Deiodinases and stem cells: an intimate relationship

Domenico Salvatore

Department of Clinical Medicine and Surgery, University of Naples “Federico II”, Naples, Italy;

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Corresponding Author :

Domenico Salvatore, MD, PhD
Department of Clinical Medicine and Surgery, University of Naples "Federico II". Via S. Pansini 5, 80131 Naples, Italy

Phone: (39) 081-7463780. Fax: (39) 081-7463668. E-mail: domenico.salvatore@unina.it

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ABSTRACT

Thyroid hormone is a major determinant of tissue functions in vivo. The deiodinase family controls tissue-specific activation or inactivation of intracellular thyroid hormones. Precise control of T3 dependent transcriptional program is required by multiple cell systems, including stem cells. In this regard, an emerging role of deiodinases is their potential implication in the definition of the stem cell biology and physiology. Several studies showed that exists a tight connection between TH and many signal pathways involved in the control of stem cell functions. These are cells that have an unlimited self-renewal capacity and the potential to differentiated into different types of mature cells. Deciphering how all these events are achieved, how T3 signal is controlled and integrated in stem cell and their niches and how it can impact on them is essentially unknown and represents a future challenge for the next years.

In these review I explore the role of deiodinases in the modulation of TH signal in stem cells of different adult tissues, namely muscle and intestine, and how their actions control the delicate balance between self-renewal, proliferation and differentiation. The molecular mechanisms presiding thyroid hormone action in stem cells may reveal unexpected therapeutical implications and a potential utilization in regenerative disease and cancer treatment.

INTRODUCTION

The active thyroid hormone (TH), T3 derives either directly from thyroid secretion or in large part through the monodeiodination of the prohormone thyroxine (T4) by one of two iodothyronine selenodeiodinases (D1 or D2) (Figure 1)(Marsili, Zavacki et al. 2011). The type 1 deiodinase is mainly expressed in the kidney, liver and thyroid and the T3 generated by this enzyme is largely released into the plasma. Type 2 deiodinase (D2) is present in the central nervous system, BAT, thyroid pituitary, retina, and in skeletal muscle (Figure 2). Much of the T3 derived from D2-mediated deiodination is thought to remain within the cell and to bind TH receptors (Bianco, Salvatore et al. 2002).

Conversely, termination of thyroid hormone signaling occurs due to the removal of a tyrosyl ring iodine by the type 3 deiodinase which converts the active hormone T3 to inactive metabolites (Figure 1).

During the last decades many studies showed that the deiodinases have the unique characteristic to modulate the intracellular TH bioavailability and then affect the TH signaling. The importance and the power of these enzymes are demonstrated by the fact that the deregulation of their expression could be involved in some pathological conditions such as cancer and cardiac diseases, but also inflammation and metabolic disorders. Furthermore, It has been demonstrated that the deiodinase system could be the direct target of different developmental pathways, such as Shh and Wnt/β-catenin, and that in many cases deiodinases play a key role as an intra-cellular hub in the cross-talk between these signals and the TH action.

An emerging role of deiodinases is their impact on the stem cell physiology and biology. These cells have an unlimited self-renewal capacity and the potential to differentiate into different types of mature cells. There are two types of stem cells: embryonic and adult. The first derive from the inner cell mass of blastocysts, and give rise to the three embryonic germ layers, while the second are responsible for the tissue homeostasis and the tissue regeneration after injury. The adult stem cells are housed in a specific microenvironment called “niche” consisting of neighboring cells such as endothelial cells fibroblast and stromal components. The niche regulates and supports stem cells and throughout the cell-cell interaction and the production of soluble factors is able to control the balance between quiescence, self-renewal and differentiation. Stem cell function is controlled by the integration of intrinsic genetic programs within the stem cell, with extracellular signals deriving from the niche.
The activation of a quiescent stem cell determines an asymmetric cell division that gives rise to one stem cell daughter (identical to the stem cell mother) and a second one called transit-amplifying (TA) cell from which derived the progenitor cells and their differentiated progenies.

The understanding of the stem cell biology received many attentions during the last years for the potential utilization in regenerative disease and the cancer treatment. Several studies showed that a tight connection exists between TH and many signal pathways involved in the control of stem cell functions.

In these review I explore the role of deiodinases in the modulation of TH signal in stem cells of different adult tissues, namely muscle and intestine, and how their actions control the delicate balance between self-renewal, proliferation and differentiation.

Deiodinases and stem cells in the skeletal muscle

Skeletal muscles, whose base functional elements are myofibers, consist of myofibers, vasculature networks, neurons, and connective tissues. This tissue is a major target of hormone action and accounts for ~40% of adult human body weight.

Fibers can be divided, relative to the different speed of contraction and overall ATP-generating capacity, into a slow-contracting/fatigue-resistant type and a fast-contracting/fatigue-susceptible type. This is reflected by the fibre-type-specific isoforms of myosin heavy chain (MyHC (fast or slow) and metabolism types (oxidative or glycolytic). The choice of myosin gene expression is under the dynamic regulation of thyroid hormone (Baldwin and Haddad 2001). In mammals, skeletal muscles are composed of varying combinations of slow and fast fibre types, with some muscles (e.g. the soleus) in which one type of fiber predominates.

Normal thyroid hormone levels are required for efficient muscle homeostasis, function and regeneration with a broad set of genes positively or negatively regulated at the transcriptional level by TH (McIntosh, Pernitsky et al. 1994; Simonides and van Hardeveld 2008). The presence and function of D2 and D3 in skeletal muscle have been hypothesized and investigated for many years but only in the last few years their specific role in muscle stem cells has been recognized.

Adult skeletal muscle in mammals is a stable tissue under normal circumstances, with terminally post-mitotic myonuclei that normally do not divide. Nonetheles, it has remarkable ability to extensive regeneration after injury. Muscle regeneration is a highly orchestrated sequence of events involving the activation of various cellular and molecular responses, among which the satellite cells (the bona fide muscle stem cells) play an indispensible role.

Satellite cells were identified based on their peculiar anatomical location: beneath the basal lamina and outside the myofiber plasma membrane. In adult skeletal muscle, all or most of satellite cells express a unique pattern of genes, which allowed the development of multiple methodologies to isolate them. Among them, and relevant to this review, satellite cells express the transcription factors Pax7 (Seale, Sabourin et al. 2000), myogenic regulatory factor Myf5 (Cornelison and Wold 1997), cell adhesion protein M-cadherin (Irintchev, Zeschnigk et al. 1994), tyrosine receptor kinase c-Met (Allen, Sheehan et al. 1995), cell surface attachment receptor α7-integrin (Burkin and Kaufman 1999; Gnocchi, White et al. 2009), and the differentiation protein CD34 (Beauchamp, Heslop et al. 2000). Of these, Pax7 is the more peculiar biomarker for satellite cells which is specifically expressed both in quiescence and during proliferation (Seale, Sabourin et al. 2000) across multiple species, including human (McLoon and Wirtschafter 2003).

We have recently demonstrated that type 2 deiodinase is expressed in satellite cells (Dentice, Marsili et al. 2010). D2 is expressed in activated satellite cells and increases during their differentiation. In this cell context, a D2- dependent and cell-autonomous amplification of thyroid hormone signaling in myoblasts is critical for proper muscle differentiation and for muscle repair.

In adults, the percentage of satellite cells can vary in different muscle and in the soleus muscle is generally two- to fourfold higher than that in tibialis anterior muscle or EDL muscle (Gibson and Schultz 1982). In such a sense, it is not a surprise that we find D2 in the soleus at the highest levels respect to the other limb muscles, maybe due to the elevated number of satellite cells present in this specific muscle.

D2 action raises intracellular T3 concentrations allowing normal MyoD expression and the programmed differentiation of these cells. Blocking D2 with reverse T3 or genetic D2 depletion prevents the increase in intracellular T3, blocks myotube formation, as well as inhibits the induction of MyoD, myogenin, and MHC, which normally occurs during the differentiation process .

So far, it is not clear which T3-dependent gene(s) requires this high degree of T3 receptor saturation for appropriate induction of the differentiation, but MyoD -given its central role in the process of myoblast differentiation and its T3 responsiveness- is certainly a target, but of course not the only one.

We show that the **Dio2 gene** in muscle cells is under the direct control of FoxO3, a forkhead box transcription factor, which regulates diverse cellular functions, such as differentiation, metabolism, proliferation and survival (Accili and Arden 2004). FoxO family members play a direct role in regulating myogenesis and, by cooperating with the PAX3/7 proteins, FoxO3 activates MyoD transcription in myoblasts (Hu, Geles et al. 2008). In light of our results, amplification of T3 signaling is also required for the execution of FoxO3 program in myogenesis. Of note, the differentiation defects caused by D2-depletion in myoblats are combined with an increase in proliferation potential.

On the opposite direction we found elevated D3 expression in proliferating myoblasts in vitro, which declines as differentiation proceeds, a pattern observed also in the C2C12 cell line. Our work demonstrated that a time-dependent and cell-autonomous degradation of thyroid hormone signaling mediated by D3 is critically required for proper myoblatsts

amplification. In the absence of D3, myoblasts undergo apoptosis making a previously unrecognized **role of D3** as a survival factor for proliferating cells. In muscle stem cells, only activated proliferating cells apoptose, not those that are quiescent or arrested in S-phase. It is not yet clear why this is the case, but we speculate that spatio-temporally excessive TH availability creates a metabolic or mitotic catastrophe, which cannot be overcome by the active proliferating cells.

What induces D3 in activated primary myoblasts? It is likely that D3 is induced by a set of integrated signals constituted by growth factors, cytokines, morphogens and other signaling pathways that are active at the injury site. Dissecting their specific role will be the object of future studies.

Multiple evidences demonstrate that satellite cells represent a heterogeneous population. However, our understanding of this heterogeneity is far from complete. An intriguing question is how satellite cells renew themselves. Stem cells can divide and self-renew by two diverse process : asymmetric cell division and symmetric cell division. In asymmetric cell division, one parental stem cell gives rise to two functionally different daughter cells: one daughter stem cell and another daughter cell destined for differentiation. In symmetric cell division, one parental stem cell divides into two daughter stem cells of equal stemness. In either fashion, the number of stem cells is maintained at a constant level.

Skeletal muscle stem cells represent a heterogeneous population of cells which span from stem-like to those more a committed towards myogenic lineage progression and differentiation. From a functional point of view, there are at least two populations of Pax-7 positive satellite cells. One population rapidly contributes to muscle repair, the second is more stem cell-like and remains longer in a quiescent state in the recipient muscle. These

functional differences are mirrored by different levels of Pax7 (Rocheteau, Gayraud-Morel et al. 2012) (Rocheteau, Gayraud-Morel et al.). In this context, Pax7-^{Hi} represents a reversible dormant stem cell state during homeostasis, while Pax7-^{Lo} are the activated ones which are prone to divide and to progress along the cell lineage cascade (Mourikis, Sambasivan et al. 2012). Here we show that T3 availability is differentially controlled in these two cell populations. During muscle homeostasis, D3 is expressed in the quiescent satellite cells that are poised for myogenic commitment (Pax7-^{Lo}). In this subset of cells, D3 expression does not simply reflect a secondary regulation of D3, but causes an intracellular attenuation of the thyroid hormone signal compared to the Pax7-^{Hi} . This is reflected in the divergent expression of oppositely regulated thyroid hormone responsive genes, which change according to the TH-signaling mediated by D3. This could correlate with the metabolic status of the cells, e.g. the Pax7-^{Lo} quiescent cells are more metabolically active than the corresponding Pax7-^{Hi} (Rocheteau, Gayraud-Morel et al. 2012). This raises intriguing issues on whether and how T3 signaling might influence stemness. Supporting this hypothesis, D2 is expressed at very high levels only in the Pax7-^{Hi} subset of cells and its expression declines with cell activation, i.e in the Pax7-$^{lo.}$

In conclusion, in muscle stem cells the deiodinase system represent a robust interlocking pathway which facilitates specific chronotropic and tissue-specific changes in T3 concentrations during periods when functional programs call for a transient increase or decrease of intracellular T3 concentrations.

Deiodinases and muscle regeneration

Mammalian skeletal muscle during adulthood is a stable postmitotic tissue with infrequent turnover of myonuclei (Schmalbruch and Lewis 2000). Minor lesions can be repaired without causing cell death, inflammatory responses, or histological changes.

Severe muscle injuries due to either traumatic lesions (e.g., extensive physical activity such as resistance training, or exposure to myotoxin) or genetic defects (e.g., muscular dystrophies) are accompanied by myofiber necrosis and inflammatory responses. This process, starting from myofiber necrosis, satellite cell activation and ending with new myofiber formation, is called muscle regeneration.

Muscle regeneration is a multistep process that includes myofiber degeneration, regeneration and remodeling (Ten Broek, Grefte et al. 2010). The repair process is characterized by the activation of the satellite cells, which give rise to activated proliferating myoblasts, followed by cell differentiation and fusion into regenerated myofibers (Figure 3) (Bentzinger, Wang et al. 2012). Satellite cells plays a critical role in this process, as proved by the completely abolished muscle regeneration upon ablation of the total satellite cell pool (all Pax7+ cells) in adulthood (Lepper, Partridge et al. 2011) (Rudnicki, Le Grand et al. 2008; Murphy, Lawson et al. 2011) (Brack and Rando 2012) (Dhawan and Rando 2005). A central role in this event is also played by the dynamic interplay between intrinsic factors within satellite cells and extrinsic factors constituting the muscle stem cell niche/microenvironment. Preliminary data from our group indicated that FAP (fibro-adipogenic precursor cells), a cell component of the stem niche, express elevated D2 and D3 levels, which indicate that a tuned control of intracellular TH is likely to be required also in the niche microenvironment.

Muscle regeneration occurs in three sequential but overlapping stages: *1*) the inflammatory response; *2*) the activation, differentiation, and fusion of satellite cells; and *3*) the maturation and remodeling of newly formed myofibers.

Under normal homeostatic conditions, satellite cells are sublaminal and mitotically quiescent cells. In this G0 state, stem cells have a low metabolism and are more resistant to DNA damage. The quiescent state is required for the long-term maintenance of muscle stem cells. The first step and the hallmark of the initial phase of muscle regeneration is extensive cell proliferation. Upon exposure to signals from a damaged environment, satellite cells exit their quiescent state and start to proliferate (satellite cell activation).

In this in vivo setting, upon injury- induced satellite cell activation, D3 is robustly induced and its expression is suppressed when cell start the differentiation phase. The D3 mRNA, protein and enzymatic activity were significantly increased at early stages after CTX injection (3-5d) and remained elevated for several days. Thereafter, D3 declined, reaching nearly normal adult levels. D3 staining showed D3 present in the progenitor Pax7-positive cells as well as in the newly regenerating fibers and also in F4/80^{+}macrophages infiltrating the injury site. Despite the presence of normal plasma T3 concentrations, selective genetic D3-depletion in the satellite cell compartment in vivo results in an intracellular thyrotoxic state followed by severe cell apoptosis, disrupting the normal pattern of tissue response to acute injury and causing a marked delay in muscle regeneration.
Thus, D3 is dynamically exploited *in vivo* to chronically attenuate TH-signaling under basal conditions while also acutely increasing gene programs required for satellite cell lineage progression as needed for skeletal muscle development or muscle repair.

After limited rounds of proliferation, the majority of satellite cells enter the myogenic differentiation program and begin to fuse. We observed that in the tibialis anterior (TA) muscle *Dio2* mRNA increased significantly and reached a peak at day 11 when most satellite cells differentiate. There after, *Dio2* mRNA declined, reaching normal levels by

day 21. No changes in serum T3 or T4 concentrations were observed at any time during the cardiotoxin experiment indicating that the D2-expression at the injury site affects only the muscle compartment. Interestingly, regeneration repair was significantly delayed in *Dio2*-null compared with wild-type mice. *Dio2* null mice have an inadequate MyoD response to muscle injury and a marked delay in the subsequent muscle regeneration, associated with an increased proliferation of activated myoblasts. Furthermore, downstream MyoD effectors, such as myogenin, MHC, as well as the regeneration marker neonatal MHC (Dentice, Marsili et al. 2010), were also dramatically reduced in the absence of D2 at both mRNA and protein level in vivo.

In agreement with in vitro data, regenerating D2-depleted muscles contain an increased number of muscle stem cells, which support the concept that reduced TH action enhanced the proliferative capacity of myoblasts in vivo.

Why should D2 inhibition and D3 induction represent an advantage for cell proliferation? In different cell context, T3 is a differentiating agent and in this sense it is associated with reduced proliferation. At a nuclear level, the bound TH receptor acts in many proliferating contexts as a tumor suppressor (Aranda, Martinez-Iglesias et al. 2009).

This reciprocal D3-D2 expression is likely to reflect a need of a different TH requirements during cell lineage progression with D3-caused low intracellular T3 required for the expansion of the satellite cell pool, while later D2 expression facilitate cell differentiation.

The reason why D2 is critical to the process of differentiation is that even in vitro, whatever intracellular T3 is provided by the FBS in the media or the serum is insufficient to induce terminal muscle differentiation. The in vivo studies also mirror this requirement for a high

intracellular T3 in that a normal concentration of circulating T3 in the *Dio2* null mice is not sufficient to support normal myogenesis and muscle repair after cardiotoxin injury.

Deiodinase-mediated control of thyroid hormone action is important in the onset of Duchenne Muscular Dystrophy (DMD)

DMD is the most common and devastating form of muscular dystrophy, with an incidence of 1 in every 3500 male births. It is characterized by progressive muscle degeneration as a result of ongoing muscle damage and incomplete regeneration.

The *mdx* mouse lacks dystrophin and is the most widely used animal model of DMD. Muscles from *mdx* mice undergo periods of muscle degeneration and regeneration, which is most extensive from about 3–8 weeks of age (Khurana and Davies 2003). Previous studies have examined the role of altered TH on the onset of skeletal muscle necrosis in dystrophin-deficient *mdx* mice. In one study, McArdle and collegues, found that treatment with PTU (an anti-thyroid drug that significantly reduces plasma TH levels) effectively prevented necrosis in the muscle of 21-day old *mdx* mice. While untreated *mdx* mice showed the characteristic 10-fold elevation in serum CK activities, this was not seen in the PTU-treated animals. Surprisingly, such positive effects were lost with longer treatment (28 days). This study provided the first demonstration that an experimental manipulation of TH levels alters the onset of necrosis in *mdx* mice (McArdle, Helliwell et al. 1998). Thyroid antagonists (PTU or MMI treatments) has also been reported to reduce the rate of muscle degeneration in avian muscular dystrophy.Previous studies demonstrated that soon after hatching, thyroidectomy combined with PTU treatment to achieve abrupt thyroid deprivation, improves muscle function in dystrophic chickens (King and Entrikin 1991). In treated animals, thyroid deprivation increases muscle function (righting ability) and reduces plasma CK activity in dystrophic chickens. In a different study, hypothyroidism was show to prolong and increase *mdx* muscle precursor cell proliferation and to delay

myotube formation in normal and dystrophic limb muscle (McIntosh and Anderson 1995). In the opposite but convergent direction, T3 supplementation for 2 weeks was found to increase the prevalence of necrosis and central nucleation in soleus and cardiac muscle of 5-or 10-week-old *mdx* mice (Anderson, Liu et al. 1994).

The finding that we transiently down-modulate MyoD by reversibly blocking D2 in myoblast precursors could have several clinical implications for patients with muscle disease. For example, myogenic precursor cells could be kept in low T3 conditions when increased proliferation and cell expansion is desired. Later, the situation could be reversed, by removing the blocking agent or by supplying T3, thus promoting myotube formation and terminal differentiation. This could significantly improve the success of myoblast transplantation protocols in which differentiation limits the degree of muscle precursor cell proliferation (Cooper, Butler-Browne et al. 2006) and could be useful in the treatment of dystrophic patients.

Deiodinase and intestinal stem cell physiology

The intestinal epithelium, responsible for the food processing and nutrient absorption, is a well-known TH tissue target. It is composed by two different compartments: the proliferative zone, called crypt, and the differentiated one, or villi. Several signaling pathways are involved in the development and the homeostasis of the intestinal epithelium, including Notch, BMP, Wnt and thyroid hormone.

In *Xenopus laevis*, during the metamorphosis, the gastrointestinal tract experiences an impressive remodeling, completely depend on thyroid hormone: the larva-proper epithelial cells undergone apoptosis and several adult stem cells appear to form the adult epithelium

(Schreiber, Cai et al. 2005). Cai and Brown showed that at metamorphosis climax, when TH level abruptly increases, D2 mRNA appears in the intestinal mesenchyme, and then its expression decreases when intestinal remodeling is completed (Cai and Brown 2004). Interestingly, the intestinal mesenchyme triggers and sustains the epithelium metamorphosis. Using an elegant system of transgenic tadpoles, it has been showed that TH signaling in intestinal mesenchymal tissue is required for the formation of the stem cells (Shi, Hasebe et al. 2011). This suggests that the D2 expressing tissue, throughout the epithelia-mesenchyme interactions plays an important role in the establishment of the specific environment called stem cell niche, an absolute requirement for the stem cell physiology.

In homeostatic conditions, intestinal epithelium is the fastest renewing tissue. In mouse, it has been demonstrated that every 3-5 days the intestinal epithelium is completely renewed. This process is highly dynamic and requires a fine coordination between cell proliferation, migration, differentiation and apoptosis along the villus axis as result of the integration of different signaling pathways. The fuel of this continuous renewal is the intestinal stem cells located at the bottom of the crypts. The multipotent intestinal stem cells give rise at the intestinal progenitors that differentiate while migrating towards the tip of the villus, where the cells die by apoptosis and are exfoliated into the lumen. During the last years, many studies showed the interplay between TH and other pathways involved in intestinal stem/progenitor cell biology, as Wnt, BMP and Notch (Fre, Pallavi et al. 2009).
Intriguing is the observation that D3 is expressed specifically in the cells at the base of the intestinal crypts of mouse intestine (where stem cells and progenitors are located), and its expression decline as the cells migrate upwards, while D2 appears (Dentice, Luongo et al. 2012). This D3 expression pattern is characteristic of the Wnt/β-catenin targets, one of the

signaling pathways involved in the intestinal development and homeostasis and moreover responsible for the stem cell self-renew capacity.

In a human colon adenocarcinoma cell lines, expressing elevated D3 levels, D3 is a direct target of Wnt/β-catenin and that the consequent intracellular low TH concentration gives to the cells a proliferative advantage (Dentice, Luongo et al. 2012). Indeed, the treatment with iopanoic acid, a deiodinase inhibitor, or the D3 knockout reduces cell proliferation rate and increases their differentiation. High level of D3 was found also in human colon cancer stem cells with β-catenin activation (Catalano, Dentice et al. 2016). In these cells D3 contributes to maintain the undifferentiated status, while T3 induces differentiation throughout the induction of the expression BMP- 4, one of the principal molecules responsible for the differentiation of normal colonic stem cell.

Another signaling pathway activated by TH and required for the intestinal cell fate determination is Notch (Fre, Huyghe et al. 2005). The activation of Notch signaling induces the cleavage of Notch-ICD, which in turns activates the Notch nuclear target genes, mainly involved in cell differentiation. It was demonstrated that Notch increases asymmetric division of the stem cells, generating one cell that maintains the stem cell proprieties and a second that is engaged in the differentiation process. TH regulates positively Notch activity by controlling the expression of several component of its pathway (i.e. Notch1, Dll1, Dll4, Hes1 and Jag1) (Sirakov, Boussouar et al. 2015). In the colon cancer stem cells, D3 by reducing intracellular T3 level, inhibits Notch signaling and increases the symmetric self-renewing division by promoting tumor growth (Catalano, Dentice et al. 2016). The deregulation of the expression of D3 is responsible also for the resistance to oxaliplatin and 5-fluorouracil, two chemotherapeutic agents routinely used for the colon cancer treatment. When the colon cancer stem cells expressing high levels of D3 are treated with T3, it is observed an increased chemotherapeutic drug-induced apoptosis (Catalano, Dentice et al. 2016). In colon cancer stem cell, the reduction of intracellular TH level,

determined by the deregulated expression of D3, affects different stem cell proprieties (asymmetric division, stem cell activation and differentiation) this give rise to an alteration of the balance between proliferation and differentiation responsible for the tumor development and chemoresistance. Understanding the molecular mechanisms that regulate this delicate balance is important to better define the stem cell biology and moreover to find new solutions for the treatment of cancer and degenerative diseases. In the future we could speculate to combine the chemotherapy with intracellular T3 treatment to enhance the curative effect of the colon cancer conventional therapy.

During the last years many evidences showed the close relationship between TH and intestinal stem cells. In particular, during the intestinal development and in adult life, in amphibian as in mammals, the coordinated spatio-temporal expression of D2 and D3 is important to define the TH local concentration and consequently to modulate the other signaling pathways involved in the definition of the intestinal stem cell niche.

Conclusions

Thyroid hormone is a key endocrine regulator that primarily functions through binding to nuclear thyroid hormone receptors and imposing a signature of gene expression. Whereas the important role of thyroid hormone signaling in different tissues and cells has been recognized for many years, the contribution of local modulation of thyroid metabolism by the deiodinases to stem cell physiology is a novel area in this field.

Deiodinase functions provides a mechanism by which thyroid hormone concentration can be modulated and controlled in a tissue-specific chronologically programmed fashion, such as during development, or in circumstances where there is a requirement for rapid control of T3 in a specific tissue.

Future *in vivo* studies are required to clarify the kinetics and coordination of DIO2 with DIO3 in other different stem cell compartments, as well as to define the role of important components that regulate thyroid hormone supply, such as the thyroid hormone transporters and thyroid hormone receptor specific expression in these crucial cells.

On a more speculative note, manipulation of deiodinase activity in the stem cells could be a potential avenue to enhance therapeutical stem cell potential, as this approach could bypass the adverse systemic effects of an increase in circulating thyroid hormones. Interestingly, reported studies identify D2 as a molecular target critical to selectively enhance muscle differentiation, or, if blocked, promote the expansion of active satellite cells for cell-based therapies. Furthermore, satellite cells as well as other tissue specific stem cells, have an intrinsic potential to differentiate into multiple mesenchymal lineages (Miladpour, Rasti et al. 2016),, i.e. myocytes, adipocytes, and osteocytes. It is not unconceivable hypothesize that modulation of intracellular thyroid hormone action by deiodinases may influence specific determination or cell fate and this may point to deiodinases as a tool to influence diverse disease progression in humans.

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Figure Legends

Fig. 1:Thyroid hormone metabolism andthe reactions catalyzed by the deiodinases type 1, 2, and 3. Deiodinases type 1 and 2 (D1 and D2) convert T4 to the active hormone T3 (3,5,3′-triiodothyronine) by removal of iodine from the outer-ring of T4 (3,5,3′,5′-tetraiodothyronine). Deiodinase type 3 (D3) generate the biologically inactive metabolites reverse T3 (3,3′,5′-triiodothyronine) and T2 (3,3′-diiodothyronine), by inner-ring deiodination of T4 and T3.

Fig. 2: Deiodinase expression in multiple tissues. D1, D2 and D3 are expressed in tissue specific manner and their expression is tightly regulated to guarantee appropriate T3 levels.

Fig. 3: Thyroid hormones and muscle regeneration. D2 and D3 are expressed differentially in muscle cells during myogenic program. These enzymes cause an increase of active thyroid hormone (T3) that control critical factors in all muscle differentiation phases. Deiodinase impact in quiescent muscle stem cells remains unknown.

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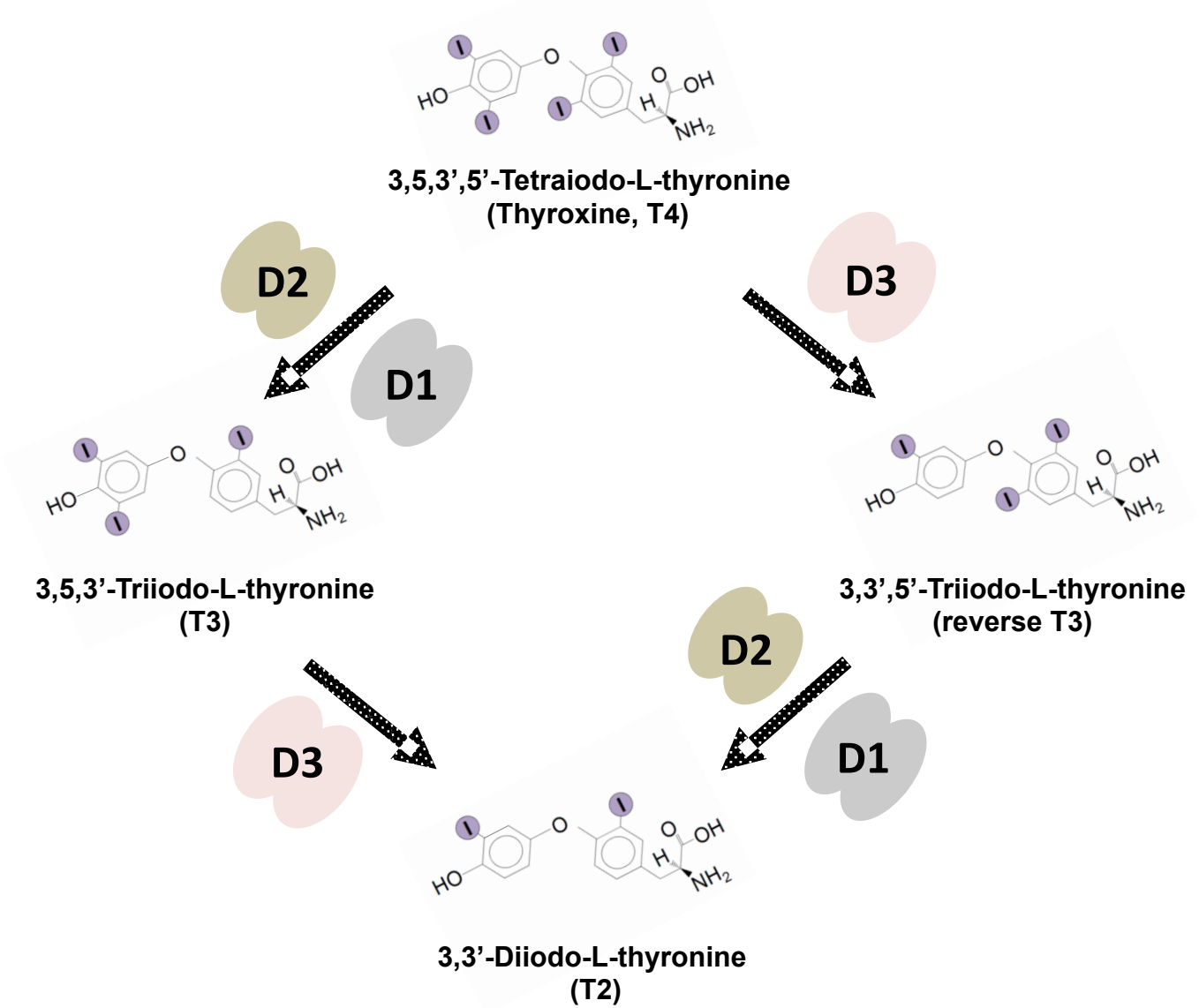
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(Thyroxine, T4)
D2
D1
D3
3,5,3'-Triiodo-L-thyronine
(T3)
3,3',5'-Triiodo-L-thyronine
(reverse T3)
D3
D2
D1
3,3'-Diiodo-L-thyronine
(T2)

Figure 1

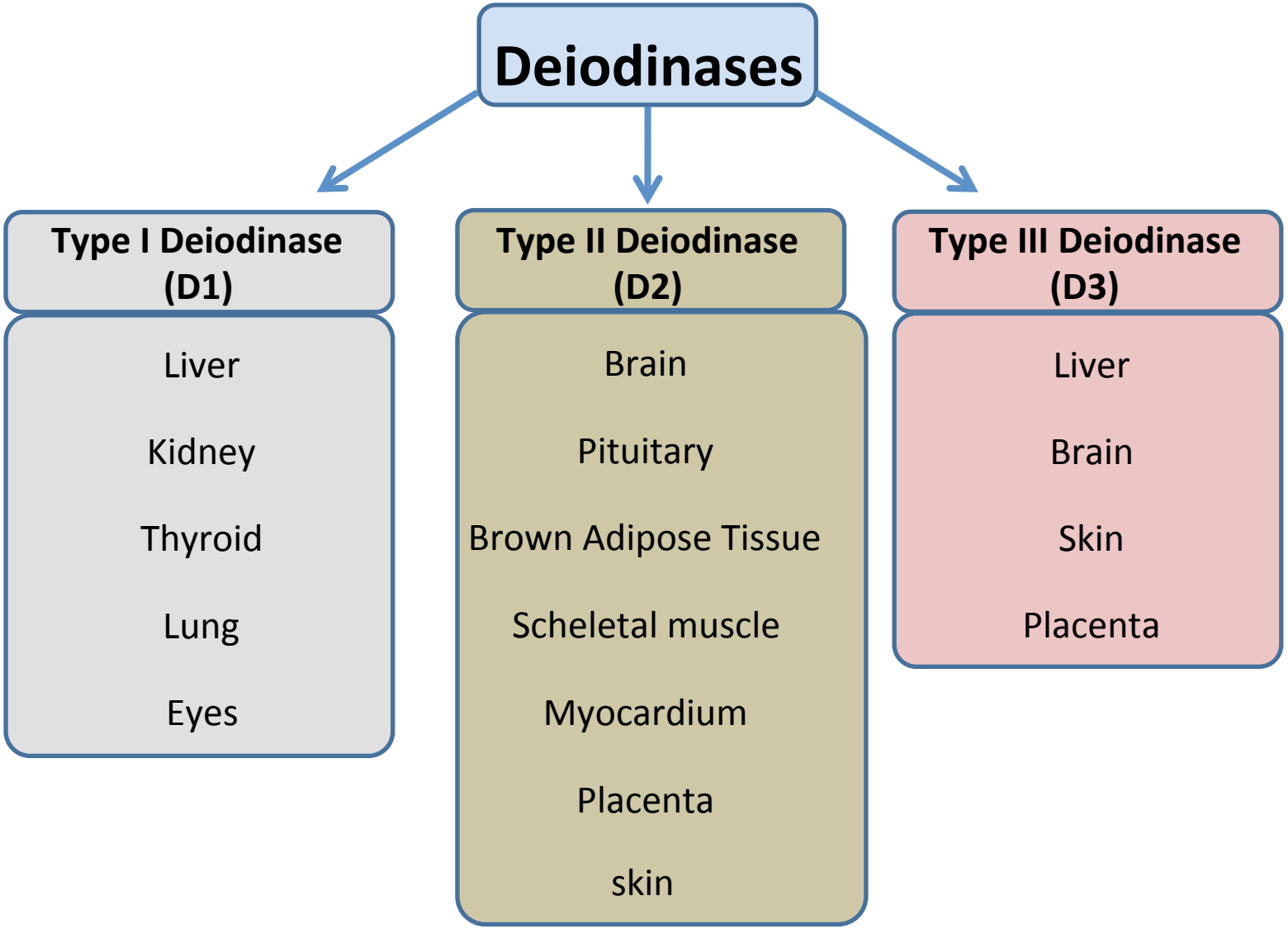
Deiodinases
Type I Deiodinase (D1)
Liver
Kidney
Thyroid
Lung
Eyes
Type II Deiodinase (D2)
Brain
Pituitary
Brown Adipose Tissue
Scheletal muscle
Myocardium
Placenta
skin
Type III Deiodinase (D3)
Liver
Brain
Skin
Placenta

Figure 2

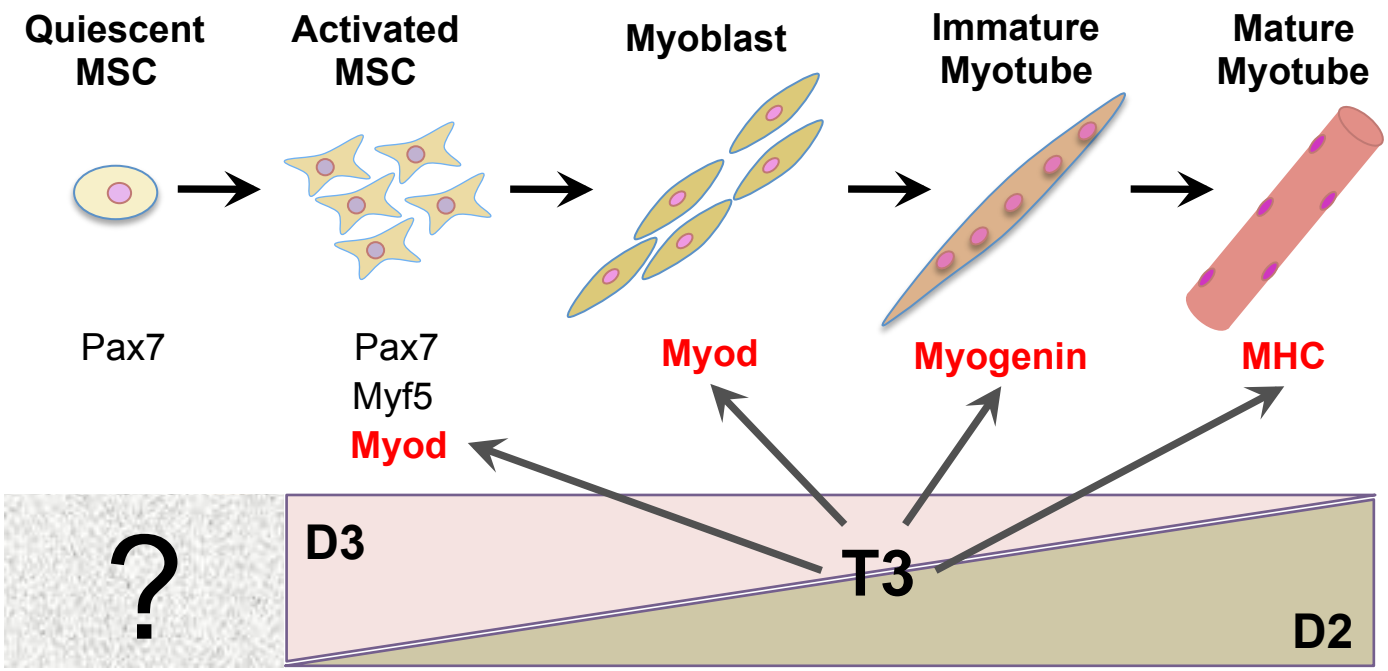
Quiescent MSC
Activated MSC
Myoblast
Immature Myotube
Mature Myotube
Pax7
Pax7
Myf5
Myod
Myod
Myogenin
MHC
?
D3
T3
D2

Figure 3