Thyroid Hormone Is a MAPK-Dependent Growth Factor for Human Myeloma Cells Acting via αvβ3 Integrin

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Abstract

Experimental and clinical observations suggest that thyroid hormone [1-thyroxine (T₄) and 3,5,3′-triiodo-l-thyronine (T₃)] can support cancer cell proliferation. T₃ and T₄ promote both tumor cell division and angiogenesis by activating mitogen-activated protein kinase (MAPK) via binding to a hormone receptor on the αvβ3 integrin, overexpressed on many cancer cells. We have studied the responsiveness of several MM cell lines to T₃ and T₄ and characterized hormonal effects on cell survival, proliferation, and MAPK activation. Overnight T₃ (1–100 nmol/L) and T₄ (100 nmol/L) incubation enhanced, up to 50% (P < 0.002), MM cell viability (WST-1 assay) and increased cell proliferation by 30% to 60% (P < 0.01). Short exposure (10 minutes) to T₃ and T₄ increased MAPK activity by 2.5- to 3.5-fold (P < 0.03). Pharmacologic MAPK inhibition blocked the proliferative action of T₃ and T₄. Antibodies to the integrin αvβ3 dimer and αv and β3 monomers (but not β1) inhibited MAPK activation and subsequent cell proliferation in response to thyroid hormone, indicating dependence upon this integrin. Moreover, tetraiodothyroacetic acid (tetrac), a non-agonist T₄ analogue previously shown to selectively block T₃/T₄ binding to αvβ3 receptor site, blocked induction of MAPK by the hormones in a dose-dependent manner. This demonstration of the role of thyroid hormones as growth factors for MM cells may offer novel therapeutic approaches. Mol Cancer Res; ©2011 AACR.

Introduction

Multiple myeloma remains an incurable disease despite remarkable improvement in survival, particularly in younger patients, in the era of the agents such as thalidomide, lenalidomide, and bortezomib. Thalidomide and lenalidomide are immunomodulatory agents with effects on the bone marrow microenvironment, rendering marrow less hospitable to myeloma plasma cells as well as inducing apoptosis of plasma cells. Bortezomib is a selective proteasome inhibitor that also exerts its effects in multiple myeloma by downregulating the nuclear factor κB pathway (1). These novel agents, when used in combination with cytotoxic agents and corticosteroids, achieve overall response rates of 60% to 90% and median progression-free survival of 2 to 3 years (2). In comparison, treatment regimens consisting only of chemotherapy and corticosteroids that were used in the past resulted in remission rates of 20% to 35% and progression-free survival of 6 to 18 months (3). Current treatment protocols have extended the overall survival of patients with multiple myeloma; however, only 68% of patients younger than 45 years survive for 5 years from the time of diagnosis, and by 10 years, only 55% remain alive (4). Ultimately the disease becomes refractory to all forms of treatment (5) and therefore drugs with new mechanisms of action are urgently needed.

Thyroid hormone has pleiotropic physiologic effects on numerous tissues and is essential for normal human development and function. Thus, it is not altogether surprising that the hormone has been shown experimentally to have trophic effects on cancer cells and influence tumor growth and progression (6–8). Indeed, a putative relationship between the thyroid gland and cancer was proposed as early as 1896. A body of epidemiologic and clinical evidence has also suggested improved survival in individuals with hypothyroidism who have a variety of tumors (reviewed in ref. 9). Enhanced response rates to radiation therapy in vitro and in vivo have been reported in hypothyroid cells (10, 11). Conversely, hyperthyroidism has sometimes been shown to be associated with an increased risk of various cancers in large population studies (12–14). An association between thyroid dysfunction and the risk of development of multiple myeloma has also been shown (15). Such an observation
does not mean that thyroid hormone is carcinogenic but does infer that the hormone supports tumor cell proliferation.

The cellular mechanisms whereby thyroid hormone stimulates cancer cell growth have recently been shown to be, at least in part, due to binding of thyroid hormone to a specific receptor site on the plasma membrane αvβ3 integrin (16, 17). The thyroid hormone receptor domain on αvβ3 is close to the RGD (Arg-Gly-Asp) recognition site (6), through which the integrin interacts with proteins of extracellular matrix (ECM). Via this receptor, thyroid hormone [1-thyroxine (T4) and 3,5,3′-triiodo-1-thyronine (T3)] activates the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) signal transduction pathways (6). Tetraiodothyroacetic acid (T3) and phosphoinositide 3-kinase (PI3K) signal pathways against product of Cell Signaling Technology. Monoclonal anti-Sigma-Aldrich. The MAPK/MEK inhibitor U0126 was a product of Cell Signaling Technology. Monoclonal anti-Sigma-Aldrich. The MAPK/MEK inhibitor U0126 was a product of Cell Signaling Technology.

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of the MAPK and PI3K cascades in many cancer cells expressed in a variety of cancer cell types (25) and the central role of the MAPK and PI3K cascades in many cancer cells (26, 27) can support concerns that thyroid hormone may promote tumor cell proliferation in the clinical setting (7, 18).

Materials and Methods

Cell lines
MM cell lines, RPMI 8226, and U266 (American Type Culture Collection) and ARP-1, ARK, and CAG cell lines (established at the Arkansas Cancer Research Center from bone marrow aspirates of patients with multiple myeloma) were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS/antibiotics and were treated with physiologic levels of T3 (100 nmol/L total hormone concentration; 100 pmol/L free hormone) and T4 (0.1 nmol/L total hormone concentration) as well as supraphysiologic concentrations.

Reagents and chemicals
T3, T4, tetra, and RGD/EG peptides were from Sigma-Aldrich. The MAPK/MEK inhibitor U0126 was a product of Cell Signaling Technology. Monoclonal antibodies against αv (clone P27) and β1 (P5D2) integrins and a β3 (clone H-96) polyclonal antibody were purchased from Santa Cruz Biotechnology. αvβ3 [LM609 unconjugated/phycoerythrin (PE)] monoclonal antibody was from Chemicon International. MAPK ERK1/2 antibodies were obtained from Cell Signaling Technology, and PCNA antibody was a product of Santa Cruz Biotechnology.

WST-1 cell viability/proliferation assay
WST-1 (Roche; 10% final concentration) was incubated with cells at 37°C for 2 hours and read with a microELISA reader at 440 nm.

Cell counts
Cells were collected in medium and counted with an ADVIA 2120 cell counter (Bayer).

Flow cytometry
Cell cycle. Cells were harvested, fixed and stained with DNA propidium iodide (PI; 50 μg/mL)/RNAse A (10 μg/mL; Sigma-Aldrich) and analyzed by fluorescence-activated cell sorting (FACS). For estimation of αvβ3 abundance in MM cells, the cells were harvested in RPMI 1640 and labeled with 10 μg/mL PE-αvβ3 antibody (LM609; Chemicon International) and analyzed by FACS (Navios Flow Cytometer; Beckman Coulter, Inc.).

Annexin/PI. Cells (10⁵) were incubated with 10 μL fluorescein isothiocyanate (FITC)-conjugated Annexin V/5 μL PI (MBL; Naka-Ku Ngoya) and analyzed by FACS as follows: Annexin V/PI−, living cells; Annexin V/PI+, early apoptosis; and Annexin V/PI−, late apoptosis.

Western blotting
Whole-cell lysates were separated on 10% to 12.5% polyacrylamide gels and analyzed by Western blotting with aforementioned primary antibodies and appropriate secondary horseradish peroxidase–conjugated antibody (Jackson ImmunoResearch Laboratories). Immunoreactive proteins were detected by chemiluminescence reagents (Pierce). α-Tubulin quantitation normalized the proteins loaded onto the membrane. Band intensity was visualized and quantified using LAS-3000 (FujiFilm).

Immunofluorescence
Cells were treated, fixed, and permeabilized with 0.1% Triton X-100 for 5 minutes at room temperature and then incubated with PE-αvβ3 antibody (LM609; Chemicon International) or rabbit anti-human pERK (Cell Signaling Technology). For pERK, a secondary FITC goat anti-rabbit IgG was used (Jackson ImmunoResearch Laboratories). Hoechst 33342 was used for nuclear staining (Sigma-Aldrich). Cells were visualized by a fluorescence microscope equipped with a camera (model IX71; Olympus) with a 20×/0.50 objective lens and Cell^A (version 3.1) Olympus software imaging.

RNA extraction
RNA was extracted using NucleoSpine RNA II Kit (Macherey-Nagel) according to the manufacturer’s instructions and eluted in 40 μL RNase-free water. RNA concentration and purity were measured with NanoDrop 1000 Spectrophotometer (Thermo Scientific).

cDNA synthesis
Two hundred nanograms of RNA was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer’s instructions.
Real-time PCR
cDNA was analyzed for apoptotic genes mRNA levels (apaf1, caspase 3, puma, bax, noxa, and fas) by 7500 Fast Real-Time PCR System (Applied Biosystems), using Applied Biosystems Fast Sybr Green Mix (list of primer sets are depicted in Supplementary Table S1). Results, normalized to β-actin, were calculated as fold change by the comparative threshold cycle method (2^−ΔΔCt) relative to control cells (i.e., controls are assigned a value of 1 by definition).

Statistical analysis
Experiments were analyzed using Student’s unpaired t tests for significance (P < 0.05).

Results
Thyroid hormones induce myeloma cell viability and proliferation
The sensitivity of myeloma cells to thyroid hormones was explored by addition of physiologic and supraphysiologic T3 and T4 concentrations (16) to several myeloma cell lines. Two adherent cell lines (CAG and ARK) and 2 nonadherent cell lines (U266 and ARP-1) were used. To adequately control for thyroid hormone concentrations in the experimental setting, cells were grown in the absence of serum for 48 hours. T3 was added at modestly supraphysiologic and pharmacologic total concentrations (1 and 100 nmol/L, respectively) and T4 at physiologic and pharmacologic total
concentrations (100 nmol/L and 1 μmol/L, respectively). Results (Fig. 1A) with physiologic and supraphysiologic thyroid hormone levels indicate a significant increase in cell viability (15%–50%). Specifically, T₃ at 1 nmol/L increased cell viability by 25% in CAG, U266, and ARK and by 42% in ARP-1 cells. Comparable results were obtained with a higher concentration of T₃ (100 nmol/L) in these cells. The addition of 100 nmol/L T₄ resulted in an increase in cell viability of 25% in CAG and ARP-1 cells, whereas a smaller, although significant, effect was seen in U266 and ARK cells. Using supraphysiologic T₄ concentrations induced an increase of 20% to 40% viability in U266, ARP-1, and ARK cells and only a 13% increase in CAG cells. We next evaluated the number of myeloma cells grown in serum-free conditions, followed by treatment with T₃ (1 nmol/L) and T₄ (100 nmol/L). Results (Fig. 1B) indicate a significant increase (30%–60%, \( P < 0.01 \)) in cell number in T₃- and T₄-treated cells, with the maximal effect observed in ARP-1 cells. Following several experiments in serum-free conditions, CAG cells exhibited the highest tolerability to these conditions in comparison with the other cell lines, as shown by a low background of nonviable cells (data not shown) and therefore most of the experiments were conducted in CAG cells.

Next, to verify the effects of thyroid hormones on myeloma cell proliferation, proliferating cell nuclear antigen (PCNA) protein, a central component in DNA synthesis and replication, was assessed by Western blotting. In accordance with the increased cell number, a parallel and significant increase in PCNA protein was observed in T₃- and T₄-treated CAG cells in a dose-dependent manner (Fig. 1C). The effect of T₄ on PCNA protein level was more potent and significant than T₃, with a maximal 2-fold effect at 100 nmol/L T₄ total concentration (\( P < 0.01 \)), which is equivalent to physiologic free hormone concentrations (16). T₃ induced PCNA expression by 20% to 50% (\( P < 0.01 \)) at supraphysiologic concentrations only (1 nmol/L–1 μmol/L). Comparable results were obtained in RPMI 8226 cells (data not shown). This increased viability and proliferation were accompanied by a reduced expression of proapoptotic genes in CAG cells (Fig. 1D). T₃ reduced the expression of apaf1 (26%, \( P < 0.004 \)), caspase 3 (26%, \( P < 0.01 \)), puma (29%, \( P < 0.01 \)), and noxa (28%, \( P < 0.01 \)) while not affecting the expression of bax and fas. T₃ treatment reduced solely the expression of apaf1 mRNA by 21% (\( P < 0.002 \)). No significant effect of T₃ on the expression of the additional genes examined was observed. Cell-cycle analysis revealed an average 30% reduction (\( P < 0.001 \)) in cell death (represented
Thyroid hormones induce MAPK activation in myeloma cells

We next determined whether the significant induction of viability and proliferation observed in T₃- and T₄-treated myeloma cells is MAPK-dependent, similar to results in other tumor types. The results are depicted in Figure 2 and show an increase in phosphorylated ERK protein (pERK) in CAG cells treated with T₃ (1 nmol/L) and T₄ (100 nmol/L). MAPK activation (2.5- to 3.5-fold) was observed as early as 10 minutes following T₃ (P < 0.003) or T₄ (P < 0.03) treatment and was blocked, expectedly, by U0126 (30 μmol/L), a potent MAPK inhibitor (Fig. 2A). The activation of MAPK was sustained for up to 24 hours (Fig. 2B) following T₃ (P < 0.01) or T₄ treatment (P < 0.05). T₃ increased pERK levels in CAG cells at 1 nmol/L concentration, after which a decline in ERK activity was observed. Activation of MAPK by T₄ occurred over a broader concentration range (10–1,000 nmol/L). Comparable results were obtained in the RPMI 8226 myeloma cell line (data not shown). This MAPK activation was evident by fluorescence microscopy, using FITC-labeled pERK antibody. Microscopy showed pERK protein in the cytoplasm of myeloma CAG cells following treatment with the 2 thyroid hormone analogues, as indicated by a fluorescent ring around the nucleus (Fig. 2C).

Induction of cell proliferation by T₃ and T₄ is MAPK dependent

Having shown that T₃ and T₄ induce both myeloma cell proliferation and MAPK activation, we next examined whether the proliferation observed was MAPK dependent. Serum-starved myeloma cells (CAG, ARK, and U266) were treated with the MAPK inhibitor U0126 (1 μmol/L) at a dose that was shown to partially inhibit MAPK (data not shown) while having a minimal effect on cell viability (Fig. 3A–B). After an overnight coincubation of U0126 with T₃ (1 nmol/L) or T₄ (100 nmol/L), cell viability and cell-cycle analyses were done. Figure 3A shows that in the presence of U0126, the increase in cell viability induced by T₃ was significantly blocked in CAG and U266 cells by 40% and 30%, respectively (P < 0.02). Similarly, in the same cell lines, the viability induced by T₄ was reversed by cotreatment with U0126 by 50% and 20%, respectively (P < 0.04). In ARK cells, a smaller effect (although significant) by U0126 on T₃- and T₄-induced cell viability was observed. Moreover, cell-cycle analyses of CAG, ARK, and U266 indicate that U0126 antagonized the cell death–lowering effect of T₃ (Fig. 3B) by 35%, 48%, and 9%, respectively (P < 0.01, not significant for U266). Similar results were obtained by T₄ cotreatment with U0126, in which the reduction in cell death was reversed by 79%, 41%, and 15%, respectively, (P < 0.04). Taken together, these experiments indicate that the cell viability produced by thyroid hormone is MAPK dependent.

αvβ3 abundance in myeloma cells is increased following thyroid hormone treatment and mediates MAPK activation

Because the αvβ3 integrin contains a receptor site for T₃ and T₄, and we have shown the effect of thyroid hormone on myeloma cells, we examined the possibility that T₃ and T₄ can affect αvβ3 integrin abundance. Myeloma cells (CAG, ARK, and ARP-1) were incubated with human monoclonal PE-labeled αvβ3 antibody (LM609) and quantified by FACS for the percentage of αvβ3-positive cells. Results (Fig. 4A) show a representative experiment in CAG cells, indicating low levels (0.75%) of αvβ3 abundance in the control cells grown in serum-free (and hormone-free) conditions and an increase of 14.5% and 11%, respectively, after exposure of cells to T₃ and T₄. Comparable results were obtained with ARK and ARP-1 cells (data not shown). The same experiment was evaluated in CAG cells by

![Graph](Image)

Figure 3. T₃ and T₄ induction of proliferation is MAPK dependent in myeloma cells. CAG, ARK, and U266 cells were seeded (80,000/96-well plate) in serum-free media for 48 hours. Cells were cotreated overnight with T₃ (1 nmol/L) and T₄ (100 nmol/L) in the presence or absence of the MAPK inhibitor U0126 (1 μmol/L) and measured for (A) cell viability (WST-1; *, P < 0.02 for T₃ and P < 0.04 for T₄) or (B) cell cycle (PI, FACS; *, P < 0.01 for T₃ and P < 0.04 for T₄). Results, presented as fold from control (DMSO), were repeated 2 to 3 times in triplicates.

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immunofluorescence, using human monoclonal PE-labeled αvβ3 antibody (LM609) and nuclear stain. Results revealed a similar increase in αvβ3 abundance in CAG cells, as indicated by the fluorescence around the nucleus (Fig. 4B). To verify identity of the integrin involved in T3 and T4 signaling via MAPK, we incubated the cells for 24 hours with 10 μg/mL blocking antibodies to different integrin subunits including αv, β3, and β1 monomers before the addition of T3 or T4. Results (Fig. 4C) show that blocking antibodies to αv and β3, but not β1, completely inhibited MAPK activation by T3 or T4. These observations confirm a role for αv and β3 integrins in T3 and T4 signaling in the tested cells. We further examined the effect of functional antibody to the αvβ3 dimer (LM609) on T3- and T4-induced MAPK activation. Figure 4D (T3) and Figure 4E (T4) show that thyroid hormone–induced MAPK activation was blocked in a concentration-dependent manner by preincubation with functional anti-αvβ3 antibody, supporting involvement of this integrin in hormone action in myeloma cells.

**RGD peptide blocks T4-induced MAPK activation and enhancement of myeloma cell viability**

The thyroid hormone–binding site on αvβ3 integrin is in close proximity to the RGD recognition site (6, 16) and we therefore determined whether increasing concentrations of RGD peptide added to CAG cells 16 hours prior to T3 or T4 treatment would block the hormone-induced activation of MAPK. The presence of RGD peptide blocked T4-induced MAPK activation (Fig. 5A, bottom) but did not affect activation of MAPK by T3 (Fig. 5A, top). This differential effect of RGD on the T4-binding domain is consistent with the subspecialization of the thyroid hormone receptor site on the integrin that has been previously described (7). As expected, control RGE peptide did not block T4- and T3-induced MAPK (Fig. 5B). Analysis of cell viability in the

**Figure 4.** Integrin αvβ3 is induced by T3 or T4 and mediates MAPK activation in myeloma cells. Serum-free CAG cells (250,000/24-well plate) were treated overnight with DMSO, T3 (1 nmol/L), or T4 (100 nmol/L), collected, and then measured for αvβ3 abundance by FACS (A) and immunofluorescence (B), using PE-conjugated anti-αvβ3 antibody (LM609). C, serum-starved CAG cells were treated overnight with 10 μg/mL functional antibodies against the monomer αv (P2W7) or β3 (H-96). β1 integrin antibody (P5D2) served as a negative control. T3 (1 nmol/L) and T4 (100 nmol/L) were added for 1 hour, and pERK and ERK levels were analyzed by Western blotting. Results were repeated 3 times. Next, serum-free CAG cells were treated overnight with functional antibody against the αvβ3 complex (LM609), 1 nmol/L T3 (D) and 100 nmol/L T4 (E) were added for 1 hour, and pERK and ERK levels were analyzed by Western blotting. Results, repeated twice, are depicted as fold from controls (DMSO). For T3 and T4, * P < 0.01 and P < 0.002, respectively.
same experiment showed that RGD treatment significantly inhibited T4-induced cell viability (Fig. 6A), whereas no effect of RGD on T3-induced cell viability was observed (Fig. 6B).

Tetrac blocks both T3- and T4-induced MAPK activation

Because tetrac, a T4 analogue with no agonist activity, was shown to selectively block T3/T4-binding site upon the αVβ3 integrin in various in vitro and in vivo cancer models (18), we further examined whether tetrac will act in MM cells in a similar manner. Serum-starved CAG cells were treated with increasing concentrations of tetrac for 30 minutes before the addition of T3 (1 nmol/L) or T4 (100 nmol/L) for 1-hour incubation. Western results show that while tetrac at 10 nmol/L concentration did not block T3-induced MAPK, at higher concentrations (100 nmol/L–100 μmol/L) ERK phosphorylation was significantly inhibited (Fig. 7A). In T4-treated cells, MAPK induction was blocked when 1 and 100 μmol/L tetrac concentrations were used (Fig. 7B). Taken together, these results indicate that in MM cells, similar to results in other tumor cell models, tetrac interrupts T3 and T4 binding to the receptor site upon the αVβ3 integrin.

Discussion

In this article, we show that thyroid hormone, acting via αVβ3 integrin and MAPK activation, induces cultured human myeloma cell proliferation. Thyroid hormones in physiologic (T4) or supraphysiologic (T3) concentrations induced myeloma cell proliferation, as evidenced by increased cell viability, cell number, and DNA synthesis. Another mechanism whereby thyroid hormones may increase cell viability can be related to the fact that thyroid hormones, particularly T4, are antiapoptotic. This antiapoptotic activity of thyroid hormone has only recently been reported in several cell and cancer models (28–30). In myeloma cells, we have shown a reduction of an array of proapoptotic genes, involved mainly in mitochondrial apoptosis.

Our findings are congruent with the recent demonstration that T3 and, with greater potency, T4 initiate cell proliferation in a number of tumor cell lines including breast cancer (31), hepatocarcinoma (32), somatotrophic tumor (33), thyroid cancer (28), sarcoma (34), and tumor-associated vascular cells via activation of a MAPK/ERK1/2-dependent pathway (16). This recently recognized role of the hormone is nongenomic in that it is initiated at a plasma
membrane receptor and does not involve the classical nuclear thyroid hormone receptors (6).

MAPK signaling seems to be critical for the proliferation of myeloma cells. It is frequently upregulated in MM cells (35), significantly contributing to MM cell survival and proliferation, as well as angiogenesis. Our investigation of thyroid hormone-stimulated proliferation in malignant plasma cells revealed rapid activation of MAPK by T3 at somewhat supraphysiologic concentrations and T4 at physiologic and supraphysiologic concentrations. Furthermore, the effects of T3 and T4 on MAPK activation last up to 24 hours after thyroid hormone treatment. This prolonged effect of thyroid hormones on MAPK activation has also been reported by Davis and colleagues (17), who observed a secondary increase in MAPK activation at 24 hours in glioma C6 cells exposed to thyroid hormone and concluded that this may in fact reflect a secondary increase in an autocrine growth factor such as FGF2. Another example of rapid and sustained MAPK activation is the initiation of angiogenesis characterized by a rapid induction of MAPK that is followed by prolonged expression of the molecule for at least 20 hours (36). The proliferative effects of thyroid hormone in myeloma cells were completely blocked by a specific MAPK inhibitor, U0126, indicating that these effects are MAPK dependent. The efficacy of U0126 was consistently higher in CAG cells than in the other cell lines used; however, because of limited data on the status of RAS mutation and MAPK activity in these cells, this effect is currently under investigation.

MAPK activation by thyroid hormones is independent of the classical nuclear thyroid hormone receptor, as the induction is very rapid (37) and does not require nuclear hormone receptor, as shown by induction of MAPK activity in HeLa and CV-1 cells that lack the classical nuclear thyroid hormone receptors (37). Such observations generated the hypothesis that this thyroid hormone-induced MAPK activity may be initiated outside the cell nucleus by a nonclassical thyroid hormone receptor. Davis and colleagues established that the activation of MAPK by thyroid hormone is initiated by binding of T3 and, with a higher affinity, T4 to the αvβ3 integrin (16) located on the plasma membrane of dividing blood vessel and tumor cells. This thyroid hormone signal transduction pathway has been reported in endothelial cells (19), fibroblasts (16), and platelets (38), as well as in a chick chorioallantoic membrane model of angiogenesis (39). The same pathway has been documented in a variety of cancer cells (6, 7, 17) but have never been studied in hematologic malignancies such as myeloma.

In the current study, we show that T3 and T4 upregulate the cell surface abundance of αvβ3 on myeloma cell lines.
This may be of importance because previous studies of αvβ3 integrin in multiple myeloma have emphasized its role in tumor proliferation and aggressiveness. Interestingly, αvβ3 integrin has been shown to be involved in myeloma cell adherence to vitronectin and fibronectin (40). It was further shown that plasma cells interacting with vitronectin and fibronectin via the αvβ3 integrin recruit MAPK/ERK1/2 and enhance proliferation and matrix metalloproteinase (MMP)-2 and MMP-9, and urokinase-type plasminogen activator secretion (41). A recent report also emphasized a role for the αvβ3 integrin in myeloma-related bone disease that is MAPK dependent (42). We show here that the αvβ3 integrin is involved in T3- and T4-induced MAPK activation and enhancement of proliferation in cultured myeloma cells.

The αvβ3 integrin binds to several ligands. Many integrins, including αvβ3, recognize the ligand tripeptide Arg-Gly-Asp (RGD; ref. 25), through which they interact with proteins of the ECM. Because the thyroid hormone receptor αvβ3 integrin is in close proximity to the RGD recognition site (6), the RGD peptide may be tested in the experimental setting as a probe for participation of the hormone receptor in cellular actions of thyroid hormone. However, misimpressions may be obtained from the use of RGD peptides as probes because the peptides alter inconsistently or only selectively inhibit actions of thyroid hormone that begin at the cell surface receptor (7). We show here that RGD peptide inhibited actions of T4 in myeloma cells but not the action of T3. These results are consistent with the subspecialization of the thyroid hormone receptor on the integrin that contains distinctive binding sites for T3 and T4 (6, 16). We also studied the action of tetrac in this work. Tetrac is a deaminated derivative of T4, which was previously shown to selectively inhibit the binding of thyroid hormones to the receptor on integrin αvβ3 (18). This effect of tetrac was examined in several models of human tumors including glioma (7, 17, 30), thyroid cancer (28), neuroblastoma, osteosarcoma, breast cancer (20), and renal cell carcinoma (21) but was never studied in myeloma.

The fact that in this work, tetrac effectively blocked induction of MAPK by T3 and T4 in myeloma cell model indicates that this action by the hormones was apparently initiated through αvβ3 integrin at the cell surface.

In addition, it is interesting to note that thyroid hormone promotes angiogenesis (42, 44), which has been shown to be of pathogenic importance in myeloma, as shown by the antiangiogenic effects of the effective anti-myeloma therapies thalidomide and lenalidomide (3). Thus, tetrac, which inhibits thyroid hormone signaling in myeloma cells, may share these antiangiogenic properties and thus be a useful adjunct to therapy in this disease.

In summary, the present work provides a cellular mechanism whereby thyroid hormones can be considered novel growth factors for myeloma cells via MAPK activation. Because the growth-promoting activity of thyroid hormone is initiated by integrin αvβ3 binding, the effects of antagonizing this molecule in myeloma should be examined.

Disclosure of Potential Conflicts of Interest

There are no potential conflicts of interest to disclose.

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