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New Drug Candidate Targeting the 4A1 Orphan Nuclear Receptor for Medullary Thyroid Cancer Therapy

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Abstract: Medullary thyroid cancer (MTC) is a relatively rare thyroid cancer responsible for a substantial fraction of thyroid cancer mortality. More effective therapeutic drugs with low toxicity for MTC are urgently needed. Orphan nuclear receptor 4A1 (NR4A1) plays a pivotal role in regulating the proliferation and apoptosis of a variety of tumor cells. Based on the NR4A1 protein structure, 2-imino-6-methoxy-2H-chromene-3-carbothioamide (IMCA) was identified from the Specs compounds database using the protein structure-guided virtual screening approach. Computationally-based molecular modeling studies suggested that IMCA has a high affinity for the ligand binding pocket of NR4A1. MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide] and apoptosis assays demonstrated that IMCA resulted in significant thyroid cancer cell death. Immunofluorescence assays showed that IMCA induced NR4A1 translocation from the nucleus to the cytoplasm in thyroid cancer cell lines, which may be involved in the cell apoptotic process. In this study, the quantitative polymerase chain reaction results showed that the IMCA-induced upregulation of sestrin1 and sestrin2 was dose-dependent in thyroid cancer cell lines. Western blot showed that IMCA increased phosphorylation of adenosine 5′-monophosphate-activated protein kinase (AMPK) and decreased phosphorylation of ribosomal protein S6 kinase (p70S6K), which is the key enzyme in the mammalian target of rapamycin (mTOR) pathway. The experimental results suggest that IMCA is a drug candidate for MTC therapy and may work by increasing the nuclear export of NR4A1 to the cytoplasm and the tumor protein 53 (p53)-sestrins-AMPK-mTOR signaling pathway.

Keywords: orphan nuclear receptor 4A1 (NR4A1); thyroid cancer; 2-imino-6-methoxy-2*H*-chromene-3-carbothioamide (IMCA)

1. Introduction

Medullary thyroid cancer (MTC) is a relatively uncommon cancer of the thyroid C cells, with about 1400 new cases per year in the U.S., yet accounts for a substantial proportion of thyroid cancer mortality [1]. Many studies have focused on MTC treatment, such as, proto-oncogene tyrosine-protein kinase receptor Ret (RET) inhibitors, Ras-like protein 1 (RAS) inhibitors, immunotherapy, and peptide receptor radionuclides. Two RET inhibitors, vandetanib and cabozantinib, are U.S. Food and Drug

Administration (FDA)-approved for the treatment of advanced MTC [2,3]. MTC is resistant to cytotoxic chemotherapy. Developing more effective drugs with higher bioactivity and fewer toxic side effects is urgently required for MTC treatment. The orphan nuclear receptor 4A1 (NR4A1) plays a pivotal role in regulating the proliferation and apoptosis in a variety of tumor cells [4]. NR4A1 may have potential for the development of new drugs for MTC treatment.

NR4A1 is a member of NR4A orphan nuclear receptor family that includes NR4A1, NR4A2, and NR4A3. NR4A1, which includes mouse homologue neuron-derived clone 77 (Nur77), human homologue testicular receptor 3 (TR3), and rat homologue nerve growth factor-induced gene B (NGFI-B), is involved in multiple physiological and pathological processes in metabolism, inflammation, vascular function, and steroidogenesis [5–7]. NR4A1 is expressed in most tumor tissues and cells. Functional studies of receptor knockdown caused by RNA interference suggest that NR4A1 knockdown reduces cellular proliferation and angiogenesis and induces intrinsic and extrinsic apoptotic pathways [8]. For instance, inhibition of NR4A1 results in decreased proliferation and increased apoptosis of pancreatic cancer cells [6]. The dual role of NR4A1 in the process of proliferation and apoptosis varies depending on the change in its intracellular location [8]. Compared with non-tumor tissues, NR4A1 is strongly expressed in the nucleus of tumor tissues, whereas inhibition of the expression of NR4A1 may decrease cell proliferation and increase cell apoptosis [9]. Translocation of NR4A1 from the nucleus to the mitochondria is a major event in NR4A1-mediated apoptosis [10]. Pro-apoptotic agents related to NR4A1, such as phorbol esters and adamantly-derived retinoids mainly induce NR4A1 expression and its translocation from the nucleus to mitochondria. NR4A1 binds with B-cell lymphoma-2 (BCL-2) to form a pro-apoptotic complex and triggers the release of cytochrome c and apoptosis [11,12]. NR4A1 is also involved in cell proliferation regulated by the mTOR signaling pathway, which is a main regulator of cell growth and metabolism [13]. Deregulation of the mTOR pathway has been closely linked to tumorigenesis [14]. In lung cancer cells, NR4A1 binds to and deactivates p53, resulting in the activation of mTOR due to decreased expression of p53-regulated sestrin1 and sestrin2 and inactivation of AMPKa [15–17]. In summary, for cancer cells, NR4A1 acts as a survival factor in the nucleus, but transforms into a killer after migrating to mitochondria. In the mitochondria, NR4A1 can interact with BCL-2, an anti-apoptotic protein, and lead to the conversion of BCL-2 from a protector to a killer that triggers the release of cytochrome c and apoptosis.

No appropriate ligand-binding cavity exists in NR4A1 to bind small molecular ligands and regulate physiological function. A review of previous studies shows that the regulation activity of NR4A receptors is ligand independent, and NR4A1 function is dependent on receptor expression and posttranslational modification [18]. However, a number of small molecule NR4A1 inhibitors of NR4A1 have been found, such as cytosporone B and related analogs [19,20], (ethyl 2-(2,3,4-trimethoxy-6-(1-octanoyl) phenyl) acetate and 1-(3,4,5-trihydroxyphenyl) no-nan-1-one), 1,1-bis(3′-indolyl)-1-(*p*-substituted phenyl) methane (C-DIM) analogs [21], 1,1-bis-(3′-indolyl)-1-(*p*-hydroxyphenyl)methane (DIM-C-pPhOH) [22,23], and ethyl 2-[2,3,4-trimethoxy-6-(1-octanoyl)phenyl]acetate (TMPA) [24]. Zhan et al first reported the crystal structure of NR4A1, and successfully screened NR4A1 small molecule ligand TMPA, which plays an important role in hepatic gluconeogenesis process mediated by NR4A1, using computer virtual screening technology [24].

In this study, IMCA was obtained from the Specs compounds database using a protein structure-guided virtual screening approach based on the NR4A1 protein structure. We focused on the effect of IMCA on thyroid cancer cell viability, apoptosis, nuclear export of NR4A1, and cell proliferation regulated by the mTOR pathway. The results demonstrate that IMCA is an anticancer drug candidate for thyroid carcinoma chemotherapy and may work as a specific antagonist of NR4A1 through the nuclear export of NR4A1 and the p53-sestrins-AMPK-mTOR signaling pathway.