Deiodinases: the balance of thyroid hormone

Local impact of thyroid hormone inactivation

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Abstract

Deiodination is a critical process by which the minimally active thyroxine (T4) molecule is converted into the favorite ligand for thyroid hormone (TH) receptors, triiodothyronine (T3). The iodothyronine deiodinases type 1, 2, and 3 (D1, D2, and D3) constitute a potent mechanism of TH activation (D1 and D2) or inactivation (D3), which functions by tissue specifically regulating TH bioavailability. D2 and D3 are widely expressed and in a dynamically and tightly coordinated fashion, thereby allowing cells to customize their own TH activity. D3, the major T3 and T4 inactivating deiodinase, catalyzes their conversion to 3,3'-diiodothyronine and to reverse T3 respectively. According to common wisdom, D3 plays a major role in lowering serum TH concentrations during development, as supported by the much wider D3 tissue expression in the embryo structures than in the adult tissues. However, several recent studies show that D3 is reexpressed in adult life in various pathophysiological contexts, which strengthens the concept that cell-specific TH inactivation is a critical mediator in cellular TH metabolism. This review focuses on the progress made in understanding the physiological function and significance of D3. It summarizes the intriguing evidence that D3 plays a pivotal role in defining local TH concentration in the developing fetus and in several conditions in adult life.


Introduction

Thyroid hormone action and deiodinases

Thyroid hormones (THs) are iodinated compounds known to influence gene expression in virtually every vertebrate cell; they exert this function through ligand-dependent transcription factors, namely, TH receptors (TRs). TH action is critically important for development, tissue differentiation, and maintenance of metabolic balance in mammals. Severe disruption of TH action during fetal and early neonatal development leads to several permanent deficits in experimental animals and humans.

Several observations indicated that TH uptake might be facilitated by different types of transporters. This was recently confirmed by the molecular identification of TH-transporting proteins (Jansen et al. 2005). These include members of the organic anion-transporting polypeptide and monocarboxylate transporter families (reviewed in Hagenbuch (2007) and Visser et al. (2007)). TH transport across the plasma membrane is a critical mechanism for TH action, and the existence of these transporter families represents a new mechanism by which TH metabolism and action are regulated.

Thyroxine (T4) can be activated to triiodothyronine (T3) in a stage- and tissue-specific manner by phenolic ring deiodination (outer ring deiodination) catalyzed by two iodothyronine deiodinases, D1 and D2, while a third deiodinase, D3, prevents T4 activation and terminates T3 action. According to the modern paradigm of TH action, because of local deiodination, TH signaling in individual tissues can change even though serum hormone concentrations are unaffected.

At nuclear level, TH signaling results from the interaction of TRs, which are members of the thyroid/steroid nuclear receptor super family, with specific genomic regions of target genes, a process that can either enhance or repress transcription. TRs recruit activators such as the SMART

This paper is one of three papers that form part of a Thematic review section on deiodinases: the balance of thyroid hormone. The Guest Editor for this section was Domenico Salvatore, University of Naples, Italy; he was not involved in the handling of this paper, on which he is listed as an author.
complex, or repressors such as the histone deacetylases (HDACs), and alter the transcription of a variety of genes (Zhang & Lazar 2000, Nishihara et al. 2004). Obviously, signaling through this pathway is sensitive to fluctuations in serum levels of TH, and the consequences of deranged thyroid function, as occur for instance in patients with Graves disease or Hashimoto thyroiditis, have been known for over 100 years.

**Type 3 iodothyronine deiodinase**

Type 3 deiodinase – the main physiological inactivator of TH – catalyzes the conversion of T₃ and T₄ to their inactive derivatives, 3,3',5'-diiodothyronine and reverse T₃ (rT₃). This enzyme is thought to control TH homeostasis by protecting tissues from excess of TH. D₃-containing microsomes in vitro exhibit a Kₘ for T₃ of 6 nM and a somewhat higher value for T₄ (37 nM). D₃ activity is insensitive to propylthiouracil inhibition, whereas it is relatively sensitive to inhibition by gold thioglucose and iodinated radiographic contrast agents such as iopanoic acid (Salvatore et al. 1995).

Similar to D₃, D₃ contains a single predicted highly conserved transmembrane domain between residues 16 and 41. D₃ is an integral membrane protein, resistant to extraction from microsomal membranes by high pH (Visser et al. 1979, Schoenmakers et al. 1995). Endogenous D₃ protein is expressed, by immunohistochemistry, on the plasma membrane of NCLP-6E cells (Baqui et al. 2000), whereas intracellularly it colocalizes with the endosomal marker EEA-1 and clathrin, and not with the two ER-resident proteins BiP and calnexin (Baqui et al. 2003). Studies conducted using fluorescence resonance energy transfer in living cells demonstrated that D₃ functions as a homodimer and that the homo- or heterodimer state is critical for its catalytic activity (Sagar et al. 2008). Its half-life is about 12 h, and there is yet no evidence of regulation of D₃ protein at the post-transcriptional level (Gereben et al. 2008a).

Thus far, D₃ activity has been identified in only a limited number of postnatal tissues, i.e. brain, skin, and pregnant uterus, whereas it is abundantly present in fetal tissues. These include liver and brain, different internal epithelia, umbilical arteries, and vein, lung, heart, intestine, and skin (Huang et al. 1986, Van der Geyten et al. 1997, Bates et al. 1999). D₃ is highly expressed in the human placenta, where it plays a role in limiting fetal tissue exposure to TH. In particular, high D₃ levels have been identified in the syncytiotrophoblast and cytotrophoblast layers, in the maternal decidua and in the amnion sheath of the umbilical cord, and in the fetal endothelium of the chorionic villi (Huang et al. 2003).

D₃ protein is present in both mouse and human skin. The skin is the largest organ in humans and serves as a metabolically active biological barrier that separates internal tissues from the external environment. The epidermis and dermis, which are the two layers constituting the adult skin, are targeted by TH. D₃ is first expressed in the mouse epidermis at E13.5; it is highly expressed in the epidermal layers and in hair follicle keratinocytes at E17.5. D₃ expression is very high in anagen (growing) phase of the hair follicle cycle, as well as in the surrounding outer root sheath. It progressively decreases during catagen and is virtually absent in telogen (Dentice et al. 2007). D₃ activity has been detected in vitro in the culture of brown preadipocytes. In this system, differentiation of precursor cells to adipocytes parallels decreased levels of D₃ expression (Hernandez et al. 2007).

Of note, several studies have revealed the reexpression of D₃ in different pathophysiological conditions, among which are cancer, cardiac hypertrophy, myocardial infarction (MI), chronic inflammation, and critical illness (Gereben et al. 2008b). Because of its presence in fetal and in malignant tissues, D₃ is referred to as an ‘oncofetal enzyme’. It is not coincidental that its highest expression has been detected in an infantile human hemangioma (Huang et al. 2000).

After the *Xenopus laevis* D₃ was cloned, the corresponding cDNAs of many species (rat, human, chicken, and tilapia) were isolated. The human D₃ mRNA encompasses 2066 nt and contains 220 bp of 5’-untranslated region (5’-UTR), an open reading frame of 834 bp, and a 3’-UTR of 1012 bp. The deduced amino acid sequence predicts a protein of 278 residues, with a molecular mass of about 32 kDa (Salvatore et al. 1995). Characterization of the mouse *Dio3* gene has shown that the coding and 3’-UTRs are contained in a single exon, ~1.9 kb long, which is transcribed using a promoter located immediately upstream (Croteau et al. 1995). All D₃ cDNAs cloned to date include a selenocysteine-encoding TGA codon and an SECIS element in the 3’-UTR. The selenocysteine residue is located in the catalytic pocket and is essential for maximal catalytic efficiency (Kuper et al. 2003).

Although the 2-3 kb band is the major mRNA in most tissues, at least four differently sized mRNAs from the rat central nervous system hybridize with the D₃ cDNA, and all are regulated by the thyroid status (Hernandez et al. 2004). Both mouse and human *Dio3* genes map to chromosomal regions that are known to include imprinted genes (chromosome 12F1 and 14q32 respectively, Fig. 1; Hernandez et al. 1998, Tsai et al. 2002). Indeed, the *Dio3* gene is subject to genomic imprinting and is preferentially expressed by the paternal allele in the mouse fetus. Alterations in genomic imprinting of chromosomes 12 and 14 in mice and humans respectively, lead to abnormal phenotypes (Sutton & Shaffer 2000). To what extent alterations in D₃ expression contribute to these abnormal phenotypes remains to be investigated. The locus containing the *Dio3* gene includes another imprinted non-coding gene, *Dio3os*, that is transcribed from the DNA strand opposite to that of *Dio3* (Fig. 1). The *Dio3os* gene partially overlaps to the coding region of the *Dio3* gene and may thus interfere with the translation of the *Dio3* transcript. In total, six different *Dio3os* transcripts have been described in different tissues and at different developmental stages in mouse (Hernandez et al. 2004). Of the six transcripts, three are most abundantly expressed in the mouse fetus and are transcribed biallelically (Tierling et al. 2006). Interestingly, Hernandez et al. (2004) have

demonstrated an inverse correlation in gene expression between the paternally imprinted Dio3 gene and the maternally imprinted Dio3os gene.

A critical role has been attributed to the genomic region containing Dio3 during development. This DNA region (about 836 kb large) is located on the long arm of mouse chromosome 12 and includes the genes Dlk1 and Dio3 at either end (Fig. 1). This region contains, besides five protein-coding genes, Delta-like homolog 1 (Dlk1); retrotransposon-like gene 1 (Rtl1/Mart1), B830012L14Rik, and Dio3; three long non-coding RNA genes, Meg3, Rian, and Mirg; one C/D box small nucleolar RNA cluster, and a cluster of 47 miRNAs (Table 1). All genes in this domain together with the 47 miRNAs are developmentally regulated and expressed in a range of embryonic and extra embryonic tissues with postnatal expression being found predominantly in the adult brain (Seitz et al. 2004, Tierling et al. 2006). Although the protein-coding genes within the Dlk1-Dio3 region are highly conserved during evolution, the miRNA cluster is conserved only in mammals (Edwards et al. 2008). The functions of these genes and miRNAs are largely unknown. Interestingly, silencing of the Dlk1-Dio3 locus is one unique feature of induced pluripotent stem cells (iPSC) clones in which Dlk1-Dio3 locus is transcriptionally silenced contributed poorly to chimeras and failed to support the development of entirely iPSC-derived animals. In contrast, iPSC clones with normal expression of the Dlk1–Dio3 cluster contributed to high-grade chimeras and generated viable all-iPSC mice. Therefore, the Dlk1–Dio3 region may serve as a marker to distinguish fully pluripotent iPSC or embryonic stem cells from partial pluripotent cells (Liu et al. 2010). The role played by the single Dio3 gene product in these complex developmental programs is unknown and requires further studies.

Mechanisms regulating type 3 deiodinase

Various agents are able to regulate D3 expression both in vitro and in vivo (Table 2). There is a strong correlation between changes in the level of D3 mRNA and changes in D3 activity. D3 cDNA cloning from X. laevis provided the first direct evidence that the Dio3 gene is positively regulated by TH (Becker et al. 1997). TH regulates D3 expression at transcriptional level in several other systems (St Germain et al. 1994, Van der Geyten et al. 1999b). In the rat, D3 activity is increased in hyperthyroidism and decreased in hypothyroidism (Mori et al. 1995, Escobar-Morreale et al. 1997). TH and retinoic acid also upregulate D3 activity and miRNA in cultured glial cells (Esfandiari et al. 1994). Being positively
regulated by TH, D3 represents a powerful mechanism by which increased TH inactivation participates in TH homeostasis in thyrotoxic states.

Several hormones and growth factors involved in the control of cell proliferation stimulate D3 expression. Estrogens and progesterones independently upregulate D3 expression in the uterus, and sex hormones upregulate D3 in the adult ovary and developing testis, all tissues that express modest amounts of D3 activity (Bates et al. 1999). Furthermore, D3 expression is strongly induced by epidermal and fibroblast growth factors (Hernandez & Obregon 1995). In astrocytes and preadipocytes, serum and phorbol esters induce D3 expression (Courtin et al. 1991), a stimulatory effect that appears to be mediated, at least in part, by the activation of the MEK/ERK signaling cascade (Pallud et al. 2013). Serum and phorbol esters of the MEK/ERK signaling cascade (Pallud et al. 2013) that appears to be mediated, at least in part, by the activation of the MEK/ERK signaling cascade (Pallud et al. 2013) has been implicated in the increase in D3 mRNA induced by fluoxetine in astrocytes (Pallud et al. 1999). D3 mRNA is transcriptionally stimulated by transforming growth factor β (TGFβ), acting via a Smad-dependent pathway, in diverse human cell types including fetal and adult fibroblasts, hemangioma cells, fetal epithelia, and skeletal muscle myoblasts (Huang et al. 2005).

Hypoxia stimulates D3 activity and mRNA through a mechanism that increases HIF-1 levels. In a rat model of cardiac failure induced by right ventricular (RV) hypertrophy, HIF-1α and D3 proteins were expressed specifically in the hypertrophic myocardium of the right ventricle, thereby resulting in an anatomically specific reduction in local T₃ content and action. These findings are consistent with a mechanism of metabolic regulation during hypoxic–ischemic injury in which HIF-1 reduces local TH signaling through induction of D3 (Simonides et al. 2008). Finally, the Shh transcriptional effector Gli2 significantly increased D3 mRNA expression, protein levels, and enzymatic activity in keratinocytes — an effect that is reversed by forskolin or cyclopamine, both of which inhibit Shh signaling (Dentice et al. 2007).

The **D3KO mouse model**

The genetic mouse model of D3 deficiency has been instructive in understanding the role of D3 with regard to fertility and developmental processes. Both male and female D3KO mice showed impaired fertility, significant perinatal mortality, and growth impairment (Hernandez et al. 2006). In addition, the developmental programming of the thyroid axis is perturbed in the D3KO mouse, presumably due to the overexposure of the animal to excessive levels of TH in utero and during the first weeks of perinatal life. Thus, the D3KO mouse manifests marked abnormalities in thyroid status and physiology, which underscores the critical role of the D3 enzyme in the development and in the function of the hypothalamic–pituitary–thyroid (HPT) axis. Interestingly, the abnormalities of the HPT resemble those observed in children born to mothers affected by hyperthyroidism during pregnancy (Dussault et al. 1982, Kempers et al. 2003). Although much remains to be explored regarding the

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**Table 1** Clusters of miRNA within the Dlk1/Dio3 region. miRNAs recently reported in the UCSC Genome Browser database (Rhead et al. 2010) are indicated in bold

<table>
<thead>
<tr>
<th>miRNAs (A)</th>
<th>miRNAs (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mir1906-1</td>
<td>Mir882</td>
</tr>
<tr>
<td>Mir1906-2</td>
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<td>Mir411</td>
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<td>Mir299</td>
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<td>Mir493</td>
<td>Mir380</td>
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<td>Mir337</td>
<td>Mir1197</td>
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<tr>
<td>Mir540</td>
<td>Mir323</td>
</tr>
<tr>
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<td>Mir758</td>
</tr>
<tr>
<td>Mir3070a</td>
<td>Mir329</td>
</tr>
<tr>
<td>Mir3070b</td>
<td>Mir494</td>
</tr>
<tr>
<td>Mir431</td>
<td>Mir679</td>
</tr>
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<td>Mir433</td>
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<td>Mir376c*</td>
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<td>Mir654</td>
</tr>
<tr>
<td>Mir1188</td>
<td>Mir376b*</td>
</tr>
<tr>
<td>Mir370</td>
<td>Mir376a*</td>
</tr>
</tbody>
</table>

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**Table 2** Transcription factors and agents regulating D3 expression

<table>
<thead>
<tr>
<th>Agent</th>
<th>Effects on D3 expression</th>
<th>Tissue or animal model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperthyroidism</td>
<td>↑</td>
<td>Xenopus leavis</td>
<td>St Germain et al. (1994) and Becker et al. (1997)</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>↑</td>
<td>Astroglial cells</td>
<td>Esfandiari et al. (1994)</td>
</tr>
<tr>
<td>Serum growth factors</td>
<td>↑</td>
<td>Rat brown adipocytes</td>
<td>Hernandez &amp; Obregon (1995)</td>
</tr>
<tr>
<td>Serum and phorbol esters</td>
<td>↑</td>
<td>Astrocytes and preadipocytes</td>
<td>Courtin et al. (1991)</td>
</tr>
<tr>
<td>Estrogens and progesterone</td>
<td>↑</td>
<td>Ovary and developing testis</td>
<td>Wasco et al. (2003)</td>
</tr>
<tr>
<td>Glucocorticoids and GH</td>
<td>↓</td>
<td>Chicken embryos</td>
<td>Van der Geyten et al. (1999a)</td>
</tr>
<tr>
<td>TGFβ</td>
<td>↑</td>
<td>Fibroblasts, myoblasts, and hemangioma cells</td>
<td>Huang et al. (2005)</td>
</tr>
<tr>
<td>HIF-1</td>
<td>↑</td>
<td>Rat heart</td>
<td>Simonides et al. (2008)</td>
</tr>
<tr>
<td>Shh/Gli2</td>
<td>↑</td>
<td>Mouse keratinocytes and BCC</td>
<td>Dentice et al. (2007)</td>
</tr>
</tbody>
</table>
Phenotypic abnormalities in the D3KO mouse, studies conducted to date clearly demonstrate the importance of D3 during development. However, refinement and optimization of the animal models, such as the generation of a conditional Dio3-KO mouse, will provide useful tools to advance our molecular understanding of D3’s role in the development and its specific role in local tissue physiology.

**Tissue-specific regulation of T₃ levels by D3**

*D3 in development and metamorphosis*

The coordination of developmental processes in all vertebrate species requires appropriate control of TH levels. During embryogenesis, insufficient levels of T₃ and/or premature exposure of the embryo to excessive or ‘adult-like’ T₃ concentrations can be detrimental and result in abnormal development (Porterfield & Hendrich 1993). During embryogenesis, D3 expression protects several organs from deleterious T₃ exposure, whereas postnatal decline in D3 expression allows later induction of tissue development and patterning. Thus, local attenuation of T₃ action by D3 is a prerequisite for correct maturation of many tissues, which vary in the threshold of T₃ requirement.

The most fascinating example of the role played by TH in development is the regulation of amphibian metamorphosis that, although complex, is initiated and orchestrated by one signal, TH. In 1912, Friedrich Guernatsch showed that feeding *Xenopus* tadpoles with the thyroid gland extracts induced metamorphosis. Shortly after, the active substance was identified as T₄. Subsequently, different studies showed that each of the components of TH signaling, namely ligand availability, expression of deiodinases, and receptors, are regulated in a tissue-specific manner in the early development of *Xenopus*, where they participate in such a complex developmental program (Marsh-Armstrong et al. 1999, Morvan-Dubois et al. 2008). In *Xenopus*, two deiodinases (D3 and D2) exert a major control of local T₃ concentrations (Morvan-Dubois et al. 2008). As discussed above, D3 plays a major role in preventing the premature exposure of selected tissues to inappropriate levels of T₃. Conversely, D2 also expressed in most tissues over a restricted period of time during *Xenopus* development, contributing to the enhanced T₃ signaling in selected cells.

In *Rana catesbeiana* tadpoles, when endogenous TH synthesis was blocked by methimazole or when D2 and D3 activities were inhibited by iopanoic acid, metamorphosis was completely blocked (Marsh-Armstrong et al. 1999). The inhibition could be overcome by the concomitant administration of replacement levels of T₃, but not T₄ (Becker et al. 1997). These results illustrate that the expression of D2 and D3 is programmed to provide the necessary amount of T₃ at the appropriate time of development. Furthermore, transgenic overexpression of D3 in metamorphosing tadpoles markedly inhibits TH-dependent proliferation of retinal cells that become resistant to exogenous TH, an effect abrogated by iopanoic acid. These results clearly demonstrate that the localized expression of D3 is sufficient to account for the asymmetric response of the retina at metamorphosis (Marsh-Armstrong et al. 1999).

How does TH trigger the complex mechanism of *Xenopus* metamorphosis and the developmental programs of several different organisms? During the adaptation from aquatic to terrestrial life, the digestive tract is drastically remodelled and most larval visceral epithelial cells undergo apoptosis, a process that can be organ-autonomously reproduced by TH (Ishizuya-Oka et al. 2010). Several studies identified a set of TH-responsive genes potentially involved in the remodeling of larval intestine in amphibian metamorphosis (Shi & Brown 1993, Buchholz et al. 2005). Although the functions of these genes remain mostly unknown, some of them are of great interest and seem to show an expression profile that is finely regulated during intestine reabsorption (Ishizuya-Oka et al. 2010). The challenge for the next years is to probe further and deeper into the molecular mechanisms downstream of TH action.

A similar tissue-specific, deiodinase-dependent differentiation program occurs in other developing organisms like neuronal and glial maturation in rat brain and in the mouse cochlea (Becker et al. 1997, Campos-Barros et al. 2000). In the retina, the development of cones, the photoreceptors for daylight, and color vision require protection from TH by over-expression of D3 (Ng et al. 2010). Excessive TH or loss of D3 in D3KO mice leads to 80% cone loss through apoptotic cell death. Cone survival could be rescued by the deletion of TRß2, demonstrating that cone survival is sensitive to TH (Ng et al. 2010). Paradoxically, proper TH signaling is required for normal cone formation, suggesting that D3 acts to limit the exposure of cones to T₃ to avoid cell death, but allowing the action of a minimal amount of T₃ necessary for cone survival and patterning.

**Role of D3 in disease**

Deiodinase activities can modulate TH signaling in different pathological conditions. Aberrant expression of individual deiodinases, single nucleotide polymorphisms in their genes, and novel regulators of expression of Dio genes all contribute to the systemic and local bioavailability of THs that can occur in pathological conditions. An impressive example is the over-expression of D3 in juvenile and adult hemangiomas that leads to ‘consumptive hypothyroidism’ due to excessive degradation of T₄ and T₃ (Huang et al. 2000). Furthermore, several pathophysiological conditions associated with severe disease are characterized by significant changes in the concentration of circulating TH, together with changes in deiodinase pathways. Such alterations may be so potent that TH signaling is compromised both locally and systemically, as happens during the low T₃ syndrome or non-thyroidal illness syndrome (NTI). The hallmark of these responses is a decrease in circulating free T₄ and an increase in total rT₃, D3
was thought not to play an important role during NTI, but recent studies show that D3 activity is upregulated in liver and skeletal muscle of critically ill patients (Peeters et al. 2003, Boelen et al. 2005), and this upregulation could contribute to the massive T₃ degradation. However, the exact role of D3 during NTI is unknown.

Although patients with genetic defects in the D3 gene have not been identified yet, intense study over the past decade has greatly expanded our knowledge about the presence and significance of altered D3 activity in different clinical settings. Below, we focus on the complex changes in TH metabolism following D3 reexpression in different pathological conditions.

D3 and the inflammation processes

The inflammatory response is associated with profound changes in TH metabolism. Inflammation is often associated with low serum T₃ and T₄ levels accompanied by changes in liver D1 and D3, muscle D2 and D3 expression (Boelen et al. 2005). Using a mouse model of acute bacterial infection, Kwakkel et al. (2010) demonstrated that D3 is highly expressed in neutrophils that infiltrate infected organs, predominantly polymorphonuclear cells (PMNs) and granulocytes. D3 induction is not pathogen specific because it occurs during peritonitis and pneumonia induced by different pathogens. The presence of D3 indicates that this enzyme is involved in the innate immune response and that it may act by reducing T₃ bioavailability or by generating iodide (I⁻) that is required by myeloperoxidase to enable PMNs to kill microbes. In fact, bacterial phagocytosis by PMNs requires iodine compounds, and T₄ and T₃ could represent an important source of iodide as leukocytes have been demonstrated to take up and deiodinate TH (Siegel & Sachs 1964).

Similarly, by inducing a turpentine-induced abscess, Boelen et al. (2005) have demonstrated induction of D3 activity in inflammatory cells that migrate to the site of inflammation. The acute inflammatory response is characterized by D3 expression in infiltrating granulocytes during chemical and bacterial peripheral inflammations (Boelen et al. 2005, 2008), suggesting that the induction of D3 might be relevant in inflammatory cells. High D3 expression during inflammation could also favor cell proliferation and tissue repair, by reducing TH signaling locally.

Several lines of evidence indicate that D3 may contribute to the pathogenesis of NTI by reducing circulating TH levels during illness when D3 activity is upregulated in the liver and skeletal muscle (Peeters et al. 2003). Recent studies have demonstrated that D3 affects the pathogenesis of NTI mostly during chronic illness, whereas its role in the T₄/T₃ changes observed during acute inflammation might be less relevant (Boelen et al. 2009). Using a mouse model of acute bacterial infection in D3-null and WT mice, Boelen et al. showed that D3 is not involved in the generation of the systemic manifestations of NTI in this model, which suggested that the changes in TH serum concentrations occurring during acute illness could be mostly due to reduced serum TBG.

While D3 mRNA is upregulated in the liver and skeletal muscle of critically ill patients, its hypothalamic concentrations decrease in the paraventricular nucleus of turpentine-injected mice (Boelen et al. 2006). Besides reducing D3 mRNA expression, turpentine-induced chronic inflammation causes an early increase of hypothalamic D2 mRNA expression, which, in turn, results in a local increase in tissue T₃ concentrations (Boelen et al. 2006). This cascade indicates that chronic inflammation exerts an inverse regulation of D2 and D3 expression in the hypothalamus (Boelen et al. 2006). Thus, infection may promote a local central hyperthyroidism by oppositely regulating D2 and D3, which in turn leads to a negative feedback control on TRH synthesis in hypothalamic neurons. In this scenario, D3 may be involved in the low TSH seen in inflammation and thus participate in the diminished TH production.

Moreover, chronic local inflammation induced by turpentine injection in the hindlimb – which results in serial activation of specific inflammatory cytokines – causes significant induction of local D3 activity in the muscle/subcutis around the resulting abscess, whereas liver D1 and D3 activities remain unchanged (Boelen et al. 2005).

Overall, several lines of evidence indicate that D3 overexpression is a common mark of inflammatory response. Taken together, these studies suggest that enhanced local degradation of T₃ during inflammation may contribute, with a not yet determined mechanism, to such a process. The increased D3 levels during inflammation could also suggest that sustained D3 is one of the leading events in the NTI syndrome, often observed during chronic inflammation.

D3 and regeneration

The contribution of deiodinases to mechanisms of tissue injury is among the most interesting recent findings regarding the impact of peripheral control of TH metabolism in the progression and outcome of disease states. A number of recent studies have demonstrated that deiodinases play a crucial role in repair mechanisms, as is the case in liver (Kester et al. 2009), brain (Li et al. 2001a,b), and muscle (Dentice et al. 2010). Such an involvement is also supported by the observation that D2 and D3 activities are regulated by a variety of growth factors and morphogens, which are important mediators of tissue injury repair.

Partial hepatectomy is commonly used to study liver regeneration in experimental animals. Kester et al. (2009) have first demonstrated the induction of D3 during liver regeneration. After massive hepatectomy, ‘stem-like’ cells switch from a quiescent state to a proliferative state and reenter the cell cycle. During these processes, many fetal genes are reactivated (Tanimizu & Miyajima 2007). Among them, D3 activity was increased tenfold and D3 mRNA expression was increased threefold 20 h after partial hepatectomy in rats. The increase in D3 expression was associated
with a maximum two- to three-fold decrease of serum and liver T3 and T4 levels. Similar inductions were also observed in a parallel experiment in mice. No significant effects on D1 and D2 activities or mRNA expression were found after partial hepatectomy (Kester et al. 2009).

It is widely accepted that THs are essential in the development of brain and repair of the nervous system (Oppenheimer & Schwartz 1997). After cryolesion, D3 is upregulated; its expression lasted 3 days in the proximal nerve segment and was sustained for 10 days in the distal segment, then declined to reach basal levels after 28 days, when recovery was fully completed (Li et al. 2001a). Studies in rats have documented a rapid increase of D3 expression in the distal and proximal segments after nerve lesion (Li et al. 2001a). D2 is also induced in injured sciatic nerve, and the induction of transient upregulation of D2 mRNA has been described in astrocytes after transient focal cerebral ischemia in rats (Margail et al. 2005). The expression of D2 and D3 is modulated by hypoxia in different models of injury to the nervous system (central and peripheral), suggesting that the coordinated expression of these enzymes may occur in these events.

In conclusion, recent studies have shown a close relationship between TH metabolism, via deiodinases expression, and the regulatory networks that control tissue repair. This leads to the concept that finely tuned TH concentration is essential in the control of the proliferation and differentiation balance necessary in the regeneration processes. In this scenario, massive induction of D3 expression in the early phases of regeneration may well correlate with a requirement of increased cellular proliferation in these circumstances.

D3 reactivation in heart diseases

D3 expression is significantly induced in several cardiac disorders. The similar changes in cardiac gene expression between pathological ventricular hypertrophy and hypothyroidism suggest that impaired cardiac TH signaling plays a role in heart failure. Induction of D3 enzyme has recently been reported in several models of ventricular remodeling and is suggested to be involved in altered TH signaling in the failing heart.

In a rat model of RV hypertrophy induced by pressure overload, D3 activity was increased by as much as fivefold in hypertrophic RV but was unaltered in the non-hypertrophic left ventricle. Stimulation of D3 activity in RV was highest in animals that developed pathological RV hypertrophy and failure, whereas D3 activity was moderately increased in animals with compensatory hypertrophy (Wassen et al. 2002). The question as to whether D3 expression in cardiac hypertrophy represents a beneficial or a maladaptive response that contributes to the development of heart failure is still open and is a suitable topic for investigation (Pol et al. 2010). Olivares et al. have reported a time-course study in rats subjected to MI by left coronary ligation. They found that D3 was expressed at high levels in infarcted cardiac tissue, thereby reinforcing the concept that D3-mediated inactivation is the main cause of the dramatic decrease in serum TH levels that occurs subsequent to MI (Olivares et al. 2007).

Hypoxia induced the expression of the Dio3 gene via a hypoxia-inducible (HIF-dependent) pathway. HIF-1α and D3 proteins were induced specifically in the hypertrophic myocardium of the RV, thereby creating an anatomically specific reduction in local T3 content and action (Simonides et al. 2008). Hypoxia-induced D3 activation leads to reduction of T3 and oxygen consumption, suggesting that D3 activity is a component of cellular responses to hypoxia (Diano & Horvath 2008).

Recently, Pol et al. have monitored D3 in mice for 8 weeks after MI. D3 activity was strongly induced in the non-infarcted area of the left ventricle, which corresponded to an increase in D3 mRNA and protein in cardiomyocytes in the first week after induction of infarction that persisted until at least 8 weeks after MI. Using an artificial TH-responsive reporter, local hypothyroidism was in the infarcted heart, which corresponded to reduced tissue T3 content, whereas plasma T3 levels were unchanged (Pol et al. 2011).

In all these circumstances, the cardiac-restricted hypothyroid condition may contribute to the complex phenotype of pathological heart remodeling as exemplified by the dramatic shift in MHC isoforms expression, which mostly depends on intracellular T3 levels.

D3 and cancer

The TH inactivation pathway via D3 has recently been implicated in the postembryonic regulation of proliferation in a number of cell types including keratinocytes and brown adipocyte precursors (Dentice et al. 2007, Olivares et al. 2007) as well as in various pathological conditions associated with enhanced proliferation, including cancer (Gereben et al. 2008a). A pathological role for the TH pathway emerged from studies showing that TR is involved in tumorigenesis and that v-erbA, the viral oncoprotein involved in avian erythroblastosis, is the cellular homolog of TRα1 (Sap et al. 1986). Furthermore, chromosomal alterations and small mutations in TR genes have been associated with several human cancers (Dentice et al. 2009).

D3 is turned on in several malignant cell lines (Kester et al. 2006) and in a number of human tumors, including astrocytomas, oligodendrogliomas, glioblastoma multiforme, and basal cell carcinomas (Gereben et al. 2008b). Tumoral D3 activity can be robust, and the elevated D3 activity reported in vascular tumors, including infantile hemangiomias and adult patients with hemangioendothelioma, supports this concept (Huang et al. 2002).

As TH is usually a differentiation agent, it is conceivable that local modulation of TH signaling plays a role in tumorigenesis, and, vice versa, its manipulation could be proposed as a tool to interfere with tumor progression. An exciting discovery in this field was the identification of the regulation of D3 expression by the Hedgehog cascade. Shh
induces proliferation in a wide variety of cellular systems including the skin, and its aberrant activation is the leading cause of several human tumors (Johnson et al. 1996). In primary proliferating keratinocytes, as well as in basal cell carcinomas, Shh increases the expression of D3, acting via a conserved Gli2 binding site on the human Dio3 promoter (Dentice et al. 2007). The Shh-mediated induction of D3 strongly suggests that Shh may induce local TH attenuation in the microenvironment. A fascinating speculation is that abrogation of TH signaling in a time- and cell type-specific fashion is essential for the biological effect of this morphogen.

The TH–Shh interaction adds an important ‘proof of concept’ for the role of D3 in the tumorigenesis of basal cell carcinoma and raises the possibility of modulating TH signaling in a specific cell compartment to modify its functional behavior. In addition, TH is involved in several systems in the control of apoptosis (Nakajima & Yaoita 2003, Puzianowska-Kuznicka et al. 2006), a process directly linked with tumor progression. Whether D3 is a secondary event or whether, alternatively, its action results in a selective advantage for growing cells has to be formally demonstrated. The restricted range of tumor associated with overexpression of D3 suggests that initiation of tumorigenesis does not necessarily require D3 and that, vice versa, D3 expression may be one of the multiple mechanisms adopted by tumoral cells to get rid of the differentiating action of T3.

In summary, local attenuation of T3 levels via D3 occurs in many human tumors. The multitude of agents, transcription factors, and morphogens that are able to induce D3 indicates that reducing TH signaling in these cells may be advantageous for proliferation. Understanding the molecular basis of this phenomenon might prompt future research to find new efficient strategies to treat human cancer.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by grants from AIRC to D S and M D.

Acknowledgements

We are grateful to Dr Raffaele Ambrosio for extracting data from the Genome Browser database. We are indebted to Jean Ann Gilder (Scientific Communication srl) for editing the text.

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Received in final form 26 February 2011
Accepted 11 March 2011
Made available online as an Accepted Preprint 11 March 2011


