REVIEW

Targeting autophagy in thyroid cancers

WeiJun Wei^{1,2}, Heather Hardin³ and Quan-Yong Luo¹¹Department of Nuclear Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China²Shanghai Jiao Tong University School of Medicine, Shanghai, China³Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USACorrespondence should be addressed to Q-Y Luo: luoqy@sjtu.edu.cn

Abstract

Thyroid cancer is one of the most common endocrine malignancies. Although the prognosis for the majority of thyroid cancers is relatively good, patients with metastatic, radioiodine-refractory or anaplastic thyroid cancers have an unfavorable outcome. With the gradual understanding of the oncogenic events in thyroid cancers, molecularly targeted therapy using tyrosine kinase inhibitors (TKIs) is greatly changing the therapeutic landscape of radioiodine-refractory differentiated thyroid cancers (RR-DTCs), but intrinsic and acquired drug resistance, as well as adverse effects, may limit their clinical efficacy and use. In this setting, development of synergistic treatment options is of clinical significance, which may enhance the therapeutic effect of current TKIs and further overcome the resultant drug resistance. Autophagy is a critical cellular process involved not only in protecting cells and organisms from stressors but also in the maintenance and development of various kinds of cancers. Substantial studies have explored the complex role of autophagy in thyroid cancers. Specifically, autophagy plays important roles in mediating the drug resistance of small-molecular therapeutics, in regulating the dedifferentiation process of thyroid cancers and also in affecting the treatment outcome of radioiodine therapy. Exploring how autophagy intertwines in the development and dedifferentiation process of thyroid cancers is essential, which will enable a more profound understanding of the physiopathology of thyroid cancers. More importantly, these advances may fuel future development of autophagy-targeted therapeutic strategies for patients with thyroid cancers. Herein, we summarize the most recent evidence uncovering the role of autophagy in thyroid cancers and highlight future research perspectives in this regard.

Key Words

- ▶ thyroid cancer
- ▶ autophagy
- ▶ radioiodine
- ▶ molecularly targeted therapy
- ▶ RR-DTC

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Introduction

Thyroid cancer is the fifth most common cancer in women in the United States and is the most commonly diagnosed cancer before the age of 30 years among women in China (Chen *et al.* 2016). Although thyroid cancer is generally considered as an indolent tumor, it has a broad range of clinical behaviors ranging from an indolent tumor under the umbrella term of differentiated thyroid cancer (DTC) to more aggressive types (i.e., anaplastic thyroid

cancer (ATC) and medullary thyroid cancer (MTC)). DTC accounts for more than 95% of thyroid cancers and contains two major subtypes (i.e., papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC)). While patients with lower risk diseases or benign thyroid nodules should not be overtreated, those with advanced diseases should be given more aggressive and efficacious treatments. Currently, a combination of surgery, radioactive iodine

(RAI, ^{131}I) and subsequent TSH suppression therapy is the mainstay of therapy for most DTC patients (Wei *et al.* 2018b). However, due to tumor dedifferentiation and concomitant loss of sodium/iodide symporter (NIS), certain thyroid cancer patients are insensitive or resistant to RAI therapy (Song *et al.* 2015). Although rare, ATC is the most aggressive form of thyroid cancers and is a clinical challenge owing to its pleomorphic histopathological features and rapid progression (Ragazzi *et al.* 2014, Keutgen *et al.* 2015, Tiedje *et al.* 2018).

The MAPK oncogenic pathway is closely involved in the proliferation, invasion and differentiation status of thyroid cancers (Fig. 1; Xing 2013). BRAF (v-raf murine sarcoma viral oncogene homolog B) is one of the well-characterized members of the MAPK pathway and somatic BRAF mutation, especially BRAF^{V600E} mutation,

occurs in up to 50% of PTCs and 45% of ATCs (Xing 2013, Landa *et al.* 2016, Molinaro *et al.* 2017, Sandulache *et al.* 2017). As such, molecularly targeted therapy suppressing oncogenic proteins like BRAF^{V600E} has increasingly been used for patients with radioiodine-refractory differentiated thyroid cancer (RR-DTC). Currently, vemurafenib and dabrafenib are two selective BRAF^{V600E} inhibitors under clinical investigation for DTC patients harboring BRAF^{V600E} mutation. In addition, two multi-kinase inhibitors with antiangiogenic properties, sorafenib and lenvatinib, have been licensed for treating RR-DTC and showed advantages in terms of progression-free survival. However, drug resistance and adverse effects associated with the single-agent treatment often lead to termination of the targeted therapy (Shen *et al.* 2014). Furthermore, the impact of these TKIs on overall survival of patients with RR-DTC

Figure 1

The MAPK and PI3K oncogenic signaling pathways are the main signaling cascades implicated in the thyroid cancer initiation, progression and dedifferentiation. The genetic mutation in the *BRAF* gene most commonly results in amino acid transformation at the 600 locus in the BRAF protein. The mutated BRAF (i.e., BRAF^{V600E}) has enzymatic function and transmits messages by phosphorylating downstream protein MEK. BRAF^{V600E} mutation in the thyroid may lead to significant reduction of proteins responsible for radioiodine uptake in both mouse models and in humans. Inhibitors blocking either mutated BRAF and/or MEK restore RAI uptake and prolong retention of radioiodine in the thyroid cancer cells. In addition to the MAPK signaling cascade, the PI3K pathway is also involved in the initiation and progression of thyroid cancers. PTEN switches off PI3K signaling by dephosphorylating PIP3 and therefore is a tumor suppressor. ALK, anaplastic lymphoma kinase; BRAF, rapidly accelerated fibrosarcoma type-B or v-raf murine sarcoma viral oncogene homolog B; MAPK, mitogen-activated protein kinase; NTRK, neurotrophic receptor tyrosine kinase; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10; RAS, rat sarcoma; RET, rearranged during transfection; RTK, receptor tyrosine kinase. A full color version of this figure is available at <https://doi.org/10.1530/ERC-18-0502>.

has not been proven yet. Meanwhile, redifferentiation of RR-DTC emerges as a very promising approach to restore NIS expression and to enable ^{131}I therapy (Ho *et al.* 2013, Rothenberg *et al.* 2015, Dunn *et al.* 2018). Nonetheless, novel strategies that can enhance the efficacy of TKIs and/or overcome resistance of TKIs are in urgent need to refine the clinical management of RR-DTCs and ATCs.

Macroautophagy (hereafter referred to as autophagy) is a process that captures and degrades intracellular components in lysosomes, thereby sustaining metabolic homeostasis and promoting the growth of a broad spectrum of cancers under certain conditions. Autophagy not only occurs upon nutrient deprivation to supply cells with building blocks but also occurs under nutrient-rich conditions to mediate the removal of superfluous or damaged organelles and potentially toxic protein aggregates (White *et al.* 2015, Klionsky *et al.* 2016). Mitophagy is a selective form of autophagy that mediates the removal of mitochondria. Loss of mitochondrial membrane potential ($\Delta\Psi\text{m}$) and mitochondrial fragmentation generally precede mitophagy (Youle & Narendra 2011). The role of autophagy in sustaining metabolism and in promoting tumorigenesis has been widely investigated in multiple types of cancers (Guo *et al.* 2013, Strohecker *et al.* 2013, Karsli-Uzunbas *et al.* 2014, Yang *et al.* 2018). Although the detailed role of autophagy in the pathogenesis of thyroid cancer is not yet elucidated (Netea-Maier *et al.* 2015), recent studies shed light on the underlying mechanisms of autophagy in regulating the development and dedifferentiation of thyroid cancers (Plantinga *et al.* 2016, Tesselaar *et al.* 2017a, Tesselaar *et al.* 2018). Besides, dysregulated autophagy is closely involved in the resistance of small-molecule drugs (e.g., vemurafenib) in thyroid cancers (Wang *et al.* 2017, Wei *et al.* 2017). We herein review the potential role of autophagy in mediating the initiation, development and progression of thyroid cancers and highlight how autophagy interferes with the dedifferentiation process of thyroid cancers. Insights from the current evidence may provide the rationale for manipulating autophagy activity in treating thyroid cancers and in redifferentiating RR-DTC either as a standalone method or in combination with clinically available TKIs.

Autophagy in initiation and development of thyroid cancers

It is conceivable that both autophagy and mitophagy are linked to the initiation and development of thyroid cancers. Several major genomic mutations and/or rearrangements accounting for the occurrence of thyroid

cancers may directly or indirectly regulate the activity of autophagy in thyroid cancers. Additionally, somatic mutations/deletions of mitochondrial DNA and alteration of mitochondrial biogenesis are associated with the carcinogenesis of a portion of Hürthle cell tumors, which are characterized by the cytoplasmic accumulation of abundant mitochondria (Maximo *et al.* 2012). As a specific type of autophagy dealing with redundant mitochondria (Youle & Narendra 2011), mitophagy plays an important role in the evolution of Hürthle cell tumors (Yi *et al.* 2017).

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor but its function is disabled in several human cancers. Although endogenous expression of HRAS^{G12V} alone is insufficient to drive thyroid tumorigenesis (Chen *et al.* 2009), and thyroid-specific knock-in of BRAF^{V600E} induces mostly benign lesions (Franco *et al.* 2011), loss of p53 or concomitant loss of function of PTEN and p53 could induce initiation and progression of ATC (Antico Arciuch *et al.* 2011, McFadden *et al.* 2014). In addition, PTEN is a critical regulator in the development and progression of FTC and follicular adenomas (Yeager *et al.* 2007, Guigon *et al.* 2009). PTEN is also closely associated with Cowden syndrome, an autosomal dominant disorder that predisposes to female breast cancer, thyroid cancer and other cancers (Zbuk & Eng 2007). PTEN-induced putative kinase 1 (PINK1)-Parkin pathway involves maintaining mitochondrial integrity and functions through triggering the mitophagy of abnormal mitochondria (Park *et al.* 2006, Grenier *et al.* 2013). Lee and colleagues found that Hürthle tumor cells had intact non-selective autophagy but ineffective mitophagy activity, which therefore resulted in decreased turnover of aberrant mitochondria and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP)-induced cell death (Lee *et al.* 2015). The authors further reported that expression of WT Parkin sensitized Hürthle cell lines to CCCP-induced cell death (Lee *et al.* 2015). Recent studies also revealed the role of autophagy in regulating the dynamics of ciliogenesis (Pampliega *et al.* 2013, Satir & Pampliega 2016). Interestingly, although PTCs have long cilia, Hürthle cells found either in benign nodules or in malignant thyroid diseases were devoid of primary cilia (Lee *et al.* 2016). Loss of cilia seemed to be a characteristic feature of Hürthle cells and was associated with high levels of autophagosome biogenesis, and inhibition of autophagy restored ciliogenesis in the Hürthle cell lines (Lee *et al.* 2016).

Succinate dehydrogenase (SDH) is a unique membrane-bound enzyme that plays dual functions in both the Krebs cycle for succinate oxidation and the

respiratory chain for electron transfer to the terminal acceptor ubiquinone. SDH contains two catalytic subunits (SDHA and SDHB) and two structural anchors (SDHC and SDHD). Generally, genes encoding the four subunits of SDH are believed to be tumor suppressor genes (Gimm *et al.* 2000). A recent study elucidated that germline SDH variants, SDHD-G12S and SDHD-H50R, may result in the oxidation and loss of function of PTEN (oxidized PTEN) in FTC cell lines (Yu *et al.* 2015). The same team further elaborately found that both normal thyroid follicles and PTC cells expressing SDHD-G12S or -H50R variants grew faster than SDHD-WT cells (Yu *et al.* 2017). Furthermore, SDHD-G12S or -H50R variants suppressed autophagy in normal thyroid follicles and PTC cells by downregulating the expression of FOXO3a target genes (*BECN1* and *ATG12* in TPC-1 cells and *BECN1* in Nthy-Ori-3 cells) in a PTEN-dependent manner (Yu *et al.* 2017). In line with previously proposed models (Netea-Maier *et al.* 2015), these results together highlight the suppressive role of autophagy in the initiation of thyroid cancer or in the early phases of its development.

Coatomer protein complex I (COP1) complex members are involved in autophagy in several cancer types including in prostate, breast and ovarian carcinoma cells (Razi *et al.* 2009, Shtutman *et al.* 2011, Claerhout *et al.* 2012). By screening with a siRNA oligonucleotide library in thyroid cancer cells (BCPAP) and normal human thyrocytes (Nthy-ori 3-1), Anania *et al.* initially found that Cyclin D1, MASTL and COPZ1 (coatomer protein complex ζ 1) are three oncogenes for thyroid cancer, and inhibition of these three genes reduced the viability of several thyroid tumor cell lines, regardless of the histotype or oncogenic lesion (Anania *et al.* 2015). Further study showed that COPZ1 silencing was associated with significantly increased LC3 puncta and conversion of the endogenous LC3-I to LC3-II in TPC-1 and 8505C cells (Anania *et al.* 2017). Actually, COPZ1 depletion caused abortive autophagy as an increase of SQSTM1/p62 in both COPZ1-depleted cell lines was observed. More importantly, COPZ1 depletion induced apoptotic cell death and suppressed tumor growth in 8505C ATC xenografts. These results indicated that downregulation of COPZ1 and concomitant disruption of autophagy may be an attractive approach for treating thyroid cancers.

Epithelial-to-mesenchymal transition (EMT) is associated with the development and invasion of thyroid cancers, especially PTC and ATC (Hardy *et al.* 2007, Hardin *et al.* 2017). EMT and autophagy are two distinct events but a cross-talk exists between these two events in a controversial way (Marcucci *et al.* 2017).

Cadherin-6 (CDH6), a type 2 cadherin, is a target of transforming growth factor- β (TGF- β) signaling pathway and therefore is a marker of EMT in thyroid cancers (Sancisi *et al.* 2013). More recently, Guignoni *et al.* reported that silencing of CDH6 expression not only profoundly attenuated EMT features but also induced LC3 and GABARAP lipidation in PTC cells, which was accompanied by a decreased level of p-AKT. In parallel, CDH6 is a potential marker for predicting distant metastases from PTC (Guignoni *et al.* 2017). These results together potentially indicate the role of CDH6 in mediating PTC progression and the underlying interaction of EMT and autophagy behind this phenotype.

One typical character of DTC is the consistent female-to-male ratio of 3:1 across almost all geographic areas and ethnicities; one of the potential factors accounting for the gender disparity is estrogen receptors (Manole *et al.* 2001). A recent study has elucidated that estrogen could induce autophagy in PTC in an estrogen receptor α dependent manner, which could, therefore, provide energy and building blocks (Fan *et al.* 2015). By binding to nuclear receptors (TRs, including TR α and TR β), thyroid hormone is a potent regulator of cellular and tissue metabolism throughout the body. Thyroid hormone, especially T₃, has been shown to exert profound effects on mitochondrial biogenesis and energy metabolism (Weitzel & Iwen 2011, Sinha *et al.* 2015). T₃ induced autophagic flux in hepatocytes and inhibited HBV-encoded X protein (HBx)-induced hepatic carcinogenesis in mice (Chi *et al.* 2017). However, thyroid hormone-related autophagy in the development of thyroid cancers remains to be determined.

Autophagy in molecularly targeted therapy of thyroid cancers

As aforementioned, numerous genetic alterations including BRAF^{V600E} mutation are involved in the tumorigenesis of thyroid cancers (Cabanillas *et al.* 2016, Fagin *et al.* 2016). In clinical trials, BRAF^{V600E} inhibitors have achieved preliminary success in the treatment of PTCs (Kim *et al.* 2013, Dadu *et al.* 2015), and the antitumor activity of vemurafenib with a partial response rate of 38.5% for patients with BRAF^{V600E}-positive PTC was observed in a recent phase 2 clinical trial (Brose *et al.* 2016). Molecularly targeted therapies also emerge as the standard of care for ATC patients either with metastatic or radiation-resistant diseases (Hanna *et al.* 2018, Iyer *et al.* 2018, Subbiah *et al.* 2018). However, there is increasing evidence that thyroid cancer patients undergoing TKI

treatment may ultimately escape the effects of therapy and then develop resistance (Kreissl *et al.* 2019).

A recent study has revealed that higher expression levels of all autophagy-related proteins were observed in BRAF^{V600E} mutation-positive PTCs compared to those without the BRAF^{V600E} mutation (Kim *et al.* 2017). Similarly, BRAF or RAS mutant thyroid cancer cell lines tend to have a higher level of autophagy following drug treatment (Bikas *et al.* 2015, Plews *et al.* 2015). Wei *et al.* recently found that following vemurafenib treatment, mitochondrial respiration and ATP production in thyroid cancer cells (K1 cells) increased significantly (Wei *et al.* 2017), indicating increased mitochondrial metabolism as a potential mechanism for vemurafenib resistance. Obatoclastax, an experimental Bcl2 inhibitor, could successfully overcome vemurafenib-induced resistance by reducing ATP production and suppressing the activity of autophagy in thyroid cancer cells (Wei *et al.* 2017). Champa *et al.* further found that obatoclastax induced necrosis of thyroid cancer cells by localizing to the lysosomes, inducing loss of acidification and blocking lysosomal fusion with autophagic vacuoles (Champa *et al.* 2016). Hydroxychloroquine (HCQ), a derivative of chloroquine (CQ), can inhibit lysosomal acidification and prevent the degradation of autophagosomes (Manic *et al.* 2014), thereby suppressing autophagy. Both CQ and HCQ are currently under clinical trials to inhibit autophagy (Levy *et al.* 2017). Wang *et al.* found that vemurafenib induced a high level of autophagy in BRAF-mutation-positive thyroid cancer cells and inhibition of autophagy by either HCQ or knockdown of essential autophagy genes augmented the efficacy of vemurafenib (Wang *et al.* 2017). In this study, the authors revealed that vemurafenib-induced autophagy was independent of the MAPK signaling pathway but was associated with endoplasmic reticulum (ER) stress response activation. These results indicate that autophagy-inhibiting strategies are thus predicted to inhibit mitochondrial metabolism or to enhance the cytotoxic effect of vemurafenib.

From current evidence, it seems that autophagy plays a double role in mediating the anti-thyroid cancer effect of sorafenib (Lin *et al.* 2012, Yi *et al.* 2018). Different from sorafenib and lenvatinib which have multiple targets, apatinib is another TKI selectively targeting vascular endothelial growth factor receptor 2 (VEGFR-2). Two recent studies have reported the potent therapeutic efficacy of apatinib in treating RR-DTCs (Lin *et al.* 2017, Zhang *et al.* 2018). Despite the exciting disease control rate (100%) and objective response rate (70.0%) for the 500mg qd schedule, the frequent and severe adverse

events may limit its consecutive clinical use (Zhang *et al.* 2018). In a preclinical setting, Feng *et al.* reported that inhibition of apatinib-induced autophagy using CQ led to increased apoptosis of ATC cells both *in vitro* and *in vivo* (Feng *et al.* 2018), indicating the superior therapeutic effect of the combinational treatment. Upon further clinical investigation, this strategy may serve as a promising treatment option for both RR-DTC and ATC, which may enhance the efficacy of apatinib while alleviating the toxicities caused by high-dose apatinib monotherapy.

It is notable that several natural compounds are capable of suppressing thyroid cancers, such as mulberry anthocyanins (Long *et al.* 2018) and flavokawain B (He *et al.* 2018). Flavokawain B-induced autophagy plays a pro-survival role in several malignancies including thyroid cancers (He *et al.* 2018, Wang *et al.* 2018a). Consequently, combinational administration of flavokawain B and CQ resulted in more profound inhibition of tumor growth in FTC models (He *et al.* 2018). Honokiol is another bioactive natural compound obtained from *Magnolia officinalis* (Prasad & Katiyar 2016). Recently, Lu *et al.* showed that honokiol treatment could suppress cell growth, induce cell cycle arrest, enhance the induction of both caspase-dependent and -independent apoptosis, and autophagy in ARO and SW579 cell lines. But honokiol treatment did not induce autophagic flux in WRO cells. The study also revealed that honokiol could affect the regulation of MAPK signaling pathways in the ATC, FTC and PDTC cells (Lu *et al.* 2017). However, a recent study indicated that the ARO cell line matches HT-29 colon cancer cell line, which was probably caused by laboratory cross-contamination (Schweppe *et al.* 2008). Therefore, key experiments using contaminated ATC cell lines might have to be repeated with better characterized ATC cell lines (Lu *et al.* 2017, He *et al.* 2018).

In recent years, accumulating evidence has indicated the potential role of miRNAs and lncRNAs as theranostic targets in thyroid cancers (Wei *et al.* 2016, Zhang *et al.* 2016). Specifically, miR-125b is downregulated in the majority of follicular thyroid carcinomas and adenomas (Wang *et al.* 2018b). Overexpression of miR-125b sensitized thyroid cancer cells to cisplatin and sorafenib. Moreover, overexpression of miR-125b enhanced the therapeutic effect of cisplatin in *in vivo* studies, which was partially mediated by downregulation of Foxp3 and the resultant increased activity of autophagy induced by stimulated Atg7 (Wang *et al.* 2018b). Similarly, overexpression of lncRNA GAS8-AS1 could inhibit the proliferation of PTC cells and induce autophagy simultaneously, and silencing of ATG5 rescued such inhibitory effect caused by

lncRNA GAS8-AS1 (Qin *et al.* 2018). In contrast, miR-30d negatively regulated cisplatin-activated autophagic response by downregulating BECN1, as a result, sensitizing ATC cells to cisplatin treatment both *in vitro* and *in vivo* (Zhang *et al.* 2014). Considering these promising yet controversial results, we would like to advocate the use of comprehensive detection and intervention methods when examining the role of autophagy in mediating the treatment outcome of small-molecular therapeutics in thyroid cancers (Wei *et al.* 2018a).

Autophagy in redifferentiation of radioiodine-refractory thyroid cancers

Findings from a large TCGA study showed that PTCs with BRAF^{V600E} mutation were associated with a less differentiated state, for instance, loss of NIS expression (Cancer Genome Atlas Research Network 2014). It is gratifying that selective MEK inhibitor selumetinib and BRAF inhibitor dabrafenib showed redifferentiation effects and enhanced ¹³¹I uptake in patients with RR-DTC (Ho *et al.* 2013, Rothenberg *et al.* 2015). More recently, Huillard and colleagues investigated the ability of vemurafenib in inducing tumor redifferentiation and in restoring RAI uptake in an 83-year-old man with BRAF^{V600E}-mutant RR-DTC and found that vemurafenib and dabrafenib could significantly enhance RAI uptake and reduce ¹⁸F-FDG uptake (Huillard *et al.* 2017). The redifferentiation effect of vemurafenib was further validated by a most recent study (Dunn *et al.* 2018), in which four of the ten evaluable RR-DTC patients responded to vemurafenib treatment likely by upregulating thyroid-specific gene expression. In a preclinical study, Chakravarty *et al.* reported the efficacy of MAPK pathway inhibitors in restoring RAI uptake in mice (Chakravarty *et al.* 2011). However, another preclinical study reported the discordance of MEK inhibition and BRAF inhibition in inducing iodine uptake in ATC models (ElMokh *et al.* 2018).

More recently, several studies elucidated the potential role of autophagy in mediating the dedifferentiation of DTC and the clinical efficacy of ¹³¹I therapy. Clinically, Plantinga *et al.* initially showed that genetic variations in ATG16L1 (300Ala (G) allele of ATG16L1) and in ATG5 (ATG5 single nucleotide polymorphisms rs2245214) were associated with a higher probability to develop thyroid carcinoma (Huijbers *et al.* 2012, Plantinga *et al.* 2014). The same group then found that although LC3-I intensity and LC3-II-positive puncta were not associated

with thyroid cancer pathology except for a small group of ATC patients, both indexes were associated with clinical response to ¹³¹I therapy. More importantly, while membranous SLC5A5 (NIS) expression was decreased in tumors with low autophagy activity, the expression of SLC5A5 was strongly elevated in tumors with high numbers of LC3-II-positive puncta, indicating autophagy has an important impact on the dedifferentiation of thyroid cancers (Plantinga *et al.* 2016).

As we all know, digitalis-like compounds are well-characterized regimens in the treatment of heart failure and atrial arrhythmia (Prassas & Diamandis 2008). Tesselaar *et al.* found that digitalis-like autophagy-activating compounds, digoxin in particular, restored NIS expression and iodide uptake in thyroid cancer cell lines, and a follow-up study revealed that upregulation of NIS was mediated by intracellular Ca²⁺ and FOS activation (Tesselaar *et al.* 2017a). Based on this preliminary evidence that digitalis-like compounds had beneficial effects in redifferentiating RR-DTC, Tesselaar *et al.* also investigated this effect using ATC cell lines (Tesselaar *et al.* 2018). The authors observed reduced autophagy activity in ATC tissues and found that while several TKIs (e.g., selumetinib) failed to restore NIS expression, most of the tested digitalis-like compounds (e.g., digoxin) were able to induce NIS at both mRNA and protein levels in the two tested ATC cell lines (Cal-62 and 8505C), which was further accompanied by increased ¹²⁵I uptake (Tesselaar *et al.* 2018).

These results together demonstrate that the activity of autophagy may serve as a marker to predict the therapeutic response of ¹³¹I treatment. Furthermore, investigation of the role of autophagy in the dedifferentiation process of thyroid cancers could potentially facilitate development of novel therapeutics to treat and to redifferentiate RR-DTCs and ATCs. Considering that digitalis-like compounds have been used in clinic for many years, they, therefore, represent promising alternates for patients with RR-DTC or ATC.

Autophagy in radiosensitization of thyroid cancers

External beam radiotherapy (EBRT) or intensity-modulated radiotherapy (IMRT) has an established role in treating ATC (Brierley 2011). Although the role of EBRT in locally advanced DTCs is controversial, a recent study elucidated that the addition of EBRT to RAI resulted in good disease control for locally advanced DTCs (Tam *et al.* 2017).

It has been reported that utilization of autophagy inhibitors could increase the radiosensitivity of several types of cancers to radiation therapy (Apel *et al.* 2008, Liang *et al.* 2015, Ondrej *et al.* 2016). However, autophagy activators, rather than inhibitors, may potentially enhance the therapeutic effects of anticancer agents which promote, or at least are compatible with, tumor-targeting immune responses (Galluzzi *et al.* 2017a). Ko *et al.* also reported that radiotherapy-induced anticancer immune response was autophagy-dependent in the immunocompetent context or, in other words, autophagy conferred immunogenic properties to tumors following radiotherapy (Ko *et al.* 2014). Consistent with this, inhibition of autophagy, either local or systemic, may trigger detrimental effects in patients with cancer since autophagy is pivotal for establishing CD8⁺ T cell memory and sustaining the development and survival of lymphocytes (Pua *et al.* 2007, Puleston *et al.* 2014). In this setting, further studies are needed to validate whether

there is any synergistic benefit by adding modulators of autophagy to radiotherapy in treating patients with advanced thyroid cancers (especially ATC).

Conclusion and future research perspectives

The tumorigenesis of the thyroid is a complex process, which is regulated by multiple cellular events including activation of oncogenes, inactivation of tumor suppressors and changes in programmed cell death pathways (Yi *et al.* 2017). The RAS-RAF-MEK-ERK and PI3K-AKT pathways are the best understood oncogenic cascades orchestrating the proliferation and differentiation of thyroid cancers (Xing 2013, Fagin *et al.* 2016). In the meantime, both signaling pathways are strongly implicated in the regulation of autophagy activity (Morani *et al.* 2014, Tesselaar *et al.* 2017b). One of the key mechanisms of how autophagy

Table 1 Summary of representative autophagy inhibitors used in preclinical and clinical studies.

Agent	Target	Tumor types	Status	References
Chloroquine (CQ)	Lysosome	Glioblastoma, brain Metastases, etc.	Clinical trials	Levy <i>et al.</i> (2017)
SBI-0206965	ULK1	Lung cancer, colorectal cancer	Preclinical	Egan <i>et al.</i> (2015)
ULK-101	ULK1	Lung cancer	Preclinical	Martin <i>et al.</i> (2018)
Hydroxychloroquine (HCQ)	Lysosome	Colorectal cancer, breast cancer, pancreatic cancer, etc.	Clinical trials	Levy <i>et al.</i> (2017), Onorati <i>et al.</i> (2018)
Quinacrine	Lysosome	Lung cancer, osteosarcoma	Clinical trials	Wang <i>et al.</i> (2015), Yan <i>et al.</i> (2018)
3-methyladenine (3-MA)	Beclin/vps34 complex	Prostate cancer, leukemia, etc.	Preclinical	Lamoureux <i>et al.</i> (2013), Missiroli <i>et al.</i> (2016)
Wortmannin	Beclin/vps34 complex	Breast cancer, melanoma, etc.	Preclinical	Abedin <i>et al.</i> (2007), Lin <i>et al.</i> (2014)
Spautin-1	Beclin/vps34 complex	Ovarian cancer, breast cancer, etc.	Preclinical	Liu <i>et al.</i> (2011), Yeo <i>et al.</i> (2018)
SAR405	vps34	Renal cancer, head and neck cancer, etc.	Preclinical	Ronan <i>et al.</i> (2014), New <i>et al.</i> (2017)
Autophinib	vps34	Breast cancer	Preclinical	Robke <i>et al.</i> (2017)
PIK-III	vps34	Pancreatic cancer, etc.	Preclinical	Dowdle <i>et al.</i> (2014)
UAMC-2526	ATG4	Colorectal cancer	Preclinical	Kurdi <i>et al.</i> (2017)
Autophagin-1	ATG4	Not reported	Preclinical	Qiu <i>et al.</i> (2016)
NSC185058	ATG4	Osteosarcoma, glioblastoma	Preclinical	Akin <i>et al.</i> (2014), Huang <i>et al.</i> (2017)
Lys05	Lysosome	Melanoma, ovarian cancer	Preclinical	McAfee <i>et al.</i> (2012), Ma <i>et al.</i> (2014)
DQ661	Lysosome	Melanoma, pancreatic cancer, and colorectal cancer	Preclinical	Rebecca <i>et al.</i> (2017), Nicastrì <i>et al.</i> (2018)
ROC-325	Lysosome	Renal cancer	Preclinical	Carew <i>et al.</i> (2017), Carew & Nawrocki (2017)
VATG-027	Lysosome	Melanoma	Preclinical	Goodall <i>et al.</i> (2014)
VATG-032	Lysosome	Melanoma	Preclinical	Goodall <i>et al.</i> (2014)
Mefloquine	Lysosome	Breast cancer, etc.	Preclinical	Sharma <i>et al.</i> (2012)
Verteporfin	Lysosome	Breast cancer, etc.	Preclinical	Donohue <i>et al.</i> (2011)
Bafilomycin A1 (BafA1)	Lysosome, endoplasmic reticulum	Thyroid cancer, etc.	Preclinical	Mauvezin <i>et al.</i> (2015)

promotes the growth and survival of thyroid cancers may lie in its ability to support cellular metabolism and mitochondrial bioenergetics (White *et al.* 2015, Yu *et al.* 2018). Mitochondria have long been associated with tumorigenesis by optimizing cancer cell environment and by mediating apoptosis (Wallace 2012). However, the mechanisms by which genetic events, such as BRAF^{V600E} mutation, regulate autophagy-related metabolism are poorly understood in thyroid cancers (Yi *et al.* 2017). In addition, the cross-talk between autophagy and apoptosis in thyroid cancers still remains to be explored (Nikoletopoulou *et al.* 2013).

As growth defects from autophagy inhibition of transplanted tumors in immunocompromised mice tend to be less impressive than that observed in autochthonous models (Eng *et al.* 2016), thyroid cancer models with a thyroid-specific endogenous expression of oncogenes may be better when examining the effects of autophagy activity on thyroid tumorigenesis (Vanden Borre *et al.* 2014). Although recent findings have indicated the regulatory role of autophagy in the dedifferentiation and concomitant development of RR-DTC, the redifferentiative effect of these autophagy-activating compounds (digitalis-like compounds and cardiac glycosides) in oncogene-induced authenticated murine thyroid cancer models, or in patient-derived tumor xenografts (Marlow *et al.* 2018), is largely unknown. Moreover, assessing the redifferentiative effect of these clinically available agents in a clinical scenario is needed to validate preclinical observations. Apart from these clinically available agents, novel chemical compounds (Galluzzi *et al.* 2017b, Chiang *et al.* 2018), or autophagy-inducing peptides (Peraro *et al.* 2017), could also be used to pharmacologically stimulate autophagy. Since BRAF and MEK inhibitors have been validated as effective in restoring radioiodine incorporation in clinical trials (Ho *et al.* 2013, Rothenberg *et al.* 2015, Huillard *et al.* 2017, Dunn *et al.* 2018), future studies may also explore if there is a synergistic effect in redifferentiating RR-DTC when a combination of molecularly targeted agent with autophagy activator is applied. Previous seminal studies have demonstrated that ¹²⁴Iodine-NaI PET could detect more lesions than ¹³¹I single photon emission computed tomography (SPECT), and the former imaging modality also allowed for quantitative dosimetry (Larson *et al.* 2017). Therefore, future studies may use ¹²⁴Iodine-NaI PET to assess the redifferentiative effect and to optimize subsequent RAI therapy.

Although there are studies reporting autophagic cell death in mediating the anti-thyroid cancer effects of several small-molecular therapeutics (Lin *et al.* 2010,

Jin *et al.* 2014, Xiang *et al.* 2018), future studies are still needed to validate such observations in thyroid cancers. When it comes to inhibiting autophagy for treating thyroid cancers, several promising inhibitors targeting the components of the autophagy pathway have been reported, including ULK1 inhibitors (Egan *et al.* 2015, Martin *et al.* 2018), Vps34 inhibitors (Liu *et al.* 2011, Ronan *et al.* 2014, Robke *et al.* 2017), ATG4B inhibitor (Vezenkov *et al.* 2015), lysosome inhibitors (McAfee *et al.* 2012, Fu *et al.* 2014, Kroemer & Galluzzi 2017, Rebecca *et al.* 2017) and CQ/HCQ (Table 1). For clinical trials, HCQ is chosen over CQ because of its relatively less toxicity than CQ at peak concentrations (Barnard *et al.* 2014, Manic *et al.* 2014). Several clinical trials involving HCQ in combination regimens or as a single-agent trial have been published (Amaravadi *et al.* 2016). Inhibition of autophagy could overcome vemurafenib resistance in several solid tumors including in thyroid cancers (Mulcahy Levy *et al.* 2017, Wang *et al.* 2017). In addition, human tumors generally possess elevated rates of basal autophagy activity, which are further correlated with poor outcome (Amaravadi *et al.* 2011, Lazova *et al.* 2012, Kim *et al.* 2017). Therefore, it is rational to design strategies inhibiting both autophagy and BRAF to enhance the efficacy of BRAF inhibitors and to overcome monotherapy-induced drug resistance in thyroid cancers. One promising future direction is to execute prospective clinical trials to assess the treatment efficacy of highly specific autophagy inhibitors, either alone or in combination with molecularly targeted agents (Mulcahy Levy *et al.* 2017).

Declaration of interest

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

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