Targeting the TSH receptor in thyroid cancer

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Abstract

Recent advances in the arena of theranostics have necessitated a re-examining of previously established fields. The existing paradigm of therapeutic thyroid stimulating hormone receptor (TSHR) targeting in the post-surgical management of differentiated thyroid cancer using levothyroxine and recombinant human thyroid stimulating hormone (TSH) is well understood. However, in an era of personalized medicine, and with an increasing awareness of the risk profile of longstanding pharmacological hyperthyroidism, it is imperative clinicians understand the molecular basis and magnitude of benefit for individual patients. Furthermore, TSHR has been recently re-conceived as a selective target for residual metastatic thyroid cancer, with pilot data demonstrating effective targeting of nanoparticles to thyroid cancers using this receptor as a target. This review examines the evidence for TSHR signaling as an oncogenic pathway, and assesses the evidence for ongoing TSHR expression in thyroid cancer metastases. Priorities for further research are highlighted.

Introduction

The thyroid stimulating hormone (TSH) receptor (TSHR) is a surface glycoprotein receptor, part of the leucine-rich repeat subfamily of G-protein-coupled receptors (LGR). It has been described as the "master switch" in regulating thyroid growth, differentiation, and thyroid hormone secretion, and is the antigenic target for Graves' disease (Davies, et al. 2005). TSHR is expressed on benign and malignant thyrocytes as the target receptor for TSH. In current clinical management of differentiated thyroid cancer (DTC), TSHR is therapeutically targeted to maximize radioiodine uptake into malignant thyrocytes by transiently upregulating the sodium-iodide symporter (NIS) through TSH stimulation, either endogenously through thyroid hormone withdrawal or exogenously with recombinant human TSH (rhTSH) (Haugen, et al. 2016). Additionally, proliferative signals to malignant thyrocytes mediated through the TSHR are therapeutically minimized by inducing pharmacologic hyperthyroidism, resulting in endogenous TSH suppression. Recent scientific

advances suggest new strategies to target TSHR in thyroid cancers, either using selective small molecule inhibitors of the TSHR to obviate the need for systemic hyperthyroidism, or using the TSHR as a target to enhance therapeutic index for drug delivery systems. This increased interest demands a critical appraisal of evidence for persistent TSHR expression in metastatic thyroid cancer. Herein we review the evidence for TSHR-signalling as a mitogenic pathway in thyroid cancers, assess the evidence for persistence of TSHR in advanced thyroid cancer, and discuss new therapeutic strategies on the horizon.

Structure and distribution of TSHR

TSHR is a 764 amino-acid 7-transmembrane domain receptor in the G-protein coupled receptor superfamily. TSHR is encoded by the gene, Thyroid Stimulating Hormone Receptor *(TSHR),* located on chromosome 14q31 and first cloned in 1989 (Parmentier, et al. 1989). Although encoded by a single gene, the gene product undergoes post-translational cleavage into an extracellular A-subunit and a largely intracellular B-subunit linked by disulfide bonds, with the exclusion of a 50 amino acid C-peptide region (Rapoport and McLachlan 2016). The A-subunit contains multiple binding sites for TSH within a leucine-rich 'binding pocket', which undergoes conformational change following binding of TSH or stimulatory autoantibodies, resulting in receptor activation (Davies and Latif 2015).

TSHR is predominantly expressed on the basolateral membrane of thyroid follicular cells. Surface expression of TSHR is estimated at 5000 receptors per cell (Rees Smith, et al. 1988). *TSHR* mRNA and protein have been detected in a variety of other human and animal tissues, including neural and immune tissues, ocular muscles and bone (Davies, et al. 2002; Williams 2011; Bassett and Williams 2016). In particular, the role of TSHR expression in orbital preadipocytes and fibroblasts in the pathogenesis of Graves' ophthalmopathy has been extensively studied (Smith 2015); and there are animal data to support TSHR-mediated bone remodeling (Abe, et al. 2003; Ma, et al. 2011). Human

data regarding *TSHR* mRNA expression and protein production in extra-thyroidal tissue are presented in Table 1. We are not aware of studies which quantify TSHR receptor density in extrathyroidal tissue. In most extrathyroidal tissues, the physiologic role of the TSHR remains unclear (Williams 2011).

TSH as a growth factor for benign and malignant thyrocytes

TSH and intracellular growth signalling

As a glycoprotein receptor in the rhodopsin family, TSHR responds to ligand binding with activation of its coupled G protein. Activation of $G\alpha_s$ stimulates adenylate cyclase and activates the cyclic adenosine monophosphate (cAMP)/Protein Kinase A (PKA) pathway with well-established mitogenic effects, while $G\alpha_q$ stimulation results in activation of Protein Kinase B and mitogen activated protein kinase (MAPK) pathways (Morshed, et al. 2009). The downstream effects are to increase transcription of thyroid-specific genes, particularly controlling iodine uptake, synthesis of thyroglobulin and thyroperoxidase, with the end result of thyroid hormone production (Roger, et al. 1988; Vassart and Dumont 1992; Bruno, et al. 2005). The regulation of thyrocyte growth has been extensively studied using *in vitro* models, however it is well recognized that human *in vivo* confirmation of any such model is paramount (Kimura, et al. 2001).

Bruno *et al.* (2005) demonstrated *in vivo* in humans that TSH regulates transcription of NIS (*SLC5A5)*, thyroglobulin (*TG)*, thyroperoxidase (*TPO)* and paired box 8 (*PAX8*) mRNA, but not *TSHR*, pendrin (*SLC26A4*) or thyroid transcription factor 1 (*NKX2-1*). Importantly, they showed that absence of TSH due to suppression from exogenous hyperthyroidism does not reduce *TSHR* mRNA. These data support observations by Shuppert *et al.* (1996) from human tissues that *TSHR* mRNA is not reduced in the presence of chronic stimulation by thyroid stimulating autoantibodies, and from Maenhaut *et al.* (1992) in dogs, confirming not only that TSHR is important in thyrocyte growth, but

that TSHR gene transcription stably continues both in the presence and absence of its ligand. This contrasts with NIS gene and protein expression, which are affected by cellular signaling from iodine and TSH (Dohán, et al. 2003).

TSHR stimulation and thyroid growth.

Human disease states of chronic TSH stimulation provide *in vivo* models of TSHR mediated thyroid growth, as seen in TSH-secreting pituitary adenomas, and Graves' disease (where TSHR stimulation occurs by thyroid stimulatory immunoglobulins (TSI) binding to TSHR). Both conditions are characterized by pathological thyroid enlargement, seen in 77% and 93% respectively (Hegedus, et al. 1983; Beck-Peccoz, et al. 2009). Additionally, chronic exposure to TSI has been associated with increased disease-specific mortality in thyroid cancer in some, but not all, studies (Belfiore, et al. 1990; Pellegriti, et al. 2013).

Recently, multiple large cohort studies have found that increased levels of serum TSH are associated with increased subsequent risk of thyroid cancer (Nieto and Boelaert 2016). The largest study, recruiting 10,178 patients presenting with nodular thyroid disease for fine needle aspiration (FNA) biopsy, found an increasing odds ratio for papillary thyroid cancer (PTC) with incremental increases of TSH within the reference range of 0.4 to 3.4 mIU/L (Fiore, et al. 2009). Both higher pathological stage of the primary tumour and the increased incidence of nodal metastases were significantly associated with higher baseline TSH levels. In this study, level of TSH, not autoantibody status, was shown to be an independent variable in predicting malignancy.

Further, several studies have now shown the importance of TSHR signaling as part of the oncogenic pathway, particularly in tumours containing mutations in B-Raf proto-oncogene (*BRAF*). Franco and colleagues (2011) showed in mice that $BRAF^{V600E}$ mutations were only oncogenic in the presence of a TSHR stimulatory pathway. This finding was confirmed by Kim and colleagues (2014), showing that TSH signaling overcomes senescence induced by $BRAF^{V600E}$ mutations in cell culture. Similarly, Lu and colleagues (2010) used a TSHR-knockout mouse model of follicular thyroid cancer

(FTC) to show that TSHR-mediated growth signaling is required for thyroid cancer to metastasise, but only in the presence of additional oncogenic mutations.

Suppressed TSH and reduced progression of thyroid cancer

A correlation between a hyperthyroid state and reduced thyroid cancer growth was noted in the 1930's by the Australian surgeon Dunhill (1937). However, it was not until the 1950s that other surgeons, notably George Crile Jr, began to widely advocate the practice of levothyroxine therapy to reduce the size of thyroid cancer metastases (Hurley 2011). Subsequently, multiple large studies, including a meta-analysis (McGriff, et al. 2002), have confirmed that levothyroxine-induced suppression of TSH is associated with reduced growth of thyroid cancers. Key studies are reviewed below.

Mazzaferri and Jhiang (1994) prospectively followed 1,355 patients with treated DTC. After 30 years, the rate of recurrence for patients treated with levothyroxine was 30%, compared to 40% in untreated patients. Baseline characteristics of these subgroups, reasons for treatment, and degree of suppression of TSH were not reported. Pujol and colleagues (1996) retrospectively compared a group of 18 patients with stable TSH <0.05 mIU/L with a group of 15 patients with stable TSH >1 mIU/L following thyroidectomy for DTC. Baseline characteristics were similar. Median relapse-free survival was twice as long in the suppressed TSH group (21.6 vs 9.3 years), with significantly fewer relapses over this period (1 vs 6 relapses). Cooper *et al.* (1998) followed 617 DTC patients from a thyroid cancer registry for median of 4.5 years, with patients grouped according to mean of measured TSH levels. In patients with advanced stage disease at diagnosis, greater TSH suppression was associated with higher rates of progression-free survival. Finally, Jonklaas and colleagues (2006) followed 2936 DTC patients from a multi-institutional registry. Patients with AJCC-TNM5 Stage 2 or higher disease had higher rates of overall survival with TSH levels below the reference range, with increased degrees of TSH suppression correlating with higher overall survival in Stage III and IV disease.

TSHR expression in *in vitro* models of thyroid cancer

Although cell lines have represented an attractive model for cancer research for decades, it is increasingly understood that due to mutation and selection pressures within culture media, these cells commonly dedifferentiate. Several independent analyses of commonly studied thyroid cancer cell lines confirm that these cell lines have largely ceased to express markers of thyrocyte differentiation, and more closely represent anaplastic thyroid cancers than DTC (Meireles, et al. 2007; van Staveren, et al. 2007; Pilli, et al. 2009). This is particularly true of TSHR expression, which is downregulated early in monolayer culture due to disruption of follicular architecture and loss of apical-basal polarity (Williams and Wynford-Thomas 1997). In a recent review, Pilli and colleagues (2009) note that TSHR expression was not detected in any of a number of commonly studied cell lines. There are conflicting data regarding TSHR expression in the FTC-133 cell line (originating from a lymph node metastases of a differentiated human follicular thyroid cancer), with some authors demonstrating mRNA expression (D'Agostino, et al. 2014) and TSH ligand binding (Paolino, et al. 2014), which may represent heterogeneity within this cell line between laboratories. Additionally, there is conflicting evidence for TSHR expression in the BCPAP cell line (derived from a poorly differentiated PTC) (Pilli et al. 2009; D'Agostino et al. 2014; Dotan, et al. 2016). Similarly, PTC cells grown in thyrosphere culture lack expression of thyroid-specific proteins, and cells fail to produce cAMP in response to TSH stimulation (Malaguarnera, et al. 2011; Giani, et al. 2015). The cell line XTC-UC1, derived from a metastatic Hurthle cell carcinoma, appeared to retain TSHR expression as well as other markers of differentiation (Zielke, et al. 1998; Meireles et al. 2007), however is no longer widely available. Consequently, cells stably transfected with *TSHR* are commonly used for *in vitro* targeting studies where reliable TSHR expression is desired.

TSHR expression in primary thyroid tumors

Following seminal work by Ichikawa and colleagues (1976), robust evidence obtained over four decades supports the continued expression of TSHR in the majority of DTC, based on studies using ligand binding, mRNA detection using Western Blotting or PCR and protein immunohistochemistry (IHC) (see Table 2). While *TSHR* mRNA and/or protein can be detected in over 90% of PTC and FTC, the relative expression of TSHR in different tumours is highly variable, both in terms of pattern and intensity of staining by IHC, and in the semi-quantitative analysis of mRNA recovery (see Table 3).

Sheils and Sweeny (1999) used PCR to semi-quantitatively study mRNA extracted from 90 formalin-fixed paraffin embedded (FFPE) thyroid tumours, expressed as a ratio of *TSHR* mRNA to the housekeeping enzyme Glyceraldehyde-3-Phosphate Dehydrogenase (*GAPDH*), and stratified by tumour type and degree of differentiation. While *TSHR* mRNA was detected in all tumours, they found a significant positive correlation between degree of differentiation and *TSHR* expression (Figure 1). Recovery of *TSHR* mRNA from medullary thyroid cancer (MTC) would not usually be expected given the neuroendocrine origin of these cells, and could suggest contamination of samples from surrounding normal thyroid tissue, however the finding is consistent with other studies suggesting MTC may in fact express the TSHR (Elisei, et al. 1994).

Tanaka and colleagues (1997) studied TSHR protein expression in 21 PTC (18 well-differentiated) and 2 FTC (both well-differentiated) using IHC. They compared receptor immunostaining intensity and distribution to surrounding thyroid tissue. They found that intensity of staining for TSHR was weaker in 43%, similar in 30% and increased in 17% of tumours, with TSHR intensity reported as homogenous in 57% and heterogenous in 43%. Tumours with weaker TSHR staining were more likely to have an aggressive clinical phenotype.

In most studies, no TSHR expression was detected in anaplastic thyroid cancer (ATC), which is consistent with the expected loss of differentiation in this phenotype (see Table 2)

TSHR expression in thyroid cancer metastases

There is a paucity of data regarding TSHR expression in thyroid cancer metastases. As is evident from Tables 2 and 3, the majority of studies of *TSHR* mRNA expression or TSHR protein levels have focused on primary thyroid tumours, with several studies including occasional metastases in their cohort, almost exclusively from neck lymph nodes. The largest and only systematic study of TSHR expression in DTC metastases is from So and colleagues (2012), who examined using immunohistochemistry the specific instance of subclinical lymph node metastases from papillary microcarcinoma, using 20 primary tumours, and 52 associated subclinical central compartment metastases. They demonstrated that in their cohort, 90% of primary tumours, and 75% of metastatic nodes stained positive for TSHR, with concordance between primary tumour and metastases demonstrated in 85% of cases. Intensity of staining was weaker in metastases than in primary tumour in 44% of cases, and similar or stronger in 56%.

Arturi and colleagues (1997) used RT-PCR to study FNAs of enlarged neck lymph nodes from 27 patients with predominantly PTC. In the 26 aspirates with adequate samples, *TSHR* mRNA was detected in all specimens. No details of patient or tumour characteristics were provided.

Evidence for persistent functional TSHR expression in thyroid cancer metastases can be inferred from detecting increased thyroglobulin production in response to TSH stimulation, as occurs in preparation for radioiodine ablation in the context of known residual disease post-thyroidectomy. Lippi and colleagues (2001) studied 12 patients with metastatic or locally invasive FTC (n=10) or PTC (n=2). For the 9 patients with data available, thyroglobulin rose by a median of 8.4-fold (range 1.3 – 29x) following TSH stimulation. Luster and colleagues (2000) report radioiodine treatment in 11 patients with advanced recurrent or residual DTC. Nine of 11 patients had previous radioiodine therapy (median 5 treatments). 10 of 11 patients had a rise in thyroglobulin following treatment with rhTSH (median 2.4x baseline, range 1.2-59.1). Jarzab and colleagues (2003) studied administration of rhTSH in 54 patients with either locoregional or distant metastatic DTC. 50

patients had received prior radioiodine ablation. Median serum thyroglobulin concentration increased 3.8-fold from baseline to day 6 post-rhTSH. Individual patient data was not reported.

In a review of the 11 largest studies of rhTSH-assisted treatment of residual or recurrent DTC (124 patients), Luster and colleagues (2005) found that serum thyroglobulin increased in response to rhTSH in at least 67% of patients for whom data was recorded. This figure is similar to the 75% prevalence of TSHR expression reported by So *et al. (2012)* in papillary microcarcinoma, and strongly suggests that TSHR expression is preserved in the majority of clinically significant DTC metastases, and persists despite previous radioiodine exposure in the majority of cases. Importantly, this 67% is likely to underestimate actual TSHR expression in clinically significant metastases, both because these case series predominantly included patients with longstanding and advanced metastatic disease, and because the surrogate outcome measure (thyroglobulin rise) relies on an intact multi-step signaling cascade from TSHR to thyroglobulin production for a positive result.

Persistence of TSHR expression in the setting of loss of other differentiation markers

TSHR expression in DTC has been compared to expression of other thyroidal markers of differentiation. Several studies have found that TSHR expression closely parallels other markers of differentiation, such as thyroglobulin and thyroperoxidase (Hoang-Vu, et al. 1992; Elisei et al. 1994; Park, et al. 2000). However evidence from a number of studies of resected thyroid tissue suggests that TSHR is more persistently expressed than other differentiation markers, including NIS, and thyroglobulin proteins (Filetti, et al. 1999; Lazar, et al. 1999; Gerard, et al. 2003), indicating not only that TSHR may remain an important signaling pathway for cellular growth, but also its utility as a conserved therapeutic target. Further *in vivo* evidence of continued TSHR expression in the absence of NIS is provided by a study of 63 patients with metastatic DTC and no radioactive iodine uptake on whole body scan, who underwent ^{18}F-fludeoxyglucose positron emission tomography (^{18}F-FDG-PET) both under basal conditions, and following rhTSH stimulation (Leboulleux, et al. 2009). This study

found that the sensitivity of FDG-PET was significantly increased following rhTSH stimulation on a per lesion basis (95% vs 81%), suggesting that these lesions continued to express TSHR in the absence of the ability to concentrate radioiodine.

Current therapeutic targeting of TSHR in DTC

Firstly, as discussed above, pharmacological TSH suppression with exogenous levothyroxine has been a mainstay of clinical DTC management for decades. However, the most recent guidelines of the American Thyroid Association now support a more individualised approach to TSH suppression, based on the likelihood of progressive residual disease and the risks associated with systemic hyperthyroidism (Haugen et al. 2016), better reflecting the paucity of evidence for benefit in low risk patients. The long-term utility of TSH suppressive therapy is limited in part by its tumoristatic, not tumoricidal, efficacy, although a survival benefit has been shown in high-risk patients (Jonklaas et al. 2006). However, in patients at lower risk of recurrence, the adverse effects of hyperthyroidism, including accelerated bone loss resulting in osteoporosis, and cardiac side effects, including atrial fibrillation, remain important caveats to universal therapy.

Secondly, TSHR-mediated upregulation in NIS expression is routinely exploited in preparation for ablative radioiodine therapy, either with endogenous TSH-stimulation by thyroxine withdrawal, or pharmacologically with rhTSH. Clinically, expression of TSHR have been shown to correlate with the degree of iodine trapping (Edmonds, et al. 1977), and with efficacy of ablation (Fallahi, et al. 2012), and both positive and negative regulation of NIS mRNA in response to TSHR stimulation has been demonstrated in human thyroid cells (Saito, et al. 1997; Bruno et al. 2005). Interestingly, TSHR-stimulation also increases the sensitivity of ^{18}F-FDG-PET imaging in the detection of residual metastatic disease, again suggesting that TSHR stimulation increases mitotic activity and glucose utilization within malignant thyrocytes (Leboulleux et al. 2009).

Novel Theranostic Exploitation of TSHR

Thus far, key characteristics have been identified that establish the TSHR as an attractive therapeutic target in DTC, namely its pivotal role in growth signaling, and its persistence as an expressed surface protein until late stages of de-differentiation. Additionally, the relative specificity of TSHR as a marker on thyroid tissue makes it an attractive potential target for novel theranostic and therapeutic agents. Over the last decade there have been several exciting developments targeting TSHR in diagnosis or treatment of thyroid malignancies (Figure 2).

TSHR as a theranostic target for drug delivery

New modalities for the localisation and treatment of metastatic DTC are required, especially for tumours that are not cured by radioiodine. Although the majority of DTC has a favourable prognosis, up to 15% of cases do not respond completely to radioiodine ablation, including 4% that exhibit no tumour reduction or progressive disease (Sciuto, et al. 2009). In cases that no longer concentrate radioiodine, the twin utility of radioiodine as a true theranostic agent (a modality with both diagnostic and therapeutic utility) is absent. Localisation of recurrent disease is then reliant on structural imaging with ultrasound, computed tomography (CT) scan or ^{18}F-FDG-PET, which although sensitive, lacks the specificity of radioiodine avidity to confirm metastatic disease. Additionally, small molecule kinase inhibitors, which have surpassed traditional cytotoxics as first line treatment for progressive radioiodine resistant DTC, are cytostatic rather than tumoricidal, and thus at best can prolong progression-free survival rather than offer a chance of cure (Haugen et al. 2016). In addition, the toxicities of such agents preclude widespread use.

Late last century, Mayo Clinic Endocrinologist John Morris (1997) suggested that TSHR may be a suitable receptor for novel targeted therapies to thyroid cancers, although identified that “no

direct data towards this goal have appeared in the literature". Although not widely cited, this article defines what has become an increasingly relevant and potentially transformative field.

An early publication by Signore's laboratory in Rome examined radiolabeled rhTSH (either with ^{123}I or ^{125}I) as a potential imaging tracer for detection of thyroid cancer metastases in a nude-mouse xenograft tumour model, demonstrating focal increase in activity at sites of tumour (Corsetti, et al. 2004). A later study similarly examined Tc^{99}m-labelled-rhTSH injected into CD-1 xenograft mice, and a dog with PTC, and demonstrated focal uptake of radioisotope in TSHR positive cells (Galli, et al. 2014).

The field of theranostics in cancer therapy has rapidly expanded over the past decade, seeking the 'holy grail' of delivery of significant concentrations of drug to target tissues (either a chemotherapeutic agent or imaging tracer) with high specificity and minimal off-target effects, with the goals of increasing therapeutic index (Sercombe, et al. 2015). Nanoliposomes offer a well-studied and attractive model for drug delivery, and can deliver large payloads per particle. Organ-specific targeting, and immune system-evasion can be modulated by molecules embedded in the lipid bilayer (Sercombe et al. 2015).

Paolino and colleagues (2014) constructed nanoliposomes coated with fragments of TSH. They demonstrated competitive binding to TSHR *in vitro*, and 3-fold selectivity in localization to thyroid tissue in Wistar rats *in vivo*. TSHR-targeted nanoliposomes loaded with the chemotherapeutic agent gemcitabine had higher efficacy against the thyroid cancer cell line FTC-133 *in vitro* than non-targeted liposomal gemcitabine and free gemcitabine. Finally, in a xenograft model, TSHR-targeted gemcitabine nanoliposomes resulted in greater reduction of FTC-133 tumour mass over 15 days of therapy than non-targeted gemcitabine liposomes. A similar study the following year confirmed these results using cisplatin (Gao, et al. 2015). These studies provide *in vivo* models to investigate the paradigm of TSHR-targeted theranostics, as liposomes can be readily adapted to deliver either a diagnostic or therapeutic load.

This paradigm was further extended by Dotan and colleagues (2016) using bio-affinity functionalized carbon-walled nanotubes targeted with either antibodies against TSHR or rhTSH. Such nanotubes are designed to convert electromagnetic energy to thermal energy, and thus deliver a cytotoxic local thermal load when stimulated by an external near-infrared light source. *In vitro* data demonstrated specific cytotoxicity against TSHR expressing cells from infrared stimulated nanotubes targeted using two commercially available antibodies against TSHR compared to controls, with similar findings using TSH and rhTSH as targeting ligands. Targeting using rhTSH and TSH resulted in greater cytotoxicity than targeting using TSHR antibody. While this study again provides *in vitro* evidence for TSHR as a specific theranostic target for thyrocytes, it is unique in suggesting a further method of localising cytotoxicity using an external infra-red light source. This additional external 'targeting' may eliminate off-target cytotoxicity that may be conferred from cytotoxic laden nanoliposomes through non-specific binding, clearance of liposomes in the reticulo-endothelial system, or low density binding to non-thyroidal TSHR.

Small molecule antagonists of TSHR

Currently, inhibition of TSHR-mediated growth signaling is achieved by pharmacologic suppression of endogenous TSH, with resultant systemic hyperthyroidism. A better system would be a pharmacological antagonist of TSHR, permitting inhibition of thyroid cancer growth while avoiding systemic side-effects that limit current widespread use of pharmacologically-induced hyperthyroidism. Such small molecule antagonists of TSHR have significant parallel interest in the treatment of Graves' disease, where stimulatory autoantibodies against TSHR induce marked hyperthyroidism. Davies and Latif (2015), in their recent appraisal of this issue, identify several molecules that have been trialed over the last decade. To date however, no molecule has been able to achieve sufficient specificity *in vivo* to substantially inhibit TSHR signaling (inhibition of cAMP production in orbital fibroblasts was 50% in one study (Neumann, et al. 2012)).

Areas for future research

The described advances suggest a future horizon of targeted therapies for thyroid cancer, where a patient's individual tumour characteristics, such as the surface expression of proteins, can be exploited for selective drug delivery. However, there remain significant obstacles to final clinical translation of these targeted therapies. Additionally, there are several fundamental questions regarding TSHR targeting in metastatic DTC that remain unanswered. Firstly, although evidence for TSHR expression on DTC metastases is compelling, it is largely indirect. The density of TSHR expression on metastatic lesions is not well studied, and whether TSHR expression would be sufficient for binding of targeted ligands is not known. Secondly, although *TSHR* mRNA and TSHR protein have been detected in a variety of tissues, our knowledge of the functional significance of these extra-thyroidal TSHR is still in its infancy. Indeed, the density of TSHR expression, and whether non-thyroidal TSHR would bind TSHR-targeted therapies in any significant quantity, would need to be carefully studied, especially in the setting of delivery of cytotoxic therapies. Finally, although there is much interest in redifferentiation therapies for thyroid cancer metastases to upregulate NIS expression, little is known about whether TSHR expression could be upregulated in DTC in a similar manner, and whether such therapies may enhance current or future treatments.

Conclusions

Although our understanding of the role of the TSHR as a target in thyroid cancer has progressed significantly since the astute clinical observations of the effects of levothyroxine on PTC in the 1930s, our therapeutic abilities to manipulate this system are largely unchanged over several decades. The era of personalized medicine and targeted therapy has cast new light on the TSHR as a potential theranostic target in metastatic DTC. While promising advances have been made in the last decade, the TSHR invites further study and the possibility of new treatment paradigms for metastastic, radioiodine resistant DTC.

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Figure 1.

Box (25-75%) and whisker (5-95%) plot of *TSHR* mRNA expression by tumour type and differentiation. Redrawn with permission from data in Sheils *et al*. (1999). TSHR mRNA expressed as a ratio of the housekeeping gene GAPDH.

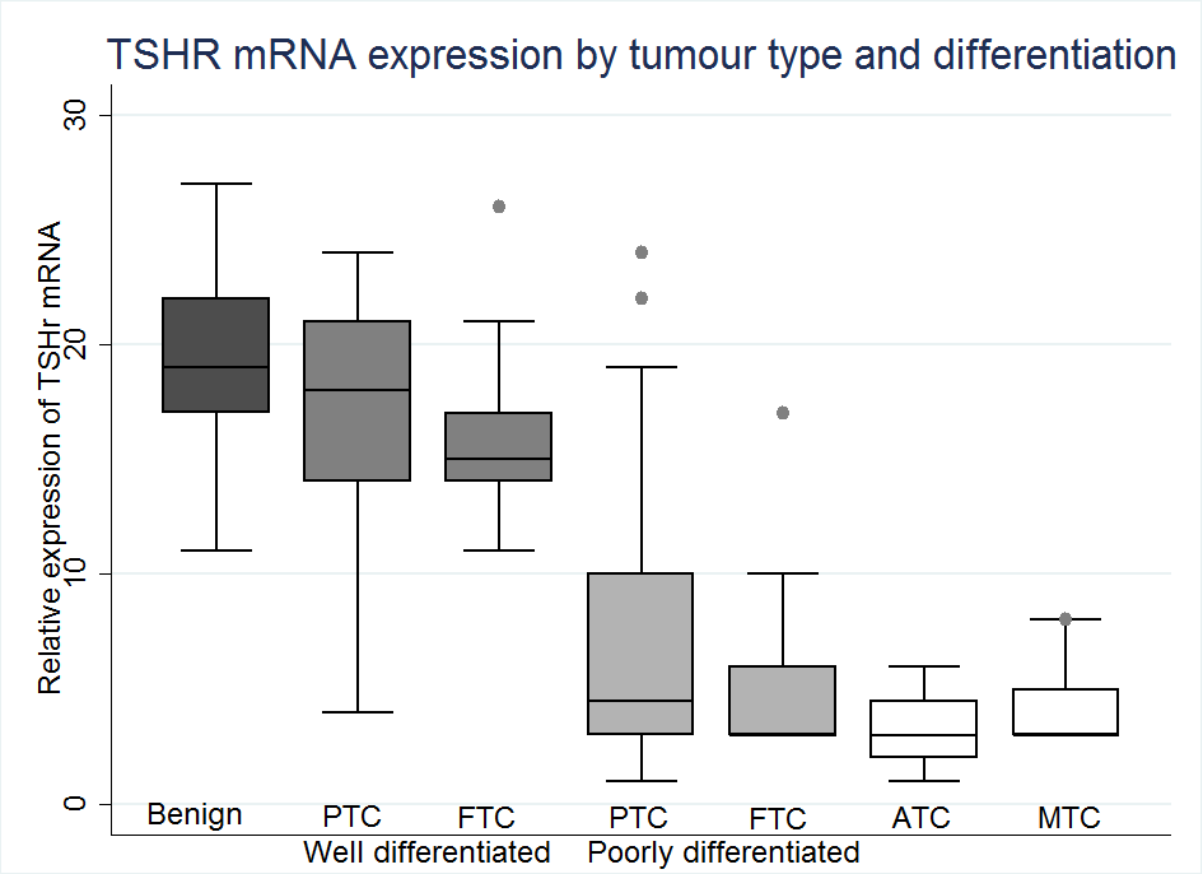
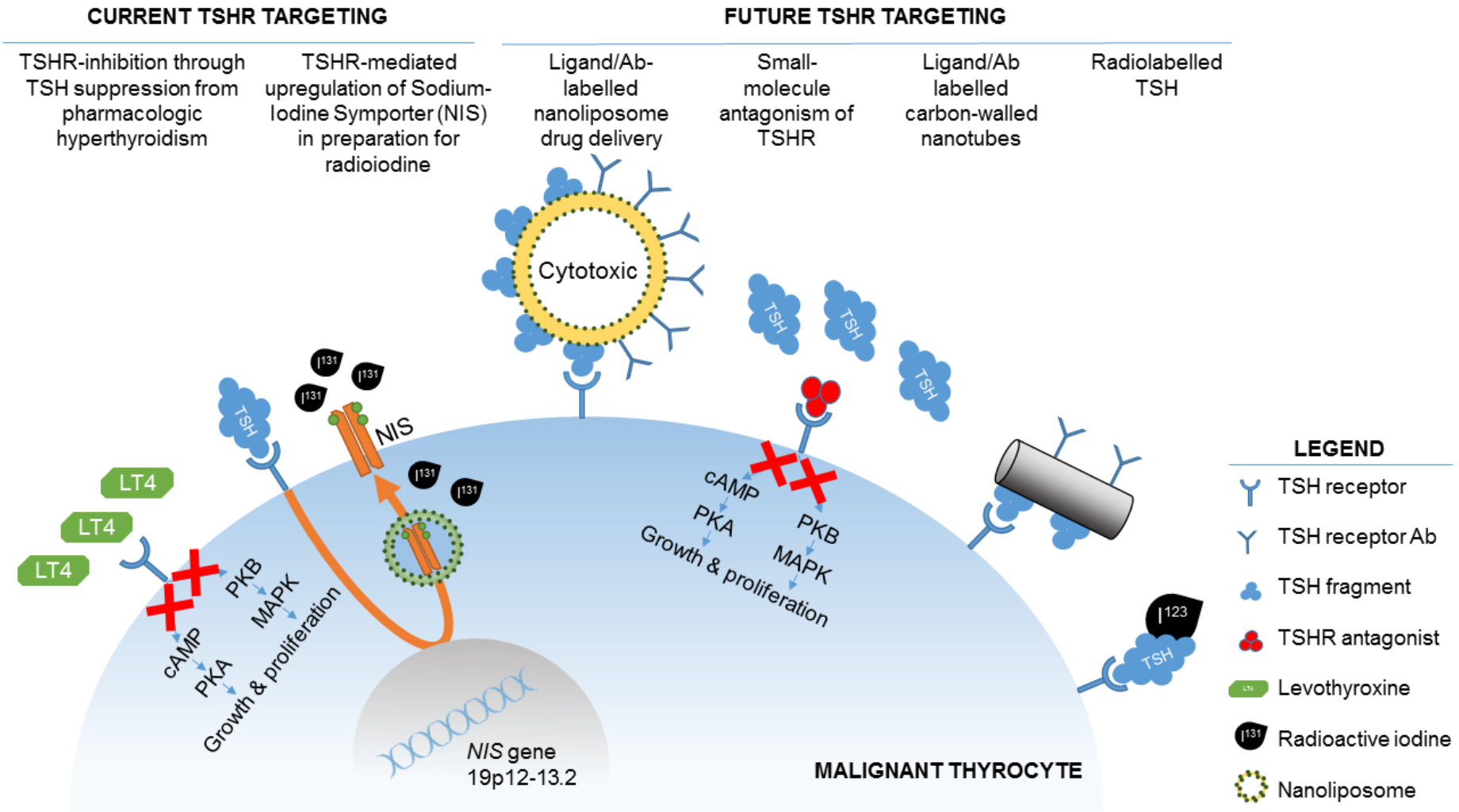


Figure 2: Schematic diagram outlining current and future theranostic targeting of TSHR in DTC.



612 **Table 1. Data from studies of human extra-thyroidal tissue expression of TSHR.**

Tissue	mRNA	Protein*	Functionality#	References
Adipose tissue	Y	Y	Y	Bell, et al. (2000); Murakami, et al. (2001); Gagnon, et al. (2014)
Adrenal	Y	Y	-	Dutton, et al. (1997)
Endometrium	Y	Y	-	Aghajanova, et al. (2011)
Erythrocytes	-	Y	Y	Balzan, et al. (2007)
Extra-ocular muscle, adipocytes	Y	Y	Y	Bahn, et al. (1998); Valyasevi, et al. (1999)
Kidney	Y	Y	Y	Dutton et al. (1997); Sellitti, et al. (2000)
Liver	Y	Y	Y	Zhang, et al. (2009)
Lymphocytes	Y	Y	-	Chabaud and Lissitzky (1977); Coutelier, et al. (1990)
Pituitary	Y	Y	-	Prummel, et al. (2000); Theodoropoulou, et al. (2000)
Hair follicles	Y	-	Y	Bodo, et al. (2009)
Thymus	Y	Y	-	Murakami, et al. (1996); Dutton et al. (1997)
Vascular smooth muscle	Y	Y	Y	Tian, et al. (2014)

*Detected by immunohistochemistry, Western Blot or ligand-binding assays. # Assessed by increased cyclic AMP production / p70 S6 kinase or Na/K ATPase in response to TSH stimulation *in vitro*. Dash: not reported.

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614 **Table 2. Evidence for TSHR expression in primary thyroid tumours and metastases**.

		TSHR expression in primary tumours								TSHR expression in lymph node metastases*							
		PTC		FTC		ATC		MTC		PTC		FTC		ATC		MTC	
Study	**Method**	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Carayon, et al. 1980	Ligand binding	6/6	100%	2/4	50%	0/4	0%	0/4	0%	5/5	100%	6/7	86%	0/1	0%	0/1	0%
Clark, et al. 1983	Ligand binding	10/10	100%	←	←	-	-	-	-	-	-	-	-	-	-	-	-
Brabant, et al. 1991	mRNA	8/8	100%	1/1	100%	1/3	33%	-	-	6/6	100%	-	-	0/1	0%	-	-
Ohta, et al. 1991	mRNA	3/3	100%	4/4	100%	-	-	-	-	-	-	-	-	-	-	-	-
Hoang-Vu, et al.1992	mRNA	19/20	95%	6/8	75%	0/5	0%	-	-	-	-	-	-	-	-		-
Shi, et al. 1993	mRNA	13/20	65%	2/2	100%	1/3	33%	-	-	-	-	-	-	-	-	-	-
Elisei, et al. 1994	mRNA	16/16	100%	2/2	100%	0/2	0%	3/6	50%	4/4	100%	-	-	-	-	0/1	0%
Arturi, et al. 1997	mRNA	-	-	-	-	-	-	-	-	23/23	100%	3/3	100%	-	-	-	-
Lazar, et al. 1999	mRNA	38/38	100%	5/5	100%	-	-	-	-	-	-	-	-	-	-	-	-
Tanaka, et al. 2000	mRNA	31/31	100%	-	-	-	-	-	-	4/4	100%	-	-	-	-	-	-
Park, et al. 2000	mRNA	23/23	100%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wang, et al. 2011	mRNA	31/32	97%	←	←	-	-	-	-	-	-	-	-	-	-	-	-
Wang, et al. 2011	IHC, frozen	32/32	100%	←	←	-	-	-	-	-	-	-	-	-	-	-	-
Tanaka, et al. 1997	IHC, frozen	21/21	100%	2/2	100%	-	-	-	-	-	-	-	-	-	-	-	-
Gerard, et al. 2003	IHC, FFPE	16/16	100%	14/14	100%	-	-	-	-	1/1	100%	2/2	100%	-	-	-	-
Matsumo, et al. 2008	IHC, FFPE	23/23	100%	←	←	0/8	0%	-	-	-	-	-	-	-	-	-	-
So, et al. 2012	IHC, FFPE	18/20	90%	-	-	-	-	-	-	39/52	75%	-	-	-	-	-	-
Lin, et al. 2016	IHC	37/46	80%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Liu, et al. 2016	IHC	102/150	68%	-	-	-	-	-	-	-	-	-	-	-	-	-	-

IHC: Immunohistochemistry. FFPE: Formalin Fixed Paraffin Embedded. Frozen: Frozen tissue. ←: Data for PTC and FTC grouped together. Dash: No Data.

* Data from Elisei *et al*. included 2 local (non-lymph node) recurrences. Location of metastases was not reported by Tanaka *et al.* or Gerard *et al.*

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616 **Table 3: Relative Expression of TSHR in Primary and Metastatic DTC.**

Author	Method	Comparison tissue	Tumoral TSHR expression relative to comparison tissue							
			Increased		Similar		Reduced		Absent	
Primary DTC										
Carayon, et al. 1980	Ligand binding	Normal	1/10	10%	2/10	20%	5/10	50%	2/10	20%
Clark, et al. 1983	Ligand binding	Normal	6/10	60%	2/10	20%	2/10	20%	0/10	0%
Brabant, et al. 1991	mRNA, frozen	Normal	-	-	-	-	5/5	100%	-	-
Shi, et al. 1993	mRNA, frozen	MNG	0/22	0%	4/22	18%	9/22	41%	9/22	41%
Tanaka, et al. 1997	IHC, frozen	Normal	4/21	19%	7/21	33%	10/21	48%	0/21	0%
Sheils, et al. 1999	mRNA, FFPE	Normal	2/76	3%	34/76	45%	40/76	53%	0/76	0%
Matsumo, et al. 2008	IHC, FFPE	Normal	16/23	70%	-	-	7/23	30%	0/23	0%
Wang, et al. 2011	mRNA/IHC, FFPE	Relative	20/32	63%	7/32	22%	-	-	5/32	16%
So, et al. 2012	IHC, FFPE	Relative	7/20	35%	9/20	45%	1/20	5%	2/20	20%
Metastases of DTC										
Carayon, et al. 1980	Ligand binding	Normal	0/14	0%	2/14	14%	9/14	64%	3/14	21%
So, et al. 2012	IHC, FFPE	Primary Tumour	23/52	44%	24/52	46%	5/52	10%	0/52	0%

DTC: Differentiated thyroid cancer. IHC: Immunohistochemistry. FFPE: Formalin Fixed, Paraffin Embedded. Frozen: Frozen tissue. MNG: Multinodular goitre. Normal: Normal thyroid tissue. Dash: no data reported.

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