Send Orders for Reprints to reprints@benthamscience.ae

Immun., Endoc. & Metab. Agents in Med. Chem., **2015**, *15*, 71-85

Thyroid Hormones Crosstalk with Growth Factors: Old Facts and New Hypotheses

Elena Candelotti[1,#], Paolo De Vito[2,#], Ragab G. Ahmed[3], Paolo Luly[2], Paul J. Davis[4,5], Jens Z. Pedersen[2], Hung-Yun Lin[5,6] and Sandra Incerpi[1,*]

[1]Department of Sciences, University Roma Tre, Rome, Italy; [2]Department of Biology, University of Rome Tor Vergata, Rome, Italy; [3]Division of Anatomy and Embryology, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt; [4]Department of Medicine, Albany Medical College, Albany, NY, USA; [5]Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Albany, NY, USA; [6]PhD Program for Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei 110, Taiwan

Sandra Incerpi

Abstract: Nongenomic effects of thyroid hormones typically start at the cell surface and do not primarily involve the classical nuclear receptors, but rather a plasma membrane receptor site identified about ten years ago on the integrin αvβ3. Transduction of the thyroid hormone signal from this integrin receptor involves activation of the MAPK pathway and may lead to events such as angiogenesis or tumor cell proliferation. This review focuses on the interaction of thyroid hormones with growth factors, in fact the integrin αvβ3 has been reported to a be a co-receptor for several growth factors such as EGF, IGF-1 and the FGF family, but also for small molecules like resveratrol. Binding of the ligand to integrin αvβ3 is inhibited by tetrac, a metabolite of L-thyroxine, and by its nanoparticulate formulation nanotetrac. Recent microarray studies on tumor cells have shown that tetrac has anti-inflammatory effects that are mediated by integrin αvβ3, and tetrac can downregulate the expression of several interleukin genes. Crosstalk between thyroid hormones and vascular growth factors is important for cell migration, vascular calcification and the angiogenic process. Thyroid hormones also show pleiotropic effects on osteoblast function and differentiation, as well as in early pregnancy. The importance of thyroid hormone interaction with neurotrophins and interleukins has also been examined. With integrin αvβ3 firmly established as the plasma membrane receptor future studies will focus on the crosstalk between thyroid hormones and growth factors in order to verify the efficiency of new pharmacological tools, such as nanotetrac.

Keywords: Early pregnancy, extravillous trophoblast, integrin αvβ3, matrix Gla protein, myokine, nerve cell, nerve growth factor, nongenomic.

INTRODUCTION

Thyroid hormones—3,5,3'-triiodo-L-thyronine (T_3) and L-thyroxine (T_4)—give rise to a wide range of effects on metabolism, growth and development [1]. T_4 represents the major form of thyroid hormone secreted by the thyroid gland, whereas T_3 is produced mainly in peripheral tissues by deiodination of T_4 and is the form of the hormone responsible for nuclear or genomic effects of the hormone [2]. The effects of T_3 are mediated by its binding to specific receptor proteins that may translocate to the cell nucleus where they regulate gene expression [1]. Two forms of thyroid hormone receptors, TRα and TRβ, have been identified and shown to belong to the steroid receptor family [1]. There is tissue specific expression of the isoforms of the thyroid receptor; for example, TRβ1 is expressed primarily in brain, liver and kidney, whereas TRα1 and TRα2 are more abundant in skeletal and cardiac muscle, but also are important in brown fat [3]. The TRβ2 receptors have the most specific expression in the adenohypophysis, hypothalamus, developing brain and inner ear [1]. The ligand-binding domains of both TRα and TRβ have been crystallized and structurally characterized in detail [4, 5]. TRs bind to DNA at thyroid hormone response elements, both as homodimers and as heterodimers with the retinoid X receptor [6]. Co-repressor proteins that bind the unliganded receptors to the ligand binding domain of genes mediate basal repression of transcription; it is the relief of this repression with binding of T_3 that results in (ligand-induced) transcriptional activity. The co-repressors NCoR (nuclear receptor co-repressor) and SMRT (silencing mediator of Retinoic Acid Receptor, RAR and TR) recruit histone deacetylases and may also directly inhibit basal transcription. Hormone binding results in a conformational change with dissociation of the co-repressors and recruitment of co-activators [7].

Nongenomic or extranuclear actions of thyroid hormones are initiated at the plasma membrane or in the cytoplasm, and do not depend primarily on the interaction of the hormone with classical nuclear receptors. Nongenomic mechanisms of thyroid hormone action rely upon transduction of the hormone signal by kinases such as mitogen-activated protein kinase (MAPK) [8-13] or phosphatidylinositol 3-kinase (PI3-K) [14-17] that are cytoplasmic in location; once activated, these kinases may move to other intracellular compartments. Activated signal transducing kinases are early (upstream) factors that may give rise to complex cellular

*Address correspondence to this author at the Department of Sciences, University Roma Tre, Viale G. Marconi, 446, 00146 Rome, Italy; Tel: 0039-06-57336335; Fax: 0039-06-57336321; E-mail: sandra.incerpi@uniroma3.it
#These authors equally contributed to this work.

1875-5844/15 $58.00+.00

events, including specific gene transcription and cell proliferation [18, 19]. Thus, nongenomic and genomic actions of thyroid hormones may engage in crosstalk. Bergh *et al*. (2005) identified the plasma membrane receptor for thyroid hormone in an integral transmembrane protein: the integrin αvβ3 [20]. This integrin has binding sites both for T_3 and for T_4, and the interaction of thyroid hormones with the integrin gives rise to a variety of physiological responses and cellular events, such as angiogenesis and tumor cell proliferation [20, 21]. Many growth factors are known to participate in the same events, and the present review focuses on the interfaces of thyroid hormone actions with the actions of growth factors, in part because of the fact that integrin αvβ3 also can act as a co-receptor for several of these growth factors, such as insulin-like growth factor-1 (IGF-1).

INTEGRIN αvβ3: STATE OF THE ART

Integrins are heterodimer receptors formed by the noncovalent association of α and β subunits to form integral plasma membrane proteins that are well conserved in evolution and are found evolutionarily from sponges to humans. They have very important interactions with extracellular matrix (ECM) proteins. Integrins have a long extracellular domain for the binding to ECM proteins, a transmembrane domain, and a short cytoplasmic domain that interacts with actin cytoskeleton and other proteins, including those involved in signal transduction [22, 23]. Integrins are formed from 18 different α subunits and at least 8 β subunits that are capable of forming 24 different αβ heterodimer proteins. Interactions of the various domains of integrin receptors, outside or inside the cell, are able to modulate a variety of cell functions, including cell differentiation, tissue development, cell proliferation, cell migration, tumor invasion, metastasis, gene expression, cell survival, angiogenesis and wound healing [24]. Dysregulation of integrins may be found in the pathogenesis of many diseases, *e.g*. autoimmune diseases, cardiovascular diseases and osteoporosis; apparently integrins are also involved in several types of tumors where integrin overexpression has been reported [25]. Viruses and bacteria often use integrins to enter the cells; the cellular uptake of human immunodeficiency virus, human parechovirus-1 and adenovirus [26, 27], and of the bacterium *Coxiella burnetii,* the etiologic agent of Q fever [28], are all mediated by integrin αvβ3.

Integrin αvβ3 is expressed on the plasma membrane of endothelial cells, smooth muscle cells, monocytes and platelets. The discovery of integrin αvβ3 as a plasma membrane receptor for thyroid hormones [20] encouraged reconsideration of these hormones—already known to be very important for the development and the maintenance of cellular steady state—as possible modulators of immune activities and cells of the immune system. But integrin αvβ3 may also be important for cancer therapy, as this integrin has been reported to be able to sensitize tumor cells to radiation [29]. Davis and coworkers have shown that tetrac, a natural metabolite of T_4 that has intracellular thyromimetic activity at high concentrations, inhibits the binding of thyroid hormone to the integrin αvβ3 and is able to increase radiosensitivity three-fold in glioma cells. Radiosensitization is achieved by short-term treatment with tetrac in the micromolar range [30], and tetrac is able to increase the number of DNA double strand-breaks in unirradiated steady-state coupled with a decreased repair rate of DSB in irradiated human glioblastoma U87MG cells [31].

About 20 years ago the group of Davis at Albany first described crosstalk between the thyroid hormone-activated MAPK pathway and the signal transducer and activator of transcription (STAT) proteins. The latter were shown to be important for the potentiation by thyroid hormone in the physiological concentration range of actions of interferon-γ (IFN-γ) and epidermal growth factor (EGF) in HeLa cells that do not contain any nuclear thyroid hormone receptors. They also established that thyroid hormone by a nongenomic mechanism caused tyrosine phosphorylation and nuclear translocation of STAT1α [32], and hypothesized that thyroid hormone-directed tyrosine phosphorylation and nuclear accumulation of STAT3 may potentiate the actions of EGF. This was shown both in CV-1 cells [33, 34], which also lack the nuclear thyroid hormone receptor, and in HeLa cells, and it has been found also to occur in TR-repleted human skin fibroblasts (BG-9). However, this direct potentiation by thyroid hormone of the STATs does not occur in all cells, and this suggests that thyroid hormones may instead potentiate the effects of other cytokines or growth factors which then cause phosphorylation of STATs [35].

INTEGRIN αvβ3 AND GROWTH FACTOR SIGNALING

A number of growth factors, such as EGF, IGF-1, Transforming growth factor α (TGF-α), the family of fibroblast growth factors (FGFs), and others, are involved in critical biological responses such as growth, differentiation and angiogenesis. The signal transduction for all these receptors have some similarities, in that they all involve a receptor tyrosine kinase that acts through receptor dimerization and subsequent receptor autophosphorylation of specific tyrosine residues in the cytoplasmic domain [36-40]. The principal signaling pathway activated is usually the Ras/MAPK pathway, after activation/phosphorylation of Src homology 2 proteins or a phosphotyrosine binding domain. Integrins directly bind and crosstalk with adjacent growth factor receptors and influence growth factor signaling, although at present the details are not fully elucidated. The signaling of integrins is very similar to the signaling of growth factor receptors and may be coupled with them. Many cell responses to growth factors such as EGF, platelet-derived growth factor (PDGF) and thrombin are dependent on the adherence of the cell to ECM ligands through integrins. Angiogenesis and cell proliferation are among the typical responses activated by growth factor-integrin interaction. It has been demonstrated by the group of Takada and Takada that an integrin-binding defective-FGF1 mutant (R50E) significantly reduced the capability of the growth factor to cause cell proliferation and migration, although R50E did give rise to FGFR1 phosphorylation and downstream Akt and ERK1/2 activation [41]. They proposed that the direct binding of integrin to FGF1 is the basis of the crosstalk between integrin and FGF [36]. The same group showed a similar situation for EGF [41] and for IGF-1 [42], concluding that the binding of integrin αvβ3 is important for growth factor signaling.

With regard to EGF signaling, the situation is complicated further by the presence of neuregulins (NRGs), other members of the EGF family that are involved in several human diseases, such as cancer, schizophrenia and cardiovascular disease. Also for NRGs the binding to integrins is essential for effective growth factor signaling, and Ieguchi and co-workers in an elegant study found that direct interaction of NRG1 with the integrin αvβ3 was critical for activation of the ErbB receptor-tyrosine kinases resulting in downstream signaling activation of Akt and ERK1/2 [41]. Thus different growth factors may compete for interaction with the same integrin.

The possible interaction between EGF and thyroid hormones was actually studied prior to the discovery of the thyroid hormone receptor on integrin αvβ3. In 2004 Shih *et al*. showed that thyroid hormone T_4 was able to increase EGF- and TGF-α-induced MAPK activation in HeLa cells that lacked TRs. The effect was mimicked by T_4-agarose, an early nanoparticulate formulation of the hormone, and was inhibited by tetrac which is known to be an inhibitor of the binding of T_4 to the plasma membrane. Studies of cell proliferation indicated that T_4 potentiated the effect of EGF, but inhibited that of TGF-α. The disparate effects of thyroid hormone on the actions of EGF and TGF-α—growth factors that share the same plasma membrane receptor—are mediated by hormone activation of cAMP-dependent protein kinase A (PKA) [43]. These authors showed in the same year that resveratrol, a naturally occurring pro-apoptotic stilbene and antioxidant, activated MAPK and caused phosphorylation and activation of p53, with consequent apoptosis. EGF inhibited the effect of resveratrol, stimulating tumor cell growth via a PKCα-mediated mechanism [44]. We now know that there also is a resveratrol receptor site on integrin αvβ3 that is distinct from the thyroid hormone receptor.

In 2009 Glinskii *et al.* [45] reported that tetrac, used as a probe for thyroid hormone effects that originate at integrin αvβ3, is able to modify expression of multiple cell survival pathway genes in ERα negative human breast cancer (MDA-MB-231) cells. The authors conducted proliferation experiments in a perfusion bellow cell culture system in the presence of tetrac and tetrac nanoparticulate (nanotetrac). The microarray experiments concentrated on a panel of survival pathway genes, and found upregulation of caspase 2 *(CASP2*) and thrombospondin 1 (*THBS1*) gene expression, the first a facilitator of apoptosis and the second an inhibitor of angiogenesis, a very important process in tumor spreading. They also found downregulation of the genes for X-linked inhibitor of apoptosis (*XIAP*) and myeloid cell leukemia sequence 1 (*MCL1*), whose gene product is also an inhibitor of apoptosis. In most cases the nanotetrac was significantly more effective than the unmodified tetrac. The family of Ras-oncogenes was differentially affected by nanotetrac, with 8 out of 13 genes downregulated [45]. These differential effects of nanotetrac are in fact quite coherent in terms of clinically desirable up- and downregulation of specific genes. Nanotetrac is more effective than unmodified tetrac with regard to the number of genes affected and drug potency. Another interesting result from the same work is the downregulation of the important gene *EGFR*, the gene for the EGF receptor. It is noteworty that nanotetrac was an efficient downregulator, whereas the unmodified tetrac gave only a trend towards a decrease. The striking effect of nanotetrac on the *EGFR* gene suggests that this nanoformulation could be a serious candidate for a new potential clinical chemotherapeutic agent.

Cancer stem cells are a population of cells capable of self-renewal and propagation. They may be resistant to chemotherapy and contribute to drug resistance, being able to escape apoptosis and survive difficult environments. Some integrins are particularly abundant in certain types of cancer and it is known that these cell surface proteins may "integrate" signals between extracellular matrix proteins and intracellular signaling pathways, supporting cell survival, proliferation and cell invasion [46, 47]. Cheresh and co-workers have noted that integrin αvβ3 has special assets in the areas of supporting cell survival and proliferation independently of cell adhesion, cell attachment or matrix ligation [46]. They found that integrin αvβ3 expression defines a cancer stem cell population involved in the resistance to EGF receptor-targeted tyrosine kinase inhibitors, and they evaluated the presence of integrin αvβ3 as a necessary and sufficient condition for stemness and EGF receptor inhibitor resistance in a variety of cancers, including pancreas, lung and breast cancer. This finding led them to propose a previously unrecognized mechanism by which integrin αvβ3, in its unliganded state, may interact with KRAS to activate in turn the RalB-TBK1-NFκB pathway that leads epithelial cancer cells to the cancer stem cell phenotype [47].

With IGF-1 the situation is similar but also of particular interest from our point of view, since IGF-1 has a high structural homology with the insulin/proinsulin system. The IGF-1 receptor is a tetramer with two α-subunits with the ligand binding domain and two β-subunits with tyrosine kinase activity that give rise to effects similar to those elicited by insulin, glucose uptake and cell proliferation. The β-subunits phosphorylate tyrosine residues on adapter proteins such as the insulin receptor substrate-1; the phosphorylation of these residues then gives rise to the activation of Akt and MAPK signaling pathways. As reported above the downstream signaling from integrin αvβ3 thyroid hormone receptor sites activates PI3-K and MAPK that give rise to a complex pattern of physiological responses among which are stimulation of angiogenesis and tumor cell proliferation [20]. We have recently reported that in L6 myoblasts in culture thyroid hormone in the physiological range is able to inhibit responses to IGF-1 such as glucose uptake and cell proliferation [48]. The effect is mediated by integrin αvβ3, since the Arg-Gly-Asp tripeptide, the disintegrin echistatin, tetrac and an antibody to αvβ3 all are able to prevent inhibition by thyroid hormone of IGF-1–mediated effects, both in short-term and long-term. The effect of thyroid hormone slowing down the typical anabolic response in L6 myoblasts can be explained as an effect intended to prepare the myoblast for the differentiation process [48].

THYROID HORMONES, VASCULAR GROWTH FACTORS AND ANGIOGENESIS

Angiogenesis, the formation of new vessels, is an important physiological event and its dysregulation occurs in the pathological states where vasculature is critical, such as reti-

nopathies or blood vessel tumors. The modulation of angiogenesis is a very complex event and depends on classical vascular growth factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), but also on EGF as mentioned above. Among the cellular processes involved in angiogenesis is the increase in migration rates of vascular smooth muscle and endothelial cells. Nonpeptide hormones such as steroids and thyroid hormones affect angiogenesis, and the role of thyroid hormone was first recognized almost 30 years ago by Chilian *et al*. [49]. Angiogenesis thus represents another example of the cellular and tissue interplay between growth factors and thyroid hormones. In studies involving a thyroid hormone analog, diiodothyropropionic acid (DITPA), it was reported that VEGF and bFGF are important for the neovascularization taking place in the area of induced infarction [50], and it is known that thyroid hormones are important for the vascularization in several pathophysiological states [51].

The molecular mechanisms of thyroid hormone action in angiogenesis are not fully understood although several recently findings point in the same direction. In fact bFGF, FGF and VEGF may mediate thyroid hormones-induced angiogenesis in the cardiac tissue [50, 52]. T_4-induced angiogenesis studied in the chick chorioallantoic membrane (CAM) model of angiogenesis was found to involve bFGF gene expression and release of FGF protein; the latter may be a secondary autocrine factor in thyroid hormone action as confirmed by the observation that anti-FGF impairs T_4-induced vascular sprouting [53]. A similar finding was reported for VEGF in the same CAM model, with small amounts of VEGF released into the medium after thyroid hormone treatment of the preparations. The downstream signaling pathway of these effects involves activation of the MAPK pathway (ERK1/2). We now know that angiogenesis depends on integrin expression at the plasma membrane of both endothelial and vascular smooth muscle cells and this information led to the finding that the integrin αvβ3 is the plasma membrane receptor for thyroid hormones [20]. The thyroid hormones and their analogues DITPA [54] and GC-1, a selective agonist of TRβ [55], have all been shown to stimulate angiogenesis through the integrin receptor in the CAM model and in human dermal microvascular endothelial cells; these effects are mediated by the MAPK pathway and inhibited by tetrac [51]. As mentioned above, EGF action may be potentiated by thyroid hormone [43] and EGF is pro-angiogenic. Therefore angiogenesis appears to be a good example of the complex interactions between thyroid hormones and growth factors.

RNA microarray studies in tumor cells have shown that tetrac has anti-inflammatory activity which is mediated by integrin αvβ3. Tetrac can downregulate the expression of several interleukin (IL) genes and of the *EGFR* gene [45]. The EGF receptor is essential for the pro-inflammatory activity of EGF. These studies indicate that thyroid hormones may also play a role in inflammation through binding to the receptor on integrin αvβ3. Thyroid hormones increase the activity of the EGF receptor [43] and this effect is inhibited by tetrac. At this point we may speculate that thyroid hormones may be contributors to the inflammatory states in different tissues and blood vessels; this may be supported by the effect of the hormone on platelet aggregation.

The relationship between calcium metabolism and thyroid hormone is very complex and has been studied quite extensively experimentally both in animals and clinically. Thyroid hormones in fact contribute to bone resorption by promoting calcium mobilization in bone [56] and this effect may be mediated by integrin αvβ3, which is known to be generously expressed in osteoclasts (this topic will be discussed further in the next section). An interesting relationship also exists between blood vessel calcification and thyroid hormones; effects of thyroid hormone T_3 on genes related to vascular calcification was described by Sato *et al*. [57] in rat aortic smooth muscle cells and also in cultured human coronary artery smooth muscle cells. Matrix Gla protein (MGP) is a secretory protein of 14 kDa expressed in a wide variety of tissues (lung, heart, kidney, skin, arterial vessels, endothelial cells, fibroblasts). It is a member of the family of proteins containing the rare modified amino acid Gla (γ-carboxyglutamate), and acts as a potent inhibitor of vascular calcification *in vivo* [58]. Physiological concentrations of T_3 (15 pmol/L free T_3) increased mRNA levels of MGP about three-fold. In addition short interference RNA directed against the TRα gene *THRA* diminished the effect of thyroid hormone on MGP expression [57]. In agreement with this result, animals with methimazole-induced hypothyroidism showed a decrease of almost 70% in MGP mRNA levels and about 30% increased calcium content. Animals with T_3-induced hyperthyroidism in contrast showed upregulation of the *MGP* gene and significantly decreased cell calcium content. The conclusion of the authors was that thyroid hormone enhances gene expression of MGP gene in rat aortic smooth muscle cells via the nuclear TRs, thus preventing smooth muscle vascular calcification [57], and tetrac could act at the level of the integrin by inhibiting the angiogenesis [51].

To understand how important this effect of the hormone on MGP protein can be from pathophysiological point of view, it is worthwhile to recall that coronary artery calcification is an important predictor of cardiovascular disease, and that MGP is a potent inhibitor of vascular calcification in many different tissues [58, 59]. The mechanisms by which MGP prevents calcification are beyond the scope of the present article; the effect is remarkable by itself, but even more when considered that at present there are no alternative mechanisms to inhibit vascular calcification [58]. It is important to recall here that a role for integrins in matrix-chondrocyte attachment was first reported about 20 years ago [60]. In fact, a cyclic peptide containing the Arg-Gly-Asp integrin recognition site sequence was shown to be capable of blocking attachment of chondrocytes to matrix proteins such as fibronectin, MGP, and type II collagen. Cations were required for chondrocyte attachment and provided a mechanism for differential regulation of attachment to ECM proteins [60]. As a working hypothesis we speculate that the effects of thyroid hormones on certain cellular processes involved in expression of genes relevant to blood vessel function or disease could be mediated by the membrane receptor on integrin αvβ3, the αv monomer of which has been shown to be internalized after binding of thyroid hormone and directed to the cell nucleus, where it can modulate specific gene expression [61].

THYROID HORMONES AND BONE

Thyroid hormones are essential regulators of skeletal development and growth. Hypothyroidism in children gives rise to retardation of bone formation. This is due to the lack of normal effects of thyroid hormones on chondrocyte proliferation and reflects impaired endochondral differentiation during ossification. In contrast, thyrotoxicosis in young persons causes premature aging of bone and can result in short stature because of accelerated closure of epiphyses. There also may be early fusion of skull sutures. T_3 stimulates FGF receptor 1 (FGFR-1) expression and activity of the latter is increased in osteoblasts by a mechanism involving TRα [62]. FGFR-1 mediates the actions of thyroid hormone on intramembranous ossification, but the molecular mechanism of hormonal regulation of endochondral ossification and linear growth of bone is still unknown. Other growth factors are involved in bone development and their action may be modulated by thyroid hormone. We would point out here that there are two basic mechanisms for bone formation: endochondral ossification and intramembranous ossification. Long bones are formed through endochondral ossification when mesenchymal stem cells differentiate into chondrocytes that proliferate and form cartilage. Central chondrocytes undergo differentiation and then apoptosis with vascular invasion and formation of the ossification center. The skull is formed by intramembranous ossification. Mesenchymal stem cells differentiate into osteoblasts, then osteocytes; they mineralize without passing through a cartilage intermediate stage. Both endochondral ossification and linear bone growth are under the control of a variety of hormones and growth factors. These include thyroid hormones, growth hormone, IGF-1, glucocorticoids and sex steroids, as well as certain cytokines and growth factors, with primary roles taken by FGF and VEGF. Chondrocyte proliferation and differentiation are tightly regulated by several growth factors and thyroid hormone may interact with some of these factors. T_3 stimulates osteoblast differentiation, bone matrix synthesis and mineralization. At the osteoblast T_3 shows pleiotropic effects; for example T_3 induces *IGF-1* transcription through its nuclear receptor and also *FGFR1* expression and activity, leading to activation of MAPK pathways and osteoblast differentiation. T_3 stimulates osteoblast function and differentiation through a complex network of interactions with cytokines and growth factors acting in autocrine or paracrine fashion [63]. With regard to osteoclasts, an excess of thyroid hormone results in increased cell numbers and activity, culminating in bone mass loss. Thyroid hormone T_3 stimulates osteoclastic bone resorption through the release of soluble mediators from osteoblasts [64]. T_3 over a wide concentration range also stimulates the production of IL-6 and causes release of cytokines from osteoblasts and from bone marrow stromal cells [65]. Osteoclasts express mRNAs for TRα1 and TRβ1, but it is uncertain whether the TRs are functional in these cells; however, osteoclasts amply express integrin αvβ3 and thus the activity of thyroid hormone on these cells might be initiated nongenomically at the cell surface receptor for the hormone. Treatment of immortalized osteoblasts with thyroid hormone results in increased expression of RANKL, IL-6, IL-8 and prostaglandin E_2, so a thyroid receptor is definitely operating in these cells [66, 67].

IGF-1 is an important modulator of endochondral ossification in conjunction with thyroid hormone, with the activity of the latter mediated by the classical nuclear receptor TRα. Mice lacking TRα1 show hypothyroid osseous features, *e.g.* delayed endochondral ossification and growth retardation. IGF-1 is actively involved in crosstalk with the Wnt/β catenin and PI3-K/Akt pathways that are activated by thyroid hormone to support growth plate ossification [68].

THYROID HORMONE AND THE IMMUNE SYSTEM

T_3 and T_4 modulate the immune response at a number of levels. Cell-mediated immunity, natural killer cell activity, and the antiviral action of interferon and proliferation of T and B lymphocytes [69] are all subject to regulation by thyroid hormones. The cells of the immune system are able to produce hormones or hormone-like molecules [70], in addition to cytokines, chemokines and eicosanoids, such as prostaglandins and leukotrienes. At the same time, thyroid hormone T_3, thyroid-stimulating hormone (TSH) and thyrotropin-releasing hormone have been found in immune cells [71-74]. T_4 can potentiate the antiviral effect of IFN-γ by a non-genomic mechanism probably mediated by the integrin αvβ3, suggesting that thyroid hormone may play a role in cellular defense mechanisms [75]. It seems possible that thyroid hormone modifies immune system activity at least in part through modulation of the effects of cytokines and growth factors.

IGF-1 is able to stimulate cell migration and is a cytokine produced by injured muscle tissue [76]. Inhibition of both IGF-1 and IGF-2 activity has been shown to be able to inhibit cell migration and invasion in breast cancer cells [77]. Taking into account that thyroid hormone is capable of modulating the growth factor responses (EGF, IGF-1 and TGF-α) in L6 myoblasts, we are pursuing the possibility of crosstalk between thyroid hormone and IGF-1 in cells that express high levels of integrin αvβ3, such as human leukemic THP-1 monocytes, inhibited by RGD and tetrac (Candelotti *et al.*, manuscript in preparation).

THYROID HORMONE INTERACTION WITH GROWTH FACTORS IN EARLY PREGNANCY

A variety of studies report alterations in fertility in hypothyroid animals and thyrotoxicosis. We have previously reported that administration of doxorubicin (DOX) to female rats during pregnancy negatively affect the thyroid-brain axis, leading to hypothyroidism and decrease of nucleotides and ATPase activities such as the Na/K-ATPase, Mg-ATPase and Ca-ATPase enzymes in fetal brain. ATP synthesis decreases significantly in the hypothyroid state. These conditions lead to impairment in nerve transmission and CNS dysfunction [78]. Administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to pregnant rats also leads to hypothyroidism and negatively affect the cholinergic system in their offsprings, whereas the γ-aminobutyric acid (GABA) is increased [79]. Variations in T_3 levels are known to affect the estrous cycle, success of pregnancy, fetal growth and lactation [80]. Menstrual disturbances are widely acknowledged to be associated with hypothyroidism and hyperthyroidism. Thyrotoxicosis in women is associated with reduced fertility although ovulation may be normal, and other factors

such as the use tobacco may interact with thyrotoxicosis in affecting the menstrual cycle. Fifty percent of thyrotoxic women who smoke have menstrual abnormalities, whereas 19% of non-smoker hyperthyroid women have abnormal cycles. Patients with abnormal menstrual cycles had higher levels of circulating T_4, and hormone levels were higher in smokers with abnormal periods. Therefore the level of T_4 appears to be an important parameter related to the disturbances of the menstrual cycle. It is interesting that no similar correlation was found for T_3 [81], indicating that these effects do not depend on the nuclear TRs. These observations by Krassas *et al*. have shed light on a topic that has not previously been broadly considered [80]. Additional studies that examine thyroid function, pregnancy and integrin αvβ3 have appeared recently. Integrins may be markers for 'unexplained infertility' [82, 83] and expression of integrins may be altered in secretory and proliferative endometrium. Cyclical changes in integrin levels have also been shown, but no variations were found in endometrium and endometriosis [84]. More recently it has been reported that increased expression of integrin αvβ3 is associated with the onset of pregnancy and this parameter can be considered a marker for successful *in vitro* fertilization [85]. A positive association of expression of leukemia inhibitory factor (LIF) with expression of integrin αvβ3 has been shown for women with unexplained infertility [86].

The expression of integrins is inversely related to the severity of endometrial cancer, although this pattern may vary with the type of tumor [87, 88]. Ceydeli *et al*. found differences in integrin αvβ3 expression in endometrial cells of 33 infertile women [89] and alteration of β3 monomer expression has also been reported in uterine endometrium in association with unexplained infertility [90]. The expression of integrin αvβ3 has been linked to infertility in connection with EGF and osteopontin; this latter factor is stimulated by progesterone, whereas EGF has been considered as a paracrine-mediated signal [91]. The αvβ3 integrin signaling pathway is also involved in IGF-1-mediated extravillous trophoblast (EVT) migration [92], and Mayama *et al*. have recently studied the effects of IGF-1 in the setting of insulin-resistance [93]. They found that exposure to insulin for 48 hours decreases both the number and the level of phosphorylation of insulin receptors, and showed that longer exposure to insulin reduces the phosphorylation of IGF-1 receptors, insulin receptor substrate-1 and Akt, and also decreases EVT migration [93]. When the cells were treated with insulin and pioglitazone, an insulin-sensitizer, phosphorylation of the proteins was restored and the migration of EVT was also partially restored. The improvement of the insulin-resistant state may in turn improve the probability of pregnancy and reduce abortion risk. Other authors have emphasized the role of integrin αvβ3 in adhesion and migration of EVT cells, and have studied the protein CD98 that is highly expressed in developing EVTs. CD98 is an amino acid transporter that is considered to be involved in cell fusion, adhesion and migration by interacting with integrin αvβ3. There is an important role of CD98-integrin αvβ3 interaction for the modulation of adhesion and migration in EVT cells [94], and T_3 downregulates Fas and Fas ligand expression, inhibits caspase 3 and PARP polymerase cleavage and inhibits apoptosis in early placental EVT cells [95].

Binding of thyroid hormone to the αvβ3 integrin typically leads to activation of the MAPK pathway, and an important role of ERK1/2 for endometrial decidualization in C57BL/6 mice and humans has been reported recently [96]. ERK1/2 is a pivotal regulator of cell adhesion, migration, cell cycle progression, proliferation and differentiation in many organs and systems. ERK1/2 belongs to the Ras/Raf/MEK pathway and is activated by phosphorylation; the phosphorylated p-MAPK in turn can phosphorylate several transcription factors modulating proliferation, differentiation, apoptosis and inflammation [97]. The expression of several genes—*FOX*, *MSK1*, *STAT1* and *STAT3*—is significantly decreased by inhibition of ERK1/2 during *in vitro* decidualization. STAT1 and STAT3 proteins are known to be activated downstream after thyroid hormone binding to the plasma membrane receptor integrin αvβ3. Also *EGFR* has an important function in modulating pregnancy and in a study applying both *EGFR*-knockout mice and primary human endometrial stromal cells with *EGFR* silenced using siRNA it was recently shown that the activity of the EGF receptor is critical for regulation of endometrial function in the early phases of pregnancy [98]. Elimination of the EGF receptors resulted in early embryo death although female fertility was unaffected and responses to steroid hormones were normal. The main problem identified was failure of blastocyst implantation and maintenance and decidualization. Among important markers of decidualization are Bmp2 and Wnt4 and these are important in the downstream signaling by the EGF receptor [98].

Coordinated actions of the EGF receptor are critical for successful progression of early pregnancy, probably because EGF is critical to multiple signaling pathways leading to gene transcription. EGF receptor signaling is also critical to survival of multiple types of tumors. *EGFR* is overexpressed in many cancers and in about 50% of endometrial tumors. In this context, it is important to mention that thyroid hormone has been reported to enhance some actions of EGF, particularly those involving the cAMP/PKA pathway [43]. Progesterone and cAMP are important factors in the decidualization process, the details of which remain incompletely understood [96, 99]. Little information is available at present on the possible roles of thyroid hormone in early pregnancy, but it has been reported that T_3 interacts with EGF in placental development and invasion of EVT cells [100]. A recent paper reported that T_3 regulates angiogenic growth factor and cytokine secretion, including IL-6, IL-8, IL-10 and IL-1β, by human decidual cells in a gestational age-dependent manner [101]. Apparently an optimal concentration of thyroid hormone is important to maintenance of a balanced inflammatory response in early pregnancy that prevents a possible immune rejection of the embryo and that stimulates optimal placenta development through the secretion of cytokines and angiogenic factors by decidual cells.

We suggest that the potential roles of thyroid hormone in early pregnancy should be more carefully investigated considering the following points: i) integrin αvβ3 is important for the fertilization process and contains the plasma membrane receptor for thyroid hormone, inhibited by tetrac and nanotetrac [43-45]; ii) ERK1/2 signaling is a downstream consequence of the interaction of thyroid hormone with integrin αvβ3 and this pathway is important for endometrial

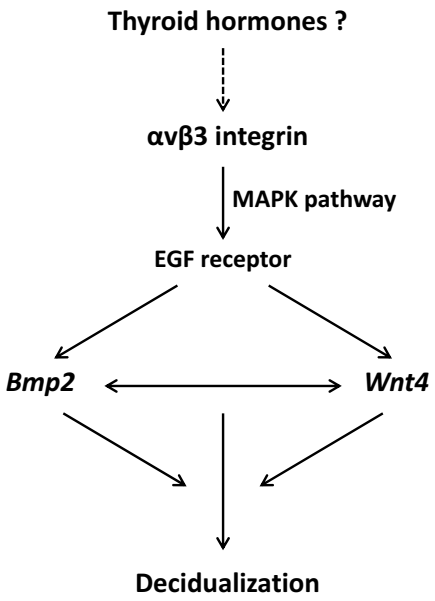

Fig. (1). A schematic representation of a hypothetical interaction between thyroid hormone and EGF receptor in the signal transduction process behind endometrial decidualization (modified from Large *et al*., 2014 [98]).

decidualization; iii) thyroid hormone is able to potentiate the effects of EGF through actions of cAMP; iv) thyroid hormone is a well established regulator of the immune system and, in particular, an important regulator of angiogenic growth factors and cytokine production by human decidual cells. A schematic representation summarizing a possible mechanism is shown in Fig. (**1**) [98].

THYROID HORMONE CROSSTALK WITH GROWTH FACTORS IN NERVE CELLS AND BRAIN

Thyroid hormones are pivotal regulators of central nervous system development, as shown dramatically by the association of hypothyroidism in children with cretinism and dwarfism [102]. Hypothyroidism inhibits the expression of genes such as myelin basic protein and others connected to the myelinization process. Thyroid hormones, principally T_3, modulates the secretion of critical growth factors in nerve cells, such as bFGF, EGF, and neuronal growth factor (NGF); bFGF and EGF are involved in ECM protein secretion and NGF regulates neurite growth and survival. These thyroid hormone effects are mainly genomic in mechanism, but nongenomic effects are critical to development and function of the central nervous system (CNS), such as the actions of T_4 or rT_3—but not T_3—on actin polymerization in astrocytes [103, 104]. Therefore also the interaction of thyroid hormones with growth factors are particularly important in the CNS. IGF-1 has been reported to be neuroprotective in the setting of neurodegenerative diseases such as Alzheimer's disease, defending neurons against β-amyloid toxicity that is mediated by the PI-3K/Akt pathway. IGF-1 is a mitogen for rat Schwann cells in the presence of high levels of cAMP [105, 106], and IGF-1R controls neuronal lifespan [107]. As mentioned above, thyroid hormones are able to modulate the activity of a variety of growth factors, particularly those whose receptors on the cell surface interact with integrin αvβ3, and IGF-1 is one such growth factor. Through the interaction with integrin αvβ3 thyroid hormone also contributes to the control of normal and abnormal angiogenesis and to tumor cell proliferation. Several angiogenic factors have important effects at the CNS. For example VEGF, whose actions are usually considered to be limited to endothelial cells, is also a neurotrophic factor that stimulates axonal growth [108]. Other growth factors with neurotrophic properties are bFGF and PDGF. VEGF is expressed in the adult mouse superior cervical ganglia and dorsal root ganglia. A receptor for VEGF, fetal liver kinase, is expressed in these neurons and also in Schwann cells. VEGF is also able to increase axonal density in a dose-dependent manner, exhibiting a maximum response at 50-100 ng/mL. NGF produces a similar effect, and when VEGF and NGF are added together there is an additive effect on axonal growth. The roles played by the two neurotrophic factors are recognized to be different, with NGF being more efficient than VEGF with respect to the induction of regenerating axons. But at variance with NGF, VEGF increased survival of both neurons and satellite-Schwann cells in the adult ganglia [108]. In 2013, Pantos and co-workers reviewed the contributions of thyroid hormone to tissue repair and adaptation to environmental stress and evolution [106]. The role of thyroid hormones in differentiation has long been recognized, such as in metamorphosis of tadpoles, and Mourouzis *et al*. suggest that thyroid hormone be considered 'the black box of repair'; these authors listed a variety of papers in which thyroid hormone has shown to participate in the repair of pancreas, lung, kidney, nerve cells and skeletal muscle [109]. The possible contribution of thyroid hormone to peripheral nerve regeneration, however, may have involved excessively high concentrations of thyroid hormones [110]. Panaite and Ibtissam studied T_3 treatment of end-plates of rat limbs that have undergone surgery [111]. Four weeks after surgery no sign of innervation was reported, but after 14 weeks there was a marked effect of T_3 in terms of enhancement of reinnervation of gastrocnemius muscle and plantar end-plates, and also increased ACh receptors at the neuromuscular junction; these results showed for the first time that T_3 can increase restoration of neuromuscular junctions and improvement in synaptic transmission [111]. These results do not seem to be mediated by neurotrophic factors, but the authors propose that TRβ might be involved as a predominant receptor in the sensory neurons. The TRα receptor may also play a role in repair, particularly in Schwann cells [112]. Therefore thyroid hormones probably make use of different pathways to promote neuronal survival and growth.

The role of thyroid hormones in tissue repair and nerve regeneration has also been studied in injured neurons in connection with crosstalk with growth factors. Contradictory results have been reported for actions of thyroid hormone on the regeneration of peripheral nerves, perhaps due to the multiple administrations of thyroid hormones made locally to achieve a high concentration of the hormone. Voinesco *et al*. [113] studied a single local administration of thyroid hormone to the axon of the rat sciatic nerve in a closed system, the surgical implantation of the sectioned terminals into silicone chambers. Four weeks after local administration of thyroid hormone T_3, there was a significant difference between control and T_3-treated myelinized axons in terms of diameter, number, higher percentage of myelinated axons

and thicker myelin sheets [113]. Peripheral and central nerve and tissue regeneration in general require the presence of growth factors released by lesioned tissues or cells. Neurotrophic soluble factors released by injured peripheral axons include NGF, brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor, glia cell line-derived neurotrophic factor, and IL-6 [114]. Lesions of the sciatic nerve give rise to a rapid activation of p38MAPK within 15 min and for a duration of up to 6 hours, with an increase in mRNA observed for NGF, IL-6, LIF and deiodinases type 2 and 3 [112]. The interaction of thyroid hormones with growth factors has actually been known since 1980, together with the effects of thyroid hormones on metabolism and calorigenesis [116]; the latter effects in particular involve somatomedins, NGF, erythropoietin and EGF. NGF, erythropoietin and EGF are particularly important to the development of both central and peripheral nervous systems. Shulga and Rivera have recently reviewed this topic [117], with special attention paid to the role of thyroid hormones on $GABA_A$ receptor-mediated transmission in development and after injury, where BDNF seems to play a particularly important role. Thyroid hormones have various roles in nerve cells, both through the classical nuclear receptors and through the hormone receptor on integrin αvβ3 that is associated with downstream signaling via MAPK [117]. The neurotrophins, in particular BDNF, are important in the course of development and are reduced in hypothyroidism [118, 119] and increased in hyperthyroidism [120, 121]. At variance with this pattern of growth factor expression is a study reporting increased NGF in hypothyroidism [118], without any change upon thyroxine treatment [122]. In analogy to BDNF also neurotrophin-3 was decreased in hypothyroidism [119] and increased in hyperthyroidism [122]. The Egr1 transcription factor is regulated by thyroid hormones [123], and Egr1 in turn regulates $p75^{NTR}$ expression in Schwann cells; thus thyroid hormone is likely to influence this modulation. When a hypothyroid state is induced during development, the signaling pathways of Akt, ERK and CREB are decreased, as is the amount of the antiapoptotic protein Bcl-2 [118].

CROSSTALK OF THYROID HORMONES WITH GROWTH FACTORS: SKELETAL MUSCLE

Skeletal muscle is today considered an endocrine organ, and this context has recently been reviewed very satisfactorily by others [124-126]; therefore we shall only briefly discuss a few particular aspects of this topic. The substantial mass of skeletal muscle in the body is now known to release growth factors or cytokines called myokines in response to exercise or injury. Among these factors are IL-6, IL-10, BDNF, IGF-1, LIF and fractalkine. It was mentioned above that in osteoclasts T_3 stimulated both bone resorption and IL-6 release; thus, there is very likely some kind of IL-6 crosstalk with thyroid hormone involved in osteoclastic bone resorption [64-66]. Against this background, we would note that IL-6 is released during muscular exercise, and several other cytokines (IL-1 receptor antagonist, ILra, IL-8, IL-10) are released after intense exercise. IL-6 levels increase exponentially during muscle exertion and reaches a peak at the end of exercise [125]. Skeletal muscle provides the major fraction of blood IL-6 associated with exercise, but is not the only source of this cytokine. IL-6 may be released from connective tissue [127, 128], brain [129] and adipose tisue [129]. As mentioned above T_3 can also modulate production of IL-6 in completely different cell types such as human decidual cells and osteoblasts, suggesting that this effect might be part of a fundamental common signaling mechanism in many cell types.

Short-term treatment of muscle cells with IL-6 increases both basal activity of the glucose transporter GLUT4 and translocation of GLUT4 to the plasma membrane. The effect is mediated by AMP-activated protein kinase (AMPK), regulated by cAMP and also by the AMP/ATP ratio. Leptin also activates AMPK in skeletal muscle. IL-6 mediates the effect on glucose uptake through the glycoprotein 130 (gp130). The latter exhibits leptin-like effects, such as activation of AMPK and stimulation of insulin signaling. BDNF is also expressed in nonneural tissues and it appears to be a regulator of metabolism in skeletal muscle [130]. We have mentioned above a relationship between thyroid hormone and BDNF. Release of BDNF can increase after exercise [131] and BDNF derives largely from the brain; in fact increased levels of BDNF are found in both hippocampus and cortex in mice after exercise. BDNF stimulates phosphorylation of AMPK and acetyl-CoA carboxylase and increases fat oxidation both *in vivo* and *in vitro* [125]. Fibroblast growth factor-21 (FGF-21) is a chemokine induced by fasting, it is produced by skeletal muscle [132] and is a local factor in liver. FGF-21 increases the hepatic expression of peroxisome proliferator-activated receptor–γ coactivator protein-1α (PGC-1α), a transcription factor that regulates energy homeostasis and increases fatty acid oxidation, the Krebs cycle and gluconeogenesis without increasing glycogenolysis. Mice devoid of *FGF21* are unable to increase PGC-1α expression in response to fasting and both ketogenesis and gluconeogenesis are impaired [133]. Increased expression of *FGF21* has been associated with chronically elevated insulin levels, primarily in men. Additional experimental evidence suggests a role of FGF-21 as an insulin-regulated myokine [134, 135]. It was recently discovered that administration of T_3 stimulates transcription of *FGF21* in liver and adipose tissue, but release or addition of FGF-21 in turn decreases the level of thyroid hormone [136]. Thus it appears that thyroid hormone and FGF-21 control each other, it is no longer a one-direction signal but a homeostatic mechanism to maintain a situation of stability, and there may be reciprocal modulation in the setting of metabolic emergency. This is the first example, to our knowledge, of an homeostatic regulation through interaction of thyroid hormone with a growth factor.

THYROID HORMONES CROSSTALK WITH GROWTH FACTORS: αvβ3 INTEGRIN A MARKER OF PHYSIOPATHOLOGICAL SITUATIONS. POSSIBLE PHARMACOLOGICAL RELEVANCE

αvβ3 integrin plays an important role in the action of thyroid hormones and growth factors. An established role of this integrin can be found in cancer cell proliferation and modulators/inhibitors of integrin αvβ3 can be used in oncotherapy. Tetrac and nanotetrac inhibit the binding of thyroid hormone to the membrane receptor on the αvβ3 integrin. In the absence of the ligand nanotetrac and tetrac show proapoptotic and anti-angiogenic actions [137-139],

Table 1. Mechanisms of reported or possible chemotherapeutic actions of tetrac/nanotetrac (modified from [142]).

Action	Examples	Effect	References
Chemosensitization	Efflux of doxorubicin, P-glycoprotein effect	↓	[137, 138]
	Efficiency of chemotherapeutic agents	↑	[137, 138]
Radiosensitization	Repair of radiation-induced DSB	↓	[30, 31]
	Radiation-induced activation of integrin αvβ3	↓	[30, 31]
Cell survival, gene expression	Antiapoptotic genes (*XIAP, MCL-1*)	↓	[45, 139]
	Proapoptotic genes (*e.g. CASP2, BC2L14*)	↑	[45, 139]
	Stress-defense genes (*e.g. HIF-1α*)	↓	[45, 139]
Cell cycle	Cyclins and cyclin-dependent protein kinase genes	↓	[45, 138, 140, 142]
Growth factors pathways	*EGFR* gene expression and function	↓	[45, 57]
	Reduction of angiogenesis	↓	[138, 140, 142]

mainly due to the cross talk of integrin with several growth factors as we have seen so far (EGF, VEGF and osteopontin) that play a role in tumor growth. We summarize here some pathological situations where the use of nanotetrac could be beneficial, more examples can be found in Table **1**: 1) Tumor cell proliferation; 2) Tumor growth and energetic metabolism: the P-glycoprotein; 3) Reproductive Physiology; 4) Vascular diseases.

1. Tumor Cell Proliferation

Cheresh and co-workers have found that integrin αvβ3 support cell survival and proliferation independently of cell adhesion, cell attachment or matrix ligation [46]. Therefore, integrin αvβ3 expression can be considered a 'marker' for cancer stem cells (CSC) and defines a cancer stem cell population involved in the resistance to EGF receptor-targeted tyrosine kinase inhibitors [47]. Nanotetrac formulation inhibiting the integrin may represent an efficient therapeutic tool for a variety of situations involving tumor growth including the downregulation of the expression of mRNA of *EFGR* in estrogen receptor-negative human breast cancer MDA-MB-231 cells [45, 139].

2. Tumor Growth and Energetic Metabolism: The P-glycoprotein

Recent experimental evidences indicate that tumorigenesis may derive from imbalance of cell metabolism with an increase of glycolitic pathway with respect to mitochondria oxidative phosphorylation. This rewiring increases the lactate production and increases intracellular acidification, affecting in turn the redox balance and the survival of the cells [141]. Tumor cells may adapt to a more acidic environment. Several transport systems are able to modulate the pH in these cells such as carbonic anhydrase, Na/H exchangers and, monocarboxylate transporters. Targeting some of these transporters, such as the Na/H exchanger or the monocarboxylate transport, may counteract tumor progression and also reflect positively on redox equilibrium. Nanotetrac inhibits the integrin αvβ3, that in turn may inhibit the Na/H exchanger giving rise to sustained intracellular acidification, that negatively affect the P-glycoprotein (P-gp glycoprotein or multidrug resistance MDR-1 or ABCB1) activity, a plasma membrane efflux pump present in normal and tumor cells, in a way to increase the residence time of antitumor drugs such as doxorubicin and etoposide substrates of P-gp [142]. The inhibition of the Na/H exchanger can be achieved also with the classical inhibitors of this transport system, such as cariporide.

3. Reproductive Physiology

Additional studies on thyroid function, pregnancy and integrin αvβ3 have appeared in the last 20 years. Integrins may be markers for 'unexplained infertility' [82, 83] and expression of integrins may be altered in secretory and proliferative endometrium, but also in normal and malignant cells [86]. The different pattern of expression of integrins and in particular of integrin αvβ3, whose expression and activity is dependent on growth factors may help to identify factors that modulate its expression and the differences in expression and activity in normal endometrium and in tumor cells. In fact, increased expression of integrin αvβ3 is associated with the onset of pregnancy and this parameter can be considered a marker for successful *in vitro* fertilization [85]. Also in this case integrin αvβ3 can be considered a main player in different physiopathological situations. Integrin αvβ3 is considered a marker for angiogenesis and endometriosis in fact has been considered an angiogenic disease since 1998, that is those diseases that depend on excessive angiogenesis, together with solid tumors, rheumatoid artritis, psoriasis, diabetic retinopathy [142]. At that time the authors of the article were looking for therapies able to inhibit the angiogenesis at the integrin level. Nowadays, tetrac nanoparticulate may represent a valid therapeutic tool, not only for endometriosis, but also for the other angiogenesis -dependent diseases mentioned above.

4. Vascular Diseases

Vascular mineralization has become a serious problem, since it leads to ectopic calcification, a predictor of vascular diseases. The vitamin K-dependent matrix Gla-protein

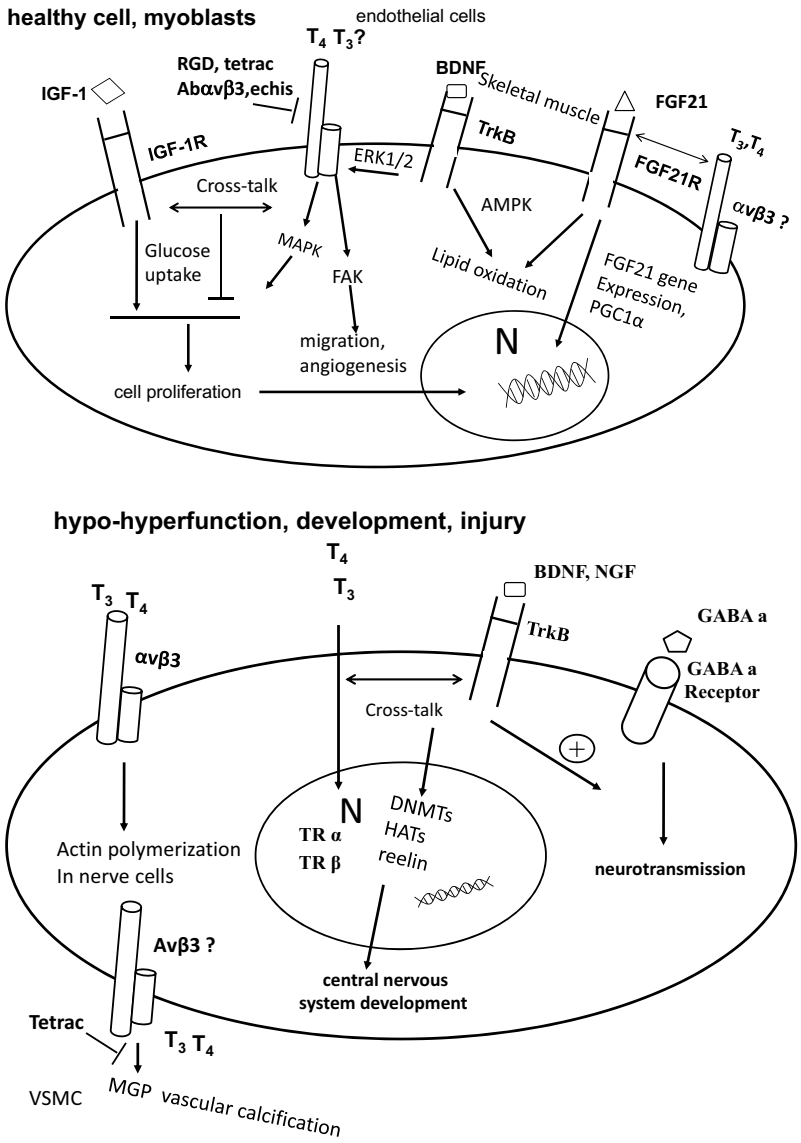

Fig. (2). Upper Panel: The cartoon shows some of the effects mediated by cross talk of thyroid hormones with growth factors, possibly mediated by integrin αvβ3. From left to right is shown the cross talk between thyroid hormones and IGF-1 in L6 myoblasts [48], then the interaction of integrin with BDNF in endothelial cells leading to migration and angiogenesis through the TrkB/integrin αvβ3/ERK1/2/FAK [150]. The role of thyroid hormones is only hypothesized here, whereas BDNF is known to be important for lipid oxidation in skeletal muscle [130]. The last part on the right shows the effect of FGF21 mediated by FGF21R and thyroid hormones leading to lipid oxidation as nongenomic effect, and *FGF21* gene expression and *PGC1α* gene expression as genomic effects [132-136]. The question mark refers to the possible role of integrin in this pathway. **Lower panel:** This cartoon refers to thyroid hypo- or hyperfunction and development. From left to right: the actin polymerization modulated by thyroid hormones in nerve cells is probably under the control of integrins (αvβ3 has a question mark). The cross talk between thyroid hormones and BDNF through TrkB is also shown, leading in hypothyroid animals to modulation of different genes [151], but also resulting in or contributing to the switch of the $GABA_A$ receptor from depolarizing postsynaptic signal in the course of development or after injury to hyperpolarization in the mature synapse [117, 121]. The lower left side of the cartoon shows the stimulation by thyroid hormones of MGP synthesis in vascular smooth muscle cells whose dysregulation may lead to vascular calcification [57-59].

(MGP) is widely recognized as an inhibitor of vascular calcification, as reported above [58]. Several atherogenic stimuli may promote vascular mineralization: inflammatory cytokines, oxidized lipids, high glucose, oxidative stress. Thyroid hormones stimulate the expression of this protein by genomic mechanisms in vascular smooth muscle cells, maybe as a part of angiogenic mechanism of these hormones. Thus thyroid hormone also serves to reduce calcification [51]. A summary of the effects of this paragraph is reported in Table **1**.

CONCLUSION

Thyroid hormone acts not only genomically through its nuclear receptors, TRα and TRβ, but also nongenomically through mechanisms that involve a plasma membrane recep-

tor on the αvβ3 integrin. Several growth factors such as EGF, FGF and IGF-1 which have tyrosine kinase receptors also crosstalk with integrins in the signaling mechanisms that lead to biological responses. In this review we have focused our attention on the interactions (crosstalk) between thyroid hormones and growth factors that may involve the αvβ3 integrin. We can draw the following conclusions from the new findings reviewed.

Thyroid hormone plays an important role in many physiological functions in metabolism and development, such as bone resorption and calcium mobilization from bone and these effects involve integrins. During skeletal development and growth, T_3 stimulates osteoblast activity, resulting in osteoblast differentiation, bone matrix synthesis and bone mineralization. Involved in this process is IGF-1 transcription that leads to Wnt and Akt pathway activation that is involved in growth plate ossification. Thyroid hormones modulate angiogenesis, and all the physiological processes related to it reported also in the previous paragraph for possible pharmacological applications involving vascular disease and reproduction. EGF plays an important role in early pregnancy, and T_3 not only interacts with EGF in placenta development and invasion of EVT, but also regulates angiogenic growth factor and cytokine secretion that ensure successful pregnancy.

Thyroid hormones also modulate immune response. T_4 potentiates the antiviral effect of IFN-γ via αvβ3 integrin. IGF-1 acts as a chemokine after an injury of muscle tissue and this is a new area that will be probably developed in the next years.

Thyroid hormones are important for the development of the central nervous system and their effects are both genomic and nongenomic; an example of the latter is actin polymerization, leading to cytoskeleton formation. T_3 modulates the secretion of bFGF, EGF and FGF and a recent study reports that T_3 is able to enhance restoration of the neuromuscular junction and synaptic transmission by an unknown mechanism. The interplay of thyroid hormones with growth factors such as NGF and EGF is well known and thyroid hormones play an important role in $GABA_A$ receptor-mediated transmission after injury, through the involvement of BDNF. In skeletal muscle, T_3 is able to increase the level of cytokines such as IL-6. The release of IL-6 also occurs with intense exercise and there is hormone crosstalk with BDNF which increases after exercise, and with the production of FGF-21, "an insulin-regulated myokine" that leads to fat oxidation. FGF-21 has received recent attention because it induces PGC1-α expression. Increased expression of this cytokine has been associated with chronically high levels of insulin and it is modulated by T_3.

Thyroid hormone involvement in a variety of metabolic and developmental processes is now widely acknowledged, and their interaction with growth factors represents an additional mechanism that may explain such processes. Understanding the role of thyroid hormones in this crosstalk may facilitate the development of new tools for therapeutic intervention, and in this context the nanotetrac formulation appears to be very promising. In this review we have focused on new developments in the field for reasons of space, but further examples can be found in recent reviews on thyroid hormone nongenomic effects concerning membrane transport systems [143], immune activities [144, 145], skeletal muscle [146], molecular aspects [147], and the possible clinical applications [148, 149]. Some of these effects are summarized in Fig. (**2**).

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists. The financial support from the Italian Ministry for University and Research, General Management for Strategies and Development of Internationalization of Scientific and Technological Research, a Lab Visit Grant from the Society for Endocrinology to Dr. R. G. Ahmed, and a grant from Roma Tre University are gratefully acknowledged.

REFERENCES

[1] Yen, P.M. Physiological and molecular basis of thyroid hormone action. *Physiol. Rev.,* **2001**, *81*, 1097-1142.

[2] Bianco, A.C.; Salvatore, D.; Gereben, B.; Berry, M.J.; Larsen, P.R. Biochemistry, cellular and molecular biology and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev*., **2002**, *23*, 38-89.

[3] Flamant, F.; Gauthier, K.; Samarut, J. Thyroid hormone signaling is getting more complex: STORMs are coming. *Mol. Endocrinol*., **2007**, *21*, 321-333.

[4] Wagner, R.I.; Apriletti, J.W.; McGrath, M.E.; West, B.L.; Baxter, J.D.; Fletterick, R.J. A structural role for hormone in the thyroid hormone receptor. *Nature,* **1995**, *378,* 690-697.

[5] Darimont, B.D.; Wagner, R.L.; Apriletti, J.W.; Stallcup, M.R.; Kushner, P.J.; Baxter, J.D.; Fletterick, R.J.; Yamamoto, K.R. Structure and specificity of nuclear receptor-coactivator interactions. *Genes Dev.,* **1998**, *12*, 3343-3356.

[6] Yen, P.M.; Ikeda, M.; Brubaker, J.H.; Forgione, M.; Sugawara, A.; Chin, W.W. Roles of v-erbA homodimers and heterodimers in mediating dominant negative activity by v-erbA. *J. Biol. Chem*., **1994**, *269*, 903-909.

[7] Bassett, J.H.; Harvey, C.B. Williams, G.R. Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. *Mol. Cell. Endocrinol*., **2003**, *213*, 1-11.

[8] Lin, H.-Y.; Davis, F.B.; Gordinier, J.K.; Martino, L.J.; Davis, P.J. Thyroid hormone induces activation of mitogen-activated protein kinase in cultured cells. *Am. J. Physiol.,* **1999**, *276*, 1014-1024.

[9] Davis, P.J.; Shih, A.; Lin, H.-Y.; Martino, L.J.; Davis, F.B. Thyroxine promotes association of mitogen-activated protein kinase and nuclear thyroid hormone receptor (TR) and causes serine phosphorylation of TR. *J. Biol. Chem.,* **2000**, *275,* 38032-38039.

[10] Shih, A.; Lin, H.-Y.; Davis, F.B.; Davis, P.J. Thyroid hormone promotes serine phosphorylation of p53 by mitogen-activated protein kinase. *Biochemistry,* **2001**, *40*, 2870-2878.

[11] Davis, P.J.; Davis, F.B.; Cody, V. Membrane receptors mediating thyroid hormone action. *Trends Endocrinol. Metab.,* **2005**, *16*, 429-435.

[12] Lei, J.; Mariash, C.N.; Bhargava, M.; Wattenberg, E.V.; Ingbar, D.H. T_3 increases Na-K ATPase activity via a MAPK/ERK1/2-dependent pathway in rat adult alveolar epithelial cells. *Am. J. Physiol. Cell Physiol*., **2008**, *294*, 749-754.

[13] D'Arezzo, S.; Incerpi, S.; Davis, F.B.; Acconcia, F.; Marino, M.; Farias, R.N.; Davis, P.J. Rapid nongenomic effects of 3,5,3'-triiodo-L-thyronine on intracellular pH of L-6 myoblasts are mediated by intracellular calcium mobilization and kinase pathways. *Endocrinology,* **2004**, *145*, 5694-5703.

[14] Moeller, L.C.; Cao, X.; Dumitrescu, A.M.; Seo, H.; Refetoff, S. Thyroid hormone mediated changes in gene expression can be initiated by cytosolic action of the thyroid hormone receptor through the phosphatidylinositol 3-kinase pathway. *Nucl. Recept. Signal,* **2006,** *4*,e020.

[15] Cao, X.; Kambe, F.; Moeller, L.C.; Refetoff, S.; Seo, H. Thyroid hormone induces rapid activation of Akt/protein kinase B-mammalian target of rapamycin-$p70^{S6K}$ cascade through phosphati-

dylinositol 3-kinase in human fibroblasts. *Mol. Endocrinol.,* **2005**, *19*, 102-112.

[16] Lei, J.; Mariash, C.N.; Ingbar, D.H. 3,3',5-Triiodo-L-thyronine up-regulation of Na,K-ATPase activity and cell surface expression in alveolar epithelial cells is Src kinase-and phosphatidylinositide 3-kinase-dependent. *J. Biol. Chem.,* **2004**, *279*, 47589-47600.

[17] Hiroi, Y.; Kim, H.H.; Furuya, F.; Huang, Z.; Simoncini, T.; Noma, K.; Ueki, K.; Nguyen. N.H.; Scanlan, T.S.; Moskowitz, M.A.; Cheng, S.Y.; Liao, J.K. Rapid nongenomic effects of thyroid hormone. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 14104-14109.

[18] Tang, H.Y.; Lin, H.-Y.; Zhang, S.; Davis, F.B.; Davis, P.J. Thyroid hormone causes mitogen-activated protein kinase-dependent phosphorylation of the nuclear estrogen receptor. *Endocrinology*, **2004**, *145*, 3265-3272.

[19] Moeller, L.C.; Dumitrescu, A.M.; Refetoff, S. Cytosolic action of thyroid hormone leads to induction of hypoxia-inducible factor-1 and glycolytic genes. *Mol. Endocrinol.,* **2005**, *19*, 2955-2963.

[20] Bergh, J.J.; Lin, H.-Y.; Lansing, L.; Mohamed, S.N.; Davis, F.B.; Mousa, S.; Davis, P.J. Integrin αVβ3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology,* **2005**, *146*, 2864-2871.

[21] Lin, H.-Y.; Sun, M.; Tang, H.Y.; Luidens, M.K.; Mousa, S.A.; Incerpi, S.; Drusano, G.L.; Davis, F.B.; Davis, P.J. L-Thyroxine vs. 3,5,3'-triiodothyronine and cell proliferation: Activation of mitogen activated protein kinase and phosphatidylinositol 3-kinase. *Am. J. Physiol. Cell. Physiol*., **2009**, *296*, 980-991.

[22] Barja-Fidalgo, C.; Coelho, A.L.J.; Saldanha-Gama, R.; Helal-Neto, E.; Mariano-Oliveira, A.; De Freitas, M.S. Disintegrins: integrin selective ligands which activate integrin-coupled signaling and modulate leukocyte functions. *Braz. J. Med. Biol. Res*., **2005**, *38*, 1513-1520.

[23] Hynes, R.O. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell,* **1992**, *69*, 11-25.

[24] Giancotti, F.G.; Ruoslahti, E. Integrin signaling. *Science*, **1999**, *285*, 1028-1032.

[25] Hosotani, R.; Kawaguchi, M.; Masui, T.; Koshiba, T.; Ida, J.; Fujimoto, K.; Wada, M.; Doi, R.; Imamura, M. Expression of integrin αvβ3 in pancreatic carcinoma: relation to MMP-2 activation and lymphonode metastasis. *Pancreas*, **2002**, *25*, 30-35.

[26] Gavrilovskaya, I.N.; Brown, E.J.; Ginsberg, M.H.; Mackow, E.R. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by β3 integrins. *J. Virol*., **1999**, *73*, 3951-3959.

[27] Ling, W.L.; Longley, R.L.; Brassard, D.L.; Armstrong, L.; Schaefer, E.J. Role of integrin αvβ3 in the production of recombinant adenovirus in HEK-293 cells. *Gene Ther*., **2002**, *9*, 907-914.

[28] Dellacasagrande, J.; Ghigo, E.; Hammami, T.; Toman, S.R.; Raoult, D.; Capo, C.; Mege, J.L. αvβ3 integrin and bacterial lipopolysaccharide are involved in *Coxiella burnetii*-stimulated production of tumor necrosis factor by human monocytes. *Infect. Immun*., **2000**, *68*, 5673-5678.

[29] Monferran, S.; Skuli, N.; Delmas, C.; Favre, G.; Bonnet, J.; Cohen-Jonathan-Moyal, E.; Toulas, C. αvβ3 and αvβ5 integrins control glioma cell response to ionising radiation through ILK and RhoB. *Int. J. Cancer*, **2008**, *123*, 357-364.

[30] Hercbergs, A.; Davis, P.J.; Davis, F.B.; Ciesielski, M.J.; Leith, J.T. Radiosensitization of GL261 glioma cells by tetraiodothyroacetic acid (tetrac). *Cell Cycle*, **2009**, *8*, 2586-2591.

[31] Hercbergs, A.H.; Lin, H.Y.; Davis, F.B.; Davis, P.J.; Leith, J.T. Radiosensitization and production of DNA double-strand breaks in U87MG brain tumor cells induced by tetraiodothyroacetic acid (tetrac). *Cell Cycle*, **2011,** *10*, 352-357.

[32] Lin, H.Y.; Thacore, H.R.; Davis, P.J.; Davis, F.B. Thyroid hormone potentiates the antiviral action of interferon-gamma in cultured human cells. *J. Clin. Endocrinol. Metab*., **1994**, *79*, 62-65.

[33] Lin, H.Y.; Thacore, H.R.; Davis, F.B.; Martino, L.J.; Davis, P.J. Potentiation by thyroxine of interferon-gamma-induced HLA-DR expression is protein kinase A and C-dependent. *J. Interferon Cytokine Res*., **1996**, *16*, 17-24.

[34] Lin, H.Y.; Shih, A.; Davis, F.B.; Davis, P.J. Thyroid hormone promotes the phosphorylation of STAT3b and potentiates the action of epidermal growth factor in cultured cells. *Biochem. J*., **1999**, *338*, 427-432.

[35] Lin, H.Y.; Martino, L.J.; Wilcox, B.D.; Davis, F.B.; Gordinier, J.K.; Davis, P.J. Potentiation by thyroid hormone of human IFN-γ-induced HLA-DR expression. *J. Immunol*., **1998**, *161*, 843-849.

[36] Mori, S.I.; Wu, C.Y.; Yamaji, S.; Saegusa, J.; Shi, B.; Ma, Z.; Kuwabara, Y.; Lam, K.S.; Isseroff, R.R.; Takada, Y.K.; Takada, Y. Direct binding of integrin αvβ3 to FGF1 plays a role in FGF1 signaling. *J. Biol. Chem*., **2008**, *283*, 18066-18075.

[37] Presta, M.; Dell'Era, P.; Mitola, S.; Moroni, E.; Ronca, R.; Rusnati, M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. *Cytokine Growth Factor Rev*., **2005**, *2*, 159-178.

[38] Ullrich, A.; Schlessinger, J. Signal transduction by receptors with tyrosine kinase activity. *Cell*, **1990**, *61*, 203-212.

[39] Powers, C.J.; McLeskey, S.W.; Wellstein, A. Fibroblast growth factors, their receptors and signaling. *Endocr. Relat. Cancer*, **2000**, *7*, 165-197.

[40] Klint, P.; Claesson-Welsh, L. Signal transduction by fibroblast growth factor receptors. *Front. Biosci*., **1999**, *4*, 165-177.

[41] Ieguchi, K.I.; Fujita, M.; Ma, Z.; Davari, P.; Taniguchi, Y.; Sekiguchi, K.; Wang, B.; Takada, Y.K; Takada, Y. Direct binding of the EGF-like domain of neuregulin-1 to integrins αvβ3 and α6β4 is involved in neuregulin-1/ErbB signaling. *J. Biol. Chem*., **2010**, *285*, 31388-31398.

[42] Saegusa, J.I.; Yamaji, S.; Ieguchi, K.; Wu, C.Y.; Lam, K.S.; Liu, F.T.; Takada, Y.K.; Takada, Y. The direct binding of insulin-like growth factor-1 (IGF-1) to integrin αvβ3 is involved in IGF-1 signaling. *J. Biol. Chem*., **2009**, *284*, 24106-24114.

[43] Shih, A.; Zhang, S.; Cao, H.J.; Tang, H.Y.; Davis, F.B.; Davis, P.J.; Lin, H.Y. Disparate effects of thyroid hormone on actions of epidermal growth factor and transforming growth factor-α are mediated by 3',5'-cyclic adenosine 5'-monophosphate-dependent protein kinase II. *Endocrinology*, **2004**, *145*, 1708-1717.

[44] Shih, A.; Zhang, S.; Cao, H.J.; Boswell, S.; Wu, Y.H.; Tang, H.Y.; Lennartz, M.R.; Davis, F.B.; Davis, P.J.; Lin, H.Y. Inhibitory effect of epidermal growth factor on resveratrol-induced apoptosis in prostate cancer cells is mediated by protein kinase C-alpha. *Mol. Cancer Therap*., **2004**, *3*, 1355-1364.

[45] Glinskii, A.B.; Glinsky, G.V.; Lin, H.Y.; Tang, H.Y.; Sun, M.; Davis, F.B.; Luidens, M.K.; Mousa, S.A.; Hercbergs, A.H.; Davis, P.J. Modification of survival pathway gene expression in human breast cancer cells by tetraiodothyroacetic acid (tetrac). *Cell Cycle*, **2009**, *8*, 3562-3570.

[46] Seguin, L.; Gozo, M.; Weis, S.M.; Cheresh, D.A. Targeting the Achilles' heel of drug-resistant cancer stem cells. *Cell Cycle*, **2014**, *13*, 2017-2018.

[47] Seguin, L.; Kato, S.; Franovic, A.; Camargo, M.F.; Lesperance, J.; Elliott, K.C.; Yebra, M.; Mielgo, A.; Lowy, A.M.; Husain, H.; Cascone, T.; Diao, L.; Wang, J.; Wistuba, I.I.; Heymach, J.V.; Lippman, S.M.; Desgrosellier, J.S.; Anand, S.; Weis, S.M.; Cheresh, D.A. An integrin β_3-KRAS-RalB complex drives tumour stemness and resistance to EGFR inhibition. *Nat. Cell. Biol*., **2014**, *16*, 457-68.

[48] Incerpi, S.; Hsieh, M.T.; Lin, H.Y.; Cheng, G.Y.; De Vito, P.; Fiore, A.M.; Ahmed, R.G.; Salvia, R.; Candelotti, E.; Leone, S.; Luly, P.; Pedersen, J.Z.; Davis, F.B.; Davis, P.J. Thyroid hormone inhibition in L6 myoblasts of IGF-I-mediated glucose uptake and proliferation: new roles for integrin αvβ3. *Am. J. Physiol. Cell Physiol*., **2014**, *307*, 150-161.

[49] Chilian, W.M.; Wangler, R.D.; Peters, K.G.; Tomanek, R.J.; Marcus, M.L. Thyroxine-induced left ventricular hypertrophy in the rat. Anatomical and physiological evidence for angiogenesis. *Circ. Res.*, **1985**, *57*, 591-598.

[50] Zheng, W.I.; Weiss, R.M.; Wang, X.; Zhou, R.; Arlen, A.M.; Lei, L.; Lazartigues, E.; Tomanek, R.J. DITPA stimulates arteriolar growth and modifies myocardial postinfarction remodeling. *Am. J. Physiol. Heart Circ. Physiol*., **2004**, *286*, 1994-2000.

[51] Luidens, M.K.; Mousa, S.A.; Davis, F.B.; Lin, H.Y.; Davis, P.J. Thyroid hormone and angiogenesis. *Vascul. Pharmacol*., **2010**, *52*, 142-145.

[52] Wang, X.; Zheng, W.; Christensen, L.P.; Tomanek, R.J. DITPA stimulates bFGF, VEGF, angiopoietin, and Tie-2 and facilitates coronary arteriolar growth. *Am. J. Physiol. Heart Circ. Physiol*., **2004**, *286*, 1994-2000.

[53] Davis, F.B.; Mousa, S.A.; O'Connor, L.; Mohamed, S.; Lin, H.Y.; Cao, H.J.; Davis, P.J. Proangiogenic action of thyroid hormone is

fibroblast growth factor-dependent and is initiated at the cell surface. *Circ. Res*., **2004**, *94*, 1500-1506.

[54] Mousa, S.A.; O'Connor, L.; Davis, F.B.; Davis, P.J. Proangiogenesis action of the thyroid hormone analog 3,5-diiodothyropropionic acid (DITPA) is initiated at the cell surface and is integrin mediated. *Endocrinology*, **2006**, *147*, 1602-1607.

[55] Mousa, S.A.; O'Connor, L.J.; Bergh, J.J.; Davis, F.B.; Scanlan, T.S.; Davis, P.J. The proangiogenic action of thyroid hormone analogue GC-1 is initiated at an integrin. *J. Cardiovasc. Pharmacol*., **2005**, *46*, 356-360.

[56] Hoffman, S.J.; Vasko-Moser, J.; Miller, W.H.; Lark, M.W.; Gowen, M.; Stroup, G. Rapid inhibition of thyroxine-induced bone resorption in the rat by an orally active vitronectin receptor antagonist. *J. Pharmacol. Exp. Ther*., **2002**, *302*, 205-211.

[57] Sato, Y.; Nakamura, R.; Satoh, M.; Fujishita, K.; Mori, S.; Ishida, S.; Yamaguchi, T.; Inoue, K.; Nagao, T.; Ohno, Y. Thyroid hormone targets matrix Gla protein gene associated with vascular smooth muscle calcification. *Circ. Res*., **2005**, *97*, 550-557.

[58] Schurgers, L.J.; Uitto, J.; Reutelingsperger, C.P. Vitamin K-dependent carboxylation of matrix Gla-protein: a crucial switch to control ectopic mineralization. *Trends Mol. Med*., **2013**, *19*, 217-226.

[59] Dalmeijer, G.W.; Van der Schouw, Y.T.; Vermeer, C.; Magdeleyns, E.J.; Schurgers, L.J.; Beulens, J.W. Circulating matrix Gla protein is associated with coronary artery calcification and vitamin K status in healthy women. *J. Nutr. Biochem*., **2013**, *24*, 624-628.

[60] Loeser, R.F. Integrins and chondrocyte–matrix interactions in articular cartilage. *Matrix Biol*., **2014**, *39*, 11–16.

[61] Lin, H.Y.; Su, Y.F.; Hsieh, M.T.; Lin, S.; Meng, R.; London, D.; Lin, C.; Tang, H.Y.; Hwang, J.; Davis, F.B.; Mousa, S.A.; Davis, P.J. Nuclear monomeric integrin αv in cancer cells is a coactivator regulated by thyroid hormone. *FASEB J*., **2013**, *27*, 3209-3216.

[62] Stevens, D.A.; Harvey, C.B.; Scott, A.J.; O'Shea, P.J.; Barnard, J.C.; Williams, A.J.; Brady, G.; Samart, J.; Chassande, O.; Williams, G.R. Thyroid hormone activates fibroblast growth factor receptor-1 in bone. *Mol. Endocrinol*., **2003**, *17*, 1751-1766.

[63] Wojcicka, A.; Bassett, J.H.; Williams, G.R. Mechanisms of action of thyroid hormones in the skeleton. *Biochim. Biophys. Acta*, **2013**, *1830*, 3979-3986.

[64] Allain, T.J.; Chambers, T.J.; Flanagan, A.M.; McGregor, A.M. Triiodothyronine stimulates rat osteoclastic bone resorption by an indirect effect. *J. Endocrinol*., **1992**, *133*, 327-31.

[65] Kim, C.H.; Kim, H.K.; Shong, Y.K.; Lee, K.U.; Kim, G.S. Thyroid hormone stimulates basal and interleukin (IL)-1-cells-induced IL-6 production in human bone marrow stromal: a possible mediator of thyroid hormone-induced bone loss. *J. Endocrinol*., **1999**, *160*, 97-102.

[66] Siddiqi, A.; Burrin, J.M.; Wood, D.F.; Monson, J.P. Triiodothyronine regulates the production of interleukin-6 and interleukin-8 in human bone marrow stromal and osteoblast-like cells. *J. Endocrinol.*, **1998**, *157*, 453-461.

[67] Miura, M.; Tanaka, K.; Komatsu, Y.; Suda, M.; Yasoda, A.; Sakuma, Y.; Ozasa, A.; Nakao, K. A novel interaction between thyroid hormones and $1,25(OH)_2D_3$ in osteoclast formation. *Biochem. Biophys. Res. Commun*., **2002**, *291*, 987-994.

[68] Wang, L.; Shao, Y.Y.; Ballock, R.T. Thyroid hormone-mediated growth and differentiation of growth plate chondrocytes involves IGF-1 modulation of β-catenin signaling. *J. Bone Miner. Res.*, **2010**, *25*, 1138-1146.

[69] Hodkinson, C.F.; Simpson, E.E.; Beattie, J.H.; O'Connor, J.M.; Campbell, D.J.; Strain, J.J.; Wallace, J.M. Preliminary evidence of immune function modulation by thyroid hormones in healthy man and woman aged 55-70 years. *J. Endocrinol*., **2009**, *202*, 55-63.

[70] Haddad, J.J.; Saadé, N.E.; Safieh-Garabedian, B. Cytokines and neuro-immune-endocrine interactions: a role for the hypothalamic-pituitary adrenal revolving axis. *J. Neuroimmunol*., **2002**, *133*, 1-19.

[71] Pállinger, E.; Csaba, G. A hormone map of human immune cell showing the presence of adrenocorticotropic hormone, triiodothyronine and endorphin in immunophenotyped white blood cells. *Immunology*, **2008**, *123*, 584-589.

[72] Csaba, G.; Kovács, P.; Pállinger, E. Effect of the inhibition of triiodothyronine (T_3) production by thiamazole on the T_3 and serotonin content of immune cells. *Life Sci*., **2005**, *18*, 2043-2052.

[73] Kamath, J.; Yarbrough, G.G.; Prange, Jr A.J.; Winokur, A. The thyrotropin-releasing hormone (TRH)-immune system homeostatic hypothesis. *Pharmacol. Ther.*, **2009**, *121*, 20-28.

[74] Csába, G.; Pállinger, E. Thyrotropic hormone (TSH) regulation of triiodothyronine (T_3) concentration in immune cells. *Inflamm. Res*., **2009**, *58*, 151-154.

[75] Lin, H.Y.; Thacore, H.R.; Davis, F.B.; Davis, P.J. Potentiation by thyroxine of interferon-γ-induced antiviral state requires PKA and PKC activities. *Am. J. Physiol. Cell. Physiol*., **1996**, *271*, 1256-1261.

[76] Pillon, N.J.; Bilan, P.J.; Fink, L.N.; Klip, A. Cross-talk between skeletal muscle and immune cells: muscle-derived mediators and metabolic implications. *Am. J. Physiol. Endocrinol. Metab*., **2013**, *304*, 453-465.

[77] Mancini, M.; Gariboldi, M.B.; Taiana, E.; Bonzi, M.C.; Craparotta, I.; Pagin, M.; Monti, E. Co-targeting the IGF system and HIF-1 inhibits migration and invasion by (triple-negative) breast cancer cells. *Br. J. Cancer*, **2014**, *110*, 2865-2873.

[78] Ahmed, R.G.; Incerpi, S. Gestational doxorubicin alters fetal thyroid-brain axis. *Int. J. Dev. Neurosci.*, **2013**, *31*, 96-104.

[79] Ahmed, R.G. Perinatal TCDD exposure alters developmental neuroendocrine system. *Food Chem. Toxicol*., **2011**, *49*, 1276-1284.

[80] Krassas, G.E.; Poppe, K.; Glinoer. D. Thyroid function and human reproductive health. *Endocr. Rev*., **2010**, *31*, 702-755.

[81] Krassas, G.E.; Pontikides, N.; Kaltsas, T.; Papadopoulou, P.; Batrinos, M. Menstrual disturbances in thyrotoxicosis. *Clin. Endocrinol*., **1994**, *40*, 641-644.

[82] Lessey, B.A. Integrins and reproduction revisited. *Eur. J. Obstet. Gynecol. Reprod. Biol*., **1995**, *62*, 264-265.

[83] Lessey, B.A.; Castelbaum, A.J.; Sawin, S.W.; Sun, J. Integrins as markers of uterine receptivity in women with primary unexplained infertility. *Fertility Steril*., **1995**, *63*, 535-542.

[84] Bridges, J.E.; Prentice, A.; Roche, W.; Englefield, P.; Thomas, E.J. Expression of integrin adhesion molecules in endometrium and endometriosis. *Br. J. Obstet. Gynaecol*., **1994**, *101*, 696-700.

[85] Lessey, B.A. Assessment of endometrial receptivity. *Fertility Steril*., **2011**, *96*, 522-529.

[86] Franasiak, J.M.; Holoch, K.J.; Yuan, L.; Schammel, D.P.; Young, S.L; Lessey, B.A. Prospective assessment of midsecretory endometrial leukemia inhibitor factor expression versus αvβ3 testing in women with unexplained infertility. *Fertility Steril*., **2014**, *101*, 1724-1731.

[87] Lessey, B.A.; Albelda, S.; Buck, C.A.; Castelbaum, A.J.; Yeh, I.; Kohler, M.; Berchuck, A. Distribution of integrin cell adhesion molecules in endometrial cancer. *Am. J. Pathol*., **1995**, *146*, 717-726.

[88] Lessey, B.A. Integrins and reproduction revisited. *Eur. J. Obstet. Gynecol. Reprod. Biol*., **1995**, *62*, 264-265.

[89] Ceydeli, N.; Kaleli, S.; Calay, Z.; Erel, C.T.; Akbas, F.; Ertungealp, E. Difference in αvβ3 integrin expression in endometrial stromal cell in subgroups of women with unexplained infertility. *Eur. J. Obstet. Gynecol. Reprod. Biol*., **2006**, *126*, 206-211.

[90] Boroujerdnia, M.G.; Nikbakht, R. Beta3 integrin expression within uterine endometrium and its relationship with unexplained infertility. *Pak. J. Biol. Sci*., **2008**, *11*, 2495-2459.

[91] Lessey, B.A. Implantation defects in infertile women with endometriosis. *Ann. N. Y. Acad. Sci*., **2002**, *955*, 265-280, discussion 293-295 and 396-406.

[92] Kabir-Salmani, M.; Shiokawa, S.; Akimoto, Y.; Sakai, K.; Nagamatsu, S.; Sakai, K.; Nakamura, Y.; Lotfi, A.; Kawakami, H.; Iwashita, M. αvβ3 integrin signaling pathway is involved in insulin-like growth factor I-stimulated human extravillous trophoblast cell migration. *Endocrinology*, **2003**, *144*, 1620-1630.

[93] Mayama, R.; Izawa, T.; Sakai, K.; Suciu, N.; Iwashita, M. Improvement of insulin sensitivity promotes extravillous trophoblast cell migration stimulated by insulin-like growth factor-I. *Endocr. J*., **2013**, *60*, 359-368.

[94] Kabir-Salmani, M.; Fukuda, M.N.; Kanai-Azuma, M.; Ahmed, N.; Shiokawa, S.; Akimoto, Y.; Sakai, K.; Nagamori, S.; Kanai, Y.; Sugihara, K.; Iwashita, M. The membrane-spanning domain of CD98 heavy chain promotes αvβ3 integrin signals in human extravillous trophoblasts. *Mol. Endocrinol*., **2008**, *22*, 707-715.

[95] Laoag-Fernandez, J.B.; Matsuo, H.; Murakoshi, H.; Hamada, A.L.; Tsang, B.K.; Maruo, T. 3,5,3'-Triiodothyronine down-regulates Fas and Fas ligand expression and suppresses caspase-3 and poly (adenosine 5'-diphosphate-ribose) polymerase cleavage and apop-

tosis in early placental extravillous trophoblasts in vitro. *J. Clin. Endocrinol. Metab*., **2004**, *89*, 4069-4077.

[96] Lee, C.H.; Kim, T.H.; Lee, J.H.; Oh, S.J.; Yoo, J.Y.; Kwon, H.S.; Kim, Y.I.; Ferguson, S.D.; Ahn, J.Y.; Ku, B.J.; Fazleabas, A.T.; Lim, J.M.; Jeong, J.W. Extracellular signal-regulated kinase 1/2 signaling pathway is required for endometrial decidualization in mice and human. *PLoS One*, **2013**, *8* e75282.

[97] Roskoski, R. Jr. ERK1/2 MAP kinases: structure, function, and regulation. *Pharmacol. Res*., **2012**, *66*, 105-143.

[98] Large, M,J.; Wetendorf, M.; Lanz, R.B.; Hartig, S.M.; Creighton, C.J.; Mancini, M.A.; Kovanci, E.; Lee, K.F.; Threadgill, D.W.; Lydon, J.P.; Jeong, J.W.; De Mayo, F.J. The epidermal growth factor receptor critically regulates endometrial function during early pregnancy. *PLoS Genet*., **2014**, *10*, e1004451.

[99] Brosens, J.J.; Hayashi, N.; White, J.O. Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. *Endocrinology,* **1999**, *140*, 4809-4820.

[100] Barber, K.J.; Franklyn, J.A.; McCabe, C.J.; Khanim, F.L.; Bulmer, J.N.; Whitley, G.S.; Kilby, M.D. The in vitro effects of triiodothyronine on epidermal growth factor-induced trophoblast function. *J. Clin. Endocrinol. Metab*., **2005**, *90*, 1655-1661.

[101] Vasilopoulou, E.; Loubière, L.S.; Lash, G.E.; Ohizua, O.; McCabe, C.J.; Franklyn, J.A.; Kilby, M.D.; Chan, S.Y. Triiodothyronine regulates angiogenic growth factor and cytokine secretion by isolated human decidual cells in a cell-type specific and gestational age-dependent manner. *Hum. Reprod.*, **2014**, *29*, 1161-1172.

[102] Mohácsik, P.; Zeöld, A.; Bianco, A.C.; Gereben, B. Thyroid hormone and the neuroglia: both source and target. *J. Thyroid Res*., **2011**, 215718.

[103] Leonard, J.L. Non-genomic actions of thyroid hormone in brain development. *Steroids*, **2008**, *73*, 1008-1012.

[104] Farwell, A.P.; Tranter, M.P.; Leonard, J.L. Thyroxine-dependent regulation of integrin-laminin interactions in astrocytes. *Endocrinology*, **1995**, *136*, 3909-3915.

[105] Schumacher, M.; Jung-Testas, I.; Robel, P.; Baulieu, E.E. Insulin-like growth factor I: a mitogen for rat Schwann cells in the presence of elevated levels of cyclic AMP. *Glia*, **1993**, *8*, 232-240.

[106] Sondell, M.; Fex-Svenningsen, A.; Kanje, M. The insulin-like growth factors I and II stimulate proliferation of different types of Schwann cells. *Neuroreport*, **1997**, *8*, 2871-2876.

[107] Puglielli L. Aging of the brain, neurotrophin signaling, and Alzheimer's disease: is IGF1-R the common culprit? *Neurobiol. Aging*, **2008**, *29*, 795-811.

[108] Sondell, M.; Lundborg, G.; Kanje, M. Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system. *J. Neurosci*., **1999**, *19*, 5731-5740.

[109] Mourouzis, I.; Politi, E.; Pantos, C. Thyroid hormone and tissue repair: new tricks for an old hormone? *J. Thyroid Res*., **2013**, 312104.

[110] Papakostas, I.D.; Macheras, G.A. Thyroid hormones and peripheral nerve regeneration. *J. Thyroid Res*., **2013**, 648395.

[111] Panaite, P.A.; Ibtissam, B.W. Thyroid hormone enhances transected axonal regeneration and muscle reinnervation following rat sciatic nerve injury. *J. Neurosci. Res*., **2010**, *88*, 1751-1763.

[112] Li, W.W.; Le Goascogne, C.; Schumacher, M.; Pierre, M.; Courtin, F. Type 2 deiodinase in the peripheral nervous system: induction in the sciatic nerve after injury. *Neuroscience*, **2001**, *107*, 507-518.

[113] Voinesco, F.; Glauser, L.; Kraftsik, R.; Barakat-Walter, I. Local administration of thyroid hormones in silicone chamber increases regeneration of rat transected sciatic nerve. *Exp. Neurol*., **1998**, *150*, 69-81.

[114] Grothe, C.; Nikkhah, G. The role of basic fibroblast growth factor in peripheral nerve regeneration. *Anat. Embryol*., **2001**, *204*, 171-177.

[115] Zrouri, H.; Le Goascogne, C.; Li, W.W.; Pierre, M.; Courtin, F. The role of MAP kinases in rapid gene induction after lesioning of the rat sciatic nerve. *Eur. J. Neurosci*., **2004**, *20*, 1811-1818.

[116] Fisher, D.A.; Hoath, S.; Lakshmanan, J. The thyroid hormone effects on growth and development may be mediated by growth factors. *Endocrinol. Exp*., **1982**, *16*, 259-271.

[117] Shulga, A.; Rivera, C. Interplay between thyroxin, BDNF and GABA in injured neurons. *Neuroscience*, **2013**, *239*, 241-252.

[118] Sinha, R.A.; Pathak, A.; Kumar, A.; Tiwari, M.; Shrivastava, A.; Godbole, M.M. Enhanced neuronal loss under perinatal hypothyroidism involves impaired neurotrophic signaling and increased proteolysis of p75(NTR). *Mol. Cell. Neurosci*., **2009**, *40*, 354-364.

[119] Koibuchi, N.; Yamaoka, S.; Chin, W.W. Effect of altered thyroid status on neurotrophin gene expression during postnatal development of the mouse cerebellum. *Thyroid*, **2001**, *11*, 205-210.

[120] Camboni, D.; Roskoden, T.; Schwegler, H. Effect of early thyroxine treatment on brain-derived neurotrophic factor mRNA expression and protein amount in the rat medial septum/diagonal band of Broca. *Neurosci. Lett*., **2003**, *350*, 141-144.

[121] Sui, L.; Ren, W.W.; Li, B.M. Administration of thyroid hormone increases reelin and brain-derived neurotrophic factor expression in rat hippocampus in vivo. *Brain Res*., **2010**, *1313*, 9-24.

[122] Lüesse, H.G.; Roskoden, T.; Linke, R.; Otten, U.; Heese, K.; Schwegler, H. Modulation of mRNA expression of the neurotrophins of the nerve growth factor family and their receptors in the septum and hippocampus of rats after transient postnatal thyroxine treatment. I. Expression of nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin 4 mRNA. *Exp. Brain Res*., **1998**, *119*, 1-8.

[123] Koibuchi N, Chin WW. Thyroid hormone action and brain development. *Trends Endocrinol. Metab*., **2000**, *11*, 123-128.

[124] Pillon, N.J.; Bilan, P.J.; Fink, L.N.; Klip, A. Cross-talk between skeletal muscle and immune cells: muscle-derived mediators and metabolic implications. *Am. J. Physiol. Endocrinol. Metab*., **2013**, *304*, 453-465.

[125] Pedersen, B.K. Muscles and their myokines. *J. Exp. Biol*., **2011**, *214*, 337-346.

[126] Pedersen, B.K. Muscle as a secretory organ. *Compr. Physiol*., **2013**, *3*, 1337-1362.

[127] Langberg, H.; Olesen, J.L.; Gemmer, C.; Kjaer, M. Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. *J. Physiol*., **2002**, *542*, 985-990.

[128] Nybo, L.; Nielsen, B.; Pedersen, B.K.; Møller, K.; Secher, N.H. Interleukin-6 release from the human brain during prolonged exercise. *J. Physiol*., **2002**, *542*, 991-995.

[129] Keller, C.; Keller, P.; Marshal, S.; Pedersen, B.K. IL-6 gene expression in human adipose tissue in response to exercise–effect of carbohydrate ingestion. *J. Physiol*., **2003**, *550*, 927-931.

[130] Matthews, V.B.; Aström, M.B.; Chan, M.H.; Bruce, C.R.; Krabbe, K.S.; Prelovsek, O.; Akerström, T.; Yfanti, C.; Broholm, C.; Mortensen, O.H.; Penkowa, M.; Hojman, P.; Zankari, A.; Watt, M.J.; Bruunsgaard, H.; Pedersen, B.K.; Febbraio, M.A. Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia*, **2009**, *52*, 1409-1418.

[131] Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **1985**, *28*, 412-419.

[132] Izumiya, Y.; Bina, H.A.; Ouchi, N.; Akasaki, Y.; Kharitonenkov. A.; Walsh, K. FGF21 is an Akt-regulated myokine. *FEBS Lett*., **2008**, *582*, 3805-3810.

[133] Potthoff, M.J.; Inagaki, T.; Satapati, S.; Ding, X.; He, T.; Goetz, R.; Mohammadi, M.; Finck, B.N.; Mangelsdorf, D.J.; Kliewer, S.A.; Burgess, S.C. FGF21 induces PGC-1α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proc. Natl. Acad. Sci*., **2009**, *106*, 10853-10858.

[134] Hojman, P.; Pedersen, M.; Nielsen, A.R.; Krogh-Madsen, R.; Yfanti, C.; Akerstrom, T.; Nielsen, S.; Pedersen, B.K. Fibroblast growth factor-21 is induced in human skeletal muscles by hyperinsulinemia. *Diabetes*, **2009**, *58*, 2797-2801.

[135] Iglesias, P.; Selgas, R.; Romero, S.; Díez, J.J. Biological role, clinical significance, and therapeutic possibilities of the recently discovered metabolic hormone fibroblastic growth factor 21. *Eur. J. Endocrinol*., **2012**, *167*, 301-319.

[136] Domouzoglou, E.M.; Fisher, F.M.; Astapova, I.; Fox, E.C.; Kharitonenkov, A.; Flier, J.S.; Hollenberg, A.N.; Maratos-Flier, E. Fibroblast growth factor 21 and thyroid hormone show mutual regulatory dependency but have independent actions *in vivo*. *Endocrinology*, **2014**, *155*, 2031-2040.

[137] Davis, P.J.; Incerpi, S.; Lin, H.Y.; Tang, H.Y.; Sudha, T.; Mousa, S.A. Thyroid hormone and P-glycoprotein in tumor cells. *BioMed Res. Int*., **2015**, *8*, 168427.

[138] Davis, P.J.; Davis, F.B; Mousa, S.A.; Luidens, M.K.; Lin, H.Y. Membrane receptor for thyroid hormone: physiologic and pharmacologic implications. *Ann. Rev. Pharmacol. Toxicol.*, **2011**, *51*, 99-115.

[139] Lin, H.Y.; Landersdorfer, C.B.; London, D.; Meng, R.; Lim, C.U.; Lin, C.; Lin, S.; Tang, H.Y.; Brown, D., Van Scoy, B.; Kulawy, R.; Queimado, L.; Drusano, G.L.; Louie, A.; Davis, F.B.; Mousa, S.A.; Davis, P.J. Pharmacodynamic modeling of anti-cancer activity of tetraiodothyroacetic acid in a perfused cell culture system. *PLoS Comput. Biol*., **2011**, *7*, e1001073.

[140] Davis, P.J.; Lin, H.Y.; Sudha, T.; Yalcin, M.; Tang, H.Y.; Hercbergs, A.; Leith, J.T.; Luidens, M.K.; Ashur-Fabian O.; Incerpi, S.; Mousa, S.A. Nanotetrac targets integrin αvβ3 on tumor cells to disorder cell defense pathways and block angiogenesis. *Onco Targets Ther.*, **2014**, *7*, 1619-1624.

[141] Michels, J.; Obrist, F.; Castedo, M.; Vitale, I.; Kroemer, G. PARP and other prospective targets for poisoning cancer cell metabolism. *Biochem. Pharmacol*., **2014**, *92*,164-171.

[142] Davis, P.J.; Glinsky, G.V.; Lin, H.Y.; Leith, J.T.; Hercbergs, A.; Tang, H.Y.; Ashur-Fabian, O.; Incerpi, S.; Mousa, S.A. Cancer Cell Gene Expression Modulated from Plasma Membrane Integrin αvβ3 by Thyroid Hormone and Nanoparticulate Tetrac. *Front. Endocrinol.*, **2015**, *5,* 240.

[143] Healy, D.L.; Rogers, P.A.; Hii, L.; Wingfield, M. Angiogenesis: a new theory for endometriosis. *Hum. Reprod. Update*, **1998**, *4*, 736-740.

[144] Farias, R.N.; Fiore, A.M.; Pedersen, J.Z.; Incerpi, S. Nongenomic actions of thyroid hormones: focus on membrane transport systems. *Immunol. Endocr. Metab. Agents Med. Chem*., **2006**, *6*, 241-254.

[145] De Vito, P.; Incerpi, S.; Pedersen, J.Z.; Luly, P.; Davis, F.B.; Davis, P.J. Thyroid hormones as modulators of immune activities at the cellular level. *Thyroid*, **2011**, *21*, 879-890.

[146] De Vito, P.; Balducci, V.; Leone, S.; Percario, Z.; Mangino, G.; Davis, P.J.; Davis, F.B.; Affabris, E.; Luly, P.; Pedersen, J.Z.; Incerpi, S. Nongenomic effects of thyroid hormones on the immune system cells: New targets, old players. *Steroids*, **2012**, *77*, 988-995.

[147] Incerpi, S.; De Vito, P.; Pedersen, J.Z.; Luly, P. Extranuclear effects of thyroid hormones in skeletal muscle. *Immunol. Endocr. Metab. Agents Med. Chem*., **2011**, *11*, 188-198.

[148] Cheng, S.Y.; Leonard, J.L.; Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr. Rev*., **2010**, *31*, 139-170.

[149] Ahmed, R.G.; Davis, P.J.; Davis, F.B.; De Vito, P.; Farias, R.N.; Luly, P.; Pedersen, J.Z.; Incerpi, S. Nongenomic actions of thyroid hormones: from basic research to clinical applications. An update. *Immunol. Endocr. Metab. Agents Med. Chem*., **2013**, *13*, 46-59.

[150] Matsuda, S.; Fujita, T.; Kajiya, M.; Takeda, K.; Shiba, H.; Kawaguchi, H.; Kurihara, H. Brain-derived neurotrophic factor induces migration of endothelial cells through a TrkB-ERK-integrin αVβ3-FAK cascade. *J. Cell. Physiol.*, **2012**, *227*, 2123-2129.

[151] Sui, L.; Li, B.M. Effects of perinatal hypothyroidism on regulation of reelin and brain-derived neurotrophic factor gene expression in rat hippocampus: Role of DNA methylation and histone acetylation. *Steroids,* **2010**, *75,* 988-997.

Received: December 11, 2014 Revised: April 02, 2015 Accepted: April 02, 2015