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Thyroid hormone-induced expression of inflammatory cytokines interfere with resveratrol-induced anti-proliferation of oral cancer cells

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ABSTRACT

Thyroid hormone, L-thyroxine (T_4), induces inflammatory genes expressions and promotes cancer growth. It also induces expression of the checkpoint programmed death-ligand 1 (PD-L1), which plays a vital role in cancer progression. On the other hand, resveratrol inhibits inflammatory genes expressions. Moreover, resveratrol increases nuclear inducible cyclooxygenase (COX)-2 accumulation, complexes with p53, and induces p53-dependent anti-proliferation. In this study, we investigated the effect of T_4 on resveratrol-induced anti-proliferation in oral cancer. T_4 increased the expression and cytoplasmic accumulation of PD-L1. Increased expressions of pro-inflammatory genes, *interleukin* (*IL*)*-1β* and *transforming growth factor* (*TGF*)*-β1*, were shown to stimulate *PD-L1* expression. T_4 stimulated pro-inflammatory and proliferative genes expressions, and oral cancer cells proliferation. In contrast, resveratrol inhibited those genes and activated anti-proliferative genes. T_4 retained resveratrol-induced COX-2 in cytoplasm and prevented COX-2 nuclear accumulation when resveratrol treated cancer cells. A specific signal transducer and activator of transcription 3 (STAT3) inhibitor, S31-201, blocked T_4-induced inhibition and restored resveratrol-induced nuclear COX-2 accumulation. By inhibiting the T_4-activated STAT3 signal transduction axis with S31-201, resveratrol was able to sequentially reestablish COX-2/p53-dependent gene expressions and anti-proliferation. These findings provide a novel understanding of the inhibitory effects of T_4 on resveratrol-induced anticancer properties via the sequential expression of *PD-L1* and inflammatory genes.

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1. Introduction

Obesity is linked with several types of diseases in which secretions of pro-inflammatory hormones and cytokines (leptin, resistin, interleukin (IL)-1β, transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, and IL-6) are increased. The reduced release of adipokines such as adiponectin and IL-10 downregulates inflammation (Mancini et al., 2016). In addition, inflammation and oxidative stress are involved in disease processes, including those of diabetes, cardiovascular diseases, and cancers. On the other hand, thyroid hormone plays an important role in regulating oxidative stress (Elnakish et al., 2015). Increased reactive oxygen species (ROS), some of the most important pro-oxidants, are well demonstrated in hyperthyroidism (Marcocci et al., 2012; Villanueva et al., 2013). Thyroxine (T_4) is also able to induce expressions of pro-inflammatory genes (Davis et al., 2016) to modulate inflammatory activities, which may be linked to cancer progression.

Programmed cell death-ligand 1 (PD-L1) plays an important role in evading immune surveillance (Guan et al., 2017). Therefore, overexpression of PD-L1 affects the cell cycle, cell proliferation, apoptosis, and carcinogenesis (Song et al., 2014). Recently, we showed that treating cancer cells with extracts of the anti-inflammatory traditional herbal medicine, *Anoectochilus formosanus,* inhibited constitutive *PD-L1* expression and its protein accumulation. It further inhibited cancer proliferation (Ho et al., 2018a). These observations suggest that PD-L1 may be involved in an inflammatory effect on cancer proliferation (Ho et al., 2018a). Evidence also indicates that pro-inflammatory cytokines, such as TGF-β (Park et al., 2016; Sun et al., 2018) and IL-1 (Guo et al., 2017), are able to induce *PD-L1* expression. TNF-α may enhance the adaptive immune resistance mediated by interferon (IFN)-γ-induced PD-L1 in hepatocellular carcinoma cells (Li et al., 2018). Several signal transduction pathways, such as activated extracellular signal-regulated kinase 1/2 (ERK1/2), phosphatidylinositol-3-kinase (PI3K), and signal transducer and activator of transcription 3 (STAT3), are involved in PD-L1 expression by different agents (Li et al., 2019; Liu et al., 2017; Wang et al., 2017). Studies conducted by our group also indicated that activated ERK1/2 (Chin et al., 2018; Lin et al., 2016a, 2018b), and PI3K (Lin et al., 2018b) were shown to play important roles in T_4-induced PD-L1 expression. However, role of STAT3 activation in T_4-induced PD-L1 is still unknown.

Resveratrol was shown to inhibit expressions of pro-inflammatory genes (Walker et al., 2014). It is also well demonstrated to induce anti-proliferative activities in different cancer cells and to inhibit cancer growth in vivo (Yousef et al., 2017). By binding to its receptor on integrin αvβ3, resveratrol activates ERK1/2. Activation of ERK1/2 is essential for resveratrol-induced expression of cyclooxygenase (COX)-2 and its nuclear accumulation to promote anti-proliferation in cancer cells. The nuclear-translocated phosphorylated ERK1/2-COX-2 complex binds with p53 to sequentially trigger phosphorylation of p53 at Ser-15 and promotes anti-proliferation (Lin et al., 2018b).

In the current study, we investigated the inhibitory action of T_4 on the anti-proliferative effect of resveratrol in oral cancer cells. Resveratrol induced anti-proliferation in two oral cancer cell lines was inhibited by T_4 via *IL-1β* or *TGF-β1* pro-inflammatory gene expression and *PD-L1* protein accumulation. Resveratrol-induced nuclear COX-2 accumulation was suppressed by co-incubation with T_4. Blocking STAT3 signaling was able to block T_4-induced expression of pro-inflammatory genes and *PD-L1*. Consequently, it reduced the inhibitory effect of T_4 on resveratrol and resumed resveratrol-induced anti-proliferation.

2. Experimental methods

2.1. Cell cultures

The human oral epidermoid carcinoma cell line, OEC-M1 (an indigenous oral cancer cell line in Taiwan), was established from gingival epidermal carcinoma of a patient in Taiwan by Dr. Ching-Liang Meng (Yang and Meng, 1994). This cell line was contributed by Dr. Hsien-Chung Chiu. Human squamous carcinoma of the tongue, SCC-25 (ATCC® CRL-1628™), was purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Cell lines were tested and authenticated by the BCRC (isoenzyme analysis, Mycoplasma, cytogenetics, tumorigenesis, and receptor expression testing). Cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified incubator with 5% CO_2. Before the study, cells were placed in 0.25% hormone-depleted serum-supplemented medium for 2 days.

2.2. Reagents

T_4 and resveratrol were obtained from Sigma-Aldrich (St Louis, MO, USA). T_4 was prepared as a stock 10^{-4} M solution in 0.04 N KOH and 4% propylene glycol. Resveratrol was dissolved in ethanol as a 100 mM stock solution. The final concentrations of solvents in which reagents were dissolved, were tested for activity and did not affect the experimental outcomes. Goat polyclonal antibody to COX-2 Polyclonal rabbit anti-COX-2 was obtained from Abcam (Cambridge, UK), monoclonal mouse anti-PD-L1 was from Cell Signaling (Beverly, MA, USA), anti-β-actin was from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA), and polyclonal rabbit anti-Lamin B1 was from GeneTex, Inc. (Hsinchu City, Taiwan). Goat anti-rabbit immunoglobulin G (IgG) and goat anti-mouse IgG were obtained from Cell Signaling, Anti-pERK1/2 was purchased from GeneTex, Inc., anti-Cyclin D1 and anti-ERK1/2 were from Santa Cruz Biotechnology, Inc., anti-α-Tubulin was obtained from Novus Biologicals (Littleton, CO, USA). Anti-PI3K, anti-pSTAT3 were supplied by Cell Signaling. Amersham ECL Western Blotting Detection Reagents were from GE Healthcare (Amersham, UK). S31-201, a specific STAT3 inhibitor, was supplied by Abcam, Millipore Immobilon-PSQ Transfer PVDF membranes and Immobilon TM Western HRP Substrate Luminol Reagent was purchased from Millipore (Billerica, MA, USA).

2.3. Cell viability assay

SCC-25 and OEC-M1 cells were plated at a density of 10^4 cells/well in 96-well plates. Cell viability was determined using the CyQUANT® NF Cell Proliferation Assay Kit (Molecular Probes, Eugene, OR, USA) (Ho et al., 2018b) at 96 h after treatment. Peptides were replaced daily with refreshed media. Briefly, medium was removed, and cells were incubated with CyQUANT® NF reagent for 1 h at 37 °C according to the manufacturer's instructions. Plates were then analyzed using a microplate reader (Varioskan™ Flash Multimode Reader, Thermo Scientific, Waltham, MA, USA) (with excitation at 485 nm and emission at 530 nm).

2.4. Quantitative real-time polymerase chain reaction (qPCR)

Total RNA was extracted with genomic DNA removed with an Illustra RNAspin Mini RNA Isolation Kit (GE Healthcare Life Sciences, Buckinghamshire, UK). One microgram of DNase I-treated total RNA was reverse-transcribed using a RevertAid H Minus First Strand cDNA Synthesis Kit (Life Technologies, Carlsbad, CA, USA) into complementary (c)DNA. cDNAs were used as the template for real-time PCRs and analyses. The real-time PCRs were conducted using a QuantiNovaTM SYBR® Green PCR Kit (Qiagen, Hilden, Germany) on a CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Reaction procedures involved initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturing at 95 °C for 5 s and combined annealing/extension at 60 °C for 10 s, as shown in detail in the manufacturer's instructions. The primer sequences were as follows: *Homo sapiens* cyclin D1 (*CCND1*), forward 5′-CAAGGCCTGAACC TGAGGAG-3′ and reverse 5′-GATCACTCTGGAGAGGAAGCG-3′

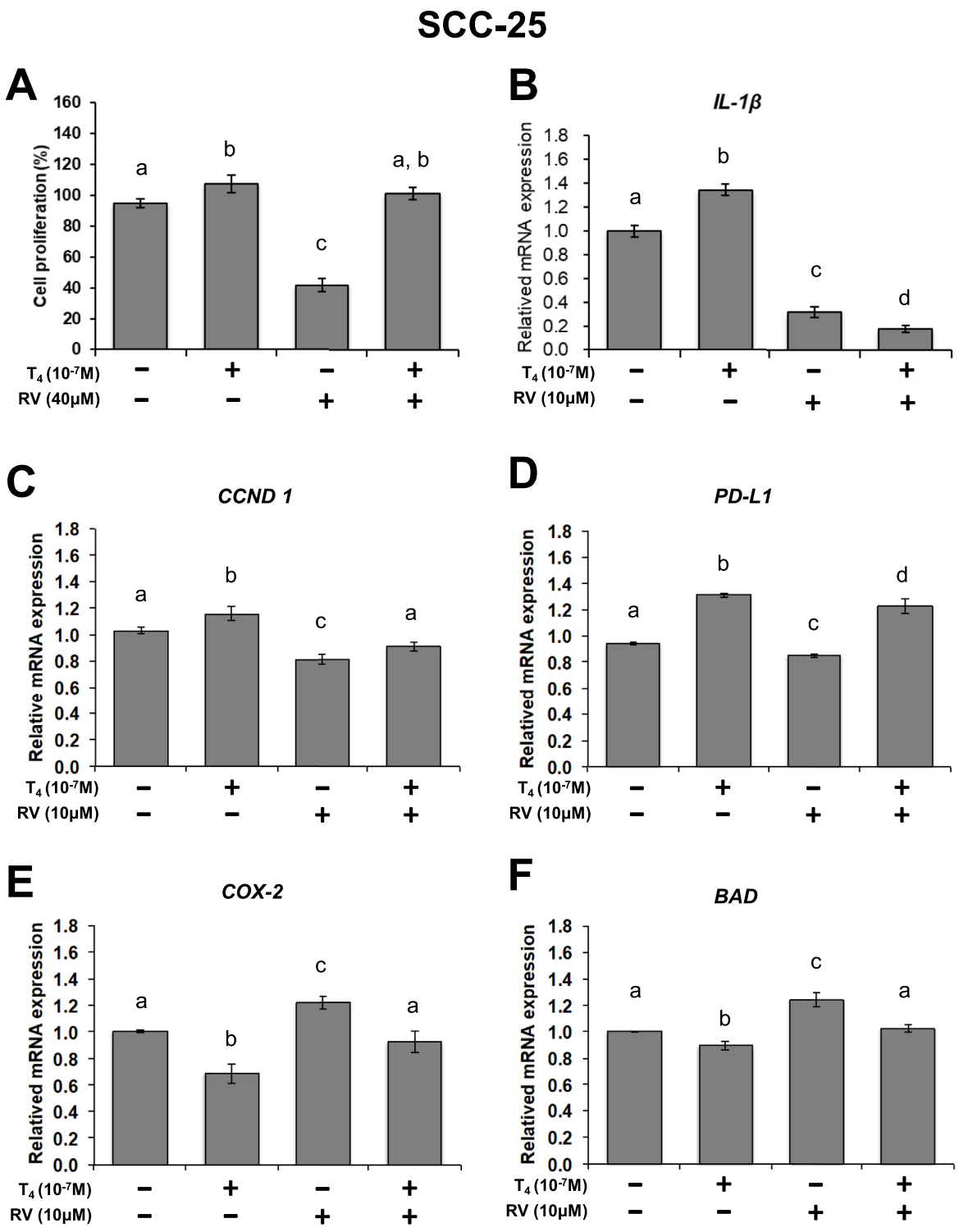

Fig. 1. Thyroxine blocks resveratrol-induced gene expression and anti-proliferation in oral cancer SCC-25 cells. (A) Oral cancer SCC-25 cells were treated with 10^{-7} M T_4, 40 μM resveratrol or their combination for 96 h and then examined by CyQUANT® NF Cell Proliferation Assay. SCC-25 cells were seeded in 6 well-tray and treated with 10^{-7} M T_4, 10 μM resveratrol or their combination for 24 h. Total RNA was extracted and qPCR of *IL-1β* **(B)**, *CCND1* **(C)**, *PD-L1* **(D)**, *COX-2* **(E)** and *BAD* **(F)** was conducted. Numbers of Independent studies (N) = 3. (Data are expressed as mean ± SD. a–d: the subsets after *post hoc* analysis of the significant differences were obtained by one-way ANOVA).

(accession no.: NM_053056); *Homo sapiens PD-L1* (CD274), forward 5′-GTTGAAGGACCAGCTCTCCC-3′ and reverse 5′-ACCCCTGCATCCTG CAATTT-3′ (accession no. AY254342.1); *Homo sapiens COX-2*, forward 5′-GCCAAGCACTTTTGGTGGAG-3′ and reverse 5′-GGGACAGCCCTTCA CGTTAT-3′ (accession no. AY462100.1); *Homo sapiens* BCL2-associated agonist of cell death (*BAD*), forward 5′-CTTTAAGAAGGGACTTCCTC GCC-3′ and reverse 5′-AAGTTCCGATCCCACCAGGA-3′ (accession no.: NM_032989.2); *Homo sapiens IL-1β*, forward 5′-CTTCGAGGCACAAGG CACA-3′ and reverse 5′-GCTTCAGACACTTGAGCAATGA-3′ (accession no.: NM_000576.2); *Homo sapiens TGF-β1*, forward 5′-GCCCTGGACAC CAACTATTGC-3′ and reverse 5′-GCTGCACTTGCAGGAGCGCAC-3' (accession no.: NM_000660.6); and *Homo sapiens* 18S ribosomal RNA

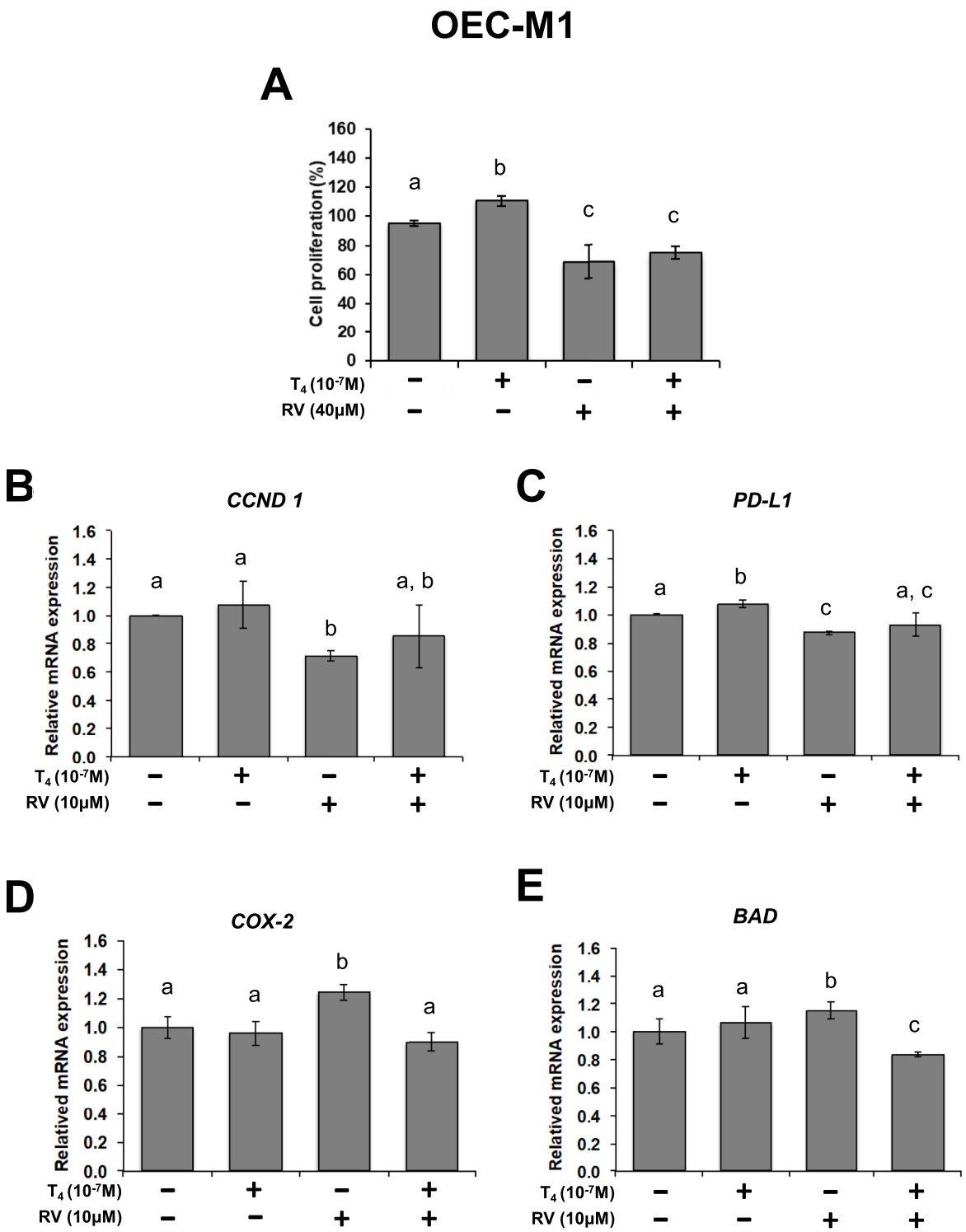

Fig. 2. Thyroxine blocks resveratrol-induced gene expression and anti-proliferation in oral cancer OCM-1 cells. (A). Oral cancer OEC-M1 cells were treated with 10^{-7} M T_4, 40 μM resveratrol or their combination for 96 h and then examined by CyQUANT® NF Cell Proliferation Assay. OEC-M1 cells were seeded in 6 well-tray and treated with 10^{-7} M T_4, 10 μM resveratrol or their combination for 24 h. Total RNA was extracted and qPCR was conducted for *CCND1* **(B)**, *PD-L1* **(C)**, *COX-2* **(D)** and *BAD* **(E)**. N = 3. (Data are expressed as mean ± SD. a–c: the subsets after *post hoc* analysis of the significant differences were obtained by one-way ANOVA).

(*18S*), forward 5′-GTAACCCGTTGAACCCCATT-3′ and reverse 5′-CCAT CCAATCGGTAGTAGCG-3′ (accession no.: NR_003286). Relative gene expressions (normalized to *18S* reference gene) were calculated according to the ΔΔCT method. The fidelity of the PCR was determined by a melting temperature analysis.

2.5. Confocal microscopy

This technique was previously described in our studies (Chin et al., 2015, 2018; Lin et al., 2018b). Briefly, exponentially growing oral cancer OEC-M1 and SCC-25 cells were seeded onto sterilized cover glasses (Paul Marienfeld, Lauda-Königshofen, Germany). Cells were

treated with resveratrol, T_4, or their combination for 24 h. Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min and then permeabilized in 0.1% Triton X-100 in PBS for 20 min. Samples were incubated with a mouse monoclonal antibody to COX-2 (1:50, Santa Cruz Biotechnology) followed by an Alexa-488-labeled goat anti-mouse antibody (1:500, Abcam) and mounted in EverBrite Hardset mounting medium with DAPI (Biotium, Fremont, CA, USA). The fluorescent signal from COX-2 was recorded and analyzed with the TCS SP5 Confocal Spectral Microscope Imaging System (Leica Microsystems, Wetzlar, Germany). The figures shown are representative of at least four fields for each experimental condition.

2.6. Western blot analyses

This method was described in our previous studies (Chin et al., 2015, 2016; Lin et al., 2016a; Yang et al., 2016). In brief, protein samples were resolved by 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A 40-μg quantity of protein was loaded in each well with 5x sample buffer, and protein samples were resolved by electrophoresis at 100 V for 2 h. The separated protein bands in gels were transferred to Millipore Immobilon-PSQ Transfer nitrocellulose membranes (Millipore) by a Mini Trans-Blot® Cell (Bio-Rad Laboratories). After completing the transfer, membranes were blocked with Tris-buffered saline containing 0.05% Tween 20 (TBST) with 2% bovine serum albumin (BSA) and then were incubated with primary antibodies to phosphorylated (p)PI3K (p85), pSTAT3, pERK1/2 (Cell Signaling Technology), or GAPDH (GeneTex International), at 4 °C with rocking overnight. Horseradish peroxidase (HRP)-conjugated secondary antibodies were either goat anti-rabbit immunoglobulin G (IgG) or goat anti-mouse IgG (1:3000, Dako, Carpentaria, CA, USA), depending on the origin of the primary antibodies. Immunoreactive proteins were detected by Immobilon™ Western HRP Substrate Luminol Reagent (Millipore). Images of the Western blots were visualized and recorded by Amersham Imager 600 (GE Healthcare Life Sciences).

2.7. Statistical analysis

All of the collected data for immunoblot and nucleotide densities were analyzed by IBM®SPSS® Statistics software vers. 19.0 (SPSS, Chicago, IL, USA). Two-tailed Student's *t*-test was conducted and considered significant at p values of < 0.05 (*, #, $, or &), 0.005 (**, ##, $$, or &&), and 0.001 (***, ###, $$$, or &&&) as the threshold for significance, and this was used to evaluate the significance of effects of the hormone, resveratrol, or inhibitors.

3. Results

3.1. T_4 inhibits resveratrol-induced anti-proliferation and gene expressions in human oral cancer cells

In order to evaluate the inhibitory effects of T_4 on resveratrol-induced anti-proliferation in human oral cancer cells, oral cancer SCC-25 cells were treated with 40 μM resveratrol for 96 h, and then cell viability was examined. T_4 alone stimulated SCC-25 cell proliferation. Conversely, cancer cell proliferation was significantly inhibited by resveratrol. Moreover, the resveratrol-induced anti-proliferative effect was inhibited by T_4 when it was present in the resveratrol-treated cell cultures (Fig. 1A). Next, expressions of genes involved in inflammation, proliferation, anti-proliferation, and immunomodulation were examined. Interestingly, the pro-inflammatory cytokine, *IL-1β,* was stimulated by T_4 alone (Fig. 1). T_4 induced expressions of *CCND1* and *PD-L1* (CD274) in SCC-25 cells. In contrast, resveratrol inhibited the proliferative genes, *CCND1* and *PD-L1* (Fig. 1C and 1D), while it induced *COX-2* and proapoptotic *BAD* expression. In co-treatment of SCC-25 cells with T_4 and resveratrol, expressions of *CCND1* and *PD-L1* by T_4 were inhibited by resveratrol (Fig. 1C and 1D). On the other hand, resveratrol-induced *COX-2* and *BAD* expressions were reversed by T_4 co-treatment.

Similar research was conducted on the other oral cancer OEC-M1 cell line. T_4 stimulated OEC-M1 cell proliferation. T_4 alone stimulated OEC-M1 cell proliferation. On the other hand, cancer cell proliferation was significantly inhibited by resveratrol. Moreover, the resveratrol-induced anti-proliferative effect was inhibited by T_4 when it was present in resveratrol-treated cell cultures (Fig. 2A). Quantitative (q)PCR studies indicated that T_4 induced expressions of *CCND1* and *PD-L1* in OEC-M1 cells. In contrast, resveratrol inhibited the proliferative genes, *CCND1* and *PD-L1* (Fig. 2B and 2C), while it induced *COX-2* and pro-apoptotic *BAD* expressions. In co-treatment of OEC-M1 cells with T_4 and resveratrol, expressions of *CCND1* and *PD-L1* by T_4 were inhibited by resveratrol (Fig. 2B and 2C). On the other hand, resveratrol-induced *COX-2* and *BAD* expressions were reversed by T_4 co-treatment.

3.2. T_4 blocks resveratrol-induced nuclear accumulation of COX-2 in human oral cancer cells

We investigated the mechanisms involved in thyroid hormone-induced interference of resveratrol-induced anti-proliferation in oral cancer cells. Confocal microscopic studies were conducted. Cells were starved for 48 h, then fed hormone-stripped serum-containing medium, and afterward treated with either 40 μM resveratrol, T_4, or their combination for another 24 h. In confocal microscopy, 94.1% (53.67/57.33) of resveratrol-treated cells exhibited nuclear COX-2 accumulation compared to the untreated control which had 38.5% (16.5/44.5) nuclear COX-2 accumulation in SCC-25 cells. Conversely, co-treatment with T_4 and resveratrol significantly reduced nuclear accumulation of COX-2 to 80% (45/56.33) compared to resveratrol treatment alone (Fig. 3). When cells were pretreated with a specific inhibitor of STAT3 (S31-201), 85.4% (37.67/44) of resveratrol-treated cells exhibited nuclear COX-2 accumulation compared to co-treatment with T_4 and resveratrol which produced 21.7% (7/31.67). T_4 reduced resveratrol-induced nuclear COX-2 accumulation by 63.7%, indicating that T_4 blocked resveratrol-induced COX-2 nuclear accumulation via activation of the STAT3 signaling pathway.

Similar results were observed in the other oral cancer cell line, OEC-M1, with 97.4% (48.33/49.67) of resveratrol-treated cells exhibiting nuclear COX-2 accumulation compared to the untreated control at 0% (0/43.67). On the other hand, co-treatment with T_4 and resveratrol significantly reduced nuclear accumulation of COX-2 to 74.9% (38.33/50.67) compared to that with resveratrol treatment alone (Fig. 4). Thus, in human oral cancer cells, T_4 inhibited both resveratrol-induced COX-2 accumulation and nuclear translocation. On the other hand, when cells were pretreated with a specific inhibitor of STAT3 (S31-201), 86% (23/27) of resveratrol-treated cells exhibited nuclear COX-2 accumulation compared to 47.3% (12.67/26) following co-treatment with T_4 and resveratrol. T_4 reduced resveratrol-induced nuclear COX-2 accumulation by 38.7%, indicating that T_4 blocked resveratrol-induced COX-2 nuclear accumulation via activation of the STAT3 signaling pathway.

3.3. Activation of the STAT3 pathway is involved in the inhibitory effect of T_4 on resveratrol-induced anti-proliferation

We further investigated the mechanisms involved in the inhibitory effect of T_4 on nuclear COX-2 accumulation by resveratrol in oral cancer cells. Because activated STAT3 plays a vital role in PD-L1 expression, we examined whether the inhibitory effect of T_4 was depleted when using a STAT3 inhibitor. OEC-M1 cells were pretreated with the STAT3 inhibitor, S31-201 (40 μM), for 30 min and then treated with T_4, resveratrol, or their combination for another 24 h. Total proteins were extracted, and Western blot analyses were conducted for pERK1/2, pPI3K and pSTAT3. Both T_4 and resveratrol activated ERK1/2 phosphorylation (Fig. 5). In addition, T_4 activated STAT3 and PI3K (Fig. 5). S31-201 inhibited activation of STAT3 and ERK1/2 by T_4 and

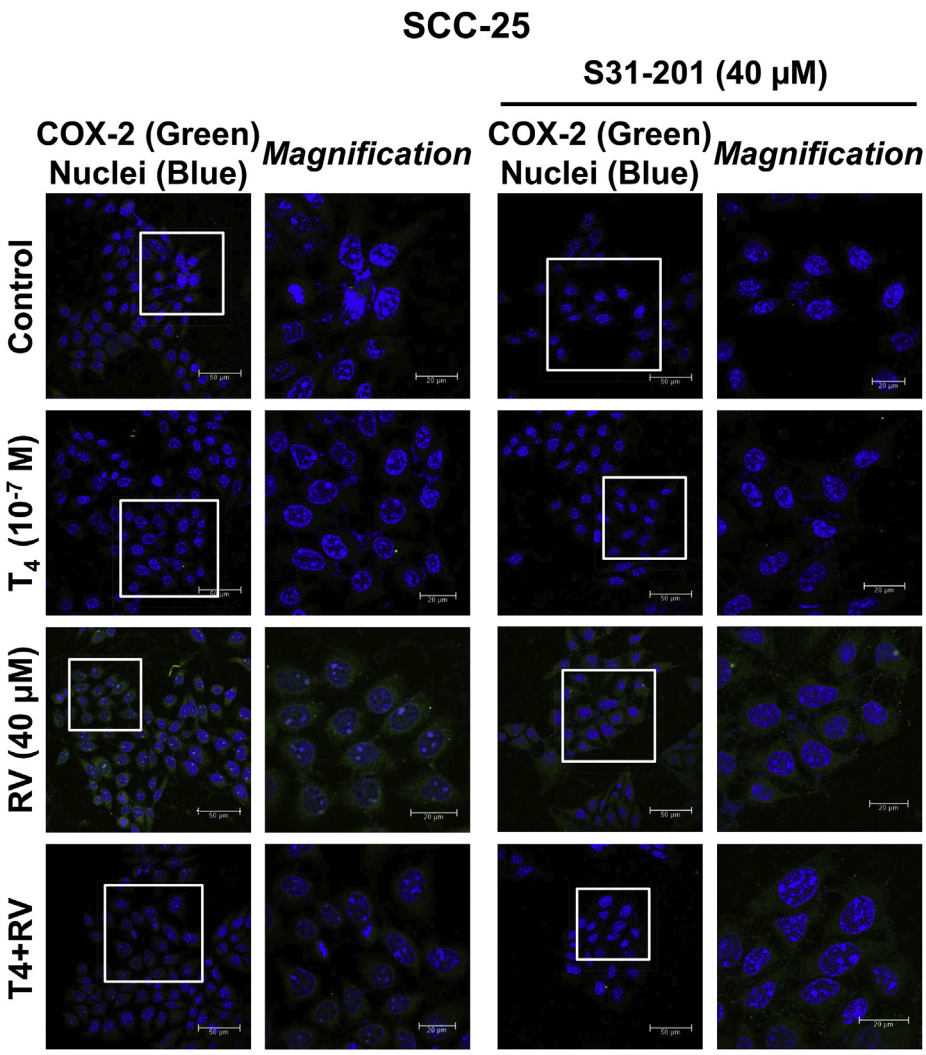

Fig. 3. Thyroxine (T_4) blocks resveratrol-induced nuclear cyclooxygenase (COX)-2 accumulation in human oral cancer SCC-25 cells. Oral cancer SCC-25 cells were seeded on a cover glass. Cells were starved for 48 h, fed hormone-stripped serum containing medium, and then treated with either 40 μM resveratrol, T_4, or their combination in the presence or absence of 40 μM S31-201 for another 24 h. Cells were fixed for confocal microscopic analysis of COX-2 expression (green color) and its nuclear accumulation. Nuclei were counter-stained with DAPI (blue color). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

resveratrol (Fig. 5). Interestingly, S31-201 enhanced PI3K activation in all treatments. These results suggest that the T_4-induced inhibitory effect on resveratrol-induced anti-proliferation may be related to STAT3 activation.

We then examined if the inhibitory effect of T_4 was depleted when using an inhibitor of STAT3. SCC-25 cells were pretreated with the STAT3 inhibitor, S31-201 (40 μM), for 30 min and were then treated with T_4, resveratrol, or their combination for another 24 h. Cells were harvested, and a qPCR was performed to examine expressions of inflammatory genes. In STAT3-inactivated cell cultures, the effect of T_4 diminished expressions of *IL-1β* and *TGF-β* (Fig. 6). On the other hand, resveratrol-induced COX-2 expression was not affected by STAT3 inhibition (Fig. 6). Similar results were observed in the other oral cancer OEC-M1 cell line. T_4 induced inflammatory genes and *PD-L1* via the STAT3-dependent signal transduction pathway. Conversely, resveratrol-regulated gene expressions were not affected by S31-201 (Fig. 7).

In summary, T_4 activated the STAT3 signal transduction pathway thereby stimulating expressions of inflammatory genes and *PD-L1* which interfered with resveratrol-induced anti-proliferation. Suppression of STAT3 activation was able to potentiate resveratrol-induced nuclear COX-2 accumulation, pro-apoptotic gene expressions, and anti-proliferation in oral cancer cells.

4. Discussion

Resveratrol was comprehensively verified to be beneficial against a range of diseases, including cancer in a variety of models; however, its translation to clinical applications still suffers from important drawbacks (Singh et al., 2015). The main concern is raised by the poor bioavailability of stilbene. No more than 1% of resveratrol is detected in the bloodstream after oral administration (Walle, 2011), and it is extensively metabolized by the small intestine and liver (Almeida et al., 2009; Cottart et al., 2010). Moreover, another concern regarding the efficacy of chemotherapeutic agents affected by the cancer cell microenvironment has recently been emphasized. The clinically desirable actions of resveratrol in cancer cells were shown to be suppressed by thyroid hormone (Chin et al., 2018; Lin et al., 2017; Nana et al., 2018), DHT (Chin et al., 2015), and estrogen (Lin et al., 2017) by interfering with resveratrol-induced nuclear COX-2 accumulation (Chin et al., 2018). Additional factors may be caused by this inhibitory effect of thyroid hormone.

Resveratrol induces COX-2 expression and phosphorylated p53-dependent antitumor activities (Lin et al., 2017). Activated ERK1/2-dependent COX-2 nuclear accumulation to form phosphorylated p53-COX-2 complexes is essential (Chin et al., 2015; Ho et al., 2017; Lin et al., 2017). In addition, resveratrol is able to suppress PD-L1 expression (Chin et al., 2018). However, in the presence of T_4, resveratrol-induced expressions of COX-2 and BAD, whose biological activities are involved in anti-proliferation (Figs. 1A, **2A**), were significantly reduced when cells were co-treated with T_4 (Figs. 1C, **2C**). Confocal microscopic studies further verified that accumulated PD-L1 by T_4 holds inducible COX-2 in the cytosol (Figs. 3, **4**) preventing its sequential nuclear

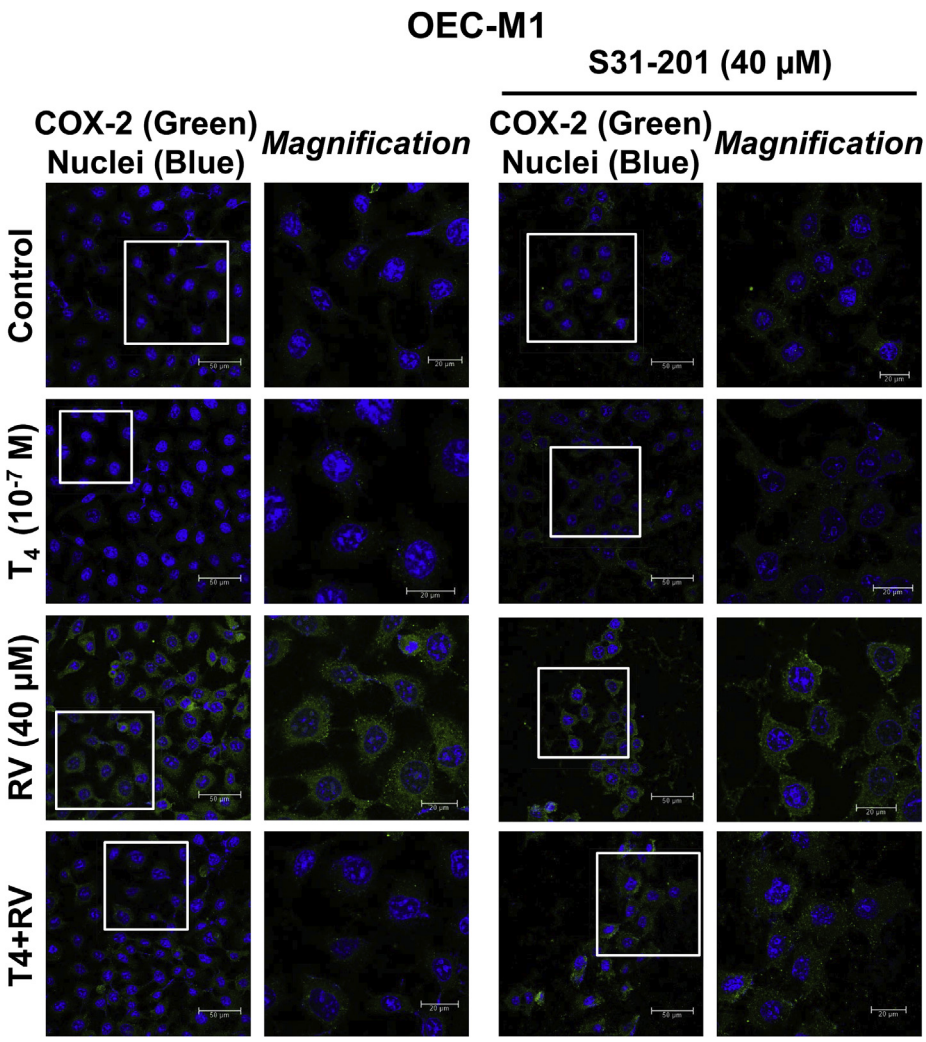

Fig. 4. Thyroxine (T_4)-induced programmed cell death-ligand 1 (PD-L1) interferes with resveratrol-induced nuclear accumulation of cyclooxygenase (COX)-2 in human oral cancer OEC-M1 cells. Oral cancer OEC-M1 cells were seeded on a cover glass. Cells were starved for 48 h, fed hormone-stripped serum containing medium, and then treated with either 40 μM resveratrol, T_4, or their combination in the presence or absence of 40 μM S31-201 for another 24 h. Cells were fixed for confocal microscopic analysis of COX-2 expression (green color) and its nuclear accumulation. Nuclei were counter-stained with DAPI (blue color). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

translocation (Chin et al., 2018). Consequently, it is most likely that T_4-induced PD-L1 accumulation blocks COX-2 nuclear translocation and preferentially occurs in settings where hyperthyroidism exists (Chin et al., 2018; Lin et al., 2018a).

Interactions between resveratrol and thyroid hormone are controversial. Treatment with resveratrol reduces expression of the sodium/iodide symporter and uptake of iodide by thyrocytes (Giuliani et al., 2014). Resveratrol also decreased expressions of thyroglobulin, thyroid peroxidase, thyroid-stimulating hormone (TSH) receptor, Nkx2-1, Foxe1, and Pax8 in the rat thyroid FRTL-5 cell line (Giuliani et al., 2014). Intraperitoneal treatment of Sprague-Dawley rats with 25 mg/kg body weight resveratrol for 60 days increased their thyroid size (Giuliani et al., 2017). Although serum TSH and thyroid hormone levels were in normal ranges, the TSH level was significantly higher in resveratrol-treated rats (Giuliani et al., 2017). Histological and immunohistochemical analyses confirmed increased proliferative activity in the thyroid of resveratrol-treated rats (Giuliani et al., 2017).

Free thyroid hormone at physiological concentrations is anti-apoptotic and pro-angiogenic. These properties are necessary in normal cells such as myocardiocytes, and neurons and in normal tissues to maintain normal physiological activities (Puzianowska-Kuznicka et al., 2006). However, they cause undesirable cancer cell proliferation (Davis et al., 2015; Lin et al., 2016b). T_4 stimulated *PD-L1* expression (Figs. 1, **2**) to retard resveratrol-induced COX-2 in the cytosol (Figs. 3, **4**) and promoted anti-proliferation in two oral cancer cell lines. T_4 was shown to inhibit the action of resveratrol by increasing *PD-L1* expression. The inhibitory effect of T_4 on resveratrol-induced anti-proliferation can be reversed by knocking down the *PD-L1* gene. These results suggest that T_4-induced *PD-L1* plays a crucial role in inhibiting resveratrol-induced anti-proliferation by blocking nuclear COX-2 accumulation in ovarian cancer cells (Chin et al., 2018) and oral cancer cells.

Expression of *PD-L1* by cancer cells is a defense mechanism to enable them to escape activated T cell-induced anticancer immune attack (Shien et al., 2016). High expression of the immunosuppressive PD-L1 protects cancer cells by allowing tumor cell growth in secrecy mode hidden from the immune system. This effect has generated much interest in developing anti-PD-L1 agents, and these were shown to have clinically important effects (Eggermont et al., 2015; Ott et al., 2013; Powles et al., 2014). In addition to resveratrol's low bioavailability, the effect of T_4-induced *PD-L1* expression on cancer cells represents a complementary hindrance that may impede the polyphenol-induced anticancer features. Furthermore, anti-resveratrol properties of PD-L1 are supported by physiological concentrations of T_4 (Chin et al., 2018) which retained resveratrol-induced nuclear COX-2 accumulation (Figs. 3, **4**). On the other hand, results also indicated that resveratrol inhibited *PD-L1* gene expression (Figs. 1, **2**). Therefore, a complex relationship exists between resveratrol and *PD-L1* that presents mutually antagonistic effects.

T_4 and resveratrol modulate *PD-L1* messenger (m)RNA expression and the PD-L1 protein level via the extracellular domain of integrin αvβ3. Conversely, susceptibilities of PD-L1 to regulation via the thyroid hormone receptor on αvβ3 vary in different types of cancers. In addition, the magnitudes of the response to T_4 of PD-L1 mRNA and protein levels are diverse among cell lines studied. From a clinical point of

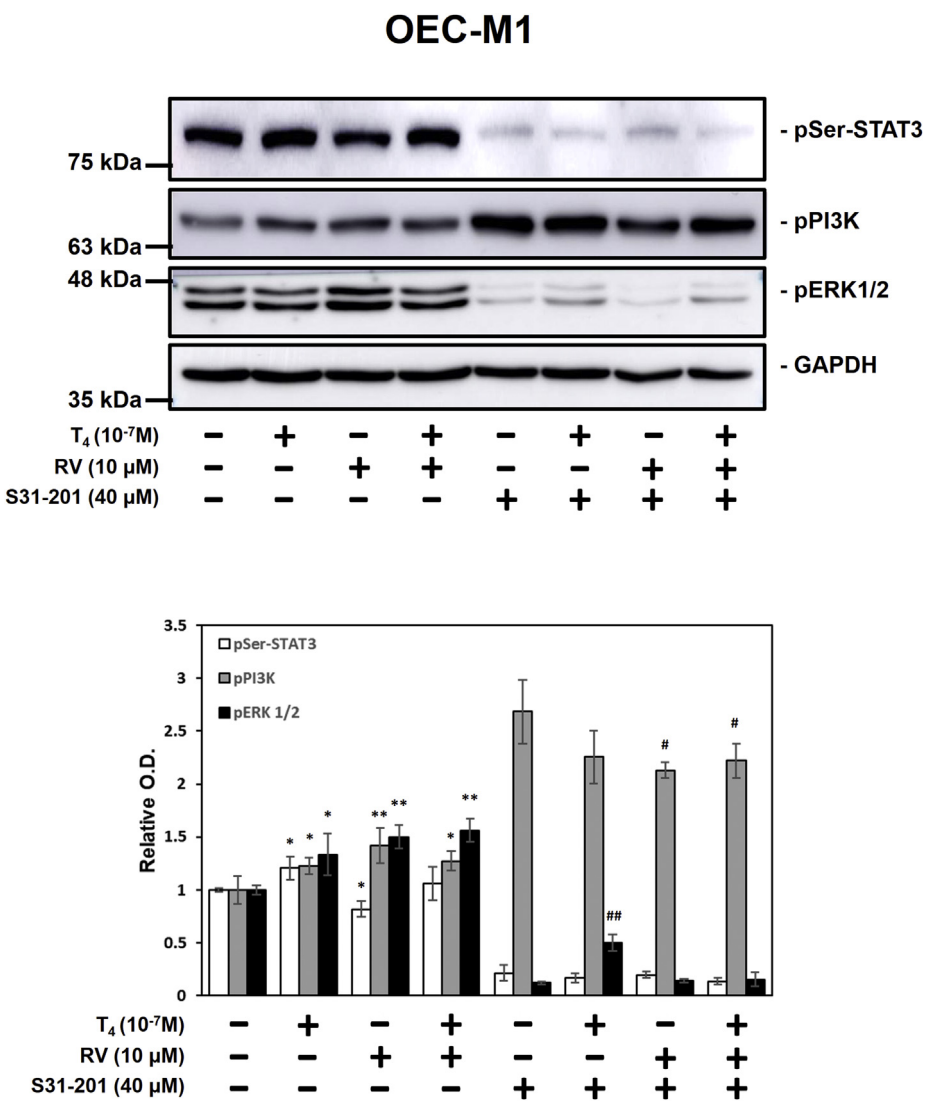

Fig. 5. Thyroxine (T_4) activates signal transducer and activator of transcription 3 (STAT3) in oral cancer cells. Oral cancer OEC-M1 cells were treated with 10^{-7} M T_4, 10 μM resveratrol, or their combination in the presence or absence of S31-201 (40 μM pretreatment) for 24 h. Total proteins were extracted for Western blotting analysis. $n = 4$. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to S31-201 treatment.

view, the thyroid hormone level is usually normal in healthy persons and even in cancer patients (Lin et al., 2016b, 2018a). Therefore, current observations suggest that activation of the PD-L1 tumor cell self-defense system may be in part due to endogenous T_4, since T_4 was added to stripped serum-containing medium in our studies. Clinical observations also suggest that endogenous thyroid hormone may support the growth of various types of cancers such as breast cancer (Hercbergs et al., 2018), renal cell carcinoma (Szymanski et al., 2016), head and neck cancers (Moeller and Fuhrer, 2013), and glioblastomas (Hercbergs et al., 2018; Lin et al., 2016b). A recent clinical observation indicates that pharmacologic elimination of T_4 but maintenance of 3,5,3′-triiodo-L-thyronine (T_3) as euthyroid hypothyroxinemia is able to prolong end-stage cancer patients' survival (Rodriguez-Molinero et al., 2018). A trophic effect of T_4 on cancer cell growth was also demonstrated in in vitro studies (Lin et al., 2016b).

In conclusion, we verified that inflammatory genes elaborated the antagonistic effect of T_4 on resveratrol-induced anti-proliferation in oral cancer cells. In addition, T_4-induced PD-L1 reduced nuclear COX-2 translocation, which is a vital mechanism of anti-proliferation by resveratrol in cancers. On the other hand, blocking expressions of inflammatory genes in oral cancer cells makes resveratrol an attractive agent that could possibly be employed in combination with other anti-STAT3 drugs. Clinical anti-inflammatory drugs are possibly suppressed by endogenous T_4, and such therapy might be improved by lowering the circulating T_4-induced inhibitory effect by pharmacologically intervening agents such as resveratrol.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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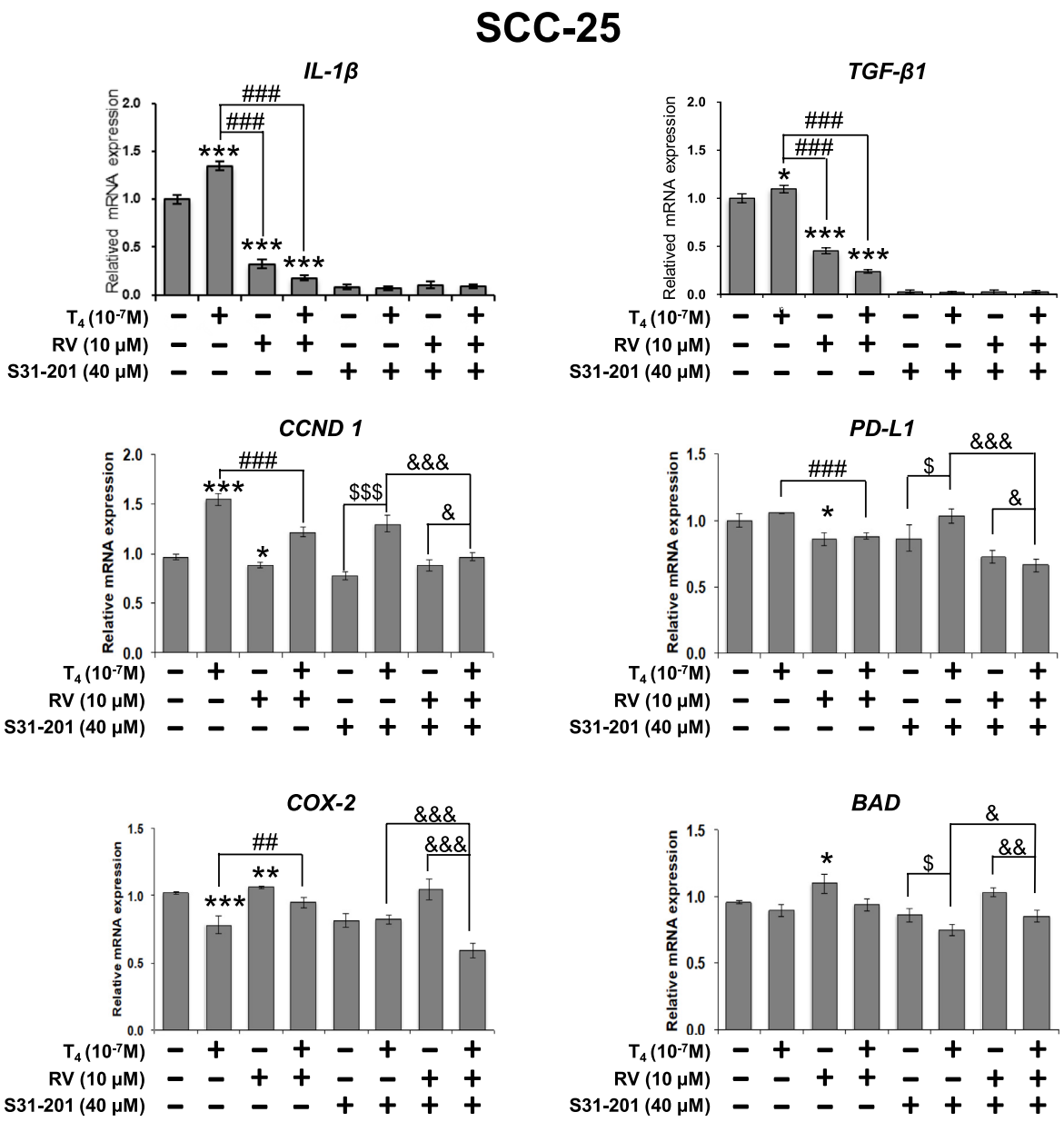

Fig. 6. Activated phosphatidylinositol-3-kinase (PI3K) plays a role in the thyroxine (T_4)-induced inhibitory effect on resveratrol-induced anti-proliferation in oral cancer SCC-25 cells. SCC-25 cells were treated with 10^{-7} M T_4, 10 μM resveratrol, or their combination in the presence or absence of S31-201 (40 μM pretreatment) for 24 h. Total RNA was extracted for analysis by a qPCR. $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the control; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared to T_4 at 10^{-7} M; $^{\$}p < 0.05$, $^{\$\$\$}p < 0.001$, compared to S31-201 treatment; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ compared to co-treatment with T_4 and S31-201.

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Conflicts of interest

All authors declare that no conflicts of interest exist.

Author contributions

YR Chen, YS Chen, ZL Li, YJ Shih, K Wang, and HY Lin conceived the experiments.

YR Chen, YS Chen, YT Chin, PJ Davis, and K Wang designed the experiments.

YR Chen, ZL Li, YJ Shih, YC Yang, CA ChangOu, PY Su, and SH Wang performed the experiments.

YR Chen, YT Chin, ZL Li, HC Chiu, YH Wu, SY Lee, SA Mousa, and HY Lin analyzed the data.

YR Chen, ZL Li, and YJ Shih prepared the figures.

YR Chen, HY Lin, LF Liu, J Whang-Peng, PJ Davis, and K Wang prepared the manuscript.

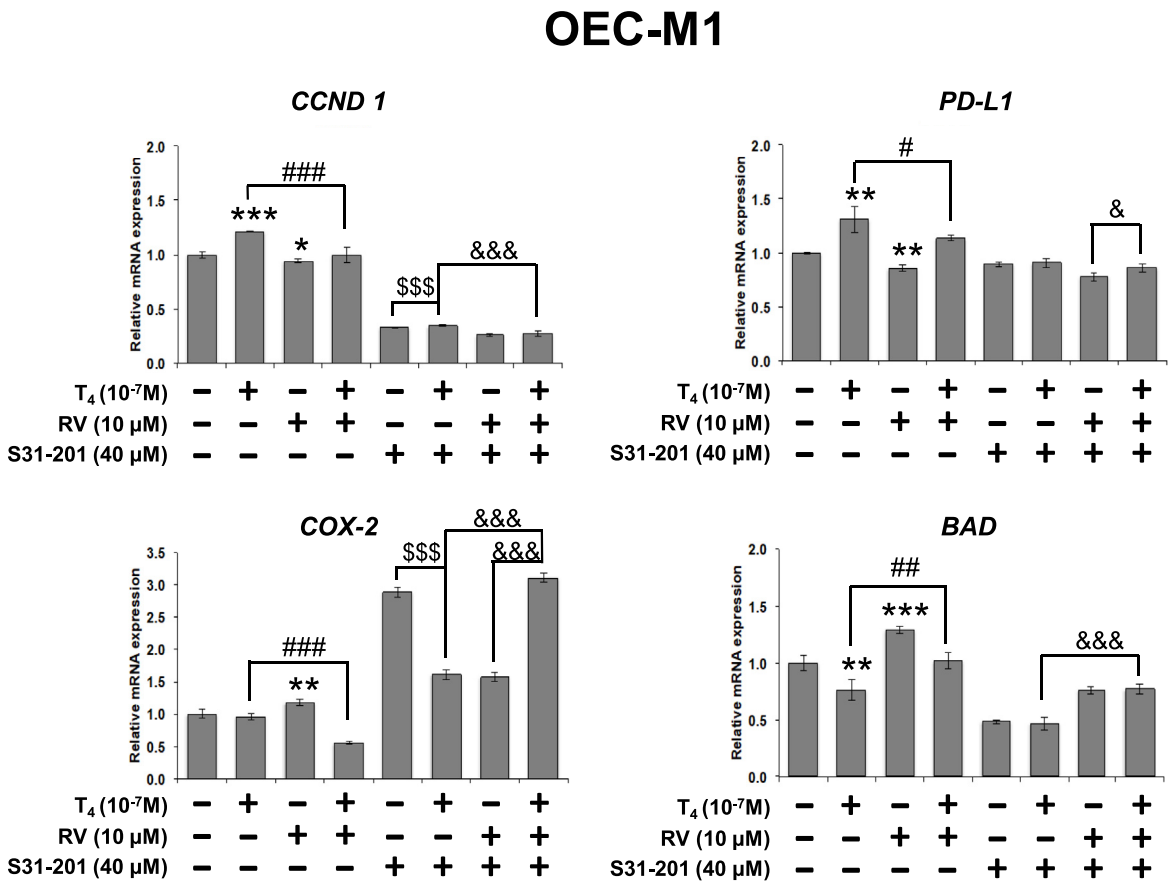

Fig. 7. Activated phosphatidylinositol-3-kinase (PI3K) plays a role in the thyroxine (T_4)-induced inhibitory effect on resveratrol-induced anti-proliferation in oral cancer OEC-M1 cells. OEC-M1 cells were treated with 10^{-7} M T_4, 10 μM resveratrol, or their combination in the presence or absence of S31-201 (40 μM pretreatment) for 24 h. Total RNA was extracted for analysis by a qPCR. $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared to T4 at 10^{-7} M; $^{\$\$\$}p < 0.001$, compared to S31-201 treatment; $^{\&}p < 0.05$, $^{\&\&\&}p < 0.001$ compared to co-treatment with T_4 and S31-201.

References

Almeida, L., Vaz-da-Silva, M., Falcao, A., Soares, E., Costa, R., Loureiro, A.I., Fernandes-Lopes, C., Rocha, J.F., Nunes, T., Wright, L., Soares-da-Silva, P., 2009. Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. Mol. Nutr. Food Res. 53 (Suppl. 1), S7–S15.

Chin, Y.T., Hsieh, M.T., Lin, C.Y., Kuo, P.J., Yang, Y.C., Shih, Y.J., Lai, H.Y., Cheng, G.Y., Tang, H.Y., Lee, C.C., Lee, S.Y., Wang, C.C., Lin, H.Y., Fu, E., Whang-Peng, J., Liu, L.F., 2016. 2,3,5,4'-Tetrahydroxystilbene-2-O-beta-glucoside isolated from polygoni multiflori ameliorates the development of periodontitis. Mediat. Inflamm. 6953459 2016.

Chin, Y.T., Wei, P.L., Ho, Y., Nana, A.W., Changou, C.A., Chen, Y.R., Yang, Y.S., Hsieh, M.T., Hercbergs, A., Davis, P.J., Shih, Y.J., Lin, H.Y., 2018. Thyroxine inhibits resveratrol-caused apoptosis by PD-L1 in ovarian cancer cells. Endocr. Relat. Cancer 25, 533–545.

Chin, Y.T., Yang, S.H., Chang, T.C., Changou, C.A., Lai, H.Y., Fu, E., HuangFu, W.C., Davis, P.J., Lin, H.Y., Liu, L.F., 2015. Mechanisms of dihydrotestosterone action on resveratrol-induced anti-proliferation in breast cancer cells with different ERalpha status. Oncotarget 6, 35866–35879.

Cottart, C.H., Nivet-Antoine, V., Laguillier-Morizot, C., Beaudeux, J.L., 2010. Resveratrol bioavailability and toxicity in humans. Mol. Nutr. Food Res. 54, 7–16.

Davis, P.J., Glinsky, G.V., Lin, H.Y., Leith, J.T., Hercbergs, A., Tang, H.Y., Ashur-Fabian, O., Incerpi, S., Mousa, S.A., 2015. Cancer cell gene expression modulated from plasma membrane integrin alphavbeta3 by thyroid hormone and nanoparticulate tetrac. Front. Endocrinol. 5, 240.

Davis, P.J., Glinsky, G.V., Lin, H.Y., Mousa, S.A., 2016. Actions of thyroid hormone analogues on chemokines. J. Immunol. Res. 3147671 2016.

Eggermont, A.M., Maio, M., Robert, C., 2015. Immune checkpoint inhibitors in melanoma provide the cornerstones for curative therapies. Semin. Oncol. 42, 429–435.

Elnakish, M.T., Ahmed, A.A., Mohler, P.J., Janssen, P.M., 2015. Role of oxidative stress in thyroid hormone-induced cardiomyocyte hypertrophy and associated cardiac dysfunction: an undisclosed story. Oxidative Med. Cell. longev. 2015, 854265.

Giuliani, C., Bucci, I., Di Santo, S., Rossi, C., Grassadonia, A., Mariotti, M., Piantelli, M., Monaco, F., Napolitano, G., 2014. Resveratrol inhibits sodium/iodide symporter gene expression and function in rat thyroid cells. PLoS One 9, e107936.

Giuliani, C., Iezzi, M., Ciolli, L., Hysi, A., Bucci, I., Di Santo, S., Rossi, C., Zucchelli, M., Napolitano, G., 2017. Resveratrol has anti-thyroid effects both in vitro and in vivo. Food Chem. Toxicol. : Int. J. Publ. Br. Ind. Biol. Res. Assoc. 107, 237–247.

Guan, J., Lim, K.S., Mekhail, T., Chang, C.C., 2017. Programmed death ligand-1 (PD-L1) expression in the programmed death receptor-1 (PD-1)/PD-L1 blockade: a key player against various cancers. Arch. Pathol. Lab Med. 141, 851–861.

Guo, B., Zhang, J., Fu, S., 2017. Inflammasomes/IL-1 pathways in myeloid cells modulate PD-1/PD-L1 checkpoint molecules. J. Immunol. 198, 124–130.124 130.

Hercbergs, A., Mousa, S.A., Leinung, M., Lin, H.Y., Davis, P.J., 2018. Thyroid hormone in the clinic and breast cancer. Horm. Cancer 9, 139–143.

Ho, Y., Chen, Y.F., Wang, L.H., Hsu, K.Y., Chin, Y.T., Yang, Y.S.H., Wang, S.H., Chen, Y.R., Shih, Y.J., Liu, L.F., Wang, K., Whang-Peng, J., Tang, H.Y., Lin, H.Y., Liu, H.L., Lin, S.J., 2018a. Inhibitory effect of Anoectochilus formosanus extract on hyperglycemia-related PD-L1 expression and cancer proliferation. Front. Pharmacol. 9, 807.

Ho, Y., Lin, Y.S., Liu, H.L., Shih, Y.J., Lin, S.Y., Shih, A., Chin, Y.T., Chen, Y.R., Lin, H.Y., Davis, P.J., 2017. Biological mechanisms by which antiproliferative actions of resveratrol are minimized. Nutrients 9.

Ho, Y., Sh Yang, Y.C., Chin, Y.T., Chou, S.Y., Chen, Y.R., Shih, Y.J., Whang-Peng, J., Changou, C.A., Liu, H.L., Lin, S.J., Tang, H.Y., Lin, H.Y., Davis, P.J., 2018b. Resveratrol inhibits human leiomyoma cell proliferation via crosstalk between integrin alphavbeta3 and IGF-1R. Food Chem. Toxicol. : Int. J. Publ. Br. Ind. Biol. Res. Assoc. 120, 346–355.

Li, N., Wang, J., Zhang, N., Zhuang, M., Zong, Z., Zou, J., Li, G., Wang, X., Zhou, H., Zhang, L., Shi, Y., 2018. Cross-talk between TNF-alpha and IFN-gamma signaling in induction of B7-H1 expression in hepatocellular carcinoma cells. Cancer Immunol. Immunother. : CII 67, 271–283.

Li, P., Huang, T., Zou, Q., Liu, D., Wang, Y., Tan, X., Wei, Y., Qiu, H., 2019. FGFR2 promotes expression of PD-L1 in colorectal cancer via the JAK/STAT3 signaling pathway. J. Immunol. 202, 3065–3075 Baltimore, Md. : 1950.

Lin, H.Y., Chin, Y.T., Nana, A.W., Shih, Y.J., Lai, H.Y., Tang, H.Y., Leinung, M., Mousa, S.A., Davis, P.J., 2016a. Actions of l-thyroxine and Nano-diamino-tetrac (Nanotetrac) on PD-L1 in cancer cells. Steroids 114, 59–67.

Lin, H.Y., Chin, Y.T., Shih, Y.J., Chen, Y.R., Leinung, M., Keating, K.A., Mousa, S.A., Davis, P.J., 2018a. In tumor cells, thyroid hormone analogues non-immunologically regulate PD-L1 and PD-1 accumulation that is anti-apoptotic. Oncotarget 9, 34033–34037.

Lin, H.Y., Chin, Y.T., Yang, Y.C., Lai, H.Y., Wang-Peng, J., Liu, L.F., Tang, H.Y., Davis,

P.J., 2016b. Thyroid hormone, cancer, and apoptosis. Compr. Physiol. 6, 1221–1237.
Lin, H.Y., Hsieh, M.T., Cheng, G.Y., Lai, H.Y., Chin, Y.T., Shih, Y.J., Nana, A.W., Lin, S.Y., Yang, Y.S.H., Tang, H.Y., Chiang, I.J., Wang, K., 2017. Mechanisms of action of nonpeptide hormones on resveratrol-induced antiproliferation of cancer cells. Ann. N. Y. Acad. Sci. 1403, 92–100.
Lin, S.J., Chin, Y.T., Ho, Y., Chou, S.Y., Sh Yang, Y.C., Nana, A.W., Su, K.W., Lim, Y.T., Wang, K., Lee, S.Y., Shih, Y.J., Chen, Y.R., Whang-Peng, J., Davis, P.J., Lin, H.Y., Fu, E., 2018b. Nano-diamino-tetrac (NDAT) inhibits PD-L1 expression which is essential for proliferation in oral cancer cells. Food Chem. Toxicol. : Int. J. Publ. Br. Ind. Biol. Res. Assoc. 120, 1–11.
Liu, S., Chen, S., Yuan, W., Wang, H., Chen, K., Li, D., Li, D., 2017. PD-1/PD-L1 interaction up-regulates MDR1/P-gp expression in breast cancer cells via PI3K/AKT and MAPK/ERK pathways. Oncotarget 8, 99901–99912.
Mancini, A., Di Segni, C., Raimondo, S., Olivieri, G., Silvestrini, A., Meucci, E., Curro, D., 2016. Thyroid hormones, oxidative stress, and inflammation. Mediat. Inflamm. 2016, 6757154.
Marcocci, C., Leo, M., Altea, M.A., 2012. Oxidative stress in graves' disease. Eur. Thyroid J. 1, 80–87.
Moeller, L.C., Fuhrer, D., 2013. Thyroid hormone, thyroid hormone receptors, and cancer: a clinical perspective. Endocr. Relat. Cancer 20, R19–R29.
Nana, A.W., Wu, S.Y., Yang, Y.S., Chin, Y.T., Cheng, T.M., Ho, Y., Li, W.S., Liao, Y.M., Chen, Y.R., Shih, Y.J., Liu, Y.R., Pedersen, J., Incerpi, S., Hercbergs, A., Liu, L.F., Whang-Peng, J., Davis, P.J., Lin, H.Y., 2018. Nano-Diamino-Tetrac (NDAT) enhances resveratrol-induced antiproliferation by action on the RRM2 pathway in colorectal cancers. Horm. Cancer 9, 349–360.
Ott, P.A., Hodi, F.S., Robert, C., 2013. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients. Clin. Cancer Res. : Off. J. Am. Assoc. Cancer Res. 19, 5300–5309.
Park, B.V., Freeman, Z.T., Ghasemzadeh, A., Chattergoon, M.A., Rutebemberwa, A., Steigner, J., Winter, M.E., Huynh, T.V., Sebald, S.M., Lee, S.J., Pan, F., Pardoll, D.M., Cox, A.L., 2016. TGFbeta1-Mediated SMAD3 enhances PD-1 expression on antigen-specific T cells in cancer. Cancer Discov. 6, 1366–1381.
Powles, T., Eder, J.P., Fine, G.D., Braiteh, F.S., Loriot, Y., Cruz, C., Bellmunt, J., Burris, H.A., Petrylak, D.P., Teng, S.L., Shen, X., Boyd, Z., Hegde, P.S., Chen, D.S., Vogelzang, N.J., 2014. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 515, 558–562.
Puzianowska-Kuznicka, M., Pietrzak, M., Turowska, O., Nauman, A., 2006. Thyroid hormones and their receptors in the regulation of cell proliferation. Acta Biochim. Pol. 53, 641–650.
Rodriguez-Molinero, A., Hercbergs, A., Sarrias, M., Yuste, A., 2018. Plasma 3,3',5-Triiodo-L-thyronine [T3] level mirrors changes in tumor markers in two cases of metastatic cancer of the breast and pancreas treated with exogenous L-T3. Cancer Biomark. : A Dis. Markers 21, 433–438.
Shien, K., Papadimitrakopoulou, V.A., Wistuba II, , 2016. Predictive biomarkers of response to PD-1/PD-L1 immune checkpoint inhibitors in non-small cell lung cancer. Lung Cancer 99, 79–87.
Singh, C.K., Liu, X., Ahmad, N., 2015. Resveratrol, in its natural combination in whole grape, for health promotion and disease management. Ann. N. Y. Acad. Sci. 1348, 150–160.
Song, X., Liu, J., Lu, Y., Jin, H., Huang, D., 2014. Overexpression of B7-H1 correlates with malignant cell proliferation in pancreatic cancer. Oncol. Rep. 31, 1191–1198.
Sun, C., Mezzadra, R., Schumacher, T.N., 2018. Regulation and function of the PD-L1 checkpoint. Immunity 48, 434–452.
Szymanski, L., Matak, D., Bartnik, E., Szczylik, C., Czarnecka, A.M., 2016. Thyroid hormones as renal cell cancer regulators. J. Signal Transduct. 1362407 2016.
Villanueva, I., Alva-Sanchez, C., Pacheco-Rosado, J., 2013. The role of thyroid hormones as inductors of oxidative stress and neurodegeneration. Oxidative Med. Cell. longev. 2013, 218145.
Walker, J., Schueller, K., Schaefer, L.M., Pignitter, M., Esefelder, L., Somoza, V., 2014. Resveratrol and its metabolites inhibit pro-inflammatory effects of lipopolysaccharides in U-937 macrophages in plasma-representative concentrations. Food & function 5, 74–84.
Walle, T., 2011. Bioavailability of resveratrol. Ann. N. Y. Acad. Sci. 1215, 9–15.
Wang, X., Yang, L., Huang, F., Zhang, Q., Liu, S., Ma, L., You, Z., 2017. Inflammatory cytokines IL-17 and TNF-alpha up-regulate PD-L1 expression in human prostate and colon cancer cells. Immunol. Lett. 184, 7–14.
Yang, C.Y., Meng, C.L., 1994. Regulation of PG synthase by EGF and PDGF in human oral, breast, stomach, and fibrosarcoma cancer cell lines. J. Dent. Res. 73, 1407–1415.
Yang, Y.C., Chin, Y.T., Hsieh, M.T., Lai, H.Y., Ke, C.C., Crawford, D.R., Lee, O.K., Fu, E., Mousa, S.A., Grasso, P., Liu, L.F., Chang, H.Y., Tang, H.Y., Lin, H.Y., Davis, P.J., 2016. Novel leptin OB3 peptide-induced signaling and progression in thyroid cancers: comparison with leptin. Oncotarget 7, 27641–27654.
Yousef, M., Vlachogiannis, I.A., Tsiani, E., 2017. Effects of resveratrol against lung cancer: in vitro and in vivo studies. Nutrients 9.