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Case Report

Analysis of the Gene Pattern Expression Pattern After Oral Administration of (Human α-lactalbumin Made Lethal to Tumor Cells (HAMLET) in a Thyroid Cancer Diagnosed Individual

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Abstract

Thyroid cancer is one of the most common endocrinological malignancies, being 1% of all malignancies. The incidence has been increasing worldwide during the last decades, especially in industrial countries, because refractory to radioiodine treatment, tumor recurrence or drug resistance. In recent years important molecular pathways contributing to tumor progression and worse survival rates have been identified in iodine-refractory differentiated thyroid carcinoma (DTC). Among the molecular therapeutics to target these specific oncogenic pathways, dehypermethylation drugs (methylation inhibitors) have showed more promising outcomes in comparison with conventional treatments. Furthermore, therapeutic effects of HAMLET have been demonstrated in human skin papillomas and bladder cancers. HAMLET limits the progression of human glioblastomas, with no evidence of toxicity for normal brain or bladder tissue. In the present study we aimed to analyze the gene expression pattern of the effect of the oral administration of HAMLET (complex of lipid-protein) in a patient with thyroid cancer before surgery and radio iodination treatment. Under the clinic settings, established for the study, and using DNA microarrays, we have determined the pattern of genes up and regulated, and the functionality of these genes through REACTOME base data. Altogether, we have found that, the effect of the oral administration of HAMLET is toward influence a higher number of down regulated genes (around 70 genes), and no difference of the up regulated genes with respect to healthy individual. The number of pathways affected by both up (n = 109), and down regulated genes (n = 41). Mostly affecting chromatin organization. Developmental biology, circadian clock, Metabolism of proteins, immune system, DNA repair, organelle biogenesis and maintenance, cell cycle, gene expression (transcription). Therefore, our data are in agreement with the fact that the integration of epigenetic and genetic targeted therapies represent a promising avenue for prophylactic treatments against tumor progression.

Keywords: Thyroid Cancer; Epigenetic and Genetic Mechanism; Hamlet; Immune System; Cell Cycle

Introduction

Cancer, a multifactorial disease characterized by a dysregulated proliferation of cells, It is a serious public health problem. It is one of the most frequent cases of death, rising to 14 million worldwide. Indeed it represents the second cause of death in developd countries and undeveloped countries [1]. Therapeutic effects of HAMLET have been demonstrated in human skin papilloma and bladder cancers. HAMLET limits the progression of human Glioblastomas, and no evidence of toxicity for normal brain or bladder tissue [2]. Amogn the factores that predisponse to cancer, the diet, the lifestyle and the genetic to name a few. The immune environment and the environment surrounding the cancerous cells plays a pivotal role in the progression of the disease. The immune environment and the environment surrounding the cancerous cells plays a pivotal role in the progression of the disease. The immune system has two major checkpoints PD1, and CTLA4 [3], In disease, CD4+ T cells of the immune system have the capacity to selectively recognize and kill pathogens or unhealthy cells, including cancer cells. T lymphocytes are able to follow a coordinated program of innate and adaptive immune response to overcome cancer antigens, and or cancer's ability to resist the immune responses [3]. But what is th PD-1/PD-L1 pathway?, the PD-1 (programmed cell death-1) recèptor is expressed on the surface of activated T cells. Its ligands, PD-L1 and PD-L2 are expressed on the surface of dendritic cells or macrophages. PD-1/PD-L1 interaction ensures that the immune system is activated only at the appropriate time in order to minimize autoimmune inflammation [2,3]. A potential therapy described since some decades ago is the alpha-lacto albumin, a small protein that possess several factty acid binding sites, a molten globule like state conformation complexed with a C18:1 fatty:acid oleic acid latter termed as human alpha-lacto albumin made lethal to tumor cells [4,5]. Current therapies are based for example on monoclonal antibody therapies against PD-1 and PD-L1, including: Nivolumab, Pembrolizumab, an anti-PD1 drug approved for previously treated metastatic melanoma and squamous non-small cell lung cancer [6,7]. Furthermore, one of the most used nowadays is the adoptive T cell therapy which involves tumor-specific T cells from patients and then expanding these *ex vivo*. Thereafter, the tumor-specific T cells can then be infused into patients to give their immune system the ability to overwhelm remaining tumor cells, and Tumor specificity must be induced in PBLs either through antigen specific expansion or genetic engineering. Another type of adoptive cell therapy is CAR T cell therapy. T cells are engineered to express chimeric antigen receptors (CARs) that recognize cancer specific antigens [8-10]. In this way, researchers can manipulate and prime it to recognize and kill tumor cells that hamper escape immune detection. Furthermore, a potential therapy described since some decades ago is the alpha-lacto albumin, a small protein that possess several factty acid binding sites, a molten globule like state conformation complexed with a C18:1 fatty:acid oleic acid latter termed as human alpha-lacto albumin made lethal to tumor cells [4,5,11]. It has several other properties, such as antimicrobial, antiviral properties, and it is able to inhibit hem agglutination mediated by the Reovirus strain type 3 Dearing (T3D) (Hakansson., *et al*. 2000; Pettersson., *et al*. 2006) [4,5]. Of relevance is that experimental evidences *in vivo* have also shown that high doses of alpha-LA have effects in signalization pathways in cellular apoptosis and necrosis, enhances the expression levesl of active caspase 3,, caspase 8. Another effect observed is that alpha-LA enhances the expression levesl of cytochrome c, exracellular signals regulated kiase (ERK1/2), and c-jun N-terminal kinase (JNK) activation. without changing the protein levels, but suppressing the protein level of Bcl2 [4,5]. The mechanism of action of HAMLET proposed is that it involves the interaction of the alpha-LA: oleic acid complex, or named also a lipoprotides with membranes increasing the fluidity of membranes, resulting in the disruption of the plasma membrane is a major factor to liporprotide toxicity towards cancer cells. Interestingly, extracellular Ca2+ influx activate the plasma membrane repair system, and the removal of Ca2+ from the medium enhanced the lipoprotides killing effect [4,5]. Finally, the complex of this proteins with lipids that possess immunomodulatory activity. Indeed, alpha-LA has a direct effect on B lymphocyte function, and is also able to suppress T cell-dependent and T cell-independent responses [12,13]. In the light of these studies, and in relation in particular to the tyroid carcinoma [14,15] recent studies have re`ported that E-Cadherin, CD56, and galectin-3 could be useful as biomarkers a marker that has been implicated in normal cellular proliferation and apoptosis [16-19]. By other hand, it is of relevance is that is in the tumor progression, iodine refractory differentiated thyroid carcinoma (DTC) that have be able to identify the molecular pathways that dictating the progression of the malignancy (20-. It has been further contributing to the learning and understanding of the molecular mechanism of both survival, resistance, mutations, with the consequent development

of molecular therapeutics to target these specific oncogenic pathways. Thus it is very interesting that for aggressive thyroid cancer (i.e. anaplastic) dehypermethylation drugs (methylation inhibitors) have showed more promising outcomes in comparison with conventional treatments [20-31], The objective of this study is to analyze the gene expression pattern before and after oral administration of HAMLET in an patient with thyroid cancer using DNA microarrays. The functionality of the genes analyzed with Reactome data base. Altogether, we found that oral administration of HAMLET in patient (s) with thyroid cancer increase the number of up and down regulation of genes, their functionality (higher number of reactions and probability to affect certain pathway, << p values), e.g. Immune system (FOXP3, IL17RF), cell cycle, chromatin organization, gene expression (transcription), metabolism of RNA, metabolism of proteins, muscle contraction, transport of small molecules, circadian clock, reactions that might serve as target to improve the hamlet therapeutic use for other cancers.

Case Report

The study and all medical research procedures with human subjects, including research with identifiable human material and data, were approved by the Research Ethics Committee of Zacatecas, "Luz González Cosió" General Hospital CONBIOETICA-32-CEI-001-20180807. Untreated healthy patient and patient with condition treated with HAMLET gave signed written consent. Adverse events and reactions were systematically monitored throughout the study. Clinic parameters (hematic biometry, urine determination of parameters were carried out in a private Lab of Clinic Analyses. LABCON, Col. Encantada. Guadalupe, Zacatecas. MX).

Procedure

Individual diagnosed with thyroid cancer were instructed to take a 30 milliliter dose HAMLET early in the morning every four days (Figure 1A) for two weeks. At each time point, feces and urine were collected for further clinical analysis. Individual were advised not to eat food before oral administration of HAMLET. Similarly, were instructed not to drink any probiotics during the study period. Blood was collected only before and after the last doses of HAMLET (Figure 1).

Gene expression pattern determination

RNA extraction from whole blood was performed using a KIT and following the manufacturer's instructions (AMBION, Life technology). The integrity of the RNA was recorded in an agarose gel 1% in TBE 0.5 X and stained with Green loading buffer. The preparation of the microarrays was carried out according to the standardized method in the Microarray Unit of the Institute of Cellular Physiology of the UNAM: Mexico City. Briefly, cDNA was prepared from the RNA of the control and test samples before and after oral administration of HAMLET. Each CDNA from the control (M1 and M3) was hybridized with the human chip (n = 10,000 genes) as well as from the problem (R1 and ·R2). The results obtained from image quantification were analyzed with Genarise. The lists of regulated UP and/or DOWN genes are reported for two Z-Score cuts (1.5 and 2.0). Statistical analyzes were performed using Graph Pad Prism 6.0 (CA, USA) using nonparametric analysis of variance (ANOVA). A $p \leq 0.05$ was considered significant.

Results

Oral administration of HAMLET induces a greater number of up-regulated (Up) than down-regulated (Down) genes in a patient with thyroid cancer.

The analysis of oral administration of HAMLET in an individual diagnosed with cancer was determined following the protocol in Figure 1 and using microarray technology. Total RNA was prepared following the manufacturer's instructions (Ambion, USA) (Figure 1). The cDNA of both the control and the problem individual, respectively, was hybridized with the DNA chip (n = 10,000 genes) (Microarray Unit, IFC.UNAM).

The first analysis of how many genes are down or up regulated after oral administration of HAMLET to an individual diagnosed with thyroid cancer, showed that most of the genes either in the control (H1 vs H1'), or the patient with thyroid cancer (P1 vs P1'), are downregulated (Table 1). Interestingly when comparing the patient before and after oral administration, the number of genes for one side the number of UP regulated genes were lowest, and after oral administration the number of genes down regulated were the highest than the rest of the compared groups. Furthermore, the while the number of up regulated genes in the healthy versus unhealthy and before oral administration (n = 232) the number of down regulated genes were the lowest (Table 1). The gene expression pattern of the healthy individual versus the patient before the oral administration with HAMLET was compared. Thus, the number of upregulated genes was greater (n = 275) than the number of downregulated genes (n = 125) while that after two weeks and at the 4th dose of administration with

HAMLET the number of genes that were upregulated versus the healthy individual was 230 compared to the number of genes that were downregulated (n = 172).

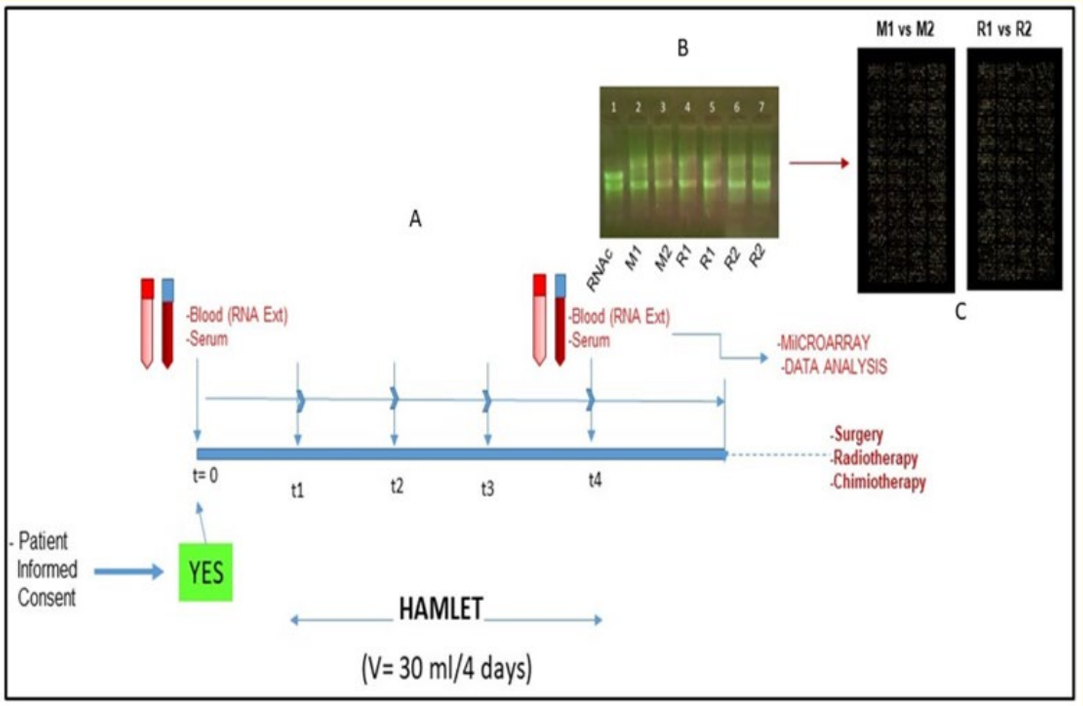

Figure 1: Scheme protocol design for the gene expression pattern before and after oral administration of HAMLET to an individual diagnosed with thyroid cancer. To approach this, and after the written consent informed of the participant (s) (health and sick individual) (A). RNA was prepared from whole blood extracted and analyzed in agarose gel 1% in TBE 0.5X and stained with Green loading buffer. (B) cDNA was prepared following manufacturer's instructions. Gene expression pattern analysed using microarrays tecnologies, crude extracted data of DNA-cDNA microarrays (C) (Unit of IFC. UNAM.).

Table 1. Most of the genes are down regulated after oral administration of HAMLET in a patient with thyroid cancer.

Sample	UP	DOWN
H1 vs H1'	252	159
P1 vs P1'	167	200
H1 vs P1	275	125
H1 vs P1'	209	181
H1' vs P1	232	83
H1' vs P1'	230	172

*Genarise program to analyze the gene expression pattern reported for two Z score cuts (2 SD) of the up and down regulated. Statiscal analyses were performed using Gtaph Pad Prism (Ca, USA). A p< 0.05 was considered significant. H1 and H1', healthy individual. P1 and P1' individual with thyroid cancer before and treatment with HAMLET.

Up and down regulation genes after oral administration of HAMLET in patient with thyroid cancer.

The effect of the oral administration of HAMLET to a patient with Thyroid cancer was determined using the microarrays technology (IFC, UNAM. CDAD MEXICO) and expressed as heat maps, indicated by the fold of change as the Z score of the UP regulated (2.87 to 4) and Down regulated (-2.0 to -4.87).

The gene expression pattern of the most up and down regulated in a healthy individual without treatment is shown in Figure 2A. Up regulated genes, from 3.5 to 4.0 encoding molecular components of the myelopoiesis (CXXC5), oxygen transport (HBD), nucleolus organization (ACTR6), cell cycle progression (SIVA), signal transduction (PDE4B), Down regulated genes with a Z values of -2.00 to -2.5encoding proteins involved in cell-cell adhesion and motility (LPP), in vesicle trafficking (FIG4), cellular response to stimuli (VN1R1), respiratory chain (NDUFAG), and actin-binding proteins (ACTR3) (see supplementary material on the identity of the ten most up or down regulated genes).

Secondly, the patient with thyroid cancer before (P1) and after treatment with HAMLET (P1'). From the analysis of the Up and down regulated genes. Up regulated genes encoding genes with a Z score value of 3.5 to 4.0 fold change, involved in Hypothyroidism disease (C10orf88), cellular response to stimuli (ARNTL2), cell cycle (CCNC), Immune system (FOXP3), transport of small molecules (SLC2AS3). Down regulated genes with a Zscore of -2.0 to -2.5, involved in signalization in cancer, marker of progressive cancer (STK17B), Immune system (IL-17RB), mitotic cycle (PTPN13), fatty acid uptake, fatty acid binding) (FABP5), small subunit processome, and RNA binding (CGI-48/UTP18) (Figure 2B).

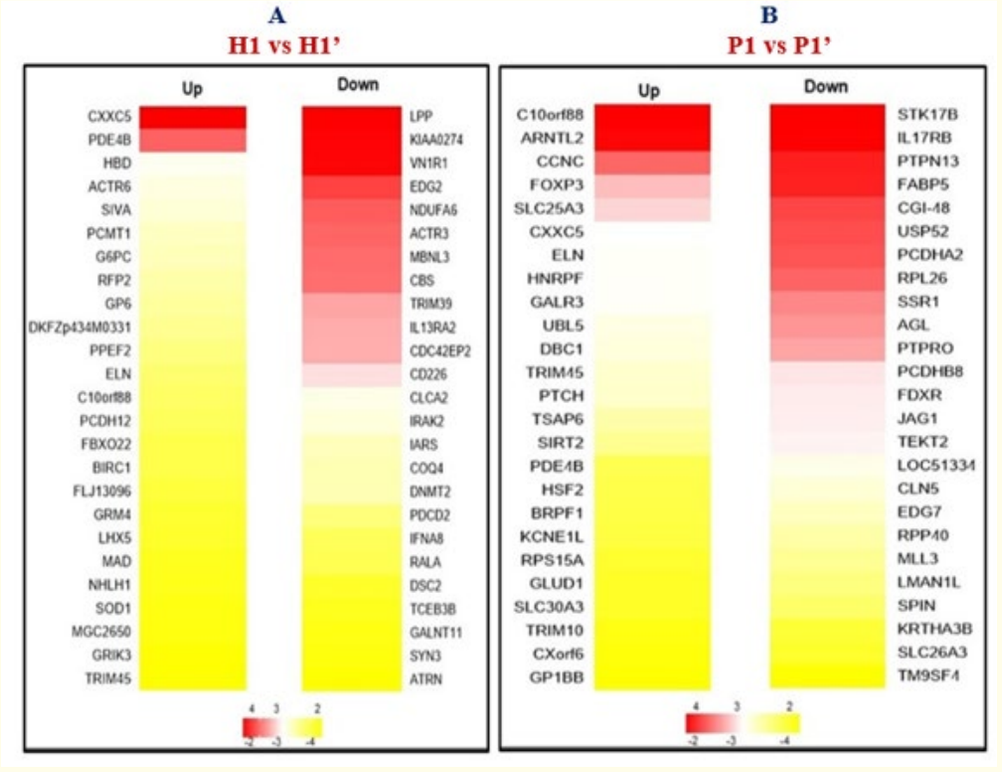

Figure 2: Heat map of the gene expression pattern of the effect of oral administration of HAMLET. A. Up regulated genes in healthy individual with Z values from 2.0 to 4.0 encoding molecular components that participate in different processes of the human biology, such as myelopoiesis (CXXC5), oxygen transport (HBD), nucleolus organization (ACTR6), cell cycle progression (SIVA), signal transduction (PDE4B), Down regulated genes with a Z values of -2.00 to -4.0 encoding proteins involved in cell-cell adhesion and motility (LPP), in vesicle trafficking (FIG4), cellular response to stimuli (VN1R1), respiratory chain (NDUFAG), and actin-binding proteins (ACTR3). B. Patient with thyroid cancer before (P1) and after oral administration of HAMLET (P1'). Up regulated genes encoding genes involved in Hypothyroidism disease(C10orf88), cellular response to stimuli (ARNTL2), cell cycle (CCNC), Immune system (FOXP3), transport of small molecules (SLC2AS3). Down regulated genes, Signal transduction (STK17B), Immune system (IL-17RB), mytotic cycle (PTPN13), FABP5 (fatty acid uptake), Small subunit process some (CGI-48).

Third

Gene expression pattern of the untreated patient with HAMLET (P1) was analyzed versus the gene expression pattern of healthy individual at time zero (Figure 3A). From the heat map we mostly pay attention to the ten of the most up and down regulated genes which identity is provided in the supplementary material. Up

regulated genes in healthy individual (H1) versus untreated patient (P1) at time zero with Z values from 2.0 to 4.0 encoding molecular components for signal transduction (ARF5), DNA binding (ETS1), tubulin superfamily, centrosome organization (TUBE1), signal transduction (AVPR2), Ribosome assembly (DDX56). Down regulated genes with a Z values of -2.00 to -4.0 encoding proteins involved in the activation of oncogenic signaling pathways (LIFR), regulate collagen fibril organization (LUM), fatty acid elongase involved in a disease related with lysosomal storage and lipid metabolism (ELOVL6). Fibroblast growth factor. Protein involved in cell multiplication and maturation, as well as the formation of new blood vessels, wound healing, and the growth, development and maintenance of bones (FGFR2). Furthermore, the gene expression of healthy individual (H1) versus the treated patient (P1') with HAMLET induced also up and down regulated genes (Figure 3B) (see suppl material for the identity of the ten most down regulated genes), is different from the untreated patient (P1). Up regulated genes with Z values from 2.0 to 4.0 encoding molecular components involved in estradiol-binding activity and can modulate intracellular estradiol levels (PDIP), stimulate migration and proliferation of monocytes and skin fibroblasts (ELN), catalyze protein synthesis (RPS28), Signal transduction, oncogenic pathways (RAB2), role in growth and development of a wide variety of tissues and species (NHLH1), Cell cycle and apoptosis regulator 2 (DBC1). Down regulated genes with a Z score value of -2.0 to -4.0 encoding molecular components involved in repressor function in several signaling pathways and may bind to RNA through interaction with spliceosome components (RBM15), polyamine metabolism (MTAP), immune system (proinflammatory cytokine TNFSF8), syalyl transferase in B cell development (SIAT48), and transport of small molecules (ABCF3) (Figure 3B).

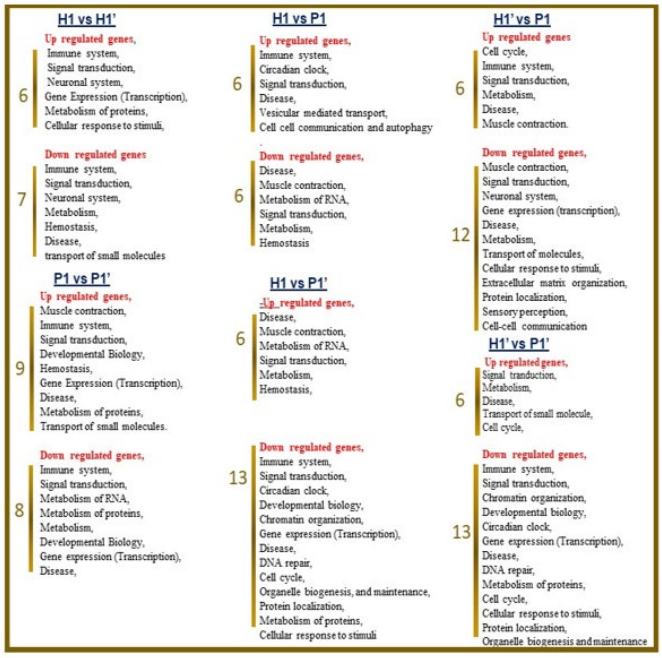

Figure 3: Reactome database of pathways and reactions in which Up and Down regulated genes encoding molecular components are affecting. The p-values represent the probability that a list of identifiers (genes up or down regulated) affected certain pathway. Thus, here it is shown a summary according to the p values from tables 2A-F, the Up and down regulated genes affecting a number of pathways in healthy individual (H1, H1') and patient with thyroid cancer (P1, P1') before or after oral administration of HAMLET. There is a differential effect between healthy individual and the patient in the number pathways affected by the down regulated genes, and most of them affect e.g. Metabolism of RNA; Metabolism of proteins, hemostasis, developmental biology, hemostasis, gene expression (transcription), muscle contraction, as well as, Chromatin organization. Circadian clock, organelle biogenesis and maintenance, cell cycle, than healthy individual (neuronal system, cellular response to stimuli).

Fourth. The gene expression pattern of up and down regulated genes in heathy individual (H1') at time of two weeks after (Figure 1) untreated versus first the untreated patient (P1) with HAMLET (Figure 4A) and second, untreated healthy individual (H1') versus treated patient with HAMLET (P1') (Figure 4B) (See supplementary material for the identity of the ten most up and down regulated genes). In the first case, up regulated genes with Z values from 2.0 to 4.0 encoding molecular components involved as nuclear protein in myelopoiesis and as activator of p53 (CXXC5), in signal transduction (PPPR5b, PDE4B), synthesis of choline or ethanolamine (CEPT1), oxygen transport (HBD). Down regulated genes with a Z score value from -2.0 to -4.0) involved in chromatin binding activity (PHC3), immune system (TGFB2), intracellular signaling in apoptosis, involved in papillary thyroid cancer (STK17B), import of protein precursor into mitochondria (TOMM40), and involved in different forms of neutrophil death depending of inflammatory microenvironment (FCAR) (Figure 4A).

In the second case, up regulated genes with Z values from 2.0 to 4.0 encoding a disulfide isomerase involved in estradiol binding activity (PDIP). A MYC associate factor an oncoproteins implicated in cell proliferation, differentiation, and apoptosis (MAX); a nucleopeptide involved in hormone secretion, cognition/memory, sensory/pain (GALR3); central/peripheric nervous system (CHRM1); as novel candidate tumor suppressor (FBLN1) (Figure 4B). Down regulated genes with Z values from 2.0 to 4.0 encoding, transcription factors involved in DNA binding (SBZF3); in histone methyl transferase, tumor suppressor (WHSC1); in synaptic junctions (PCDHAR); histone deacetylase 3 regulation of osteoblast differentiation and bone formation (HDAC3), and a membrane-bound receptor tyrosine kinase involved in cell multiplication and maturation, as well as the formation of new blood vessels, wound healing, and the growth, development and maintenance of bones (FGFR2) (Figure 4B).

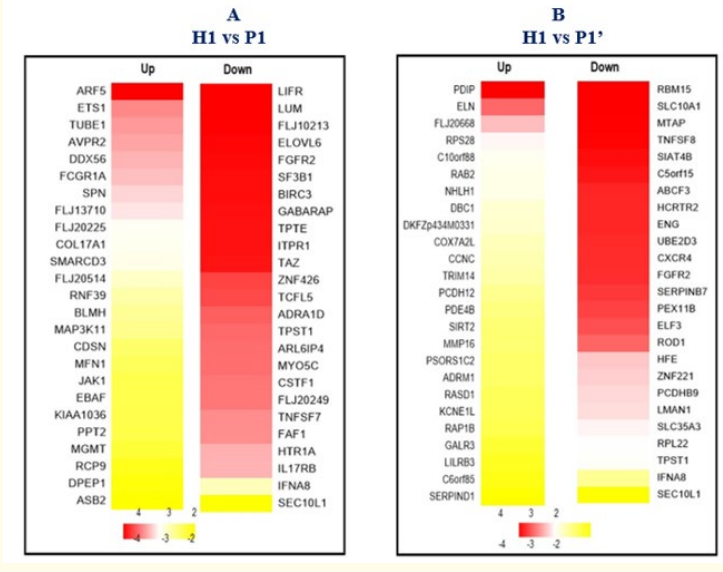

Figure 4: Gene expression pattern between healthy individual and the diagnosed patient with Thyroid Cancer. A. Up regulated genes in healthy individual (H1) versus untreated patient (P1) at time zero with Z values from 2.0 to 4.0 encoding molecular components for signal transduction (ARF5, AVPR2), DNA binding (ETS1), centrosome organization (TUBE1), Ribosome assembly (DDX56). Down regulated genes with a Z values of -2.00 to -4.0 encoding proteins involved in the activation of oncogenic signaling pathways (LIFR), regulate collagen fibril organization (LUM). Fibroblast growth factor, involved in cell multiplication and maturation, as well as the formation of new blood vessels, wound healing, and the growth, development and maintenance of bones (FGFR2). B. Up regulated genes in healthy individual (H1) versus treated patient (P1') with HAMLET two weeks after with Z values from 2.0 to 4.0 encoding molecular components involved in estradiol-binding activity and can modulate intracellular estradiol levels (PDIP), stimulate migration and proliferation of monocytes and skin fibroblasts (ELN), oncogenic pathways (RAB2). Cell cycle and apoptosis regulator 2 (DBC1). Down regulated genes with a Z score value of -2.0 to -4.0 encoding molecular components involved in repressor function in several signaling pathways and may bind to RNA through interaction with spliceosome components (RBM15), polyamine metabolism (MTAP), immune system (TNFSF8), syalyltransferase in B cell development (SIAT48), transport of small molecules (ABCF3).

The Up and down regulated genes participate in different signaling pathways important for the homeostasis of the cell cycle, immune system, endocrine system.

The analysis of the functionality of the up and down regulated genes before and after oral administration of HAMLET using the REACTOME base data, highlight the pathways after the HAMLET administration in a patient with thyroid cancer were hit by at least by one of the genes. This is calculated based on the probability score, which is corrected for false discovery rate using the Benjamin-Hochberg method.

In the-the healthy individual a time zero (H1) and at two weeks later (H1').influence the following pathways, as follow, Up regulated genes, Immune system, Signal transduction, Neuronal system, Gene Expression (Transcription), Metabolism of proteins, Cellular response to stimuli, Down regulated genes participate in Immune system, Signal transduction, Neuronal system, Metabolism, Hemostasis, Disease, transport of small molecules (Table 2A).

Table 2A. Up and down genes (n= 25) with high probability to be involved in the different pathways

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
FOXO-mediated transcription of oxidative stress, metabolic and neuronal genes	2 / 49	0.003	0.004	0.166	2 / 34	0.002
Glycogen storage disease type Ia (G6PC)	1 / 3	1.93e-04	0.006	0.166	1 / 1	6.72e-05
Detoxification of Reactive Oxygen Species	2 / 67	0.004	0.007	0.166	10 / 34	0.002
Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	2 / 73	0.005	0.009	0.166	1 / 36	0.002
Interleukin-12 signaling	2 / 84	0.005	0.011	0.172	1 / 56	0.004
Interleukin-12 family signaling	2 / 96	0.006	0.015	0.177	1 / 114	0.008
FOXO-mediated transcription	2 / 110	0.007	0.019	0.19	2 / 85	0.006
Activation of Ca-permeable Kainate Receptor	1 / 13	8.35e-04	0.025	0.205	2 / 2	1.34e-04
Ionotropic activity of kainate receptors	1 / 14	8.99e-04	0.027	0.205	2 / 4	2.69e-04
Platelet Adhesion to exposed collagen	1 / 17	0.001	0.032	0.205	3 / 8	5.37e-04
Estrogen-dependent gene expression	2 / 153	0.01	0.035	0.205	2 / 66	0.004
Protein repair	1 / 19	0.001	0.036	0.205	1 / 7	4.70e-04
Glycogen storage diseases	1 / 22	0.001	0.042	0.205	1 / 10	6.72e-04
Presynaptic function of Kainate receptors	1 / 23	0.001	0.043	0.205	2 / 2	1.34e-04
Factors involved in megakaryocyte development and platelet production	2 / 194	0.012	0.054	0.205	1 / 43	0.003
ER Quality Control Compartment (ERQC)	1 / 33	0.002	0.062	0.205	1 / 9	6.05e-04
Activation of kainate receptors upon glutamate binding	1 / 34	0.002	0.063	0.205	4 / 6	4.03e-04
DARPP-32 events	1 / 35	0.002	0.065	0.205	2 / 12	8.06e-04
Hemostasis	4 / 804	0.052	0.067	0.205	10 / 342	0.023
Molecules associated with elastic fibres	1 / 37	0.002	0.069	0.205	3 / 10	6.72e-04
NFE2L2 regulating anti-oxidant/detoxification enzymes	1 / 39	0.003	0.072	0.205	1 / 28	0.002
Calnexin/calreticulin cycle	1 / 41	0.003	0.076	0.205	1 / 13	8.73e-04
GPVI-mediated activation cascade	1 / 43	0.003	0.08	0.205	5 / 25	0.002

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
TRAF6 mediated IRF7 activation	2 / 44	0.003	0.003	0.144	2 / 13	8.73e-04
Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	2 / 73	0.005	0.008	0.144	1 / 36	0.002
Interleukin-12 signaling	2 / 84	0.005	0.01	0.144	1 / 56	0.004
Interleukin-12 family signaling	2 / 96	0.006	0.013	0.144	1 / 114	0.008
DDX58/IFIH1-mediated induction of interferon-alpha/beta	2 / 105	0.007	0.015	0.144	2 / 53	0.004
Cysteine formation from homocysteine	1 / 10	6.42e-04	0.018	0.144	1 / 2	1.34e-04
Stimuli-sensing channels	2 / 121	0.008	0.02	0.144	5 / 33	0.002
IRAK2 mediated activation of TAK1 complex	1 / 12	7.71e-04	0.021	0.144	5 / 5	3.36e-04
Signaling by Interleukins	4 / 658	0.042	0.029	0.144	7 / 505	0.034
IRAK2 mediated activation of TAK1 complex upon TLR2/6 or 9 stimulation	1 / 18	0.001	0.032	0.144	5 / 5	3.36e-04
SARS-CoV-2 activates/modulates innate and adaptive immune responses	2 / 160	0.01	0.033	0.144	2 / 47	0.003
p38MAPK events	1 / 19	0.001	0.034	0.144	2 / 5	3.36e-04
Lysosphingolipid and LPA receptors	1 / 19	0.001	0.034	0.144	1 / 5	3.36e-04
TRAF6-mediated induction of TAK1 complex within TLR4 complex	1 / 19	0.001	0.034	0.144	1 / 6	4.03e-04
Immune System	9 / 2,663	0.171	0.039	0.144	56 / 1,717	0.115
Synthesis of PIPs at the late endosome membrane	1 / 22	0.001	0.039	0.144	3 / 7	4.70e-04
Serotonin Neurotransmitter Release Cycle	1 / 23	0.001	0.041	0.144	2 / 4	2.69e-04
Selenoamino acid metabolism	2 / 180	0.012	0.041	0.144	2 / 33	0.002
Cytokine Signaling in Immune system	5 / 1,099	0.071	0.044	0.144	20 / 785	0.053
JNK (c-Jun kinases) phosphorylation and activation mediated by activated human TAK1	1 / 26	0.002	0.046	0.144	1 / 3	2.02e-04
Signalling to RAS	1 / 26	0.002	0.046	0.144	2 / 10	6.72e-04
Factors involved in megakaryocyte development and platelet production	2 / 194	0.012	0.047	0.144	3 / 43	0.003

UP

12 of 24 Identifiers Were found in the Reactome were 76 were hit by at least one of them

DOWN

20 of 24 Identifiers Were found in the Reactome were 161 were hit by at least one of them

Note: Data from the untreated healthy individual (H1) and healthy individual (H1') with an interval of two weeks. A low values of p (< values <<<), higher probability that the up(LEFT) or down (right) regulated genes are affected or influence a certain pathway.

Table 2a

The patient before and after (at time of two weeks later) administration of HAMLET, as P1 and P1'

Up regulated genes, muscle contraction, Immune system, Signal transduction, Developmental Biology, hemostasis, Gene Expression (Transcription), Disease, Metabolism of proteins, transport of small molecules. Down regulated genes, Immune system, Signal transduction, Metabolism of RNA, Metabolism of proteins, Metabolism, Developmental Biology, Gene expression (Transcription), Disease, (Table 2B).

Table 2B. Up and down genes (n= 25) with high probability to be involved in the different pathways

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
Vpr-mediated induction of apoptosis by mitochondrial outer membrane permeabilization	2 / 4	2.57e-04	4.13e-05	0.008	1 / 2	1.34e-04
RUNX1 and FOXP3 control the development of regulatory T lymphocytes (Tregs)	2 / 17	0.001	7.33e-04	0.048	13 / 20	0.001
Interferon gamma signaling	4 / 177	0.011	7.36e-04	0.048	2 / 23	0.002
Mitochondrial protein degradation	3 / 104	0.007	0.002	0.086	4 / 20	0.001
TP53 Regulates Transcription of Genes Involved in Cytochrome C Release	2 / 33	0.002	0.003	0.086	3 / 25	0.002
Transport of nucleosides and free purine and pyrimidine bases across the plasma membrane	2 / 33	0.002	0.003	0.086	1 / 16	0.001
Interactions of Vpr with host cellular proteins	2 / 47	0.003	0.005	0.125	1 / 4	2.69e-04
NOTCH1 Intracellular Domain Regulates Transcription	2 / 57	0.004	0.008	0.125	11 / 18	0.001
Influenza Virus Induced Apoptosis	1 / 4	2.57e-04	0.009	0.125	1 / 2	1.34e-04
Constitutive Signaling by NOTCH1 PEST Domain Mutants	2 / 68	0.004	0.011	0.125	11 / 21	0.001
Signaling by NOTCH1 HD+PEST Domain Mutants in Cancer	2 / 68	0.004	0.011	0.125	11 / 21	0.001
Signaling by NOTCH1 PEST Domain Mutants in Cancer	2 / 68	0.004	0.011	0.125	11 / 21	0.001
Constitutive Signaling by NOTCH1 HD+PEST Domain Mutants	2 / 68	0.004	0.011	0.125	11 / 21	0.001
Signaling by NOTCH1 in Cancer	2 / 68	0.004	0.011	0.125	11 / 39	0.003
Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	2 / 73	0.005	0.012	0.125	1 / 36	0.002
Interferon Signaling	4 / 397	0.025	0.013	0.125	2 / 119	0.008
Zinc efflux and compartmentalization by the SLC30 family	1 / 6	3.85e-04	0.014	0.125	1 / 7	4.70e-04
TP53 Regulates Transcription of Cell Death Genes	2 / 83	0.005	0.016	0.125	3 / 68	0.005
Enhanced binding of GP1BA variant to VWF multimer:collagen	1 / 7	4.50e-04	0.016	0.125	1 / 1	6.72e-05
Defective F9 activation	1 / 7	4.50e-04	0.016	0.125	1 / 1	6.72e-05

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
RUNX3 regulates NOTCH signaling	2 / 16	0.001	5.13e-04	0.043	2 / 7	4.70e-04
Major pathway of rRNA processing in the nucleolus and cytosol	4 / 189	0.012	5.95e-04	0.043	6 / 7	4.70e-04
rRNA processing in the nucleus and cytosol	4 / 207	0.013	8.34e-04	0.043	7 / 15	0.001
Nephron development	2 / 23	0.001	0.001	0.043	1 / 17	0.001
rRNA processing	4 / 245	0.016	0.002	0.051	7 / 21	0.001
SRP-dependent cotranslational protein targeting to membrane	3 / 119	0.008	0.002	0.051	5 / 5	3.36e-04
Interleukin-37 signaling	2 / 36	0.002	0.003	0.058	1 / 14	9.40e-04
Lewis blood group biosynthesis	2 / 39	0.003	0.003	0.059	5 / 13	8.73e-04
Blood group systems biosynthesis	2 / 52	0.003	0.005	0.093	5 / 22	0.001
Kidney development	2 / 75	0.005	0.01	0.117	1 / 50	0.003
Signaling by NOTCH1 t(7;9)(NOTCH1:M1580_K2555) Translocation Mutant	1 / 8	5.14e-04	0.016	0.117	1 / 5	3.36e-04
Constitutive Signaling by NOTCH1 t(7;9)(NOTCH1:M1580_K2555) Translocation Mutant	1 / 8	5.14e-04	0.016	0.117	1 / 5	3.36e-04
XBP1(S) activates chaperone genes	2 / 95	0.006	0.016	0.117	1 / 47	0.003
Peptide chain elongation	2 / 97	0.006	0.017	0.117	4 / 5	3.36e-04
Defective CYP11A1 causes AICSR	1 / 9	5.78e-04	0.018	0.117	1 / 1	6.72e-05
Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)	2 / 101	0.006	0.018	0.117	1 / 1	6.72e-05
HIF1alpha activates chaperones	2 / 101	0.006	0.018	0.117	1 / 53	0.004
Eukaryotic Translation Elongation	2 / 102	0.007	0.019	0.117	4 / 9	6.05e-04
Eukaryotic Translation Termination	2 / 106	0.007	0.02	0.117	3 / 5	3.36e-04
Formation of a pool of free 40S subunits	2 / 106	0.007	0.02	0.117	1 / 2	1.34e-04
Electron transport from NADPH to Ferredoxin	1 / 10	6.42e-04	0.02	0.117	2 / 2	1.34e-04
Selenocysteine synthesis	2 / 112	0.007	0.022	0.117	2 / 7	4.70e-04
Viral mRNA Translation	2 / 114	0.007	0.023	0.117	2 / 2	1.34e-04
Response of EIF2AK4 (GCN2) to amino acid deficiency	2 / 115	0.007	0.023	0.117	4 / 16	0.001

UP

24 of 28 Identifiers Were found in the Reactome were 195 were hit by at least one of them

DOWN

20 of 26 Identifiers Were found in the Reactome were 160 were hit by at least one of them

Note: Data from the untreated patient (P1) and treated patient with HAMLET (P1') with an interval of two weeks. A low values of p (< values <<<), higher probability that the up(LEFT) or down (right) regulated genes are affected or influence a certain pathway.

Table 2b

The untreated healthy individual (H1) and the untreated patient (P1)

Up regulated genes, immune system, circadian clock, signal transduction, disease, vesicular mediated transport, cell cell communication and autophagy. Down regulated genes, Disease, muscle contraction, Metabolism of RNA, Signal transduction, Metabolism, Hemostasis (Table 2C).

Table 2C. Up and down genes (n= 25) with high probability to be involved in the different pathways

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
Interferon gamma signaling	3 / 177	0.011	0.002	0.116	17 / 23	0.002
Defective AVP does not bind AVPR2 and causes neurohypophyseal diabetes insipidus (NDI)	1 / 2	1.28e-04	0.003	0.116	1 / 1	6.72e-05
Interleukin-12 signaling	2 / 84	0.005	0.006	0.116	5 / 56	0.004
Interleukin-12 family signaling	2 / 96	0.006	0.008	0.116	29 / 114	0.008
Vasopressin-like receptors	1 / 6	3.85e-04	0.008	0.116	4 / 7	4.70e-04
COPI-dependent Golgi-to-ER retrograde traffic	2 / 107	0.007	0.01	0.116	5 / 11	7.39e-04
COPI-mediated anterograde transport	2 / 107	0.007	0.01	0.116	5 / 12	8.06e-04
IFNG signaling activates MAPKs	1 / 10	6.42e-04	0.014	0.116	3 / 3	2.02e-04
Nef Mediated CD4 Down-regulation	1 / 10	6.42e-04	0.014	0.116	1 / 5	3.36e-04
Type I hemidesmosome assembly	1 / 11	7.06e-04	0.015	0.116	5 / 6	4.03e-04
Interleukin-9 signaling	1 / 11	7.06e-04	0.015	0.116	7 / 13	8.73e-04
RHOT2 GTPase cycle	1 / 11	7.06e-04	0.015	0.116	1 / 3	2.02e-04
Interleukin-21 signaling	1 / 12	7.71e-04	0.017	0.116	5 / 5	3.36e-04
MAPK1 (ERK2) activation	1 / 12	7.71e-04	0.017	0.116	1 / 3	2.02e-04
Miro GTPase Cycle	1 / 12	7.71e-04	0.017	0.116	1 / 6	4.03e-04
Interferon Signaling	3 / 397	0.025	0.018	0.116	34 / 119	0.008
Interleukin-27 signaling	1 / 13	8.35e-04	0.018	0.116	9 / 20	0.001
MAPK3 (ERK1) activation	1 / 13	8.35e-04	0.018	0.116	1 / 4	2.69e-04
Golgi-to-ER retrograde transport	2 / 148	0.01	0.018	0.116	5 / 18	0.001
Interleukin-2 signaling	1 / 14	8.99e-04	0.02	0.116	12 / 19	0.001
ER to Golgi Anterograde Transport	2 / 164	0.011	0.022	0.116	5 / 39	0.003
Interleukin-15 signaling	1 / 16	0.001	0.022	0.116	14 / 17	0.001
Interleukin-35 Signalling	1 / 16	0.001	0.022	0.116	15 / 26	0.002
Regulation of IFNG signaling	1 / 16	0.001	0.022	0.116	2 / 4	2.69e-04
IL-6-type cytokine receptor ligand interactions	1 / 17	0.001	0.024	0.116	10 / 14	9.40e-04

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
FGFR2 mutant receptor activation	6 / 43	0.003	9.58e-10	2.32e-07	17 / 18	0.001
Signaling by FGFR2 IIIa TM	5 / 24	0.002	3.64e-09	4.40e-07	2 / 2	1.34e-04
Signaling by FGFR2 in disease	6 / 58	0.004	5.62e-09	4.50e-07	27 / 28	0.002
Signaling by FGFR in disease	6 / 82	0.005	4.31e-08	2.59e-06	27 / 99	0.007
Constitutive Signaling by Aberrant PI3K in Cancer	6 / 96	0.006	1.08e-07	5.20e-06	1 / 2	1.34e-04
Phospholipase C-mediated cascade; FGFR2	4 / 25	0.002	4.21e-07	1.47e-05	3 / 3	2.02e-04
PI3K/AKT Signaling in Cancer	6 / 124	0.008	4.80e-07	1.47e-05	1 / 21	0.001
FGFR2 ligand binding and activation	4 / 26	0.002	4.91e-07	1.47e-05	4 / 5	3.36e-04
PI3P, PP2A and IER3 Regulate PI3K/AKT Signaling	6 / 129	0.008	6.03e-07	1.57e-05	1 / 7	4.70e-04
Negative regulation of the PI3K/AKT network	6 / 137	0.009	8.53e-07	2.05e-05	1 / 10	6.72e-04
PI-3K cascade:FGFR2	4 / 31	0.002	9.84e-07	2.17e-05	6 / 7	4.70e-04
SHC-mediated cascade:FGFR2	4 / 33	0.002	1.26e-06	2.52e-05	4 / 4	2.69e-04
FRS-mediated FGFR2 signaling	4 / 34	0.002	1.42e-06	2.55e-05	9 / 9	6.05e-04
Negative regulation of FGFR2 signaling	4 / 41	0.003	2.96e-06	5.03e-05	3 / 14	9.40e-04
Downstream signaling of activated FGFR2	4 / 42	0.003	3.26e-06	5.21e-05	22 / 23	0.002
PI3K Cascade	4 / 58	0.004	1.15e-05	1.61e-04	1 / 6	4.03e-04
IRS-mediated signalling	4 / 65	0.004	1.80e-05	2.16e-04	1 / 9	6.05e-04
Intracellular signaling by second messengers	7 / 367	0.024	2.23e-05	2.49e-04	9 / 116	0.008
IRS-related events triggered by IGF1R	4 / 69	0.004	2.27e-05	2.49e-04	1 / 12	8.06e-04
Activated point mutants of FGFR2	3 / 23	0.001	2.41e-05	2.54e-04	9 / 10	6.72e-04
IGF1R signaling cascade	4 / 72	0.005	2.67e-05	2.54e-04	1 / 17	0.001
Insulin receptor signalling cascade	4 / 72	0.005	2.67e-05	2.54e-04	1 / 25	0.002
Signaling by Type 1 Insulin-like Growth Factor 1 Receptor (IGF1R)	4 / 73	0.005	2.82e-05	2.54e-04	1 / 19	0.001
Diseases of signal transduction by growth factor receptors and second messengers	8 / 541	0.035	3.32e-05	2.99e-04	42 / 516	0.035
Signaling by FGFR2	4 / 88	0.006	5.80e-05	4.64e-04	29 / 46	0.003

UP

12 of 19 Identifiers Were found in the Reactome were 180 were hit by at least one of them

DOWN

18 of 25 Identifiers Were found in the Reactome were 235 were hit by at least one of them

Note: Data from the untreated patient (H1) and treated patient with HAMLET (P1) with an interval of two weeks. A low values of p (< values <<<), higher probability that the up(LEFT) or down (right) regulated genes are affected or influence a certain pathway.

Table 2c

The untreated healthy individual (H1) and the treated patient with HAMLET (P1')

Up regulated genes, Disease, muscle contraction, Metabolism of RNA, Signal transduction, Metabolism, Hemostasis, Down regulated genes, immune system, signal transduction, circadian clock, developmental biology, chromatin organization, Gene expression (Transcription), Disease, DNA repair, cell cycle, organelle biogenesis, and maintenance, protein localization, Metabolism of proteins, cellular response to stimuli (Table 2D).

Table 2D. Up and down genes (n= 25) with high probability to be involved in the different pathways

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
Hedgehog 'on' state	2 / 92	0.006	0.007	0.164	9 / 37	0.002
Ligand-receptor interactions	1 / 8	5.14e-04	0.011	0.164	3 / 4	2.69e-04
GLI proteins bind promoters of Hh responsive genes to promote transcription	1 / 8	5.14e-04	0.011	0.164	1 / 4	2.69e-04
Hedgehog 'off' state	2 / 124	0.008	0.013	0.164	3 / 32	0.002
Degradation of the extracellular matrix	2 / 148	0.01	0.018	0.164	6 / 105	0.007
Signaling by Hedgehog	2 / 168	0.011	0.023	0.164	12 / 82	0.006
Golgi Cisternae Pericentriolar Stack Reorganization	1 / 17	0.001	0.024	0.164	2 / 6	4.03e-04
P2Y receptors	1 / 18	0.001	0.025	0.164	1 / 13	8.73e-04
Interferon gamma signaling	2 / 177	0.011	0.026	0.164	2 / 23	0.002
Activation of SMO	1 / 20	0.001	0.028	0.164	1 / 9	6.05e-04
Nucleotide-like (purinergic) receptors	1 / 23	0.001	0.032	0.164	1 / 15	0.001
Initiation of Nuclear Envelope (NE) Reformation	1 / 27	0.002	0.037	0.164	1 / 7	4.70e-04
DARPP-32 events	1 / 35	0.002	0.048	0.164	2 / 12	8.06e-04
Activation of Matrix Metalloproteinases	1 / 35	0.002	0.048	0.164	4 / 27	0.002
Molecules associated with elastic fibres	1 / 37	0.002	0.051	0.164	3 / 10	6.72e-04
SARS-CoV-1 modulates host translation machinery	1 / 41	0.003	0.056	0.164	1 / 3	2.02e-04
SMAD2/SMAD3:SMAD4 heterotrimer regulates transcription	1 / 44	0.003	0.06	0.164	1 / 24	0.002
Elastic fibre formation	1 / 45	0.003	0.062	0.164	6 / 17	0.001
Formation of the ternary complex, and subsequently, the 43S complex	1 / 54	0.003	0.074	0.164	1 / 3	2.02e-04
NOTCH1 Intracellular Domain Regulates Transcription	1 / 57	0.004	0.078	0.164	2 / 18	0.001
Extracellular matrix organization	2 / 328	0.021	0.078	0.164	12 / 319	0.021
Translation initiation complex formation	1 / 62	0.004	0.084	0.164	2 / 2	1.34e-04
SARS-CoV-2 modulates host translation machinery	1 / 62	0.004	0.084	0.164	1 / 6	4.03e-04

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
FGFR2 mutant receptor activation	6 / 43	0.003	5.58e-10	1.19e-07	17 / 18	0.001
Signaling by FGFR2 IIIa TM	5 / 24	0.002	2.33e-09	2.33e-07	2 / 2	1.34e-04
Signaling by FGFR2 in disease	6 / 58	0.004	3.28e-09	2.33e-07	27 / 28	0.002
Signaling by FGFR in disease	6 / 82	0.005	2.53e-08	1.34e-06	27 / 99	0.007
Phospholipase C-mediated cascade; FGFR2	4 / 25	0.002	2.97e-07	1.21e-05	3 / 3	2.02e-04
FGFR2 ligand binding and activation	4 / 26	0.002	3.46e-07	1.21e-05	4 / 5	3.36e-04
PI-3K cascade:FGFR2	4 / 31	0.002	6.95e-07	2.08e-05	6 / 7	4.70e-04
SHC-mediated cascade:FGFR2	4 / 33	0.002	8.89e-07	2.30e-05	4 / 4	2.69e-04
FRS-mediated FGFR2 signaling	4 / 34	0.002	1.00e-06	2.30e-05	9 / 9	6.05e-04
Negative regulation of FGFR2 signaling	4 / 41	0.003	2.09e-06	4.37e-05	3 / 14	9.40e-04
Downstream signaling of activated FGFR2	4 / 42	0.003	2.30e-06	4.37e-05	22 / 23	0.002
PI3K Cascade	4 / 58	0.004	8.16e-06	1.31e-04	1 / 6	4.03e-04
IRS-mediated signalling	4 / 65	0.004	1.27e-05	1.78e-04	1 / 9	6.05e-04
IRS-related events triggered by IGF1R	4 / 69	0.004	1.61e-05	2.00e-04	1 / 12	8.06e-04
Activated point mutants of FGFR2	3 / 23	0.001	1.86e-05	2.00e-04	9 / 10	6.72e-04
IGF1R signaling cascade	4 / 72	0.005	1.90e-05	2.00e-04	1 / 17	0.001
Insulin receptor signalling cascade	4 / 72	0.005	1.90e-05	2.00e-04	1 / 25	0.002
Signaling by Type 1 Insulin-like Growth Factor 1 Receptor (IGF1R)	4 / 73	0.005	2.00e-05	2.00e-04	1 / 19	0.001
Signaling by FGFR2	4 / 88	0.006	4.13e-05	4.13e-04	29 / 46	0.003
Signaling by FGFR2 amplification mutants	2 / 5	3.21e-04	5.75e-05	5.17e-04	4 / 4	2.69e-04
Constitutive Signaling by Aberrant PI3K in Cancer	4 / 96	0.006	5.78e-05	5.20e-04	1 / 2	1.34e-04
Signaling by Insulin receptor	4 / 102	0.007	7.30e-05	5.84e-04	1 / 34	0.002
DDX58/IFIH1-mediated induction of interferon-alpha/beta	4 / 105	0.007	8.16e-05	6.53e-04	3 / 53	0.004
Signaling by FGFR	4 / 109	0.007	9.41e-05	7.53e-04	29 / 142	0.01
PI3K/AKT Signaling in Cancer	4 / 124	0.008	1.54e-04	0.001	1 / 21	0.001

UP

12 of 19 Identifiers Were found in the Reactome were 132 were hit by at least one of them

DOWN

22 of 28 Identifiers Were found in the Reactome were 276 were hit by at least one of them

Note: Data from the untreated patient (H1) and treated patient with HAMLET (P1') with an interval of two weeks. A low values of p (< values <<<), higher probability that the up(LEFT) or down (right) regulated genes are affected or influence a certain pathway.

Table 2D

The untreated healthy individual (H1') (at time of two weeks later) and the untreated patient with HAMLET (P1)

Up regulated genes cell cycle, immune system, signal transduction, Metabolism, Disease, muscle contraction. Down regulated genes, muscle contraction, signal transduction, Neuronal system, Gene expression (transcription), Disease, Metabolism, transport of molecules, cellular response to stimuli, extracellular matrix organization, protein localization, sensory perception, cell-cell communication (Table 2E).

The untreated healthy individual (H1') (at time of two weeks later) and the treated patient with HAMLET (P1')

Up regulated genes, signal tranduction, metabolism, disease, transport of small molecule, cell cycle, Down regulated genes,

Table 2E. Up and down genes (n= 25) with high probability to be involved in the different pathways

Pathway name	Entities found	Entities ratio	Entities p-value	Entities FDR*	Reactions found	Reactions ratio
Factors involved in megakaryocyte development and platelet production	3/194	0.012	0.003	0.097	2/43	0.003
Hedgehog 'on' state	2/92	0.006	0.009	0.097	9/37	0.002
XBP1(S) activates chaperone genes	2/95	0.006	0.009	0.097	1/47	0.003
IRE1alpha activates chaperones	2/101	0.006	0.011	0.097	1/53	0.004
Muscarinic acetylcholine receptors	1/7	4.50e-04	0.011	0.097	2/5	3.36e-04
COPI-dependent Golgi-to-ER retrograde traffic	2/107	0.007	0.012	0.097	5/11	7.39e-04
COPI-mediated anterograde transport	2/107	0.007	0.012	0.097	5/12	8.06e-04
Ligand-receptor interactions	1/8	5.14e-04	0.012	0.097	3/4	2.69e-04
GLI proteins bind promoters of Hh responsive genes to promote transcription	1/8	5.14e-04	0.012	0.097	1/4	2.69e-04
Nef Mediated CD4 Down-regulation	1/10	6.42e-04	0.015	0.097	1/5	3.36e-04
Hedgehog 'off' state	2/124	0.008	0.016	0.097	3/32	0.002
Truncations of AMER1 destabilize the destruction complex	1/14	8.99e-04	0.021	0.097	1/1	6.72e-05
AXIN missense mutants destabilize the destruction complex	1/14	8.99e-04	0.021	0.097	1/1	6.72e-05
APC truncation mutants have impaired AXIN binding	1/14	8.99e-04	0.021	0.097	1/1	6.72e-05
Signaling by APC mutants	1/14	8.99e-04	0.021	0.097	1/2	1.34e-04
Golgi-to-ER retrograde transport	2/148	0.01	0.022	0.097	5/18	0.001
Signaling by GSK3beta mutants	1/15	9.63e-04	0.023	0.097	1/1	6.72e-05
Signaling by AXIN mutants	1/15	9.63e-04	0.023	0.097	1/2	1.34e-04
Signaling by AMER1 mutants	1/15	9.63e-04	0.023	0.097	1/2	1.34e-04
Estrogen-dependent gene expression	2/153	0.01	0.023	0.097	2/66	0.004
Unfolded Protein Response (UPR)	2/156	0.01	0.024	0.097	1/99	0.007
Signaling by CTNNB1 phospho-site mutants	1/16	0.001	0.024	0.097	4/4	2.69e-04
CTNNB1 S45 mutants aren't phosphorylated	1/16	0.001	0.024	0.097	1/1	6.72e-05
CTNNB1 S37 mutants aren't phosphorylated	1/16	0.001	0.024	0.097	1/1	6.72e-05

Pathway name	Entities found	Entities ratio	Entities p-value	Entities FDR*	Reactions found	Reactions ratio
GRB7 events in ERBB2 signaling	2/6	3.85e-04	6.85e-05	0.017	1/1	6.72e-05
Signaling by PTK6	3/71	0.005	3.87e-04	0.024	4/53	0.004
Signaling by Non-Receptor Tyrosine Kinases	3/71	0.005	3.87e-04	0.024	4/53	0.004
Downregulation of ERBB2:ERBB3 signaling	2/16	0.001	4.81e-04	0.024	2/8	5.37e-04
ERBB2 Activates PTK6 Signaling	2/18	0.001	6.08e-04	0.024	2/2	1.34e-04
ERBB2 Regulates Cell Motility	2/19	0.001	6.76e-04	0.024	2/2	1.34e-04
PI3K events in ERBB2 signaling	2/22	0.001	9.03e-04	0.027	4/7	4.70e-04
Signaling by ERBB2 TMD/JMD mutants	2/30	0.002	0.002	0.04	6/13	8.73e-04
MECP2 regulates neuronal receptors and channels	2/32	0.002	0.002	0.04	2/26	0.002
Signaling by ERBB2 KD Mutants	2/35	0.002	0.002	0.04	8/17	0.001
SHC1 events in ERBB2 signaling	2/36	0.002	0.002	0.04	4/6	4.03e-04
Downregulation of ERBB2 signaling	2/36	0.002	0.002	0.04	4/14	9.40e-04
Signaling by ERBB2 in Cancer	2/36	0.002	0.002	0.04	14/62	0.004
Lewis blood group biosynthesis	2/39	0.003	0.003	0.044	5/13	8.73e-04
Blood group systems biosynthesis	2/52	0.003	0.005	0.073	5/22	0.001
Defective SLC35A3 causes arthrogryposis, mental retardation, and seizures (AMRS)	1/3	1.93e-04	0.006	0.083	1/1	6.72e-05
Non-integrin membrane-ECM interactions	2/61	0.004	0.007	0.086	8/22	0.001
Defective F8 sulfation at Y1699	1/4	2.57e-04	0.008	0.095	1/1	6.72e-05
Signaling by ERBB2	2/68	0.004	0.008	0.098	24/46	0.003
ECM proteoglycans	2/79	0.005	0.011	0.115	2/23	0.002
Signaling by ERBB4	2/82	0.005	0.012	0.115	12/52	0.003
Signaling by MET	2/88	0.006	0.013	0.115	2/51	0.003
PTK6 Down-Regulation	1/7	4.50e-04	0.014	0.115	2/3	2.02e-04
XBP1(S) activates chaperone genes	2/95	0.006	0.015	0.115	1/47	0.003
Constitutive Signaling by Aberrant PI3K in Cancer	2/96	0.006	0.016	0.115	1/2	1.34e-04

UP

15 of 23 Identifiers Were found in the Reactome were 118 were hit by at least one of them

DOWN

20 of 25 Identifiers Were found in the Reactome were 235 were hit by at least one of them

Note: Data from the untreated patient (H1') and treated patient with HAMLET (P1) with an interval of two weeks. A low values of p (< values <<<), higher probability that the up(LEFT) or down (right) regulated genes are affected or influence a certain pathway.

Table 2E

immune system, signal transduction, Chromatin organization, Developmental biology, Circadian clock, Gene expression (Transcription), Disease, DNA repair, Metabolism of proteins, cell cycle, cellular response to stimuli, protein localization, organelle biogenesis and maintenance (Table 2F).

Table 2F. Up and down genes (n= 25) with high probability to be involved in the different pathways

Pathway name	Entities found	Entities ratio	Entities p-value	Entities FDR*	Reactions found	Reactions ratio
Cyclin E associated events during G1/S transition	2/73	0.006	0.01	0.149	3/20	0.001
Muscarinic acetylcholine receptors	1/5	4.21e-04	0.01	0.149	2/5	3.28e-04
Cyclin A:Cdk2-associated events at S phase entry	2/75	0.006	0.011	0.149	2/19	0.001
Loss of MECP2 binding ability to the NCoR/SMRT complex	1/8	6.74e-04	0.017	0.149	1/1	6.55e-05
Glycosphingolipid transport	1/8	6.74e-04	0.017	0.149	1/6	3.93e-04
Cell Cycle, Mitotic	4/524	0.044	0.023	0.149	18/357	0.023
PPARA activates gene expression	2/118	0.01	0.025	0.149	2/41	0.003
Regulation of lipid metabolism by PPARalpha	2/120	0.01	0.026	0.149	2/45	0.003
G1/S Transition	2/121	0.01	0.027	0.149	3/61	0.004
Loss of function of MECP2 in Rett syndrome	1/13	0.001	0.027	0.149	1/5	3.28e-04
Disorders of Developmental Biology	1/13	0.001	0.027	0.149	1/5	3.28e-04
Pervasive developmental disorders	1/13	0.001	0.027	0.149	1/5	3.28e-04
Disorders of Nervous System Development	1/13	0.001	0.027	0.149	1/5	3.28e-04
Golgi Cisternae Pericentriolar Stack Reorganization	1/14	0.001	0.029	0.149	2/6	3.93e-04
Synthesis of IP2, IP, and Ins in the cytosol	1/14	0.001	0.029	0.149	2/14	9.17e-04
Mitotic G1 phase and G1/S transition	2/139	0.012	0.034	0.149	3/101	0.007
Transcription of E2F targets under negative control by DREAM complex	1/19	0.002	0.039	0.149	1/12	7.86e-04
S Phase	2/150	0.013	0.039	0.149	5/55	0.004
Initiation of Nuclear Envelope (NE) Reformation	1/20	0.002	0.041	0.149	1/7	4.59e-04
M Phase	3/380	0.032	0.045	0.149	4/96	0.006
Cell Cycle	4/658	0.055	0.047	0.149	20/457	0.03
DARPP-32 events	1/24	0.002	0.049	0.149	2/12	7.86e-04
Complex III assembly	1/25	0.002	0.051	0.149	1/10	6.55e-04
BMAL1:CLOCK,NPAS2 activates circadian gene expression	1/27	0.002	0.055	0.149	20/20	0.001
G0 and Early G1	1/27	0.002	0.055	0.149	1/27	0.002

Pathway name	Entities found	Entities ratio	Entities p-value	Entities FDR*	Reactions found	Reactions ratio
FGFR2 mutant receptor activation	6/43	0.003	8.05e-10	2.53e-07	17/18	0.001
Signaling by FGFR2 IIIa TM	5/24	0.002	3.15e-09	4.95e-07	2/2	1.34e-04
Signaling by FGFR2 in disease	6/58	0.004	4.73e-09	4.96e-07	27/28	0.002
Signaling by FGFR in disease	6/82	0.005	3.63e-08	2.83e-06	27/99	0.007
Phospholipase C-mediated cascade; FGFR2	4/25	0.002	3.76e-07	2.28e-05	3/3	2.02e-04
FGFR2 ligand binding and activation	4/26	0.002	4.39e-07	2.28e-05	4/5	3.36e-04
PI-3K cascade:FGFR2	4/31	0.002	8.79e-07	3.96e-05	6/7	4.70e-04
SHC-mediated cascade:FGFR2	4/33	0.002	1.13e-06	4.39e-05	4/4	2.69e-04
FRS-mediated FGFR2 signaling	4/34	0.002	1.27e-06	4.43e-05	9/9	6.05e-04
Negative regulation of FGFR2 signaling	4/41	0.003	2.65e-06	8.15e-05	3/14	9.40e-04
Downstream signaling of activated FGFR2	4/42	0.003	2.91e-06	8.15e-05	22/23	0.002
PI3K Cascade	4/58	0.004	1.03e-05	2.47e-04	1/6	4.03e-04
IRS-mediated signalling	4/65	0.004	1.61e-05	3.38e-04	1/9	6.05e-04
IRS-related events triggered by IGF1R	4/69	0.004	2.03e-05	3.79e-04	1/12	8.06e-04
Activated point mutants of FGFR2	3/23	0.001	2.22e-05	3.79e-04	9/10	6.72e-04
IGF1R signaling cascade	4/72	0.005	2.39e-05	3.79e-04	1/17	0.001
Insulin receptor signalling cascade	4/72	0.005	2.39e-05	3.79e-04	1/25	0.002
Signaling by Type 1 Insulin-like Growth Factor 1 Receptor (IGF1R)	4/73	0.005	2.52e-05	3.79e-04	1/19	0.001
Signaling by FGFR2	4/88	0.006	5.20e-05	7.80e-04	29/46	0.003
Signaling by FGFR2 amplification mutants	2/5	3.21e-04	6.45e-05	9.03e-04	4/4	2.69e-04
Constitutive Signaling by Aberrant PI3K in Cancer	4/96	0.006	7.27e-05	9.45e-04	1/2	1.34e-04
Signaling by Insulin receptor	4/102	0.007	9.17e-05	0.001	1/34	0.002
Signaling by FGFR	4/109	0.007	1.18e-04	0.001	29/142	0.01
RUNX1 regulates transcription of genes involved in interleukin signaling	2/7	4.50e-04	1.26e-04	0.002	2/5	3.36e-04
PI3K/AKT Signaling in Cancer	4/124	0.008	1.93e-04	0.002	1/21	0.001

UP

18 of 25 Identifiers Were found in the Reactome were 227 were hit by at least one of them

DOWN

19 of 25 Identifiers Were found in the Reactome were 273 were hit by at least one of them

Note: Data from the untreated patient (H1') and treated patient with HAMLET (P1') with an interval of two weeks. A low values of p (< values <<<), higher probability that the up(LEFT) or down (right) regulated genes are affected or influence a certain pathway.

Table 2f

Table 3. Reactome analysis of the pathways influenced by the up and down regulated genes after oral administration of HAMLET in a patient with thyroid cancer.

Sample	UP	DOWN
H1 vs H1'	76 (12/24)	161(20/24)
P1 vs P1'	195 (24/28)	160(20/26)
H1 vs P1	180(12/19)	235(18/25)
H1 vs P1'	132(12/19)	276(22/28)
H1' vs P1	118(15/23)	235(20/25)
H1' vs P1'	227(18/25)	273(19/25)

- According to the p values from the table 2(A-F), at low values of p (< values <<<), higher probability that the up(LEFT) or down (right) regulated genes are affected or influence a certain pathway. For example, proportion of identifiers **(x/x)** found in the Reactome where i.e. a number of pathways **(X)** were hit by at least one of them (Fabregat et al., 2016; 2017). Recatome pathwasy are enriched in the submitte data (n= 25 genes). My list contain more proteins **(x/x)** for pathway **X** tan would be expected by chance.

Table 3

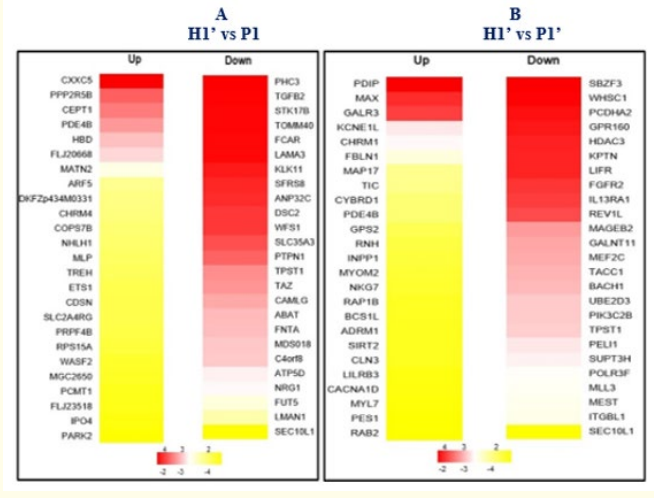

Figure 5: Gene expression pattern between healthy individual (H1') and the diagnosed patient with Thyroid Cancer. (P1') A. Up regulated genes in healthy individual (H1') versus untreated patient (P1) at time zero with Z values from 2.0 to 4.0 encoding molecular components involved as nuclear protein in myelopoiesis and as activator of p53 (CXXC5), in signal transduction (PPPR5b, PDE4B), synthesis of choline or ethanolamine (CEPT1), oxygen transport (HBD). Down regulated genes with a Z score value from -2.0 to -4.0) involved in chromatin binding activity (PHC3), immune system (TGFB2), intracellular signaling in apoptosis, involved in papillary thyroid cancer (STK17B). B. Up regulated genes in healthy individual (H1') versus treated patient (P1') with Z values from 2.0 to 4.0 encoding a disulfide isomerase involved in estradiol binding activity (PDIP), a MYC associate factor an oncoproteins implicated in cell proliferation, differentiation, and apoptosis (MAX); a nucleopeptide involved in hormone secretion, cognition/memory, sensory/pain (GALR3); central/peripheral nervous system (CHRM1); as novel candidate tumor suppressor (FBLN1). Down regulated genes in healthy individual (H1') versus treated patient (P1') with Z values from 2.0 to 4.0 encoding, transcription factors involved in DNA binding (SBZF3); in histone methyl transferase, tumor suppressor (WHSC1); in synaptic junctions (PCDHAR); histone deacetylase 3 regulation of osteoblast differentiation and bone formation (HDAC3), and FGFR2 involved in cell multiplication and maturation, as well as the formation of new blood vessels, wound healing, and the growth, development and maintenance of bones.

Discussion

The aim of the present study is to analyze the expression gene pattern before and after oral administration of HAMLET in an individual diagnosed with thyroid cancer. DNA microarrays (heat maps) and REACTOME data base were used to determine the gene expression pattern and their functionality in the different pathways of the human biology. Thyroid cancer is the most common endocrine malignancy, representing 1% of all human malignancies, and a progressively increasing incidence rate has been observed in recent years, with 95% of thyroid cancer represented by differentiated thyroid carcinomas [20-23] (Abdel-Rahman., *et al*. 2015; Ananas., *et al*. 2020; Huang., *et al*. 2021). It is a highly heterogeneous malignancy characterized by genetic, cytological and histological alterations. Several histological variants such as papillary and follicular thyroid carcinoma, while Differentiated thyroid cancer (DTC) is the most common endocrinological malignancy. The pathogenesis and molecular mechanisms of thyroid cancer remain to be elucidated, despite the fact that the genetics and epigenetics of thyroid cancer are gradually increasing, and gene mutations and methylation changes play an important roles in its occurrence and development (Huang., *et al*. 2021) [23]. Indeed, aggressiveness of thyroid tumors is closely linked to specific gene alterations Epigenetic modification refer to genetic modification that does not change the DNA sequence of a gene but causes heritable phenotypic changes in its expression. Epigenetic modification mainly includes four aspects: DNA methylation, chromatin remodelling, noncoding RNA regulation, and histone modification [26-29]. Several molecular markers for diagnostic and prognostic have been reported such as BRAF and RAS point mutations; RET/PTC and PAX8/PPARγ gene rearrangements; mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/AKT signaling pathways, p53, Wnt-beta catenin, HIF1α and NF-kappaB signaling pathways; microRNA profiles (Oh and Ahn., 2021; Fagin., *et al*. 2923) [24,25]. Interestingly, methylation have been demonstrated in more than 70% of DTC, and it has been identified genes whose expression is associated with the methylation status of their promoters, strengthening the role of the DNA methylation in well-differentiated and development thyroid cancer. At the same time allows optimization of novel markers that are associated and indicative of recurrence, high risks of mortality, and metastatic potential. In addition to consider these agents for diagnosis and treatment of radioiodine refractory and inoperable DTC patients. At this point, for example it has been shown that the upregulation of LncRNA GHET1 was significantly associated with tumor invasion, gender, and lymph node metastasis in patients with thyroid cancer [30] (Liu., *et al*. 2021). The overexpression of LncRNA GHET1 promoted cell proliferation, invasion, and migration, inhibited cell apoptosis, and increased cell population at the S phase in TPC cells. In addition, small ubiquitin-like modifier (SUMO) modification is increasingly recognized as critical in tumorigenesis and progression. This study identifies biomarkers (BMP8A, RGS8, and SERPIND1) linked to SUMOylation in papillary thyroid carcinoma (PTC) aiming to advance therapeutic and prognostic strategies (Li., *et al*. 2024) [31]. Their linkage to immune response and drug sensitivity highlights their importance as targets for therapeutic intervention and prognosis in PTC research. A positive correlation has been found between expression of vascular endothelial growth factor (VEGF) and a more aggressive phenotype of DTC (Abdel-Rahman., *et al*. 2015) [20,22].

By another hand by approaching the causes of the incidence of thyroid cancer has been increasing worldwide during the last decades for four main reasons: tumor progression, tumor recurrence, tumor refractory to radioiodine, and drug resistance, mutations, which have allowed to identify the molecular pathways that dictamine the progression of the malignancy. A deeper understanding of the genetic (e.g. BRAF/V600E, PIK3CA, TP53 mutation, etc.) and epigenetic (e.g. histone methylation, histone de-acetylation, microRNA) regulations that could be driving tumor progression, in addition to further contributing to the learning and understanding of the molecular mechanism involved, resulting in the development of molecular therapeutics to target these specific oncogenic pathways, and/or inhibitors of any of these processes, i.e. dehypermethylation drugs (methylation inhibitors) [26-29].

In addition to these lights of the literature, it has been reported that alpha-lactalbumin (α) (alpha-LA) a small (Mr 14,200) acidic (pI 4–5) Ca2+-binding protein, which serves primarily as a substrate for lactate synthase, a component of the lactose synthase enzyme system [4,5]. Studies from the literature have described that partially unfolded complexes of α-LA with oleic acid showed significant cytotoxicity to various tumor and bacterial cells [4,5]. Oleic acid and protein form a common nuclear shell structure, called lipoproteins (lipids and partially denatured

proteins) considered as molten globular containers filled with toxic oil whose action begins by stabilizing the unfolded protein into the protein complex: followed by insertion and integration of fatty acid in the membrane, resulting in membrane alteration, internalization of HAMLET, selection of cellular components and finally, activation of different signaling pathways such as apoptosis, caspase, Ras, c-Myc pathways and cell death [32,33]. From *in vitro* studies, it has been found that Treatment of RAW 264.7 cells with a high concentration of alpha-LA (100 g/mL) results in a time- and dose-dependent decrease in growth activity, morphological changes, and increased hypoglycemia [34]. In fact, a high dose of this protein induces cell apoptosis and necrosis. It activates several signaling pathways, such as activation of cytochrome c, caspase 3, caspase 8, extracellular signal-regulated kinase (ERK1/2), and cJun N-terminal kinase (JNK); however, it suppresses the protein level of Bcl-2. In fact, it has been suggested that long-term consumption of alpha-LA reduces the risk of colon cancer, possible through an inhibition of cyclooxygenase-2 [35]. Of note is the finding that even a single exposure to culture medium containing alpha-LA from an active batch for a period as short as 30 minutes is sufficient to cause cell death, possibly through apoptosis [25,36]. Furthermore, animal models, the oral administration of alfa-LA has a marked suppressive effect on liver fibrosis through a nitric oxide-mediated mechanism in rats [37]. Highlighted by these studies, in the present work we aimed to analyze the effect of the oral administration of the Human α-lactalbumin Made Lethal to Tumor cells (HAMLET) under a clinic protocol (Figure 1) at the level of gene expression (Up and Down regulated genes (Figure 2-4) and functional analysis of these genes (Table 2A-F, Table 3) (Figure 3). Of relevance is that the oral administration of HAMLET induced a decrease the number of up regulated genes with respect to healthy individual and an increase in the down regulated genes (Table 1). The gene expression pattern influenced by the HAMLET oral administration is characterized by the Up regulation of genes with Z values of 2.0 to 4.0) in the patient (P1, P1') with thyroid cancer before (P1) and after oral administration of HAMLET (P1') encoding genes involved in Hypothyroidism disease (C10orf88), cellular response to stimuli (ARNTL2), cell cycle (CCNC), Immune system (FOXP3), transport of small molecules (SLC2AS3). Down regulated genes, Signal transduction (STK17B), Immune system (IL-17), mytotic cycle (PTPN13), FABP5 (fatty acid uptake), Small subunit process some (CGI-48). Whilein healthy individual (H1, H1'), up regulated genes involved in myelopoiesis (CXXC5), oxygen transport (HBD), nucleolus organization (ACTR6), cell cycle progression (SIVA), signal transduction (PDE4B), Down regulated genes with a Z values of -2.00 to -4.0 encoding proteins involved in cell-cell adhesion and motility (LPP), vesicle trafficking (Figure 4), cellular response to stimuli (VN1R1), respiratory chain (NDUFAG), and actin-binding proteins (ACTR3) (Figure 2A-D). In agreement with these highlights from the literature we found that oral administration of HAMLET to a patient (P1, P1') most of the genes are down regulated than up regulated (Table 1). Remarkably, the gene expression pattern of up and down regulated genes of the untreated healthy individual (Figure 1) compared with untreated patient (Figure 4A) revealed some interesting up or down regulated genes. Thus, up regulated genes encoding molecular components involved in the synthesis of choline or ethanolamine (CEPT1). The Down regulated genes encoding molecular components involved in chromatin binding activity (PHC3), immune system (TGFB2), intracellular signaling in apoptosis, involved in papillary thyroid cancer (STK17B) (Figure 4A) More interesting, the Up regulated genes in healthy individual (H1') versus treated patient (P1') encoding a disulfide isomerase involved in estradiol binding activity (PDIP), a MYC associate factor an oncoproteins implicated in cell proliferation, differentiation, and apoptosis (MAX); a nucleopeptide involved in hormone secretion, cognition/memory, sensory/pain (GALR3); central/peripheral nervous system (CHRM1); as novel candidate tumor suppressor (FBLN1). Down regulated genes encoding, transcription factors involved in DNA binding (SBZF3); in histone methyl transferase, tumor suppressor (WHSC1); histone deacetylase 3 regulation of osteoblast differentiation and bone formation (HDAC3), and the fibroblast growth factor involved in cell multiplication and maturation, as well as the formation of new blood vessels, wound healing, and the growth, development and maintenance of bones. (FGFR2) (Figure 4B). This data are in agreement with the number of pathways in which down regulated genes are influenced by these genes after oral administration of HAMLET in the patient (P1') (Table 2A-F). For example in healthy individual up regulated genes influence 76 pathways, and down regulated genes influence 161 pathways. Remarkably, before and after oral administration in the patient (P1 and P1'), Up regulated genes influence 195 while down regulated genes are the same number of pathways than healthy individual. When the healthy individual vs patient at time zero 235 pathway are influence by the down regulated genes. And 180 of the Up regulated genes. And after oral administration, 276

pathways are influenced versus 161 in healthy individual. The most strikingly difference was after the fourth dose, in which 227 pathways form the up regulated genes while 273 were influenced by the down regulated genes. Moreover, after oral administration of HAMLET, the number of reactions that were hit by at least one the genes was higher in the patient than in healthy individual. 24/28 up regulated genes versus 12 /24 in the healthy individual. 20/24 I healthy individual versus 20/26 in the patient treated with HAMLET (Table 3, Figure 3).

In summary, DNA microarrays (represented as the heat maps), continue be a promising and potential tool to determine the signature of the up and down regulated genes in the different pathways of the human biology. Furthermore, the results of the present clinic report using the aforementioned technology and the REACTOME data base, allow to conclude that oral administration of HAMLET down regulated genes that play a role the progression of the thyroid cancer (STK17B). Down regulated inflammatory and autoimmune responses through IL17RB, and down regulated signalization in in cell growth, mitotic cycle, differentiation (PTPN13), fatty acid binding proteins (FABP5), RNA binding (UTP18), synaptic junctions, (PCHA2, PCDHB8). Of note is that up regulated genes encoding markers of hypothyroidism as C16orf88. Up regulate to the cyclic involved in cell cycle (CCNC), and transcriptional regulators of the immune system (FOXP3). In addition, up regulate the cellular response (ARNTL2) to stimuli and transport of small molecules (SLC25A3). We propose that all these encoding molecular components could be targeted in patients with thyroid cancer before radioiodine treatment and surgery with oral administration of HAMLET as a prophylactic treatment.

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Declarations Statement

The authors declare no conflict of interest.

Ethics Approval and Consent to Participate

The study and all the procedures for medical research involving human subjects, including research on identifiable human material and data were performed under the principles of the Declaration of Helsinki, and approved by the Ethic Committee in Research of the Zacatecas, General Hospital "Luz Gonzalez Cosio" CONBIOETICA-32-CEI-001-20180807.

Consent for Publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review.

Competing Interests

The authors declare no competing of interests.

Availability of Data and Material

Data will be shared following institutional guidelines. The review of the literature was based on search and data from PubMed database without limitation to 2024.

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Author's Contributions

G.G.G.M. and A.A.C. Conceptualization, G.G.G.M. Methodology, analysis, and writing. D.C.R.M. D.C.S. P.R.M. Collaboration in patient's contact, clinic lab samples analysis, discussion. A.E.T. Methodology. All authors have read and approved the manuscript.

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