Pathological processes and therapeutic advances in radioiodide refractory thyroid cancer

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Abstract

While in most patients with non-medullary thyroid cancer (TC), disease remission is achieved by thyroidectomy and ablation of tumor remnants by radioactive iodide (RAI), a substantial subgroup of patients with metastatic disease present tumor lesions that have acquired RAI resistance as a result of dedifferentiation. Although oncogenic mutations in *BRAF*, *TERT* promoter and *TP53* are associated with an increased propensity for induction of dedifferentiation, the role of genetic and epigenetic aberrations and their effects on important intracellular signaling pathways is not yet fully elucidated. Also immune, metabolic, stemness and microRNA pathways have emerged as important determinants of TC dedifferentiation and RAI resistance. These signaling pathways have major clinical implications since their targeting could inhibit TC progression and could enable redifferentiation to restore RAI sensitivity. In this review, we discuss the current insights into the pathological processes conferring dedifferentiation and RAI resistance in TC and elaborate on novel advances in diagnostics and therapy to improve the clinical outcome of RAI-refractory TC patients.

Key Words

- thyroid cancer
- radioiodide resistance
- dedifferentiation
- hNIS
- tumor microenvironment

*Journal of Molecular
Endocrinology*
(2017) **59**, R141–R154

Introduction

Non-medullary thyroid cancer (TC) is the most frequent endocrine tumor accounting for 90% of all endocrine cancer cases. Several histological TC subtypes are distinguished as papillary TC (PTC) as the most frequent, followed by follicular TC (FTC), invasive follicular-variant papillary TC and poorly differentiated TC and anaplastic TC (ATC) as the most rare subtypes (Xing 2013, Fagin & Wells 2016). Patient prognosis is different for these TC subtypes; in most cases, PTC and FTC patients have a favorable prognosis after performing thyroidectomy and effective ablation of (metastatic) tumor remnants that have retained the capacity for iodide uptake and, hence,

displayed high sensitivity to radioactive iodide (RAI) therapy. In contrast, poorly differentiated TC and ATC are often highly progressive, metastatic and resistant to RAI therapy at diagnosis due to loss of thyroid-specific gene expression. For the latter group of patients, treatment options are rarely curative and patients face an increased risk of death. While poorly differentiated TC patients with RAI-refractory tumor lesions have a variable prognosis depending on location and progression of metastases, ATC patients have a poor median survival of 5 months and a 1-year survival chance of less than 20% (Smallridge *et al.* 2012, Cabanillas *et al.* 2016).

Recently, studies into the molecular characteristics of TC have revealed genetic and transcriptomic hallmarks associated with the observed phenotypic differences between TC subtypes and have indicated that poorly differentiated TC and ATC originate from well-differentiated tumors by the accumulation of additional genetic and epigenetic aberrations, resulting in the loss of thyroid-specific gene expression and development of resistance to RAI therapy. At the molecular level, this is corroborated by the Thyroid Differentiation Score (TDS), which consists of expression values of 16 thyroid-specific genes that stratify TC tumors based on their differentiation state. In ATC, the expression of virtually all TDS included genes is diminished, whereas in poorly differentiated TC, the human sodium iodide symporter (hNIS/SLC5A5) expression is specifically reduced and leaving expression of the other genes largely unaffected (Cancer Genome Atlas Research Network 2014, Landa *et al.* 2016, Yoo *et al.* 2016). In addition to genetic and epigenetic events that lead to a diminished expression of thyroid-specific genes, also immunometabolic networks, cancer stem cells (CSCs) and microRNAs (miRNAs) play a critical role in TC dedifferentiation resulting in aggressive and RAI-resistant TC with a poor clinical outcome. In this review, an integrated overview of the current knowledge on the role of these pathways in TC and future prospects for clinical implementation in personalized diagnostics and therapy are presented.

Oncogenic pathways

Genetic factors

It is well established that initial TC development is mostly caused by oncogenic hotspot mutations in the serine/threonine-protein kinase *BRAF*, the intracellular signal transducer *HRAS/KRAS/NRAS* or genetic rearrangements of the tyrosine-protein kinase receptor *RET* proto-oncogene, which has culminated into the general concept of TC development driven by RAS-RAF-MEK-ERK and PI3K-Akt-mTOR oncogenic signaling pathways (Xing 2013, Petrulea *et al.* 2015). In addition, genome-wide association studies have uncovered several germline-encoded genetic variants that predispose to TC development, including the cancer-associated genes *MAGI3*, *SMAD3* and *TERT* (Gudmundsson *et al.* 2012, 2017, Medici *et al.* 2014). Less well defined are the underlying mechanisms contributing to the transition from well differentiated to poorly differentiated TC or ATC. Mutations in *BRAF*, *CTNNB1*, *TP53*, *EIF1AX*, *PTEN*, *PIK3CA*, the SWI/SNF chromatin remodeling complex,

mismatch repair genes and histone methyl transferases are frequently found in specifically poorly differentiated and ATC tumor subtypes, providing diagnostic markers and mechanistic insights into the dedifferentiation process (Garcia-Rostan *et al.* 1999, Hou *et al.* 2007, Santarpia *et al.* 2008, Nikiforov & Nikiforova 2011). Furthermore, *TERT* promoter mutations are associated with more aggressive behavior and with RAI resistance (Melo *et al.* 2014, Landa *et al.* 2016, Liu *et al.* 2016, Yang *et al.* 2017).

Epigenetic factors

Because of their central role in RAI responsiveness, the regulation of hNIS, TSH receptor (TSHR), thyroglobulin (TG) and thyroid peroxidase (TPO) expression has been extensively investigated. Expression of these thyroid-specific genes is predominantly driven by thyroid-specific transcription factors NKX2-1 (TTF-1), FOXE1 (TTF-2), PAX8 and/or HHEX (Fernandez *et al.* 2015). However, despite the occurrence of TC dedifferentiation, the expression of these transcription factors remains largely unaltered, indicating that other intracellular aberrations are responsible for the diminished expression of hNIS, TSHR, TG and TPO. Accordingly, epigenetic mechanisms have been elucidated that influence the functional expression of thyroid-specific genes by modulation of DNA methylation or histone acetylation marks. In this respect, epigenetic modifications at the level of both DNA and histones are important regulators of gene expression; DNA methylation generally leads to gene silencing, whereas histone modifications modulate chromatin accessibility for binding of transcription factors. In previous studies, the role of DNA methylation in TC pathology and progression has been investigated. Several gene promoters and enhancers have been identified of which CpG islands are aberrantly methylated, such as *PTEN*, *RAP1GAP* and *CITED1* (Alvarez-Nunez *et al.* 2006, Xing 2007, Zuo *et al.* 2010, Sassa *et al.* 2011). Furthermore, global DNA hypomethylation and specific DNA hypermethylation of several gene promoters and enhancers, including those driving expressions of hNIS, TSHR and tumor suppressor genes, have been observed in *BRAF* V600E-mutant PTCs and are associated with a silenced expression of the corresponding genes, resulting in less-differentiated tumor cells (Hu *et al.* 2006, Lee *et al.* 2008, Hou *et al.* 2011, Rodriguez-Rodero *et al.* 2013, Choi *et al.* 2014). These epigenetic changes could also lead to a dysregulated expression of pituitary tumor transforming gene (PTTG) and PTTG binding factor, factors that are known to repress hNIS expression, impair RAI uptake and to predict

poor clinical outcome (Read *et al.* 2011, 2017). Moreover, histone deacetylase inhibitors have been demonstrated to elicit beneficial effects on hNIS expression and iodide uptake capacity *in vitro*, which however was not clinically validated (Hou *et al.* 2010, Zhang *et al.* 2014, Cheng *et al.* 2016, Nilubol *et al.* 2017). Despite these compelling findings, the clinical significance of DNA methylation of specific genes and modification of specific gene-associated histones remain to be investigated. Furthermore, since histone deacetylase inhibitors non-selectively influence histone marks of multiple genes, insights into gene-specific histone (de)acetylation effects could provide explanations for the observed differences between *in vitro* models and patient studies and could revive the clinical potential of targeting gene-specific histone modifications.

Transcriptional regulation by miRNAs

miRNAs are a large array of short non-coding oligonucleotides that inhibit the gene expression at a posttranscriptional level by labeling target mRNA sequences for degradation, thereby influencing a large variety of biological pathways including apoptosis, proliferation and differentiation (Bartel 2004). Accumulating evidence indicates that miRNA expression profiles potentially influence the cellular phenotype, both in physiological and pathological processes, and represents a promising therapeutic target for a wide range of malignancies (Rupaimoole & Slack 2017). In TC, differences in miRNA expression profiles are associated with tumor histology and have emerged as potential biomarkers to distinguish between (1) PTC vs normal thyroid tissue (He *et al.* 2005, Pallante *et al.* 2006, Tetzlaff *et al.* 2007), (2) benign follicular adenoma versus malignant FTC (Rossing *et al.* 2012) and (3) classical FTC vs oncocytic-variant FTC (Nikiforova *et al.* 2008). Predominantly, the miRNAs miR-146b, miR-181b, miR-221, miR-222 and miR-224 are highly upregulated in PTC as compared to normal thyroid tissue (Agretti *et al.* 2012, Dettmer *et al.* 2013, Swierniak *et al.* 2013, Acibucu *et al.* 2014). In contrast, predictive miRNA biomarkers for FTC are upregulation of miR-181 and miR-200 and downregulation of miR-199 (Mancikova *et al.* 2015). Different miRNA expression profiles have been observed in ATC, with elevated expressions of miR-17, miR-221, miR-222 and decreased let-7, miR-30 and miR-29 expressions as compared to normal thyroid tissue (Mancikova *et al.* 2015). Moreover, the differential expression of some of these and other miRNAs is associated with TC aggressiveness, epithelial–mesenchymal transition, vascular invasion,

lymph node dissemination, risk of recurrence and dedifferentiation, including increased expression of miR-9, miR-21 and miR-146b, HMGB1-induced miR-221 and miR-222 upregulation and miR-204 downregulation (Braun *et al.* 2010, Mardente *et al.* 2012, Chou *et al.* 2013, Cancer Genome Atlas Research Network 2014, Guo *et al.* 2015, Sondermann *et al.* 2015). By specifically downregulating hNIS expression, miR-339 and miR-146b are identified to promote TC dedifferentiation and RAI resistance (Lakshmanan *et al.* 2015, Riesco-Eizaguirre *et al.* 2015). Accordingly, inhibition of these miRNAs by specific inhibitors results in improved RAI sensitivity (Lakshmanan *et al.* 2015, Li *et al.* 2015, Riesco-Eizaguirre *et al.* 2015). All together, miRNA dysregulation in TC provides mechanistic insights into TC development and progression and represents potential targets for improving TC diagnostics, prognosis and therapy.

In Fig. 1, a comprehensive overview of activated oncogenic pathways is provided which is involved in tumorigenesis, progression and RAI resistance of TC driven by genetic and epigenetic aberrations and by dysregulated expression of miRNAs and their target genes.

Immunometabolic networks

Despite the identification of genetic, epigenetic and miRNA aberrations that predispose to TC dedifferentiation and RAI refractoriness, still extensive heterogeneity exists within tumor subgroups regarding differentiation status and clinical RAI response. Hence, these factors only partially explain the occurrence of TC dedifferentiation and provide limited sensitivity and specificity to predict RAI responsiveness and clinical outcome. Other mechanisms involved in this phenomenon include the interaction of TC with its tumor microenvironment (TME). Within the TME of TC, tumor cells interact with immune cell infiltrates and orchestrate immune responses favoring tumor proliferation, dedifferentiation and progression (Galdiero *et al.* 2016, French *et al.* 2017). Some of the most abundant immune cells in TC are tumor-associated macrophages (TAMs). While in well-differentiated TC, only few TAMs are present, advanced TC tumors are heavily infiltrated by TAMs, which proportionally increases along the spectrum of dedifferentiation. Hence, the intratumoral density of TAMs is associated with poor prognosis (Ryder *et al.* 2008, Kim *et al.* 2013, 2016). Furthermore, inhibition of intratumoral TAM recruitment is shown to ameliorate TC progression, indicating the important functional role of TAMs in promoting TC aggressiveness (Ryder *et al.* 2013). Recently, by exploring the functional characteristics

Figure 1

Schematic overview of activated oncogenic pathways involved in tumorigenesis, progression and radioactive iodide resistance in non-medullary thyroid cancer driven by genetic and epigenetic aberrations and by dysregulated expression of microRNAs and their target genes. Genetic aberrations comprise point mutations and rearrangements in oncogenes and tumor suppressor genes and induce malignant transformation and/or dedifferentiation. Epigenetic aberrations include silencing of cancer-associated and thyroid-specific genes by DNA methylation and by modulation of histone acetylation or methylation of H3K4, H3K9 or H3K27 and other lysine residues of histones. Expression of several microRNAs is dysregulated in advanced thyroid cancer (upregulated microRNAs in green, downregulated microRNAs in red), leading to reciprocally regulated expression of their target genes. MMR, mismatch repair genes; HMTs, histone methyltransferases.

of TAMs, it was observed that the glycolytic metabolite lactate is an important driver of the TAM pro-tumorigenic phenotype (Colegio *et al.* 2014, Arts *et al.* 2016). Lactate is a pleiotropic factor influencing many cancer-associated pathways of immune evasion, angiogenesis, invasiveness and dissemination (San-Millan & Brooks 2017). Lactate concentrations are highly elevated in TC tumors because of aerobic glycolysis as a predominant metabolic pathway inside tumor cells. Aerobic glycolysis, also designated as the Warburg effect, has been established as a central and generic hallmark of malignant cells during proliferation, although under normoxic conditions, oxidative phosphorylation is reduced and, alternatively, cancer cells favor the oxygen-independent glycolysis pathway for fast generation of ATP and building blocks while reducing production of reactive oxygen species (Gatenby & Gillies 2004, Hanahan & Weinberg 2011, Lunt & Vander Heiden 2011). Aerobic glycolysis is a specific feature of malignant cells and facilitates the activation of Hypoxia-Inducible Factor 1 α (HIF-1 α) signaling, tumor growth, invasiveness and immune evasion in aggressive TC subtypes with lactate and HMGB1 as major intermediate factors (Koperek *et al.* 2013, Lee *et al.* 2015, Mardente *et al.* 2015, Gill *et al.* 2016). In turn, the HIF-1 α activity in tumor cells induces production and signaling of vascular endothelial growth factor (VEGF), facilitating angiogenesis and activation of effector mechanisms further promoting invasion, metastatic potential and dedifferentiation of TC (de Araujo-Filho *et al.* 2009, Karaca *et al.* 2011, Galdiero *et al.* 2016).

In addition, also other immunomodulatory factors are demonstrated to be involved in aggressiveness and RAI sensitivity of TC. One of these factors is the NF- κ B pathway, of which genetic variation in the NF- κ B inhibitor alpha (NFKBIA) gene is associated with decreased sensitivity to RAI therapy and with elevated production of interleukin (IL)-1 β (Plantinga *et al.* 2017). Interestingly, IL-1 β has been demonstrated to decrease hNIS and TG expression in thyrocytes and TC cell lines (Yamashita *et al.* 1989, Kimura *et al.* 1992, Ohta *et al.* 1996). Another pro-inflammatory protein involved in TC dedifferentiation is IL-32, which activates pro-survival pathways driven by IL-8 (Heinhuis *et al.* 2016). Accordingly, inhibition of IL-8 signaling results in decreased survival and proliferation of TC tumor cells *in vitro* and *in vivo* (Liotti *et al.* 2017). Furthermore, genetic variation in IL32, leading to an elevated expression of the IL-32 γ isoform and increased production of the pro-inflammatory cytokines TNF α , IL-1 β and IL-6, contributes to susceptibility to TC development and RAI resistance (Plantinga *et al.* 2013).

These findings corroborate the biological connection between the role of aerobic glycolysis and HIF-1 α signaling in TC tumor cells on the one hand with infiltration of macrophages that consequently acquire pro-tumorigenic features on the other. Based on this extensive body of evidence, the interplay between tumor cells and TAMs has emerged as an important mechanism in RAI resistance and poor clinical outcome of TC patients, as illustrated in Fig. 2 (Scarpino *et al.* 2004, Ryder *et al.* 2008,

Figure 2

Schematic overview of interconnected immunometabolic pathways elicited in tumor cells and tumor-associated macrophages (TAMs) in the tumor microenvironment of radioiodide refractory thyroid cancer that collectively promote tumor initiation, progression and dedifferentiation. Soluble factors produced by either tumor cells and/or TAMs activate intracellular pathways that contribute to proliferation, angiogenesis, pro-tumorigenic inflammation, immune evasion and epithelial–mesenchymal transition of non-medullary thyroid cancer.

Burrows *et al.* 2010, Klaus *et al.* 2017). Future studies are warranted to identify appropriate targets for therapy to counteract the pro-tumorigenic intercellular interactions between tumor cells and TAMs.

It is also well established that several other specialized immune cell types are present in TC tumors, including dendritic cells, myeloid-derived suppressor cells, natural killer cells and regulatory T-cells that express inhibitory immune checkpoints such as Programmed cell death protein 1 (PD-1) or Programmed death-ligand 1 (PD-L1) (extensively reviewed in French *et al.* (2017)). Although elevated numbers of these cell types are observed either in the TME or in peripheral blood of TC patients, their clinical significance and their contribution to TC dedifferentiation and RAI resistance still remain unexplored.

Autophagy

Another mechanism involved in TC dedifferentiation is autophagy, an intracellular degradation machinery influencing survival, proliferation and differentiation pathways (Bincoletto *et al.* 2013, Netea-Maier *et al.* 2015). Autophagy is a central process downstream of both PI3K-Akt-mTOR and BRAF-RAS-MEK-ERK oncogenic signaling and is therefore strongly implicated in thyroid carcinogenesis, proliferation and dedifferentiation. In physiology, autophagy facilitates intracellular degradation of cytoplasmic components, including organelles and large protein complexes, in order to maintain cellular homeostasis and to generate energy and building blocks for protein synthesis (Mizushima & Komatsu 2011, Feng *et al.* 2015). In cancer, autophagy is known as a double-edged sword with both cancer-promoting and cancer-inhibiting features. By its role as a survival mechanism

in hypoxic, nutrient-deprived or otherwise threatening conditions, autophagy promotes resistance to chemo- and radiotherapy. In contrast, beneficial effects of autophagy-driven survival are rendered by potent inhibition of cell proliferation, thereby blocking tumor growth (Gewirtz 2014, Duffy *et al.* 2015, Kumar *et al.* 2015). In addition to effects on survival and proliferation, autophagy is also involved in cellular differentiation (Mizushima & Levine 2010, Helgason *et al.* 2013). In TC specifically, autophagy has been shown to intertwine with oncogenic signaling and *in vitro* activation of autophagy is demonstrated to increase sensitivity of TC to treatment with chemotherapy, radiotherapy, TRAIL and mTOR kinase inhibitors (Lin *et al.* 2010, Jin *et al.* 2014, Morani *et al.* 2014, Yi *et al.* 2014, Fan *et al.* 2015, Plews *et al.* 2015) and to promote resistance to BRAF inhibitors (Wang *et al.* 2017). Furthermore, germline-genetic variants in autophagy genes, specifically *ATG5* (rs2245214) and *ATG16L1* p.Thr300Ala (rs2241880), known to functionally impair the autophagy machinery, are associated with genetic susceptibility to TC development and/or resistance to RAI treatment (Huijbers *et al.* 2012, Plantinga *et al.* 2014b). Accordingly, loss of autophagy activity was demonstrated to be associated with RAI resistance in TC patients (Plantinga *et al.* 2016). These findings indicate the important role of autophagy in the regulation of TC proliferation and differentiation.

Epithelial-mesenchymal transition (EMT)

One of the mechanisms that enables epithelial cell-derived tumor cells to invade other tissues and to establish lymphatic or hematogenous metastases is EMT. By this process, tumor cells gain mesenchymal properties including enhanced migration, invasiveness and elevated

resistance to anoikis and apoptosis and display augmented production of extracellular matrix components (Vasko *et al.* 2007, Kalluri & Weinberg 2009). EMT occurring in TC and other epithelial cell-derived tumors, designated as type III EMT, is considered to be elicited by similar genetic and epigenetic changes that also drive primary tumor development (Knauf *et al.* 2011, Baquero *et al.* 2013, Mitchell *et al.* 2016, Anelli *et al.* 2017). In addition, EMT is often accompanied by dedifferentiation and gain of stem cell properties, representing another cause of acquired RAI resistance of metastatic TC lesions in lymph nodes and at distal locations (Riesco-Eizaguirre *et al.* 2009, Liu & Brown 2010, Hardin *et al.* 2013, Lan *et al.* 2013). Once tumor cells have achieved colonization at the metastatic location, tumor cells reverse EMT by mesenchymal–epithelial transition (MET), explaining the resemblance of morphological characteristics shared with the primary tumor. Despite the transition of metastatic tumor lesions to the original tumor cell phenotype by MET, the loss of differentiation markers is considered irreversible resulting in RAI-refractory metastases. Molecular pathways driving EMT in TC involve zinc finger E-box binding homeobox 1 (ZEB1), Smad7 and Slug and loss of E-cadherin expression is shown to predominantly occur in metastatic PTC and ATC tissues (Montemayor-Garcia *et al.* 2013, Wang *et al.* 2013, 2014, Guo *et al.* 2014, Jung *et al.* 2015, Li *et al.* 2016). Important growth factors promoting EMT in TC are transforming growth factor β (TGF- β) and platelet-derived growth factor (PDGF), which are demonstrated to activate the expression of vimentin, Slug, Snail, Twist, PRRX1, High Mobility Group A1 (HMGA1), HMGB1 and the stem cell marker SOX9 and to inhibit E-cadherin and hNIS, leading to enhanced dedifferentiation and increased *in vivo* tumor growth and spread (Costamagna *et al.* 2004, Mardente *et al.* 2012, Hardin *et al.* 2014, Baquero *et al.* 2016, Ekpe-Adewuyi *et al.* 2016, Lv *et al.* 2016, Huang & Guo 2017, Zhong *et al.* 2017). HIF-1 α signaling and inflammatory mediators are demonstrated to evoke the expression of EMT markers in TC (Lv *et al.* 2015, Yang *et al.* 2015, Klaus *et al.* 2017). Moreover, previous studies indicate that abrogation of autophagy in TC is an important mechanism contributing to EMT induction, which is counteracted by activation of the AMPK-autophagy pathway (Han *et al.* 2015, Cazarin *et al.* 2016, Gugnoni *et al.* 2017). In addition to EMT occurring in tumor cells intrinsically, surrounding cells in the TME also contribute to EMT induction in TC. Tumor-infiltrating mast cells have been demonstrated to promote TC aggressiveness by increasing proliferation, invasiveness and vascularization as a result of EMT induction by signaling through IL-8,

Akt and Slug (Melillo *et al.* 2010, Visciano *et al.* 2015). All together, these findings illustrate an interconnected signaling network of pathways driving angiogenesis, tumor cell survival, proliferation, dedifferentiation and EMT, thereby evoking invasion, dissemination and RAI resistance of TC tumors (illustrated in Fig. 2).

CSCs

The thyroid gland is capable of regenerating itself, at least to some extent, which is thought to be facilitated by stem or progenitor cells present inside the adult gland located in a niche designated as solid cell nests (Coclet *et al.* 1989, Gibelli *et al.* 2009). Thyroid stem cells are characterized by the expression of p63, Oct-4, hNanog, CD44 and SCA-1 and are able to differentiate into fully functional thyroid follicular cells (Hoshi *et al.* 2007, Lan *et al.* 2007, Klonisch *et al.* 2009, Ahn *et al.* 2014). Also, the expression of the stem cell marker aldehyde dehydrogenase together with enhanced cell proliferation and invasion is observed in small populations of tumor cells in PTC, FTC and ATC (Todaro *et al.* 2010). Therefore, an alternative hypothesis of the etiology of poorly differentiated TC and ATC, opposed to gradual loss of differentiation in pre-existing PTC or FTC by accumulation of additional genetic aberrations, is malignant transformation of thyroid stem or precursor cells into CSCs (Takano & Amino 2005, Zhang *et al.* 2006, Takano 2014). In this scenario, specifically thyroid stem or precursor cells acquire genetic and epigenetic aberrations and initiate tumor development. This hypothesis has been especially postulated as a potential origin of ATC. However, this could also explain the occurrence of poorly differentiated TC originating from thyroid precursor cells, including thyroblasts or prothyrocytes (Hoshi *et al.* 2007, Lan *et al.* 2007, Fagman & Nilsson 2011, Zane *et al.* 2016). This is supported by the striking resemblance between thyroid-specific gene expression profiles of well-differentiated TC, poorly differentiated TC and ATC on the one hand and end-stage differentiated thyroid follicles, thyroid precursor and thyroid progenitor cells on the other. Fully differentiated follicular cells and well-differentiated TC exhibit the physiological expression of TG, TPO and hNIS, whereas thyroid stem cells, poorly differentiated TC and ATC are all characterized by the lack of end-stage differentiation markers (Takano 2014, Landa *et al.* 2016). To improve diagnostics and therapy for TC patients with tumors driven by CSCs, validation of specific biomarkers and further insights into the pathological mechanisms of TC development, progression and RAI resistance elicited by thyroid CSCs are warranted.

Emerging treatments for RAI-refractory TC

Since RAI-refractory TC also displays diminished susceptibility to other conventional anticancer treatments, i.e. chemo- and radiotherapy, treatment options are limited (Pacini *et al.* 2012, De Bernardi *et al.* 2014, Romesser *et al.* 2014). Over the last decades, adjunctive therapies have been investigated with the purpose to restore RAI sensitivity in poorly differentiated TC, termed as redifferentiation. Initially, these were vitamin A derivatives (retinoic acid, bexarotene), PPAR γ activators (thiazolidinediones), inhibitors of iodide release (lithium) and histone deacetylase inhibitors (valproic acid, depsipeptide, trichostatin A), which, however, were minimally effective in clinical trials (Liu *et al.* 2006, 2008, Kim *et al.* 2009, Rosenbaum-Krumme *et al.* 2012a,b, Nilubol *et al.* 2017). More recently, kinase inhibitors have been demonstrated to be more effective, with inhibitors of BRAF and MEK kinases in combination with RAI treatment reaching temporarily improved clinical responses in 40–60% of RAI-refractory TC patients. Furthermore, two other targets have been identified to induce effective redifferentiation *in vitro*: inhibition of the mTOR kinase and activation of autophagy and cFOS by Na⁺/K⁺ ATPase inhibitors (Plantinga *et al.* 2014a, Tesselaar *et al.* 2017). The currently available evidence of these emerging redifferentiation therapies is summarized in Table 1.

MEK inhibition

For over a decade, MEK kinase inhibitors have been regarded as an important target for treating RAI-resistant TC. Numerous *in vitro* and *in vivo* mouse studies have shown the capacity of these agents to inhibit tumor progression and to augment thyroid-specific gene expression (Fenton *et al.* 2010, Henderson *et al.* 2010, Chakravarty *et al.* 2011, Nagarajah *et al.* 2016). In the clinical setting, the MEK inhibitor selumetinib was observed to profoundly increase RAI avidity in metastatic poorly differentiated TC, resulting in increased iodide accumulation in target

lesions in 12 out of 20 patients and reaching a clinical response rate in eight of these patients (Ho *et al.* 2013). Recently, a phase III clinical trial (ClinicalTrials.gov number, NCT01843062) is initiated to validate these beneficial effects of adjunctive selumetinib treatment in a larger cohort of RAI-refractory TC patients.

BRAF inhibition

The V600E hotspot mutation in the *BRAF* oncogene has been well established as an oncogenic driver of PTC. As a targeted treatment, small molecules have been developed and experimentally tested that specifically inhibit the V600E-mutated BRAF kinase, including sorafenib, vemurafenib and dabrafenib (Liu *et al.* 2012). Currently, sorafenib (simultaneously inhibiting V600E mutant BRAF, VEGFR and PDGFR) and lenvatinib (multiple kinase inhibitor of VEGFR, FGFR, PDGFR, c-Kit and RET) are approved for treatment of RAI-refractory TC patients (Brose *et al.* 2014). Despite its specificity for V600E mutant *BRAF*, sorafenib appears equally effective for treatment of advanced *BRAF* wild-type PTC, questioning the contribution of V600E mutant BRAF inhibition to the observed clinical effects of sorafenib. In another clinical trial, benefits of vemurafenib treatment for RAI-refractory TC patients were studied, which was shown to establish partial responses in 38.5% of the patients without administration of additional RAI treatments (Brose *et al.* 2016). In addition, the redifferentiation potential of the BRAF V600E inhibitors vemurafenib and dabrafenib has been assessed in RAI-refractory TC patients. Strikingly, out of ten treated patients with dabrafenib in combination with RAI, six patients exhibited increased intratumoral iodide accumulation resulting in either partial response or stable disease (Rothenberg *et al.* 2015). Another study reported one single PTC patient with metastatic disease that reached a complete response by treatment with vemurafenib followed by RAI (Huillard *et al.* 2016). In contrast, no increased iodide avidity was reached with sorafenib treatment (Hoftijzer *et al.* 2009). The potential

Table 1 Overview of currently available evidence on emerging non-medullary thyroid cancer redifferentiation therapies.

Therapeutic target	Drug name	Preclinical data		Clinical response rates			
		<i>In vitro</i>	<i>In vivo</i>	PD (%)	SD (%)	PR (%)	CR (%)
MEK	Selumetinib	X	X	0	37.5	62.5	0
BRAF	Dabrafenib	X	X	10	70	20	0
	Vemurafenib	X	X	Single case study, CR was reached			
mTOR	Everolimus	X	–	–	–	–	–
Autophagy	Digoxin	X	–	–	–	–	–

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

of lenvatinib to establish TC redifferentiation in RAI-refractory TC patients has not been investigated so far.

Notably, not all RAI-refractory TC patients benefit from the above-mentioned adjunctive treatments for improving RAI response, indicating that between patients, clinically significant phenotypic differences exist that are decisive for treatment response. Additional efforts are therefore warranted to characterize tumor-associated factors that counteract or disable TC redifferentiation in order to further optimize the clinical efficacy of these targeted therapies.

mTOR inhibition

The mTOR (mammalian target of rapamycin) kinase is an important factor in oncogenic signaling downstream of PI3K and Akt, one of the pathways heavily implicated in TC pathogenesis (Netea-Maier *et al.* 2015, Petrulea *et al.* 2015). Inhibitors of mTOR have been developed that are demonstrated to ameliorate tumor growth in a number of malignancies. In TC specifically, the mTOR inhibitor rapamycin was observed to restore hNIS expression and iodide uptake capacity *in vitro* in TC cell lines (Plantinga *et al.* 2014a). Clinically, treatment with the mTOR inhibitor everolimus or temsirolimus, alone or in combination with sorafenib, has been shown to result in stable disease in about 50% of the TC patients with advanced disease and, rarely, in partial responses without reaching complete responses (Lim *et al.* 2013, Wagle *et al.* 2014, Schneider *et al.* 2017, Sherman *et al.* 2017). Unfortunately, a potentially increased RAI avidity induced in patient tumors by treatment with mTOR inhibitors has not been investigated in these trials.

Autophagy activation

The functional role of autophagy in RAI resistance was investigated by assessing the capacity to increase RAI sensitivity by treatment with autophagy-activating drugs. By testing five different classes of autophagy activators, it was demonstrated that Na⁺/K⁺ ATPase inhibitors such as digoxin, also designated as digitalis-like compounds or cardiac glycosides, are capable of restoring hNIS expression and iodide uptake in TC cell lines (Tesselaar *et al.* 2017). Interestingly, in other cancer types, these agents have been shown to inhibit other mechanisms that are also involved in TC aggressiveness and RAI resistance, including aerobic glycolysis, TERT activity, EMT, angiogenesis and growth factor signaling, indicating its multifaceted effects as promising anticancer treatment (Prassas & Diamandis 2008).

Future studies in mouse models and TC patients are required for assessing the clinical utility of digoxin and its analogs as adjunctive treatment to improve RAI sensitivity in TC.

Concluding remarks

Although non-medullary TC patients generally have a good prognosis and a high propensity to reach long-term remission, high-quality diagnostics and effective treatment options for patients with metastatic RAI-refractory TC are still lacking leading to high morbidity and mortality rates in this subset of patients. Recent advances in understanding the underlying mechanisms of TC dedifferentiation and RAI resistance have generated the required insights into the identification of novel clinically relevant biomarkers and therapeutic targets, especially MEK, BRAF and mTOR kinases and autophagy that harbor the promising potential to achieve effective patient-tailored diagnosis and therapy of RAI-refractory TC.

Declaration of interest

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

Funding

T S Plantinga was supported by a Veni grant of the Netherlands Organization for Scientific Research (NWO; grant number 016.136.065) and by the Alpe d'HuZes fund of the Dutch Cancer Society (grant number KUN2014-6728). R T Netea-Maier was supported by a grant from the Dutch Cancer Society (grant number #10559).

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Received in final form 12 September 2017

Accepted 20 September 2017

Accepted Preprint published online 20 September 2017