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Thyroid hormone receptor inhibits hepatoma cell migration through transcriptional activation of Dickkopf 4

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ABSTRACT

Triiodothyronine (T_3) is a potent form of thyroid hormone mediates several physiological processes including cellular growth, development, and differentiation via binding to the nuclear thyroid hormone receptor (TR). Recent studies have demonstrated critical roles of T_3/TR in tumor progression. Moreover, long-term hypothyroidism appears to be associated with the incidence of human hepatocellular carcinoma (HCC), independent of other major HCC risk factors. Dickkopf (DKK) 4, a secreted protein that antagonizes the canonical Wnt signaling pathway, is induced by T_3 at both mRNA and protein levels in HCC cell lines. However, the mechanism underlying T_3-mediated regulation of DKK4 remains unknown. In the present study, the 5′ promoter region of DKK4 was serially deleted, and the reporter assay performed to localize the T_3 response element (TRE). Consequently, we identified an atypical direct repeat TRE between nucleotides −1645 and −1629 conferring T_3 responsiveness to the *DKK4* gene. This region was further validated using chromatin immunoprecipitation (ChIP) and electrophoretic mobility shift assay (*EMSA*). Stable *DKK4* overexpression in SK-Hep-1 cells suppressed cell invasion and metastatic potential, both *in vivo* and*in vitro*, via reduction of matrix metalloproteinase-2 (*MMP-2*) expression. Our findings collectively suggest that DKK4 upregulated by T_3/TR antagonizes the Wnt signal pathway to suppress tumor cell progression, thus providing new insights into the molecular mechanism underlying thyroid hormone activity in HCC.

1. Introduction

Thyroid hormone, 3,3′-5-triiodo-l-thyronine (T_3), is a potent mediator of numerous physiological processes, including embryonic development, cell differentiation, metabolism, and cell proliferation [1,2]. Genes encoding two thyroid hormone receptor isoforms, TRα and TRβ, are located on human chromosomes 17 and 3, respectively. TRs belong to the steroid hormone and retinoic acid receptor superfamily. These receptors are ligand-dependent transcription factors that comprise modular functional domains mediating hormone binding (ligands), DNA binding, receptor homo- and heterodimerization, and interactions with other transcription factors and cofactors [3].

Accumulating evidence has confirmed important roles of TR as a potent suppressor of tumorigenesis, invasiveness, and metastasis formation [4,5]. V-erbA, a mutant form of TR with loss of ligand binding ability, triggers hepatocellular carcinoma (HCC) in transgenic mice [6,7]. Earlier experiments by our group and others have revealed that TRα and TRβ cDNAs are truncated or mutated at high frequencies in human HCCs [8–10]. Notably, long-term hypothyroidism is associated with incidence of HCC, independent of other major HCC risk factors. In view of the significance of T_3/TR in HCC, we focused on the downstream signals of T_3 leading to suppression of tumorigenesis. Previously, we reported a positive correlation between TR and DKK4 protein expression in HCC patients. Additionally, T_3 induced DKK4 expression in a TR-dependent manner in hepatoma cell lines *in vitro*. However, the mechanisms of DKK4 regulation by TR and the downstream pathway are yet to be elucidated.

The Dickkopf (DKK) family includes secreted antagonists of Wnt signaling, and comprises four members, each possessing an N-terminal signal peptide with two conserved cysteine-rich domains separated by a linker region [11]. These proteins bind to LRP5/6 and Kerman, which inhibit functional complex formation

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between Wnt-Frizzled-LRP5/6 receptor, thereby suppressing the Wnt/β-catenin signaling pathway [12]. DKKs are frequently hypermethylated in gastrointestinal cancer, whereas knockdown of DKK4 enhances the growth and invasiveness of esophageal and colorectal cancer cells [13,14].

Experiments from the current investigation showed that T_3 upregulates DKK4 transcription in a TR-dependent manner. Furthermore, the DR4-like TRE region of the *DKK4* promoter mediating the effect of T_3 on *DKK4* expression is located at nucleotide positions −1645 to −1629. DKK4 induced by T_3 exerts an anti-metastatic effect on hepatoma cells.

2. Materials and methods

2.1. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Previously established HepG2 sublines (HepG2-TRα1 and HepG2-TRβ1) overexpressing wild-type TRα1 or TRβ1 protein were used [15]. Total RNA was purified using TRIzol, and cDNA synthesized using the Superscript III kit for RT-PCR (Life Technologies, Karlsruhe, Germany), as described previously [16]. Real-time qRT-PCR was conducted in a 15 μl reaction mixture containing 50 nM forward and reverse primers and 1× SYBR Green reaction mix, using the ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA) and comparative Δ^{Ct} analysis. The following primers were used for qRT-PCR: DKK4 (70 bp product) forward, 5′-CCAGCGAGATGCCATGTG-3′, and reverse, 5′-TGCATC TTCCATCGTA GTACAAA-3′. The ribosomal binding protein (*Ribo-L35A*) gene was employed as the endogenous control.

2.2. Chromatin immunoprecipitation (ChIP) assays

ChIP assays were performed to determine the interactions of TR with TRE within the *DKK4* promoter region. Proteins were cross-linked to DNA by adding formaldehyde directly to medium to a final concentration of 1% for 10 min at room temperature, as described previously [17]. The antibody against TR was obtained from S -Y Cheng at the National Cancer Institute. Other antibodies used were anti-RXRα (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-IgG (R&D Systems, Inc., Minneapolis, MN). The 130 bp *DKK4* promoter fragment containing the predicted TRE binding site was identified with PCR using the following primers: forward. 5′-GCTGTTTGTGCCGTGTTACAGG-3′, and reverse, 5′-GGGTTCTGTCTCTGCCG GGT-3′. Amplified products were analyzed using 2% agarose gel electrophoresis.

2.3. Immunoblot analysis

Cell supernatant or lysates were fractionated with 10% SDS–PAGE, and the separated proteins transferred to a nitrocellulose membrane (pH 7.9, Amersham Pharmacia Biotech Inc., Piscataway, NJ). Subsequent procedures were performed according to previous reports [18]. Goat polyclonal antibodies to DKK4 (1:1000 dilution in TBS) were purchased from Abcam (Cambridge, UK). The mouse anti-human fascin monoclonal antibody was obtained from Santa Cruz Biotechnology. Immune complexes were visualized with an enhanced chemiluminescence detection kit (Amersham). The intensities of immunoreactive bands were quantified via analysis with Image Gauge software (Fuji Film, Tokyo, Japan).

2.4. Cloning of DKK4 promoter fragments and measurement of activity

Fragments of the *DKK4* promoter (−2071 to −1) were inserted into the pA3TK vector (Promega Corp., Madison, WI). Serial deletion mutants of the promoter were amplified using PCR, and promoter construct sequences confirmed via automatic DNA sequencing. To determine the transcriptional activity of TREs within the*DKK4* promoter, HepG2-TRα1 or HepG2-TRβ1 stable cell lines (7×10^5 cells/24-well dish) were transfected with 0.2 μg pA3TK vector containing *DKK4* promoter sequences and 0.05 μg SVβ, a β-galactosidase expression vector (Clontech, Palo Alto, CA), using TurboFectin reagent (Fermentas, Glen Burnie, MD). 24 h after transfection, these cells received 0 or 10 nM T_3. Transfected and untransfected cells were incubated for an additional 24 h, and lysed to measure luciferase and β-galactosidase activities [19]. Luciferase activity was normalized to that of β-galactosidase.

2.5. Electrophoretic mobility shift assay (EMSA)

The *DKK4* promoter fragment (positions −1700 to −1571) was amplified and labeled with [α-^{32}P] dCTP (3000 Ci/mmol; Amersham) via PCR for use as a probe. For EMSA, equal amounts of *in vitro*-translated (Promega) TR and RXR proteins were incubated with [α-^{32}P]-labeled *DKK4* fragments, as described elsewhere [20]. The C4 antibody against TR was used to confirm the identity of the protein complex. Mopc21, a mouse monoclonal antibody, was employed as the negative control (Sigma–Aldrich, St. Louis, MO, USA) [15].

2.6. In vitro assay of invasive activity

SK-Hep-1 cell density was adjusted to approximately 5×10^4 -cells, and 200 μl of the suspension added to wells coated with Matrigel (Becton–Dickinson, Franklin Lakes, NJ, USA) in triplicate. The medium in the upper chamber was serum-free DMEM, and that in the lower chamber was DMEM supplemented with 10% FBS. After incubation for 24 h at 37 °C, the number of viable cells that had traversed the filter to the lower chamber was measured.

2.7. Statistical analysis

Statistical analyses were performed using means and standard deviations, one-way analysis of variance (ANOVA), and Tukey's honestly significant difference post hoc test.

2.8. Xenograft models of tumor progression and metastasis

Five-week-old, male C.B17/Icr-*Prkdc*^{scid}/CrlNarl SCID mice were divided into two groups for tail vein injection using control or DKK4-expressing SK-Hep1 (1×10^6/200 μl PBS) cells. All animals were sacrificed by CO_2 asphyxiation 6 weeks after tumor inoculation and lungs were removed for tumor biopsy. All procedures of animal experiments were in accordance with United States National Institutes of Health guidelines, and the Guide for Care and Use of Laboratory Animals issued by the Chang-Gung Institutional Animal Care and Use Committee. This study was conducted under the approval of Chang Gung Institutionally Animal Care and Use Committee (IACUC Approval No. CGU08-05).

3. Results

3.1. T_3 induces DKK4 expression in HepG2-TR cells at the transcriptional level

To assess DKK4 induction by T_3 in TR-overexpressing hepatoma cells, three isogenic HepG2 cell lines were used (HepG2-Neo, HepG2-TRβ1, and HepG2-TRα1, Fig. 1A). *DKK4* mRNA levels increased by 7.8 to 17-fold following incubation of HepG2-TR cells with 10 nM T_3 for 24 h, as observed from qRT-PCR analysis.

However, *DKK4* expression remained unaffected following T_3 treatment in HepG2-Neo cells (Fig. 1B).

The effect of T_3 on DKK4 protein expression was additionally assessed in HepG2 isogenic cell lines incubated with medium containing 0 or 10 nM T_3 (Fig. 1C). The DKK4 protein level increased 9.5- to 6.3-fold following incubation of HepG2-TRα1 and TRβ1 cells with 10 nM T_3 for 24 h. These results confirm that T_3 enhances both DKK4 mRNA and protein expression in HepG2 isogenic cell lines (Fig. 1C). In contrast, DKK4 protein was not induced in the HepG2-Neo control cell line by T_3.

To examine whether T_3 affects *DKK4* transcription, we cloned a 2.1 kb fragment of the human *DKK4* promoter. The promoter region, encompassing nucleotides −2071 to −1 (−1 is relative to the translational initiation site), was cloned and inserted into the luciferase reporter plasmid. In addition, various deletion mutants of the promoter region from positions −2071 to −1 were prepared to localize potential thyroid response elements (TREs). Using these reporter constructs, the effect of transactivation of TR via T_3 stimulation on the *DKK4* 5′-flanking regions was determined.

In the reporter activity assay, luciferase activity of the −2071 to −1 region (containing six putative TREs, fragment b) was increased by approximately three-fold in the presence of T_3 in HepG2-TRβ1 cells (Fig. 1D. However, the −699 to −1 region (fragment c) lost T_3-induced *DKK4* promoter activity. Treatment with T_3 enhanced promoter activity of the −2071 to −459 region (fragment d) by 3.8-fold (Fig. 2A). Fragment (d) was further divided into three (e, f, and g). Interestingly, the effect of T_3 was only prevalent in fragment (g). Mutation of TRE1 in fragment (g), yielding fragment (h) (Fig. 1D), led to loss of T_3-induced *DKK4* promoter activity. Similar results were observed with the TRE1 deletion mutant (fragment i, Fig. 1D).

Our results collectively show that TRE1, located between positions −1648 and −1632, binds to and mediates the effect of T_3 on *DKK4* promoter activity. Further analysis of the GGGCCTttggTAACCT sequence revealed an atypical direct repeat TRE with a spacing of four base pairs (DR4). Analogous results were obtained with a TRα1 stable cell line (data not shown).

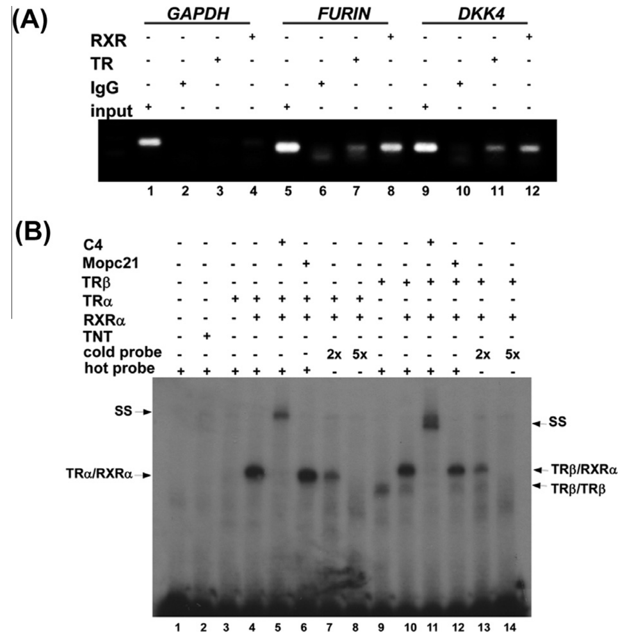

Fig. 2. T_3-mediated DKK4 transcription is dependent on direct binding of TR proteins to the TRE regions of the *DKK4* promoter. (A) ChIP assays demonstrated that TR is recruited to the *DKK4* 5′-flanking region together with RXR. One set of primers for each *DKK4* TRE, positive control TRE (*furin*), and negative control (*GAPDH*) were prepared. ChIP assay results were evaluated via PCR and gel electrophoresis. All ChIP assays were repeated at least three times. (B) EMSA analysis of binding of TR proteins to positions −1700 to −1571 of the *DKK4* promoter.

3.2. TR binds to nucleotides −1645 to −1629 of the DKK4 promoter

To determine the precise binding site of TR within the *DKK4* promoter, immunoprecipitation (ChIP) assays were performed. Both TR and retinoid X receptor (RXR) were clearly recruited to TRE-binding site 1 (Fig. 2A, lanes 11 and 12), whereas the assay with

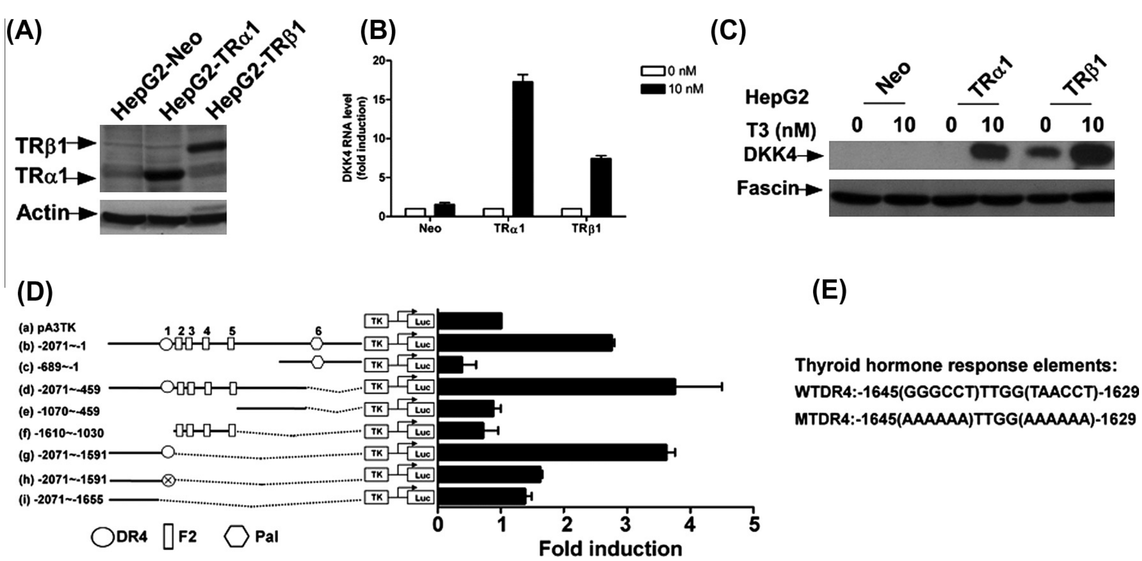

Fig. 1. T_3/TR-dependent up-regulation of the *DKK4* at transcriptional level. (A) TRs protein expression were assessed in HepG2-Neo, -TRα1#1 and TRβ1 cell lines. (B,C) HepG2-Neo, HepG2-TRα1#1 and HepG2-TRβ1 cell lines were incubated for 24 h in T_3-depleted regular medium. Then, T_3-depleted regular medium were replaced with serum-free medium supplemented with or without T_3 (10 nM) for another 24 h. Total RNA and supernatant fractions of these cells were isolated and *DKK4* expression analyzed using qRT-PCR and immunoblot analysis. Values (means ± SEM) are presented as fold induction, compared with 0 nM T_3 control. All assays were repeated at least three times. (D) HepG2-TRβ1 cells were transfected with a luciferase reporter plasmid driven by the *DKK4* 5′-flanking region (−2071 to −1), which contained six putative TREs with minimal thymidine kinase promoter (pA3TK-Luc), or vectors expressing β-galactosidase (transfection efficiency control). Cells were incubated for 24 h in the presence or absence of T_3 (10 nM) before harvesting to measure luciferase activity. Luciferase activity was normalized to that of β-galactosidase. Various deletion mutants of the *DKK4* 5′-flanking region were generated based on the pA3TK-Luc vector, and transfected into cells. The promoter regions contained in these mutants are shown. Data are presented as means ± SEM of values from three independent experiments performed in triplicate. (E) Sequence and location of putative wild-type and mutant TREs.

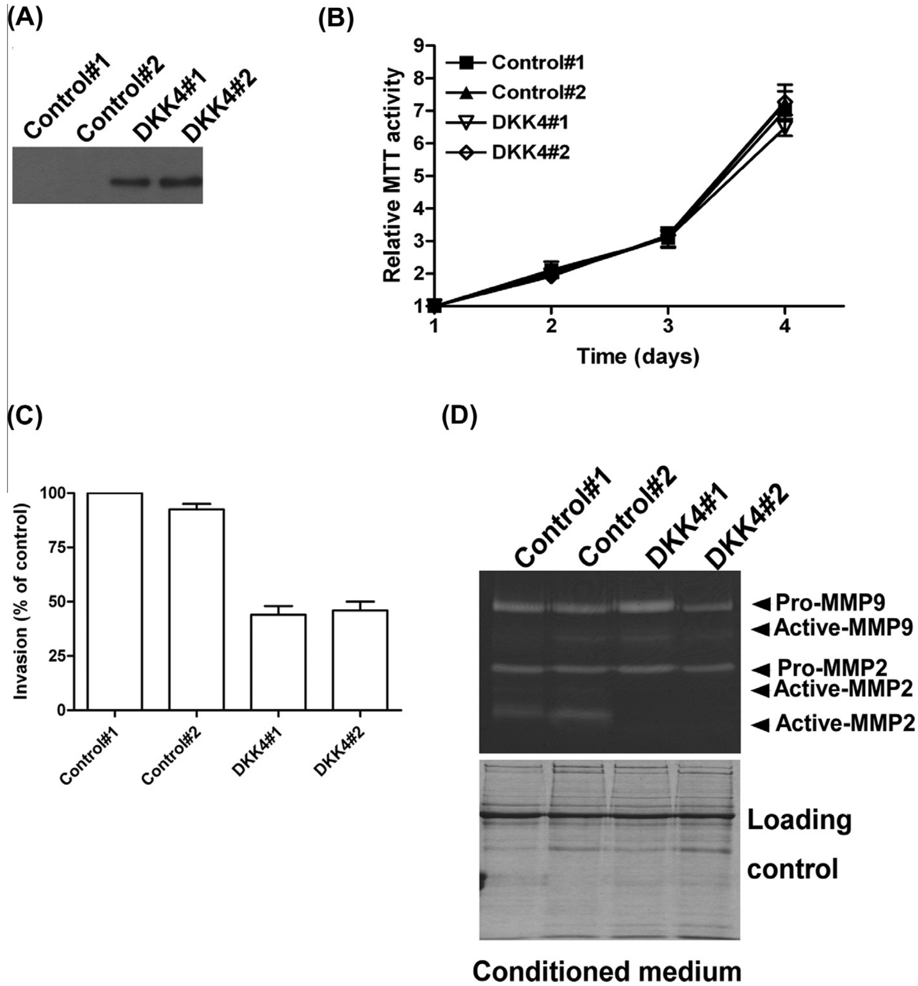

Fig. 3. Overexpression of DKK4 represses SK-Hep-1 cell invasion *in vitro*. (A) DKK4 protein expression levels in two DKK4-expressing SK (SK-DKK4 #1 and SK-DKK4 #2) and control cell lines (SK-control#1 and SK-control#2). (B) MTT assay for two stable and two control lines. (C) Invasion properties of two DKK4-expressing and two control cell lines are shown. Cells were added to the upper chamber of Transwell units coated with Matrigel and incubated for 24 h. Relative invasion of cells were measured as the number of cells traversing the filter to the lower chamber. (D) MMP activity was detected using zymography. Data are presented as means ± SEM of values from three independent experiments. Coomassie blue-stained conditioned medium proteins represent the internal control. **P* < 0.05 (one-way ANOVA with Tukey's multiple comparison test), compared with control cell lines.

control IgG revealed only background levels (Fig. 2A, lane 10). Conversely, a set of primers for the negative control (Fig. 3C, lanes 3–4, human *GAPDH*) did not yield detectable bands. We used the *furin* gene, which is regulated by T_3,[21] as a positive control (Fig. 2A, lanes 7 and 8). Lanes 1, 5, and 9 represent the 5% input controls.

To confirm that TR directly binds the −1645 to −1629 fragment within the *DKK4* promoter, the electrophoretic mobility shift assay (EMSA) was conducted using the fragment as a probe. T7 Quick Coupled Transcription/Translation (TNT) protein did not bind the [α-^{32}P]-labeled −1645/−1629 fragment (Fig. 2B, lane 2). The TRα1/TRα1 homodimer was not detected (lanes 3). However, TRβ1 protein produced a predominant band representing the TRβ/TRβ homodimer (lanes 9). RXRα can form TRα/RXRα or TRβ/RXRα heterodimers (lanes 4 and 10). The specific TR/TRE complex was supershifted following the addition of TR-specific antibody, C4 (lanes 5 and 11), but not the non-specific antibody, Mopc21 (lanes 6 and 12). Furthermore, the specific TR/TRE complex could be competed out by the addition 2- to 5-fold higher concentrations of specific cold competitors (lanes 7–8 and 13–14).

3.3. DKK4 overexpression suppresses hepatoma cell invasion in vitro

Wnt signals promote proliferation, transformation, and migration in some cancer types, such as breast and prostate cancer [22,23]. DKK4 is a Wnt antagonist protein involved in the Wnt signaling pathway [24]. To explore the specific biological role of DKK4 in hepatoma cells, we established DKK4-expressing (SK-DKK4) and control cell lines (SK-control). Western blot analysis disclosed that SK-DKK4 cells secrete more DKK4 into culture medium than SK-control cells (Fig. 3A). Overexpression of DKK4 did not affect cellular proliferation, relative to SK control cells (Fig. 3B). The effects of *DKK4* on cell invasiveness were measured *in vitro* using Matrigel Transwell invasion assays. Notably, the invasive ability of DKK4-expressing SK cells was inhibited by 50–55%, compared with control cells (Fig. 3C).

Matrix metallopeptidases (MMPs) are zinc- and calcium-dependent proteinases that degrade extracellular matrix components and numerous other proteins [25]. Overexpression of DKK4 suppressed active MMP-2 expression by 85–90% in SK cells (Fig. 3D).

3.4. DKK4 reduces metastasis formation in vivo

To determine whether the *in vitro* results could be replicated *in vivo*, we investigated the effect of DKK4 on tumor invasiveness in SCID mice. Lung metastatic foci were observed in animals injected with SK-DKK4 cells, compared to those administered SK-Hep1 control cells. All lines of SCID mice developing multiple tumor nodules, detected based on H&E staining, are indicated by

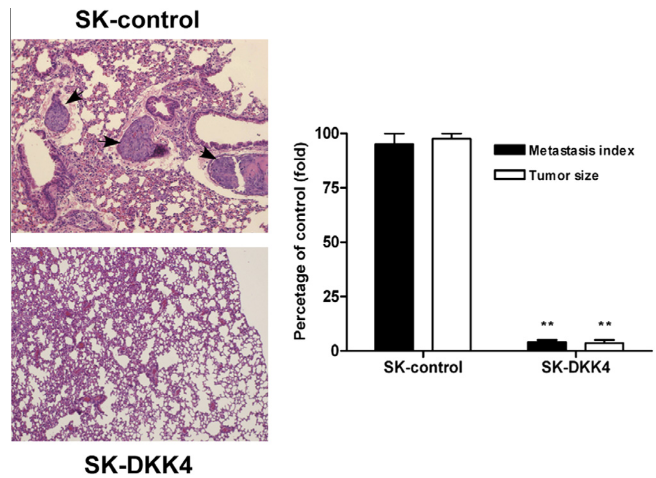

Fig. 4. Effect of DKK4 on SK-Hep-1 metastasis in SCID mice. SCID mice were inoculated i.v. with control or DKK4-expressing SK-Hep-1 cell lines (1×10^6). All analyses were performed six weeks after inoculation with tumor cells. Mice were sacrificed, and their lungs examined. H&E staining of metastasized SK-control and SK-DKK4 cells in lung. The average of the metastasis index (fold density of tumor numbers in SK-DKK4/SK-control per cm^2) or relative tumor size (fold decrease) in the lung (average tumor size in SK-DKK4/SK-control cells per cm^2 area) (right panel). Subcutaneous and i.v. injections. $n = 4$ in each group. $^{**}P < 0.01$.

arrowheads in the lung (Fig. 4A). Significantly, average metastasis index and tumor size were reduced by 90–95% in animals injected with SK-DKK4 cells, compared with those with SK-control cells (Fig. 4B).

Our data clearly indicate that TRs play a tumor suppressor role by inducing the expression of DKK4 that suppresses metastasis formation in hepatoma cells.

4. Discussion

Accumulating evidence from recent reports clearly supports an association between aberrant TR regulation or *TR* mutation and human hepatocellular carcinoma [26]. For instance, TR-mutated (v-erbA) transgenic mice have been shown to develop hepatocellular carcinoma. Moreover, aberrant expression of TR is implicated in tumor development and progression [6,9,27,28]. Epidemiological studies further suggest that long-term hypothyroidism is associated with HCC, independent of other major HCC risk factors [29–31]. These findings indicate that loss of normal TR function caused by a decrease in expression or complete loss of normal TR activity provides an opportunity for tumors to proliferate, metastasize, and invade other tissues.

Studies by our group previously showed that TR suppresses Wnt signals via DKK4 induction, leading to inhibition of liver cancer progression, although the underlying mechanisms were unclear. In the current investigation, we attempted to elucidate the molecular mechanism underlying *DKK4* regulation by T_3 and the downstream effects in isogenic HepG2 cell lines. Our results demonstrate that *DKK4* is induced by T_3 at the transcriptional level, and DR4-like TRE is located at nucleotides −1645 to −1629 within the *DKK4* promoter. Moreover, T_3 regulated DKK4 suppresses hepatoma cell metastasis.

The effects of DKK4 on tumor progression appear multifaceted. Earlier research documented that DKK4 is induced via the TCF/β-catenin pathway in colon cancer [32]. DKK4 overexpression was reported to enhance the migration and invasive abilities of colon cancer cells. Expression of DKK4 was suppressed by 1α, 25- dihydroxyvitamin D3 (1,25(OH)2D3) in SW480 cells. In contrast, another study showed that DKK4 is a potent inhibitor of TCF-dependent signaling and growth in colorectal cancer cells [33]. Consequently, upregulation of DKK4 may provide a negative feedback loop for inhibition of the Wnt/β-catenin pathway in colon cancer. The studies collectively demonstrate a dual role of DKK4 in different tissue, microenvironment, and cell types.

DKK4 performs a critical function in tumor progression, and regulation of DKK4 expression is crucial for tumorigenesis. Previous studies have reported that DKK4 expression is silenced in colorectal and liver cancers [14,34]. The *DKK4* gene is hypermethylated as determined from epigenetic analysis. Moreover, DKK4 expression is activated in colorectal cancer cell lines following treatment with trichostatin A (TSA), a histone deacetylase inhibitor with antitumor ability [33]. Ligand-bound TRs act as transcriptional activators to induce promoter-proximal chromatin remodeling and histone acetylation. Data from the current study showed that DKK4 expression is low in hepatoma cells, but activated by T_3/TR upon binding to the −1645 to −1629 region on the *DKK4* promoter. DKK4 induced by TR exerted anti-metastatic activity in hepatoma cell lines. Notably, cell metastatic ability, as well as activity of MMP-2, a member of the matrix metalloprotease family, was decreased significantly in SK-Hep1 cells expressing DKK4. MMP-2 presents a key factor in the enhancement of invasion and metastasis in several malignant tumor cells [35].

In conclusion, DR4-like TRE located within the *DKK4* promoter is responsible for T_3-dependent DKK4 transcription. Expression of DKK4, in turn, leads to suppression of hepatoma cell metastasis via inhibition of MMP-2 activity. Our novel findings provide a mechanistic link for aberrant regulation of TR and DKK4 in HCC.

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References

[1] A. Aranda, A. Pascual, Nuclear hormone receptors and gene expression, Physiol. Rev. 81 (2001) 1269–1304.
[2] Y.H. Huang, M.M. Tsai, K.H. Lin, Thyroid hormone dependent regulation of target genes and their physiological significance, Chang Gung Med. J. 31 (2008) 325–334.
[3] S.Y. Cheng, Multiple mechanisms for regulation of the transcriptional activity of thyroid hormone receptors, Rev. Endocr. Metab. Disord. 1 (2000) 9–18.
[4] O. Martinez-Iglesias, S. Garcia-Silva, S.P. Tenbaum, J. Regadera, F. Larcher, J.M. Paramio, B. Vennstrom, A. Aranda, Thyroid hormone receptor beta1 acts as a potent suppressor of tumor invasiveness and metastasis, Cancer Res. 69 (2009) 501–509.
[5] X.G. Zhu, L. Zhao, M.C. Willingham, S.Y. Cheng, Thyroid hormone receptors are tumor suppressors in a mouse model of metastatic follicular thyroid carcinoma, Oncogene 29 (2010) 1909–1919.
[6] C. Barlow, B. Meister, M. Lardelli, U. Lendahl, B. Vennstrom, Thyroid abnormalities and hepatocellular carcinoma in mice transgenic for v-erbA, EMBO J. 13 (1994) 4241–4250.
[7] J. Sap, A. Munoz, K. Damm, Y. Goldberg, J. Ghysdael, A. Leutz, H. Beug, B. Vennstrom, The c-erb-A protein is a high-affinity receptor for thyroid hormone, Nature 324 (1986) 635–640.
[8] K.H. Lin, H.Y. Shieh, S.L. Chen, H.C. Hsu, Expression of mutant thyroid hormone nuclear receptors in human hepatocellular carcinoma cells, Mol. Carcinog. 26 (1999) 53–61.
[9] I.H. Chan, M.L. Privalsky, Thyroid hormone receptors mutated in liver cancer function as distorted antimorphs, Oncogene 25 (2006) 3576–3588.
[10] I.H. Chan, M.L. Privalsky, Thyroid hormone receptor mutants implicated in human hepatocellular carcinoma display an altered target gene repertoire, Oncogene 28 (2009) 4162–4174.
[11] V.E. Krupnik, J.D. Sharp, C. Jiang, K. Robison, T.W. Chickering, L. Amaravadi, D.E. Brown, D. Guyot, G. Mays, K. Leiby, B. Chang, T. Duong, A.D. Goodearl, D.P. Gearing, S.Y. Sokol, S.A. McCarthy, Functional and structural diversity of the human Dickkopf gene family, Gene 238 (1999) 301–313.
[12] J.M. Gonzalez-Sancho, O. Aguilera, J.M. Garcia, N. Pendas-Franco, C. Pena, S. Cal, A. Garcia de Herreros, F. Bonilla, A. Munoz, The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is downregulated in human colon cancer, Oncogene 24 (2005) 1098–1103.

[13] T. Maehata, H. Taniguchi, H. Yamamoto, K. Nosho, Y. Adachi, N. Miyamoto, C. Miyamoto, N. Akutsu, S. Yamaoka, F. Itoh, Transcriptional silencing of Dickkopf gene family by CpG island hypermethylation in human gastrointestinal cancer, World J. Gastroenterol. 14 (2008) 2702–2714.

[14] H. Sato, H. Suzuki, M. Toyota, M. Nojima, R. Maruyama, S. Sasaki, H. Takagi, Y. Sogabe, Y. Sasaki, M. Idogawa, T. Sonoda, M. Mori, K. Imai, T. Tokino, Y. Shinomura, Frequent epigenetic inactivation of DICKKOPF family genes in human gastrointestinal tumors, Carcinogenesis 28 (2007) 2459–2466.

[15] K.H. Lin, H.Y. Shieh, H.C. Hsu, Negative regulation of the antimetastatic gene Nm23-H1 by thyroid hormone receptors, Endocrinology 141 (2000) 2540–2547.

[16] P.J. Tai, Y.H. Huang, C.H. Shih, R.N. Chen, C.D. Chen, W.J. Chen, C.S. Wang, K.H. Lin, Direct regulation of androgen receptor-associated protein 70 by thyroid hormone and its receptors, Endocrinology 148 (2007) 3485–3495.

[17] C.H. Liao, S.C. Yeh, Y.H. Huang, R.N. Chen, M.M. Tsai, W.J. Chen, H.C. Chi, P.J. Tai, C.J. Liao, S.M. Wu, W.L. Cheng, L.M. Pai, K.H. Lin, Positive regulation of spondin 2 by thyroid hormone is associated with cell migration and invasion, Endocr. Relat. Cancer 17 (2010) 99–111.

[18] C.S. Liao, P.J. Tai, Y.H. Huang, R.N. Chen, S.M. Wu, L.W. Kuo, C.T. Yeh, M.M. Tsai, W.J. Chen, K.H. Lin, Regulation of AKR1B1 by thyroid hormone and its receptors, Mol. Cell. Endocrinol. 307 (2009) 109–117.

[19] Y.H. Huang, C.Y. Lee, P.J. Tai, C.C. Yen, C.Y. Liao, W.J. Chen, C.J. Liao, W.L. Cheng, R.N. Chen, S.M. Wu, C.S. Wang, K.H. Lin, Indirect regulation of human dehydroepiandrosterone sulfotransferase family 1A member 2 by thyroid hormones, Endocrinology 147 (2006) 2481–2489.

[20] S.M. Wu, Y.H. Huang, Y.H. Lu, L.F. Chien, C.T. Yeh, M.M. Tsai, C.H. Liao, W.J. Chen, C.J. Liao, W.L. Cheng, K.H. Lin, Thyroid hormone receptor-mediated regulation of the methionine adenosyltransferase 1 gene is associated with cell invasion in hepatoma cell lines, Cell Mol. Life Sci. 20 (2010) 1831–1843.

[21] R.N. Chen, Y.H. Huang, Y.C. Lin, C.T. Yeh, Y. Liang, S.L. Chen, K.H. Lin, Thyroid hormone promotes cell invasion through activation of furin expression in human hepatoma cell lines, Endocrinology 149 (2008) 3817–3831.

[22] H. Yamamoto, N. Oue, A. Sato, Y. Hasegawa, H. Yamamoto, A. Matsubara, W. Yasui, A. Kikuchi, Wnt5a signaling is involved in the aggressiveness of prostate cancer and expression of metalloproteinase, Oncogene 29 (2010) 2036–2046.

[23] K. Benhaj, K.C. Akcali, M. Ozturk, Redundant expression of canonical Wnt ligands in human breast cancer cell lines, Oncol. Rep. 15 (2006) 701–707.

[24] Y. Kawano, R. Kypta, Secreted antagonists of the Wnt signalling pathway, J. Cell Sci. 116 (2003) 2627–2634.

[25] M.D. Sternlicht, Z. Werb, How matrix metalloproteinases regulate cell behavior, Annu. Rev. Cell Dev. Biol. 17 (2001) 463–516.

[26] J.M. Gonzalez-Sancho, V. Garcia, F. Bonilla, A. Munoz, Thyroid hormone receptors/THR genes in human cancer, Cancer Lett. 192 (2003) 121–132.

[27] K.H. Lin, X.G. Zhu, H.C. Hsu, S.L. Chen, H.Y. Shieh, S.T. Chen, P. McPhie, S.Y. Cheng, Dominant negative activity of mutant thyroid hormone alpha1 receptors from patients with hepatocellular carcinoma, Endocrinology 138 (1997) 5308–5315.

[28] S.Y. Cheng, Thyroid hormone receptor mutations in cancer, Mol. Cell. Endocrinol. 213 (2003) 23–30.

[29] T.A. Dragani, Risk of HCC: genetic heterogeneity and complex genetics, J. Hepatol. 52 (2010) 252–257.

[30] M.M. Hassan, A. Kaseb, D. Li, Y.Z. Patt, J.N. Vauthey, M.B. Thomas, S.A. Curley, M.R. Spitz, S.I. Sherman, E.K. Abdalla, M. Davila, R.D. Lozano, D.M. Hassan, W. Chan, T.D. Brown, J.L. Abbruzzese, Association between hypothyroidism and hepatocellular carcinoma: a case-control study in the United States, Hepatology 49 (2009) 1563–1570.

[31] A. Reddy, C. Dash, A. Leerapun, T.A. Mettler, L.M. Stadheim, K.N. Lazaridis, R.O. Roberts, L.R. Roberts, Hypothyroidism: a possible risk factor for liver cancer in patients with no known underlying cause of liver disease, Clin. Gastroenterol. Hepatol. 5 (2007) 118–123.

[32] N. Pendas-Franco, J.M. Garcia, C. Pena, N. Valle, H.G. Palmer, M. Heinaniemi, C. Carlberg, B. Jimenez, F. Bonilla, A. Munoz, J.M. Gonzalez-Sancho, DICKKOPF-4 is induced by TCF/beta-catenin and upregulated in human colon cancer, promotes tumour cell invasion and angiogenesis and is repressed by 1alpha,25-dihydroxyvitamin D3, Oncogene 27 (2008) 4467–4477.

[33] S. Baehs, A. Herbst, S.E. Thieme, C. Perschl, A. Behrens, S. Scheel, A. Jung, T. Brabletz, B. Goke, H. Blum, F.T. Kolligs, Dickkopf-4 is frequently down-regulated and inhibits growth of colorectal cancer cells, Cancer Lett. 276 (2009) 152–159.

[34] C.H. Liao, C.T. Yeh, Y.H. Huang, S.M. Wu, H.C. Chi, M.M. Tsai, C.Y. Tsai, C.J. Liao, Y.H. Tseng, Y.H. Lin, C.Y. Chen, I.H. Chung, W.L. Cheng, W.J. Chen, K.H. Lin, Dickkopf 4 positively regulated by the thyroid hormone receptor suppresses cell invasion in human hepatoma cells, Hepatology 55 (2012) 910–920.

[35] M.D. Sternlicht, Z. Werb, How matrix metalloproteinases regulate cell behavior, Annu. Rev. Cell Dev. Biol. 17 (2001) 463–516.