

Autophagy in endocrine tumors

Andrea Weckman¹, Fabio Rotondo², Antonio Di Ieva¹, Luis V Syro³, Henriett Butz², Michael D Cusimano¹ and Kalman Kovacs²

¹Division of Neurosurgery, Department of Surgery, and ²Division of Pathology, Department of Laboratory Medicine, St Michael's Hospital, 30 Bond Street, Toronto, Ontario, M5B 1W8 Canada

³Department of Neurosurgery, Hospital Pablo Tobon Uribe and Clínica Medellín, Medellín, Colombia

Correspondence should be addressed to F Rotondo
Email
rotondf@smh.ca

Abstract

Autophagy is an important intracellular process involving the degradation of cytoplasmic components. It is involved in both physiological and pathological conditions, including cancer. The role of autophagy in cancer is described as a 'double-edged sword,' a term that reflects its known participation in tumor suppression, tumor survival and tumor cell proliferation. Available research regarding autophagy in endocrine cancer supports this concept. Autophagy shows promise as a novel therapeutic target in different types of endocrine cancer, inhibiting or increasing treatment efficacy in a context- and cell-type-dependent manner. At present, however, there is very little research concerning autophagy in endocrine tumors. No research was reported connecting autophagy to some of the tumors of the endocrine glands such as the pancreas and ovary. This review aims to elucidate the roles of autophagy in different types of endocrine cancer and highlight the need for increased research in the field.

Key Words

- ▶ autophagy
- ▶ autophagy modulation
- ▶ endocrine disease
- ▶ neoplasia
- ▶ therapeutic potential

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Introduction

The word 'autophagy' derives from the Greek roots 'auto,' or self, and 'phagy,' to eat (Levine & Klionsky 2004) – in other words, self-cannibalism or self-eating. Autophagy is a genetically programmed and evolutionarily conserved intracellular process. Different types of autophagy, such as macroautophagy, microautophagy and chaperone-mediated autophagy, have been described, each with different specific functions and slightly different mechanisms. All types of autophagy, however, share the end result of lysosome-mediated degradation of cytoplasmic components (Weckman *et al.* 2014). The ubiquitous cellular process of autophagy has been explored in a wide array of different contexts, including both physiological and pathological conditions. More recently, autophagy has been implicated in the pathogenesis of cancer and is commonly referred to as a 'double-edged sword' for its role

in both tumor progression and tumor suppression (White & DiPaola 2009).

Relative to other types of neoplasia, endocrine tumors occur more rarely (Table 1). Endocrine tumors are defined as neoplasia of the hormone-secreting cells of the classic endocrine glands, including the pituitary, thyroid, parathyroid and adrenal glands, as well as the ovaries and testes. The apparent rareness of endocrine tumors is partly due to the fact that many pituitary, adrenal, parathyroid and thyroid tumors remain undiagnosed (Nicholson 2008). However, since more and more evidence is emerging on the importance of autophagy in cancer, its role must be investigated in endocrine cancers as well. Our paper aims to review the function of autophagy in oncogenesis and consolidate existing research regarding the role of autophagy in endocrine tumors.

Table 1 Epidemiology of endocrine cancers. The inconsistency in format and relative lack of epidemiological data reflect the rarity of endocrine neoplasms and resulting lack of motivation to study them

Tumor type	Epidemiological data
Pituitary	10–15% of intracranial neoplasms ^a
Adrenal	Adrenal cortical adenomas: incidence unknown ^a Adrenal cortical carcinomas: 1/million per year ^a Pheochromocytomas: 2–9/million per year ^b
Thyroid	Most common endocrine neoplasm: 1% of all cancer ^a 0.8–5.0/100 000 per year (male) ^a 1.9–19.4/100 000 per year (female) ^a
Parathyroid	Primary hyperparathyroidism: 17.7 cases/million per year ^a Parathyroid carcinoma: 0.005% of all cancers ^b
Endocrine pancreas	1–2% of pancreatic neoplasms ^a 4–12/million per year ^b
Endocrine ovary	1% of ovarian cancers ^c
Endocrine testis	1% of testicular cancers ^c

^aDeLellis (2004).

^bSturgeon (2009).

^cRies *et al.* (2007).

Autophagy

The discovery of the involvement of autophagy in a wide array of both physiological and pathological conditions such as cardiac, pulmonary and liver diseases, neurodegeneration, infection, immunity and cancer (Shintani & Klionsky 2004, Levine & Kroemer 2008, Mizushima *et al.* 2008, Choi *et al.* 2013) has led to a recent increase in interest in the multifaceted role of autophagy in humans. Consequently, the precise mechanisms and molecular players in mammalian autophagy have been extensively reviewed (Levine & Klionsky 2004, Mizushima 2007, Yang & Klionsky 2010, Tanida 2011) (Fig. 1A). The autophagic process, most commonly stimulated by nutrient deprivation (Mizushima 2007), initiates with the engulfment of specific cytoplasmic components by a phagophore or isolation membrane to form a double-membraned autophagosome. The autophagosome proceeds to fuse with a lysosome in order to expose its contents to the lysosomal degradative enzymes and break down the engulfed cytoplasmic constituents.

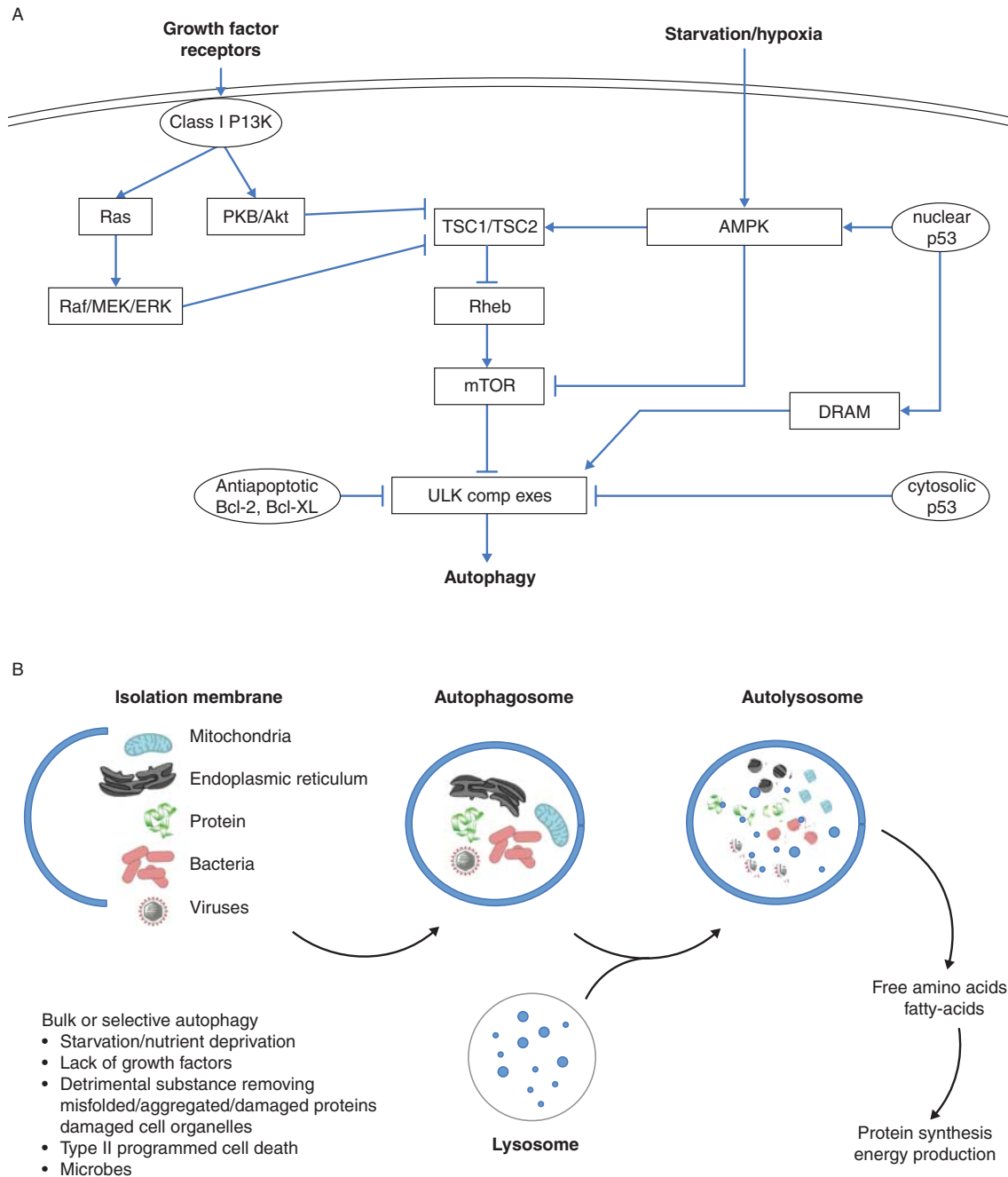
Autophagy has various physiological functions. Under normal conditions, autophagy plays a cellular ‘housekeeping’ role, responsible for removing detrimental substances such as long-lived, misfolded, aggregated or

otherwise damaged proteins and organelles. During periods of metabolic stress (i.e., nutrient deprivation, hypoxic conditions and/or lack of growth factors), autophagy breaks down proteins into the basic amino acids essential to survival. These amino acids are then used in various crucial processes such as synthesizing proteins important for the cell to adapt to stress, or the Krebs cycle to produce ATP for cellular energy (Mizushima 2007, Levine & Kroemer 2008) (Fig. 1B). Autophagy has also been implicated in protecting the genome from genetic instability and DNA mutations that ultimately result in tumor development (Mathew *et al.* 2007a, Levine & Kroemer 2008). Paradoxically to its cell-survival-oriented functions, autophagy is also involved in type II programmed cell death. Type II programmed cell death is distinguishable from type I programmed cell death (apoptosis) partly by the accretion of autophagic vacuoles, as well as the use of endogenous lysosomal enzymes for degradation of the dying cell, rather than lysosomal enzymes from phagocytes (Shintani & Klionsky 2004). Interestingly, both the cell survival and cell death features of autophagic function have been implicated in the development, progression and treatment efficacy in cancer cases.

Autophagy and cancer

The function of autophagy in cancer has been extensively reviewed over the past decade (Kondo *et al.* 2005, Hippert *et al.* 2006, Jin & White 2007, Mathew *et al.* 2007b, Morselli *et al.* 2009, White & DiPaola 2009, Kimmelman 2011, Yang *et al.* 2011, White 2012, Kubisch *et al.* 2013, Lu & Harrison-Findik 2013). Autophagy plays a dual, context-dependent role in tumor suppression and tumor survival, a paradox that is widely accepted while its mechanism remains largely unexplained.

The role of autophagy as a cellular housekeeper is linked to its proposed role as a tumor suppressor. The importance of autophagy in tumor suppression was suggested after the observation that *beclin 1*^{+/-} (a gene linked to autophagy) mice had increased rates of tumor development (Qu *et al.* 2003, Yue *et al.* 2003). Moreover, monoallelic loss of *beclin 1* is prevalent in human prostate, ovarian and breast cancers (Aita *et al.* 1999, Liang *et al.* 1999) and restoring expression of *beclin 1*, in human breast carcinoma cells results in inhibition of *in vitro* tumorigenesis and cellular proliferation (Liang *et al.* 1999). Although this is the most well known tumor suppressor gene associated with autophagy activation, there are many others that have also been reported in the

**Figure 1**

The process of autophagy in mammalian cells. (A) In this complex process, a series of protein complexes and autophagy-related proteins are involved in the multi-step process of autophagy. (B) Autophagy is initiated with the engulfment of a portion of cytoplasmic components or selective cargo by a phagophore to form an autophagosome. The outer membrane of the

autophagosome proceeds to fuse with lysosome. The contents within the autophagosome are then exposed to lysosomal degradative enzymes, which act to degrade these cytoplasmic constituents. The resulting biomolecules are eventually recycled back to the cytoplasm. A full colour version of this figure is available at <http://dx.doi.org/10.1530/ERC-15-0042>.

literature (Morselli *et al.* 2009). Upstream of autophagy, the PI3K pathway is constitutively activated by mutations in many cancers, leading to the activation of mTOR and consequent inhibition of autophagy (Shaw & Cantley

2006, Mathew *et al.* 2007b, Morselli *et al.* 2009). Without autophagy, the build-up of toxic cellular constituents such as damaged mitochondria and p62, a signaling adaptor protein, leads to oxidative stress and accumulation of ROS,

which activates a DNA damage response and ensuing genomic instability (Karantza-Wadsworth *et al.* 2007, Mathew *et al.* 2007a, 2009). Thus, despite the loss of autophagy's cellular survival mechanism, this increased rate of genomic mutation and instability brought about by the suppression of autophagy fuel tumor development and progression (Mathew *et al.* 2007b). Furthermore, chronic tissue damage from the accumulation of intracellular toxic elements induces the production of chemokines and cytokines in an inflammatory response that also contributes to tumor progression (White 2012). Finally, the induction of autophagic programmed cell death in cells with an intact autophagy pathway may be a plausible tumor suppressor mechanism. While persistently increased autophagy in highly mutated cells could be the direct cause of cell death, there remains the possibility that autophagy is not actually the reason behind cell death, but rather a final increased attempt to save the cell (White 2014). The issue of autophagic cell death remains a controversial topic and requires further investigation in order to make conclusions about its role in mammalian physiology, and more specifically cancer biology (Denton *et al.* 2012). Although existing research convincingly implicates autophagy in tumor suppression, the complete picture is unclear, and additional evidence is required before this knowledge becomes therapeutically useful.

Contradictory to its tumor suppression activity, autophagy also acts in a pro-survival fashion. Under stressful conditions (i.e., hypoxia, nutrient deprivation), autophagy breaks down proteins and organelles to ensure that the cell has enough self-supplied nutrients to adapt to the stress and survive. For example, during stressful conditions, autophagy uses the amino acids from degraded proteins to sustain the production of ATP through the Krebs cycle in mitochondria (White 2012). Since tumor cells are more susceptible to cellular stresses due to higher frequencies of hypoxic conditions, metabolic stress from intense cellular proliferation, and adverse conditions caused by treatment attempts, the induction of autophagy is essential for their survival (Mathew *et al.* 2007b). Autophagy is also essential for the recovery of cells in cases where nutrient access is regained after prolonged periods of starvation (Degenhardt *et al.* 2006, Mathew *et al.* 2007b). In fact, there is preliminary evidence that autophagy may be connected to the process of cellular senescence, with the ultimate goal of protecting the cell from detrimental conditions such as chemotherapy or radiation, as well as telomeric shortening and the activation of oncogenes (Gewirtz 2013). This could serve as a tumor suppressor function, whereby cells with newly

activated oncogenes enter senescence to prevent the development of cancer, or as a pro-tumor mechanism, whereby tumor cells survive chemotherapy in a senescent state. In this case, autophagy induced in residual or metastatic tumor cells in response to prolonged adverse conditions and stress can keep the cancerous cells alive but dormant for weeks and help them and regrow once conditions ameliorate (Mathew *et al.* 2007b). Autophagy as a mechanism of survival is especially prominent in apoptosis-defective lines of cancer cells (Lum *et al.* 2005, Degenhardt *et al.* 2006, Mathew *et al.* 2007b). Loss of autophagic activity inhibits the ability of cells to survive metabolic stress, recover from it if they survive and, in tumor cells without apoptosis, ends in necrotic cell death (Degenhardt *et al.* 2006, Mathew *et al.* 2007b). Interestingly, it has also been suggested that cancer cells have the ability to activate autophagy in neighboring stromal cells in order to gain the nutrients they need to survive (Lisanti *et al.* 2010, Martinez-Outschoorn *et al.* 2010).

It is clear that autophagy is required for survival of cancer cells under adverse metabolic conditions (Lum *et al.* 2005, Degenhardt *et al.* 2006, Mathew *et al.* 2007b), and yet deficiencies in the mechanisms of autophagy have been shown to induce tumor progression (Aita *et al.* 1999, Liang *et al.* 1999, Qu *et al.* 2003, Yue *et al.* 2003). Several theories have attempted to resolve the apparent inconsistency of autophagy in cancer. First, autophagy may play a dynamic, stage-dependent role in cancer biology whereby it initially plays a tumor suppressor role by attempting to prevent the occurrence of oxidative stress, DNA mutations and genomic instability, but converts to a tumor cell survival role in the later stages of tumor progression (Lu & Harrison-Findik 2013). Thus, autophagic deficiencies in the initial stages of tumorigenesis would induce tumor progression. In later stages of autophagy-deficient cancer, the loss of a major tumor cell survival mechanism may be dwarfed by the increased genomic instability promoting rapid tumor progression and occurrence of necrotic cell death, which initiates an inflammatory response associated with increased tumorigenesis (Degenhardt *et al.* 2006, White & DiPaola 2009). Although lack of autophagy makes it more difficult for cancerous cells to survive metabolic stress, it also speeds up their progression. Further confounding the role of autophagy in cancer are studies in several different types of cancer, including endocrine cancers, indicating that both autophagy inducers and autophagy enhancers work as adjunctive chemotherapies, depending on the type of cancer. Martinez-Outschoorn *et al.* (2010) proposed that this paradox could be explained by evidence indicating that

the occurrence of autophagy in tumor stromal cells promotes tumor progression, while the occurrence of autophagy in the tumor cells themselves has an anticancer effect. Thus, autophagy seems to act differentially in different tissue types and different types of cancer. If this is the case, for the full potential of autophagy-targeted cancer therapy to be realized, autophagy inhibitors and inducers will have to be investigated systematically in a context-dependent manner. For effective anticancer drugs to take advantage of autophagy, it is essential to precisely define the details of the process(es).

Although much of the research concerning cancer in human endocrine glands has been done in non-endocrine cells of the endocrine glands (i.e., epithelial ovarian cancer, pancreatic adenocarcinoma, etc.), there has been some investigation into autophagy in endocrine cell cancers. The following sections will discuss existing research on autophagy in endocrine cancers in the context of each endocrine gland.

Autophagy in pituitary tumors

Much like research into autophagy in the normal pituitary gland, recent research into autophagy in pituitary tumors is scarce or nonexistent, and much of what can be found manifests in the form of case studies. The first finding related to autophagy in pituitary adenomas was the electron microscopic discovery of accumulating pigment granules formed via crinophagic or autophagic degradation of secretory granules, as a recurring feature of spontaneous prolactin cell adenomas in rats (Kovacs *et al.* 1977). The subsequent discovery of crinophagy in a human silent corticotroph adenoma suggested a more functional role for autophagy in pituitary adenomas (Kovacs *et al.* 1978). An increase in intracellular degradation of secretory granules by means of autophagy and crinophagy was proposed to account for the lack of adrenocorticotrophin hypersecretion from the 'silent' adenoma cells (Kovacs *et al.* 1978). In a case study of 300 pituitary adenomas, 17 of which were determined to be silent corticotrophs, however, only two showed signs of increased autophagy (Horvath *et al.* 1980) suggesting there were 15 adenomas for which autophagy was not the cause of their hormonal inactivity. Since there are so many steps in the production, packaging, storing and secretion of hormones that can go wrong in silent pituitary adenoma cells (Horvath *et al.* 1980), this small percentage in which autophagy was involved implies that it is one of many components or one of many ways to produce 'silence,' rather than being the chief explanation for silent pituitary

adenomas. A case study of a pituitary adenoma-causing acromegaly revealed another circumstance involving autophagy in pituitary tumors. The paradoxical occurrence of relatively low serum growth hormone (GH) in the context of a large adenoma and evident acromegaly pointed to a case of 'burnt-out' acromegaly, which is usually caused by spontaneous infarction of the pituitary adenoma (Mashiter *et al.* 1982). In this case, no infarction had occurred and the development of increased crinophagy within the somatotrophs was put forward as an explanation for the conflicting combination (Mashiter *et al.* 1982). The trigger behind the spontaneous decline of GH secretion due to increased crinophagy was not discussed. These observations of autophagy in different types of pituitary adenomas were not conclusive; single, unrepeated case studies are insufficient to make any valid statements about autophagy in pituitary adenomas and more research is necessary to elucidate the full extent of its role.

Occurrences of autophagy in response to therapies have also been documented in pituitary adenomas. Acrylonitrile is known to prevent the occurrence of spontaneous pituitary adenomas (especially prolactinomas) in rats (Kamijo *et al.* 1986). After 24 h of treatment, acrylonitrile induced autophagy in both prolactin and GH cells of the rat pituitary, suggesting that this preventative effect may in some fashion be mediated via autophagy (Kamijo *et al.* 1986). No direct connection was established, however, given the known cytoprotective roles of autophagy, it is feasible that acrylonitrile-induced autophagy could protect the cells from the manifestation of adverse cellular events that would otherwise cause adenomas. In a human study, the exposure of GH-secreting pituitary adenomas to SMS 201-995, a somatostatin analogue, for 10 days in several patients with acromegaly resulted in suppression of GH secretion (George *et al.* 1987, Lamberts *et al.* 1987), as well as the manifestation of crinophagy in many of the somatotroph adenoma cells (George *et al.* 1987). These findings give the impression that SMS 201-995 could be regulating the intracellular degradation of GH secretory granules through crinophagy, and thus modifying GH serum levels, to achieve its therapeutic effect.

There has been a significant deficiency in research concerning autophagy in pituitary adenomas, and autophagy in pituitary carcinomas has not been investigated at all. One limit placed on investigating autophagy in pituitary adenomas resides in the fact that such tumors are benign and slow-growing, and their importance has been overshadowed by more aggressive types of cancer with a more pressing need to investigate new treatments.

Whatever the reason, rapidly expanding research suggesting an important role for autophagy in cancer in general justifies the re-opening of exploration into the role of autophagy in the underlying pathophysiology of pituitary tumors. This line of investigation would allow for the potential to take advantage of autophagy in order to enhance existing treatments and efficacy of care in the treatment of pituitary neoplasms, as has been done in various other endocrine cancers.

Autophagy in adrenal cancer

Whereas studies concerning autophagy in pituitary tumors are mainly outdated, research into autophagy in several types of adrenal cancer is recent and focuses on the role of autophagy in the context of mechanism of action of pharmacological treatments. In an effort to optimize treatment of adrenocortical carcinomas, a rare endocrine malignancy of the adrenal cortex, Cerquetti *et al.* (2011) investigated the anticancer mechanism of a PPAR- γ agonist, rosiglitazone (RGZ), and discovered that RGZ inhibits cell growth in the H295R adrenocortical carcinoma cell line by inducing autophagic cell death. Specifically, RGZ stimulates autophagy via AMPK activation, increased ROS formation and the up-regulation of several proteins known to be involved in the autophagic process such as beclin 1 and LAMP-1 (Cerquetti *et al.* 2011). Although adrenocortical adenomas are relatively common, to our knowledge there has been no investigation into the role of autophagy in their pathophysiology or treatment.

Autophagy was also recently implicated in the action of several drugs being investigated in the treatment of pheochromocytomas, a rare adrenal medulla cancer. Unlike RGZ-induced autophagy in adrenocortical carcinoma cells, however, current research suggests that chemically induced autophagy in the rat pheochromocytoma cell line, PC12, promotes cell survival (Ikeda *et al.* 2013, Fabrizi *et al.* 2014). For example, sunitinib, a known anticancer drug, induces both apoptosis and autophagy in PC12 cells, most likely through the direct inhibition of mTOR (Saito *et al.* 2012, Ikeda *et al.* 2013). The inhibition of autophagy, however, increased the apoptotic and anti-proliferative effects of sunitinib treatment in PC12 cells (Ikeda *et al.* 2013), implying that in this case autophagy is acting against the anticancer effects of sunitinib and in favor of PC12 cell survival. This finding suggests that the targeted inhibition of autophagy may be a therapeutic option for increasing the effectiveness of and surmounting resistance to sunitinib in the treatment of pheochromocytomas (Ikeda *et al.* 2013). Although lithium was also

proposed as a treatment option for pheochromocytomas due to its ability to inhibit PC12 cell growth in culture (Kappes *et al.* 2007), the doses used in that paper were well outside the established therapeutic window for lithium use. When given in dosages within its therapeutic window, lithium promoted the proliferation of PC12 cells in culture and protected them from toxin-induced cell death (Fabrizi *et al.* 2014). Moreover, the protective effect of lithium seemed to be mediated through its induction of autophagy as a mechanism to cope with cell stress in response to a lack of nutrients due to overgrowth, as well as toxic compounds (Fabrizi *et al.* 2014). This discrepancy between the findings of Kappes *et al.* (2007) and Fabrizi *et al.* (2014) may be indicative of the fine line between autophagy as a protective mechanism and overstimulation of autophagy leading to programmed cell death. Due to its propensity for activating autophagy as a defense mechanism at therapeutically viable doses, contrary to the suggestion of Kappes *et al.* (2007), the use of lithium as a treatment for pheochromocytomas is unlikely to be effective.

The findings relating to autophagy in the adrenal gland clearly show the dual nature of autophagy in cancer. In the treatment of one adrenocortical carcinoma cell line, RGZ appears to exert its anticancer effects via the induction of autophagy (Cerquetti *et al.* 2011). In the treatment of pheochromocytomas, however, autophagy acts as a protective mechanism for the PC12 cells in the face of cytotoxic treatments (Ikeda *et al.* 2013, Fabrizi *et al.* 2014). Even between two different cell lines of one subtype of adrenal cancer autophagy is differentially involved in treatment mechanism. In SW13 cells, another adrenocortical carcinoma cell line, RGZ did not induce autophagy, but rather elicited cell growth inhibition via cell-cycle dysregulation (Cerquetti *et al.* 2011). Thus, although autophagy may be an effective target for some types of adrenal cancer, it is certainly not a universally applicable approach. Taken together, the few papers concerning autophagy in the treatment of adrenal cancer emphasize its diverse roles within the cancer cell. With only three studies on the topic, however, it is impossible to generalize the results to any useful effect. Furthermore, all of the studies were conducted *in vitro* on cell lines that, in the case of the rat PC12 cells, are known to imprecisely represent malignant pheochromocytoma cells (Saito *et al.* 2012). Future research is required to elucidate the specifics of autophagy in the different types of adrenal cancer *in vitro* and *in vivo* and take full advantage of its therapeutic potential as a target for induction or inhibition, as indicated.

Autophagy and cancer of the endocrine pancreas

A literature search for autophagy and pancreatic cancer revealed the existence of many publications concerning autophagy and exocrine pancreatic cancer but, to our knowledge, no papers directly covering the topic of autophagy in endocrine pancreatic cancer. Several studies investigate autophagy in insulinoma cell lines, a β -cell-derived tumor that secretes insulin, but all have been done in the context of diabetes research, not cancer research (Kaniuk *et al.* 2007, Choi *et al.* 2009, Fujimoto *et al.* 2009, Martino *et al.* 2012). The lack of inquiry into autophagy and cancer of the endocrine pancreas reflects its relatively low percentage of all pancreatic cancers. Interestingly, dysregulation of major pathways upstream of autophagy (i.e., PTEN, AKT) are common in neuroendocrine tumors such as pancreatic neuroendocrine tumors (Wang *et al.* 2002, Shah *et al.* 2006, Zitzmann *et al.* 2007), suggesting that therapeutic modulation of autophagy may be a promising field of research. In fact, there have been several studies and clinical trials exploring the use of the mTOR inhibitor RAD001 (or everolimus), as well as the receptor tyrosine kinase inhibitor (TKIs) sunitinib against pancreatic neuroendocrine tumors (Yao 2007, Zitzmann *et al.* 2007, Yao *et al.* 2011) with successful anticancer effects. Although these papers did not directly discuss autophagy, since both of these drugs were shown to induce autophagy in thyroid cancer and modulation of autophagy as an adjunctive therapy improved their anticancer effects (Lin *et al.* 2012), it may be speculated that this would be the case for endocrine pancreatic cancer as well. Research into the direct occurrence of autophagy in cancer of the endocrine pancreas is required to develop any concrete conclusions about its role and determine whether it can be exploited for therapeutic purposes.

Autophagy in thyroid cancer

Thyroid cancer is the most common endocrine cancer (Nicholson 2008) and, as such, the role of autophagy in its treatment has been more extensively explored and reviewed (Li *et al.* 2014) than in most endocrine cancers. There are many potential genetic and epigenetic associations between the pathogenesis of thyroid cancer and the pathways of autophagy regulation (Morani *et al.* 2013). A recent study actually discovered a direct connection between a single nucleotide polymorphism in the ATG5 gene and an increased susceptibility for non-medullary thyroid carcinoma (MTC) (Plantinga *et al.* 2014).

These connections, combined with a lack of effective treatment options for thyroid cancers, has led to a rapidly expanding line of investigation into the modulation of autophagy as a potential therapeutic target. There are four main types of thyroid cancer: papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC) and anaplastic thyroid carcinoma (ATC) – all of which arise from follicular cells of the thyroid gland – and MTC, which arises from the parafollicular C-cells of the thyroid (Gimm 2001). As discussed in the case of adrenal cancer, the occurrence of autophagy in response to the same drug can vary even between different cell lines of the same type of cancer (Cerquetti *et al.* 2011). Accordingly, since each type of thyroid cancer should be considered a separate disease, autophagy is often investigated in the treatment of one type at a time and has been presented below with that in mind.

PTC is the most common type of thyroid cancer, with a relatively good prognosis (Gimm 2001). Current publications concerning autophagy in PTC point predominantly to the therapeutic induction of autophagy as an anticancer mechanism. Doxorubicin and external beam radiation, two therapies used to treat advanced PTC, both induce autophagy in PTC cells (Lin *et al.* 2009), and the inhibition of autophagy using 3-MA in those cells treated with doxorubicin or external beam radiation increased their chemo- and radioresistance respectively (Lin *et al.* 2009). Moreover, the use of RAD001, an mTOR inhibitor that induces autophagy in PTC cells, in conjunction with doxorubicin or external beam radiation, increased their anticancer effects (Lin *et al.* 2010). The elimination of RAD001-mediated sensitization to doxorubicin and radiation when PTC cells were also treated with ATG-5 siRNAs to block autophagy implicates autophagy induction as the primary mechanism for this improved therapeutic effectiveness (Lin *et al.* 2010). Similarly, treatment of a PTC cell line with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) increased autophagy, and autophagic inhibition with ATG-7 siRNA increased cell resistance to TRAIL-mediated apoptosis, suggesting a pro-death role of autophagy in TRAIL-treated PTC cells (Jin *et al.* 2014). The occurrence of autophagy as a pro-death mechanism in response to TRAIL may explain why PTC seems to be the most susceptible thyroid cancer to TRAIL-induced cell death. Interestingly, treatment of a PTC cell line with rosuvastatin demonstrated a shift from autophagy to apoptosis in a dose-dependent manner (Zeybek *et al.* 2011). This finding could be explained by the idea that autophagy acts as a pro-survival mechanism in the face of low-dose toxic stress, but prolonged or excessive

stimulation of autophagy results in activation of apoptosis or autophagic cell death (Zeybek *et al.* 2011).

FTC also arises from the follicular cells of the thyroid, and it is the least studied type of thyroid cancer in terms of autophagy. In human FTC cells treated with reversine, a small-molecule drug previously shown to inhibit thyroid cancer cell growth (Hua *et al.* 2012), autophagy is induced, most likely via suppression of the AKT/mTOR pathway (Lu *et al.* 2012). Treatment of FTC cells with either reversine or rapamycin, both known autophagy inducers, significantly decreased levels of cellular proliferation, and a combination of the two produced a synergistic effect on the depression of FTC cell viability (Lu *et al.* 2012). This research, however, does not properly delineate between autophagy as a pro-survival and pro-death mechanism. Although this paper attributed the occurrence of autophagy to a pro-death mechanism, there remains the possibility that autophagy is induced as a method of cell survival in response to reversine toxicity alone, and its overstimulation when a direct autophagy inducer (i.e., rapamycin) is added leads to the induction of autophagic cell death.

MTC arises from the parafollicular C-cells of the thyroid gland, has no known curative treatment other than surgery, and can be either hereditary or sporadic (Gimm 2001). All hereditary and many sporadic cases of MTC are characterized by mutations that activate the ret protooncogene (RET), but attempts at treating MTC with RET activity inhibitors have proven to stabilize the disease rather than cure it (Lin *et al.* 2012). Although their original hypothesis postulated that autophagy inhibition would increase the anticancer effects of RET-targeting TKIs such as sunitinib and sorafenib, Lin *et al.* (2012) discovered the opposite. Both sunitinib and sorafenib activate autophagy in MTC cells (Lin *et al.* 2012). In concordance with the effects of RAD001 on chemotherapies in PTC discussed above (Lin *et al.* 2010), the use of everolimus (RAD001) in addition to sunitinib or sorafenib treatment in an MTC cell line potentiated their anticancer effects and knocking down ATG5 reversed this effect (Lin *et al.* 2012). An mTOR-independent autophagic activator produced the same results, suggesting that autophagic influence on the efficacy of sunitinib and sorafenib does not depend on the mTOR pathway (Lin *et al.* 2012). Together these findings implicate pathway-independent amplification of autophagy as a method of overcoming the stabilizing effects and increasing the cytotoxicity of RET-targeting chemotherapeutic treatments for patients with MTC.

The search for an effective treatment other than surgery for MTC has yielded another potential treatment

target in microRNA-183, which is overexpressed in sporadic MTC (Abraham *et al.* 2011). Antagonizing miRNA-183 in an MTC cell line revealed a decrease in viable cancer cells in the absence of apoptosis marker changes, as well as a simultaneous increase in autophagy (Abraham *et al.* 2011). This finding suggests, but does not prove, that autophagy is the cause of cell death. As with most of the cases discussed here, it remains possible that autophagy is up-regulated as a survival attempt and that cell death occurs in spite of autophagy rather than because of it. In fact, another microRNA, miR-9-3p was shown to be down-regulated in sporadic MTC (Abraham *et al.* 2011) and its transfection into MTC cell lines reduced cell viability, at least partially, via a decrease in autophagic flux and autophagy-related gene expression and a concurrent increase in apoptotic flux (Gundara *et al.* 2015). Interestingly, Gundara *et al.* (2015) also investigated the mRNA autophagy gene profile in clinical samples of both sporadic and hereditary MTC. Levels of autophagy gene mRNAs were increased in sporadic MTC compared to hereditary MTC, a finding that correlates well with decreased miR9-3-p levels in sporadic MTC. Specifically, beclin 1, a mammalian orthologue of yeast Atg6 that plays a principal role in autophagy, was highly overexpressed in sporadic MTC and was shown to positively correlate with residual disease. From this, Gundara *et al.* (2015) suggested that beclin 1 expression may be a useful biomarker for persistent or recurrent disease. Authors concluded that in MTC, autophagy appears to be a mechanism for tumor cell survival rather than cell death, and its inhibition may be of therapeutic advantage.

Finally, ATC is a relatively rare but extremely aggressive form of thyroid cancer of the follicular cells with a poor prognosis and no reliably successful treatment (Gimm 2001). Resistance of ATC cells to current treatments is one reason they are ineffective (Zhang *et al.* 2014), and recent findings suggest that autophagy-mediated cancer cell survival in response to chemotherapy may underlie this resistance. In two ATC cell lines, autophagy levels increased in response to treatment with cisplatin, a chemotherapeutic agent that is commonly employed in the treatment of ATC (Zhang *et al.* 2014). Inhibition of this drug-induced autophagy in ATC cell lines treated with cisplatin using a microRNA-30d mimic that prevents the expression of beclin 1 caused an increase in apoptotic cell death (Zhang *et al.* 2014). miRNA-30d-mediated inhibition of autophagy also sensitized ATC cells to cisplatin in an ATC xenograft mouse model (Zhang *et al.* 2014). These findings indicate that resistance to the antitumor effects of cisplatin in ATC cell lines *in vitro* and *in vivo* is partially due

to the protective role of autophagy. Furthermore, miR-30d is naturally down-regulated in ATC (Visone *et al.* 2007), contributing to the advancement of the disease and suggesting that ATC cells actively de-repress autophagy as a survival mechanism. ATC is also resistant to TRAIL, a chemotherapeutic drug that induces tumor cell-specific apoptosis (Walczak *et al.* 1999, Jin *et al.* 2014). Although treatment of a human ATC cell line with TRAIL did not significantly change autophagy levels, inhibition of autophagy with ATG7 siRNA sensitized the ATC cell line to the apoptotic effects of TRAIL (Jin *et al.* 2014). Conversely, Yeung *et al.* (2007) observed that treatment of an ATC mouse xenograft with CA4P induced autophagy in the cancer cells and partially attributed the antitumor effect of CA4P to the induction of autophagy-mediated cell death. The authors failed to experimentally differentiate between autophagic cell death and autophagy induced as a survival mechanism by the threat of CA4P cytotoxicity, however, making their conclusion about the nature of the involvement of autophagy questionable.

When the publications concerning autophagy in PTC, FTC and MTC are considered together, the enhancement of autophagy with adjunct treatments appears to be a novel and promising target to increase the anticancer activity of proposed drugs. In the case of ATC, however, autophagy appears to be conferring therapeutic resistance and adjunctive inhibition of autophagy increases the anticancer activity of various chemotherapies. Thus, as in the case of autophagy in adrenal cancers, the proposed roles of autophagy in the progression and treatment of different types of thyroid cancer illustrate its reputation as a double-edged sword. The findings presented here are in their preliminary stages (i.e., mainly *in vitro*), however, and further investigation *in vivo* is required to fully elucidate the potential for targeting autophagy in the treatment of different thyroid cancers.

Autophagy in parathyroid cancer

In contrast to thyroid neoplasia, there is very little research to be found regarding autophagy in parathyroid cancer. An electron microscopic study of three cases of parathyroid carcinomas revealed autophagy in one case, as well as an abundance of secretory granules and lipid droplets in conjunction with autophagy in another case (Altenähr & Saeger 1973). The latter involved a silent parathyroid carcinoma with no signs of the hyperparathyroidism that is commonly associated with parathyroid cancer (Altenähr & Saeger 1973). Since the hormone synthesis and packaging organelles all appeared intact, the

lack of hormone secretion could be explained in several ways (Altenähr & Saeger 1973). The lipid droplets could be interpreted as remnants of autophagic degradation of secretory granules leading to the conclusion that perhaps overactive autophagy was responsible for the silence. Alternatively, the remaining number of secretory granules suggests that there was a problem with the cancer cell's secretion mechanism and autophagy was simply induced as a natural response to accumulating granules. Thus, analogous to silent pituitary adenomas, autophagy has been implicated in the pathway behind hormonal inactivity in parathyroid carcinomas.

A more recent study suggested a connection between CDC73, a tumor suppressor gene whose mutation is implicated in human hyperparathyroidism-jaw tumor syndrome and an elevated risk of malignant as well as sporadic parathyroid cancer, and de-repression of autophagy via the mTOR pathway (Zhang *et al.* 2012). Zhang *et al.* (2012) showed that flies with a mutation in the *Drosophila* CDC73 homologue were resistant to starvation, implicating autophagic pathways in the process and suggesting that resistance to starvation contributes to parathyroid malignancy. There was no direct connection to autophagy or starvation resistance in the actual cancer cells however, and investigations into this pathway in human cancer cells are needed to confirm the theory.

As with many of the other endocrine cancer types, research regarding autophagy in parathyroid cancer is sorely lacking. The implication of autophagy in hormonal silence is an interesting parallel to the same suggestion in pituitary adenomas, and the genetic connection to autophagy in parathyroid carcinomas could be promising in the treatment of this rare condition. With only one publication discussing each of these findings, however, it is impossible to draw any concrete conclusions about the role of autophagy in parathyroid cancer.

Autophagy in ovarian and testicular cancer

To our knowledge, there are no publications discussing the role of autophagy in endocrine-type ovarian cancer. This is most likely due to the fact that cancer of the endocrine ovarian cells, classified as ovarian sex-cord stromal tumors, is extremely rare and accounts for a very small percentage of all ovarian cancers (Nicholson 2008). Conversely, since epithelial ovarian carcinomas account for ~90% of all ovarian malignant cancers and remain one of the most common causes of cancer-related deaths in women (Ries *et al.* 2007), there has been an upsurge of recent research into autophagy and epithelial ovarian cancer. It appears

that autophagy may have a pivotal role in enhancing treatments and overcoming treatment resistance for epithelial ovarian cancer, and perhaps if investigations were to be carried out regarding autophagy in ovarian sex-cord stromal tumors, comparable results would be forthcoming.

Similarly to ovarian cancer, endocrine cell-derived testicular cancers, such as Leydig cell tumors or Sertoli cell tumors, make up a very small percentage of all testicular cancers. In one study examining the role of retinoic acids in cancerous Leydig cell lines, endogenous levels of retinoic acid were found to enhance cellular proliferation while higher pharmacological doses had an anti-proliferative effect, inducing apoptotic cell death (Perri *et al.* 2010). Interestingly, evidence of autophagy was found at a borderline level between these two contrasting effects (Perri *et al.* 2010), suggesting that it acts as a tipping-point mechanism between cellular survival efforts and programmed cell death in Leydig tumor cells. This finding corresponds well to the generally accepted notion of the complex, dual-faced functions of autophagy and to findings concerning the role of autophagy in other endocrine cancers.

The lack of research investigating autophagy in endocrine-type ovarian and testicular cancer reported here lays bare a large gap in current knowledge concerning underlying cellular mechanisms in the pathology and potential treatment targets of these two types of cancer. Since the involvement of autophagy in both physiological conditions and non-cancer pathologies of the ovaries and testes has been reported (Weckman *et al.* 2014), and since autophagy appears to be important in non-endocrine ovarian cancer as well as in cancer of many other endocrine glands, it is likely that much remains to be discovered in this area.

Future directions and conclusions

With increasing evidence of autophagy's function in the pathophysiology of many diseases, including cancer, the field has garnered increasing attention in the scientific community. In several endocrine cancers such as endocrine-type pancreatic and ovarian cancers, however, there is no direct research linking autophagy to their pathophysiology. Even in the remaining types of endocrine cancer, there are still large gaps in our knowledge of autophagy's part in the underlying disease mechanism, and thus in its potential use as a treatment target. Based on recent advances in other types of cancer, an improved understanding of the function of autophagy in endocrine

cancers may allow for the discovery of more targeted and effective treatments. There are many studies concerning autophagy in other, more aggressive and more common types of non-endocrine cancers of the endocrine glands (i.e., epithelial ovarian cancer, pancreatic adenocarcinomas, etc.), and many of them simultaneously investigate different cell lines for the same type of cancer. In the future, the incorporation of even a single endocrine-type cancerous cell line in these multiple cell line experiments would likely generate important contributions to our understanding of autophagy in endocrine cancers.

In endocrine cancer cell lines, both autophagy inhibitors and autophagy enhancers work as adjunct therapies in a cell-type specific manner (Table 2). For example, the anticancer effects of sunitinib were enhanced with an autophagy inhibitor in the treatment of pheochromocytoma, while in MTC, it was sunitinib combined with an autophagy inducer that increased its anticancer effects (Lin *et al.* 2012, Ikeda *et al.* 2013). Even within one cancer subtype, a single drug had differential effects on autophagy in two different cell lines (Cerquetti *et al.* 2011). Thus, there is an inconsistency in the actions and effective modulations of autophagy for treatment in different types of endocrine cancer cell lines. This inconsistency could be explained in several possible ways. Perhaps the differences in the benefits of autophagy enhancement or inhibition between cancers are explained by the stages of tumor progression at which the therapy was applied. For example, if an autophagy inhibitor were applied early in tumorigenesis, it would most likely inhibit the housekeeping role of autophagy leading to increased DNA mutation and more rapid tumor progression. If it were applied later in tumorigenesis, it can be speculated that it would inhibit the cell survival role of autophagy in stressed tumor cells leading to increased tumor cytotoxicity. Another possibility was put forward whereby enhancing autophagy within tumor cells would be cytotoxic, while enhancing autophagy within neighboring tumor stromal cells would support the survival of tumor cells by providing the nutrients they need to survive (Martinez-Outschoorn *et al.* 2010). Future research should aim to delineate these discrepancies in autophagy-targeting treatments between different endocrine cancer types. If the underlying physiology or pathophysiology behind these tissue-specific discrepancies could be elucidated in endocrine cancers, it is possible that the findings could be transferable to other more common and more aggressive cancers as well. It must also be noted that many of the autophagy inhibitors and enhancers used in the publications covered in this review have diverse effects

Table 2 Therapeutic potential of autophagy modulation

Type of cancer	Therapy	Independent effect on autophagy?	Combinatorial effect with autophagy-targeted drugs?	Inhibition or enhancement of autophagy as target?
Spontaneous pituitary adenomas	Acrylonitrile (Kamijo <i>et al.</i> 1986)	Yes, induced autophagy	NA	Proposed enhancement as method of prevention
Pituitary adenoma (GH secreting)	SMS 201–995 (George <i>et al.</i> 1987)	Yes, induced autophagy	NA	Proposed enhancement as method of reducing GH secretion
Adrenocortical carcinoma	RGZ (Cerquetti <i>et al.</i> 2011)	Yes, induced autophagy	NA	Enhancement
Pheochromocytoma	Sunitinib (Saito <i>et al.</i> 2012, Ikeda <i>et al.</i> 2013)	Yes, induced autophagy	Yes, autophagy inhibitor enhances anti-cancer effect	Inhibition
Anaplastic thyroid cancer	Cisplatin (Zhang <i>et al.</i> 2014)	Yes, induced autophagy	Yes, autophagy inhibitor increases apoptotic cell death	Inhibition
	TRAIL (Jin <i>et al.</i> 2014)	No	Yes, autophagy inhibition sensitized ATC cell line to apoptotic effects of TRAIL	Inhibition
Papillary thyroid cancer	Doxorubicin (Lin <i>et al.</i> 2009, Lin <i>et al.</i> 2010)	Yes, induced autophagy	Yes, autophagy inhibition increased their chemoresistance; autophagy enhancement increased their anticancer effects	Enhancement
	External beam radiation (Lin <i>et al.</i> 2009, 2010)	Yes, induced autophagy	Yes, autophagy inhibition increased their radioresistance, autophagy enhancement increased its anticancer effects	Enhancement
	TRAIL (Jin <i>et al.</i> 2014)	Yes, induced autophagy	Autophagy inhibition increased resistance to TRAIL	Enhancement
Follicular thyroid cancer	Reversine (Lu <i>et al.</i> 2012)	Yes, induced autophagy	Yes, autophagy inducer increases depression of cell viability	Enhancement
Medullary thyroid cancer	Sunitinib and Sorafenib (Lin <i>et al.</i> 2012)	Yes, induced autophagy	Yes, autophagy inducer increases anticancer effects	Enhancement

within the cell and do not act exclusively on autophagy. Thus, although many of the drugs modulate autophagy as part of their effect, it cannot be said with any certainty that their anti- or pro-cancer effects could be attributed solely to autophagic manipulation. This fact could also account for the differential effects of treatments in different types of cancer and even different cell lines.

Future studies should focus on establishing a standardized, systematic method of investigating the role of autophagy modulation as an adjunctive treatment in endocrine cancers. They should also aim to shed more light on whether the manipulation of autophagy may be used as a new treatment option, or with new combinations of treatments, and whether genetic information can be used to better tailor therapies to individual patients. Many of the studies reported in this review investigate either the inhibition or the enhancement of autophagy as an adjunctive treatment, but not both. It is difficult to

compare or contrast the results of two studies employing opposite approaches with any confidence, especially in the case of such a versatile, two-faced process as autophagy. In addition, a more conclusive method of analyzing the specificity of drug effects and measuring whether cell death is directly caused by autophagy or whether cell death occurs in spite of up-regulated, survival-oriented autophagy would aid in understanding the potential for modulation of autophagy in endocrine cancer treatments. Since there is controversy surrounding autophagic cell death in general (Denton *et al.* 2012), clarifying this process and a method of measuring it would be beneficial for research regarding all cancers, not just endocrine types. Perhaps most importantly, there exist only *in vitro* studies to support the idea that autophagy may be a successful adjunct target in the treatment of endocrine cancers. Since an *in vitro* tumor environment differs greatly from an *in vivo* tumor environment, it is

impossible to conclude with any certainty that targeting autophagy would be clinically useful as an adjunctive treatment.

The role of autophagy in general has still to be elucidated in the whole physiopathological spectrum of conditions, including endocrine tumors. Autophagy has thus far proven to be a promising therapeutic target in the treatment of endocrine cancers *in vitro*, but much more research is needed to take full advantage of this fascinating biological process and determine whether these findings will hold true clinically.

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

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Author contribution statement

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