심포지움 1-2

New Promising Therapies for Thyroid Cancers : Combination Treatment with Histone Deacetylase Inhibitor and Retinoic Acid Targeting Up-regulation of RAR β Expression

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Most patients with thyroid cancer have well differentiated tumors that usually respond to conventional therapy including total or near total thyroidectomy, radioiodine ablation and TSH suppression. About 10% of patients, however, have aggressive cancers as a consequence of de-differentiation. During de-differentiation, thyroid cancers not only show more mitosis, fibrosis, and altered cell structure, they also lose thyroid-specific functions (iodine uptake, TSH receptor expression, and thyroglobulin production). These poorly differentiated tumors mostly fail to take up radioiodine and are responsible for most deaths from thyroid cancer. New therapies need to be developed for patients with these types of tumors.

The histone deacetylase inhibitors are among the most promising new antineoplastic therapies for poorly differentiated or undifferentiated thyroid cancer. These drugs have been shown to inhibit growth and induce apoptosis and redifferentiation in a variety of hematologic and solid cancer cell lines and animal models. Retinoic acids (RAs) have been used as a redifferentiation therapy for various human cancers. Promising therapeutic effects of RAs have been demonstrated in acute promyelocytic leukemia, skin cancer, breast cancer, and head and neck cancer. RAs also have some therapeutic effects in thyroid cancers. Recently, investigations reported the combination treatment with RA and histone deacetylase inhibitors showed favorable therapeutic effects in various human cancers.

Histone Deacetylase Inhibitors

Histones are nuclear proteins that organize DNA into nucleosomes, which are the repeating structural elements in nuclear chromatin. The basic nucleosome structure consists of a core formed by four pairs of histone proteins, around which DNA is wrapped. The four pairs of core histone proteins have lysine-rich amino-terminal tails that can be modified by attaching or removing acetyl groups. The acetylation status of the histone tails determines the conformation of DNA in the nucleosome, and thereby plays an important role in the modulation of gene expression; the genes regulated by the histone deacetylases have been shown to be active in cellular growth, cell cycle control, differentiation, and apoptosis. The acetylation status of histones is determined by the activity of two classes of enzymes, the histone acetyltransferases and histone deacetylases, which function by modifying the configuration and number of lysine-rich acetyl groups in the histone tails. The histone acetyltransferases act in conjunction with a complex array of other nuclear factors to activate transcription of genes controlling cellular growth and differentiation. In contrast, the histone deacetylases cause compaction of the nuclear chromatin and are primarily associated with transcriptional repression of genes controlling cellular growth and differentiation. Several related transcriptional repressors and corepressors have been identified that recruit histone deacetylases to specific promoter regions. Aberrant acetylation of histones has been implicated in the development of various types of solid and hematologic cancers. In recent years much attention has been focused on the role of histone deacetylases in modulating the expression of oncogenes causing several types of cancer, including non-Hodgkin's lymphoma and acute myeloid leukemia.

Recruitment of histone deacetylases to oncogenic promoter regions has been shown to result in unbalanced histone acetylation, changes in chromatin structure, and subsequent abnormalities in growth, differentiation, cell cycle control, and apoptosis. In the past decade several types of drugs that inhibit histone deacetylases have been identified and studied in vitro and in vivo for their ability to inhibit growth and induce redifferentiation, cell cycle arrest and apoptosis in neoplastic cells ; the different histone deacetylase inhibitors have emerged as among the most promising classes of anticancer therapeutics.

The histone deacetylase inhibitors are a heterogeneous group of structurally dissimilar compounds whose precise mechanisms of action have yet to be elucidated. The different types of histone deacetylase inhibitors appear to share the ability to alter the chromatin structure of the 2% of the human genome regulating growth, differentiation, and apoptosis. Some of the types of drugs with histone deacetylase-inhibiting activity include short-chain fatty acids (including butyrate and valproic acid), hydroxamic acids (trichostatin A, SAHA, suberoylanilide hydroxamic acid), cyclic tetrapeptides (depsipeptide, also known as FK228), and benzamide-containing compounds (MS-275). Of these different types of drugs, we have been investigating the effects of trichostatin A (also known as TSA), depsipeptide (FK228) and valproic acid in thyroid cancer cells. We have started a pilot study of valproic acid in patients with refractory thyroid cancer.

Retinoic Acid

Retinoic acids (RAs), biologically active metabolites of vitamin A, play an important role in morphogenesis, proliferation and differentiation in vertebrates. RAs have been used as a re-differentiation therapy for various human cancers including thyroid cancers. In vitro and in vivo studies demonstrate that RAs inhibits tumor growth, increases Iodide uptake, re-expresses differentiation genes and induces apoptosis in thyroid cancers. However, RA effects differed between thyroid cancer cell lines and in clinical trials using 13-cis RA, only 20−40% of patients with advanced thyroid cancers responded to RA therapy.

The action of RAs require the effective signaling pathway involving nuclear receptors, the retinoic-acid receptors (RARα, RARβ, RARγ) and retinoid-X receptors (RXRα, RXRβ, RXRγ), to be active in the target tissues. It is well established that RAR/RXR heterodimers or RXR homodimers are the functional units that transduce the retinoid signal and activate gene transcription by binding to the RA response elements located in the promoter region of the RA-inducible target gene. In vitro studies have shown variable expression of mRNA and protein for the isoforms of retinoid receptors in thyroid cancer cell lines and tissues, as well as variable anti-tumor growth effects by RAs. Retinoid receptor expression could be one of the molecular mechanisms involved in thyroid tumor proliferation and differentiation, and documenting alteration in retinoid receptor expression in thyroid tumors might be useful in predicting responsiveness to RA re-differentiation therapy.

Among the isoforms of the retinoid receptor, RARβ is considered to play an anti-oncogenic role and dysregulation of RARβ seems to be involved in the pathogenesis of various human epithelial cancers. RARβ expression is required for retinoid-mediated growth inhibition; however, it is downregulated in breast carcinoma, head and neck cancer, oral cancer, and thyroid cancer. RA treatment results in re-expression of the RARβ gene and induces an anti-tumor growth effect in these cancers. We found that RARβ expression was consistently lower in different subtypes of thyroid cancer when compared with normal thyroid tissues and loss of RARβ expression showed a correlation with progression of thyroid disease and with de-differentiation of cancer cells. These results suggest that down-regulation of RARβ gene expression may play an important role in the pathogenesis of thyroid cancer.

Combination Treatment with Histone Deacetylase Inhibitor and Retinoic Acid for Thyroid Cancer

Dysregulation of the retinoic acid receptor (RAR) β seems to be involved in the pathogenesis of various human epithelial cancers. RARβ expression is required for retinoid-mediated growth inhibition and is also associated with cellular sensitivity to retinoid. Therefore, investigators tried to find new compound which can induce RARβ re-expression.

Loss of RARβ expression has also been attributed to dysregulation of histone acetylation/deacetylation, which modulates chromatin structure and gene transcription, as well as hypermethylation of the RARβ promoter. The Histone deacetylase (HDAC) inhibitors re-express RARβ gene in various human cancer cells by inhibiting HDAC activity and increasing histone acetylation with restoration of RA-induced RARβ transcription and increase sensitivity to retinoic acid. The combination treatment with histone deacetylase inhibitor and retinoic acid showed more therapeutic effect than single treatments did in head and neck squamous carcinoma, renal cell cancer, acute myeloblastic leukemia and myelodysplastic syndrome.

Of the subtypes of thyroid cancer, Anaplastic thyroid carcinoma (ATC), an undifferentiated tumor, accounts for less than 5% of non-medullary thyroid carcinoma but it is the most lethal form of thyroid malignancy with a median survival of 2−6 months after diagnosis. ATC eventually lost thyroid-specific functions (iodine uptake, TSH receptor expression, and thyroglobulin production) by de-differentiation of tumor cells. Because no effective therapy is available for patients with ATC, it is necessary to develop more effective therapeutic modalities for this cancer. ATC exhibited more resistance to RAs in inhibiting growth or in re-expressing differentiation genes than well differentiated thyroid cancer cells did and showed lower RARβ expression than well differentiated cancers did. It might be important to identify new compounds and induce RARβ re-expression so that RAs could be more effective in the treatment of patients with this aggressive cancer.

We examined anticancer effects of trichostatin A (TSA) alone or in combination with retinoic acid (RA) on growth inhibition and redifferentiation in anaplastic thyroid cancer cells by up-regulation of RARβ expression. We could found that TSA showed therapeutic effects and the combination treatment with all-trans RA and TSA showed more favorable therapeutic effects in up-regulating RARβ expression, inhibiting cell growth, inducing redifferentiation and apoptosis than single treatment of all-trans RA or TSA in anaplastic cancer cells.

Summary

New therapies are needed for poorly differentiated and undifferentiated thyroid cancers that exhibit dysregulated growth and poor uptake of radioactive iodine. The combination treatment with retinoic acid and histone deacetylase inhibitors, which targets RARß reexpression, is one of the new promising therapies for refractory thyroid cancers and further laboratory investigation for these modality is necessary.

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