldChange.

**∞: infinite: ratio between the number of sequences in normal tissue equal to 0 over the number of sequences found for multinodular goiter.

https://doi.org/10.1371/journal.pone.0268354.t001
2) reduced apoptotic pathway activity; 3) increased inflammatory activity; and 4) H19 gene expression with possible inhibitory activity of the p53 proto-oncogene.

The presence of H19 gene products hyper-expressed in multinodular goiter, a non-malignant disease with different forms of presentation in endemic regions (small and large multinodular goiters), contributes to the understanding of the genesis of multinodular goiter and its possible roles as a risk factor for malignant lesions and as a possible molecular marker.

Fig 2. Protein-protein interaction analysis of 63 differentially expressed functional genes using the GeneMANIA software. A total of 423 gene interactions (more than 50% co-expression) of the five genes (TNF-RSF19, AC2413771 (HOTS gene), RP11-514P8.6, C1QL4, and SPHKAP) had no interactions identified with the others.

https://doi.org/10.1371/journal.pone.0268354.g002
Further studies in endemic areas with more replicates for NGS analysis and a better understanding of the function of ncRNAs in the development of the disease will be necessary to confirm the hypothesis of multinodular goiter as a pro-tumor state of the thyroid.

Previous findings in the literature have described the low expression of the \(H19\) gene and its products in thyroid cancer \[46\], which contradicts the high expression of this sequence in the multinodular goiter samples used in this study. This suggests that high \(H19\) gene expression may be used in conjunction with other molecular markers as a diagnostic tool in deciding between conservative and/or surgical treatment for multinodular goiter patients in endemic areas, such as the Amazon.

Future studies should further elucidate the molecular profile of multinodular goiter, deepen the understanding of the functions of non-coding RNA in malignant and non-malignant nodular diseases, and facilitate the development of rapid and cost-effective diagnostic protocols that consider the level of \(H19\) gene expression in patients with multinodular goiter.

**Acknowledgments**

to the Adriano Jorge Hospital Foundation: Fundação Hospital Adriano Jorge (FHAJ)
to Federal University of Amazonas: Universidade Federal do Amazonas (UFAM)
to the University of Amazonas State: Universidade do Estado do Amazonas (UEA)
and to Amazonas State Research Support Foundation: Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM)
for their institutional support.

**Author Contributions**

**Conceptualization:** Paulo Rodrigo Ribeiro Rodrigues, Diego Monteiro de Carvalho, Spartaco Astolfi Filho, João Bosco Lopes Botelho.

**Data curation:** Lorena Naciff Marçal, Enedina Nogueira de Assunção, Paulo Rodrigo Ribeiro Rodrigues, Diego Monteiro de Carvalho, Spartaco Astolfi Filho, João Bosco Lopes Botelho.

**Formal analysis:** Lorena Naciff Marçal, Enedina Nogueira de Assunção, Paulo Rodrigo Ribeiro Rodrigues, Diego Monteiro de Carvalho, Spartaco Astolfi Filho, João Bosco Lopes Botelho.

**Funding acquisition:** Lorena Naciff Marçal, Paulo Rodrigo Ribeiro Rodrigues.

**Investigation:** Enedina Nogueira de Assunção, Paulo Rodrigo Ribeiro Rodrigues, Diego Monteiro de Carvalho, Spartaco Astolfi Filho, João Bosco Lopes Botelho.

**Methodology:** Enedina Nogueira de Assunção, Diego Monteiro de Carvalho, Spartaco Astolfi Filho, João Bosco Lopes Botelho.

**Project administration:** Enedina Nogueira de Assunção, Adolfo José da Mota, Diego Monteiro de Carvalho, Spartaco Astolfi Filho, João Bosco Lopes Botelho.

**Resources:** Deborah Laredo Jezini, Adolfo José da Mota, Diego Monteiro de Carvalho, João Bosco Lopes Botelho.

**Software:** Eraldo Ferreira Lopes, Deborah Laredo Jezini, Lorena Naciff Marçal, Adolfo José da Mota, Diego Monteiro de Carvalho, João Bosco Lopes Botelho.

**Supervision:** Eraldo Ferreira Lopes, Deborah Laredo Jezini, Lorena Naciff Marçal, Paulo Rodrigo Ribeiro Rodrigues, Adolfo José da Mota, Diego Monteiro de Carvalho, João Bosco Lopes Botelho.
Validation: Eraldo Ferreira Lopes, Deborah Laredo Jezini, Lorena Naciff Marçal, Paulo Rodrigo Ribeiro Rodrigues, Adolfo José da Mota, Diego Monteiro de Carvalho, João Bosco Lopes Botelho.

Visualization: Wenberger Lanza Daniel de Figueiredo, Eraldo Ferreira Lopes, Deborah Laredo Jezini, Diego Monteiro de Carvalho, João Bosco Lopes Botelho.

Writing – original draft: Wenberger Lanza Daniel de Figueiredo, Eraldo Ferreira Lopes, Deborah Laredo Jezini, Diego Monteiro de Carvalho.

Writing – review & editing: Wenberger Lanza Daniel de Figueiredo, Diego Monteiro de Carvalho.

References

1. Can AS, Rehman A. goiter [Internet]. StatPearls [Internet]. StatPearls Publishing; 2021 [citado 22 de agosto de 2021]. https://www.ncbi.nlm.nih.gov/books/NBK562161/

2. Fama F, Sindoni A, Ciccio M, Polito F, Piccard A, Saint-Marc O, et al. Preoperatively undiagnosed papillary thyroid carcinoma in patients thyroidectomized for benign multinodular goiter. Arch Endocrinol Metab [Internet]. 23 de março de 2018 [citado 11 de agosto de 2021]; Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2359-39972018000200139&lng=en&nrm=iso&tlng=en PMID: 29641730

3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 38 cancers in 185 countries. CA Cancer J Clin. novembro de 2018; 68(6):394–424. https://doi.org/10.3322/caac.21492 PMID: 30207588

4. Cha YJ, Koo JS. Next-generation sequencing in thyroid cancer. J Transl Med. dezembro de 2016; 14(1):322. https://doi.org/10.1186/s12967-016-1074-7 PMID: 27871285

5. Zhou CK, Check DP. Fama F, Sindoni A, Ciccio M, Polito F, Piccard A, Saint-Marc O, et al. Preoperatively undiagnosed papillary thyroid carcinoma in patients thyroidectomized for benign multinodular goiter. Arch Endocrinol Metab [Internet]. 23 de março de 2018 [citado 11 de agosto de 2021]; Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2359-39972018000200139&lng=en&nrm=iso&tlng=en PMID: 29641730

6. Luan W, Zhou Z, Ni X, Xia Y, Wang J, Yan Y, et al. Long non-coding RNA H19 promotes glucose metabolism and cell growth in malignant melanoma via miR-106a-5p/E2F3 axis. J Cancer Res Clin Oncol. março de 2018; 144(3):531–42. https://doi.org/10.1007/s00432-017-0560-x PMID: 29094204

7. Morishita Y, Kabil O, Young KZ, Kellogg AP, Chang A, Arvan P. Thyrocyte cell survival and adaptation to chronic endoplasmic reticulum stress due to misfolded thyroglobulin. J Biol Chem. 15 de maio de 2020; 295(20):20876–87. https://doi.org/10.1074/jbc.RA120.012856 PMID: 32241916

14. Abdullah Mt, Junit SM, Ng KL, Jayapalani JJ, Kanikalan B, Hashim OH. Papillary Thyroid Cancer: Genetic Alterations and Molecular Biomarker Investigations. Int J Med Sci. 2019; 16(3):450–60. https://doi.org/10.7150/ijms.29835 PMID: 30911279

15. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. abril de 2012; 9(4):357–8. https://doi.org/10.1038/nmeth.1923 PMID: 22386286
16. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics. dezembro de 2011; 12(1):323.

17. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. outubro de 2010; 11(10):R106. https://doi.org/10.1186/gb-2010-11-10-r106 PMID: 20979621

18. Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, et al. PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res. 4 de janeiro de 2017; 45(D1):D183–9. https://doi.org/10.1093/nar/gkw1138 PMID: 27899595

19. Wheeler DL. Database resources of the National Center for Biotechnology Information: update. Nucleic Acids Res. 1o de janeiro de 2004; 32(90001):35D–40. https://doi.org/10.1093/nar/gkh073 PMID: 14681353

20. Zuberi K, Franz M, Rodriguez H, Montojo J, Lopes CT, Bader GD, et al. GeneMANIA Prediction Server 2013 Update. Nucleic Acids Res. 1o de julho de 2013; 41(W1):W115–22. https://doi.org/10.1093/nar/gkt533 PMID: 23794635

21. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res. 1o de julho de 2010; 38(suppl_2):W214–20 . https://doi.org/10.1093/nar/gkq537 PMID: 20576703

22. Hutchins JRA. What's that gene (or protein)? Online resources for exploring functions of genes, transcripts, and proteins. Kellogg D, organizador. Mol Biol Cell. 15 de abril de 2014; 25(8):1187–201. https://doi.org/10.1091/mbc.E13-10-0602 PMID: 24723265

23. Linkov F, Ferris RL, Yurkovetsky Z, Marrangoni A, Velikokhatnaya L, Gooding W, et al. Multiplex analysis of cytokines as biomarkers that differentiate benign and malignant thyroid diseases. PROTEOMICS—Clin Appl. dezembro de 2008; 2(12):1575–85. https://doi.org/10.1002/prca.200780095 PMID: 19234619

24. Nagar S, Ahmed S, Peeples C, Urban N, Boura J, Thibodeau A, et al. Evaluation of genetic biomarkers for distinguishing benign from malignant thyroid neoplasms. Am J Surg. abril de 2014; 207(4):596–601. https://doi.org/10.1016/j.amjsurg.2013.06.012 PMID: 24713092

25. Yao Y, Chen X, Yang H, Chen W, Qian Y, Yan Z, et al. Hsa_circ_0058124 promotes papillary thyroid cancer tumorigenesis and invasiveness through the NOTCH3/GATAD2A axis. J Exp Clin Cancer Res. 19 de julho de 2019; 38(1):318. https://doi.org/10.1186/s13046-019-1321-x PMID: 31324198

26. Lan X, Bao H, Ge X, Cao J, Fan X, Zhang Q, et al. Genomic landscape of metastatic papillary thyroid carcinoma and novel biomarkers for predicting distant metastasis. Cancer Sci. 2020; 111(6):2163–73. https://doi.org/10.1111/cas.14389 PMID: 32187423

27. Stolf BS, Abreu CM, Mahler-Araujo MB, Dellamano M, Martins WK, de Carvalho MB, et al. Expression profile of malignant and non-malignant diseases of the thyroid gland reveals altered expression of a common set of genes in goiter and papillary carcinomas. Cancer Lett. setembro de 2005; 227(1):59–73. https://doi.org/10.1016/j.canlet.2004.11.050 PMID: 16051032

28. Stolf BS, Carvalho AF, Martins WK, Runza FB, Brun M, Hirata R, et al. Differential expression of IGFBP-5 and two human ESTs in thyroid glands with multinodular goiter, adenoma and papillary or follicular carcinomas. Cancer Lett. março de 2003; 191(2):193–202. https://doi.org/10.1016/s0304-3835(02)00679-1 PMID: 12618333

29. Knobel M, Medeiros-Neto G. Moléstias associadas à carência crônica de iodo. Arq Bras Endocrinol Metabol. fevereiro de 2004; 48(1):53–61. https://doi.org/10.1590/s0004-27302004000100007 PMID: 15611818

30. Knobel M. Etiopathology, clinical features, and treatment of diffuse and multinodular nontoxic multinodular goiters. J Endocrinol Invest. abril de 2016; 39(4):357–73. https://doi.org/10.1007/s40618-015-0391-7 PMID: 26392367

31. Chow LS, Gharib H, Goellner JR, van Heerden JA. Nondiagnostic Thyroid Fine-Needle Aspiration Cytology: Management Dilemmas. Thyroid. dezembro de 2001; 11(12):1147–51. https://doi.org/10.1089/10507250152740993 PMID: 12186502

32. Grani G, Sponziello M, Pecce V, Ramundo V, Durante C. Contemporary Thyroid Nodule Evaluation and Management. J Clin Endocrinol Metab. 1o de setembro de 2020; 105(9):2869–83. https://doi.org/10.1210/clinem/dgaa322 PMID: 32491169

33. Kihara M, Hirokawa M, Matsumoto H, Yabuta T, Shindo H, Higashiyama T, et al. Evaluation of cytologically benign solitary thyroid nodules by ultrasonography: A retrospective analysis of 1877 cases. Auris Nasus Larynx. junho de 2013; 40(3):308–11. https://doi.org/10.1016/j.anl.2012.09.007 PMID: 23103151
34. Tan H, Li Z, Li N, Qian J, Fan F, Zhong H, et al. Thyroid imaging reporting and data system combined with Bethesda classification in qualitative thyroid nodule diagnosis. Medicine (Baltimore). dezembro de 2019; 98(50):e18320. https://doi.org/10.1097/MD.00000000000018320 PMID: 31852120

35. Onyango P, Feinberg AP. A nucleolar protein, H19 opposite tumor suppressor (HOTS), is a tumor growth inhibitor encoded by a human imprinted H19 antisense transcript. Proc Natl Acad Sci. 4 de outubro de 2011; 108(40):16759–64. https://doi.org/10.1073/pnas.110904108 PMID: 21940503

36. Duan C, Zhang C, Peng Z. Functions and mechanisms of long noncoding RNAs in lung cancer. Onco-Targets Ther. julho de 2016; Volume 9:4411–24. https://doi.org/10.2147/OTT.S109549 PMID: 27499635

37. Pope C, Mishra S, Russell J, Zhou Q, Zhong X-B. Targeting H19, an Imprinted Long Non-Coding RNA, in Hepatic Functions and Liver Diseases. 8 de março de 2017; 5(1):11. https://doi.org/10.3390/diseases5010011 PMID: 28933364

38. FAN M, LI X, JIANG W, HUANG Y, LI J, WANG Z. A long non-coding RNA, PTCSC3, as a tumor suppressor and a target of miRNAs in thyroid cancer cells. Exp Ther Med. abril de 2013; 5(4):1143–6. https://doi.org/10.3892/etm.2013.933 PMID: 23599737

39. Li Z, Gao B, Hao S, Tian W, Chen Y, Wang L, et al. Knockdown of lncRNA-PANDAR suppresses the proliferation, cell cycle and promotes apoptosis in thyroid cancer cells. EXCLI J. 23 de março de 2017; 16:354–62. https://doi.org/10.17179/excli2017-113 PMID: 28507479

40. Hegedüs L, Brixi TH, Paschke R. Etiology of Simple goiter. Thyroid. março de 2009; 19(3):209–11. https://doi.org/10.1089/thy.2009.0047 PMID: 19265491

41. Abdullah MI, Lee CC, Mat Junit S, Ng KL, Hashim OH. Tissue and serum samples of patients with papillary thyroid cancer with and without benign background demonstrate different altered expression of proteins. PeerJ. 13 de setembro de 2016; 4:e2450. https://doi.org/10.7717/peerj.2450 PMID: 27672505

42. Mathai AM, Preetha K, Valsala Devi S, Vicipili S, Pradeep R, Shaick A. Analysis of Malignant Thyroid Neoplasms with a Striking Rise of Papillary Microcarcinoma in an Endemic goiter Region. Indian J Otolaryngol Head Neck Surg. outubro de 2019; 71(Suppl 1):121–30. https://doi.org/10.1007/s12070-017-1156-8 PMID: 31741946

43. Jiang X, Feng X, Li G, Zhao Q, Yin H. Differential expression of connexin 43 in human autoimmune thyroid disease. Acta Histochem. maio de 2010; 112(3):278–83. https://doi.org/10.1016/j.acthis.2009.02.001 PMID: 19321193

44. Dominguez C, Karayan-Tapon L, Desurmont T, Gibelin H, Crespin S, Fromont G, et al. Altered Expression of the Gap Junction Protein Connexin43 Is Associated with Papillary Thyroid Carcinomas When Compared with Other Noncancer Pathologies of the Thyroid. Thyroid. outubro de 2011; 21(10):1057–66. https://doi.org/10.1089/thy.2011.0041 PMID: 21875346

45. Xu L, Chen S-W, Qi X-Y, Li X-X, Sun Y-B. Ginsenoside improves papillary thyroid cancer cell malignancies partially through upregulating connexin 31. Kaohsiung J Med Sci. 2018; 34(6):313–20. https://doi.org/10.1016/j.kjms.2017.12.006 PMID: 29747774

46. Lan X, Sun W, Dong W, Wang Z, Zhang T, He L, et al. Downregulation of long noncoding RNA H19 contributes to the proliferation and migration of papillary thyroid carcinoma. Gene. 10 de março de 2018; 646:98–105. https://doi.org/10.1016/j.gene.2017.12.051 PMID: 29287713