Deiodinases and their intricate role in thyroid hormone homeostasis

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Abstract

The deiodinases are a family of enzymes that mediate the activation and inactivation of thyroid hormone. Their role in the regulation of systemic thyroid hormone concentration is well established and underpins the treatment of common thyroid diseases. Interest in this field was recently renewed when the deiodinases were implicated in tissue development and homeostasis, and also in a wide range of human diseases. Three deiodinases have been identified, namely, D1, D2 and D3, which differ in their structure, catalytic properties, and tissue distribution. Notably, the expression of these enzymes changes during life in relation to the different needs of each organ and to ageing. The systemic homeostatic role of deiodinases clearly emerges during changes in serum thyroid hormone concentrations as seen in patients with thyroid dysfunction. On the other hand, the role played by the deiodinases at tissue level represents a mechanism whereby thyroid hormone signaling can be fine-tuned within a given cell in a precise time-space window without perturbing serum thyroid hormone concentrations. This review maps the overall functional role of the deiodinases and explores challenges and novel opportunities arising from the expanding knowledge of these "master" components of the thyroid homeostatic system.

Introduction

Thyroid hormones (THs) influence the differentiation, growth and energy metabolism of virtually all cells and tissues. The biological action of THs is mediated through the interaction of T3 with nuclear TH receptors, and their binding to chromatin[1]. In healthy individuals, the thyroid gland produces T3 (the most biologically active form of thyroid hormone) and T4 (a weaker receptor ligand than T3) in a ratio of approximately 1:14. T3 plasma homeostasis in vertebrates is guaranteed by two potent systems that control the synthesis, secretion and metabolism of T4 and T3, and therefore their circulating concentration. They also allow the modulation of TH availability in target cells[2]. One system is based on central regulation operated by the hypothalamus-pituitary-thyroid axis (HPT) that maintains serum TH levels at remarkably stable levels through the actions of thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH). The HPT axis is negatively controlled by T4 and T3 in the circulation. Suppression of TSH and TRH secretion is mostly mediated by T4 and its intracellular conversion to T3 in pituitary cells and in hypothalamic TRH neurons respectively[3,4,5,6]. An increase in circulating T4 and the consequent increase in intracellular T3 concentration in hypothalamic TRH neurons and in pituitary thyrotrophs shuts down TRH and TSH gene expression, whereas a drop in circulating T4 results in an opposite effect[4]. The second system operates at intracellular level when T3 and T4 enter the cell via TH transporters[7]. At least 20 cell-specific TH transporters have been identified so far[7]. Once inside the cell, the concentration of TH is tightly controlled by three iodothyronine deiodinase enzymes (D1, D2 and D3), which catalyze the removal of iodine atoms at the phenolic ring (activation pathway) or at the tyrosil ring (inactivation pathway) of T4 and T3 (Figure 1)[2]. The three deiodinases belong to the thioredoxin fold superfamily and share a highly conserved active site containing the rare selenocysteine amino acid, which is critical for enzymatic activity[8]. While D1 and D3 are located in the plasma membrane, D2 is an endoplasmic reticulum-resident protein[8]. D1 catalyzes both phenolic and tyrosil ring deiodination and is expressed in the thyroid, liver and kidneys, while D2 catalyzes only phenolic ring deiodination and thus contributes to T3 production in the central nervous system, thyroid, skeletal muscle and brown adipose tissue[9]. D3, the physiological terminator of THs, inactivates both T3 and T4 through tyrosil ring deiodination[10]. Under normal conditions, D3 is expressed in the brain, placenta and pancreas, although under pathophysiological conditions, it can be induced in other tissues[11]. The coordinated activity of the three deiodinases impacts on the serum and tissue levels of both T4 and T3[12].

T3 exerts its biological activity either by binding to nuclear receptors and regulating target gene expression (genomic action, or Type I action) or by binding to cytosolic partners and activating

intracellular cascades (non-genomic or Type II action)[13]. TH receptors (TRs) belong to the superfamily of nuclear hormone receptors that function as ligand-modulated transcription factors[14]. Transcription of target genes occurs upon binding of the T3-TR complexes to TH response elements (TREs) within the chromatin and is regulated by an exchange of co-repressor (CoR) and co-activator (CoA) complexes. Indeed, in the absence of the T3 ligand, TRs bind TREs in association with CoR proteins. The binding of T3 to TR induces CoR release and CoA recruitment and consequently a net shift from transcriptional repression to transcriptional activation - a process known as the genomic effects of THs[15,16,17]. The mechanisms of genomic TH action differ from the effects of THs that occur within seconds or minutes. The latter actions constitute the non-genomic effects of THs, and comprise the binding of T3 or T4 to cellular proteins and interactions of TRs with cellular partners[17]. Importantly, although these cytosolic interactions of TH or TRs are collectively called "non-genomic actions", they may culminate in transcriptional activation of target genes[18].

Control of T3 concentration in the circulation

T3 is the major biologically active thyroid hormone[19] and, as mentioned above, its serum concentration remains relatively stable throughout life thanks to the coordinated action of the HPT axis, the TH transporters and the peripheral deiodinase activity. The finding that the human thyroid secretes only 20% of the daily T3 requirement[20] (versus about 50% of the daily requirement in rats[21]), while peripheral conversion of T4-to-T3 provides the remaining 80%, supports the concept that deiodinases play a preeminent role in T3 plasma homeostasis in humans[22]. Notably, the gold standard treatment for hypothyroidism is T4. The rationale for this treatment is that the T4-to-T3 conversion is sufficient to provide the total T3 requirement. Finally, the existence of a considerable extrathyroidal T4 pool that could be used by deiodinases in situations of TH insufficiency (e.g., iodine deficiency) to ensure a stable T3 level, is in line with the evolutionary development of a peripheral control of plasma T3 concentrations that could be triggered when the HPT axis is insufficient or failing[23].

D1 and D2 are able to convert T4-to-T3, albeit with a different enzymatic efficiency. The Km of D2 for T4 is in nanomolar range (1-4 nM) while the Km of D1 is in micromolar range (1-10 microM)[9]. This means that, at physiological concentrations, T4 is a much better substrate for D2 than for D1.

This consideration is based on the concept of "free hormone concentration" and neglects the issues of cellular compartmentalization of substrates and ligands regulated by membrane transporters and intracellular deiodinases at cell level. We cannot exclude that, locally, this compartmentalization could be strategical to generate areas with a non-uniform concentration of the substrates. In this scenario, the Km values measured in vitro, in the absence of binding proteins and transporters, might not reflect the physiological role of deiodinases due to the potentially different local intracellular in vivo concentrations of TH. Accordingly, T3 production may be greater in a large but low affinity D1-expressing tissue (i.e., liver, kidney or thyroid) than in a D2-expressing tissue, despite the higher in vitro affinity of D2 for T4.

It is difficult to define the precise percentage of the total plasma T3 produced by D1 and D2 respectively in humans. A healthy adult requires about 30 µg T3 daily, of which about 5 µg are directly secreted by the thyroid gland. The remaining 25 µg are obtained via peripheral deiodination. Several groups have tried to quantify the contribution of D1 to the production of circulating T3 by taking advantage of its specific sensitivity to propylthiouracil (PTU)[24],[25],[26]. In these studies, PTU reduced T3 plasma levels by about only 20-30% in euthyroid patients suggesting that D1 is not the major plasma T3 producer. However, we cannot exclude that these studies underestimated the role of D1 in T3 homeostasis due to a not completely effective PTU dose or to a contemporary reduction of T3 clearance. *Vice versa*, in hyperthyroid patients PTU decreased T3 plasma levels by about 50%, which indicates that, in this condition, D1 has a greater impact on T3 production than in euthyroid patients[27,28].

Currently, D2 is believed to produce most of the T3 daily requirement, namely, 20 µg/day, and a small aliquot (5 µg/day) is provided by D1[20]. However, D1, because of its subcellular localization and abundant expression, is likely to contribute also significantly to circulating T3 levels[29,30,31]. In D1-expressing tissues, T3 rapidly equilibrates between the intracellular and extracellular space, whereas in D2-expressing tissues (that express D2 in the endoplasmic reticulum[31]), T3 is much slower in equilibrating between the two locations. The T3 produced by D1[20,32,33,34] exits cells in about 30 minutes, while D2-generated T3 remains in the cell for about 8 hours[33]. Of course, since the discovery of several TH transporters differentially expressed among various tissues, the precise kinetics of T3 production, tissue distribution and equilibrium with plasma does not depend only on deiodinase actions and may differ among tissues.

D1 plays also an important role in iodine recycling by acting as a scavenger enzyme[35]. Mice lacking D1 showed, compared to WT mice, a reduced urinary excretion of iodide and an increased fecal

excretion of iodiothyronines[36]. The role played by D2 in the homeostatic control of plasma T3 is supported by its negative regulation by THs at transcriptional and post-transcriptional level[9]. D2 has a very short half-life (about 20 minutes) and can vary depending on whether T4 is available or not[9]. Thyroid hormones regulate D1 and D2 expression in an opposite fashion: hyperthyroidism increases D1 and decreases D2 whereas hypothyroidism decreases D1 and increases D2, thereby supporting a role of D2 in controlling plasma T3 homeostasis[9,37]. In hypothyroidism, the peripheral conversion of T4-to-T3 is increased consequent to D2 induction. In fact, it is estimated that D2 accounts for about 70% of the extrathyroidal production of T3 in hypothyroidism versus about 30% in hyperthyroidism[20,25,38]. In conclusion, the properties of D1 and D2 support the concept that, in thyroid hormone homeostasis, D1 exerts a more "systemic action" whereas D2 exerts "local action".

The generation of mouse models lacking D1 and/or D2 led to the unexpected observation that deiodinase activity is not essential for T3 plasma homeostasis in mice[36,39]. The double D1-D2KO mouse has normal plasma T3 levels, thanks to an increased TSH level and the consequent greater secretion of T3 from the thyroid[35]. These observations indicated that, at least in rodents, a deficit in the activating deiodinases can be overcome by multiple mechanisms including the presence of an intact HPT axis and altered clearance of the iodothyronines. Furthermore, it is not inconceivable that the mild impact of the absence of D1 and D2 on T3 plasma concentrations in rodents may also reflect the greater contribution of the thyroid gland itself in T3 production in rodents versus humans[21,22, 40,41].

Polymorphisms in the type 2 deiodinases (*DIO*2) gene and clinical implications

Several polymorphisms have been identified in the *DIO* genes[42,43,44] (Table 1). Some of them have been shown to affect TH plasma levels probably by modifying deiodinase activity or expression. In particular, a *DIO1* polymorphism (C785T) is associated with higher T4 and rT3 and lower T3 plasma levels, which suggests that C785T results in decreased D1 activity[42]. On the other hand, the *DIO1* A1814G polymorphism is associated with increased T3 levels and a higher T3/rT3 ratio, which suggests increased D1 activity. The *DIO3* T154G polymorphism does not affect TH plasma levels[42] (34). The *DIO*2 Thr92Ala polymorphism that occurs in about 15-30% of Caucasians is of particular interest[45]. Studies in primary culture of pituitary and skeletal muscle cells demonstrated that the Thr92Ala enzyme is less efficient than the wild-type enzyme in converting T4-to-T3[43,46], and, moreover, that it has a longer half-life and accumulates in the Golgi complex[47]. In addition, in

a study conducted with knock-in mouse models, mice with the Thr92Ala substitution manifested hypothyroidism in distinct brain areas, which was reversed by administration of T3[48]. Clinical studies suggest that this single-nucleotide polymorphism is associated with metabolic disease, mental disorders, a higher odds of developing Alzheimer's disease (in African Americans) and an altered HPT axis[43,45,49,50,51,52]. However, the role of Ala92 and its clinical implications are controversial as many other studies failed to confirm these data [53,54,55]. Two studies conducted in healthy subjects did not find a correlation between THs levels and the Thr92Ala polymorphism[42,56]. Castagna et al., in athyreotic subjects, found that plasma FT3 levels were lower in patients carrying the Thr92Ala polymorphism, probably as a result of the lower efficiency of the conversion of exogenous T4-to-T3[43] and therefore, the need for higher LT4 doses to normalize TSH levels. In an earlier study, the Thr92Ala polymorphism was not associated with the T4 dose in 154 athyreotic subjects[57].

The Thr92Ala polymorphism has also been associated with insulin resistance[50,58,59]. It has been suggested that decreased D2 activity in adipose tissue and skeletal muscle (two insulin-sensitive tissues) determines local hypothyroidism that, in turn, causes the decreased expression of the insulin-sensitive glucose transporter GLUT4 (a T3 target gene), thus leading to insulin resistance[46]. These effects were not confirmed in subsequent studies, probably due to a low statistical power, a different ethnic background and the genetic complexity of the explored pathological conditions[60,61]. However, even in the study[43] in which the polymorphism was associated with a low T3 level, a percentage of D2 Thr92Ala carriers had T3 levels within the reference range. It is likely that Thr92Ala is associated with, but is not the only player, in determining T3 plasma levels in athyreotic patients, and that other genetic actors could be involved in this process[62]. The Thr92Ala polymorphism and the Trp64Arg β3 variant of the adrenergic receptor (ADRB3) were strongly associated with obesity in 135 Caucasian non-diabetic women[50]. Moreover, in a population of type 2 mellitus diabetic patients, insulin resistance was more severe in carriers of both D2 Ala/Ala and PPARγ2 Ala12 alleles than in those with other D2/PPARγ2 genotype combinations[63].

Another interesting D2 polymorphism has been identified in one of the three open reading frames of the 5' UTR of *DIO*2, which is the ORF responsible for the inhibitory effect of the 5' UTR on D2 expression[44]. In young subjects, the D2 ORFa-Gly3Asp polymorphism is associated with decreased levels of T4 and rT3 and consequently with increased T3/T4 and T3/rT3 ratios, conditions that reflect increased D2 activity[44]. Moreover, in athyreotic subjects D2 ORFa-Gly3Asp influences the

set point of the HPT axis, reducing sensitivity to T4[64]. Although this polymorphism affects THs metabolism, it is not related to insulin resistance or hypertension[55].

Hopefully, with time, novel genetic markers, e.g. a *DIO*2 polymorphism will be found that could help clinicians to identify populations that have a low T3 plasma concentration under thyroxine treatment and clinically fail to respond optimally to it.

Monotherapy with levothyroxine versus combined T3 and T4

The gold standard treatment of hypothyroidism is monotherapy with levothyroxine (LT4), even though the thyroid gland, as largely discussed above, secretes both T4 and T3. Levothyroxine is among the five most commonly prescribed drugs worldwide. It is considered a safe and well-tolerated drug that is able to restore a normal TSH level as well as biochemical and clinical euthyroidism [65].

The main advantage of monotherapy with LT4 is its long half-life (6-8 days)[66]. Consequently, a single daily administration ensures stable levels of plasma T4, with a relatively small plasma T4 peak a few hours after ingestion[67]. However, about 10-15 % of hypothyroid patients, albeit treated with LT4 and with normal TSH levels, still complain of hypothyroidism symptoms[68]. Escobar-Morreale and colleagues reported that administration of LT4 alone in rats normalized T4 and T3 plasma but not tissue concentrations[69]. Only combined T3 and T4 treatment normalized concomitantly plasma and tissue T4 and T3 levels[69]. Athyreotic patients treated with LT4 lack 20% of the T3 produced directly by the thyroid gland, which should, at least in theory, be compensated for at peripheral level. In a large retrospective study, Gullo et al. demonstrated that although LT4 monotherapy normalized TSH levels, it reduced FT3 levels (by about 20%) and slightly increased FT4 levels in about 20% of subjects[70]. This finding was confirmed in a study of 469 LT4-treated patients in which T4-treated patients differed in 12 of 52 metabolic and clinical well-being parameters, thereby indicating that a normal TSH level does not guarantee normal metabolic conditions[71]. In support of this concept, clinical biomarkers of peripheral euthyroidism in athyreotic patients treated with T4 were associated more directly to FT3 levels than to TSH levels, which again suggested that normal TSH levels do not always equal tissue euthyroidism[72]. Interestingly, Werneck de Castro et al. showed that the disjunction between TSH and T3 levels depends on a different hypothalamic D2 degradation in response to T4 compared to other tissues, which also allows the normal execution of the T4-mediated feedback mechanism regulation of TRH-TSH secretion[73]. Therefore, the current view is that standard T4 monotherapy (at a TSH-euthyroid dose)

does not restore euthyroidism and clinical well-being in all hypothyroid patients. This scenario has led physicians to evaluate the clinical potential of combined T3 and T4 treatment. Unlike LT4, T3 is not a manageable drug because of its very short T3 half-life (one day). In a T3 monotherapy regimen, at least three daily doses are required to achieve stable normal T3 plasma levels[67]. A study conducted in hypothyroid patients with the *DIO*2 polymorphism revealed that symptoms improved when liothyronine (LT3) was added to LT4 therapy[74]. Combined T4+T3 treatment improved cognitive function and body weight in some studies but not in others[75-82]. Discordance among studies might be related to incorrect selection of patients, and/or to the administration of a non-physiologic T4/T3 ratio. Another important limitation in trials with combined T3 and T4 therapy is the wide plasma T3 fluctuations that occur due to the short T3 half-life. Indeed, T3 tissue uptake might result in tissue-selective (transient) hyperthyroidism. Recently, a more stable slow-release form of T3 has been synthesized[83]. This is a poly-zinc-liothyronine (PZL) which is a metal coordinated form of LT3 (Zn[T3][H_2O])n. Hypothyroid rats treated with PZL showed a lower T3 peak and a longer T3 half-life compared to the standard T3 treated rats[83]. These results are very encouraging for a future application of PZL in a clinical setting.

In a small cohort of 45 hypothyroid patients, Carlé et al. reported an association between D2 92Ala and the MCT10 single nucleotide polymorphism rs17606253[84]. MCT10 is a plasma membrane amino acid transporter, similar to MCT8, that facilitates the uptake and efflux of T3 and T4 across the plasma membrane. Subjects harboring both polymorphisms preferred L-T4 plus L-T3 therapy to L-T4 alone[84]. It is tempting to speculate that, in a rapidly evolving era of personalized medicine, the treatment of hypothyroidism should not forcefully titrate everyone regardless of his or her individual (genetic) predisposition into putatively "target values"**.**

Currently, given the lack of conclusive data regarding combined therapy, and the conflicting results of clinical trials, the European Thyroid Association and American Thyroid Association recommend LT4 monotherapy as the treatment of choice for hypothyroidism[85,65]. However, combined therapy can be considered for poor-responder T4-treated patients and in experimental settings[65].

The deiodinases in the control of T3 in "peripheral tissues"

In the following two subsections we provide two examples of how the deiodinases control intracellular TH action in a physiological (muscle stem cells) and in a pathological (skin cancer) context.

Muscle tissue and muscle stem cells as a paradigm of the control of intracellular TH action

During embryo development, adequate levels of THs are also essential for the formation and differentiation of muscle progenitor cells from the endoderm and subsequently for the shift of neonatal to adult myosin isoforms[86]. In adult life, T3 regulates muscle homeostasis by ensuring the correct expression of structural and metabolic muscle proteins[87,88]. D2 and D3 are expressed at a very low level in healthy adult muscle. Consequently, the role of the deiodinases in this tissue has been largely neglected. Recently, we found that D2 and D3 are abundantly expressed in muscle stem cells and play a major role in muscle regeneration and in the formation and maturation of new fibers[89,90].

Muscle stem cells (aka "satellite cells") are the key players in muscle regeneration[91]. They are normally dormant in adults and are located beneath the basal lamina in the quiescent phase of the cell cycle[91]. Upon damage, satellite cells become activated, enter the cell cycle and activate a differentiation program that is orchestrated by the sequential expression of a hierarchy of muscle-specific transcriptional factors[92]. T3 induces satellite cell differentiation[93,94] (Figure 2). Accordingly, D2 is specifically up-regulated at the beginning of the differentiation process, and its catalytic activity is essential for the correct formation and fusion of new myofibers, as demonstrated by the sharp delay in the regeneration process in D2KO mice[93]. Importantly, studies conducted with mouse models of muscle stem cell-specific genetic D2- and D3-knock out showed that the muscle regeneration process is compromised in the absence of D2 or D3[93,95,94]. Intriguingly, while the differentiation program of satellite cells is delayed in the absence of D2, cell proliferation is considerably enhanced[93]. Differently, D3 is highly expressed in the very early phases of muscle regeneration, thereby enabling T3 inactivation, which in turn is required for satellite cell proliferation[95]. Loss of D3 satellite cells disrupts the fine control of T3 availability and prematurely exposes satellite cells to the "normal but spatio-temporally excessive" T3 concentration in serum. This "cascade" is in line with a novel concept in which D2 and D3 are silent in resting muscle conditions, but are readily available to switch on action in such critical circumstances as muscle damage or muscle disease (Figure 2). Derangement of this delicate equilibrium can lead to delayed myotube differentiation (in case of reduced D2 expression), or to a dramatic deficit in regeneration (in case of reduced D3 expression) with deleterious consequences for muscle health and homeostasis. Intriguingly, when D2 was specifically depleted in adult muscle fibers, the muscle physiology and performance were not significantly affected, thus leading to the conclusion that D2 is not relevant for thyroid hormone modulation in adult skeletal muscle[96,97]. Together with the results obtained by the satellite cell-specific D2-depletion, these data indicate that the contribution

of D2 to muscle is not relevant in adult mature fibers whereas it is significant in muscle stem cell physiology. This suggests that TH metabolism by D2 plays a role in myogenesis under normal (muscle homeostasis) or pathologic conditions (muscle dystrophy), and likely in all events in which muscle stem cells play a major role.

Deiodinases and cancer

Thyroid hormones are classical regulators of cancer growth, differentiation and metabolism[11]. Accordingly, deregulation of the TH signal is a peculiar trait of many human solid tumors[11]. The growth of several tumors is positively or negatively regulated by THs[98,99]. In cancer, the action of TH is largely due to its impact on such oncogenic pathways as the Shh[100], Wnt[101], BMP[101], Notch[102], PI3K[103]and Ras pathways[104]. Thus, many carcinogenic programs are associated with changes in intracellular TH status, which are often due to alteration of deiodinase expression[101, 105]. Interestingly, TH impacts on diverse oncogenic and tumor suppressor pathways, and these pathways are often involved in altering the balanced expression of D2 and/or D3, thus resulting in a fine circular loop which, in turn, amplifies or attenuates tumor growth (Figure 3). The first deiodinase to be associated with cancer formation was D3[10], which is overexpressed in basal cell carcinomas (BCC)[105,106], gliomas[107], colon cancer[108] and hepatic adenomas[109], thus establishing D3 as an oncofetal protein[11]. Basal cell carcinomas are clear-cut examples of how D3-induced attenuation of TH signaling promotes and sustains cancer cell proliferation and progression (Figure 4). Proliferation of BCC cells drastically drops when D3 is depleted or when external T3 is provided, thus leading to a parallel increase in apoptotic cell death and massive reduction of BCC cell growth *in vitro* and *in vivo*[105,100,106]. Importantly, genetic D3-depletion in a mouse model that spontaneously forms BCC, as well as treatment of BCC tumors with topical T3, confirmed the druggable application of increased local T3 to attenuate BCC cancer growth[105,106]. Besides being effective on the bulk of tumoral cells, TH activation also affects the stemness of cancer stem cells and their transition to a more committed phase[101]. Indeed, treatment of colon cancer stem cells with T3 confers differentiation potential to these cells, and enhances their sensitivity to chemotherapy[14, 101].

D1 and D2 actions have also been related to tumorigenesis[110,111]. D1 is down-regulated in renal cancer, hepatic adenocarcinoma, papillary thyroid carcinoma (PTC) and in lung cancer compared to the counterpart healthy tissues[112,109,113,114]. Forced re-expression of D1 in renal cancer cells resulted in downregulation of several oncoproteins (e.g., STAT3, ANPEP, TGFBI and TGM2), thereby

supporting the concept that suppression of D1 might potentiate the proliferation and tumorigenesis of cancer tissues and cell lines. Differently, D1 is expressed in follicular thyroid neoplasias, and in some of these tumors the elevated expression of D1 and D2 might be responsible for the increased plasma T3/T4 ratio[114,115].

D2 is up- or down-regulated in various types of cancer. High D2 expression is a trait of many brain tumors, namely, astrocytomas, glioblastomas and oligodendrogliomas, as well as pituitary tumors and follicular thyroid tumors[107,116,117,118]. Interestingly, D2 is expressed in BCC cells in parallel with D3[119,120]. In this context, the outstanding questions are: (i) is D2 expression simply a bystander effect of local intracellular hypothyroidism driven by elevated D3 or does it play a functional role in the tumoral context? and (ii) how can we explain the concomitant expression of two functionally opposite enzymes within a tumor? Studies performed in colon cancer have indicated that D2 and D3 are not expressed simultaneously in the same cancer cell, while they are differentially modulated in specific stages of carcinogenesis[101]. It is tempting to speculate that these two enzymes are maintained in a fine-tuned balance within any single cell and that specific signals direct the balance versus a "dominant" deiodinase, which directs activation or inactivation of the hormone above the plasma steady state.

Therefore, the data available suggest that the expression of D2 and D3 could be either specifically localized to different cells within the tumor, or in a different subcellular localization within the same cell thus creating different territories of T3 concentrations within that cell. These remain open questions and are object of intensive studies in different laboratories. Nevertheless, the role of D2 in tumorigenesis is still poorly understood, as is the contribution of the D2-generated T3 to the tumorigenic program. It is feasible that besides regulating cancer cell proliferation, TH is also involved in the complex multistep events of cancer progression *in vivo*.

Conclusions

Notwithstanding normal TSH levels, a significant proportion of hypothyroid patients on LT4 replacement therapy complain of the persistence of symptoms of hypothyroidism. From a biochemical viewpoint, therapy with LT4 is associated with slightly more elevated serum FT4 levels and reduced serum FT3 and FT3/FT4 ratios versus healthy subjects, in about 15% and 30% of patients respectively.

It is conceivable that polymorphisms in genes involved in thyroid hormone metabolism, i.e. transporters and deiodinases, are causally related to persistent complaints under LT4 therapy. However, it remains to be established whether combined T4 + T3 treatment could relieve such symptoms.

We believe that understanding the molecular mechanisms underlying the persistence of symptoms during T4 therapy will lead to new therapeutic approaches for the millions of patients suffering from hypothyroidism, and provide the rationale for clinical studies designed to assess the efficacy of customized TH replacement. Obviously, a major treatment advance in hypothyroid patients would be to deliver T3 in ways that provide stable plasma levels while maintaining serum TSH within normal range thereby limiting the adverse effects related to systemic thyrotoxicosis.

On the other hand, the deiodinases constitute a powerful system that controls TH concentration at plasma and tissue-specific intracellular level. TH-based therapy is a promising approach to customized treatment of various human diseases. However, off-target effects constitute a major issue for tissues such as bone and cardiac muscle in which an increased or decreased TH concentration have deleterious consequences. A common goal of many research groups is to modulate the action of TH in time and space in order to therapeutically affect specific cell behavior in pathological conditions. In this regard, the deiodinases are a promising tool with which to manipulate TH levels. As far as the muscle system is concerned, manipulation of deiodinase levels has the potential to lead to therapies that will help promote normal muscle regeneration and muscle homeostasis under conditions in which these processes are suboptimal, such as muscular dystrophies. Similarly, in the early phases of tumor formation and progression, blocking the action of the oncofetal protein D3 may be a tool with which to block cell proliferation and induce apoptosis.

Given the findings that have accumulated over recent years in relation to this complex family of enzymes, the development of strategies for deiodinase manipulation can be a future challenge for therapeutic interventions in clinical medicine.

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Author Contributions

C.L. and M.D. provided input on the manuscript and wrote the paper, D.S. designed the overall review, supervised and wrote the paper.

REFERENCES

1. Brent, G.A. The molecular basis of thyroid hormone action. *N Engl J Med* **331**, 847-53 (1994).
2. Gereben, B. et al. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr Rev* **29**, 898-938 (2008).
3. Silva, J.E. & Larsen, P.R. Pituitary nuclear 3,5,3'-triiodothyronine and thyrotropin secretion: an explanation for the effect of thyroxine. *Science* **198**, 617-20 (1977).
4. Christoffolete, M.A. et al. Atypical expression of type 2 iodothyronine deiodinase in thyrotrophs explains the thyroxine-mediated pituitary thyrotropin feedback mechanism. *Endocrinology* **147**, 1735-43 (2006).
5. Fonseca, T.L. et al. Coordination of hypothalamic and pituitary T3 production regulates TSH expression. *J Clin Invest* **123**, 1492-500 (2013).
6. Luongo, C. et al. The selective loss of the type 2 iodothyronine deiodinase in mouse thyrotrophs increases basal TSH but blunts the thyrotropin response to hypothyroidism. *Endocrinology* **156**, 745-54 (2015).
7. Visser, W.E., Friesema, E.C. & Visser, T.J. Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol Endocrinol* **25**, 1-14 (2011).
8. Gereben, B., Zeold, A., Dentice, M., Salvatore, D. & Bianco, A.C. Activation and inactivation of thyroid hormone by deiodinases: local action with general consequences. *Cell Mol Life Sci* **65**, 570-90 (2008).
9. 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* **23**, 38-89 (2002).
10. Dentice, M. & Salvatore, D. Deiodinases: the balance of thyroid hormone: local impact of thyroid hormone inactivation. *J Endocrinol* **209**, 273-82 (2011).
11. Dentice, M., Antonini, D. & Salvatore, D. Type 3 deiodinase and solid tumors: an intriguing pair. *Expert Opin Ther Targets* **17**, 1369-79 (2013).

12. Bianco, A.C. & Silva, J.E. Cold exposure rapidly induces virtual saturation of brown adipose tissue nuclear T3 receptors. *Am J Physiol* **255**, E496-503 (1988).
13. Flamant, F. et al. Thyroid Hormone Signaling Pathways: Time for a More Precise Nomenclature. *Endocrinology* **158**, 2052-2057 (2017).
14. Brent, G.A. Mechanisms of thyroid hormone action. *J Clin Invest* **122**, 3035-43 (2012).
15. Yen, P.M. Physiological and molecular basis of thyroid hormone action. *Physiol Rev* **81**, 1097-142 (2001).
16. Ortiga-Carvalho, T.M., Sidhaye, A.R. & Wondisford, F.E. Thyroid hormone receptors and resistance to thyroid hormone disorders. *Nat Rev Endocrinol* **10**, 582-91 (2014).
17. Cheng, S.Y., Leonard, J.L. & Davis, P.J. Molecular aspects of thyroid hormone actions. *Endocr Rev* **31**, 139-70 (2010).
18. 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 beta through the phosphatidylinositol 3-kinase pathway. *Nucl Recept Signal* **4**, e020 (2006).
19. Vella, K.R. & Hollenberg, A.N. The actions of thyroid hormone signaling in the nucleus. *Mol Cell Endocrinol* **458**, 127-135 (2017).
20. Maia, A.L., Kim, B.W., Huang, S.A., Harney, J.W. & Larsen, P.R. Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. *J Clin Invest* **115**, 2524-33 (2005).
21. Chanoine, J.P. et al. The thyroid gland is a major source of circulating T3 in the rat. *J Clin Invest* **91**, 2709-13 (1993).
22. Pilo, A. et al. Thyroidal and peripheral production of 3,5,3'-triiodothyronine in humans by multicompartmental analysis. *Am J Physiol* **258**, E715-26 (1990).
23. Schreiber, G. The evolutionary and integrative roles of transthyretin in thyroid hormone homeostasis. *J Endocrinol* **175**, 61-73 (2002).
24. Geffner, D.L., Azukizawa, M. & Hershman, J.M. Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secretion in man. *J Clin Invest* **55**, 224-9 (1975).
25. Saberi, M., Sterling, F.H. & Utiger, R.D. Reduction in extrathyroidal triiodothyronine production by propylthiouracil in man. *J Clin Invest* **55**, 218-23 (1975).
26. LoPresti, J.S. et al. Alterations in 3,3'5'-triiodothyronine metabolism in response to propylthiouracil, dexamethasone, and thyroxine administration in man. *J Clin Invest* **84**, 1650-6 (1989).
27. Croxson, M.S., Hall, T.D. & Nicoloff, J.T. Combination drug therapy for treatment of hyperthyroid Grave's disease. *J Clin Endocrinol Metab* **45**, 623-30 (1977).
28. Laurberg, P., Torring, J. & Weeke, J. A comparison of the effects of propylthiouracil and methimazol on circulating thyroid hormones and various measures of peripheral thyroid hormone effects in thyrotoxic patients. *Acta Endocrinol (Copenh)* **108**, 51-4 (1985).
29. Koenig, R.J. Regulation of type 1 iodothyronine deiodinase in health and disease. *Thyroid* **15**, 835-40 (2005).
30. Leonard, J.L. & Rosenberg, I.N. Subcellular distribution of thyroxine 5'-deiodinase in the rat kidney: a plasma membrane location. *Endocrinology* **103**, 274-80 (1978).
31. Baqui, M.M., Gereben, B., Harney, J.W., Larsen, P.R. & Bianco, A.C. Distinct subcellular localization of transiently expressed types 1 and 2 iodothyronine deiodinases as determined by immunofluorescence confocal microscopy. *Endocrinology* **141**, 4309-12 (2000).
32. Silva, J.E., Dick, T.E. & Larsen, P.R. The contribution of local tissue thyroxine monodeiodination to the nuclear 3,5,3'-triiodothyronine in pituitary, liver, and kidney of euthyroid rats. *Endocrinology* **103**, 1196-207 (1978).

33. Bianco, A.C. & Silva, J.E. Nuclear 3,5,3'-triiodothyronine (T3) in brown adipose tissue: receptor occupancy and sources of T3 as determined by in vivo techniques. *Endocrinology* **120**, 55-62 (1987).
34. Silva, J.E. & Matthews, P. Thyroid hormone metabolism and the source of plasma triiodothyronine in 2-week-old rats: effects of thyroid status. *Endocrinology* **114**, 2394-405 (1984).
35. Galton, V.A., Schneider, M.J., Clark, A.S. & St Germain, D.L. Life without thyroxine to 3,5,3'-triiodothyronine conversion: studies in mice devoid of the 5'-deiodinases. *Endocrinology* **150**, 2957-63 (2009).
36. Schneider, M.J. et al. Targeted disruption of the type 1 selenodeiodinase gene (Dio1) results in marked changes in thyroid hormone economy in mice. *Endocrinology* **147**, 580-9 (2006).
37. Lum, S.M., Nicoloff, J.T., Spencer, C.A. & Kaptein, E.M. Peripheral tissue mechanism for maintenance of serum triiodothyronine values in a thyroxine-deficient state in man. *J Clin Invest* **73**, 570-5 (1984).
38. Abuid, J. & Larsen, P.R. Triiodothyronine and thyroxine in hyperthyroidism. Comparison of the acute changes during therapy with antithyroid agents. *J Clin Invest* **54**, 201-8 (1974).
39. Schneider, M.J. et al. Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. *Mol Endocrinol* **15**, 2137-48 (2001).
40. Abrams, G.M. & Larsen, P.R. Triiodothyronine and thyroxine in the serum and thyroid glands of iodine-deficient rats. *J Clin Invest* **52**, 2522-31 (1973).
41. Nguyen, T.T., Chapa, F. & DiStefano, J.J., 3rd. Direct measurement of the contributions of type I and type II 5'-deiodinases to whole body steady state 3,5,3'-triiodothyronine production from thyroxine in the rat. *Endocrinology* **139**, 4626-33 (1998).
42. Peeters, R.P. et al. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *J Clin Endocrinol Metab* **88**, 2880-8 (2003).
43. Castagna, M.G. et al. DIO2 Thr92Ala Reduces Deiodinase-2 Activity and Serum-T3 Levels in Thyroid-Deficient Patients. *J Clin Endocrinol Metab* **102**, 1623-1630 (2017).
44. Peeters, R.P. et al. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. *Am J Physiol Endocrinol Metab* **289**, E75-81 (2005).
45. Dora, J.M., Machado, W.E., Rheinheimer, J., Crispim, D. & Maia, A.L. Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case-control study and meta-analysis. *Eur J Endocrinol* **163**, 427-34 (2010).
46. Canani, L.H. et al. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* **90**, 3472-8 (2005).
47. McAninch, E.A. et al. Prevalent polymorphism in thyroid hormone-activating enzyme leaves a genetic fingerprint that underlies associated clinical syndromes. *J Clin Endocrinol Metab* **100**, 920-33 (2015).
48. Jo, S. et al. Type 2 deiodinase polymorphism causes ER stress and hypothyroidism in the brain. *J Clin Invest* (2018).
49. Gumieniak, O. et al. Ala92 type 2 deiodinase allele increases risk for the development of hypertension. *Hypertension* **49**, 461-6 (2007).
50. Mentuccia, D. et al. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. *Diabetes* **51**, 880-3 (2002).
51. McAninch, E.A. et al. A Common DIO2 Polymorphism and Alzheimer Disease Dementia in African and European Americans. *J Clin Endocrinol Metab* **103**, 1818-1826 (2018).

52. McAninch, E.A., Rajan, K.B., Miller, C.H. & Bianco, A.C. Systemic Thyroid Hormone Status During Levothyroxine Therapy In Hypothyroidism: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab* (2018).
53. Maia, A.L. et al. Lack of association between the type 2 deiodinase A/G polymorphism and hypertensive traits: the Framingham Heart Study. *Hypertension* **51**, e22-3 (2008).
54. Grarup, N. et al. Studies of the common DIO2 Thr92Ala polymorphism and metabolic phenotypes in 7342 Danish white subjects. *J Clin Endocrinol Metab* **92**, 363-6 (2007).
55. van der Deure, W.M. et al. Impact of thyroid function and polymorphisms in the type 2 deiodinase on blood pressure: the Rotterdam Study and the Rotterdam Scan Study. *Clin Endocrinol (Oxf)* **71**, 137-44 (2009).
56. Wouters, H.J. et al. No Effect of the Thr92Ala Polymorphism of Deiodinase-2 on Thyroid Hormone Parameters, Health-Related Quality of Life, and Cognitive Functioning in a Large Population-Based Cohort Study. *Thyroid* **27**, 147-155 (2017).
57. Heemstra, K.A. et al. Thr92Ala polymorphism in the type 2 deiodinase is not associated with T4 dose in athyroid patients or patients with Hashimoto thyroiditis. *Clin Endocrinol (Oxf)* **71**, 279-83 (2009).
58. Yalakanti, D. & Dolia, P.B. Association of Type II 5' Monodeiodinase Thr92Ala Single Nucleotide Gene Polymorphism and Circulating Thyroid Hormones Among Type 2 Diabetes Mellitus Patients. *Indian J Clin Biochem* **31**, 152-61 (2016).
59. Zhang, X. et al. The Type 2 Deiodinase Thr92Ala Polymorphism Is Associated with Worse Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. *J Diabetes Res* **2016**, 5928726 (2016).
60. Maia, A.L. et al. The type 2 deiodinase (DIO2) A/G polymorphism is not associated with glycemic traits: the Framingham Heart Study. *Thyroid* **17**, 199-202 (2007).
61. Mentuccia, D. et al. The Thr92Ala deiodinase type 2 (DIO2) variant is not associated with type 2 diabetes or indices of insulin resistance in the old order of Amish. *Thyroid* **15**, 1223-7 (2005).
62. Medici, M., Chaker, L. & Peeters, R.P. A Step Forward in Understanding the Relevance of Genetic Variation in Type 2 Deiodinase. *J Clin Endocrinol Metab* **102**, 1775-1778 (2017).
63. Estivalet, A.A. et al. D2 Thr92Ala and PPARgamma2 Pro12Ala polymorphisms interact in the modulation of insulin resistance in type 2 diabetic patients. *Obesity (Silver Spring)* **19**, 825-32 (2011).
64. Hoftijzer, H.C. et al. The type 2 deiodinase ORFa-Gly3Asp polymorphism (rs12885300) influences the set point of the hypothalamus-pituitary-thyroid axis in patients treated for differentiated thyroid carcinoma. *J Clin Endocrinol Metab* **96**, E1527-33 (2011).
65. Wiersinga, W.M., Duntas, L., Fadeyev, V., Nygaard, B. & Vanderpump, M.P. 2012 ETA Guidelines: The Use of L-T4 + L-T3 in the Treatment of Hypothyroidism. *Eur Thyroid J* **1**, 55-71 (2012).
66. Anbar, M., Guttmann, S., Rodan, G. & Stein, J.A. The determination of the rate of deiodination of thyroxine in human subjects. *J Clin Invest* **44**, 1986-91 (1965).
67. Celi, F.S. et al. The pharmacodynamic equivalence of levothyroxine and liothyronine: a randomized, double blind, cross-over study in thyroidectomized patients. *Clin Endocrinol (Oxf)* **72**, 709-15 (2010).
68. Peterson, S.J. et al. An Online Survey of Hypothyroid Patients Demonstrates Prominent Dissatisfaction. *Thyroid* **28**, 707-721 (2018).
69. Escobar-Morreale, H.F., Obregon, M.J., Escobar del Rey, F. & Morreale de Escobar, G. Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. *J Clin Invest* **96**, 2828-38 (1995).
70. Gullo, D. et al. Levothyroxine monotherapy cannot guarantee euthyroidism in all athyreotic patients. *PLoS One* **6**, e22552 (2011).

71. Peterson, S.J., McAninch, E.A. & Bianco, A.C. Is a Normal TSH Synonymous With "Euthyroidism" in Levothyroxine Monotherapy? *J Clin Endocrinol Metab* **101**, 4964-4973 (2016).
72. Ito, M. et al. Biochemical Markers Reflecting Thyroid Function in Athyreotic Patients on Levothyroxine Monotherapy. *Thyroid* **27**, 484-490 (2017).
73. Werneck de Castro, J.P. et al. Differences in hypothalamic type 2 deiodinase ubiquitination explain localized sensitivity to thyroxine. *J Clin Invest* **125**, 769-81 (2015).
74. Panicker, V. et al. Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. *J Clin Endocrinol Metab* **94**, 1623-9 (2009).
75. Grozinsky-Glasberg, S., Fraser, A., Nahshoni, E., Weizman, A. & Leibovici, L. Thyroxine-triiodothyronine combination therapy versus thyroxine monotherapy for clinical hypothyroidism: meta-analysis of randomized controlled trials. *J Clin Endocrinol Metab* **91**, 2592-9 (2006).
76. Valizadeh, M. et al. Efficacy of combined levothyroxine and liothyronine as compared with levothyroxine monotherapy in primary hypothyroidism: a randomized controlled trial. *Endocr Res* **34**, 80-9 (2009).
77. Nygaard, B., Jensen, E.W., Kvetny, J., Jarlov, A. & Faber, J. Effect of combination therapy with thyroxine (T4) and 3,5,3'-triiodothyronine versus T4 monotherapy in patients with hypothyroidism, a double-blind, randomised cross-over study. *Eur J Endocrinol* **161**, 895-902 (2009).
78. Appelhof, B.C. et al. Combined therapy with levothyroxine and liothyronine in two ratios, compared with levothyroxine monotherapy in primary hypothyroidism: a double-blind, randomized, controlled clinical trial. *J Clin Endocrinol Metab* **90**, 2666-74 (2005).
79. Saravanan, P., Simmons, D.J., Greenwood, R., Peters, T.J. & Dayan, C.M. Partial substitution of thyroxine (T4) with tri-iodothyronine in patients on T4 replacement therapy: results of a large community-based randomized controlled trial. *J Clin Endocrinol Metab* **90**, 805-12 (2005).
80. Bunevicius, R., Kazanavicius, G., Zalinkevicius, R. & Prange, A.J., Jr. Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism. *N Engl J Med* **340**, 424-9 (1999).
81. Bunevicius, R. et al. Thyroxine vs thyroxine plus triiodothyronine in treatment of hypothyroidism after thyroidectomy for Graves' disease. *Endocrine* **18**, 129-33 (2002).
82. Clyde, P.W., Harari, A.E., Getka, E.J. & Shakir, K.M. Combined levothyroxine plus liothyronine compared with levothyroxine alone in primary hypothyroidism: a randomized controlled trial. *JAMA* **290**, 2952-8 (2003).
83. Da Conceicao, R.R., Fernandes, G.W., Fonseca, T.L., Bocco, B. & Bianco, A.C. Metal Coordinated Poly-Zinc-Liothyronine Provides Stable Circulating Triiodothyronine Levels in Hypothyroid Rats. *Thyroid* **28**, 1425-1433 (2018).
84. Carle, A., Faber, J., Steffensen, R., Laurberg, P. & Nygaard, B. Hypothyroid Patients Encoding Combined MCT10 and DIO2 Gene Polymorphisms May Prefer L-T3 + L-T4 Combination Treatment - Data Using a Blind, Randomized, Clinical Study. *Eur Thyroid J* **6**, 143-151 (2017).
85. Jonklaas, J. et al. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. *Thyroid* **24**, 1670-751 (2014).
86. Sugie, H. & Verity, M.A. Postnatal histochemical fiber type differentiation in normal and hypothyroid rat soleus muscle. *Muscle Nerve* **8**, 654-60 (1985).
87. Simonides, W.S. & van Hardeveld, C. Thyroid hormone as a determinant of metabolic and contractile phenotype of skeletal muscle. *Thyroid* **18**, 205-16 (2008).

88. Salvatore, D., Simonides, W.S., Dentice, M., Zavacki, A.M. & Larsen, P.R. Thyroid hormones and skeletal muscle--new insights and potential implications. *Nat Rev Endocrinol* **10**, 206-14 (2014).
89. Ambrosio, R., De Stefano, M.A., Di Girolamo, D. & Salvatore, D. Thyroid hormone signaling and deiodinase actions in muscle stem/progenitor cells. *Mol Cell Endocrinol* **459**, 79-83 (2017).
90. Marsili, A. et al. Type 2 iodothyronine deiodinase levels are higher in slow-twitch than fast-twitch mouse skeletal muscle and are increased in hypothyroidism. *Endocrinology* **151**, 5952-60 (2010).
91. Yin, H., Price, F. & Rudnicki, M.A. Satellite cells and the muscle stem cell niche. *Physiol Rev* **93**, 23-67 (2013).
92. Conboy, I.M., Conboy, M.J., Smythe, G.M. & Rando, T.A. Notch-mediated restoration of regenerative potential to aged muscle. *Science* **302**, 1575-7 (2003).
93. Dentice, M. et al. The FoxO3/type 2 deiodinase pathway is required for normal mouse myogenesis and muscle regeneration. *J Clin Invest* **120**, 4021-30 (2010).
94. Salvatore, D. Deiodinases and stem cells: an intimate relationship. *J Endocrinol Invest* **41**, 59-66 (2018).
95. Dentice, M. et al. Intracellular inactivation of thyroid hormone is a survival mechanism for muscle stem cell proliferation and lineage progression. *Cell Metab* **20**, 1038-48 (2014).
96. Ignacio, D.L. et al. Early Developmental Disruption of Type 2 Deiodinase Pathway in Mouse Skeletal Muscle Does Not Impair Muscle Function. *Thyroid* **27**, 577-586 (2017).
97. Werneck-de-Castro, J.P. et al. Thyroid Hormone Signaling in Male Mouse Skeletal Muscle Is Largely Independent of D2 in Myocytes. *Endocrinology* **156**, 3842-52 (2015).
98. Boelaert, K. et al. Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. *J Clin Endocrinol Metab* **91**, 4295-301 (2006).
99. Fiore, E. et al. Lower levels of TSH are associated with a lower risk of papillary thyroid cancer in patients with thyroid nodular disease: thyroid autonomy may play a protective role. *Endocr Relat Cancer* **16**, 1251-60 (2009).
100. Luongo, C. et al. The sonic hedgehog-induced type 3 deiodinase facilitates tumorigenesis of basal cell carcinoma by reducing Gli2 inactivation. *Endocrinology* **155**, 2077-88 (2014).
101. Catalano, V. et al. Activated Thyroid Hormone Promotes Differentiation and Chemotherapeutic Sensitization of Colorectal Cancer Stem Cells by Regulating Wnt and BMP4 Signaling. *Cancer Res* **76**, 1237-44 (2016).
102. Sirakov, M. et al. The thyroid hormone nuclear receptor TRalpha1 controls the Notch signaling pathway and cell fate in murine intestine. *Development* **142**, 2764-74 (2015).
103. Hiroi, Y. et al. Rapid nongenomic actions of thyroid hormone. *Proc Natl Acad Sci U S A* **103**, 14104-9 (2006).
104. Garcia-Silva, S. & Aranda, A. The thyroid hormone receptor is a suppressor of ras-mediated transcription, proliferation, and transformation. *Mol Cell Biol* **24**, 7514-23 (2004).
105. Dentice, M. et al. Sonic hedgehog-induced type 3 deiodinase blocks thyroid hormone action enhancing proliferation of normal and malignant keratinocytes. *Proc Natl Acad Sci U S A* **104**, 14466-71 (2007).
106. Di Girolamo, D. et al. Reciprocal interplay between thyroid hormone and microRNA-21 regulates hedgehog pathway-driven skin tumorigenesis. *J Clin Invest* **126**, 2308-20 (2016).
107. Mori, K. et al. Thyroxine 5-deiodinase in human brain tumors. *J Clin Endocrinol Metab* **77**, 1198-202 (1993).
108. Dentice, M. et al. beta-Catenin regulates deiodinase levels and thyroid hormone signaling in colon cancer cells. *Gastroenterology* **143**, 1037-47 (2012).

109. Sabatino, L., Iervasi, G., Ferrazzi, P., Francesconi, D. & Chopra, I.J. A study of iodothyronine 5'-monodeiodinase activities in normal and pathological tissues in man and their comparison with activities in rat tissues. *Life Sci* **68**, 191-202 (2000).
110. Casula, S. & Bianco, A.C. Thyroid hormone deiodinases and cancer. *Front Endocrinol (Lausanne)* **3**, 74 (2012).
111. Maia, A.L., Goemann, I.M., Meyer, E.L. & Wajner, S.M. Deiodinases: the balance of thyroid hormone: type 1 iodothyronine deiodinase in human physiology and disease. *J Endocrinol* **209**, 283-97 (2011).
112. Wawrzynska, L., Sakowicz, A., Rudzinski, P., Langfort, R. & Kurzyna, M. The conversion of thyroxine to triiodothyronine in the lung: comparison of activity of type I iodothyronine 5' deiodinase in lung cancer with peripheral lung tissues. *Monaldi Arch Chest Dis* **59**, 140-5 (2003).
113. Pachucki, J. et al. Type I 5'-iodothyronine deiodinase activity and mRNA are remarkably reduced in renal clear cell carcinoma. *J Endocrinol Invest* **24**, 253-61 (2001).
114. de Souza Meyer, E.L., Dora, J.M., Wagner, M.S. & Maia, A.L. Decreased type 1 iodothyronine deiodinase expression might be an early and discrete event in thyroid cell dedifferentation towards papillary carcinoma. *Clin Endocrinol (Oxf)* **62**, 672-8 (2005).
115. Schreck, R., Schnieders, F., Schmutzler, C. & Kohrle, J. Retinoids stimulate type I iodothyronine 5'-deiodinase activity in human follicular thyroid carcinoma cell lines. *J Clin Endocrinol Metab* **79**, 791-8 (1994).
116. Murakami, M. et al. Expression of type II iodothyronine deiodinase in brain tumors. *J Clin Endocrinol Metab* **85**, 4403-6 (2000).
117. Nauman, P., Bonicki, W., Michalik, R., Warzecha, A. & Czernicki, Z. The concentration of thyroid hormones and activities of iodothyronine deiodinases are altered in human brain gliomas. *Folia Neuropathol* **42**, 67-73 (2004).
118. Tannahill, L.A. et al. Dysregulation of iodothyronine deiodinase enzyme expression and function in human pituitary tumours. *Clin Endocrinol (Oxf)* **56**, 735-43 (2002).
119. Miro, C. et al. The Concerted Action of Type 2 and Type 3 Deiodinases Regulates the Cell Cycle and Survival of Basal Cell Carcinoma Cells. *Thyroid* **27**, 567-576 (2017).
120. Cicatiello, A.G., Ambrosio, R. & Dentice, M. Thyroid hormone promotes differentiation of colon cancer stem cells. *Mol Cell Endocrinol* **459**, 84-89 (2017).

Key Points

- The coordinated action of the HPT axis and deiodinases is critical to ensure a stable T3 plasma concentration in euthyroid conditions and to counteract TH alterations under pathological circumstances.

- Deiodinases confer tissues the capacity to customize (enhancing or reducing) the intracellular concentration of thyroid hormone at single cell level and independently from plasma.

- Deiodinase action is essential to ensure optimal intracellular T3 concentration at single cell level, in a time-temporal dependent window.

- The common *DIO*2 Thr92Ala polymorphism affects enzyme stability and activity. This alters thyroid hormone metabolism thereby exposing subjects to reduced T4-to-T3 conversion which could be critical in athyreotic patients.

- In TH-treated athyreotic patients, normal TSH levels do not invariably equal tissue euthyroidism. Therefore, clinical or metabolic biomarkers of peripheral euthyroidism are required, particularly for symptomatic but biochemically euthyroid patients.

- Studies are required to identify genetic or metabolic traits in hypothyroid patients in whom LT4 treatment alone does not suffice to restore tissue euthyroidism and clinical wellbeing.

Figure legends

Figure 1. Deiodinase thyroid hormone action.

The pro-hormone T4 is activated to T3 by monodeiodination of the phenolic thyronine ring by D1 or D2 to form T3. Deiodination of the tyrosyl ring by D1 or D3 inactivates T4 and T3.

Figure 2. Thyroid hormone metabolism is dynamically regulated during linear progression of satellite cells.

In myoblasts, D3 is abundantly expressed in the early phase of satellite cell activation. This results in reduced TH action and enables rapid amplification of satellite cells. At the opposite, *the* surge of D2 expression in the later stages of myocyte differentiation leads to TH activation and expression of muscle-specific transcriptional factors.

Figure 3. The interplay between thyroid hormone and oncogenic signaling pathways in cancer cells.

Thyroid hormone directly regulates different cancer-related pathways. In parallel, these pathways regulate deiodinase gene expression, thereby resulting in activation or attenuation of the thyroid hormone signal at intracellular level.

Figure 4. Tumorigenesis of basal cell carcinoma (BCC) is highly sensitive to thyroid hormone action.

The TH inactivating enzymeD3 is potently up-regulated during BCC tumorigenesis, and leads to reduced TH intracellular availability and enhanced proliferation of BCC cells. D3-depletion or TH treatment drastically reduces BCC growth by inducing BCC cell apoptosis.

Table 1. Polymorphisms identified in *DIO* genes and their correlation with the deiodinase activities and TH levels

Polymorphism	Deiodinase activity	TH	Reference
D1			
C785T	Decreased	Increased rT3 levels Reduced T3/rT3 ratio	41
A1814G	Increased	Increased T3 levels Increased T3/rT3 ratio	41
D2			
T92A	Decreased	Increased T4 Reduced T3 Reduced T3/T4	42
ORFa-G3A	Increased	Reduced T4 Increased T3/T4	43
D3			41
T154G	Unchanged	-	

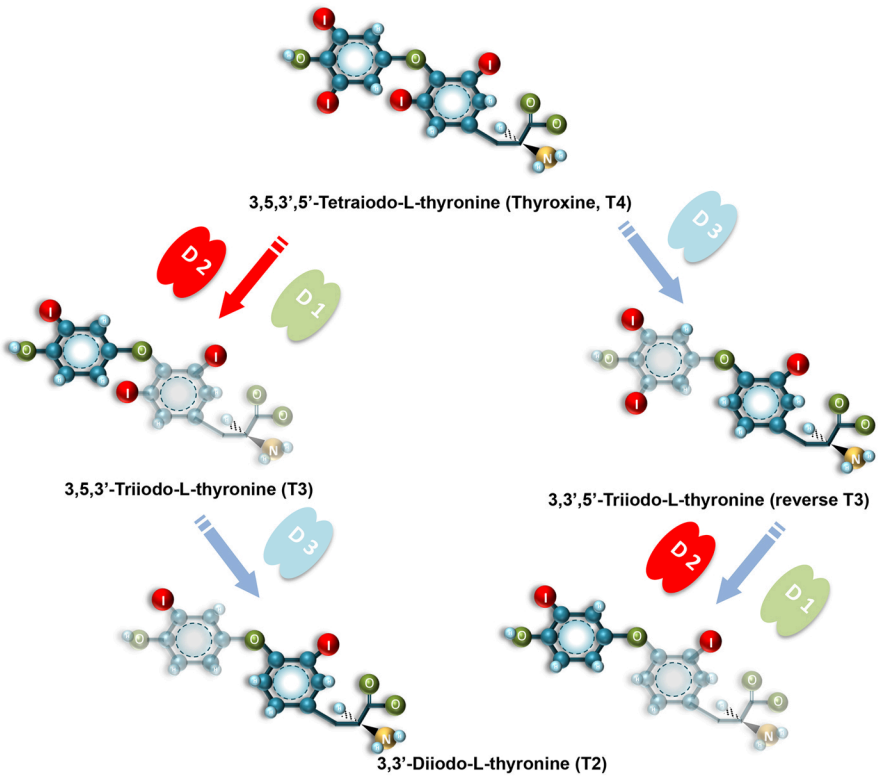
3,5,3',5'-Tetraiodo-L-thyronine (Thyroxine, T4)
D2
D1
D3
3,5,3'-Triiodo-L-thyronine (T3)
3,3',5'-Triiodo-L-thyronine (reverse T3)
D3
D2
D1
3,3'-Diiodo-L-thyronine (T2)

Figure 1

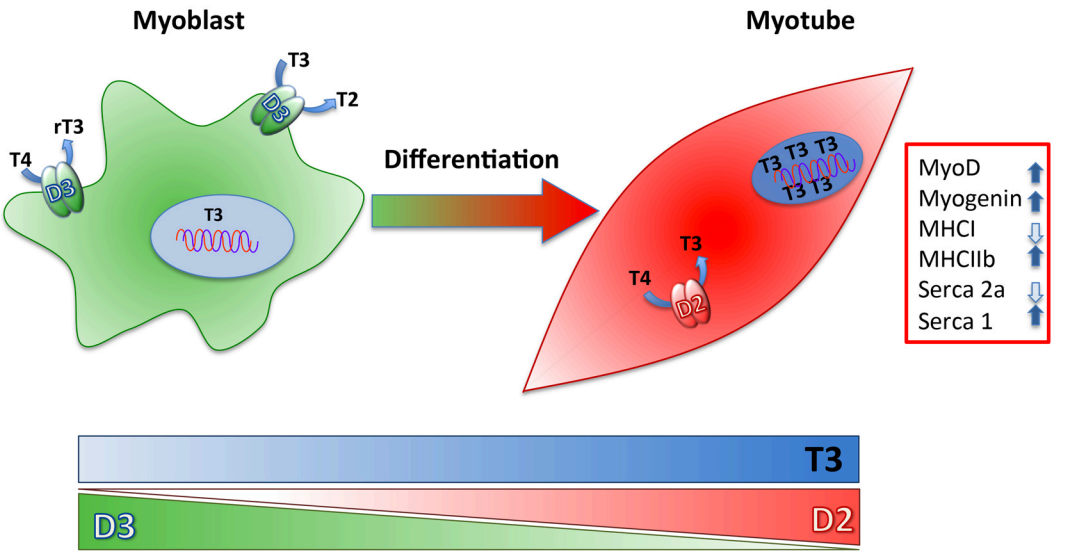
Myoblast
Myotube
T3
T2
rT3
T4
D3
T3
Differentiation
T3 T3 T3
T3 T3
T3
T4
D2
MyoD
Myogenin
MHCI
MHCIIb
Serca 2a
Serca 1
T3
D2
D3

Figure 2

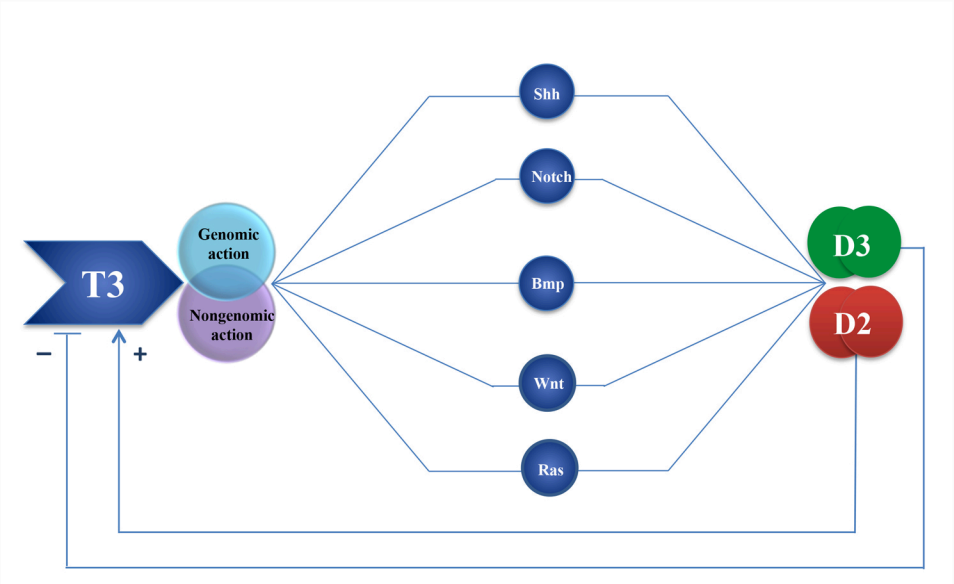
T3
Genomic action
Nongenomic action
−
+
Shh
Notch
Bmp
Wnt
Ras
D3
D2

Figure 3

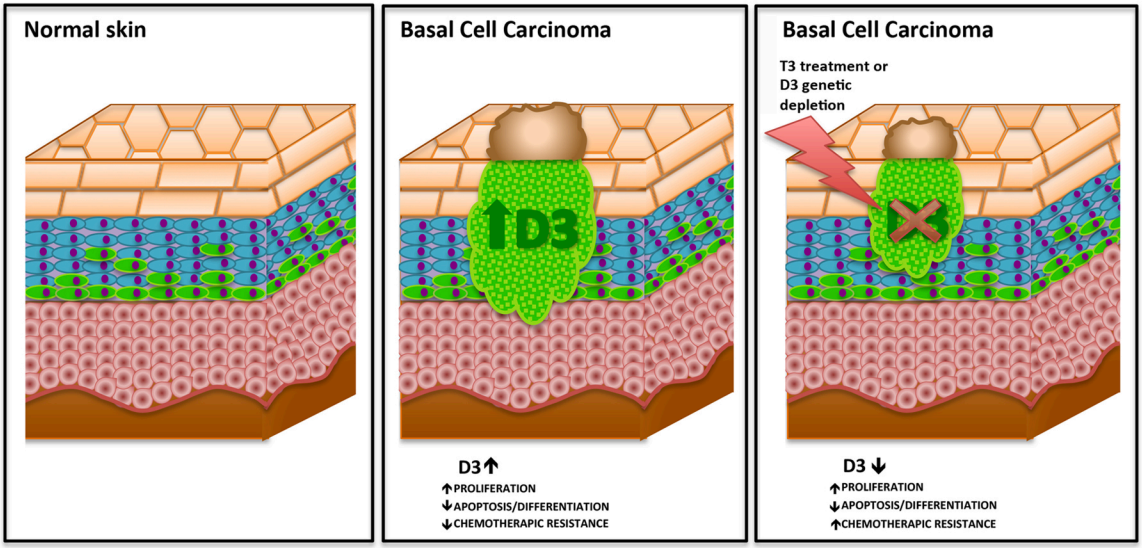
Normal skin
Basal Cell Carcinoma
↑D3
D3↑
↑PROLIFERATION
↓APOPTOSIS/DIFFERENTIATION
↓CHEMOTHERAPIC RESISTANCE
Basal Cell Carcinoma
T3 treatment or
D3 genetic
depletion
D3 ↓
↑PROLIFERATION
↓APOPTOSIS/DIFFERENTIATION
↑CHEMOTHERAPIC RESISTANCE
D3

Figure 4