Pathol. Oncol. Res. (2013) 19:597–604
DOI 10.1007/s12253-013-9625-1

RESEARCH

Candidate Agents for Papillary Thyroid Cancer Identified by Gene Expression Analysis

Wei Zhu · Chen Li · Zhilong Ai

Received: 10 December 2012 / Accepted: 6 March 2013 / Published online: 22 March 2013

Abstract A better understanding of the molecular mechanisms involved in papillary thyroid cancer (PTC) is needed to manage these patients effectively. Our objectives were to expand our understanding of this disease, and to identify biologically active small molecules capable to reverse PTC. We downloaded gene expression data of PTC from Gene Expression Omnibus database and employed computational bioinformatics analysis to compare gene expression patterns with normal tissues. Small molecules that induced inverse gene changes to the PTC were identified. A total of 2,154 differentially expressed genes (DEGs) with a false discovery rate of 0.01 were identified. These 2,154 DEGs were significantly enriched in 17 pathways, including pathways associated with signal transduction, tumorigenesis and lipid or amino acid metabolism. In addition, we identified large amount of small molecules that capable to reverse PTC. We found a group of small molecules that can provide new ideas for the therapeutic studies in PTC. These drugs are clearly a direction that warrants additional consideration.

Keywords Papillary thyroid carcinoma · Differentially expressed genes · Small molecules

Introduction

Papillary thyroid carcinoma (PTC) belongs to well-differentiated thyroid cancer, which also include Follicular and Hurthle cell carcinoma. It is the most common type of thyroid cancer, representing 75 % to 85 % of all thyroid cancer cases [1, 2]. PTC can occur at any age, and its incidence has been increasing over the last few decades in many areas of the world [2–8]. Most patients with PTC have good prognosis after surgical treatment, with a overall 5-year survival rate of 96–97 % [9]. However, there are still many patients die of localized disease or distant metastasis [10]. In addition, chemotherapy with cisplatin or doxorubicin has limited efficacy, producing occasional objective responses (generally for short durations) [11]. Consequently, a standard protocol for chemotherapeutic management is desperate need for these patients.

Molecular studies performed in the last decades, have elucidated in part the molecular mechanisms underlying thyroid cancer initiation and progression [12]. Genetic alterations, including BRAF and RAS point mutations [13], rearrangements of the tyrosine kinase domains of the RET gene with amino-terminal sequence of an unrelated gene [14, 15], PAX8/PPARγ rearrangements [16, 17] and p53 inactivation [18] underlines the molecular mechanisms resulting in thyroid cancer. These genetic alterations are found in more than 70 % of PTC. Abnormalities in the RET/RAS/B-RAF/MAP kinase pathway are found in 80 % of cases with no important overlap [19, 20].

The molecular mechanisms underlying sporadic PTC are not fully being understood. In this study, we aimed to explore the molecular mechanisms of PTC using a computational bioinformatics analysis of gene expression, and to identify small molecules for the treatment of this disease. Candidate agents identified by our approach may provide the ground work for a new therapy approach for PTC.

Materials and Methods

Affymetrix Microarray Data

We downloaded the gene expression profile data on PTC patients with normal controls from Gene Expression Omnibus

Wei Zhu and Chen Li should be regard as cofirst authors.

W. Zhu · C. Li · Z. Ai (✉)
Department of General Surgery, Zhongshan Hospital Affiliated to Fudan University, No.180 Fenglin Road, Xuhui District, Shanghai 200032, China
e-mail: aizhilongazl@hotmail.com

(GEO), which was based on HG-U133 Plus 2 platform (Affymetrix Human Genome U133 Plus 2.0 Array). These expression data were deposited by He and colleagues (Accession number: GSE3467) [21]. A total of 18 genechips obtained from patients with sporadic PTC undergoing surgical resection were available for further analysis, including 9 genechips from tumor tissue of PTC and 9 genechips from normal thyroid tissue adjacent to PTC tumors.

We downloaded the raw CEL data and the annotation file for probes of the platform from GEO. Then, we used R package (v.2.13.0) [22] to analyze the gene expression profile. The CEL source files were divided into two groups: the PTC group and the normal group, and normalization was performed using RMA (Robust Multi-array Average) algorithm [23]. The probability of genes being differentially expressed between PTC and normal control was computed using the limma package [24]. To circumvent the multi-test problem which might induce too much false positive results, the BH method [25] was used to adjust the raw *p*-values into false discovery rate (FDR). We defined FDR <0.01 to be statistically significant.

Gene Ontology Analysis of the Differentially Expressed Genes

The Gene Ontology (GO) is a collaborative effort to address the need for consistent descriptions of gene products in different databases and utilizes a controlled GO vocabulary in a curated database [26]. GO provides three structured networks of defined terms to describe gene product attributes: biological process, molecular function, and cellular compartment. To functional classify the differentially expressed genes, we performed GO enrichment analysis using clusterProfiler [27] and searched for over-represented GO terms in these three ontologies, respectively. The *p*-value <0.1 and count number >2 were selected as cutoff criteria.

Pathway Enrichment Analysis of the Differentially Expressed Genes

The KEGG (Kyoto Encyclopedia of Genes and Genomes) PATHWAY database records networks of molecular interactions in the cells, and variants of them specific to particular organisms [28]. To explore the dysfunctional pathways in PTC tissues, we inputted the DEGs into DAVID (Database for Annotation, Visualization and Integrated Discovery) for pathway enrichment analysis. The DAVID now provides a comprehensive set of function annotation tools for investigators to understand biological meaning behind large list of genes [29].

Identification of Candidate Compounds

The connectivity map (Cmap, version 2) comprises genomic profiling data from 6,100 treatment-control pairs (instances) involving 1,309 bioactive molecules (perturbagens). It can be used to find connections among small molecules sharing a mechanism of action, chemicals and physiological processes, and diseases and drugs [30].

We first divided the DEGs into two groups: up-regulation group and down-regulation group. Then, we performed gene set enrichment analysis of the DEGs compared to the differential gene expression of the treatment-control pair (instance) in CMap database. The output consisted of a group of chemical perturbagens with an enrichment score ranging from +1 and −1. The score represented the correlation between the query signature profile and the gene profile of a treatment-control pair. A high positive connectivity score indicates that the corresponding perturbagen induced the expression of the query signature, whereas a high negative enrichment score indicated reversal of expression of the query signature by the perturbagen. A zero or "null" enrichment score indicated that no effect upon expression of the query signature was recorded.

Results

Differentially Expressed Genes Between PTC Tissue and Normal Control Tissue

The gene expression profile of GSE3467 was downloaded from GEO database and empirical bayes methods in limma package was used to identify differentially expressed genes in PTC tissues compared with normal controls. At a FDR of 0.01, a total of 3,096 probe sets were differentially expressed between PTC tissues and normal controls. By mapping these probe sets to NCBI entrez genes, we obtained a total of 2,154 DEGs.

Table 1 Classification of differentially expressed genes (DEGs) according to cellular component ontology

GO-ID	Description	*p*-value	DEGs
GO:0042613	MHC class II protein complex	0.02015	HLA-DQB1,CD74,HLA-DQA1,HLA-DRB1,HLA-DOA,HLA-DMA, HLA-DPA1,HLA-DOB,HLA-DPB1,HLA-DRA,HLA-DMB
GO:0030061	mitochondrial crista	0.061163	FDX1,LYN,AKAP1,OPA1,COX6B2
GO:0030992	intraflagellar transport particle B	0.061163	HSPB11,IFT88,IFT74,IFT27,IFT52
GO:0035985	senescence-associated heterochromatin focus	0.083802	HMGA2,HMGA1,CDKN2A

Table 2 Classification of DEGs according to molecular function ontology

GOID	Description	*p*-value	DEGs
GO:0005220	inositol 1,4,5-trisphosphate-sensitive calcium-release channel activity	0.053905	ITPR1,CYTH3,ITPR2,ITPR3
GO:0048154	S100 beta binding	0.053905	S100A11,S100A6,S100A1,S100B
GO:0050544	arachidonic acid binding	0.053905	PPARG,ALOX5AP,SNCA,STX
GO:0051731	polynucleotide 5′-hydroxyl-kinase activity	0.053905	PNKP,N4BP2,NOL9,CLP1
GO:0004029	aldehyde dehydrogenase (NAD) activity	0.073221	ALDH1A1,ALDH7A1,ALDH1A3,ALDH9A1, ALDH1B1,ALDH2,ALDH4A1
GO:0004716	receptor signaling protein tyrosine kinase activity	0.091522	ERBB4,KIT,TYRO3,EGFR,ERBB3,LYN,SYK,KDR
GO:0004075	biotin carboxylase activity	0.093265	PC,PCCA,ACACB,BTD,MCCC1

Modulated GO Categories in PTC

To determine the modulated GO categories in PTC tissue, the DEGs were mapped to the three ontologies in GO (Tables 1, 2 and 3). From Table 1, we could find that four categories were significantly enriched in cellular component ontology, including MHC class II protein complex (*p*-value=0.02015), mitochondrial crista (*p*-value=0.061163), intraflagellar transport particle B (*p*-value=0.061163) and senescence—associated heterochromatin focus (*p*-value=0.083802). In ontology of molecular function, 7 GO categories were enriched, including inositol 1,4,5-trisphosphate-sensitive calcium-release channel activity, S100 beta binding and arachidonic acid binding. Several GO category of biological process were enriched, such as antigen processing and presentation of peptide or polysaccharide antigen via MHC class II, establishment of mitotic spindle localization and positive regulation of cell aging.

Dysregulated Pathways in PTC

In order to identify the dysregulated pathways in PTC tissues, we performed pathway enrichment analysis using the online biological classification tool DAVID. A total of 17 pathways with *p*-value less than 0.05 were enriched (Table 4). The most significant pathway was cell adhesion molecules with *p*-value=1.80E-06. The other significant pathways included valine, leucine and isoleucine degradation (*p*-value=4.75E-05), propanoate metabolism (*p*-value=9.53E-05) and tryptophan metabolism (*p*-value=0.003512).

Identification of Candidate Small Molecules to Reverse PTC

In order to identify candidate small molecules capable to reverse PTC, we performed computational bioinformatics analysis of the derived gene signature using the Connectivity Map. The perturbagens from the CMap were analyzed according to their permutated results, *p*-values, and enrichment scores. A search against 6,100 treatment- control pairs representing 1,309 bioactive small molecules identified large amount small molecules which exhibited positive or negative correlation to the query signature. The top 20 significant small molecules were listed in Table 5. From Table 5, we could find that 7 perturbagens were enriched with highly significant negative scores (enrichment score <−0.900) : camptothecin

Table 3 Classification of DEGs according to biological process ontology

GOID	Description	*p*-value	DEGs
GO:0002504	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	0.022196	FCER1G,HLA-DQB1,MARCH8,MARCH1,CD74,HLA-DQA1, HLA-DRB1,HLA-DOA,HLA-DMA,HLA-DPA1,HLA-DOB, HLA-DPB1,HLA-DRA,THBS1,HLA-DMB
GO:0040001	establishment of mitotic spindle localization	0.03436	ESPL1,HTT,NUSAP1,CDK5RAP2,NDC80,CENPA,PAFAH1B1, DYNLT1,NDE1
GO:0090343	positive regulation of cell aging	0.064211	HMGA2,HMGA1,LMNA,CDKN2A
GO:0033233	regulation of protein sumoylation	0.064742	HDAC4,RASD2,PIAS1,PIAS3,EGR1,CDKN2A
GO:0042487	regulation of odontogenesis of dentine-containing tooth	0.064742	RUNX2,DICER1,TNFRSF11B,IFT88,NGFR,WNT10A
GO:0070723	response to cholesterol	0.076295	TGFBR1,TGFB1,ACSL5,INHBB,LRP6,AACS,HMGCS1, INHBA,SMAD2
GO:0000132	establishment of mitotic spindle orientation	0.093207	HTT,CDK5RAP2,NDC80,CENPA,PAFAH1B1,DYNLT1,NDE1
GO:0051294	establishment of spindle orientation	0.093207	HTT,CDK5RAP2,NDC80,CENPA,PAFAH1B1,DYNLT1,NDE1

Table 4 The dysregulated pathways in PTC (p-value<0.05)

KEGG-ID	Term	Count	p-value	DEGs
hsa04514	Cell adhesion molecules (CAMs)	35	1.80E-06	HLA-DQB1, CLDN16, CLDN8, CLDN9, HLA-DRB1, CLDN10, ITGB2, CLDN11, CDH2, SDC4, CDH3, CDH4, SDC2, ITGAM, NRCAM, PVRL3, ESAM, CD4, CNTNAP1, HLA-DOA, SELPLG, NEGR1, ICAM1, PTPRF, SELL, CD276, NLGN2, CD40, HLA-DQA1, NCAM1, SIGLEC1, CD86, CD58, CLDN1, VCAN
hsa00280	Valine, leucine and isoleucine degradation	16	4.75E-05	BCAT1, ALDH6A1, ACADSB, ACADM, EHHADH, IL4I1, ACAT1, ALDH7A1, MUT, IVD, ALDH1B1, MCEE, AOX1, ALDH2, ALDH9A1, PCCA
hsa00640	Propanoate metabolism	13	9.53E-05	ALDH6A1, ACADM, SUCLG1, EHHADH, ACACB, ACAT1, ALDH7A1, MUT, ALDH1B1, MCEE, ALDH2, ALDH9A1, PCCA
hsa00380	Tryptophan metabolism	12	0.003512	WARS, TDO2, ALDH7A1, CYP1B1, ALDH1B1, EHHADH, AOX1, OGDHL, ALDH2, IL4I1, ACAT1, ALDH9A1
hsa00071	Fatty acid metabolism	12	0.003512	ACADSB, ALDH7A1, ACSL1, ACADM, ALDH1B1, EHHADH, ALDH2, ADH5, ADH1B, ACAT1, ALDH9A1, ACSL5
hsa05200	Pathways in cancer	53	0.005205	E2F1, PDGFA, PPARG, TGFB1, ACVR1B, CDKN2B, PAX8, TGFA, RARA, CCNA1, FGF1, TPR, PRKCA, EGFR, WNT10A, RELA, RUNX1T1, RXRG, CCND1, CRKL, MAPK8, LAMC2, LAMC1, BID, FGFR2, KITLG, BCL2L1, KIT, TCF7L1, LAMB3, BCL2, AXIN2, TRAF6, RUNX1, TRAF5, CSF1R, PIK3R2, FN1, FZD8, EPAS1, TGFBR1, MET, ITGA2, BIRC5, ITGA3, MAPK10, STAT1, FZD5, DVL1, RASSF5, CBLB, LAMA5, BAX
hsa04512	ECM-receptor interaction	18	0.010575	TNC, ITGB4, ITGA2, ITGA3, SDC4, SDC2, CD47, LAMB3, CD36, CD44, LAMA5, COMP, ITGB6, LAMC2, AGRN, LAMC1, COL1A1, FN1
hsa05222	Small cell lung cancer	18	0.010575	E2F1, FHIT, RELA, RXRG, ITGA2, ITGA3, BCL2L1, CCND1, LAMB3, CDKN2B, LAMA5, BCL2, LAMC2, LAMC1, TRAF6, TRAF5, FN1, PIK3R2
hsa00903	Limonene and pinene degradation	6	0.014691	ALDH7A1, ALDH1B1, EHHADH, ALDH2, LCLAT1, ALDH9A1
hsa04920	Adipocytokine signaling pathway	15	0.014783	NFKBIE, RELA, RXRG, ADIPOR2, ACACB, MAPK10, PPARGC1A, IRS1, PRKCQ, TNFRSF1A, ACSL1, CD36, MAPK8, PRKAA2, ACSL5
hsa00310	Lysine degradation	11	0.021106	ALDH7A1, SETDB2, ALDH1B1, EHHADH, SETMAR, OGDHL, ALDH2, ACAT1, ALDH9A1, SUV420H1, AASDH
hsa05210	Colorectal cancer	17	0.022446	EGFR, FZD8, TGFBR1, MET, BIRC5, MAPK10, FZD5, TCF7L1, TGFB1, DVL1, ACVR1B, CCND1, BAX, BCL2, MAPK8, AXIN2, PIK3R2
hsa00561	Glycerolipid metabolism	11	0.024512	DGKA, LPL, ALDH7A1, ALDH1B1, LIPG, ALDH2, LCLAT1, DGKI, AGPAT4, GPAM, ALDH9A1
hsa00620	Pyruvate metabolism	10	0.029766	ME1, ALDH7A1, ME3, ALDH1B1, ALDH2, ACYP1, ACACB, ACAT1, ALDH9A1, PC
hsa04060	Cytokine-cytokine receptor interaction	40	0.036841	CXCL1, TNFRSF21, PDGFA, TNFRSF12A, TNFRSF25, CCR1, IL21R, KITLG, KIT, CCL28, TGFB1, TNFRSF1A, ACVR1B, CCL22, TNFRSF11B, IL4R, IL1RAP, TPO, GHR, CSF1R, EGFR, TGFBR1, MET, LIFR, CD40, TNFSF9, CCL18, INHBB, TNFRSF10A, TNFRSF10C, CCL13, TNFRSF10B, TNFSF11, CXCL14, IL20RA, CXCL16, NGFR, BMP7, MPL, BMPR1A
hsa04670	Leukocyte trans-endothelial migration	21	0.037441	PRKCA, CLDN8, CLDN16, ICAM1, CLDN9, NCF2, GNAI1, SIPA1, ACTN1, CLDN10, ITGB2, CLDN11, ITGAM, RASSF5, CYBB, MAPK13, PTK2B, CLDN1, ESAM, MSN, PIK3R2
hsa05416	Viral myocarditis	14	0.049521	BID, HLA-DQB1, ICAM1, HLA-DRB1, ITGB2, CD40, HLA-DQA1, CCND1, CD86, CD55, FYN, SGCD, HLA-DOA, MYH10

(enrichment score = −0.983), daunorubicin (enrichment score = −0.932), mitoxantrone (enrichment score = −0.975), GW-8510 (enrichment score = −0.914), alsterpaullone (enrichment score = −0.963), doxorubicin (enrichment score = −0.962) and irinotecan (enrichment score = −0.952). The small molecules of trihexyphenidyl (enrichment score = 0.919) and sulmazole (enrichment score = 0.912) were associated with highly significant positive score.

Discussion

In this study, we re-analyzed the gene expression data in PTC tissues with normal controls downloaded from GEO database, in an attempt to expanding our understanding of this disease, and then identified biologically active small molecules capable to reverse PTC using computational bioinformatics methods. Results show that expressions of total

Table 5 The top 20 significant small molecules

Perturbagen	Enrichment score	*p*-value
Genistein	0.545	0
LY-294002	0.289	0
Camptothecin	−0.983	0.00002
Daunorubicin	−0.932	0.00002
Mitoxantrone	−0.975	0.00004
GW-8510	−0.914	0.0001
Alsterpaullone	−0.963	0.00014
Doxorubicin	−0.962	0.00016
Irinotecan	−0.952	0.00022
Mebendazole	−0.847	0.00024
CP-690334-01	0.693	0.00028
Medrysone	−0.757	0.0004
Trihexyphenidyl	0.919	0.00114
Ginkgolide A	−0.839	0.00119
Anisomycin	0.828	0.00133
Thioridazine	−0.408	0.00143
Sulmazole	0.912	0.00146
Thiamazole	0.706	0.00173
Remoxipride	−0.819	0.00201
Diethylstilbestrol	0.695	0.00209
Cinchonine	0.813	0.00235

2,154 genes were altered in PTC at a FDR of 0.01. These 2,154 DEGs were significantly enriched in 17 pathways, including pathways associated with signal transduction, tumorigenesis and lipid or amino acid metabolism. In addition, we identified large amount of small molecules which can provide new ideas for the therapeutic studies in PTC.

From the result of GO enrichment analysis in molecular function ontology, we could observe an effect of PTC on members of S100 protein family (S100A11, S100A6, S100A1 and S100B). S100 proteins are a family of intracellular calcium-binding proteins ability to form homodimers, heterodimers and oligomeric assemblies and are characterized by tissue and cell-specific expression [31–33]. There is growing evidence that expression of S100 proteins is altered in many tumors, often in association with tumor progression, and they are therefore potentially important tumor biomarkers and therapeutic targets.

S100A11 is overexpressed in uterine smooth muscle tumors [34], anaplastic large cell lymphomas [35] and pancreatic tumors [36], while significantly down-regulated in bladder tumors [37]. S100A6 is overexpressed in melanoma, pancreatic and colorectal cancers, and expression has been shown to correlate with tumor growth and metastatic progression suggesting a potential role for S100A6 in the development of malignancy [38, 39]. It is however down-regulated in prostate cancer and medulloblastoma. Expression of S100A1 is low in most normal tissues, but up-regulated un cancers of kidneys, skin and ovary [40, 41]. S100B is over-expressed in anaplastic astrocytomas and glioblastomas [42], and melanomas [39]. It is one of the best-studied biomarker for melanoma and has been validated in a clinical setting [43].

We also observed the enrichment of HMGA (high-mobility group A) family genes (HMGA1 and HMGA2) involved in cell aging. HMGA proteins have roles in assembling or modulating macromolecular complexes that are involved in various biological processes; they can bind to specific structures in DNA, modifying its conformation and consequently facilitating the binding of a group of transcription factors [44]. HMGA proteins have been found to be abundant in several malignant neoplasias, including colorectal, prostate, cervical, lung, breast and thyroid carcinoma [45, 46]. Overexpression of HMGA1 has been suggested to be a diagnostic indicator for human prostate tumors, thyroid neoplasia, colorectal cancers and breast carcinoma [47–49]. It has been suggested that the aberrant expression of HMGA play a role in inhibiting the functions of p53 family members in thyroid cancer cells [50].

The most obvious and well-known action of thyroid is an increase in basal energy expenditure obtained acting on protein, carbohydrate and lipid metabolism [51]. As expected, pathway enrichment analysis revealed that many metabolic pathways were dysregulated in PTC tissue, such as valine, leucine and isoleucine degradation, tryptophan metabolism, and fatty acid metabolism and so on. The dysregulated pathways may lead to the difference of metabolin between PTC patients and normal controls, and then lead to early diagnosis of this disease.

There are several important implications of this work. The identification of a group of small molecules with potential therapeutic efficacy for PTC is an important observation. From Table 5, the small molecules of camptothecin, daunorubicin, mitoxantrone, GW-8510, alsterpaullone, doxorubicin and irinotecan was associated with highly significant negative score, suggesting that these small molecules were capable of targeting PTC. Most of these small molecules were reported to have anti-tumor effect and some of them has been applied in clinical treatment.

Camptothecin class of compounds has been demonstrated to be effective against a broad spectrum of tumors [52]. Two camptothecin analogues, topotecan and irinotecan have been approved and are used in cancer chemotherapy today [53]. Daunorubicin is chemotherapeutic of anthracycline family that is given as a treatment for some types of cancer. It is most commonly used to treat specific types of leukaemia, such as acute myeloid leukemia and acute lymphocytic leukemia [54, 55]. Mitoxantrone is an anthracenedione antineoplastic agent which used in the treatment of certain types of cancer, mostly metastatic breast cancer, acute myeloid leukemia and multiple sclerosis [56–58]. GW8510 is a 3′substituted indolone that was development as an inhibitor of cyclin-dependent kinase [59]. A recent study reported

GW8510 may increase insulin expression in pancreatic alpha cells [60]. Alsterpaullone is also a cyclin-dependent kinase inhibitor. It induces apoptosis by early activation of both caspase-8 and -9 [61]. Doxorubicin is commonly used in the treatment of a wide range of cancers, including thyroid cancer [62, 63]. Further study of these small molecules may provide the groundwork for developing new therapies for treatment of PTC.

Overall, we have demonstrated that 2,154 genes which involved in metabolism and protein binding were differentially expressed in PTC samples compared to normal controls. Pathways associated with signal transduction, tumorigenesis and lipid or amino acid metabolism were dysregulated in PTC samples. Besides, we identified a group of small molecules which may be exploited as adjuvant drug to improve therapeutic effect for PTC. These drugs are clearly a direction that warrants additional consideration.

Declaration of Conflicting Interests The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Hundahl SA, Fleming ID, Fremgen AM, Menck HR (1998) A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985–1995. Cancer 83(12):2638–2648. doi:10.1002/(SICI)1097-0142(19981215)83:12<2638::AID-CNCR31>3.0.CO;2-1
2. Leenhardt L, Grosclaude P, Cherie-Challine L (2004) Increased incidence of thyroid carcinoma in france: a true epidemic or thyroid nodule management effects? Report from the French Thyroid Cancer Committee. Thyroid 14(12):1056–1060. doi:10.1089/thy.2004.14.1056
3. Davies L, Welch HG (2006) Increasing incidence of thyroid cancer in the United States, 1973–2002. JAMA 295(18):2164–2167. doi:10.1001/jama.295.18.2164
4. Wang Y, Wang W (2012) Increasing incidence of thyroid cancer in Shanghai, China, 1983–2007. Asia Pac J Public Health. doi:10.1177/1010539512436874
5. Olaleye O, Ekrikpo U, Moorthy R, Lyne O, Wiseberg J, Black M, Mitchell D (2011) Increasing incidence of differentiated thyroid cancer in South East England: 1987–2006. Eur Arch Otorhinolaryngol 268(6):899–906. doi:10.1007/s00405-010-1416-7
6. Ramirez-Vick M, Nieves-Rodriguez M, Lugaro-Gomez A, Perez-Irizarry J (2011) Increasing incidence of thyroid cancer in Puerto Rico, 1985–2004. P R Health Sci J 30(3):109–115
7. Capezzone M, Morabito E, Bellitti P, Giannasio P, De Sanctis D, Bruno R (2007) Increasing incidence of thyroid cancer in Basilicata: an Italian study. J Endocrinol Invest 30(6):507–512
8. Hall SF, Walker H, Siemens R, Schneeberg A (2009) Increasing detection and increasing incidence in thyroid cancer. World J Surg 33(12):2567–2571. doi:10.1007/s00268-009-0226-9
9. Sanabria A, Dominguez LC, Vega V, Osorio C (2011) Prognosis of patients with thyroid cancer who do not undergo surgical treatment: a SEER database analysis. Clin Transl Oncol 13(9):692–696. doi:10.1007/s12094-011-0716-8
10. Cady B, Rossi R (1988) An expanded view of risk-group definition in differentiated thyroid carcinoma. Surgery 104(6):947–953
11. Santacroce L, Gagliardi S, SA K (2009) Thyroid, papillary carcinoma: treatment & medication. eMedicine Oncol 12:17
12. Legakis I, Syrigos K (2011) Recent advances in molecular diagnosis of thyroid cancer. J Thyroid Res 2011:384213. doi:10.4061/2011/384213
13. Xing M (2007) BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev 28(7):742–762. doi:10.1210/er.2007-0007
14. Fusco A, Grieco M, Santoro M, Berlingieri MT, Pilotti S, Pierotti MA, Della Porta G, Vecchio G (1987) A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. Nature 328(6126):170–172. doi:10.1038/328170a0
15. Santoro M, Melillo RM, Grieco M, Berlingieri MT, Vecchio G, Fusco A (1993) The TRK and RET tyrosine kinase oncogenes cooperate with ras in the neoplastic transformation of a rat thyroid epithelial cell line. Cell Growth Differ 4(2):77–84
16. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW 2nd, Tallini G, Kroll TG, Nikiforov YE (2003) RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab 88(5):2318–2326
17. Placzkowski KA, Reddi HV, Grebe SK, Eberhardt NL, McIver B (2008) The role of the PAX8/PPARgamma fusion oncogene in thyroid cancer. PPAR Res 2008:672829. doi:10.1155/2008/672829
18. Malaguarnera R, Vella V, Vigneri R, Frasca F (2007) p53 family proteins in thyroid cancer. Endocr Relat Cancer 14(1):43–60. doi:10.1677/erc.1.01223
19. Knauf JA, Kuroda H, Basu S, Fagin JA (2003) RET/PTC-induced dedifferentiation of thyroid cells is mediated through Y1062 signaling through SHC-RAS-MAP kinase. Oncogene 22(28):4406–4412. doi:10.1038/sj.onc.1206602
20. Mitsutake N, Miyagishi M, Mitsutake S, Akeno N, Mesa C Jr, Knauf JA, Zhang L, Taira K, Fagin JA (2006) BRAF mediates RET/PTC-induced mitogen-activated protein kinase activation in thyroid cells: functional support for requirement of the RET/PTC-RAS-BRAF pathway in papillary thyroid carcinogenesis. Endocrinology 147(2):1014–1019. doi:10.1210/en.2005-0280
21. He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A (2005) The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci U S A 102(52):19075–19080. doi:10.1073/pnas.0509603102
22. Team RDC (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing.
23. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4(2):249–264. doi:10.1093/biostatistics/4.2.249
24. Smyth GK (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Statistical applications in genetics and molecular biology 3:Article3. doi:10.2202/1544-6115.1027
25. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B (Methodological) 57:289–300
26. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25(1):25–29. doi:10.1038/75556
27. Yu G, Wang LG, Han Y, He QY (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 16(5):284–287. doi:10.1089/omi.2011.0118

28. Kanehisa M (2002) The KEGG database. Novartis Found Symp 247:91–101, discussion 101-103, 119-128, 244-152
29. da Huang W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4(1):44–57. doi:10.1038/nprot.2008.211
30. Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J, Brunet JP, Subramanian A, Ross KN, Reich M, Hieronymus H, Wei G, Armstrong SA, Haggarty SJ, Clemons PA, Wei R, Carr SA, Lander ES, Golub TR (2006) The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science 313(5795):1929–1935. doi:10.1126/science.1132939
31. Donato R (2001) S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 33(7):637–668
32. Heizmann CW, Fritz G, Schafer BW (2002) S100 proteins: structure, functions and pathology. Front Biosci 7:d1356–1368
33. Roth J, Vogl T, Sorg C, Sunderkotter C (2003) Phagocyte-specific S100 proteins: a novel group of proinflammatory molecules. Trends Immunol 24(4):155–158
34. Kanamori T, Takakura K, Mandai M, Kariya M, Fukuhara K, Sakaguchi M, Huh NH, Saito K, Sakurai T, Fujita J, Fujii S (2004) Increased expression of calcium-binding protein S100 in human uterine smooth muscle tumours. Mol Hum Reprod 10(10):735–742. doi:10.1093/molehr/gah100
35. Rust R, Visser L, van der Leij J, Harms G, Blokzijl T, Deloulme JC, van der Vlies P, Kamps W, Kok K, Lim M, Poppema S, van den Berg A (2005) High expression of calcium-binding proteins, S100A10, S100A11 and CALM2 in anaplastic large cell lymphoma. Br J Haematol 131(5):596–608. doi:10.1111/j.1365-2141.2005.05816.x
36. Ohuchida K, Mizumoto K, Ohhashi S, Yamaguchi H, Konomi H, Nagai E, Yamaguchi K, Tsuneyoshi M, Tanaka M (2006) S100A11, a putative tumor suppressor gene, is overexpressed in pancreatic carcinogenesis. Clin Cancer Res 12(18):5417–5422. doi:10.1158/1078-0432.CCR-06-0222
37. Memon AA, Sorensen BS, Meldgaard P, Fokdal L, Thykjaer T, Nexo E (2005) Down-regulation of S100C is associated with bladder cancer progression and poor survival. Clin Cancer Res 11(2 Pt 1):606–611
38. Lesniak W, Slomnicki LP, Filipek A (2009) S100A6—new facts and features. Biochem Biophys Res Commun 390(4):1087–1092. doi:10.1016/j.bbrc.2009.10.150
39. Salama I, Malone PS, Mihaimeed F, Jones JL (2008) A review of the S100 proteins in cancer. Eur J Surg Oncol 34(4):357–364. doi:10.1016/j.ejso.2007.04.009
40. DeRycke MS, Andersen JD, Harrington KM, Pambuccian SE, Kalloger SE, Boylan KL, Argenta PA, Skubitz AP (2009) S100A1 expression in ovarian and endometrial endometrioid carcinomas is a prognostic indicator of relapse-free survival. Am J Clin Pathol 132(6):846–856. doi:10.1309/AJCPTK87EMMIKPFS
41. Cossu-Rocca P, Contini M, Brunelli M, Festa A, Pili F, Gobbo S, Eccher A, Mura A, Massarelli G, Martignoni G (2009) S-100A1 is a reliable marker in distinguishing nephrogenic adenoma from prostatic adenocarcinoma. Am J Surg Pathol 33(7):1031–1036. doi:10.1097/PAS.0b013e31819c6ff9
42. Camby I, Nagy N, Lopes MB, Schafer BW, Maurage CA, Ruchoux MM, Murmann P, Pochet R, Heizmann CW, Brotchi J, Salmon I, Kiss R, Decaestecker C (1999) Supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas are characterized by a differential expression of S100 proteins. Brain Pathol 9(1):1–19
43. Gogas H, Eggermont AM, Hauschild A, Hersey P, Mohr P, Schadendorf D, Spatz A, Dummer R (2009) Biomarkers in melanoma. Ann Oncol 20(Suppl 6):vi8–13. doi:10.1093/annonc/mdp251
44. Thanos D, Du W, Maniatis T (1993) The high mobility group protein HMG I(Y) is an essential structural component of a virus-inducible enhancer complex. Cold Spring Harb Symp Quant Biol 58:73–81
45. Fusco A, Fedele M (2007) Roles of HMGA proteins in cancer. Nat Rev Cancer 7(12):899–910. doi:10.1038/nrc2271
46. Peluso S, Chiappetta G (2010) High-mobility group A (HMGA) proteins and breast cancer. Breast Care (Basel) 5(2):81–85. doi:10.1159/000297717
47. Abe N, Watanabe T, Sugiyama M, Uchimura H, Chiappetta G, Fusco A, Atomi Y (1999) Determination of high mobility group I(Y) expression level in colorectal neoplasias: a potential diagnostic marker. Cancer Res 59(6):1169–1174
48. Tamimi Y, van der Poel HG, Karthaus HF, Debruyne FM, Schalken JA (1996) A retrospective study of high mobility group protein I(Y) as progression marker for prostate cancer determined by in situ hybridization. Br J Cancer 74(4):573–578
49. Masciullo V, Baldassarre G, Pentimalli F, Berlingieri MT, Boccia A, Chiappetta G, Palazzo J, Manfioletti G, Giancotti V, Viglietto G, Scambia G, Fusco A (2003) HMGA1 protein over-expression is a frequent feature of epithelial ovarian carcinomas. Carcinogenesis 24(7):1191–1198. doi:10.1093/carcin/bgg075
50. Frasca F, Rustighi A, Malaguarnera R, Altamura S, Vigneri P, Del Sal G, Giancotti V, Pezzino V, Vigneri R, Manfioletti G (2006) HMGA1 inhibits the function of p53 family members in thyroid cancer cells. Cancer Res 66(6):2980–2989. doi:10.1158/0008-5472.CAN-05-2637
51. Pucci E, Chiovato L, Pinchera A (2000) Thyroid and lipid metabolism. Int J Obes Relat Metab Disord 24(Suppl 2): S109–112
52. Liu LF, Desai SD, Li TK, Mao Y, Sun M, Sim SP (2000) Mechanism of action of camptothecin. Ann N Y Acad Sci 922:1–10
53. Bomgaars L, Berg SL, Blaney SM (2001) The development of camptothecin analogs in childhood cancers. Oncologist 6(6):506–516
54. Souhami RL, Prankerd TA (1970) Daunorubicin in acute leukaemia. Postgrad Med J 46(535):272–275
55. Gottlieb AJ, Weinberg V, Ellison RR, Henderson ES, Terebelo H, Rafla S, Cuttner J, Silver RT, Carey RW, Levy RN et al (1984) Efficacy of daunorubicin in the therapy of adult acute lymphocytic leukemia: a prospective randomized trial by cancer and leukemia group B. Blood 64(1):267–274
56. Fox EJ (2006) Management of worsening multiple sclerosis with mitoxantrone: a review. Clin Ther 28(4):461–474. doi:10.1016/j.clinthera.2006.04.013
57. Periti P, della Cuna GR, Pannuti F, Mazzei T, Preti P, Martoni A, Mini E (1985) First-line combination chemotherapy with mitoxantrone and cyclophosphamide in advanced breast cancer. Invest New Drugs 3(2):167–171
58. Ho AD, Lipp T, Ehninger G, Illiger HJ, Meyer P, Freund M, Hunstein W (1988) Combination of mitoxantrone and etoposide in refractory acute myelogenous leukemia—an active and well-tolerated regimen. J Clin Oncol 6(2):213–217
59. Johnson K, Liu L, Majdzadeh N, Chavez C, Chin PC, Morrison B, Wang L, Park J, Chugh P, Chen HM, D'Mello SR (2005) Inhibition of neuronal apoptosis by the cyclin-dependent kinase inhibitor GW8510: identification of 3′ substituted indolones as a scaffold for the development of neuroprotective drugs. J Neurochem 93(3):538–548. doi:10.1111/j.1471-4159.2004.03004.x
60. Fomina-Yadlin D, Kubicek S, Vetere A, He KH, Schreiber SL, Wagner BK (2012) GW8510 increases insulin expression in pancreatic alpha cells through activation of p53 transcriptional activity. PLoS One 7(1):e28808. doi:10.1371/journal.pone.0028808

61. Lahusen T, De Siervi A, Kunick C, Senderowicz AM (2003) Alsterpaullone, a novel cyclin-dependent kinase inhibitor, induces apoptosis by activation of caspase-9 due to perturbation in mitochondrial membrane potential. Mol Carcinog 36(4):183–194. doi:10.1002/mc.10114
62. Shimaoka K, Schoenfeld DA, DeWys WD, Creech RH, DeConti R (1985) A randomized trial of doxorubicin versus doxorubicin plus cisplatin in patients with advanced thyroid carcinoma. Cancer 56(9):2155–2160
63. Williams SD, Birch R, Einhorn LH (1986) Phase II evaluation of doxorubicin plus cisplatin in advanced thyroid cancer: a Southeastern Cancer Study Group Trial. Cancer Treat Rep 70(3):405–407