Oncophagy: harnessing regulation of autophagy in cancer therapy

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Abstract

Autophagy is an increasingly well-characterised process of cell component auto-digestion and recycling thought necessary for cellular subsistence. As we gain a more thorough understanding of the mechanisms underlying autophagy, its relevance to human disease and therapeutic potential are being clarified. This review summarises the evidence implicating autophagy in the pathogenesis and potential treatment of malignant disease. In addition, we explore the molecular role of microRNAs as key regulators in what we propose should now become known as ‘oncophagy’.

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Introduction

Autophagy is a eukaryotic cell process of self-catabolic degradation originally described in the 1960’s (Deter & De Duve 1967). The term autophagy is derived from Greek terminology ‘auto’ (meaning self) and ‘phagy’ (eating) to describe the process of cell component auto-digestion and recycling required for cellular survival. Beyond such origins, dual utility has also been proposed whereby autophagy plays somewhat of a Janus-like role in cell fate (Liu et al. 2011) being as important to cell death as it is to survival. Now that we are gaining a more thorough understanding of the mechanisms underlying autophagy, its relevance to human disease and burgeoning therapeutic potential are being unravelled (Ding et al. 2008, Chen et al. 2009, Li et al. 2009, 2012, Chen & White 2011, Gundara et al. 2011). Here, we review the potential pathogenic, prognostic and therapeutic roles of autophagy within the context of human malignancy and with a special reference to microRNA-mediated regulation.

Autophagy: the mechanism

Cellular homeostasis is a complex process dependent on close regulation of synthesis and degradation of both structural and functional elements. The ubiquitin–proteasome pathway is one such mechanism whereby cellular proteins can be broken down and disposed of or recycled. Alternatively, the autophagy–lysosome pathway maintains particular importance in management of longer lived or larger proteins, potentially including entire organelles such as mitochondria (Klionsky & Emr 2000).

Autophagy has been sub-classified into three discrete sub-types (macro-, micro- and chaperone-mediated autophagy), the central theme involving proteolysis of cytosol components at the lysosome (Glick et al. 2010). The majority of current scientific interest focuses on macroautophagy and its relation to cellular processes in health and disease (Glick et al. 2010). As such, the term autophagy is generally considered to be a reference to macroautophagy specifically (Klionsky et al. 2008).

Autophagy commences with the phagophore (an isolation membrane) that originates from the endoplasmic reticulum, golgi apparatus and endosomes. The phagophore consumes intra-cellular, cytoplasmic contents (damaged/dysfunctional DNA, cell organelles and proteins) and sequesters them within the double-membrane autophagosome. This then fuses with the lysosome and lysosomal acid proteases induce degradation of autophagosomal contents (thence known as...
the autolysosome). The resulting component nucleotides, fatty acids and amino acids may then be recycled as cellular building blocks or removed as a waste product. Given such a mechanism, autophagy has been known as a ‘recycling factory’, enabling efficient energy production and removal of potentially toxic waste products of metabolic processes (Glick et al. 2010). Therein lies the importance of autophagy, which, when defective, can result in a multitude of human diseases (White et al. 2010).

**Autophagy: regulation**

Regulation of autophagy is complex. However, a basic understanding of the key regulatory steps is crucial to an understanding of potential autophagy-based biomarkers and therapies. In their review of autophagy regulation, Glick et al. (2010) describe five key points at which regulation is imposed:

1. phagophore formation,
2. Atg5–Atg12 conjugation, interaction with Atg16L and multimerisation at the phagophore,
3. LC3 processing and insertion into the extending phagophore membrane,
4. capture of random or selective targets for degradation,
5. fusion of the autophagosome with the lysosome, followed by proteolytic degradation by lysosomal proteases of engulfed molecules.

Numerous key regulators are involved in maintenance of the pathway from phagophore to autolysosome. Within this regulatory milieu reside ATG genes or autophagy-related genes and their associated Atgs (autophagy gene products). Our understanding of autophagy regulation was relatively superficial until the 1990’s when ATGs were discovered and have formed the crux of our understanding of regulatory processes since (Li et al. 2012). Beclin-1 was the first ATG discovered to be of importance in mammalian autophagy (Liang et al. 1999) and there are now over 30 ATGs known to be involved. ATGs (and their protein products) have subsequently been the target of specific investigation in an attempt to further delineate, and employ, the molecular utility of autophagy (Li et al. 2012).

Embedded within the myriad of regulatory processes governing autophagy is mammalian target of rapamycin (mTOR), which itself is part of the class I phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/Akt) signalling pathway. mTOR acts to inhibit autophagy under growth-promoting conditions, and repression of its activity has been found in states of nutrient deprivation and hypoxia, conditions also associated with autophagy (Yang & Klionsky 2010). More specifically, class I PI3K is activated by growth factor receptors, which then lead to downstream mTOR activation and ultimately autophagy inhibition (Yang & Klionsky 2010). Thus, it can be reasoned that in states of nutrient deprivation, a lack of growth factors will release mTOR inhibitory signalling and result in autophagy induction to recycle cellular constituents and maintain cell viability.

An understanding of the mechanistic pathways involved is of great interest as it opens opportunities for potential therapeutic intervention. The ability to manipulate these complex pathways is, however, dependent on an understanding of the clinical importance of autophagy in disease, and cancer in particular. Beyond a homoeostatic role, autophagy has been shown to be of importance in normal development (Kuma et al. 2004), and from a clinicopathologic viewpoint, it has been associated with neurodegenerative (Yang & Mao 2010), metabolic (Jung & Lee 2010), infectious (Gutierrez et al. 2007), inflammatory (White et al. 2010) and malignant diseases (Chen et al. 2009, White et al. 2010, Li et al. 2012).

The harnessing of a self-catabolic process like autophagy against malignancy is a novel concept and is the one that is fast gaining broad scientific and clinical interest (White & DiPaola 2009, Ravikumar et al. 2010, White et al. 2010, Li et al. 2012). The remainder of this review focuses primarily on our current understanding of the role of autophagy in cancer biology and the steps that have already been taken to utilise this process as a potential therapeutic strategy in cancer treatment.

**Autophagy: role in oncogenesis and cancer therapy**

Currently, conflicting evidence regarding the role of autophagy in tumour biology has led to a likening of this process to a double-edged sword (White & DiPaola 2009). Autophagy has been shown to be both inhibitory and beneficial to the malignant process (Ravikumar et al. 2010). Some investigators have suggested that it may suppress early tumour development, serving as an anti-neoplastic, biological defence mechanism in the early stages of oncogenesis (Karantza-Wadsworth et al. 2007), while paradoxically supporting tumour cell survival later, once true malignancy has evolved (White et al. 2010, Yang & Kimmelman 2011). Enhanced sensitivity of cancer
cells to chemo- and radiotherapy has also been associated with enhanced autophagic flux (Mukubou et al. 2010), although discerning objectively what reflects an efficacious therapeutic effect, rather than evidence of collateral cellular stress is a contentious issue (Klionsky et al. 2008, Shen et al. 2012). Additionally, direct quantification of basal tumour tissue autophagic flux has also proven difficult and has led some investigators to suggest that direct evidence linking reduced autophagic flux to tumorigenesis is still lacking (Chen & Debnath 2010). These observations reinforce the notion that the role of autophagy in oncogenesis is not only highly context dependent (White et al. 2010), but it is also still poorly understood.

**Autophagic cell death**

The association between oncogenesis and autophagy is typified by the finding of several ATG proteins that have been touted as potential tumour suppressors. Beclin-1 (Qu et al. 2003), PTEN (Arico et al. 2001) and p53 (Feng et al. 2005) have been found to be significant in autophagy pathways, with Beclin-1 gene suppression, for example, being linked directly to tumorigenesis (Qu et al. 2003). More specifically, reduced Beclin-1 expression has been identified in sporadic human breast cancers (Liang et al. 1999), higher grade brain tumours (Miracco et al. 2007) and epithelial ovarian cancers (Shen et al. 2008). The Beclin-1 genomic locus (chromosome 17q21) has also been shown to be deleted in up to 50% of breast (Saito et al. 1993), 75% of ovarian (Russell et al. 1990, Saito et al. 1993) and 40% of prostate (Gao et al. 1995) cancers. Conversely, elevated levels of Beclin-1 expression correlate significantly with disease-free and overall survival in colon (Li et al. 2009), hepatocellular (Ding et al. 2008) and oesophageal (squamous cell) cancers (Chen et al. 2009), and functional experimentation demonstrates that Beclin-1 overexpression can reduce in vitro colon (Koneri et al. 2007) and cervical (Wang et al. 2007) cancer cell proliferation and in vivo xenograft tumour growth (Wang et al. 2007). This effect is reversed when Beclin-1 is suppressed using RNA interference methods (Wang et al. 2007).

It has also been suggested that the tumour suppressor role of PTEN is enacted indirectly through positive regulation of autophagy (Arico et al. 2001). This is thought to occur secondary to PTEN inhibition of the PI3K/Akt pathway, as originally demonstrated in a colon cancer cell line by Arico et al. (2001). Consistent with this mechanism, heterozygous loss of PTEN in mice has been shown to lead to breast and thyroid tumours, among others (Kishimoto et al. 2003). Additionally, PTEN mutations have also been identified in a range of hereditary cancer syndromes (Pezzolesi et al. 2008) and aggressive sporadic malignancies, such as castration-resistant prostate cancer (Grasso et al. 2012). A similar positive regulatory role in autophagy has also been proven for the commonly implicated tumour suppressor, p53 (Feng et al. 2005).

These findings support the theory that ongoing and continual autophagic flux may be tumoricidal and necessary to avoid neoplastic transformation, during the earliest stages of cancer initiation (Shen et al. 2008, White et al. 2010). However, if autophagy fails to maintain this housekeeper function, neoplasia ensues. This effect has been demonstrated in breast cancer and defines the role of Beclin-1 as a haplo-insufficient tumour suppressor (Karantza-Wadsworth et al. 2007). Having demonstrated such an effect, Karantza-Wadsworth et al. (2007) concluded that defective autophagy leaves cells susceptible to metabolic stress and DNA damage that ultimately leads to genomic instability and cancer initiation. It may be that many tumour suppressors, not unlike Beclin-1, PTEN or p53, exert their ‘suppressor’ effect through such an autophagic avenue.

In keeping with this theoretical continuum, associations between therapeutic response to cytotoxic agents and elevations in markers of autophagic flux have led some investigators to claim that iatrogenic autophagy induction may be a valid therapeutic strategy, by taking advantage of what was previously labelled ‘autophagic cell death’ (Ullen et al. 2010, Abraham et al. 2011). Ullen et al. (2010), in examining the in vitro efficacy of the tyrosine kinase inhibitor, sorafenib on prostate cancer cells, identified a dose-dependent impact on cell viability in parallel with evidence of increased autophagic flux. This led them to suggest that autophagy manipulation (in combination with established therapies) may be a valid approach to treating cancer.

Numerous other malignancies have also been investigated regarding the potential therapeutic harnessing of autophagic cell death. Using a variety of agents, claims of autophagic cell death have been made in a vast range of malignancies including hepatocellular carcinoma (HCC; Wang et al. 2010), oestrogen receptor-negative breast cancer (Vanderlaag et al. 2010), non-small-cell lung cancer (Li et al. 2010b), ovarian cancer (Le et al. 2010), pancreatic cancer (Mujumdar et al. 2010), gastric cancer (Hashimoto et al. 2008), malignant glioma (Takeuchi et al. 2005) and papillary thyroid cancer (Lin et al. 2009). Many of these investigations demonstrate...
an apoptosis-independent therapeutic in vitro effect on cellular proliferation in association with elevations in markers of autophagy. In reasoning such results, investigators have previously concluded that autophagic cell death is the mechanism by which therapeutic efficacy is being enacted.

The role of the tyrosine kinase receptor pathway and autophagy has also been highlighted more recently by Xu & Weiha (2011), who demonstrated that EGFR receptor knockdown leads to autophagy induction. However, and in direct contrast to Ullen et al., they concluded that the beneficial effect on prostate cancer cells related to EGFR protein suppression rather than inhibition of tyrosine kinase activity.

Table 1 summarises a broad, representative selection of basic scientific and clinicopathologic literature examining the pathophysiological, prognostic and therapeutic themes of what can now be labelled ‘oncophagy’ (i.e. cancer-related autophagy).

The alternative hypothesis

One of the lasting problems in identification of what may be considered autophagic cell death is confirming that this is the definitive, lethal process cells are committing to, rather than being an observation of a collateral response to stress that accompanies cellular demise. This is a present-day challenge for autophagy investigators and is a concept that has recently been challenged strongly (Shen et al. 2012).

Shen et al. (2012) questioned whether or not autophagic cell death exits at all. Following exhaustive experimentation involving treatment of osteosarcoma cells with 1400 different agents, they were unable to identify definitive evidence of autophagic cell death (Shen et al. 2011). They concluded that markers of autophagic flux may well be present within the context of experimentation attempting to demonstrate a therapeutic effect, but this does not constitute evidence of cell death ‘by’ autophagy, but rather, death ‘with’ autophagy. This is an important point, and one that has led to abandonment of older terminology whereby autophagy was otherwise known as programmed cell death type II (Kroemer et al. 2009). Despite this, the possibility of autophagy still being intimately involved in ‘lethal signalling’ pathways towards cell death is likely, but the precise role it plays requires further investigation and definition.

Given that the case for induced autophagic cell death is becoming less compelling, an alternative hypothesis requires consideration. In keeping with the homoeostatic role of autophagy in cellular health, there is evolving evidence that this may also be a potent strategy employed by tumour cells to ensure survival, thus paving the way for therapeutic manipulation (White & DiPaola 2009). White & DiPaola articulate this concept well by suggesting that tumour cells, with their rapid proliferation and poor blood supply, are some of the most metabolically stressed. This is justified by data showing that tumour tissues generally possess an increased state of autophagic flux (Degenhardt et al. 2006), therefore supporting the theory of a heightened reliance on mechanisms such as autophagy for survival and ultimately enhanced levels of stress tolerance when compared to cells in health (White & DiPaola 2009). Suppressing or removing this tumour cell survival mechanism would thus seem an attractive therapeutic manoeuvre.

The at times conflicting, two faced nature of autophagy involvement in oncogenesis is exemplified by the tumour suppressor gene ARHI (DIRAS3) (Lu et al. 2008). In attempting to explore ARHI gene suppression in ovarian cancer, Lu et al. induced re-expression of ARHI in a cell culture model and demonstrated cell death in association with autophagy induction. When cells were xenotransplanted into a mouse model without ARHI induction, tumours predictably grew, and when ARHI was induced tumour growth was inhibited, as expected. When ARHI induction was withdrawn after 30 days, tumours began to grow promptly indicating that xenografted cells had remained dormant but still viable and capable of proliferation. Additional experimentation with autophagy blockade (using chloroquine) led to retarded tumour growth following ARHI induction withdrawal. Cells were again viable but apparently dormant. The authors suggest that this is indicative of the establishment of an adaptive dormancy state that is dependent on ARHI-mediated autophagy. Lu et al. concluded that autophagy, while maintaining an efficacious tumoricidal effect in vitro, seemingly appears to aid maintenance of tumour cell dormancy in the in vivo model, thus implicating it as a tumour cell survival strategy. This provided some of the first evidence of therapeutic benefit when employing autophagy blockade, rather than induction, in a model of established malignancy.

A role for autophagic tumour cell survival has also been investigated in HCC cell lines and xenografts (Ding et al. 2011). Ding et al. treated HCC cell lines with oxaliplatin, a known chemotherapeutic agent efficacious in HCC. Treated cells and mouse model xenografts demonstrated evidence of autophagy induction in association with cell death and tumour
regression. However, when treatment was supplemented with autophagy blockade (using pharmacological and small interfering RNA methods), oxaliplatin treatment was potentiated with an increase in cell death and tumour regression being observed. These results suggest that autophagy may well be an adaptive tumour cell survival mechanism rather than promoting cell death. Similar findings have been reported by Li et al. (2010a) when examining the increased effect of 5-FU on colon cancer cell lines and xenografts with autophagy blockade (employing 3-methyladenine and siRNA to Atg7). Interestingly, this was also observed in association with an increase in flux through the apoptotic pathway, implying a degree of crosstalk between these two pathways of cell fate, a link that has been suggested previously (Shen et al. 2011). In exploring the cross talk between apoptosis, autophagy and necrosis, Shen et al. (2012) also showed enhanced cellular toxicity during treatment with autophagy blockade (using siRNA to ATG5 and ATG7) to further reinforce the theory of autophagy being a cytoprotective mechanism. The alternative hypothesis therefore follows: namely that if this cytoprotective mechanism is disabled, tumour cells are vulnerable to toxic insults and death.

This novel theory has been expanded on both in the laboratory and also clinically through the commencement of several clinical trials that are attempting to exploit pharmacologic autophagy blockade in combination with recognised chemotherapeutics for cancer. Adjutant autophagy blockade has been shown to be therapeutic during in vitro studies investigating various treatments for myeloid leukaemia (Bellodi et al. 2009), colon cancer (Carew et al. 2010), glioma (Lomonaco et al. 2009) and breast cancer (Vazquez-Martín et al. 2009). In addition, and while it is beyond the scope of this review to go into detail, it is important to note that such discoveries are now being translated into several phase II trials that are underway and are employing chloroquine as the autophagy blocking agent of choice. Efficacy in a number of cancer types is being explored, ranging from haematological malignancies to solid adult tumours, prostate and lung cancer (White & DiPaola 2009). The specifics of these trials are summarised in White & DiPaola (2009).

Thus, it would appear that there are a number of important factors within the realm of experimental therapeutic oncophagy that investigators should be aware of. The cell, tissue or tumour type; stage of malignant transformation and nature of treatment employed are important contextual factors of significance. The White et al. group focus on these concepts in their eloquent reviews of autophagy in inflammation and cancer (White & DiPaola 2009, White et al. 2010). They also promote the ‘double-edged’ nature of autophagy by suggesting that autophagy generally promotes cellular survival and an anti-neoplastic housekeeping function that, when defective, is potentially carcinogenic. More specifically, they cite the inability of an impaired autophagic process to clear damaged proteins and organelles, leaving a toxic stimulus within the cellular milieu that may induce genetic instability and ultimately malignant transformation. They go on to suggest that while autophagy blockade may be therapeutically useful in managing progression of malignant disease, it may also be logical and advantageous to induce autophagy as a protective strategy in the early stages of pre-malignant transformation. This could even be considered in a prophylactic context.

The supposed paradoxical nature of autophagy manipulation may therefore be a redundant concept, or rather, it should be recognised that autophagy is a dynamic biological process. Autophagy induction is prophylactic before malignancy evolves, and blockade is therapeutic for established disease, which may well be the mantra of oncophagy as we continue exploration in an attempt to harness this normal physiological process for management of a highly abnormal one.

**Novel therapeutic approaches: the autophagamiR**

While the genetic and molecular basis of autophagy is now coming under increasing scrutiny, there remains a dearth of knowledge regarding the influence of microRNA’s (miRNAs). miRNAs are short (~22nt) non-coding RNAs that affect regulation at a post-transcriptional level through target mRNA suppression (Shenouda & Alahari 2009). This is achieved through recognition of the miRNA response element present in the 3’ UTR domain of the target mRNA. Given the exponential increase in interest regarding miRNA profiles of malignant diseases (and associated mRNA targets), the question regarding miRNA regulation of autophagy within the cancer context requires clarification (Gundara et al. 2011). More specifically, the negative regulatory nature of miRNA function is especially attractive within the domain of oncophagy, given increasing interest in strategies employing blockade of cytoprotective, autophagic flux.

One of the earliest studies considering this issue demonstrated a role for miR-30a targeting of Beclin-1 and subsequently autophagic flux in breast, brain and
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<td>SIRT1 expression induces autophagy; homozygous deletion of SIRT1 in mouse model reduces autophagic flux in association with prostate intraepithelial neoplasia and Gleason grade of disease.</td>
<td>Shen et al. (2011)</td>
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<td>Prostate</td>
<td>Oncogenesis</td>
<td>Examined prostate cancers for ATG5 expression using IHC (n = 107); correlated expression with clinical outcome</td>
<td>ATG5 IHC positivity found in 100% of prostate intraepithelial neoplasia and 90% of cancers; expression did not correlate with tumour size, Gleason grade or stage of disease.</td>
<td>Kim et al. (2011)</td>
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<td>Prostate</td>
<td>Therapy</td>
<td>Treatment of prostate cancer cell lines (DU145 and PC3); effect on cell proliferation and autophagic flux examined</td>
<td>Treatment of DU145 cells with 140 different agents and autophagic flux examined (using GFP-LC3); autophagic flux examined with ATG5-7 knockdown followed by treatment with 80 different autophagy inducers.</td>
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<td>Prostate</td>
<td>Therapy</td>
<td>Treatment of U2OS cells with 140 different agents (DU145 and PC3); effect on cell proliferation and autophagic flux examined</td>
<td>Treatment of U2OS cells with 140 different agents (DU145 and PC3); effect on cell proliferation and autophagic flux examined.</td>
<td>Shen et al. (2011)</td>
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<td>Osteosarcoma</td>
<td>Therapy</td>
<td>Treatment of osteosarcoma cells with 140 different agents and autophagic flux examined (using GFP-LC3); ATG5-7 knockdown followed by treatment with 80 different autophagy inducers.</td>
<td>Evidence of true autophagic flux with 59 different agents and ATG5-7 knockdown followed by treatment with autophagy inducers reduced GFP-LC3 puncta formation in association with an increase in markers of autophagy.</td>
<td>Shen et al. (2011)</td>
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lung cancer cell lines (Zhu et al. 2009). Functional experimentation showed that miR-30a negatively regulated Beclin-1 mRNA and protein expression and subsequently inhibited autophagic flux. This effect was maintained despite attempts to induce autophagy with rapamycin. Unfortunately, the downstream influence on cellular proliferation was not examined in this study, making it difficult to comment further regarding the theory of autophagy blockade as a therapeutic strategy. However, Zou et al. (2012) have recently confirmed this hypothesis by showing that miR-30a inhibition of Beclin-1 can enhance the efficacy of platinum-based chemotherapy in vitro, effectively sensitising cancer cells to therapy through active inhibition of autophagy.

Subsequent to the original studies of Zhu et al., Frankel et al. (2011) have demonstrated that miR-101 negatively regulates autophagy in MCF-7, breast cancer cells. Overexpression of miR-101 was shown to inhibit autophagy, and when knocked down, autophagy induction was observed. Importantly, Frankel et al. were also able to identify several miR-101 targets. This was achieved through an mRNA array study, 48 h post-miR-101 transfection of MCF-7 cells. Following filtering, normalisation, statistical analyses and qPCR validation, 14 potential targets were identified. Those deemed to be of interest within the context of miRNA regulation of autophagy (STMN1, RAB5A and ATG4D) were then knocked down using siRNAs in an attempt to mimic the effect of miR-101 on MCF-7 cells. As hypothesised, knockdown of gene targets inhibited basal levels of autophagy and also blocked rapamycin-induced autophagy to a similar extent to that induced by miR-101 transfection. These studies reinforce the therapeutic benefit of miRNA-mediated inhibition of autophagy.

miR-375 has also been shown to regulate the response to hypoxic stress-induced autophagy (Chang et al. 2012). Using HCC cell lines and mouse model xenografts, Chang et al. demonstrated that hypoxia induced evidence of autophagic flux that was abolished with transfection of a miRNA shown to be down-regulated in HCC cell lines (i.e. miR-375). The ability of cancer cells to employ autophagy as a tumour cell survival strategy was similarly compromised following miR-375 transfection and numbers of viable cells were reduced accordingly. Additionally, a predicted miR-375 target, ATG7, was validated through luciferase reporter experimentation.

Similar findings that support the importance of what are now known as autophagamiRs (Gundara et al. 2011) have been reported more recently, further
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<td>miR30a differential expression with autophagy induction; predicted Beclin-1 target validated with functional experiments; miR-30a negatively regulates Beclin-1 expression and autophagic flux</td>
<td>Zhu et al. (2009)</td>
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<td>Brain</td>
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<td></td>
<td>Array identified significant under + overexpression of miR-9* + miR-206 respectively; pre-miR-9* cell line transfection → cell cycle arrest, reduced DNA synthesis, reduced proliferation, increased apoptosis and elevated LC3B protein expression; conclude that miR-9* transfection reduces cell proliferation possibly through autophagic cell death</td>
<td>Roccaro et al. (2010)</td>
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<td>Lung</td>
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<td>Reduced cell proliferation post-treatment with anti-miR-183 in association with altered LC3B expression and no change in markers of apoptosis</td>
<td>Abraham et al. (2011)</td>
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<td>Imatinib therapy of CML cell lines induces autophagic flux + suppresses miR-30a expression; miR-30a transfection specifically reduces Beclin-1/Atg5 expression and inhibits autophagic flux; miR-30a mimic or Beclin-1/Atg5 knockdown → enhanced imatinib-mediated cytotoxicity and elevated apoptosis; anti-miR-30a → increased autophagic flux and reduces the effect of imatinib; conclude that miR-30a dysregulation (and effect on autophagic flux) may contribute to the efficacy of imatinib-mediated apoptosis</td>
<td>Yu et al. (2012)</td>
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<tr>
<td>Chronic myeloid leukaemia</td>
<td>Therapy</td>
<td>Quantification of K562 cell line and primary CML cell miR-30a expression; functional experimentation using a variety of combinatory treatment with imatinib +/- miR-30a/anti-miR-30a and knockdown of Beclin-1 and Atg5</td>
<td>Imatinib therapy of CML cell lines induces autophagic flux + suppresses miR-30a expression; miR-30a transfection specifically reduces Beclin-1/Atg5 expression and inhibits autophagic flux; miR-30a mimic or Beclin-1/Atg5 knockdown → enhanced imatinib-mediated cytotoxicity and elevated apoptosis; anti-miR-30a → increased autophagic flux and reduces the effect of imatinib; conclude that miR-30a dysregulation (and effect on autophagic flux) may contribute to the efficacy of imatinib-mediated apoptosis</td>
<td>Yu et al. (2012)</td>
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<td>Hepatocellular carcinoma</td>
<td>Oncogenesis</td>
<td>Functional miR-376b experimentation using MCF-7 + Huh-7 cells with autophagy induction (starvation + rapamycin)</td>
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<td>Korkmaz et al. (2012)</td>
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<td>Hepatocellular carcinoma</td>
<td>Oncogenesis</td>
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<td>miR-375 is down-regulated in HCC tissue and cell lines; miR-375 transfection → negatively regulates Atg7, reduces autophagic flux and slows HCC cell line proliferation under hypoxic conditions</td>
<td>Chang et al. (2012)</td>
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validating this concept. Roles for miR-130a and miR-143 have been demonstrated in chronic lymphocytic leukaemia, with autophagy inhibition shown to be affected through repression of validated miR-130a gene targets, ATG2B and DICER1 (Kovaleva et al. 2012). miR-376b has also been shown to inhibit autophagy through ATG4C and BECN1 repression in both breast cancer (MCF-7) and hepatoma (Huh-7) cell lines (Korkmaz et al. 2012). Comparable results have been reproduced by other groups using miR-181a, a result facilitated through ATG5 inhibition of a cisplatin-resistant squamous cell carcinoma cell line (Huang et al. 2012). Furthermore, colon cancer miR-502 under-expression, when ‘replaced’ using both in vitro and in vivo models, has shown evidence of autophagy inhibition and cell death through Rab1B repression (shown to be overexpressed in human colon cancer tissue samples) (Zhai et al. 2012). Lastly, a role for miR-9* regulation of autophagy has also been reported as one mechanism for cell toxicity in a cell culture model of Waldentsrom macroglobulinaemia; a form of B-cell lymphoma (Roccaro et al. 2010).

Huang et al., while demonstrating the link between miR-181a and ATG5, were also able to identify the role of autophagamiR in drug resistance. More specifically, they showed that exposure of a squamous cell carcinoma cells to cisplatin resulted in altered expression of a number of markers of autophagic flux, an effect mediated by p-ΔNp63α-dependent transcriptional regulation. Additionally, miR-181a was also shown to be a downstream p-ΔNp63α effector target. Taken together, these results have revealed some of the first evidence of autophagamiR-mediated drug resistance mechanisms, thus enabling further work geared towards manipulation of these evolving treatment resistance strategies.

Similarly, the studies of Zou et al. have also showed that platinum-based chemotherapy leads not only to evidence of autophagic flux but also to reduced miR-30a tumour expression. Forced expression of miR-30a in cell culture and xenograft-treated cells subsequently resulted in enhanced treatment response, in association with reduced autophagic flux (Zou et al. 2012). Similar themes have been explored by Yu et al. (2012) in chronic myeloid leukaemia, whereby a role for miR-30a-induced autophagic repression in enhancing the response to imatinib therapy (an autophagy inducing insult) has also been proven. These findings reaffirm suggestions of tumour cell drug resistance mechanisms being reliant on autophagy, which, when manipulated through an improved understanding of miRNA regulation, results in enhanced treatment sensitivity.

Table 2 continued

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<td>Colon cancer</td>
<td>Oncogenesis</td>
<td>Functional cell line + xenograft studies employing miR-502 transfection and a number of downstream assays performed</td>
<td>Cell line + xenograft treatment with platinum-based chemotherapy; effect on autophagy and miRNA expression examined; subsequent miR-30a transfection of a breast cancer cell line (MCF-7); effect on cell viability assessed</td>
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<td>Head-neck (SCC)</td>
<td>Oncogenesis</td>
<td>Treated an SCC cell line (JHU029) with cisplatin therapy</td>
<td>Chemotherapy treatment of cell lines / autophagic flux in association with reduced miR-30a expression; translation of miR-30a → reduced autophagic flux; improved treatment response; concluded that miR-30a inhibition of autophagy can sensitize tumour cells to chemotherapy</td>
</tr>
<tr>
<td>Breast</td>
<td>Therapy</td>
<td>Cell line + xenograft treatment with platinum-based chemotherapy; effect on autophagy and miRNA expression examined; subsequent miR-30a transfection of a breast cancer cell line (MCF-7); effect on cell viability assessed</td>
<td>Cell line + xenograft treatment with platinum-based chemotherapy; effect on autophagy and miRNA expression examined; subsequent miR-30a transfection of a breast cancer cell line (MCF-7); effect on cell viability assessed</td>
</tr>
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References:

Zhai et al. (2012)
Huang et al. (2012)
Kovaleva et al. (2012)
Korkmaz et al. (2012)
Huang et al. (2012)
Roccaro et al. (2010)
Zou et al. (2012)
Clearly then, investigation of miRNAs that govern autophagy is well and truly in evolution and the present body of evidence is in favour of miRNA inhibition of autophagic flux as the mechanism of therapeutic benefit. Not only is autophagy an important process within tumour biology but miRNAs are now becoming important players in regulation of what may ultimately be shown to be a fundamental tumour cell survival strategy and one that may even be of importance in acquired drug resistance. The translational impact of these findings is now more obvious than ever and miRNAs may represent the key to clinical application of the principles governing oncophagy. A summary of studies focusing on investigation of the autophagamiR in cancer is detailed in Table 2.

Conclusion

Our philosophical interpretation of the aforementioned findings is of great significance. Focussing too heavily on the inner conflict regarding whether autophagy is pro- or anti-tumour is to lose sight of the ultimate objective, namely, harnessing therapeutic potential regardless of the specific mechanism or method employed. An alternative viewpoint would maintain that these results do not provide contradictory evidence, but rather unveil the theme of manipulating autophagy dynamics within context, meaning that the self-catabolic process should not necessarily be inextricably linked to cell death, but rather be seen as a process that is intrinsic to the cell’s response to any foreign (or native) insult. Autophagy induction has been associated with therapeutic benefit and this may well prove to be an effective strategy, but we must also take advantage of a context in which autophagy is cytoprotective and employ blockade as an alternative treatment strategy. The negative regulatory nature of miRNAs makes them a logical and important candidate for blockade initiation. The overriding challenge, however, lies in identifying which approach to employ, and when.

Declaration of interest

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

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