T₃/TRs axis in hepatocellular carcinoma: new concepts for an old pair

Andrea Perra^{1,*}, Michelina Plateroti^{2,*} and Amedeo Columbano^{1,*}

¹Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy

²Cancer Research Center of Lyon INSERM U1052, CNRS UMR5286, Université de Lyon, Université Lyon 1, Centre Léon Bérard, Département de la Recherche, Lyon, France

*(A Perra, M Plateroti and A Columbano contributed equally to this work)

Correspondence should be addressed to A Perra or M Plateroti or A Columbano

Email

andreaperra@omeca.it or michelina.plateroti@univ-lyon1.fr or columbano@unica.it

Abstract

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide, and its burden is expected to further increase in the next years. Chronic inflammation, induced by multiple viruses or metabolic alterations, and epigenetic and genetic modifications, cooperate in cancer development via a combination of common and distinct aetiology-specific pathways. In spite of the advances of classical therapies, the prognosis of this neoplasm has not considerably improved over the past few years. The advent of targeted therapies and the approval of the systemic treatment of advanced HCC with the kinase inhibitor sorafenib have provided some hope for the future. However, the benefits obtained from this treatment are still disappointing, as it extends the median life expectancy of patients by only few months. It is thus mandatory to find alternative effective treatments. Although the role played by thyroid hormones (THs) and their nuclear receptors (TRs) in human cancer is still unclear, mounting evidence indicates that they behave as oncosuppressors in HCC. However, the molecular mechanisms by which they exert this effect and the consequence of their activation following ligand binding on HCC progression remain elusive. In this review, we re-evaluate the existing evidence of the role of TH/TRs in HCC development; we will also discuss how TR alterations could affect fundamental biological processes, such as hepatocyte proliferation and differentiation, and consequently HCC progression. Finally, we will discuss if and how TRs can be foreseen as therapeutic targets in HCC and whether selective TR modulation by TH analogues may hold promise for HCC treatment.

Key Words

- ▶ thyroid hormone receptor
- ▶ triiodothyronine
- ▶ hepatocellular carcinoma
- ▶ microRNA
- ▶ TR β agonists
- ▶ local hypothyroidism
- ▶ cell differentiation

Endocrine-Related Cancer
(2016) **23**, R353–R369

TH/TR axis

Thyroid hormones, namely 3,5,3'-triiodo-L-thyronine (T₃) and 3,5,3',5'-tetraiodo-L-thyronine (thyroxine or T₄), influence a variety of physiological processes, including development, metabolism, cell growth and proliferation. THs are produced in response to signals deriving from the hypothalamus, which synthesises the thyrotrophin-releasing hormone (TRH). TRH induces the expression

of the thyroid-stimulating hormone (TSH) which, in turn, stimulates the follicular cells of the thyroid gland to secrete mainly T₄. T₄ is then transported across the cell membrane of responsive cells by specific transporters, including the monocarboxylate anion transporters 8 and 10 (MCT8 and MCT10) (Heuer & Visser 2009, van der Deure *et al* 2010), and is converted

into the active T₃ hormone in peripheral tissues, such as liver and kidney. This conversion, carried out by type I 5'-deiodinase (DIO1) and, to a lesser extent, by DIO2 (Gereben *et al.* 2008), leads to increased levels of circulating T₃. On the opposite, type III deiodinase (DIO3) is responsible for thyroid hormone inactivation as it converts T₄ and T₃ to the inactive metabolites rT₃ and 3,3'-diiodothyronine (T₂), respectively.

Although it has been proposed that rapid non-genomic mechanisms initiated at the cell membrane could be involved in mediating the actions of thyroid hormones (for a review, see Davis *et al.* 2016), most of the effects of THs on cellular proliferation and differentiation are mediated through the thyroid hormone nuclear receptors (Brent 1994, Cheng *et al.* 2010). TRs are members of the steroid/thyroid superfamily of nuclear hormone receptors, which are ligand-modulated transcription factors (Mangelsdorf *et al.* 1984, Lazar 1993, Forrest & Vennstrom 2000). The human TRs are encoded by two genes, *THRA* (*NR1A1*) and *THRB* (*NR1A2*), localised in human chromosomes 17 and 3, respectively. Both genes encode for several mRNA isoforms, generated by use of different promoters and alternative splicing (Mitsuhashi *et al.* 1988, Chassande *et al.* 1997, Harvey *et al.* 2007). Although the TR isoforms are widely distributed, differences exist concerning their expression in various tissues and/or during developmental stages. For example, TRα1 is the dominant receptor in the brain and skeletal system and mediates most of the synergism between T₃ and the sympathetic signalling pathway in the heart; TRβ, abundant in liver, is responsible for most of T₃ effects on lipid metabolism and on metabolic regulation (Hsu & Brent 1998, Weiss & Murata 1998, Wikstrom *et al.* 1998, Brent 2000, Kaneshige *et al.* 2000). All TRs have a similar domain organisation that is shared by all nuclear hormone receptors: an amino-terminal A/B domain which recruits co-regulatory proteins; a central DNA-binding domain (DBD), or C region, consisting of two zinc-finger motifs intercalating with the major and minor grooves of DNA; a linker D region, necessary for nuclear translocation of the receptor; and a carboxy-terminal ligand-binding domain (LBD), forming a pocket that binds T₃ (Yen 2001). Acting as transcription factors, TRs bind to specific DNA sequences known as thyroid hormone response elements (TREs), located in the regulatory regions of T₃-target genes, activating or repressing transcription in response to the hormone. *In vivo*, TRs bind TREs predominantly as heterodimers with the retinoid X receptor (RXR), another member of the nuclear hormone receptor superfamily (Lazar 2003).

TH/TRs and cancer

In the last years, emerging evidence has shown that TRs and THs are implicated in cancer. The first evidence came from the demonstration that the *v-ErbA* oncogene, isolated from an avian retrovirus, is an altered form of the *TRα* gene (Thormeyer & Baniahmad 1999) that antagonises TRs activity by competing for TREs or co-activator binding (Yen 1994). Later on, several studies reported TR lower and/or aberrant expression and/or somatic mutations in human cancers (Table 1), supporting the hypothesis that they might play a role in tumour development. Further evidence that TRβ1 acts as a tumour suppressor came from the observation that transgenic mice harbouring a dominant negative mutant *TRβ* (*TRβPV*), originally identified in a patient with thyroid hormone resistance (RTH), spontaneously develop thyroid cancer (Suzuki *et al.* 2002). Moreover, Zhu and coworkers reported that mice devoid of functional TRs (*TRα1*^{-/-}/*TRβ1*^{-/-}) spontaneously develop follicular thyroid cancer and lung metastases (Zhu *et al.* 2010). On the contrary, thyroid hormones and TRα1 receptor promote intestinal tumourigenesis, as they control intestinal epithelial cell proliferation and expression of components of the Wnt pathway (Kress *et al.* 2009, 2010). Thus, while the body of evidence indicates that partial or complete loss of TRs function stimulates the proliferative, invasive and metastatic capacity of tumour cells, other findings show that TRs activation may facilitate tumour progression. Until now, these often contradictory data did not allow to clearly establish whether TRs play an oncogenic or a tumour suppressor role. It is, however, worth underlying that TRs play tissue-specific functions (Kress *et al.* 2009), probably accounting for these contradictory results.

TRs alterations and HCC

Hepatocellular carcinoma (HCC) is the second cause of cancer-related death worldwide, and its burden is expected to increase further in the next future (Jemal *et al.* 2011, Njei *et al.* 2015). While HCC has historically been more common in the developing world, its incidence in developed countries has almost doubled in the last two decades, largely as a result of liver cirrhosis. Since the efficacy of traditional chemotherapeutic agents and of new developed drugs and their ability to produce a significant survival benefit is questionable, there is, thus, an urgent need to develop novel molecular targeted therapies for HCC.

Table 1 Different cancer types displaying *TRβ* downregulation/mutation.

Type of cancer	Species	Regulation	Reference
Breast cancer	Human	Loss of heterozygosity	Ali et al. (1989), Chen et al. (1994)
Breast cancer	Human	A variable degree of <i>TRβ1</i> promoter hypermethylation	Li et al. (2002)
Breast cancer	Human	Lack of <i>TRβ1</i> nuclear staining	Li et al. (2002)
Breast cancer	Human	↓ <i>TRβ1</i> transcripts in breast cancer cell lines	Li et al. (2002)
Breast cancer	Human	↓ <i>TRβ1</i> RNA (semi-quantitative RT-PCR)	Silva et al. (2002)
Breast cancer	Human	↓ protein levels (Western blot)	Silva et al. (2002)
Breast cancer	Human	Tumour-specific truncated <i>TRβ1</i> RNA	Silva et al. (2002)
Breast cancer	Human	↓ <i>TRβ</i> mRNA (RT-PCR)	Ling et al. (2010)
Breast cancer	Human	Hypermethylation in cancer tissues and plasma samples	Ling et al. (2010)
Breast cancer	Human	Low <i>TRβ</i> mRNA levels associated with poor outcome	Gu et al. (2015)
Colon cancer	Human	↓ <i>TRβ</i> mRNA levels (Northern blot)	Markowitz et al. (1989)
Colon cancer	Human	↓ nuclear TRβ1 (IHC)	Hörkkö et al. (2006)
Colon cancer	Human	↓ TRβ1 protein levels (WB)	Hörkkö et al. (2006)
Hepatocellular carcinoma	Human	Point mutations in <i>TRβ1</i>	Lin et al. (1999)
Hepatocellular carcinoma	Human	↓ <i>TRβ1</i> mRNA levels (qRT-PCR)	Frau et al. (2015), Martínez-Iglesias et al. (2016)
Hepatocellular carcinoma	Human	↓ TRβ protein levels (WB)	Liao et al. (2012)
Lung carcinoma	Human	Loss of heterozygosity	Leduc et al. (1989)
Lung carcinoma	Human	Absent TRβ1 expression (RT-PCR)	Iwasaki et al. (2010)
Lung carcinoma	Human	TRβ1 promoter methylation	Iwasaki et al. (2010)
Renal carcinoma	Human	↓ mRNA levels (Northern blot)	Puzianowska-Kuznicka et al. (2000)
Renal carcinoma	Human	↓ protein levels (WB)	Puzianowska-Kuznicka et al. (2000)
Renal carcinoma	Human	<i>TRβ1</i> mutations	Kamiya et al. (2002)
Renal carcinoma	Human	↓ <i>TRβ1</i> mRNA levels (qRT-PCR)	Master et al. (2010)
Renal carcinoma	Human	↓ TRβ1 protein levels (WB)	Master et al. (2010)
Renal carcinoma	Human	↓ <i>TRβ</i> mRNA levels (qRT-PCR)	Wojcicka et al. (2014)
Pituitary tumour	Human	Somatic mutation in the ligand-binding domain of <i>TRβ</i>	Ando et al. (2001a)
Pituitary tumour	Human	Alternative splicing of <i>TRβ2</i> mRNA	Ando et al. (2001b)
Thyroid cancer	Human	↓ mRNA levels (Northern blot)	Wallin et al. (1992), Brönnegård et al. (1994)
Thyroid cancer	Human	↓ mRNA levels (Northern blot)	Puzianowska-Kuznicka et al. (2002)
Thyroid cancer	Human	<i>TRβ</i> mutations	Puzianowska-Kuznicka et al. (2002)
Thyroid cancer	Human	Hypermethylation of the <i>TRβ</i> gene in tumour samples and in thyroid tumour cell lines	Joseph et al. (2007)
Thyroid cancer	Human	Loss of heterozygosity	Joseph et al. (2007)
Thyroid cancer	Human	↓ mRNA levels (qRT-PCR)	Takano et al. (2003), Rosignolo et al. (2015)
Uveal melanoma	Human	Loss of heterozygosity (LOH)	Sisley et al. (1993)
Human hepatocarcinoma and breast cancer cells inoculated into nude mice	Mouse	TRβ1 re-expression reduces tumour growth and has an inhibitory effect on invasiveness, extravasation and metastasis formation	Martínez-Iglesias et al. (2009)
Skin tumours (chemical skin carcinogenesis in mice)	Mouse	↓ TRβ expression (IHC)	Martínez-Iglesias et al. (2009)
Mammary tumour	Mouse	<i>TRβ</i> mutations increase the risks of mammary hyperplasia and tumour	Guigon et al. (2010)
Mammary tumour	Mouse	Mutation of a single copy of <i>TRβ</i> doubles the percentage of Pten ^{+/-} females to develop mammary tumours	Guigon et al. (2010)
Thyroid carcinoma	Mouse	Mice deficient in total functional TRs or with a targeted homozygous mutation of the <i>TRβ</i> gene spontaneously develop metastatic thyroid carcinoma	Suzuki et al. (2002), Kato et al. (2004), Zhu et al. (2010)
Hepatocellular carcinoma	Rat	↓ mRNA levels (qRT-PCR)	Frau et al. (2015)
Hepatocellular carcinoma	Rat	↓ protein levels (WB)	Frau et al. (2015)

TR mutation

Search of activating mutations in *TR* genes in HCC gave rise to contrasting findings. Lin and coworkers (1996) showed that 65% of the tumours analysed display mutations in *TRα* and 76% in *TRβ* genes, with a subgroup exhibiting mutations in both loci (Lin et al. 1999). The majority of the mutated TRs act as dominant negative molecules, hampering the activity of the wild-type receptor, leading to impaired transcriptional activation; moreover, many of them display defects in T₃-dependent co-repressor release and/or co-activator binding (Chan & Privalsky 2006). Notably, these TR mutants activate several pro-proliferative genes, such as colony-stimulating factor 1 (*CSF1*), neuronal cell adhesion molecule (*NRCAM*) and repress tumour suppressor genes, such as dickkopf-related protein 1 (*DKK1*) and tissue inhibitor of metalloproteinase 3 (*TIMP3*) (Yang & Privalsky 2001). Although these findings suggest that *TR* mutations may play an oncogenic role in HCC development, evidence for such a high frequency of *TR* mutations in human HCC has not been confirmed in more recent studies. Indeed, several works based on the powerful deep sequencing analysis did not detect any mutation in TRs, although they were able to detect mutations in other genes, at frequencies as low as 1% (Guichard et al. 2012, Cleary et al. 2013, Ahn et al. 2014, Totoki et al. 2014, Schulze et al. 2015). In addition, publicly available RNAseq data for TRs (442 human HCC) (<http://www.cbioportal.org/index.do>) reported only two mutations for *TRβ* and none for *TRα*, further confirming that *Tr* mutations are virtually absent in human HCC. Moreover, no TR mutations have been described in experimentally induced HCCs, including a recent study aimed at detecting *Trs* mutations in chemically induced rat HCC (Frau et al. 2015).

Methylation

Alternative to mutations, dysregulation of TR expression could be a mechanism by which these receptors play a suppressive role in the carcinogenic process. Hypermethylation of the *TRβ* gene promoter region is frequent in several human cancers and leads to *TRβ* silencing (Joseph et al. 2007, Dunwell et al. 2009, Iwasaki & Sunaga 2010, Ling et al. 2010), while reactivation of the silenced murine *Trβ* gene delays thyroid tumour progression (Kim et al. 2013). Unfortunately, data on the possible role of *TR* methylation in human HCC are only available from the Liver Hepatocellular Carcinoma (TGCA, Provisional), which contains methylation data for 379 of

442 samples. This topic is particularly relevant since *TRβ1* mRNA levels were found significantly downregulated in the vast majority of HCCs compared with matched cirrhotic tissues, in two independent studies on human patients (Frau et al. 2015, Martínez-Iglesias et al. 2016). Decreased levels of *Trβ1* induced target genes, such as *DIO1*, and glucose-6-phosphatase (*G6PC*) were detected in the same HCCs samples. However, in neither of these studies, the methylation status of the TR promoters was investigated. In the only extensive study of DNA methylation in human HCC (Villanueva et al. 2015), no aberrant methylation of TRs has been reported. Thus, whether hypermethylation of *TR* promoters might contribute to the decreased expression of the receptors and affect HCC onset and progression is still an unsolved question.

Animal studies confirmed a highly significant downregulation of *Trβ1* expression in HCCs, both at mRNA and protein levels (Frau et al. 2015). As expected, decreased levels of target genes positively regulated by TRβ1, such as *Dio1*, *G6pc* and *Spot14* (Feng et al. 2000), and upregulation of *App*, a gene negatively regulated by TRβ1 (O'Barr et al. 2006), were associated with *Trβ1* downregulation. When methylation of *Tr* promoter was analysed in these rodent HCCs, no difference was found compared with normal liver. Thus, whether hypermethylation occurs in human HCC and plays a role in the downregulation of TRβ1 observed in these tumours still remains an unsolved question.

microRNA

microRNAs (miRNAs) are small non-coding RNAs, which negatively control gene expression by binding to complementary sequences present in untranslated regions of the target transcripts. The involvement of microRNAs in cancer pathogenesis is well established as they behave as oncogenes or tumour suppressor genes depending on the cellular function of their mRNA targets (Calin & Croce 2006, Lujambio & Lowe 2012). The importance of microRNAs in cancer progression is also underlined by the observation that they can influence both the response to chemotherapy and the development of drug resistance (Tomokuni et al. 2011, Zhou et al. 2011, Giordano & Columbano 2013).

Recently, a number of studies provided evidence that *TRβ* expression could be repressed through microRNA regulatory mechanisms (Master et al. 2010, Jazdzewski et al. 2011, Nishi et al. 2011, Ruiz-Llorente et al. 2014). In a study on papillary thyroid cancer (PTC) patients, lower

levels of TR β transcripts were observed in most tumour samples (Jazdzewski et al. 2011), and TR β downregulation was associated with high levels of miR-21, -146a, -181a and -221, all predicted to target TR β . Moreover, while downregulation of TR β expression in human clear cell renal carcinomas (ccRCC) is not associated with changes in DNA methylation of TR β promoter region, it is inversely correlated with the levels of miR-204 (for which a putative interaction site was identified in the TR β 1 3'UTR) (Master et al. 2010).

Unfortunately, not many studies have investigated the possible role of miRNAs in the regulation of TR expression in HCC. We have recently analysed in rat HCCs the expression of miRNAs (miR-21, miR-27a, miR-181a, miR-221, miR-146a and miR-204) known to target *Trp1* (Master et al. 2010, Jazdzewski et al. 2011, Nishi et al. 2011, Tomokuni et al. 2011). Interestingly, miR-27a, miR-146a, miR-181a and miR-204 were upregulated in rat HCCs displaying *Trp1* downregulation (Frau et al. 2015). Among these miRNAs, miR-27a showed an inverse relationship with TR β expression in human HCCs and in the five HCC cell lines examined, suggesting that this miRNA might negatively regulate *Trp1* expression in human HCC. Accordingly, transfection of HuH7, HepG2 and Mahlavu cells with an miR-27a mimic, led to a significant decrease in *Trp1* expression. MiRNAs may modulate TH-mediated effects also indirectly, i.e. by acting on enzymes involved in the T₄–T₃ conversion or on their degradation. Interestingly, tumour-specific changes in intracellular T₃ concentration correlate with changes in the *DIO1*-targeting miR-224 (Boguslawska et al. 2011). Notably, this miRNA is one of the most upregulated in human and rat HCC (Imbeaud et al. 2010, Giordano & Columbano 2013), and the inverse relationship between miR-224 levels and *Trp1* expression is present since the very early stages of experimental models of hepatocarcinogenesis (Petrelli et al. 2014, Frau et al. 2015).

Not only miRNAs regulate the expression of TRs, but T₃/TRs receptor signalling is, in turn, able to regulate miRNA expression (Diniz et al. 2013, 2015, Huang et al. 2013, Lin et al. 2013, Lu et al. 2013, Yap et al. 2013, Ruiz-Llorente et al. 2014). In fact, T₃ treatment of HepG2-TR-expressing cells stimulates miR-21 expression with subsequent T-cell lymphoma invasion and metastasis 1 (TIAM1) suppression and promotion of hepatoma cell migration and invasion (Huang et al. 2013). Work by the same group also showed that T₃ downregulates miR-17 expression in HepG2-TR-expressing cells and that the T₃/TR axis promotes cell migration through miR-17 downregulation (Lin et al. 2013). Based on these data, the authors proposed a novel

metastatic pathway involving the activities of T₃/TR, miRNA-17, p-AKT and metallo-proteinase3 (MMP3) in hepatoma cells. In the same context, but with opposite conclusions, studies from Ruiz-Llorente and coworkers (Ruiz-Llorente et al. 2014) showed that T₃ increases the levels of miR-424 and miR-503 in SK-TR HCC cells. These authors postulated that this induction plays an important role in the anti-tumourigenic and anti-invasive actions mediated by T₃. T₃-induced expression of these miRNAs was also found in non-transformed hepatocytes and in MDA-TR β overexpressing breast cancer cells, indicating that the phenomenon is not specific for the HCC cell line. Furthermore, miR-424 or miR-503 depletion enhanced extravasation to the lungs of hepatocarcinoma cells injected in the tail vein of mice (Ruiz-Llorente et al. 2014).

Although we have a yet limited knowledge about the relevance of the cross-regulation between miRNAs and TRs, nevertheless, the impact of this mutual regulation on HCC development and metastatic capacity represents a very promising topic that it is worth to be actively pursued (Table 2).

TRs, hypo- and hyperthyroidism and HCC

An aberrant activity of TRs is associated with several human cancers; however, it is still unclear whether changes in thyroid hormone levels affect cancer development and progression. Indeed, conflicting results are reported. Population-based case–control studies of risk factors associated to development of ovarian and pancreatic cancers found that hyperthyroidism is associated with a two-fold increase in cancer risk (Ness et al. 2000, Ko et al. 2007). Moreover, while hyperthyroidism is associated with more advanced clinical stage and higher risk of recurrence in prostate cancer (Lehrer et al. 2002), hypothyroidism is associated with a lower risk of carcinoma and a reduced progression to more invasive stages for mammary cancer (Cristofanilli et al. 2005). Consistent with hypothyroidism being beneficial, pharmacologically induced hypothyroidism, together with tamoxifen treatment, resulted in better survival of glioblastoma patients, and a significantly longer survival was observed in patients with recurrent high-grade gliomas treated with tamoxifen and the anti-thyroid drug propylthiouracil (Hercbergs et al. 2003).

An opposite conclusion stems from two case–control studies suggesting that hypothyroidism represents a risk factor for HCC development. In one of these studies, women with a history of hypothyroidism had a 2.8-fold higher risk of HCC (Hassan et al. 2009); in the second

Table 2 miRNAs and TRs/T₃ axis.

	Tissue	Species	miRNA	Regulation	Reference
TR α	Breast	Human	miR-10a	Positive correlation between miR-10a and THR α gene expression	Khan et al. (2015)
TR α	Liver	Human	miR-17	miR-17 expression negatively associated with TR α 1	Lin et al. (2013)
TR α	Liver	Human	miR-21	In patients with increased TR α 1 expression in tumour tissues, miR-21 is concomitantly increased	Huang et al. (2013)
		Rat			
		Mouse			
TR β	Heart	Rat	miR-27a	TR β 1 is a target of miR-27a	Nishi et al. (2011)
		Human			
TR β	Heart	Rat	miR-27a	miR-27a regulates β -MHC gene expression by targeting TR β 1 in cardiomyocytes	Nishi et al. (2011)
		Human			
TR β	Heart	Mouse	miR-208a miR-199a	miR-208a and miR-199a contribute to THR β -mediated cardiac hypertrophy	do Império et al. (2015)
TR β	Liver	Rat	miR-27a miR-146a miR-181a miR-204	Upregulation in HCC displaying TR β 1 downregulation	Frau et al. (2015)
TR β	Liver	Human	miR-27a	Inverse correlation between TR β 1 and miR-27a in HCC cell lines	Frau et al. (2015)
TR β	Liver	Human	miR-181a	Inverse relationship between TR β 1 and miR-181a in human cirrhotic peritumoural tissue	Frau et al. (2015)
TR β	Kidney	Human	miR-204	miR-204 reported as a candidate regulator of TR β 1 expression	Master et al. (2010)
TR β	Kidney	Human	miR-155 miR-425	THR β is targeted by miR-155 and miR-425	Wojcicka et al. (2014)
TR β	Kidney	Human	miR-155 miR-425	Inverse correlation between TR β and miRs in clear cell renal cell carcinoma	Wojcicka et al. (2014)
TR β	Kidney	Human	miR-452	miR-452 directly regulates the expression of TR β 1 in renal cancer cells	Boguslawska et al. (2014)
TR β	Thyroid	Human	miR-21 miR146a miR181a miR-221	An inverse correlation between TR β and miRs in papillary thyroid carcinoma tumours	Jazdzewski et al. (2011)
TR β	Thyroid	Human	miR-21 miR-146a miR-181a miR-221	An inverse correlation between TR β and miRs in papillary thyroid carcinomas	Rosignolo et al. (2015)
TH	Breast cancer cells, hepatocarcinoma cells expressing TR β	Human	miR-424 miR-503	T ₃ treatment induces miR-424 and miR-503	Ruiz-Llorente et al. (2014)
TH	Breast cancer cells, hepatocarcinoma cells expressing TR β	Human	miR-424 miR-503	Reduced expression of miR-424 and miR-503 in tumours developed in hypothyroid mice	Ruiz-Llorente et al. (2014)
TH	Heart	Rat	miR-208a miR-208b	miR-208a is upregulated in response to T ₄ treatment	Diniz et al. (2013)
TH	Heart	Rat	miR-208a miR-208b	miR-208b is downregulated in response to T ₄ treatment	Diniz et al. (2013)
TH	Heart	Rat	miR-133	miR-133 expression is reduced in TH-induced cardiac hypertrophy	Diniz et al. (2015)
TH	Heart	Rat	miR-133	miR-133 mimic prevents the cardiomyocyte hypertrophy in response to T ₃ <i>in vitro</i>	Diniz et al. (2015)
TH	Hepatic cell line	Human	miR-181d	T ₃ treatment increases miR-181d expression	Yap et al. (2013)
TH	Liver	Mouse	miR-1 miR-206 miR-133a miR-133b	miRs upregulation in hypothyroid mice	Dong et al. (2010)
TH	Proximal tubular epithelial cell line	Human	miR-34a	T ₃ treatment induces miR-34a expression	Lu et al. (2013)

study, hypothyroidism was significantly more prevalent in patients with HCC of unknown aetiology than in HCC patients with alcoholic liver disease or HCV. Thus, these results suggest that hypothyroidism may be a permissive factor for the development of HCC (Reddy *et al.* 2007).

A hypothyroid status of HCC has been described in human HCC (Liao *et al.* 2012, Frau *et al.* 2015, Martinez-Iglesias *et al.* 2016). Moreover, animal studies showed that downregulation of TRs, especially *Trβ1*, associated to severely reduced *Dio1* expression, is a very early event in the multistage process of hepatocarcinogenesis and precedes neoplastic transformation (Frau *et al.* 2015). The latter results suggest that alterations of *Trβ1* expression may play a critical role not only in the progression but also in the onset of HCC. Based on these observations, a regulatory loop can be hypothesised, wherein decreased expression of *Trβ1* leads to diminished transcription of its classical target gene *Dio1*; in turn, *Dio1* inhibition leads to reduced T₄ to T₃ conversion causing local hypothyroidism (Fig. 1). The data obtained from the

rat model suggest that this loop is an early event and may be critical in conferring a growth advantage to preneoplastic hepatocytes. Notably, downregulation of *Trβ1* is particularly evident in the most aggressive lesions, endowed with a higher proliferative capacity, further supporting the relevance of the hypothyroid status in cancer development.

Collectively, these studies demonstrate that TRβ1 downregulation is associated to HCC onset and progression and that local hypothyroidism takes place in a species- and aetiology-independent fashion.

In this context, a very interesting study unveiled a possible mechanism by which astrocyte-elevated gene-1 (AEG-1), an oncogene overexpressed and an independent prognostic factor in HCC (Jung *et al.* 2015), links non-thyroidal illness syndrome (NTIS) to cancer development. NTIS, a condition often associated with malignancy, is characterised by low serum T₃, but normal T₄ levels due to decreased *DIO1* activity in the liver (Srivastava *et al.* 2015). In a study, aimed at investigating the role of AEG-1 in NTIS in the context

Figure 1

Hypothyroid status of preneoplastic/neoplastic hepatocytes and effect of T₃ and/or TRβ agonists. (A) In normal hepatocytes, T₄ is converted in T₃ by deiodinase 1 (DIO1). Binding of T₃ to TR activates transcription of TR-target genes, including DIO1, and stimulates transcription of *Trβ*. (B) In preneoplastic and neoplastic hepatocytes, downregulation of *TRβ* due to different mechanisms (methylation of promoter gene, upregulation of TR-targeting miRNAs) results in decreased expression of *DIO1* and consequently, in an impaired conversion of T₄ into T₃. This generates a hypothyroid status in preneoplastic/neoplastic hepatocytes that favors cancer progression. (C) Exogenous T₃ or TRβ agonists bind and activate TRβ. TRβ activation leads to its increased expression as well as to enhanced expression of its target genes, including *DIO1* which, unlike in hypothyroid pre/neoplastic hepatocytes (B), is now able to convert T₄ into T₃. The hyperthyroid status induced by T₃ or TRβ1 agonists and the consequent robust activation of TRβ triggers several biological processes, such as proliferation, senescence and differentiation, which negatively interfere with cancer progression. Arrow width is representative of the modifications of the activity of the TH/TRβ axis.

of HCC, the authors found that AEG-1 inhibits ligand-dependent TR/RXR activity and downstream *DIO1* gene expression in human HCC cells and AEG-1 transgenic hepatocytes. Moreover, an inverse correlation was observed between AEG-1 and *DIO1* levels in human HCC patients. AEG-1 thus links NTIS with cancer development, including HCC.

In agreement with the possible critical role of local hypothyroidism in HCC development, the switch from hypothyroid to hyperthyroid status of preneoplastic lesions caused by T₃ administration was associated with the disappearance of most preneoplastic lesions and with a significant inhibition of HCC development and lung metastases (Ledda-Columbano *et al.* 2000).

T₃ and HCC

While a general consensus exists regarding the oncosuppressor role of TRβ1 on HCC, controversial data have been reported about its role upon T₃ activation. *In vivo* studies showed that 1-week treatment with T₃ accelerates the regression of chemically induced hepatic preneoplastic lesions in rats subjected to the resistant hepatocyte (RH) model of hepatocarcinogenesis (Ledda-Columbano *et al.* 2000). Moreover, repeated cycles of T₃ reduced the frequency of HCC by 50% and completely inhibited lung metastases. Similarly, a short T₃ treatment leads to the disappearance of preneoplastic hepatic foci in rats exposed to another well-established model of rat hepatocarcinogenesis, namely the choline-deficient diet model (Perra *et al.* 2009). These results, together with the finding that TRβ and *DIO1* are profoundly downregulated in preneoplastic lesions and in rat and human HCC, and that an increase in their levels upon T₃ treatment is associated with preneoplastic nodule regression, suggest that reactivation of the T₃-TR axis may impact on the fate of preneoplastic and neoplastic lesions (Fig. 1). It follows that increasing the intracellular levels of T₃ might represent a novel therapeutic approach.

An anti-tumourigenic action of T₃ was also described by Liao and coworkers (2012); in this work, the authors proposed that T₃ stimulates the expression of suppressor genes, such as Dickkopf 4 (DKK4), a secreted protein that antagonises the Wnt signal pathway (Liao *et al.* 2012). Intriguingly, however, in two other studies, the same authors suggested that T₃ promotes cancer progression inducing either lipocalin-2 (Chung *et al.* 2015) or furin (Huang *et al.* 2012). To explain these contrasting results, the authors hypothesise that T₃ may inhibit cancer

cell proliferation at early stages of HCC development, while it promotes cancer cell migration and invasion in malignant tumours or late-stage cancer. Although conceptually valid, this hypothesis is not yet supported by experimental data suggesting a dual role of T₃ in different steps of the hepatocarcinogenic process; moreover, in the latter studies, the effect of T₃ was investigated only at the final step of the tumourigenic process, namely in fully transformed HCC cell lines.

In addition, it is unclear why in the study showing an anti-tumourigenic activity of T₃ (Liao *et al.* 2012) TR levels were downregulated in HCC compared with non-tumoural liver, while in the one where T₃ was shown to exert a pro-tumourigenic effect, TR levels were higher in the tumours and correlated with a poor survival (Chung *et al.* 2015). Thus, whether T₃ inhibits or promotes HCC development has still to be clarified.

T₃/TRs axis and hepatocyte proliferation

Increased cell proliferation has long been associated to cancer development. Therefore, it is not surprising that many studies aimed to investigate the effect of the TH/TR axis on liver cell proliferation. TR downregulation occurs concomitantly with increased DNA synthesis in rat liver regeneration after 2/3 partial hepatectomy (PH) and returns towards control values once proliferation ceases (Frau *et al.* 2015). These *in vivo* data are in agreement with those, *in vitro*, showing that Trβ, when not bound to T₃, acts as a cell cycle inhibitor by inhibiting Cyclin D1 expression (González-Sancho *et al.* 2002) or repressing its induction by the oncogene Ha-Ras^{val12} (García-Silva *et al.* 2004). However, they are in contrast with the finding that a significant delay in the restoration of liver mass post-PH occurs in mice lacking *Tra1*, *Trβ1* or both receptors (López-Fontal *et al.* 2010).

Activation of TR by T₃ has been long recognised as a potent hepatomitogenic event (Short *et al.* 1972, Francavilla *et al.* 1994, Pibiri *et al.* 2001). Although the molecular mechanisms through which T₃ induces hepatocyte proliferation are unclear, it is of interest to note that its mitogenic effect occurs in the absence of activation of transcription factors such as AP-1, NF-κB or STAT₃, and it is not associated with an increased expression of *c-fos*, *c-jun* or *c-myc* proto-oncogenes (Pibiri *et al.* 2001). On the other hand, Cyclin D1 mRNA and protein levels increase very rapidly after T₃ treatment, suggesting that Cyclin D1 could be implicated in the T₃-triggered rapid entry into S phase of hepatocytes (Pibiri *et al.* 2001). An additional mechanism responsible

for T₃-induced mitogenesis has recently implicated β -catenin, an important nuclear effector of the Wnt signalling pathway (Nejak-Bowen & Monga 2011). Indeed, while in wild-type mice administration of T₃ induces a robust wave of hepatocyte proliferation, no mitogenic response is seen in the hepatocyte-specific β -catenin knockout mice (Fanti et al. 2014).

Importantly, T₃ is not only mitogenic for intact liver, but it also improves the regenerative response of rodent livers after 70 or 90% hepatectomy and stimulates the regenerative response of the liver of old rats when given before 70% PH (Bockhorn et al. 2007, Columbano et al. 2008, Malik et al. 2008, Taki-Eldin et al. 2011).

Liver expresses both TR α and TR β receptors; however, although TR α is predominant in the hepatocyte precursors and in the stellate cells, and could play a critical role in hepatocyte maturation during the perinatal period (Rodd et al. 1992), TR β 1 is the predominant T₃ receptor in adult liver (Schwartz et al. 1992). Gene profiling of livers from *Tr α* or *Tr β* KO mice identified a large number of differentially regulated genes, revealing a clear predominance of *Tr β* over *Tr α* in adult liver function (Flores-Morales et al. 2002, Yen et al. 2003). Accordingly, T₃ is able to induce proliferation in KO mice devoid of *Tr α* (Kowalik et al. 2010), and the TR β -specific agonist GC-1 mimics the effect of T₃ on hepatocyte proliferation (Columbano et al. 2006), thus indicating that the mitogenic activity of T₃ on fully differentiated hepatocytes mainly depends on TR β .

Collectively, these studies provide substantial evidence that T₃ induces hepatocyte proliferation both in intact and injured liver, leading to the hypothesis that while in the absence of its ligand TR β exerts an antiproliferative effect on hepatocytes, its activation by T₃ rapidly stimulates their proliferation. They also suggest that the possible use of T₃ or its analogues could represent a useful tool in pathological conditions characterised by an impaired regenerative ability (i.e. aged livers) or when a rapid growth stimulation of the liver is required (i.e. size transplantation).

While the mitogenic effect of T₃ on normal hepatocytes seems unquestionable, several *in vitro* works have reported its inhibitory role on cancer cells. Indeed, TR β stably expressing hepatocarcinoma cell line SK-hep1 is poorly able to grow in soft agar when treated with T₃ (Martinez-Iglesias et al. 2009), and thyroid hormone inhibits proliferation of a HepG2-TR α cell line, possibly by stimulating the expression of transforming growth factor- β (TGF- β) (Yen et al. 2006). However, it should be noted that, *in vivo*, T₃ maintains its powerful mitogenic

activity for preneoplastic hepatic nodules (Ledda-Columbano et al. 2000).

In conclusion, even though it is unclear whether T₃ exerts an opposite effect on normal and transformed hepatocytes, it is clear that T₃ is a strong inducer of hepatocyte proliferation. It follows that while in the absence of ligand TR β acts an oncosuppressor, it delivers mitogenic signals once activated by binding to T₃.

T₃/TRs axis and cell differentiation

T₃-induced regression of hepatic preneoplastic lesions occurs concomitantly with an increased proliferative activity (Ledda-Columbano et al. 2000). Since no signs of increased apoptosis are present, a possible explanation for such a paradoxical result is that T₃ exerts both pro-proliferative and pro-differentiating effects on preneoplastic hepatocytes. In several models of rat hepatocarcinogenesis, the biochemical phenotype of preneoplastic hepatocytes closely resembles that of foetal or neonatal hepatocytes; indeed, they lack the expression of enzymes normally present in differentiated hepatocytes (P-450, ATPase, glucose-6-phosphatase) (Roomi et al. 1985), while exhibiting high levels of enzymes expressed at a low level or absent in fully differentiated hepatocytes (γ -glutamyl transpeptidase, glutathione S-transferase P, glucose-6-phosphate dehydrogenase, α -fetoprotein). During the carcinogenic process, most preneoplastic lesions show a slow regression over time and their immature phenotype is replaced by the acquisition of adult differentiated features (Enomoto & Farber 1982). These findings suggest the existence of an active remodelling, which involves the modulation of specific genes through a genetically programmed process. Notably, a rapid loss of several markers associated with preneoplasia occurs after treatment with T₃ or TR β agonists (Perra et al. 2009), suggesting that T₃-induced mitogenesis is associated with – or followed by – a process of differentiation.

Interestingly, T₃ induces differentiation of oval cells (considered a progenitor type) to hepatocytes, as shown by the loss of oval cell markers and the acquisition of mature hepatocyte markers (László et al. 2008). Even more intriguingly, a recent work (Catalano et al. 2016) showed that T₃ treatment induces differentiation of colorectal cancer stem cells (CR-CSCs), by increasing the levels of the bone morphogenetic protein (BMP4, a known promoter of differentiation in normal colonic epithelium) and of its downstream targets. The same work also showed that increasing intracellular levels of T₃ results in reduced

clonogenic and tumourigenic potential and establishes a higher sensitivity of CR-CSCs to chemotherapy, supporting the hypothesis that T₃ may inhibit tumour development by activating a differentiation program. Since high doses of exogenous BMP4 promote CD133-positive HCC-CSC differentiation and inhibit the self-renewal and tumourigenic capacity of these cells (Zhang et al. 2012), one can speculate that treatment with T₃ may also induce differentiation of highly aggressive HCC-CSC and reduce their tumourigenic capacity by inducing BMP4 levels.

The potential relevance of T₃-induced differentiation in modulating tumour development is also highlighted by a work implicating Krüppel-like factors (KLFs) in TR-induced differentiation (Cvoro et al. 2015). KLFs are classified as a part of Sp1/KLF family of zinc-finger-containing transcription factors and play an important role in proliferation, differentiation, apoptosis, inflammation and development (Cao et al. 2010). They act as transcriptional activators or repressors, depending on the type and developmental stage of the cell. TR actions on neuronal differentiation in mammalian and amphibian are mediated by KLF9 induction (Denver et al. 1999, Dugas et al. 2012). KLF9 and KLF4 control NOTCH1 gene expression and exert opposite effects on its transcription, thereby influencing the Notch signalling pathway (Ying et al. 2011). Notch signalling, in turn, cross-reacts with other signalling pathways including Wnt, FGF, TGFβ/BMP and Hedgehog (Katoh et al. 2007) and converges on a transcriptional network that involves OCT4, NANOG and SOX2 to regulate stem cell maintenance and differentiation (Schnersch et al. 2010). Thus, alterations in KLF9 levels could greatly influence cell differentiation processes. In line with these findings, TRs induce KLF9 in several normal and transformed liver cell lines and/or progenitors, including HepG2 cells, non-transformed liver cells, human induced pluripotent stem cells (hiPSC) and human embryonic stem cells (hESC).

It is worth to underline that complex cross-regulations between T₃/TRα and key signalling pathways including Wnt, Notch and BMP have been largely described in the intestinal epithelium, where T₃/TRα controls the balance between cell proliferation and cell differentiation of the crypt precursor cells (Plateroti et al. 2006, Kress et al. 2009, 2010, Sirakov et al. 2015). Interestingly, studies in human HCC showed that KLF9 expression is downregulated compared with the normal liver counterpart, and that exogenous KLF9 expression inhibits proliferation of HCC cell lines and their tumourigenic capacity when xenografted in immunodepressed mice (Sun et al. 2014). However, the

relationship between T₃/TRs and KLF9 in this same setting was not investigated. Given the importance of KLF9 and Notch signalling in regulating the balance between cell proliferation and cell differentiation, further studies aimed at investigating how the T₃/TR axis in conjunction with KLF9 regulates HCC development are of paramount importance. Towards this direction, preliminary data provide evidence for a decrease in *Klf9* mRNA levels in rat preneoplastic lesions exhibiting reduced *Trβ* expression, and its strong upregulation following T₃ treatment (A Columbano and A Perra, personal communications). These findings indicate an important avenue for future investigations on the T₃/TR/KLF9 axis in preneoplastic and neoplastic hepatocytes to obtain more insights into the ways by which TRs modulate HCC development by reactivating a differentiation programme.

TRs and T₃ analogues

Hyperthyroidism is associated with a wide range of harmful effects, in particular cardiac dysfunction, i.e. tachycardia, arrhythmias and precipitation of ischaemic episodes or heart failure. These and other adverse effects have strongly limited the possible use of T₃ as a therapeutic agent. Therefore, several efforts have been made to develop T₃ analogues that could induce some beneficial effects (triglyceride, cholesterol, obesity and body mass lowering), without most of the adverse T₃/TR-dependent side effects. This purpose was achieved by the synthesis of TRβ agonists. Among the several analogues so far generated, GC-1, KB2115 and the Hep-Direct prodrug MBO07811 have reproduced most of the beneficial effects of T₃, in the absence of deleterious effects (Chiellini et al. 1998, Erion et al. 2007, Berkenstam et al. 2008). Experimental studies showed that treatment with GC-1 causes a reduction in triglyceride levels greater than that produced by equimolar doses of T₃ (Grover et al. 2004). These effects were achieved at doses devoid of relevant side effects on heart rate and that did not cause muscle loss or an increase in the overall catabolic state (Trost et al. 2000). GC-1 also prevents the development and progression of rat hepatic steatosis by increased mitochondrial and peroxisomal fatty acid β-oxidation and reduced levels of inflammatory markers (Perra et al. 2008). Similarly, MB07811 exhibits anti-steatotic activity in different animal models, through increased fatty oxidation in rats and mice (Cable et al. 2009). Interestingly, analogues such as GC-1, KB2115 and MB07344 display liver selectivity (Martínez et al. 2009). Due to their beneficial effects, two of the aforementioned designed analogues, GC-1

and KB2115, commercially known as Sobetirome and Eprotirome, respectively, entered human clinical trials for dyslipidaemia, displaying encouraging results in the absence of harmful effects typically associated with TH high levels (for reviews, see [Tancevski *et al.* 2011](#), [Meruvu *et al.* 2013](#)). Unfortunately, no phase II trials for GC-1 are planned. As to KB2115, a phase III trial was terminated due to unexpected effects on animal studies; in addition, reduction in T₃, as well as some degree of liver toxicity, occurred in homozygous patients affected by familial hypercholesterolaemia, treated with 50 or 100 µg of eprotirome for only 6 weeks ([Sjouke *et al.* 2014](#)).

Although caution about the use of these analogues should be maintained, as they could have some deleterious effects, still the possibility of their therapeutic use in a disease that currently does not offer any satisfactory alternative, such as HCC, might be considered. Indeed, animal studies support the possible use of T₃ analogues to interfere with HCC development and progression. A short-term treatment with GC-1 of rats carrying chemically induced preneoplastic nodules induces a rapid disappearance of the vast majority of these lesions in two distinct experimental protocols ([Perra *et al.* 2009](#)), one of which characterised by extensive fatty liver, a condition often preceding HCC development in humans. Similar to what observed with T₃, regression of preneoplastic hepatocytes occurred concomitantly with GC-1-induced proliferation and is associated to downregulation of preneoplastic markers; these results again suggest that, when activated by ligand binding, TRβ exerts both pro-proliferative and pro-differentiating effects on preneoplastic hepatocytes. In a quite similar way, treatment with KAT-68, a liver-selective thyromimetic with hypocholesterolaemic properties and devoid of the cardiotoxicity elicited by T₃, shows inhibitory effects in the early and late phases of hepatocarcinogenesis ([Hayashi *et al.* 2005](#)). Indeed, in this study, KAT-68 treatment was shown to induce a reduction in the number and mean size of preneoplastic lesions when given early in the tumourigenic process. Notably, similar to the GC-1 study ([Perra *et al.* 2009](#)), the reduction in preneoplastic lesions induced by KAT-68 was associated with enhanced cell proliferation.

Based on these results, it is conceivable that treatment with T₃ analogues may prove to be beneficial in HCC therapy. Assuming that a condition of local hypothyroid status exists in both rodent and human HCCs, it will be critical to investigate whether these agents can induce a differentiation programme not only in preneoplastic lesions, but also in fully transformed

Table 3 Open questions.

Is methylation of the TR promoter responsible for the downregulation of thyroid hormone receptors commonly observed in HCC?
Do microRNAs play a role in downregulating TR expression?
Is the differentiating effect of T ₃ intimately associated with its mitogenic activity?
Can T ₃ agonists represent possible therapeutic drugs in HCC?

HCCs. Our preliminary results (A C and A P) indicate that T₃ is indeed able to elicit, in early HCC, a modification of genes/pathways similar to that observed in preneoplastic nodules (i.e. a switch from a local hypothyroid to hyperthyroid status, downregulation of the NRF2-Keap1 pathway, loss of the putative progenitor cell marker cytokeratin-19). If T₃ analogues prove to be able to induce the same effects, one could seriously consider the possibility of interfering with HCC progression by inducing a differentiation programme to which even fully transformed cells may not be resistant ([Fig. 1](#)).

Conclusions

Emerging evidence highlights the relevance of the T₃/TRs axis in the regulation of HCC development. Activation of TRs is required for normal growth and proliferation of liver cells, but the exact role played in the tumourigenic process by their unliganded vs liganded form is unclear. Absence or low expression of TRs seems to be a common event in many human cancers, including HCC, and in experimental studies; downregulation of TRs is observed at very early stages of the tumourigenic process, suggesting its critical role in HCC development. However, the identification of the regulatory mechanisms responsible for their altered expression (gene promoter methylation? microRNAs?) remains a still unsolved topic that needs to be carefully addressed ([Table 3](#)). Although several targets and mechanisms of anti-tumourigenic actions of TRs have been hypothesised to date, several issues remain elusive. In particular, the effect of TR activation following T₃-binding on proliferation and differentiation of normal and genetically altered hepatocytes, the mechanisms by which activated TRs trigger these two fundamental processes and the impact of these effects on HCC progression remain to be clarified. Undoubtedly, elucidation of the consequences of T₃/TRs signalling crosstalk with other pathways and specific co-regulators may help in clarifying the anti-tumourigenic effects exhibited by T₃ in experimental studies and in determining the crucial therapeutic implications.

Finally, the availability of a number of recently developed TR β 1 agonists, devoid of T₃-induced adverse side effects, offers the fascinating perspective to consider their usefulness as therapeutic cancer drugs.

Declaration of interest

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

Funding

This work was supported by Associazione Italiana Ricerca sul Cancro (AIRC, Grants IG-15279 to A C), Ministero Università e Ricerca Scientifica (PRIN-2010LC747T to A C) Fondation ARC pour la Recherche sur le Cancer (grant No PGA1201402000834) and by the Département du Rhône de la Ligue contre le cancer (grant No 137838) to M P.

Acknowledgements

The authors thank V P Leoni, R Loi and M A Kowalik for their constructive and helpful suggestions in the preparation of this manuscript.

References

- Ali IU, Lidereau R & Callahan R 1989 Presence of two members of c-erbA receptor gene family (c-erbA beta and c-erbA2) in smallest region of somatic homozygosity on chromosome 3p21-p25 in human breast carcinoma. *Journal of the National Cancer Institute* **81** 1815–1820. (doi:10.1093/jnci/81.23.1815)
- Ahn SM, Jang SJ, Shim JH, Kim D, Hong SM, Sung CO, Baek D, Haq F, Ansari AA, Lee SY, et al. 2014 Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. *Hepatology* **60** 1972–1982. (doi:10.1002/hep.27198)
- Ando S, Sarlis NJ, Oldfield EH & Yen PM 2001a Somatic mutation of TRbeta can cause a defect in negative regulation of TSH in a TSH-secreting pituitary tumor. *Journal of Clinical Endocrinology and Metabolism* **86** 5572–5576. (doi:10.1210/jc.86.11.5572)
- Ando S, Sarlis NJ, Krishnan J, Feng X, Refetoff S, Zhang MQ, Oldfield EH & Yen PM 2001b Aberrant alternative splicing of thyroid hormone receptor in a TSH-secreting pituitary tumor is a mechanism for hormone resistance. *Molecular Endocrinology* **15** 1529–1538. (doi:10.1210/mend.15.9.0687)
- Berkenstam A, Kristensen J, Mellström K, Carlsson B, Malm J, Rehnmark S, Garg N, Andersson CM, Rudling M, Sjöberg F, et al. 2008 The thyroid hormone mimetic compound KB2115 lowers plasma LDL cholesterol and stimulates bile acid synthesis without cardiac effects in humans. *PNAS* **105** 663–667. (doi:10.1073/pnas.0705286104)
- Bockhorn M, Frilling A, Benko T, Best J, Sheu SY, Trippler M, Schlaak JF & Broelsch CE 2007 Tri-iodothyronine as a stimulator of liver regeneration after partial and subtotal hepatectomy. *European Surgical Research* **39** 58–63. (doi:10.1159/000098443)
- Brönnegård M, Törning O, Böös J, Sylven C, Marcus C & Wallin G 1994 Expression of thyrotropin receptor and thyroid hormone receptor messenger ribonucleic acid in normal, hyperplastic, and neoplastic human thyroid tissue. *Journal of Clinical Endocrinology and Metabolism* **79** 384–389. (doi:10.1210/mend.15.9.0687)
- Boguslawska J, Wojcicka A, Piekliko-Witkowska A, Master A & Nauman A 2011 MiR-224 targets the 3'UTR of type 1 5'-iodothyronine deiodinase possibly contributing to tissue hypothyroidism in renal cancer. *PLoS ONE* **6** e24541. (doi:10.1371/journal.pone.0024541)
- Boguslawska J, Piekliko-Witkowska A, Wojcicka A, Kedzierska H, Poplawski P & Nauman A 2014 Regulatory feedback loop between T3 and microRNAs in renal cancer. *Molecular and Cellular Endocrinology* **384** 61–70. (doi:10.1016/j.mce.2014.01.006)
- Brent GA 1994 The molecular basis of thyroid hormone action. *New England Journal of Medicine* **331** 847–853. (doi:10.1056/NEJM199409293311306)
- Brent GA 2000 Tissue-specific actions of thyroid hormone: insights from animal models. *Reviews in Endocrine and Metabolic Disorders* **1** 27–34. (doi:10.1023/A:1010056202122)
- Cable EE, Finn PD, Stebbins JW, Hou J, Ito BR, van Poelje PD, Linemeyer DL & Erion MD 2009 Reduction of hepatic steatosis in rats and mice after treatment with a liver-targeted thyroid hormone receptor agonist. *Hepatology* **49** 407–417. (doi:10.1002/hep.22572)
- Calin GA & Croce CM 2006 MicroRNA signatures in human cancers. *Nature Reviews Cancer* **6** 857–866. (doi:10.1038/nrc1997)
- Cao Z, Sun X, Icli B, Wara AK & Feinberg MW 2010 Role of Kruppel-like factors in leukocyte development, function, and disease. *Blood* **116** 4404–4414. (doi:10.1182/blood-2010-05-285353)
- Catalano V, Dentice M, Ambrosio R, Luongo C, Carollo R, Benfante A, Todaro M, Stassi G & Salvatore D 2016 Activated thyroid hormone promotes differentiation and chemotherapeutic sensitization of colorectal cancer stem cells by regulating Wnt and BMP4 signaling. *Cancer Research* **76** 1237–1244. (doi:10.1158/0008-5472.CAN-15-1542)
- Chan IH & Privalsky ML 2006 Thyroid hormone receptors mutated in liver cancer function as distorted antimorphs. *Oncogene* **25** 3576–3588. (doi:10.1038/sj.onc.1209389)
- Chassande O, Fraichard A, Gauthier K, Flamant F, Legrand C, Savatier P, Laudet V & Samarut J 1997 Identification of transcripts initiated from an internal promoter in the c-erbA alpha locus that encode inhibitors of retinoic acid receptor alpha and triiodothyronine receptor activities. *Molecular Endocrinology* **11** 1278–1290. (doi:10.1210/me.11.9.1278)
- Chen LC, Matsumura K, Deng G, Kurisu W, Ljung BM, Lerman MI, Waldman FM & Smith HS 1994 Deletion of two separate regions on chromosome 3p in breast cancers. *Cancer Research* **54** 3021–3024.
- Cheng SY, Leonard JL & Davis PJ 2010 Molecular aspects of thyroid hormone actions. *Endocrine Reviews* **31** 139–170. (doi:10.1210/er.2009-0007)
- Chiellini G, Aprelletti JW, Yoshihara HA, Baxter JD, Ribeiro RC & Scanlan TS 1998 A high-affinity subtype-selective agonist ligand for the thyroid hormone receptor. *Chemistry & Biology* **5** 299–306. (doi:10.1016/s1074-5521(98)90168-5)
- Chung IH, Chen CY, Lin YH, Chi HC, Huang YH, Tai PJ, Liao CJ, Tsai CY, Lin SL, Wu MH, et al. 2015 Thyroid hormone-mediated regulation of lipocalin 2 through the Met/FAK pathway in liver cancer. *Oncotarget* **6** 15050–15064. (doi:10.18632/oncotarget)
- Cleary SP, Jeck WR, Zhao X, Chen K, Selitsky SR, Savich GL, Tan TX, Wu MC, Getz G, Lawrence MS, et al. 2013 Identification of driver genes in hepatocellular carcinoma by exome sequencing. *Hepatology* **58** 1693–1702. (doi:10.1002/hep.26540)
- Columbano A, Simbula M, Pibiri M, Perra A, Deidda M, Locker J, Pisanu A, Ucheddu A & Ledda-Columbano GM 2008 Triiodothyronine stimulates hepatocyte proliferation in two models of impaired liver regeneration. *Cell Proliferation* **41** 521–531. (doi:10.1111/j.1365-2184.2008.00532.x)
- Columbano A, Pibiri M, Deidda M, Cossu C, Scanlan TS, Chiellini G, Muntoni S & Ledda-Columbano GM 2006 The thyroid hormone receptor-beta agonist GC-1 induces cell proliferation in rat liver and pancreas. *Endocrinology* **147** 3211–3218. (doi:10.1210/en.2005-1561)
- Cristofanilli M, Yamamura Y, Kau SW, Bevers T, Strom S, Patangan M, Hsu L, Krishnamurthy S, Theriault RL & Hortobagyi GN 2005

- Thyroid hormone and breast carcinoma. Primary hypothyroidism is associated with a reduced incidence of primary breast carcinoma. *Cancer* **103** 1122–1128. (doi:10.1002/cncr.20881)
- Cvoro A, Devito L, Milton FA, Noli L, Zhang A, Filippi C, Sakai K, Suh JH, Sieglaff DH, Dhawan A, et al. 2015 A thyroid hormone receptor/KLF9 Axis in human hepatocytes and pluripotent stem cells. *Stem Cells* **33** 416–428. (doi:10.1002/stem.1875)
- Davis PJ, Goglia F & Leonard JL 2016 Nongenomic actions of thyroid hormone. *Nature Reviews Endocrinology* **12** 111–121. (doi:10.1038/nrendo.2015.205)
- Denver RJ, Ouellet L, Furling D, Kobayashi A, Fujii-Kuriyama Y & Puymirat J 1999 Basic transcription element binding protein is a thyroid hormone-regulated gene in the developing central nervous system: evidence for a role in neurite outgrowth. *Journal of Biological Chemistry* **274** 23128–23134. (doi:10.1074/jbc.274.33.23128)
- Diniz GP, Takano AP & Barreto-Chaves ML 2013 MiRNA-208a and miRNA-208b are triggered in thyroid hormone-induced cardiac hypertrophy – role of type 1 Angiotensin II receptor (AT1R) on miRNA-208a/α-MHC modulation. *Molecular Cell Endocrinology* **374** 117–124. (doi:10.1016/j.mce.2013.04.010)
- Diniz GP, Lino CA, Guedes EC, Moreira Ldo N & Barreto-Chaves ML 2015 Cardiac microRNA-133 is down-regulated in thyroid hormone-mediated cardiac hypertrophy partially via Type 1 Angiotensin II receptor. *Basic Research in Cardiology* **110** 49. (doi:10.1007/s00395-015-0504-7)
- do Império GE, Ramos IP, Santiago LA, Pereira GE, dos Santos Almeida NA, Fuziwara CS, Pazos-Moura CC, Kimura ET, Olivares EL & Ortiga-Carvalho TM 2015 The impact of a non-functional thyroid receptor beta upon triiodotironine-induced cardiac hypertrophy in mice. *Cellular Physiology and Biochemistry* **37** 477–490. (doi:10.1159/000430370)
- Dong H, Paquette M, Williams A, Zoeller RT, Wade M & Yauk C 2010 Thyroid hormone may regulate mRNA abundance in liver by acting on microRNAs. *PLoS ONE* **5** e12136. (doi:10.1371/journal.pone.0012136)
- Dugas JC, Ibrahim A & Barres BA 2012 The T3-induced gene KLF9 regulates oligodendrocyte differentiation and myelin regeneration. *Molecular and Cellular Neuroscience* **50** 45–57. (doi:10.1016/j.mcn.2012.03.007)
- Dunwell TL, Hesson LB, Pavlova T, Zabarovska V, Kashuba V, Catchpoole D, Chiamonte R, Brini AT, Griffiths M, Maher ER, et al. 2009 Epigenetic analysis of childhood acute lymphoblastic leukemia. *Epigenetics* **4** 185–193. (doi:10.4161/epi.4.3.8752)
- Enomoto K & Farber E 1982 Kinetics of phenotypic maturation of remodeling of hyperplastic nodules during liver carcinogenesis. *Cancer Research* **42** 2330–2335.
- Erion MD, Cable EE, Ito BR, Jiang H, Fujitaki JM, Finn PD, Zhang BH, Hou J, Boyer SH, van Poelje PD, et al. 2007 Targeting thyroid hormone receptor-beta agonists to the liver reduces cholesterol and triglycerides and improves the therapeutic index. *PNAS* **104** 15490–15495. (doi:10.1073/pnas.0702759104)
- Fanti M, Singh S, Ledda-Columbano GM, Columbano A & Monga SP 2014 Tri-iodothyronine induces hepatocyte proliferation by protein kinase A-dependent β-catenin activation in rodents. *Hepatology* **59** 2309–2320. (doi:10.1002/hep.26775)
- Feng X, Jiang Y, Meltzer P & Yen PM 2000 Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. *Molecular Endocrinology* **14** 947–955. (doi:10.1210/mend.14.7.0470)
- Flores-Morales A, Gullberg H, Fernandez L, Stahlberg N, Lee NH, Vennström B & Norstedt G 2002 Patterns of liver gene expression governed by TRbeta. *Molecular Endocrinology* **16** 1257–1268. (doi:10.1210/me.16.6.1257)
- Forrest D & Vennstrom B 2000 Functions of thyroid hormone receptors in mice. *Thyroid* **10** 41–52. (doi:10.1089/thy.2000.10.41)
- Francavilla A, Carr BI, Azzarone A, Polimeno L, Wang Z, Van Diehl DH, Subbotin V, Prelich JG & Starzl TE 1994 Hepatocyte proliferation and gene expression induced by triiodothyronine in vivo and in vitro. *Hepatology* **20** 1237–1241. (doi:10.1016/0270-9139(94)90763-3)
- Frau C, Loi R, Petrelli A, Perra A, Menegon S, Kowalik MA, Pinna S, Leoni VP, Fornari F, Gramantieri L, et al. 2015 Local hypothyroidism favors the progression of preneoplastic lesions to hepatocellular carcinoma in rats. *Hepatology* **61** 249–259. (doi:10.1002/hep.27399)
- García-Silva S & Aranda A 2004 The thyroid hormone receptor is a suppressor of ras-mediated transcription, proliferation, and transformation. *Molecular and Cellular Biology* **24** 7514–7523. (doi:10.1128/MCB.24.17.7514-7523.2004)
- Gereben B, Zeöld A, Dentice M, Salvatore D & Bianco AC 2008 Activation and inactivation of thyroid hormone by deiodinases: local action with general consequences. *Cellular and Molecular Life Science* **65** 570–590. (doi:10.1007/s00018-007-7396-0)
- Giordano S & Columbano A 2013 MicroRNAs: new tools for diagnosis, prognosis, and therapy in hepatocellular carcinoma? *Hepatology* **57** 840–847. (doi:10.1002/hep.26095)
- González-Sancho JM, Figueroa A, López-Barahona M, López E, Beug H & Muñoz A 2002 Inhibition of proliferation and expression of T1 and cyclin D1 genes by thyroid hormone in mammary epithelial cells. *Molecular Carcinogenesis* **34** 25–34. (doi:10.1002/mc.10046)
- Grover GJ, Egan DM, Sleph PG, Beehler BC, Chiellini G, Nguyen NH, Baxter J & Scanlan TS 2004 Effects of the thyroid hormone receptor agonist GC-1 on metabolic rate and cholesterol in rats and primates: selective actions relative to 3,5,3'-triiodo-L-thyronine. *Endocrinology* **145** 1656–1661. (doi:10.1210/en.2003-0973)
- Gu G, Gelsomino L, Covington KR, Beyer AR, Wang J, Rechoum Y, Huffman K, Carstens R, Andò S & Fuqua SA 2015 Targeting thyroid hormone receptor beta in triple-negative breast cancer. *Breast Cancer Research and Treatment* **150** 535–545. (doi:10.1007/s10549-015-3354-y)
- Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, et al. 2012 Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nature Genetics* **44** 694–698. (doi:10.1038/ng.2256)
- Guigon CJ, Kim DW, Willingham MC & Cheng SY 2011 Mutation of thyroid hormone receptor-β in mice predisposes to the development of mammary tumors. *Oncogene* **30** 3381–3390. (doi:10.1038/onc.2011.50)
- Harvey CB, Bassett JH, Maruvada P, Yen PM & Williams GR 2007 The rat thyroid hormone receptor (TR) Deltabeta3 displays cell-, TR isoform-, and thyroid hormone response element-specific actions. *Endocrinology* **148** 1764–1773. (doi:10.1210/en.2006-1248)
- Hassan MM, Kaseb A, Li D, Patt YZ, Vauthey J-N, Thomas MB, Curley SA, Spitz MR, Sherman SI, Abdalla EK, et al. 2009 Association between hypothyroidism and hepatocellular carcinoma: a case-control study in the United States. *Hepatology* **49** 1563–1570. (doi:10.1002/hep.22793)
- Hayashi M, Tamura T, Kuroda J, Ohnoda H, Shibata N, Akahane M, Kashida Y & Mitsumori K 2005 Different inhibitory effects in the early and late phase of treatment with KAT-681, a liver-selective thyromimetic, on rat hepatocarcinogenesis induced by 2-acetylaminofluorene and partial hepatectomy after diethylnitrosamine initiation. *Toxicological Sciences* **84** 22–28. (doi:10.1093/toxsci/kfi044)
- Hercbergs AA, Goyal LK, Suh JH, Lee S, Reddy CA, Cohen BH, Stevens GH, Reddy SK, Peereboom DM, Elson PJ, et al. 2003 Propylthiouracil-induced chemical hypothyroidism with high-dose tamoxifen prolongs survival in recurrent high grade glioma: a phase I/II study. *Anticancer Research* **23** 617–626.
- Heuer H & Visser TJ 2009 Minireview: pathophysiological importance of thyroid hormone transporters. *Endocrinology* **150** 1078–1083. (doi:10.1210/en.2008-1518)

- Hörkkö TT, Tuppurainen K, George SM, Jernvall P, Karttunen TJ & Mäkinen MJ 2006 Thyroid hormone receptor beta1 in normal colon and colorectal cancer-association with differentiation, polypoid growth type and K-ras mutations. *International Journal of Cancer* **118** 1653–1659. (doi:10.1002/ijc.21556)
- Hsu J-H & Brent GA 1998 Thyroid hormone receptor gene knockouts. *Trends in Endocrinology and Metabolism* **9** 103–112. (doi:10.1016/S1043-2760(98)00026-5)
- Huang YH, Lin KH, Liao CH, Lai MW, Tseng YH & Yeh CT 2012 Furin overexpression suppresses tumor growth and predicts a better postoperative disease-free survival in hepatocellular carcinoma. *PLoS ONE* **7** e40738. (doi:10.1371/journal.pone.0040738)
- Huang YH, Lin YH, Chi HC, Liao CH, Liao CJ, Wu SM, Chen CY, Tseng YH, Tsai CY, Lin SY, et al. 2013 Thyroid hormone regulation of miR-21 enhances migration and invasion of hepatoma. *Cancer Research* **73** 2505–2517. (doi:10.1158/0008-5472.CAN-12-2218)
- Imbeaud S, Ladeiro Y & Zucman-Rossi J 2010 Identification of novel oncogenes and tumor suppressors in hepatocellular carcinoma. *Seminars in Liver Disease* **30** 75–86. (doi:10.1055/s-0030-1247134)
- Iwasaki Y, Sunaga N, Tomizawa Y, Imai H, Iijima H, Yanagitani N, Horiguchi K, Yamada M & Mori M 2010 Epigenetic inactivation of the thyroid hormone receptor beta1 gene at 3p24.2 in lung cancer. *Annals of Surgical Oncology* **17** 2222–2228. (doi:10.1245/s10434-010-0956-9)
- Jazdzewski K, Boguslawska J, Jendrzewski J, Liyanarachchi S, Pachucki J, Wardyn KA, Nauman A & de la Chapelle A 2011 Thyroid hormone receptor beta (THRB) is a major target gene for microRNAs deregulated in papillary thyroid carcinoma (PTC). *Journal of Clinical Endocrinology and Metabolism* **96** E546. (doi:10.1210/jc.2010-1594)
- Jemal A, Bray F, Center MM, Ferlay J, Ward E & Forman D 2011 Global cancer statistics. *CA: A Cancer Journal for Clinicians* **61** 69–90. (doi:10.3322/caac.20107)
- Joseph B, Ji M, Liu D, Hou P & Xing M 2007 Lack of mutations in the thyroid hormone receptor (TR) alpha and beta genes but frequent hypermethylation of the TRbeta gene in differentiated thyroid tumors. *Journal of Clinical Endocrinology and Metabolism* **92** 4766–4770. (doi:10.1210/jc.2007-0812)
- Jung HI, Ahn T, Bae SH, Chung JC, Kim H, Chin S, Jeong D, Cho HD, Lee MS, Kim HC, et al. 2015 Astrocyte elevated gene-1 overexpression in hepatocellular carcinoma: an independent prognostic factor. *Annals of Surgical Treatment and Research* **88** 77–85. (doi:10.4174/ast.2015.88.2.77)
- Kamiya Y, Puzianowska-Kuznicka M, McPhie P, Nauman J, Cheng SY & Nauman A 2002 Expression of mutant thyroid hormone nuclear receptors is associated with human renal clear cell carcinoma. *Carcinogenesis* **23** 25–33. (doi:10.1093/carcin/23.1.25)
- Kaneshige M, Kaneshige K, Zhu Z, Dace A, Garrett L, Carter TA, Kazlauskaitė R, Pankratz DG, Wynshaw-Boris A, Refetoff S, et al. 2000 Mice with a targeted mutation in the thyroid hormone beta receptor gene exhibit impaired growth and resistance to thyroid hormone. *PNAS* **97** 13209–13214. (doi:10.1073/pnas.230285997)
- Kato Y, Ying H, Willingham MC & Cheng SY 2004 A tumor suppressor role for thyroid hormone beta receptor in a mouse model of thyroid carcinogenesis. *Endocrinology* **145** 4430–4438. (doi:10.1210/en.2004-0612)
- Katoh M 2007 Networking of WNT, FGF, Notch, BMP, and Hedgehog signaling pathways during carcinogenesis. *Stem Cell Reviews* **3** 30–38. (doi:10.1007/s12015-007-0006-6)
- Kim WG, Zhu X, Kim DW, Zhang L, Kebebew E & Cheng SY 2013 Reactivation of the silenced thyroid hormone receptor b gene expression delays thyroid tumor progression. *Endocrinology* **154** 25–35. (doi:10.1210/en.2012-1728)
- Khan S, Wall D, Curran C, Newell J, Kerin MJ & Dwyer RM 2015 MicroRNA-10a is reduced in breast cancer and regulated in part through retinoic acid. *BMC Cancer* **2** 345. (doi:10.1186/s12885-015-1374-y)
- Ko AH, Wang F & Holly EA 2007 Pancreatic cancer and medical history in a population-based case-control study in the San Francisco Bay Area, California. *Cancer Causes & Control* **18** 809–819. (doi:10.1007/s10552-007-9024-6)
- Kowalik MA, Perra A, Pibiri M, Cocco MT, Samarut J, Plateroti M, Ledda-Columbano GM & Columbano A 2010 TRbeta is the critical thyroid hormone receptor isoform in T3-induced proliferation of hepatocytes and pancreatic acinar cells. *Journal of Hepatology* **53** 686–692. (doi:10.1016/j.jhep.2010.04.028)
- Kress E, Rezza A, Nadjar J, Samarut J & Plateroti M 2009 The frizzled-related sFRP2 gene is a target of thyroid hormone receptor alpha1 and activates beta-catenin signaling in mouse intestine. *Journal of Biological Chemistry* **284** 1234–1241. (doi:10.1074/jbc.M806548200)
- Kress E, Samarut J & Plateroti M 2009 Thyroid hormones and the control of cell proliferation or cell differentiation: paradox or duality? *Molecular and Cellular Endocrinology* **313** 36–49. (doi:10.1016/j.mce.2009.08.028)
- Kress E, Skah S, Sirakov M, Nadjar J, Gadot N, Scoazec JY, Samarut J & Plateroti M 2010 Cooperation between the thyroid hormone receptor TRalpha1 and the WNT pathway in the induction of intestinal tumorigenesis. *Gastroenterology* **138** 1863–1874. (doi:10.1053/j.gastro.2010.01.041)
- László V, Dezso K, Baghy K, Papp V, Kovalszky I, Sáfrány G, Thorgerisson SS, Nagy P & Paku S 2008 Triiodothyronine accelerates differentiation of rat liver progenitor cells into hepatocytes. *Histochemistry and Cell Biology* **130** 1005–1014. (doi:10.1007/s00418-008-0482-z)
- Lazar MA 1993 Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocrine Review* **14** 184–193. (doi:10.1210/er.14.2.184)
- Lazar MA 2003 Thyroid hormone action: a binding contract. *Journal of Clinical Investigation* **112** 497–499. (doi:10.1172/JCI19479)
- Ledda-Columbano GM, Perra A, Loi R, Dore M, Shinozuka H & Columbano A 2000 Cell proliferation induced by triiodothyronine in rat liver is associated with nodule regression and reduction of hepatocellular carcinomas. *Cancer Research* **60** 603–609.
- Leduc F, Brauch H, Hajj C, Dobrovic A, Kaye F, Gazdar A, Harbour JW, Pettengill OS, Sorenson GD, van den Berg A, et al. 1989 Loss of heterozygosity in a gene coding for a thyroid hormone receptor in lung cancers. *American Journal of Human Genetics* **44** 282–287.
- Lehrer S, Diamond EJ, Stone NN, Droller MJ & Stock RG 2002 Serum triiodothyronine is increased in men with prostate cancer and benign prostatic hyperplasia. *Journal of Urology* **168** 2431–2433. (doi:10.1016/S0022-5347(05)64161-4)
- Li Z, Meng ZH, Chandrasekaran R, Kuo WL, Collins CC, Gray JW & Dairkee SH 2002 Biallelic inactivation of the thyroid hormone receptor beta1 gene in early stage breast cancer. *Cancer Research* **62** 1939–1943.
- Liao CH, Yeh CT, Huang YH, Wu SM, Chi HC, Tsai MM, Tsai CY, Liao CJ, Tseng YH, Lin YH, et al. 2012 Dickkopf 4 positively regulated by the thyroid hormone receptor suppresses cell invasion in human hepatoma cells. *Hepatology* **55** 910–920. (doi:10.1002/hep.24740)
- Lin KH, Zhu XG, Shieh HY, Hsu HC, Chen ST, McPhie P & Cheng SY 1996 Identification of naturally occurring dominant negative mutants of thyroid hormone alpha 1 and beta 1 receptors in a human hepatocellular carcinoma cell line. *Endocrinology* **137** 4073–4081. (doi:10.1210/en.137.10.4073)
- Lin KH, Shieh HY, Chen SL & Hsu HC 1999 Expression of mutant thyroid hormone nuclear receptors in human hepatocellular carcinoma cells. *Molecular Carcinogenesis* **26** 53–61. (doi:10.1002/(sici)1098-2744(199909)26:1<53::aid-mc7>3.0.co;2-z)
- Lin YH, Liao CJ, Huang YH, Wu MH, Chi HC, Wu SM, Chen CY, Tseng YH, Tsai CY, Chung IH, et al. 2013 Thyroid hormone receptor represses miR-17 expression to enhance tumor metastasis

- in human hepatoma cells. *Oncogene* **32** 4509–4518. (doi:10.1038/onc.2013.309)
- Ling Y, Xu X, Hao J, Ling X, Du X, Liu X & Zhao X 2010 Aberrant methylation of the THRB gene in tissue and plasma of breast cancer patients. *Cancer Genetics Cytogenetics* **196** 140–145. (doi:10.1016/j.cancergencyto.2009.09.010)
- López-Fontal R, Zeini M, Través PG, Gómez-Ferrera M, Aranda A, Sáez GT, Cerdá C, Martín-Sanz P, Hortelano S & Boscá L 2010 Mice lacking thyroid hormone receptor beta show enhanced apoptosis and delayed liver commitment for proliferation after partial hepatectomy. *PLoS ONE* **5** e8710. (doi:10.1371/journal.pone.0008710)
- Lu X, Chen Z, Liang H, Li Z, Zou X, Luo H, Guo W & Xu L 2013 Thyroid hormone inhibits TGFβ1 induced renal tubular epithelial to mesenchymal transition by increasing miR34a expression. *Cellular Signalling* **25** 1949–1954. (doi:10.1016/j.cellsig.2013.06.005)
- Lujambio A & Lowe SW 2012 The microcosmos of cancer. *Nature* **482** 347–355. (doi:10.1038/nature10888)
- Malik R, Habib M, Tootle R & Hodgson H 2008 Exogenous thyroid hormone induces liver enlargement whilst maintaining regenerative potential. Xenobiotic mitogens and liver regeneration. A study relevant to donor preconditioning. *American Journal of Transplantation* **5** 1801–1807. (doi:10.1111/j.1600-6143.2005.00949.x)
- Mangelsdorf DJ, Umesono K & Evans RM 1984 The retinoid receptors. In *The Retinoids: Biology, Chemistry and Medicine*, edn 2, pp 319–349. Eds MB Sporn, AB Roberts & DS Goodman. New York, NY, USA: Raven Press.
- Markowitz S, Haut M, Stellato T, Gerbic C & Molkentin K 1989 Expression of the ErbA-beta class of thyroid hormone receptors is selectively lost in human colon carcinoma. *Journal of Clinical Investigation* **84** 1683–1687. (doi:10.1172/JCI114349)
- Martínez L, Nascimento AS, Nunes FM, Phillips K, Aparicio R, Dias SM, Figueira AC, Lin JH, Nguyen P, Apriletti JW, et al. 2009 Gaining ligand selectivity in thyroid hormone receptors via entropy. *PNAS* **106** 20717–20722. (doi:10.1073/pnas.0911024106)
- Martínez-Iglesias O, García-Silva S, Tenbaum SP, Regadera J, Larcher F, Paramio JM, Vennström B & Aranda A 2009 Thyroid hormone receptor beta1 acts as a potent suppressor of tumor invasiveness and metastasis. *Cancer Research* **69** 501–509. (doi:10.1158/0008-5472.can-08-219)
- Martínez-Iglesias OA, Alonso-Merino E, Gómez-Rey S, Velasco-Martín JP, Martín Orozco R, Luengo E, García Martín R, Ibáñez de Cáceres I, Fernández AF, Fraga MF, et al. 2016 Autoregulatory loop of nuclear corepressor 1 expression controls invasion, tumor growth, and metastasis. *PNAS* **113** 328–337. (doi:10.1073/pnas.1520469113)
- Master A, Wójcicka A, Piekietko-Witkowska A, Bogusławska J, Popławski P, Tański Z, Darras VM, Williams GR & Nauman A 2010 Untranslated regions of thyroid hormone receptor beta1 mRNA are impaired in human clear cell renal cell carcinoma. *Biochimica et Biophysica Acta* **1802** 995–1005. (doi:10.1016/j.bbdis.2010.07.025)
- Meruvu S, Ayers SD, Winnier G & Webb P 2013 Thyroid hormone analogues: where do we stand in 2013? *Thyroid* **23** 1333–1344. (doi:10.1089/thy.2012.0458)
- Mitsuhashi T, Tennyson GE & Nikodem VM 1988 Alternative splicing generates messages encoding rat c-erbA proteins that do not bind thyroid hormone. *PNAS* **85** 5804–5808. (doi:10.1073/pnas.85.16.5804)
- Nejak-Bowen KN & Monga SP 2011 Beta-catenin signaling, liver regeneration and hepatocellular cancer: sorting the good from the bad. *Seminars in Cancer Biology* **21** 44–58. (doi:10.1016/j.semcancer.2010.12.010)
- Ness RB, Grisso JA, Cottreau C, Klapper J, Vergona R, Wheeler JE, Morgan M & Schlesselman JJ 2000 Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. *Epidemiology* **11** 111–117. (doi:10.1097/00001648-200003000-00006)
- Njei B, Rotman Y, Ditah I & Lim JK 2015 Emerging trends in hepatocellular carcinoma incidence and mortality. *Hepatology* **61** 191–199. (doi:10.1002/hep.27388)
- Nishi H, Ono K, Horie T, Nagao K, Kinoshita M, Kuwabara Y, Watanabe S, Takaya T, Tamaki Y, Takanabe-Mori R, et al. 2011 MicroRNA-27a regulates beta cardiac myosin heavy chain gene expression by targeting thyroid hormone receptor beta1 in neonatal rat ventricular myocytes. *Molecular and Cellular Biology* **31** 744–755. (doi:10.1128/MCB.00581-10)
- O'Barr SA, Oh JS, Ma C, Brent GA & Schultz JJ 2006 Thyroid hormone regulates endogenous amyloid-precursor protein gene expression and processing in both *in vitro* and *in vivo* models. *Thyroid* **16** 1207–1213. (doi:10.1089/thy.2006.16.1207)
- Perra A, Simbula G, Simbula M, Pibiri M, Kowalik MA, Sulas P, Cocco MT, Ledda-Columbano GM & Columbano A 2008 Thyroid hormone (T₃) and TRbeta agonist GC-1 inhibit/reverse nonalcoholic fatty liver in rats. *FASEB Journal* **22** 2981–2989. (doi:10.1096/fj.08-108464)
- Perra A, Kowalik MA, Pibiri M, Ledda-Columbano GM & Columbano A 2009 Thyroid hormone receptor ligands induce regression of rat preneoplastic liver lesions causing their reversion to a differentiated phenotype. *Hepatology* **49** 1287–1296. (doi:10.1002/hep.22750)
- Petrelli A, Perra A, Cora D, Sulas P, Menegon S, Manca C, Migliore C, Kowalik MA, Ledda-Columbano GM, Giordano S, et al. 2014 MicroRNA/gene profiling unveils early molecular changes and nuclear factor erythroid related factor 2 (NRF2) activation in a rat model recapitulating human hepatocellular carcinoma (HCC). *Hepatology* **59** 228–241. (doi:10.1002/hep.26616)
- Pibiri M, Ledda-Columbano GM, Cossu C, Simbula G, Menegazzi M, Shinozuka H & Columbano A 2001 Cyclin D1 is an early target in hepatocyte proliferation induced by thyroid hormone (T₃). *FASEB Journal* **15** 1006–1013. (doi:10.1096/fj.00-0416com)
- Plateroti M, Kress E, Mori JI & Samarut J 2006 Thyroid hormone receptor alpha1 directly controls transcription of the beta-catenin gene in intestinal epithelial cells. *Molecular and Cellular Biology* **26** 3204–3214. (doi:10.1128/MCB.26.8.3204-3214.2006)
- Puzianowska-Kuznicka M, Nauman A, Madej A, Tanski Z, Cheng S & Nauman J 2000 Expression of thyroid hormone receptors is disturbed in human renal clear cell carcinoma. *Cancer Letters* **155** 145–152. (doi:10.1016/S0304-3835(00)00416-X)
- Puzianowska-Kuznicka M, Krystyniak A, Madej A, Cheng SY & Nauman J 2002 Functionally impaired TR mutants are present in thyroid papillary cancer. *Journal of Clinical Endocrinology and Metabolism* **87** 1120–1128. (doi:10.1210/jcem.87.3.8296)
- Reddy A, Dash C, Leerapun A, Mettler TA, Stadheim LM, Lazaridis KN, Roberts RO & Roberts LR 2007 Hypothyroidism: a possible risk factor for liver cancer in patients with no known underlying cause of liver disease. *Clinical Gastroenterology and Hepatology* **5** 118–123. (doi:10.1016/j.cgh.2006.07.011)
- Rodd C, Schwartz HL, Strait KA & Oppenheimer JH 1992 Ontogeny of hepatic nuclear triiodothyronine receptor isoforms in the rat. *Endocrinology* **131** 2559–2564. (doi:10.1210/en.131.6.2559)
- Roomi MW, Ho RK, Sarma DS & Farber E 1985 A common biochemical pattern in preneoplastic hepatocyte nodules generated in four different models in the rat. *Cancer Research* **45** 564–571.
- Rosignolo F, Maggisano V, Sponziello M, Celano M, Di Gioia CR, D'Agostino M, Giacomelli L, Verrienti A, Dima M, Pecce V, et al. 2015 Reduced expression of THRβ in papillary thyroid carcinomas: relationship with BRAF mutation, aggressiveness and miR expression. *Journal of Endocrinological Investigation* **38** 1283–1289. (doi:10.1007/s40618-015-0309-4)
- Ruiz-Llorente L, Ardila-González S, Fanjul LF, Martínez-Iglesias O & Aranda A 2014 microRNAs 424 and 503 are mediators of the anti-proliferative and anti-invasive action of the thyroid hormone receptor beta. *Oncotarget* **5** 2918–2933. (doi:10.18632/oncotarget)
- Schnerch A, Cerdan C & Bhatia M 2010 Distinguishing between mouse and human pluripotent stem cell regulation: the best laid plans of mice and men. *Stem Cells* **28** 419–430. (doi:10.1002/stem.298)

- Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S, Couchy G, Meiller C, Shinde J, Soysouvanh F, et al. 2015 Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nature Genetics* **47** 505–511. (doi:10.1038/ng.3252)
- Schwartz HL, Strait KA, Ling NC & Oppenheimer JH 1992 Quantitation of rat tissue thyroid hormone binding receptor isoforms by immunoprecipitation of nuclear triiodothyronine binding capacity. *Journal of Biological Chemistry* **267** 11794–11799.
- Short J, Brow RF, Husakova A, Gilbertson JR, Zemel R & Lieberman I 1972 Induction of deoxyribonucleic acid synthesis in the liver of intact animal. *Journal of Biological Chemistry* **247** 1757–1766.
- Silva JM, Domínguez G, González-Sancho JM, García JM, Silva J, García-Andrade C, Navarro A, Muñoz A & Bonilla F 2002 Expression of thyroid hormone receptor/erbA genes is altered in human breast cancer. *Oncogene* **21** 4307–4316. (doi:10.1038/sj.onc.1205534)
- Sirakov M, Boussouar A, Kress E, Frau C, Lone IN, Nadjar J, Angelov D & Plateroti M 2015 The thyroid hormone nuclear receptor TR α 1 controls the Notch signaling pathway and cell fate in murine intestine. *Development* **142** 2764–2774. (doi:10.1242/dev.121962)
- Sisley K, Curtis D, Rennie IG & Rees RC 1993 Loss of heterozygosity of the thyroid hormone receptor B in posterior uveal melanoma. *Melanoma Research* **3** 457–461. (doi:10.1097/00008390-199311000-00009)
- Sjouke B, Langslet G, Ceska R, Nicholls SJ, Nissen SE, Öhlander M, Ladenson PW, Olsson AG, Hovingh GK & Kastelein JJ 2014 Eprotrirome in patients with familial hypercholesterolaemia (the AKKA trial): a randomised, double-blind, placebo-controlled phase 3 study. *Lancet Diabetes & Endocrinology* **2** 455–463. (doi:10.1016/s2213-8587(14)70006-3)
- Srivastava J, Robertson CL, Gredler R, Siddiq A, Rajasekaran D, Akiel MA, Emdad L, Mas V, Mukhopadhyay ND, Fisher PB, et al. 2015 Astrocyte elevated gene-1 (AEG-1) contributes to non-thyroidal illness syndrome (NTIS) associated with hepatocellular carcinoma (HCC). *Journal of Biological Chemistry* **29** 15549–15558. (doi:10.1074/jbc.m115.649707)
- Sun J, Wang B, Liu Y, Zhang L, Ma A, Yang Z, Ji Y & Liu Y 2014 Transcription factor KLF9 suppresses the growth of hepatocellular carcinoma cells in vivo and positively regulates p53 expression. *Cancer Letters* **355** 25–33. (doi:10.1016/j.canlet.2014.09.022)
- Suzuki H, Willingham MC & Cheng SY 2002 Mice with a mutation in the thyroid hormone receptor beta gene spontaneously develop thyroid carcinoma: a mouse model of thyroid carcinogenesis. *Thyroid* **12** 963–969. (doi:10.1089/105072502320908295)
- Takano T, Miyauchi A, Yoshida H, Nakata Y, Kuma K & Amino N 2003 Expression of TRbeta1 mRNAs with functionally impaired mutations is rare in thyroid papillary carcinoma. *Journal of Clinical Endocrinology and Metabolism* **88** 3447–3449. (doi:10.1210/jc.2003-030012)
- Taki-Eldin A, Zhou L, Xie HY, Chen KJ, Zhou WH, Zhang W, Xing CY, Yang Z, Zhang K & Zheng SS 2011 Tri-iodothyronine enhances liver regeneration after living donor liver transplantation in rats. *Journal of Hepato-Biliary-Pancreatic Sciences* **18** 806–814. (doi:10.1007/s00534-011-0397-2)
- Tancevski I, Rudling M & Eller P 2011 Thyromimetics: a journey from bench to bed-side. *Pharmacology & Therapeutics* **131** 33–39. (doi:10.1016/j.pharmthera.2011.04.003)
- Thormeyer D & Baniahmad A 1999 The v-erbA oncogene. *International Journal of Molecular Medicine* **4** 351–358.
- Tomokuni A, Eguchi H, Tomimaru Y, Wada H, Kawamoto K, Kobayashi S, Marubashi S, Tanemura M, Nagano H, Mori M, et al. 2011 miR-146 suppresses the sensitivity to interferon-alpha in hepatocellular carcinoma cells. *Biochemical and Biophysical Research Communications* **414** 675–680. (doi:10.1016/j.bbrc.2011.09.124)
- Totoki Y, Tatsuno K, Covington KR, Ueda H, Creighton CJ, Kato M, Tsuji S, Donehower LA, Slagle BL, Nakamura H, et al. 2014 Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nature Genetics* **46** 1267–1273. (doi:10.1038/ng.3126)
- Trost SU, Swanson E, Gloss B, Wang-Iverson DB, Zhang H, Volodarsky T, Grover GJ, Baxter JD, Chiellini G, Scanlan TS, et al. 2000 The thyroid hormone receptor β -selective agonist GC-1 differentially affects plasma lipids and cardiac activity. *Endocrinology* **141** 3057–3064. (doi:10.1210/en.141.9.3057)
- van der Deure WM, Peeters RP & Visser TJ 2010 Molecular aspects of thyroid hormone transporters, including MCT8, MCT10, and OATPs, and the effects of genetic variation in these transporters. *Journal of Molecular Endocrinology* **44** 1–11. (doi:10.1677/JME-09-0042)
- Villanueva A, Portela A, Sayols S, Battiston C, Hoshida Y, Méndez-González J, Imbeaud S, Letouze E, Hernandez-Gea V, Cornella H, et al. 2015 DNA methylation-based prognosis and epidrivers in hepatocellular carcinoma. *Hepatology* **61** 1945–1956. (doi:10.1002/hep.27732)
- Wallin G, Brönnegård M, Grimelius L, McGuire J & Tørring O 1992 Expression of the thyroid hormone receptor, the oncogenes c-myc and H-ras, and the 90 kD heat shock protein in normal, hyperplastic, and neoplastic human thyroid tissue. *Thyroid* **2** 307–313. (doi:10.1089/thy.1992.2.307)
- Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H & Refetoff S 1998 Thyroid hormone action on liver, heart, and energy expenditure in thyroid hormone receptor b-deficient mice. *Endocrinology* **139** 4945–4952. (doi:10.1210/en.139.12.4945)
- Wikstrom L, Johansson C, Salto C, Barlow C, Campos Barros A, Baas F, Forrest D, Thoren P & Vennström B 1998 Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1. *EMBO Journal* **17** 455–461. (doi:10.1093/emboj/17.2.455)
- Wojcicka A, Piekliko-Witkowska A, Kedzierska H, Rybicka B, Poplawski P, Boguslawska J, Master A & Nauman A 2014 Epigenetic regulation of thyroid hormone receptor beta in renal cancer. *PLoS ONE* **9** e97624. (doi:10.1371/journal.pone.0097624)
- Yang Z & Privalsky ML 2001 Isoform-specific transcriptional regulation by thyroid hormone receptors: hormone-independent activation operates through a steroid receptor mode of co-activator interaction. *Molecular Endocrinology* **15** 1170–1185. (doi:10.1210/mend.15.7.0656)
- Yap CS, Sinha RA, Ota S, Katsuki M & Yen PM 2013 Thyroid hormone negatively regulates CDX2 and SOAT2 mRNA expression via induction of miRNA-181d in hepatic cells. *Biochemical and Biophysical Research Communications* **440** 635–639. (doi:10.1016/j.bbrc.2013.09.116)
- Yen CC, Huang YH, Liao CY, Liao CJ, Cheng WL, Chen WJ & Lin KH 2006 Mediation of the inhibitory effect of thyroid hormone on proliferation of hepatoma cells by transforming growth factor-beta. *Journal of Molecular Endocrinology* **36** 9–21. (doi:10.1677/jme.1.01911)
- Yen PM, Ikeda M, Brubaker JH, Forgione M, Sugawara A & Chin WW 1994 Roles of v-erbA homodimers and heterodimers in mediating dominant negative activity by v-erbA. *Journal of Biological Chemistry* **269** 903–909.
- Yen PM 2001 Physiological and molecular basis of thyroid hormone action. *Physiological Reviews* **81** 1097–1142.
- Yen PM, Feng X, Flamant F, Chen Y, Walker RL, Weiss RE, Chassande O, Samarut J, Refetoff S & Meltzer PS 2003 Effects of ligand and thyroid hormone receptor isoforms on hepatic gene expression profiles of thyroid hormone receptor knockout mice. *EMBO Reports* **4** 581–587. (doi:10.1038/sj.embor.embor862)
- Ying M, Sang Y, Li Y, Guerrero-Cazares H, Quinones-Hinojosa A, Vescovi AL, Eberhart CG, Xia S & Lateral J 2011 Kruppel-like family of transcription factor 9, a differentiation-associated transcription factor, suppresses Notch1 signaling and inhibits glioblastoma-initiating stem cells. *Stem Cells* **29** 20–31. (doi:10.1002/stem.561)
- Zhang L, Sun H, Zhao F, Lu P, Ge C, Li H, Hou H, Yan M, Chen T, Jiang G, et al. 2012 BMP4 administration induces differentiation of CD133+ hepatic cancer stem cells, blocking their contributions to

hepatocellular carcinoma. *Cancer Research* **72** 4276–4285.

(doi:10.1158/0008-5472.CAN-12-1013)

Zhou C, Liu J, Li Y, Liu L, Zhang X, Ma CY, Hua SC, Yang M & Yuan Q
2011 microRNA-1274a, a modulator of sorafenib induced a
disintegrin and metalloproteinase 9 (ADAM9) down-regulation in

hepatocellular carcinoma. *FEBS Letters* **585** 1828–1834.

(doi:10.1016/j.febslet.2011.04.040)

Zhu XG, Zhao L, Willingham MC & Cheng SY 2010 Thyroid hormone
receptors are tumor suppressors in a mouse model of metastatic follicular
thyroid carcinoma. *Oncogene* **29** 1909–1919. (doi:10.1038/onc.2009.476)

Received in final form 24 June 2016

Accepted 27 June 2016

Accepted Preprint published online 27 June 2016