Identification and Functions of the Plasma Membrane Receptor for Thyroid Hormone Analogues

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Abstract: Integrin $\alpha v\beta 3$ is a heterodimeric structural protein of the plasma membrane that bears a cell surface receptor for thyroid hormone. The functions of this receptor are distinct from those of the classical nuclear receptor (TR) for thyroid hormone. The integrin is expressed primarily by cancer cells, dividing endothelial and vascular smooth muscle cells, and osteoclasts. The hormone receptor on $\alpha v\beta 3$ enables L-thyroxine (T_4) and 3, 5, 3'-triiodo-L-thyronine (T_3) to stimulate cancer cell proliferation and angiogenesis and to regulate the activity of certain membrane ion pumps. Bound to the receptor, the hormone ligand also stimulates protein trafficking within the cell. A deaminated derivative of T_4, tetraiodothyroacetic acid (tetrac), blocks binding and actions of T_4 and T_3 at the receptor on $\alpha v\beta 3$; tetrac also has anti-proliferative actions at the integrin thyroid hormone receptor beyond the effects of antagonizing actions of agonist thyroid hormone analogues at the receptor. The structure-activity relationships of hormone analogues at the receptor have been computer-modeled and indicate that the receptor includes a site that binds T_3 and a site that binds both T_4 and T_3. Mathematical modeling of the kinetics of hormone-binding also suggests the existence of two sites. Cell proliferation is modulated from the T_4/T_3 site. Tetrac has been re-formulated as a nanoparticle (nanotetrac) that acts exclusively at the $\alpha v\beta 3$ receptor and does not enter cells. Nanotetrac disrupts expression of genes in multiple cancer cell survival pathways. The tetrac formulations block human cancer cell proliferation *in vitro* and in tumor xenografts. Nanotetrac and tetrac inhibit the pro-angiogenic actions *in vitro* of vascular endothelial growth factor, basic fibroblast factor, and other growth factors. Thus, the receptor described on integrin $\alpha v\beta 3$ for T_4 and T_3, the function of which is materially affected by tetrac and nanotetrac, provides insight into tumor cell biology and vascular biology. [*Discovery Medicine* 11(59):337-347, April 2011]

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Thyroid hormone has pro-angiogenic activity that was shown in experimental animal models more than 20 years ago (Chilian et al., 1985). The pro-angiogenic effect of the hormone — L-thyroxine (T_4) and 3, 5, 3'-triiodo-L-thyronine (T_3) (Figure 1A) — was confirmed in the chick chorioallantoic membrane (CAM) system in 2004 (Davis et al., 2004) and was mimicked by T_4 covalently bonded to agarose, a formulation of the hormone that does not gain access to the cell interior and to classical nuclear thyroid hormone receptor (TR) proteins. The angiogenic action of T_4 was blocked by PD98059, an inhibitor of the mitogen-activated protein kinase (MAPK) signal transduction pathway, and protein kinase C was also implicated in hormone action (Davis et al., 2004; 2011). Basic fibroblast growth factor (bFGF, FGF2) gene expression was a downstream

consequence of hormone action, as was cellular release of pro-angiogenic FGF2 (Davis et al., 2004). Thus, thyroid hormone was shown to be angiogenic by a novel mechanism that began at the cell surface, rather than in the cell nucleus, and that required activation of MAPK and specific gene expression.

Identification of the Cell Surface Receptor for Thyroid Hormone

An obvious question raised by these angiogenic experiments was the identity of the receptor for thyroid hor-
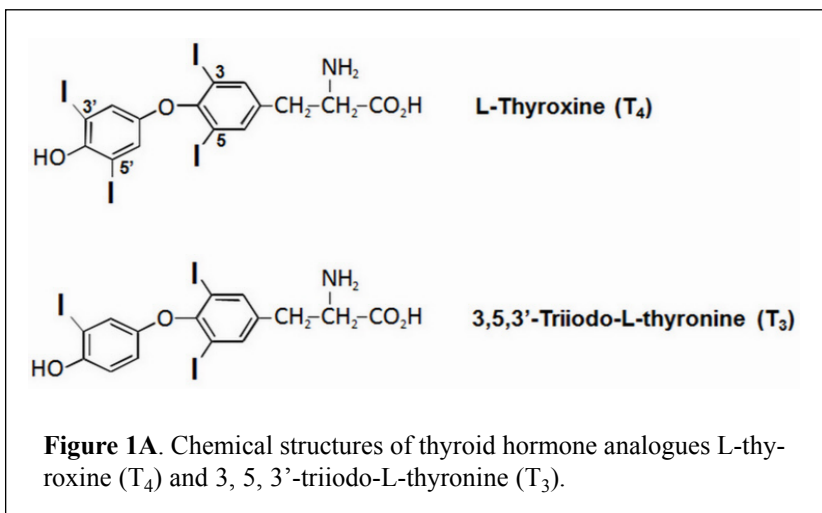

Figure 1A. Chemical structures of thyroid hormone analogues L-thyroxine (T_4) and 3, 5, 3'-triiodo-L-thyronine (T_3).
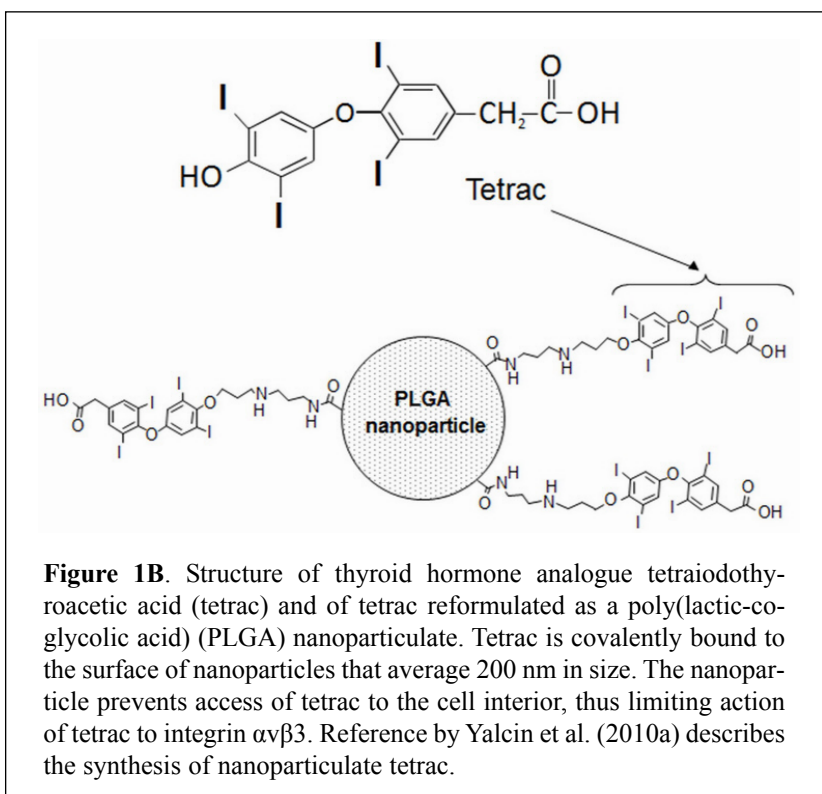

Figure 1B. Structure of thyroid hormone analogue tetraiodothyroacetic acid (tetrac) and of tetrac reformulated as a poly(lactic-co-glycolic acid) (PLGA) nanoparticulate. Tetrac is covalently bound to the surface of nanoparticles that average 200 nm in size. The nanoparticle prevents access of tetrac to the cell interior, thus limiting action of tetrac to integrin αvβ3. Reference by Yalcin et al. (2010a) describes the synthesis of nanoparticulate tetrac.

mone. The CAM model responds to a variety of blood vessel growth factors, such as vascular endothelial growth factor (VEGF) and bFGF. The model involves crosstalk between the specific plasma membrane receptors for growth factors — VEGFR and bFGFR — and a structural heterodimeric protein of the cell membrane, integrin αvβ3 (Sahni and Francis, 2004; De et al., 2005). The integrin has multiple functions, including recognizing specific extracellular matrix (ECM) proteins that are important to guiding cell movement (Plow et al., 2000). Among several integrins, αvβ3 bears an Arg-Gly-Asp (RGD) recognition site that has a permissive function in angiogenesis, verifying the RGD sequence that is found in multiple vascular growth factors.

Against this background, it was imperative to probe the pro-angiogenic action of agonist iodothyronines in the CAM model of angiogenesis with RGD peptide and with monoclonal integrin αvβ3 antibody (Bergh et al., 2005; Davis et al., 2011). Both agents inhibited thyroid hormone-induced angiogenesis, indicating not only that the hormone receptor was on αvβ3, but that the binding site for the hormone was either at or near the RGD recognition site or was obscured by allosteric change in the integrin that might occur with binding of the RGD peptide. Specific, high affinity-binding of radiolabeled hormone to the purified integrin was demonstrated. To complete the identification and function of the receptor, we also showed that siRNA knockdown of this specific integrin eliminated transduction of the hormone signal into MAPK activation.

In a series of studies published a decade or more before integrin αvβ3 was shown to bear a thyroid hormone receptor, we had shown that tetraiodothyroacetic acid (tetrac), a deaminated derivative of T_4 (Figure 1B), inhibited certain thyroid hormone actions, the onset of which was sufficiently rapid to exclude participation of TR and gene expression or transcription (Mylotte et al., 1985; Lin et al., 1998; 1999; Davis et al., 2000). When we tested tetrac for activity at the integrin αvβ3 receptor for T_4 and T_3, we found not surprisingly that tetrac blocked binding and

actions of T_4 and T_3 (Bergh et al., 2005; Davis et al., 2006). Acting alone at the receptor in the absence of T_4 and T_3, tetrac lacked agonist activity and did not suppress basal angiogenesis in the CAM (Davis et al., 2004; Bergh et al., 2005; Davis et al., 2006). Remarkably, however, tetrac inhibited the angiogenic activities of VEGF and bFGF added to the assay system (Mousa et al., 2008). This set of observations indicated that tetrac was more than inhibitor of agonist thyroid hormone analogue-binding to $\alpha v\beta 3$ and affected crosstalk between the integrin, presumably via the RGD recognition site, and nearby VEGF or bFGF receptors (De et al., 2005; Mahabeleshwar et al., 2007; Sahni and Francis, 2004; Somanath et al., 2009). We have subsequently described complex, coherent actions of tetrac via the integrin thyroid hormone receptor on gene expression relevant to cancer cell survival pathways (Cheng et al., 2010; Davis et al., 2011) (see below). Tetrac has also been re-formulated as a nanoparticle (Figure 1B) that does not gain access to the interior of cells and acts exclusively at the plasma membrane thyroid hormone receptor on integrin $\alpha v\beta 3$.

Thus, a small number of experiments in the CAM system and in the human dermal microvascular endothelial cell (HDMEC) microtubule assay system permitted the identification of a cell surface receptor for thyroid hormone that had previously escaped recognition and provided a possible mechanistic basis for the description of thyroid hormone-induced angiogenesis in animal models.

It may be useful to point out that the induction by thyroid hormone of bone demineralization in the rat had been shown by Hoffman et al. (2002) to be inhibited by an RGD peptide 15 years before integrin $\alpha v\beta 3$ was found to bear an iodothyronine receptor. Intriguing today is the knowledge that expression of integrin $\alpha v\beta 3$ is particularly generous on osteoclasts and we can propose that thyroid hormone-induced bone demineralization is driven by stimulation of osteoclastic activity through an integrin that bears a cell membrane receptor for the hormone.

Further, the conversion of soluble actin to fibrous actin by T_4 that was described in the 1990's by Farwell, Leonard and co-workers in glial cells had also been found to be susceptible to inhibition by an RGD peptide (Farwell et al., 1995). Thus, these processes of thyroid hormone-induced and RGD peptide-inhibitable bone resorption and cytoskeletal maturation in retrospect inferred the existence of an integrin receptor for the hormone. However, there was little basis to suspect at the time the experiments were conducted that these

complex cellular systems required a plasma membrane receptor for T_4 or T_3.

Because the principal ligands of the integrins are proteins, the concept that a receptor for a small molecule like thyroid hormone existed on integrin $\alpha v\beta 3$ was surprising, if not heretical. Subsequently, however, it has been appreciated that receptors for the stilbene, resveratrol (Lin et al., 2006), and for dihydrotestosterone (DHT) (Lin et al., 2009) are present on $\alpha v\beta 3$. The actions of resveratrol and of DHT also are blocked by an RGD peptide and thus the receptors are proximate to the RGD recognition site or are obscured by allosteric changes elsewhere in $\alpha v\beta 3$ that result from binding of an RGD peptide.

Structural Requirements for Binding and Activity of Thyroid Hormone Analogues at the $\alpha v\beta 3$ Receptor

Integrins are α/β heterodimeric type I membrane receptors that mediate divalent cation-dependent interactions with components of the extracellular environment (Plow et al., 2000; Hynes, 2002). Structural data have been reported for the extracellular portion of the $\alpha v\beta 3$ integrin in its unliganded state and in complex with a cyclic RGD peptide (Xiong et al., 2001; 2002), More recently, the structure of the ectodomain of $\alpha v\beta 3$ (1TM-$\alpha v\beta 3$) has been determined (Xiong et al., 2009). These data reveal that the $\alpha v\beta 3$ integrin heterodimer consists
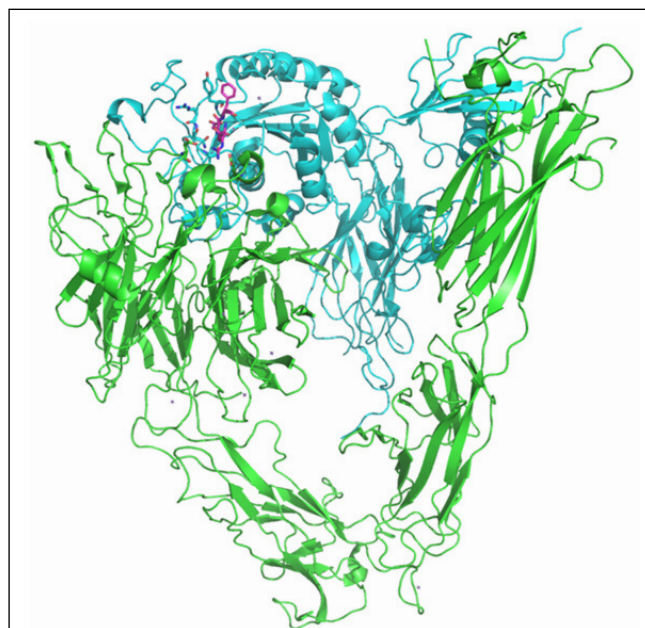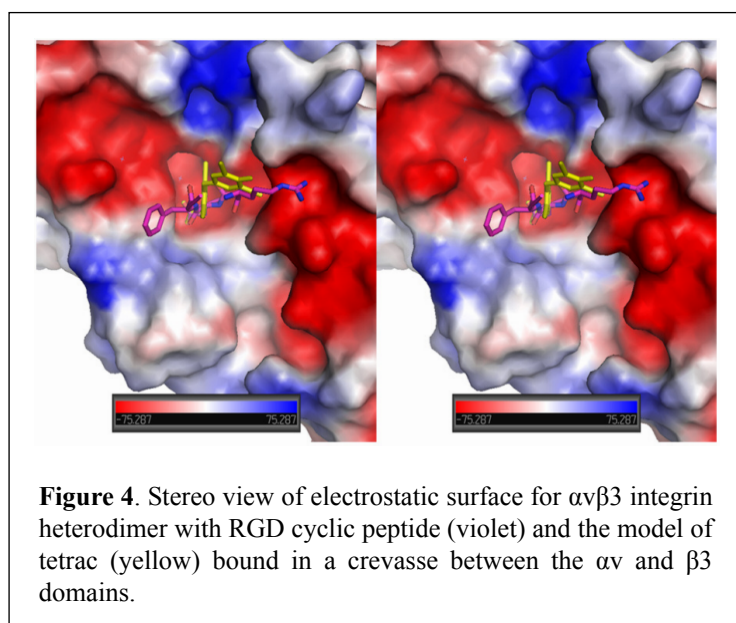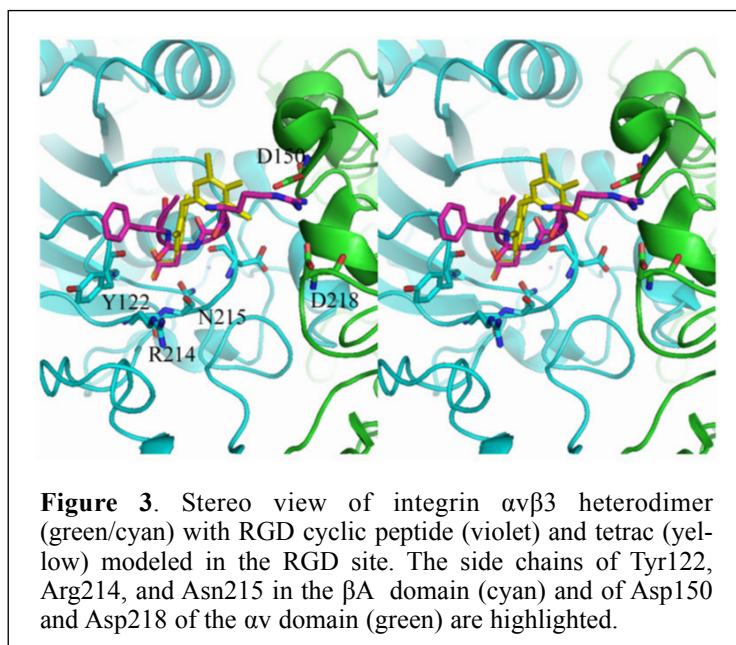

Figure 2. Crystal structure of $\alpha v\beta 3$ (1LSG) with αv (green) and $\beta 3$ (cyan) (Xiong et al., 2002). Also shown is the RGD cyclic peptide (violet).

of 12 domains assembled into an ovoid "head" and two "tails" (Figure 2). The head consists of a 7-bladed β-propeller from the αv and βA domains (Xiong et al., 2001; 2002). The β3 portion of the integrin head is composed of the βA and hybrid domains. The shape of αvβ3 integrin places the 4-domain αv subunit and the 8-domain β3 subunit in a bent profile with an approximate 135-degree angle. Overall, the αvβ3 integrin resembles the structure observed for G-proteins (Xiong et al., 2003). The RGD cyclic peptide presenting the Arg-Gly-Asp sequence inserts into a crevice between the propeller and the βA domains on the integrin head

(Xiong et al., 2002). These data show that the RGD peptide Asp carboxylate oxygens interact with the backbone of Tyr122 and Asn215 and the side chain of Arg214, while the Arg side chain inserts into a narrow groove at the top of the propeller domain and form salt bridges to Asp150 and Asp218 (Figure 3). Compared with the apo αvβ3 integrin structure, there are small conformational changes primarily affecting the βA domain. These changes also affect the metal (Ca^{2+} or Mn^{2+}) binding site in this region.

In order to understand how thyroid hormone analogues interact with integrin, models of their potential interactions in αvβ3 integrin were carried out (Cody et al., 2007a). In the case of tetrac-binding, the acetic acid moiety was mapped to that of Asp in the RGD cyclic peptide (Figure 3). In this model, most of the hormone interactions are with the βA domain of the integrin. Similar models were made with T_4 and T_3, and with the stilbene, resveratrol, and estradiol. These modeling data indicated that there was sufficient space in the cavity for the hormones to bind (Figure 4). In the case of the more planar steroid-like molecules, the modeling data indicate a second, smaller binding pocket present near the RGD recognition site as illustrated in the electrostatic surface computed for the αvβ3 integrin bound with an RGD peptide (Figure 4) (Cody et al., 2007a).

Figure 3. Stereo view of integrin αvβ3 heterodimer (green/cyan) with RGD cyclic peptide (violet) and tetrac (yellow) modeled in the RGD site. The side chains of Tyr122, Arg214, and Asn215 in the βA domain (cyan) and of Asp150 and Asp218 of the αv domain (green) are highlighted.

To more quantitatively model potential interactions of αvβ3 integrin with thyroid hormone (T_4, T_3) and the analogue tetrac, molecular docking experiments using quantum chemical calculations (QM/MM) were carried out in the presence of Ca^{2+} or Mg^{2+} near the RGD recognition site (Cody et al., 2007b), as observed in their crystal structures (Xiong et al., 2002). These computational results indicated a strong electronic contribution to the binding energies by the presence of Ca^{2+} or Mg^{2+} near the active site that impacts ligand-binding. These calculations also showed that there were significant differences in the binding orientation of similar ligands, as illustrated by the two different orientations for the binding of T_3 shown in Figure 5. These computational results showed that the preferential binding of T_4 and tetrac to the RGD recognition site was similar. Computational data reveals that the phenolic ring of T_3 (T_3-Ca^{2+}) occupies an alternate binding pocket near the RGD peptide site (Figure 6). These data support the binding kinetics data

Figure 4. Stereo view of electrostatic surface for αvβ3 integrin heterodimer with RGD cyclic peptide (violet) and the model of tetrac (yellow) bound in a crevasse between the αv and β3 domains.
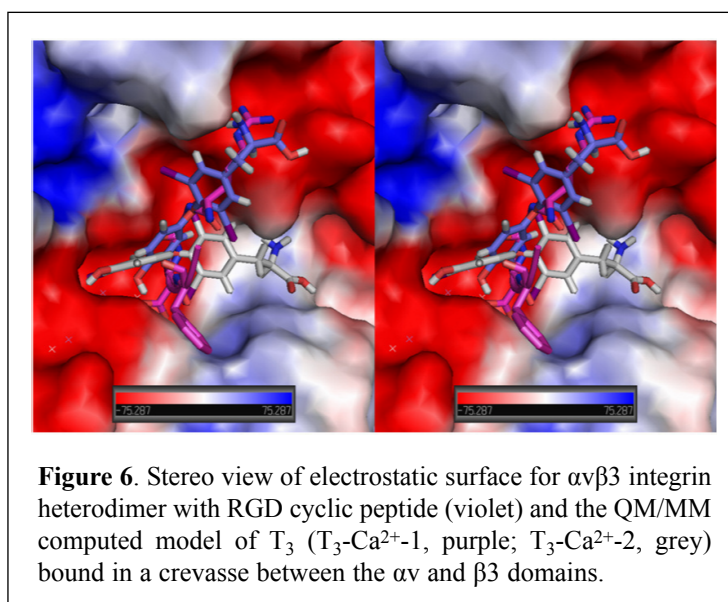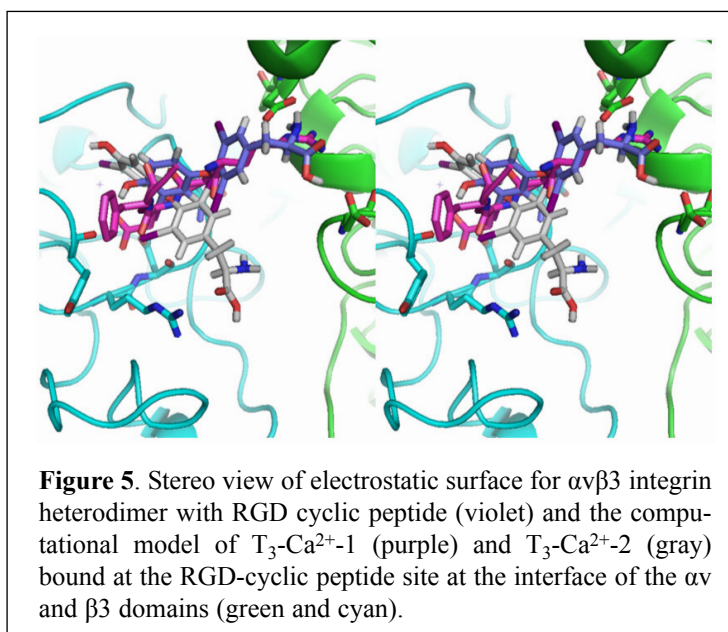
that are consistent with the presence of two discrete binding sites for T_3 that control distinct downstream signal transduction pathways (Davis et al., 2011).

Cells That Express Integrin αvβ3 and the Cell Surface Receptor for Thyroid Hormone

The CAM results that originally disclosed the existence of the thyroid hormone receptor on αvβ3 were consistent with the already appreciated presence of this integrin in abundance on dividing blood vessel cells, that is, endothelial cells and vascular smooth muscle cells. In physiological concentrations, T_4 and T_3 both have pro-angiogenic activity. In addition, cancer cells generously express integrin αvβ3. In a variety of human cancer cell lines studied *in vitro,* T_4 and T_3 have been shown to stimulate cell proliferation via the integrin receptor for thyroid hormone (Davis et al., 2006; Lin et al., 2007; 2009b). In physiological concentrations, T_4 is active in the cancer cell proliferation system, whereas T_3 is less potent *in vitro* as a stimulator of cancer cell division. These observations are particularly worrisome because of a prevailing clinical view that normal levels of thyroid hormone in the cells of the intact organism support essential, healthy gene expression and metabolism. Studies by Goodman and colleagues 30 years ago and by Borek and Guernsey and their co-workers that described support by thyroid hormone of tumor growth have not been widely cited or widely accepted (Goodman et al., 1980; Borek et al., 1983; Guernsey et al., 1981). The possibility that induction of hypothyroidism may retard tumor progression, e.g., of glioblastoma (Hercbergs et al., 2003) or of renal cell carcinoma (Riesenbeck et al., 2010; Baldazzi et al., 2010; Schmidinger et al., 2011) has been explored and would appear to be an important issue to consider further. The molecular basis of the actions of thyroid hormone on certain tumor cells *in vitro* and on tumor cell xenografts is reviewed in a later section.

Thus, generous expression of integrin αvβ3 occurs on tumor cells and dividing blood vessel cells (Dijkgraaf et al., 2009; Dimastromatteo et al., 2010; Yoshimoto et al., 2008) and, as noted above, on osteoclasts (Nakamura et al., 2007). This integrin is also found in smaller quantities on platelets where T_4, but not T_3 or other agonist thyroid hormone analogues, can induce platelet aggregation via the iodothyronine receptor (Mousa et al., 2010). αvβ3 is present on neurons,

where Yonkers and Ribera (2008) have shown that T_4 can affect excitability, increasing sodium current (INa) in the zebrafish sensory neuron, raising the possibility that physiologic concentrations of thyroid hormone do contribute to the basal state of ion transport in excitable cells. Thyroid hormone enhances the motility of human white blood cells *in vitro,* where the cue is an ECM protein, and this action is inhibited by an RGD peptide and by tetraiodothyroacetic acid (SA Mousa, unpublished observations). On hepatic stellate cells, integrin αvβ3 appears to mediate a pro-fibrotic action of thyroid hormone in the liver, as shown by Zvibel (2010). The activity of the Na^+/H^+ exchanger (NHE1) in mouse myoblasts has been reported by Incerpi and co-workers

Figure 5. Stereo view of electrostatic surface for αvβ3 integrin heterodimer with RGD cyclic peptide (violet) and the computational model of T_3-Ca^{2+}-1 (purple) and T_3-Ca^{2+}-2 (gray) bound at the RGD-cyclic peptide site at the interface of the αv and β3 domains (green and cyan).

Figure 6. Stereo view of electrostatic surface for αvβ3 integrin heterodimer with RGD cyclic peptide (violet) and the QM/MM computed model of T_3 (T_3-Ca^{2+}-1, purple; T_3-Ca^{2+}-2, grey) bound in a crevasse between the αv and β3 domains.

to be stimulated by T_3 and inhibitable by tetrac (D'Arezzo et al., 2004). Thus, the integrin, albeit in smaller amounts than in cancer cells or dividing blood vessel cells, is the site of initiation of several recently recognized actions of iodothyronines.

Although more than 20 integrins have been described and a number of them have RGD recognition sites (Plow et al., 2000), our own extraction of plasma membrane proteins and a search for additional hormone-binding sites have revealed that only the αvβ3 moiety is capable of binding thyroid hormone analogues. The observation that an integrin can have small molecule ligands was somewhat surprising, given the extensive literature on large molecule-integrin interactions and the critical role played by the protein in interpreting the ECM (Plow et al., 2000). Since the identification of a thyroid hormone receptor on integrin αvβ3, however, cases have been made for the existence of other small molecule receptor sites on the integrin. The receptor for resveratrol on αvβ3 can mediate anti-cancer actions of this agent on cancer cells, such as p53-dependent apoptosis (Tang et al., 2006; Lin et al., 2011a). The receptor for DHT on αvβ3 is responsible for the proliferative effect of the androgen on ERα-negative human breast cancer cells (Lin et al., 2009a). RGD peptides interfere with the effects of resveratrol and of DHT. The actions of the latter agents are not affected by tetrac, indicating that while these receptors all appear to be near the RGD recognition site of αvβ3, the function of the sites are discrete.

Regulation of Gene Expression from the Plasma Membrane Thyroid Hormone Receptor on αvβ3

αvβ3 and other integrins are highly plastic molecules that can alter their molecular postures with the binding of specific protein ligands and in response to Ca^{2+} and Mn^{2+} (Plow et al., 2000). The binding of such large molecule ligands by αvβ3 has been shown to result in expression of certain genes (Lossner et al., 2009; Mi et al., 2009; Rusnati et al., 1997; Zhang et al., 2003). Despite such observations, it was surprising to find that the thyroid hormone analogue, tetrac, and its nanoparticulate formulation, nanotetrac, had broad effects on tumor cell gene expression that coherently worked to disable tumor cell survival pathways (Glinskii et al., 2009; Yalcin et al., 2010a). The nanoparticulate formulation consists of tetrac covalently bound to the surface of the particle, thus preventing access of tetrac to the cell interior where the agent is, undesirably, a low-grade thyromimetic, rather than the antagonist that it is at the αvβ3 hormone receptor site.

A summary of cancer cell genes affected by tetrac is presented in Table 1. When first observed, the spectrum of genes affected by tetrac was surprising because the effects of the thyroid hormone analogue T_3 on gene expression have been extensively described (Feng et al., 2000; Miller et al., 2004; Moeller et al., 2004) and are genomic, that is, they require the interaction of T_3 with classical TR isoforms in the cell nucleus. The identification of the αvβ3 receptor for iodothyronines disclosed that thyroid hormone can affect gene transcription without entering the cell. Thus, hormone analogues can have effects on gene expression that are invoked nongenomically.

The action of thyroid hormone ana-

Table 1. Modulation of Gene Expression by Nanoparticulate Tetrac and Tetrac in Human Breast Cancer Cells (MDA-MB-231)

Gene Abbreviation	Gene Full Name	Direction of Change
CDKN2C	Cyclin-dependent kinase inhibitor	↑
Cyclins	Cell cycle regulators	↓
XIAP	X-linked inhibitor of apoptosis protein	↓
MCL1	Myeloid cell leukemia-1 (factor), prevents MOMP*	↓
CASP2	Caspase 2, promotes apoptosis	↑
BCL2L14	B cell lymphoma-2, promotes apoptosis	↑
THBS1	Thrombospondin, inhibits angiogenesis	↑
CXCL10	Anti-endothelial cell chemokine	↑
EDN1	Endothelin-1	↑
CTNNA1	Catenin (Wnt oncogene pathway)	↓
CTNNA2	Catenin (Wnt oncogene pathway)	↓
CBY1	Catenin inhibitor	↑
NR1D1	Nuclear receptor Rev-erbα, orphan nuclear receptor, regulator of circadian rhythm	↓
* MOMP, mitochondrial outer membrane permeabilization.		

logues on gene expression initiated at αvβ3 has been primarily studied via the use of tetrac. The actions of analogues T_3 or T_4 on gene transcription that are definitively initiated at αvβ3 have not been systematically surveyed. T_3 is capable of stimulating expression of several genes by nongenomic mechanisms that either begin in cytoplasm or at the cell membrane. For example, T_3 was shown to enhance the expression of hypoxia-inducible factor-1α (*HIF-1α*) nongenomically (Moeller et al., 2005) and we found subsequently that this effect can involve αvβ3 (Lin et al., 2009b). Expression of sarcoplasmic reticulum Ca^{2+}-ATPase (*SERCA*) and α- and β-myosin heavy chain (*MHC*) in cardiac myocytes can also be nongenomically initiated by T_3 (Iordanidou et al., 2010) via kinase activities regulated from the cell surface, but the possible origin of the hormone signal at the αvβ3 receptor has not yet been studied in this model.

In analyzing the effects of tetrac and nanotetrac on tumor cell gene expression (Glinskii et al., 2009), a number of conclusions may be drawn. First, the actions of these agents at the level of the genome are pro-apoptotic or suppress the anti-apoptotic (tumor cell defense) process. Second, factors important to the conduct of cell division, such as cyclins, are downregulated. Third, the Wnt/β-catenin-dependent gene activation important to tumor cell survival is decreased by expression of the *CBY1* gene. The β-catenin pathway is of current interest as a target in oncology. Fourth, although there is extensive overlap of the patterns or signatures of gene expression of the two agents, there are significant differences. For example, the nanoparticulate form of tetrac can stimulate the transcription of thrombospondin 1(*TBSP1*) by tumor cells and can suppress expression of epidermal growth factor receptor (*EGFR*). Unmodified tetrac lacks these effects. Both of these actions of nanotetrac are inimical to tumor cell survival, in that the TBSP1 protein is anti-angiogenic and the epidermal growth factor protein is an important supporter of tumor cell division and is pro-angiogenic. The fact that nanotetrac and tetrac differ in their effects on gene expression suggests that the fit into the receptor groove of tetrac covalently bound to the surface of the nanoparticle is different from the fit of unmodified tetrac. Initiated at the plasma membrane integrin receptor for thyroid hormone analogues on cancer cells and endothelial cells, the actions of nanotetrac and tetrac on gene expression are selective and coherent. The actions include stimulation of apoptosis, suppression of anti-apoptotic defense pathways, and inhibition of angiogenesis.

Such effects of tetrac and nanotetrac have encouraged testing of the drugs against a variety of tumor xenografts. In xenografts of human breast (Rebbaa et al., 2008), kidney (Yalcin et al., 2009), follicular thyroid cancer (Yalcin et al., 2010a), medullary thyroid cancer cells (Yalcin et al., 2010b), lung (M Yalcin and SA Mousa, unpublished observations), and pancreas (M Yalcin and SA Mousa, unpublished observations), the agents are anti-proliferative at the level of tumor cells and significantly anti-angiogenic.

There is no significant action of tetrac on the *in vitro* proliferation of immortalized nonmalignant cells, such as human 293T and monkey CV-1 kidney cells (Lin et al., 2011c). Actions of the agent on gene expression in these cells have not yet been studied, but, given the absence of an effect of tetrac on proliferation, the action of the hormone analogue on gene expression may be modest.

Other Cellular Effects of Thyroid Hormone Directed from the Cell Surface Receptor on Integrin αvβ3

Intracellular protein trafficking can be regulated from the thyroid hormone receptor on αvβ3. For example, nuclear import of TRβ1 that is resident in cytoplasm is stimulated by thyroid hormone; the hormone-induced TR trafficking requires phosphorylation of TR by MAPK (Davis et al., 2000; Lin et al., 2003; Davis et al., 2008) and uptake of TR by the nucleus probably occurs with the receptor in a complex with the kinase in thyroid hormone-treated cells (Cao et al., 2009; Davis et al., 2008). Estrogen receptor-α (ERα) is similarly caused to move into the nucleus from the cytoplasm by αvβ3-mediated thyroid hormone action. Signal transducer and activator of transcription-1α (STAT1α) is involved in the conversion of cytokine signals into cellular actions and its nuclear uptake is also promoted nongenomically by T_4 via the integrin. The potentiation by thyroid hormone of the STAT1α-dependent action of interferon-γ on HLA-DR expression may be a function of the effect of the hormone on the trafficking of STAT1α (Lin et al., 1998). Like TR, ERα (Tang et al., 2004) and STAT1α (Lin et al., 1998) are both subject to phosphorylation of specific serines by MAPK (extracellular regulated protein kinase 1/2, ERK1/2). Other proteins whose shuttling into the nucleus occurs under the direction of thyroid hormone include the oncogene suppressor protein, p53 (Shih et al., 2001), STAT3 (Lin et al., 1999), Trip230 (Chen et al., 1999), and the internalized αv monomer (see below). The insertion of Na, K-ATPase protein into the lung alveolar cell plasma membrane is induced nongenomically by thyroid hor-

mone and involves PI3K (Bhargava et al., 2007), a signal transducing enzyme that can be controlled from the cell surface by T_3 at the αvβ3 receptor for the hormone (Lin et al., 2009).

It is clear that thyroid hormone has "outside-in" effects on the integrin to which it binds, activating the MAPK and PI3K cascades inside cells to modify a spectrum of cellular actions. It is now appreciated that the binding of thyroid hormone influences the internalization of integrin αvβ3. There are novel features to this process. The αv and β3 monomers separate inside the thyroid hormone-treated cell and β3 is limited to cytoplasm, whereas nuclear import of αv occurs (Lin et al., 2007). Within the nucleus of cells exposed to iodothyronines, αv is found in association with MAPK and with specific nucleoproteins, such as p300 and other coactivators. Indeed, αv may be clustered with other nucleoproteins at the cyclooxygenase-2 (*COX-2*) gene, raising the remarkable possibility that the αv monomer of a classical membrane protein may directly influence gene transcription. This observation raises the possibility that increased expression of *COX-2* as a marker of cancer cell aggressiveness may be permissively supported by agonist thyroid hormone analogues. It is not known whether the internalization of the αvβ3 heterodimer that is regulated by thyroid hormone may include protein ligands bound to the integrin.

There is another and very separate link between thyroid hormone action and COX-2 that involves the COX-2 protein. We have shown that agonist thyroid hormones are anti-apoptotic in tumor cells and that tetrac is pro-apoptotic. The anti-apoptotic action of T_4 was first disclosed in studies of the action of a pro-apoptotic stilbene, resveratrol, in cancer cells. Resveratrol treatment of cancer cells induces a *nuclear* complex of MAPK and COX-2 protein that is required for subsequent activation of the oncogene suppressor protein, p53 (Tang et al., 2006; Lin et al., 2008; 2011b). Agonist thyroid hormone analogues disrupt formation of the nuclear complex of MAPK and COX-2 and this is essential to the anti-apoptotic effects of the hormone (Lin et al., 2011b). Tetrac not unexpectedly blocks this effect of T_4 (Lin et al., 2008; 2011b). These observations distinguish between *constitutive* expression of *COX-2* as a marker of cancer cell invasiveness and *inducible COX-2,* the gene product of which accumulates in the tumor cell nucleus to support p53-dependent apoptosis.

Another action of tetrac that is mediated by the hormone receptor on αvβ3 is radiosensitization of cancer cells, as demonstrated *in vitro* (Hercbergs et al.,

2009; 2011). The mechanism of this effect of tetrac is interference with repair of double-strand DNA breaks (Hercbergs et al., 2011), although the molecular basis for this interference is not yet understood.

Finally, the possibility that the binding of thyroid hormone by integrin αvβ3 might modulate the interactions of the integrin with ECM proteins is an interesting one. The possibility has not yet been explored, but modulation of such interactions by agonist thyroid hormone analogues or by tetrac would affect cell motility and vectors of migration that are keyed to ECM protein ligands of αvβ3. Vascular growth factors are of course found in ECM and we commented earlier on the effects of iodothyronine analogues on the crosstalk between the hormone receptor on the integrin and the specific receptors for several vascular growth factors that are nearby.

Conclusions

A receptor for thyroid hormone analogues recently defined on cell surface protein integrin αvβ3 has revealed the existence of several nongenomic functions of the hormone not previously appreciated. These include stimulation of 1) tumor cell proliferation, 2) angiogenesis, and 3) intracellular trafficking of a variety of proteins important to hormone and cytokine actions. The trafficking includes nuclear uptake of the internalized αv monomer. Tetraiodothyroacetic acid (tetrac) inhibits the agonist functions of T_4 and T_3 at the plasma membrane integrin, but tetrac and a nanoparticulate formulation of tetrac also have discrete functions of their own in the absence or presence of T_4 or T_3. These functions include the disabling of expression of specific genes important to cancer cell survival pathways and inhibition of repair of double-stranded DNA breaks.

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Disclosure

The authors report no conflicts of interest.

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