Combined effects of all-trans-retinoic acid and trichostatin A on the induction of differentiation of thyroid carcinoma cells

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[Abstract] Background and Objective: The effectiveness rate of all-trans-retinoic acid (RA) is only about 30% in the clinical application of inducing thyroid carcinoma differentiation. In addition, there are severe toxic side effects, which limit its clinical application. Phase I–III clinical studies have been conducted on the combined application of two or more kinds of inducers in tumors. Nevertheless, the combination of RA with histone deacetylase inhibitors is rarely reported. This study aimed to evaluate the effects of differentiation for papillary thyroid carcinoma and follicular thyroid carcinoma cell lines induced by RA combined with trichostatin A (TSA), enhancing the effect of induction, while reducing the toxic side effects of a single drug, to provide a theoretical basis for preclinical trials.

Methods: After incubation with RA combined with TSA, K1 and FTC-133 were grouped into Group 1 (RA 10⁻⁴ mol/L plus TSA 1.65 x 10⁻² mol/L), Group 2 (RA 1 x 10⁻⁴ mol/L plus TSA 3.31 x 10⁻² mol/L), Group 3 (RA 1 x 10⁻³ mol/L plus TSA 1.65 x 10⁻² mol/L), Group 4 (RA 1 x 10⁻³ mol/L plus TSA 3.31 x 10⁻² mol/L) by four varied concentrations and three time points (12 h, 24 h, and 48 h). The cell proliferation, conformation, toxic effect, and induced differentiation on K1 and FTC-133 cell lines were studied microscopically with hematoxylin-eosin (HE) to observe cell quantity and morphology, methyl-thiazolyl-tetrazolium (MTT) to calculate cell survival rates, and electrochemiluminescence analysis measuring in vitro thyroglobulin (Tg) levels.

Results: The research showed that K1 and FTC-133 cells had cell spacing increases, with an outer edge of smooth, nuclear chromatin condensation after RA combined with TSA. Survival rate was assessed by an analysis of variance (ANOVA) by concentration and time point, F values of K1 and FTC-133 were 23.52 and 170.14, and 57.09 and 224.35, respectively. There were significant differences for both cells (P < 0.01). The SNK analysis indicated that survival rates were in the order of Group 2 < Group 1 < Group 4 < Group 3, Tg was also assessed by ANOVA, F values of K1 were 69.63 and 101.07, and F values of FTC-133 were 79.77 and 81.72 (P < 0.01). Group 1 was compared with Group 3 of K1 and FTC-133 by the least significant difference (LSD) method, and there was no statistical difference between the two groups (P = 0.06, 0.2, respectively; P > 0.05), yet a significant difference was seen between the other groups.

Conclusions: Lower concentrations of RA combined with lower concentrations of TSA have both inhibited cell proliferation, decreased toxicity of the drugs, and increased the effect of K1 and FTC-133 cell differentiation. The mechanism of action may be that TSA has pretranscription DNA regulation and that RA has posttranscriptional signal regulation to enhance the effects of inhibited proliferation and differentiation of cells by transcription systems.

Key words: All-trans-retinoic acid (RA), trichostatin A (TSA), differentiated thyroid carcinoma (DTC)
inhibiting proliferation in a variety of tumor cells. Preclinical trials have been carried out\(^5\), including studies of trichostain A (TSA).

In addition to a single application of RA or TSA as an antitumor drug, the combined application of two or more inducers can enhance the effect of induction at multiple stages of tumorigenesis and some breakthroughs have been achieved in both basic research and clinical trials\(^6\). The combined application of induced drugs of molecular targets could become a new antitumor clinical program. The differences of the combined effects on the inhibition of proliferation, cell toxicity, and the induction of differentiation between the papillary thyroid cancer cell lines K1 and the follicular thyroid carcinoma cell line FTC-133 were compared to provide evidence for clinical applications of combined inducing drugs in antitumor treatment.

**Materials and methods**

**Cell cultures: K1 and FTC-133 cell lines**

Both cell lines were purchased from the European Collections of Cell Cultures (ECACC). After the culture medium was added, the cells were injected into the culture bottles and kept in 5% CO\(_2\) and saturated humidity at 37°C in the dark. K1 cell lines were maintained in Dulbecco’s modified eagle medium (DMEM):Ham’s F12:MCDB105 (2:2:1, Invitrogen Co., Sigma-Aldrich) containing 10% fetal bovine serum (HyClone), and FTC-133 were maintained in DMEM:Ham’s F12 (1:1) containing 10% fetal bovine serum; 1 x 10\(^5\) U/L of penicillin and 100 mg/mL of streptomycin were supplemented with pH values adjusted to 7.2–7.4.

**Preparation of RA and TSA**

RA and TSA (Sigma-Aldrich) were dissolved in anhydrous ethanol and saved at -20°C. The final concentrations of RA were 1 x 10\(^{-4}\) mol/L and 1 x 10\(^{-5}\) mol/L, and those of TSA were 1.65 x 10\(^{-3}\) mol/L, 3.3 x 10\(^{-7}\) mol/L. The concentrations of the combined groups were as follows: Group 1 with RA 1 x 10\(^{-4}\) mol/L + TSA 1.65 x 10\(^{-7}\) mol/L, Group 2 with RA 1 x 10\(^{-4}\) mol/L + TSA 3.3 x 10\(^{-7}\) mol/L, Group 3 with RA 1 x 10\(^{-5}\) mol/L + TSA 1.65 x 10\(^{-7}\) mol/L, and Group 4 with RA 1 x 10\(^{-5}\) mol/L + TSA 3.3 x 10\(^{-7}\) mol/L. The control group had the same amount added of 2 μL of anhydrous ethanol (< 0.1% V/V). All experiments mentioned above were carried out in dim light.

**Observation of K1 and FTC-133 morphology**

K1 and FTC-133 cells were digested and adjusted to 1 x 10\(^{3}\)/mL by cell counting, and were then seeded into 96-well plates. Each group has 3 wells and the control group was set up. A total of 150 μL of culture medium of K1 and FTC-133 was added. After 24 h, the cells adhered to the wall. The culture medium was changed with a serum-free medium. The combined drugs for Groups 1–4 were added and cells were incubated for 12, 24, and 48 h under the same conditions. Afterwards, cells in the experimental groups and the control group were fixed with 4% paraformaldehyde in situ for 10 min at room temperature followed by hematoxylin-eosin staining (HE). Real-time image analysis system by microscopy (Olympus, IX71) was used for the image analysis.

**Fluorescent staining of K1 and FTC-133 cell nuclei**

Cultured with the methods mentioned above, K1 cells, FTC-133 cells, and cells in the control group were fixed with 4% paraformaldehyde in situ for 10 min at room temperature. Cells were washed 3 times for 5 min each in phosphate buffered saline (PBS), incubated in 0.3% H\(_2\)O\(_2\) for 30 min at room temperature, washed in PBS 3 times again, and shaken on a rotary shaker for 5 min each. Cells were then stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (1:500) for 5–10 min, washed 3 times in PBS, shaken for 5 min each, dried, and mounted with 30% glycerol. Real-time image analysis system by microscopy (Olympus, IX71) was used in the image analysis.

**Detection of thyroglobulin (Tg) levels**

Cultured according to the method mentioned above, the supernatant fluids of the experimental groups for K1 and FTC-133 and the control group were collected and detected with electrochemiluminescence immunoassay (ECLIA). The reagent kit was all provided by Roche (US). The ranges for Tg measurement was 0.1–1000 μg/L. The intergroup and intragroup differences were 4.2% and 7.8%, respectively.

**Statistical analysis**

SPSS version 13.0 was used for statistical analysis. ANOVA and orthogonal comparisons were used to analyze the combined effects of the drugs on the induction of differentiation in tumor cells.

**Results**

**Effects of the combined RA and TSA treatment on the morphology and apoptosis of K1 and FTC-133**

*Observation of morphology and apoptosis of K1 cells* K1 cells in the control group showed irregular and pleomorphic morphology. As time went on, the number of cells increased and cells formed multilayers, with heap-like aggregation and high-speed growth. At the same time, the membranes stretched to the extensions of the surrounding regions and nucleocytoplasmic atypia increased. In addition, adhesion between cells became obvious and there were cell protrusions in varying numbers (Figure 1). After treatment with combined RA
and TSA, the number of floating dead cells increased significantly. Specifically, the toxicity of Group 4 was the highest, with cells growing in a poor state. Cells became round and smaller with smooth edges and cell protrusions reduced. The morphology tended to be regular and normalized. The number of nuclear pyknosis increased, intercellular spaces became larger and small flake-free cell growth areas were present. A small amount of apoptotic bodies could be observed. K1 nuclei showed diffuse, homogeneous blue fluorescence and the morphology of the nuclei reduced significantly, while DNA fragments were rarely seen.

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Figure 1 The morphology of K1 cells and cell nuclei with RA combined TSA
A, B, blank K1 cells with HE staining and DAPI staining, respectively. C, D, K1 cells after RA 1 × 10⁻⁴ mol/L plus TSA 1.65 × 10⁻² mol/L, respectively with HE staining and DAPI staining. A, B shows that K1 cells had polymorphism, heterogeneity, cell processes, heap-like aggregations, and diffuse uniform blue fluorescence, respectively (amplification factor × 100, 48 h). C, D shows that K1 cells had a split-phase, apoptotic bodies, cell spacing increases, and diffuse uniform blue fluorescence, but apoptotic bodies were not obvious, respectively (amplification factor × 100, 48 h).

**Observation of morphology and apoptosis of FTC-133 cells**

FTC-133 cells of the control group appeared shuttle-like with much irregular morphology. Adhesions between cells were obvious. There were cell protrusions in varying numbers. As time went on, the number of cells increased and cells formed multilayers with heap-like aggregations (Figure 2). After treatment with combined RA and TSA, intercellular spaces became larger and cells became smaller with decreased protrusions and smooth edges. The number of cells decreased and polynucleation and heterogeneity reduced. Nuclei showed diffuse, homogeneous blue fluorescence and the morphology of the nuclei reduced significantly, while DNA fragments were rarely seen.

![Figure 2](image)

**Effects of combined RA and TSA treatment on proliferation and toxicity**

**Effects on the proliferation of K1** Survival rate variance analysis was performed according to group and time. The main intersubjective effects were analyzed: \( F = 900.374, P < 0.001 \), showing significant differences among the means of all groups. Comparison of survival rates among groups with different concentrations: \( F = 23.52, P < 0.001 \), showing significant differences. Comparison of survival rates among groups with different times: \( F = 170.14, P < 0.001 \), showing significant differences. SNK analysis of the role of combined treatment showed the cell survival rates from low to high were as follows: Group 2 < Group 1 < Group 4 < Group 3. The higher the drug concentration, the lower the cell survival rate and the stronger the inhibition of cell proliferation. The higher the drug concentration, the higher the cell mortality and the greater the cell toxicity. The most toxic group was the one with the highest concentration of RA combined with TSA. Groups with low concentrations of RA combined with TSA showed less cell inhibition and lower toxicity (Table 1).

**Effects on the proliferation and toxicity of FTC-133** Survival rate variance analysis was performed according to group and time. The main intersubjective effects were analyzed: \( F = 1965.76, P < 0.001 \), showing significant differences among all groups. Comparison of survival rates among groups with different concentrations: \( F = 57.09, P < 0.001 \), showing significant differences. Comparison of survival rates among groups with different times: \( F = 224.35, P < 0.001 \), showing significant differences. SNK analysis of the role of combined treatment showed that the cell survival rates from low to high were as

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The strongest inhibition of cell proliferation and toxicity was the group with the highest concentration of RA combined with TSA. Groups with low concentrations of RA combined with TSA showed less cell inhibition and lower toxicity (Table 2).

**Effects of combined RA and TSA treatment on Tg secretion in K1 and FTC-133**

**Effect on Tg secretion in K1 cells**

Tg variance analysis was performed. The main intersubjective effects were analyzed: $F = 1270.61, P < 0.001$, showing significant differences among means in all groups. Comparison of Tg among groups with different concentrations: $F = 69.63, P < 0.001$, showing significant differences. Comparison of survival rates among different times: $F = 101.07, P < 0.001$, showing significant differences.

Groups were compared in pairs with LSD: Group 1/Group 3, $P = 0.33$, no significance, showing similar effects of induction. SNK analysis of the effect of drugs on Tg secretion in K1 cells showed the effect from low to high as follows: Group 4 < Group 2 < Group 3 < Group 1. The effect of RA combined with low concentrations of TSA was the best. SNK analysis of different times showed that the effect of treatment for 48 h was the strongest. As time went on, Tg levels increased in a time-dependent manner (Table 3).

**Effect on Tg secretion in FTC-133 cells**

Tg variance analysis was performed. Main intersubjective effect was analyzed: $F = 1512.27, P < 0.001$, showing significant differences among means in all groups. Comparison of Tg among groups with different concentrations: $F = 79.77, P < 0.001$, showing significant differences. Comparison of survival rates among groups with different times: $F = 81.72, P < 0.001$, showing significant differences. Groups were compared in pairs with LSD: Group 1/Group 3, $P = 0.20$, no significance, showing similar effects of induction. There were significant differences among other groups. SNK analysis of the effect of drugs on Tg secretion of FTC-133 cells showed the effect from low to high as follows: Group 4 < Group 2 < Group 3 < Group 1. The effect of RA combined with low concentrations of TSA was the best. SNK analysis of different times showed that the effect of treatment for 48 h was the strongest. As time went on, Tg levels increased in

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**Table 1** The effects of RA combined with TSA on the survival rate of K1 (x ± s, 3 samples per concentration point)

<table>
<thead>
<tr>
<th>Group</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>SR(%)</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>0.81 ± 0.00</td>
<td>63.38</td>
<td>0.62 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>0.71 ± 0.02</td>
<td>55.47</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.91 ± 0.02</td>
<td>71.31</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>0.84 ± 0.02</td>
<td>65.99</td>
<td>0.69 ± 0.02</td>
</tr>
</tbody>
</table>

Group 1, RA $1 \times 10^{-4}$ mol/L plus TSA $1.65 \times 10^{-7}$ mol/L; Group 2, RA $1 \times 10^{-4}$ mol/L plus TSA $3.31 \times 10^{-7}$ mol/L; Group 3, RA $1 \times 10^{-5}$ mol/L plus TSA $1.65 \times 10^{-7}$ mol/L; Group 4, RA $1 \times 10^{-5}$ mol/L plus TSA $3.31 \times 10^{-7}$ mol/L. A significant difference in the effects of the test subjects between groups was observed ($P < 0.01$). There was also a significant difference between different concentrations and different times ($P < 0.001, P < 0.01$). SR, survival rate.

**Table 2** The effect of RA combined with TSA on the survival rate of FTC-133 (x ± s, 3 samples per concentration point)

<table>
<thead>
<tr>
<th>Group</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>SR(%)</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>0.52 ± 0.05</td>
<td>63.59</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>0.41 ± 0.06</td>
<td>50.06</td>
<td>0.36 ± 0.02</td>
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<tr>
<td>3</td>
<td>0.61 ± 0.07</td>
<td>74.49</td>
<td>0.65 ± 0.09</td>
</tr>
<tr>
<td>4</td>
<td>0.57 ± 0.08</td>
<td>68.72</td>
<td>0.59 ± 0.08</td>
</tr>
</tbody>
</table>

Group 1, RA $1 \times 10^{-4}$ mol/L plus TSA $1.65 \times 10^{-7}$ mol/L; Group 2, RA $1 \times 10^{-4}$ mol/L plus TSA $3.31 \times 10^{-7}$ mol/L; Group 3, RA $1 \times 10^{-5}$ mol/L plus TSA $1.65 \times 10^{-7}$ mol/L; Group 4, RA $1 \times 10^{-5}$ mol/L plus TSA $3.31 \times 10^{-7}$ mol/L. The combined application of high RA and TSA can enhance the inhibition effect on FTC-133, and survival rates (SR) can reach the lowest levels ($P < 0.001, P < 0.01$). The combined application of low RA and TSA had an inhibition effect on FTC-133, which was weaker than that of the combined application of high RA and TSA.

**Table 3** The secretion levels of Tg in K1 and FTC-133 with RA combined with TSA (x ± s, 3 samples per concentration point)

<table>
<thead>
<tr>
<th>Group</th>
<th>K1 Tg 12 h</th>
<th>FTC</th>
<th>K1 Tg 24 h</th>
<th>FTC</th>
<th>K1 Tg 48 h</th>
<th>FTC</th>
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<tr>
<td></td>
<td>1.43 ± 0.03</td>
<td>1.36 ± 0.00</td>
<td>1.80 ± 0.02</td>
<td>1.54 ± 0.01</td>
<td>2.09 ± 0.01</td>
<td>1.84 ± 0.01</td>
</tr>
<tr>
<td>1</td>
<td>2.78 ± 0.02</td>
<td>2.79 ± 0.01</td>
<td>3.93 ± 0.04</td>
<td>3.42 ± 0.01</td>
<td>4.24 ± 0.07</td>
<td>4.38 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>2.64 ± 0.01</td>
<td>2.62 ± 0.01</td>
<td>2.85 ± 0.04</td>
<td>2.91 ± 0.01</td>
<td>3.15 ± 0.06</td>
<td>3.16 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>2.56 ± 0.00</td>
<td>2.93 ± 0.02</td>
<td>3.76 ± 0.01</td>
<td>3.12 ± 0.01</td>
<td>4.02 ± 0.02</td>
<td>4.16 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>0.33 ± 0.01</td>
<td>2.33 ± 0.01</td>
<td>2.45 ± 0.01</td>
<td>2.44 ± 0.02</td>
<td>2.78 ± 0.01</td>
<td>2.74 ± 0.02</td>
</tr>
</tbody>
</table>

Group 0, the control group; Group 1, RA $1 \times 10^{-4}$ mol/L plus TSA $1.65 \times 10^{-7}$ mol/L; Group 2, RA $1 \times 10^{-4}$ mol/L plus TSA $3.31 \times 10^{-7}$ mol/L; Group 3, RA $1 \times 10^{-5}$ mol/L plus TSA $1.65 \times 10^{-7}$ mol/L; Group 4, RA $1 \times 10^{-5}$ mol/L plus TSA $3.31 \times 10^{-7}$ mol/L. A significant difference in the effects of the test subjects between groups was observed ($P < 0.001, P < 0.01$). There was also a significant difference between different concentrations and different times ($P < 0.001, P < 0.01$).
a time-dependent manner.

Discussion

RA, the bioactive metabolite of vitamin A, plays an important role in basic biologic activities, such as the development of vertebrate embryo and cell growth. It combines with a super gene family (steroid/thyroid hormone/RA) of retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (with 3 subtypes of α, β, and γ) to regulate transcription factors, resulting in the induction of cell differentiation and the regulation of cell proliferation. As an induction agent in the treatment of squamous cell carcinoma and acute promyelocytic leukemia (APL), RA has been successfully applied in the treatment of APL, with a remission rate more than 90%[7]. However, in the clinical induction of differentiation in the thyroid, its efficiency was about 30%. In addition, the drug side effects limit its clinical application, which is still in Phase I clinical trials[8-11]. We have studied the clinical effect of RA and its application was also limited because of its toxic side effects[12].

TSA is an antifungal drug, which can inhibit histone deacetylases (HDACs) according to studies in vivo and in vitro. The histone code is a new gene regulation mechanism. As proteins of acetylation and deacetylation, TSA can bring functional genes into active or inhibited states to regulate gene transcription and expression. The development of drugs that can inhibit HDACs is a promising direction for the development of anticancer drugs, playing an important role in antiproliferation and induction of differentiation.

MTT is a dye that can accept a hydrogen atom. Succinate dehydrogenase in the mitochondria of living cells can reduce exogenous MTT to a water-insoluble blue-purple formazan result in its precipitation in cells, while dead cells do not have such functions. DMSO can also dissolve blue-purple crystal masses (formazan) in cells. The determination of its absorbance value can indirectly reflect the number of living cells. Within a certain range, the amount of MTT crystals is directly proportional to the number of cells. Survival rate is directly proportional to cell proliferation inhibition of drugs. When the survival rate is high, the mortality rate is low and the cytotoxicity is weak; vice versa, the cytotoxicity is strong. Experimental results have shown that survival rates of groups with high concentrations of RA combined with high or low concentrations of TSA were lower than those with low concentrations of RA combined with high or low concentrations of TSA. It has been suggested that the effect of high concentrations of RA combined with high or low concentrations of TSA on the inhibition of cell proliferation was strong. While in terms of drug toxic effects, after combined treatment with high concentrations of RA, the survival rate was low, suggesting that RA has strong toxic effects. The toxic effects of groups with low concentrations of RA combined with low concentration TSA was weak, but there was still a certain degree of inhibition in cell proliferation. It not only inhibited tumor cell proliferation, but also reduced the toxic effects of the drugs.

Combined effects showed transformation of malignant cells from poorly differentiated cells to well-differentiated cells, with cell morphology tending to be regular and smaller. In addition, the speed of proliferation reduced, grew in poor conditions, cells became round, nuclear pyknosis increased, a small amount of cell apoptosis was seen, and intercellular space became larger, showing a significant induction of apoptosis.

In the presence of RA, RARs, and RXRs RA signal transduction systems form activated conjugate complexes, including activators of histone acetyltransferase, opening DNA chains, and regulating promoters of functional genes through transcription factors. On one hand, RA regulates growth, differentiation, and apoptosis through a signal transduction system by tyrosine protein kinase (mitogen-activated protein kinase, MAPK), a phosphoinositide signal system (Inositol trisphosphate, IP3), cell cycle regulators (cyclin Cyc, cell cyclin-dependent kinases, and their inhibitors CDI), apoptotic genes (B-cell lymphoma Bcl), and so on[7]. On the other hand, RA increased the iodide uptake capacity through upregulating the expression of the functional genes and proteins of NIS, 5’DI, and Tg[13,14]. Van Herle et al.[15] found that the iodide uptake capacity of FTC cells treated by RA was dependent on drug concentration, which was enhanced with increases in drug concentration. Schreck et al.[13] found that the expression of 5’DI in FTC cells treated by RA increased and cell proliferation was inhibited. TSA inhibited cell proliferation by regulating cell cycle regulatory factors, such as CDK, CDI: p21, and p27[16]. The results of animal experiments suggested that TSA could selectively inhibit growth of tumor grafts without the destruction of normal cells[13], but its mechanism was unclear. Some scholars[17] have proposed that RA induced differentiation and apoptosis of tumor cells sharing a common pathway. Apoptosis occurred after terminal differentiation of cells and it was likely the pattern of the death of cells that were induced to differentiate to become mature. Apoptosis was the outcome of the induction of differentiation. In our experiments, we observed that only a small number of cell underwent apoptosis due to the restrictions of the experimental method without flow cytometry or an apoptosis staining kit. Kitazono et al.[16] used Depsipeptide (FK228) to induce FTC and anaplastic thyroid cancer cells and found that histone acetylation, gene expression of NIS, and Tg and iodine uptake increased.

In almost all mammals, thyroid hormones regulate body metabolism and gene expression. Tg, the raw material for hormone synthesis, is the specific marker secreted by thyroid tissue derived from epithelial tissue differentiation. It was reported that normal thyroid could synthesize Tg and the expression of Tg was different in DTC cells. Tg had a certain expression, in the well-differentiated DTC cells while it had a low expression in dDTC or poorly differentiated cells. Studies in vivo and in vitro suggested that Tg was the differentiation marker of DTC. The expression of Tg increased after treatment by an inducer. Tg can be used as a quantitative indicator of differentiation in DTC.

Experiments showed that tumor cells treated by high concentrations of RA combined with low concentrations of TSA secrete high levels of Tg. However, the level of Tg secreted by tumor cells treated by low concentrations of RA combined with...
low concentrations only slightly lower than that by tumor cells treated by high concentrations of RA combined with low concentrations of TSA. Despite that, due to the inhibition of cell growth, drug toxicities could be reduced and the effectiveness of cell induction could be improved. According to drug toxicity, cell survival, and Tg levels in the experiment, low concentrations of RA combined with low concentrations of TSA not only can inhibit the proliferation of K1 and FTC-133 and induce drug toxicity, but also can enhance the differentiation of tumor cells. In combination therapy, Tg levels increased when only RA increased, while Tg levels decreased when only TSA increased, suggesting that RA rather than TSA plays an important role in this induction. However, TSA play an important role in the launch of the induction.

The possible mechanism is that TSA might act on the preregulation of DNA in transcription, inhibit HDACs, and open the DNA chain through the acetylation of histones, while RA might act on following the regulation of transcription, form activators of HDACs, and regulate the promoters of histone acetylation through transcription factors, both of which could enhance promoters before transcription. Cell proliferation could be inhibited and induction of differentiation could be enhanced through a posttranscriptional co-conduction system. At present, according to the hypothesis and the reports (1), various combined applications of drugs have been carried out in phase I, II, and III clinical trials.

The possible mechanisms of action of RA may be related to its combination with two kinds of nuclear receptors, RARs and RXRs. It combined with an RA response element (RARE) at the initiation region of the target gene, and downregulated the expression of nuclear transcription factors, such as TTF-1, TTF-2, and Pax-8. Tg cannot regulate the gene expression of Tg, TPO, NIS, or TSHR by itself, depending on the regulation of TSH. In-vitro experiments have shown that there is no certain evidence how RA regulates the expression of Tg synthesis after undergoing regulation pathways of transcription factors RARE without a TSH-stimulating factor in the culture medium. TSA inhibits the deacetylation of histones to make transcription factors active. The mechanism of a TSA-induced differentiation of DTC is still unclear.

RA combined with TSA-enhanced induction of the differentiation of DTC, indicating that low concentrations of TSA maintain the acetylation of histones to activate transcription factors, which is helpful to RA to enhance the gene expression of transcription factors. Even if the concentration of RA increased again, an increase of Tg levels was not obvious. The induction effect of high concentrations of TSA combined with RA in DTC was lower than that of low concentrations of TSA and RA, which may be caused by an increase in the drug toxicity of cells treated by high concentration of TSA combined with RA and an increase in cell death, and as a result, the overall Tg levels decreased.

The diversity of the DTC phenotype indicates complex and inconsistent induction of DTC. There may be many differences in the regulation pathways of Tg, NIS, TPO, and TSHR, as well as different rate-limiting enzymes and signal transduction systems. Through a single method of induction, you may encounter many obstacles. Using a combination to regulate the effective and key stages before and after transcription may be a direction for inducing differentiation of DTC in the future.

References