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Review Article

The role of autophagy in hypoxia-induced radioresistance

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ABSTRACT

Radiotherapy is a widely used treatment modality against cancer, and although survival rates are increasing, radioresistant properties of tumours remain a significant barrier for curative treatment. Tumour hypoxia is one of the main contributors to radioresistance and is common in most solid tumours. Hypoxia is responsible for many molecular changes within the cell which helps tumours to survive under such challenging conditions. These hypoxia-induced molecular changes are predominantly coordinated by the hypoxia inducible factor (HIF) and have been linked with the ability to confer resistance to radiation-induced cell death. To overcome this obstacle research has been directed towards autophagy, a cellular process involved in self degradation and recycling of macromolecules, as HIF plays a large role in its coordination under hypoxic conditions. The role that autophagy has following radiotherapy treatment is conflicted with evidence of both cytoprotective and cytotoxic effects. This literature review aims to explore the intricate relationship between radiotherapy, hypoxia, and autophagy in the context of cancer treatment. It provides valuable insights into the potential of targeting autophagy as a therapeutic strategy to improve the response of hypoxic tumours to radiotherapy.

Introduction

Radiotherapy (ionising radiation; IR) remains a significant treatment modality to fight cancer and is estimated to be used in at least two thirds of treatment regimes in Western countries [1]. Despite the overall survival rate of cancer patients treated with IR has significantly improved, there remains many tumour types which possess radioresistant characteristics. Hypoxia is defined as a reduction in oxygen availability and is a common hallmark within solid tumours because of the chaotic and disordered vascularisation to a rapidly growing cancer. Consequently, a compromised blood supply leads to areas of variable hypoxia. The presence of hypoxia can profoundly affect a tumours aggressiveness as well as dictating the response to treatment modalities, in particular its response to radiotherapy.

The impact of hypoxia inducing radioresistance was first discovered by Gray *et al.* in 1953 [2]. This study demonstrated that severely hypoxic tissue required a radiation dose three times greater than that of normal tissue to generate a similar level of damage. Hypoxia is now a well-established negative prognostic factor for radiotherapy treatment and the mechanisms behind this are still to be fully elucidated [3]. Hypoxia is responsible for molecular changes within the cell and notably the activation of Hypoxia-Inducible Factor (HIF), which is a transcription factor that helps cells survive under low oxygen conditions. HIF acts as a heterodimer, consisting of an oxygen dependent α-subunit (HIF-1α, HIF-2α and HIF-3α) and a constitutively expressed β-subunit (HIF-1β) which is also known as aryl hydrocarbon nuclear translocator (ARNT) [4,5]. HIF is a master transcription factor of the hypoxic response and is an endogenous marker of hypoxia. HIF-1α is constantly expressed by cells however it is subjected to degradation under normoxic conditions. HIF-1α has an oxygen dependent degradation domain (ODDD) and under normoxic conditions hydroxylation occurs via prolyl-4-hydroxylases (PHDs) which subsequently increases the affinity of the protein for the von Hippel-Lindau (VHL) tumour suppressor protein [6,7]. VHL promotes HIF-1α ubiquitination and subsequent degradation by the ubiquitin-proteosome pathway. Additionally, factor-inhibiting HIF (FIH) catalyses the oxygen dependent asparaginyl hydroxylation in the transcriptional activation domain which blocks the interaction of HIF-1α with the p300/CBP transcriptional co-activator proteins [8]. During hypoxia, both PHD and FIH activity is supressed which allows HIF-1α accumulation and subsequent translocation to the nucleus. Here it dimerises with the constitutively expressed HIF-1β protein, generating the HIF complex, which binds to hypoxia response elements (HRE) on

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target genes to stimulate their transcription. Interestingly, it has been estimated that greater than 2% of genes in the human genome are either directly or indirectly regulated by HIF-1α [9]. HIF target genes encompass a variety of biological pathways which enable cellular survival under oxygen deprived conditions. HIF upregulation is associated with many pro-tumorigenic effects and has been proposed to play an important role in tumour therapeutic resistance and subsequent poor patient prognosis [10]. The most well characterised HIF-regulated genes are involved with the regulation of oxygen supply to the cell via angiogenesis, and metabolic reprogramming to shift the cells energy dependence towards glycolysis [11]. In addition, other important HIF target genes are involved in apoptosis, proliferation, redox homeostasis, metastatic potential and notably autophagy [11]. Autophagy is a well-known cellular survival mechanism which is active under hypoxic conditions. Autophagy is a process by which cells degrade and recycle their own components. As a consequence, autophagy has been implicated in the radioresistance of hypoxic tumours and targeting this pathway may therefore represent a potential strategy for overcoming hypoxia-induced radioresistance in cancer therapy, which is a focus of this review.

Autophagy mechanism

Autophagy is an evolutionary preserved process which can be termed as 'self-eating', where the cell digests proteins and damaged organelles to maintain cellular homeostasis. There are three known types of autophagy: microautophagy, chaperone-mediated autophagy and macroautophagy [12]. Microautophagy refers to the direct engulfment of cytoplasmic contents into lysosomes, which are then degraded by lysosomal hydrolases. Chaperone-mediated autophagy is a highly specific process in which proteins are recognized and targeted for degradation [12]. The most studied type is macroautophagy, hereafter referred to as autophagy, which involves the formation of cytosolic vesicles which fuse together with lysosomes for degradation [12]. Autophagy responds to a variety of cellular stresses such as organelle damage, nutrient deprivation, abnormal protein accumulation, hypoxia and IR. Although autophagy is generally considered a protective mechanism, excessive autophagic events can actually lead to cell death [13].

Autophagy is characterised by the sequestration of target cellular components via the formation of a double membrane vesicle known as the autophagosome (Fig. 1). Autophagosome initiation occurs via a complex of unc-51-like kinase (ULK1), ATG13 and 200 kDa focal adhesion kinase family-interacting protein (FIP200); ULK:ATG13:FIP200 [14]. The kinase mammalian Target of Rapamycin (mTOR) is a key inhibitor of autophagy, where It acts by phosphorylating ULK and preventing the complex activation [14]. The ULK:ATG13:FIP200 complex activates the downstream regulatory complex consisting of vesicular protein sorting 34 (VPS34), BECLIN1 and VPS15 [14,15]. Other regulatory proteins are involved, namely Activating molecule in BECLIN1 autophagy protein (AMBRA1), ATG14L, and Ultraviolet

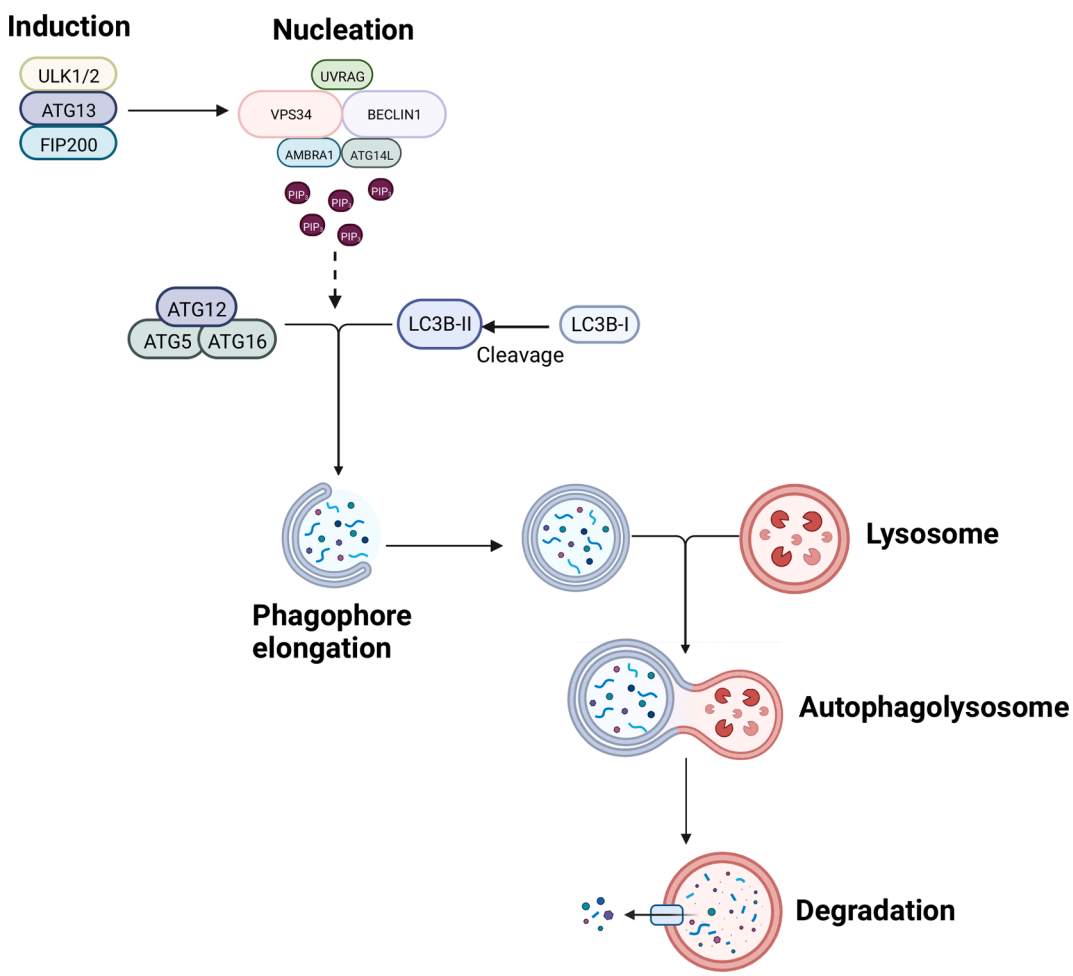

Fig. 1. Autophagy induction begins with the activation of the ULK1/2, ATG13 and FIP200 complex, which then activates the downstream nucleation complex composed of UVRAG, VPS34, BECLIN 1, AMBRA1 and ATG14L. This whole complex generates phosphatidylinositol-3-phosphate (PtdIns3P) required for autophagosome formation. Autophagosome formation is reliant ATG12-ATG5-ATG16L recruiting LC3B-II to the site of membrane elongation. The autophagosome then fuses with lysosomes to form an autophagolysosome which subjects the contents to degradation. Figure created with BioRender.com.

irradiation resistance associated gene (UVRAG) [16]. This VPS34: BECLIN1:VPS15 complex is responsible for generating phosphatidylinositol-3-phosphate (PtdIns3P) required for autophagosome formation.

Autophagosome formation is dependent on the ATG12-ATG5-ATG16L complex which recruits the microtubule-associated protein 1 light chain 3-II (LC3B-II) to the site of membrane elongation [17]. LC3B-II is created by ATG3 and ATG7 activating LC3B-I and conjugating it to phosphatidylethanolamine (PE). The exact mechanism involving the fusion of the autophagosome with lysosomes to generate autophagolysosomes is unclear. However, some key membrane proteins have been identified, which include RAB7, lysosomal membrane associated proteins (LAMPs) and soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors (SNAREs) [18]. Furthermore, new evidence has emerged that autophagy may be more selective than previously thought, when orchestrated by p62 (Sequestosome 1/SQSTM1). p62 has been identified as an important protein that is phosphorylated by ULK1, which then recognises ubiquitinated proteins and subsequently brings them to the autophagosome for degradation via the autophagy process [19,20].

Autophagy and cancer

The role that autophagy plays in cancer can be described as a double-edged sword, as it has been identified as acting as both a tumour suppressor and a tumour promoter. Basal levels of autophagy exist in all cells and is beneficial in preventing tumour development. Autophagy plays a role in preventing genotoxic stress, such as through reactive oxygen species (ROS), and thus to prevent the accumulation of mutations and genetic instability. Furthermore, autophagy removes damaged organelles which again prevents damage accumulation and maintains cellular homeostasis [21]. Heterozygous loss of BECLIN1, a key player in the promotion of autophagy, has been shown to promote tumorigenesis in mice and BECLIN1 gene deletions are common in breast, ovarian and prostate cancers [22]. Furthermore, BECLIN1 levels have been shown to be decreased in glioblastoma (GBM), ovarian cancer and liver cancer, which suggests that autophagy plays an important role in preventing tumorigenesis [23–25]. Additionally in breast cancer cells, an overexpression of BECLIN1 has been demonstrated to prevent proliferation and clonogenicity of the cells and inhibited tumour development in nude mice [26]. Aside from BECLIN1, it has also been reported in mouse knockouts of both ATG5 and ATG7 that there was an increase in development of liver cancer due to a deficiency in autophagy and subsequent increase in oxidative stress [27]. These studies suggest that autophagy plays an important role in preventing tumour development.

Interestingly, there is alternative evidence that once the tumour is established, autophagy may actually play a role in sustaining and promoting cancer cell proliferation. Tumour cells are regularly exposed to adverse conditions and autophagy can help to support proliferation through recycling macromolecules to help fuel the cell as well as preventing damage accumulation [28]. However, it should be noted that some cancer cells have higher than normal basal levels of autophagy which could act as a mechanism to fuel their high metabolic demands [28]. Therefore, autophagy is likely to have multiple roles in tumour survival. Autophagy has also been linked with aiding cancer cell migration and invasion particularly in hepatocellular carcinoma, as well as helping to maintain cancer stem cell properties in breast cancer cell lines [29,30]. Greater LC3B-II expression has been shown in colorectal tumour tissue compared to adjacent normal tissue and LC3B-II expression was correlated with a more aggressive tumour [31]. There is evidence that some cancers may rely on autophagy more than others and are said to be autophagy dependent [32]. It has been shown that cancers which have a mutation in the RAS pathway are particularly associated with having an increased reliance on autophagy to maintain high metabolic demands [33]. For example, pancreatic cancer is known to have high mutation rates in the RAS pathway which has been subsequently linked with increased activity of autophagy promoting transcription factors [34]. However even within the same cancer types there is a lot of heterogeneity in the reliance on autophagy for survival. shRNA knockdown of autophagy regulators in a panel of breast cancer cell lines revealed that some cell lines relied on autophagy more than others to survive [35]. These data demonstrate the complexity of autophagy regulation in cancer and indicates that some cancers rely more heavily on autophagy for survival which could have implications for autophagy modulation. As well as aiding survival, autophagy has also been linked with promoting therapy resistance. For example, BECLIN1 expression was found to be associated with poor survival in colorectal patients receiving fluorouracil (5-FU) [36]. Additionally, in oesophageal cancer cells and following cisplatin treatment, BECLIN1 was observed to be upregulated which increased autophagy thus acting as a protective mechanism [37].

Radiotherapy

Radiotherapy is frequently used in the treatment of cancer, either alone or in combination with chemotherapy and/or surgery [38]. Radiotherapy commonly utilises low-LET photons that leads to the therapeutic effect largely through DNA damage [39]. At a cellular level DNA damage from IR occurs through two mechanisms: direct and indirect damage. Direct damage occurs when IR directly interacts with DNA causing disruption to the molecular structure of DNA [40]. Indirect damage is the predominant cause of IR-induced DNA damage and occurs when IR interacts with water molecules which produces free radicals such as hydroxyl ($^{\bullet}$HO) and alkoxy ($^{\bullet}$RO2) radicals. These free radicals interact with DNA molecules causing damage in the form of oxidised DNA bases, single and double strand breaks, as well as clustered/complex DNA damage which contains multiple DNA lesions in close proximity which is a hallmark of IR exposure and contributes to IR-induced cell death [41]. The presence of oxygen at the point of irradiation has a significant impact on the effectiveness of radiotherapy treatment, as demonstrated by the fact that hypoxic tumours require a radiation dose three times higher than that of comparative normoxic tumours to achieve similar damage, this increase is described as the oxygen enhancement ratio (OER) [2]. There are primarily two different aspects by which hypoxia contributes to radioresistance. The first is explained by the oxygen fixation hypothesis (OFH). Here, IR induces the production of DNA radicals, either by direct or indirect ionisation generated from water radiolysis, and in the presence of oxygen the indirect DNA damage becomes fixed. This prevents the chemical restitution of the DNA lesions which can ultimately lead to cell death [42]. Conversely, in the absence of sufficient oxygen, these lesions can be restituted therefore decreasing DNA damage and cell death. Experiments performed in cells, yeast and bacteria have shown a relatively similar general OER curve which suggests a roughly hyperbolic relationship with oxygen tension [43–45]. This concept is important in radiobiology allowing the potential increase in radiotherapy efficacy up to threefold. While the OFH provides a clear mechanistic explanation for hypoxia-induced radioresistance, it is acknowledged that the complete picture is more intricate. It is argued that OFH does not fully explain the physiological processes, and the complex molecular pathways that induce radioresistance, particularly at milder levels of hypoxia. Consequently, strategies to overcome hypoxic radioresistance (e.g. nimorazole and carbogen), have yielded disappointing results [10]. Despite this, the importance of HIF in cellular responses to hypoxia is unquestionable and therefore a significant number of studies continue to investigate HIF-dependent radioresistance mechanisms [46]. One interesting and promising avenue that is being explored is the role of autophagy in the context of hypoxia-induced radioresistance.

Autophagy induced by tumour hypoxia

Autophagy is particularly prevalent in tumour cells located in the

hypoxic centre, as opposed to cells at the periphery, indicating a relationship between hypoxia and autophagy [47,48]. Furthermore, in a variety of cell lines including, GBM, hepatocellular carcinoma, oral keratinocytes and cervical cancer, autophagy was shown to be induced at 1% oxygen [48–50]. The link between autophagy and its role as a survival mechanism in hypoxia has been demonstrated previously in a variety of cell lines (cancer and normal cells), where both 1% and 0.1% oxygen induced autophagy without triggering cell death [51]. Furthermore, studies in colorectal cancer cells have shown that inhibiting autophagy under hypoxic conditions induces cell death via apoptosis [48,52,53]. Therefore, autophagy may play a vital role in promoting cell survival and preventing apoptosis under hypoxia. Moreover, it is known that cells under hypoxia cannot maintain adequate antioxidant capacity which leads to ROS accumulation. Conversely, autophagy has been suggested as an important mechanism to prevent ROS accumulation, through eliminating damaged mitochondria via a process termed mitophagy [54].

HIF-dependent autophagy regulation

HIF-1α has been particularly linked with playing an important role in promoting hypoxia-induced autophagy in cancer cells [52,53,55]. An important target gene of HIF-1α is BCL-2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3), which is involved in autophagy coordination. BNIP3-induced autophagy was originally thought to act as a cell death mechanism, however more recent studies in mouse embryo fibroblasts (MEFs) have revealed its role as an adaptive survival mechanism following hypoxic exposure [56]. BNIP3L (Bcl-2/adenovirus E1B 19 kDa interacting protein 3 like, also known as NIX) has been identified as a homolog of BNIP3 that shares over 50 % sequence similarity with BNIP3. BNIP3L regulation is also hypoxia dependent and predominantly driven by HIF-1α [57]. The purpose and interplay between these two proteins in hypoxia-dependent autophagy regulation remains to be fully understood. However, and in order to achieve full ablation of autophagy, knockdowns of both BNIP3 and BNIP3L are required, indicating redundancy and overlap in function between the two proteins [58]. The exact mechanism of both BNIP3 and BNIP3L inducing autophagy is still to be fully elucidated, however more recently it has been suggested that they compete with the binding of BCl2 to BECLIN 1, thus freeing BECLIN1 to induce autophagy [59]. Additionally, HIF-1α may coordinate autophagy independent of BNIP3 and BNIP3L. It was shown in triple negative breast cancer cells that HIF-1α increased the expression of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), which promoted the invasion and proliferation of the cells by activating autophagy [60].

HIF-independent autophagy regulation

Although HIF plays an important role in autophagy regulation, there is also evidence of HIF-independent mechanisms under hypoxic conditions. p62, as a transporter for polyubiquitinated proteins to be subjected to degradation via autophagy, has under hypoxic conditions been shown to be downregulated due to autophagy-induced clearance, whereas autophagy inhibitors prevented this reduction [61]. This increase in autophagy under hypoxia was shown to be independent of HIF expression, demonstrating that the regulation of autophagy under hypoxia is multifaceted [61]. Furthermore, hypoxia has been shown to lead to the oxygen-sensitive demethylation of ULK1, leading to the downstream phosphorylation of Beclin 1 and ATG13 [49]. As previously mentioned, mTOR is an important autophagy regulator [62] and under metabolically normal states this associates with the ULK1-ATG13 complex rendering its activity and prevents the activation of the downstream autophagy proteins [63]. However, under hypoxic conditions, the cells are in a nutrient deprived state and the metabolic state of the cells switches to a glycolytic metabolic pathway. As a result, the mTOR pathway is deactivated, due to reduced signalling from the upstream mitogen activated protein kinase (MAPK) and phosphoinositide3 kinase (PI3K) pathway, and it dissociates from the ULK1/ATG13 complex subsequently triggering autophagy [63].

Another HIF-independent process of autophagy induction is through the unfolded protein response (UPR). There have been several reports that indicate that the UPR helps hypoxic tumour cells to carry out autophagy. The endoplasmic reticulum (ER) is an important organelle involved in the maturation of proteins and under conditions of ER stress, such as hypoxia, an accumulation of misfolded proteins occurs which activates the UPR to maintain ER homeostasis [64]. The UPR is initiated by three key sensors inositol-requiring protein 1 (IRE1), protein kinase RNA-like ER kinase (PERK) and activating transcription factor 6 (ATF6) [65]. PERK is involved with mediating the transcriptional activity of activating transcriptional factor 4 (ATF4) and CCAAT/enhancer-binding protein-homologous protein (CHOP) which increase the transcription LC3B and ATG5 [66]. Although LC3B and ATG5 are not required for activation of autophagy, it has been observed that the replenishment of these proteins is required to maintain hypoxia-induced autophagy. For example, it was shown that hypoxic cells deficient in PERK signalling failed to maintain LC3B levels sufficient to sustain hypoxia induced autophagy [66]. Overall, it is clear that hypoxia increases autophagy and although HIF plays a major role there are HIF-independent mechanisms of autophagy which work to maintain cellular homeostasis under such conditions.

Autophagy and hypoxia induced radioresistance

Many studies have shown that autophagy is induced in response to IR, however the exact role that autophagy plays in the cellular radiation response remains controversial [67–71]. As highlighted above, autophagy can either be a cell survival or a cell death mechanism, and the same principles follow in response to IR. A number of different factors, including cell/tumour type and dose/type of radiation, will contribute to the possible role and effect of autophagy post-irradiation. Aside from DNA damage, IR induces oxidative stress which in turn can cause protein misfolding, endoplasmic reticulum stress and compromised mitochondrial function, all of which are known to induce autophagy [72]. Conversely, cells have an increased radioresistance under hypoxic conditions, therefore autophagy may be a factor contributing to this increased survival. Indeed, the use of chloroquine (CQ), an autophagy inhibitor, has been shown to radiosensitise lung cancer and osteosarcoma cell lines under hypoxia [73,74]. Furthermore, in breast cancer cell lines under hypoxia, siRNA-mediated knockdown of BECLIN1 radiosensitised cells due to a delay in DSB repair, evident through γH2AX foci analysis [75]. However, there is limited evidence to suggest that hypoxia-induced autophagy may lead to cell death following IR. One example is in renal carcinoma, where autophagy was shown to induce radiosensitisation under hypoxia with a similar response found in hypoxic breast cancer cells treated with an mTOR inhibitor [76]. However, caution should be taken as this is a small body of evidence. Additionally, as mentioned autophagy is upregulated under hypoxia, therefore autophagy inhibition may contribute to hypoxic radiosensitisation by selectively killing hypoxic tumour cells. Thus, reducing the hypoxic fraction of the tumour rather than altering the intrinsic radioresistance of the cells [66].

It has been reported that under hypoxic conditions, HIF-1α may be a key factor coordinating the radioprotective autophagic response (Fig. 2). A positive correlation was found in osteosarcoma cell lines between HIF-1α and LC3B-II, which promoted autophagy and protected the cells from radiotherapy-induced ROS [74]. In colon cancer cells, HIF-1α has been shown to be responsible for inducing autophagy which increased the radioresistance of these tumour cells [77]. Similarly, in a breast cancer cell line, silencing of HIF-1α increased radiosensitivity due to a reduction in autophagy [78]. Mechanistically, HIF-1α contributes to autophagy activation and subsequent radioresistance through the transcription of genes such as BNIP3 or BNIP3L. BNIP3 was shown to

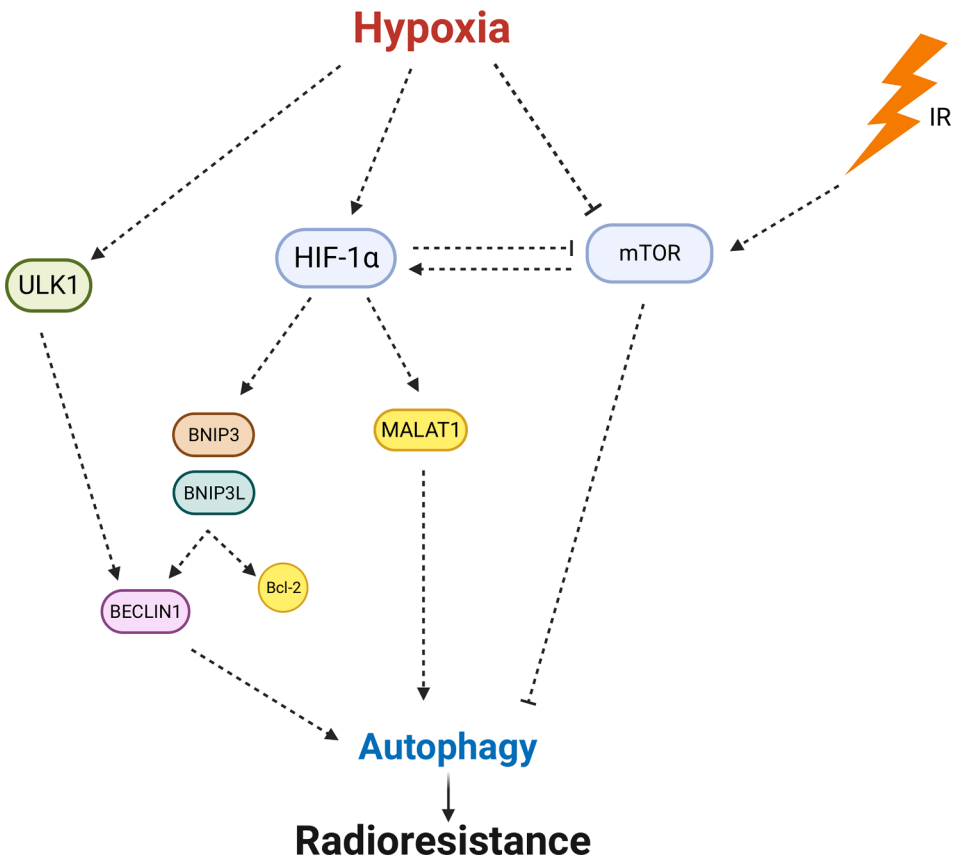

Fig. 2. A schematic diagram of the potential relationships and interplay between hypoxia, IR and autophagy with solid arrows representing activation and flat arrows representing inhibition. Figure created with BioRender.com.

promote autophagy under hypoxia and induce radioresistance in a variety of cancer cell lines [79]. It is hypothesised that BNIP3 and BNIP3L disrupts the BCL-2/BECLIN1 1 complex, which releases BECLIN1 to induce autophagosome development, as well as inhibiting the mTOR pathways (Fig. 2) [51,80]. HIF-1α signalling pathway has been shown to promote BNIP3 dependent autophagy and prevent apoptosis in liver cancer cells [81]. Additionally, evidence suggests that HIF-1α can promote the expression of BECLIN1 which leads to increased radioresistance in lung cancer cells [73]. In oesophageal cancer, the protein butyrophilin subfamily 3 member A1 was shown to be a direct target of HIF-1α which induces autophagy via the phosphorylation of ULK1 and this promoted radioresistance [82]. Overexpression of HIF-1α has been demonstrated to inhibit mTOR and activate autophagy which promoted radioresistance in mesenchymal stem cells [83].

The complex relationship between HIF and mTOR following radiotherapy treatment is an area needing further research. It is known that following IR the mTOR pathway is activated which in turn leads to increased expression of HIF-1α. Despite this, the two pathways have different roles in autophagy activation, with mTOR inhibiting autophagy whereas HIF-1α activates the pathway. It has also been found that HIF-1α works as a negative regulator of the mTOR (Fig. 2) [84]. The relationship between mTOR and HIF-1α could be deciding factor as to whether the cell utilises autophagy as a protective or cell death mechanism, but which will no doubt be dependent on factors such as the radiation conditions and the cellular background.

Clinical studies

Based on preclinical evidence suggesting that the modulation of autophagy can improve radiosensitivity in specific types of cancer, several clinical trials have been conducted as a strategy to enhance radiotherapy efficacy. It should be noted though, that these studies primarily focus on evaluating modulation of autophagy to improve radiosensitivity, and do not directly assess hypoxia-induced radioresistance [85,86]. However, it is possible to extrapolate the results of these trials to potentially evaluate hypoxia-induced radioresistance and autophagy modulation [85–89]. This is because the effects of autophagy modulation on radiosensitivity and radiotherapy efficacy affects common pathways linked to hypoxia such as the PI3K-AKT-mTOR pathway [90], but also these trials have been performed in tumours which are well documented to develop areas of hypoxia [91].

The majority of current clinical studies that have investigated modulation of autophagy in radiotherapy treatment have assessed patients with glioblastoma, notably characterised by a high degree of hypoxia [86–89,92,93]. As previously mentioned, CQ and its derivative hydroxychloroquine (HCQ), can act as autophagy inhibitors and are an exciting prospect since they are already licensed for use as anti-malarial agents making them an attractive target for drug repurposing. A 3 + 3 phase I clinical trial of patients with GBM has been performed to determine optimum dosing of HCQ prior to radiotherapy [89]. This was followed by a non-comparative phase II trial to assess HCQ in combination with radiotherapy and TMZ, however their results did not demonstrate improved survival compared to a historical cohort of patients. Autophagy inhibition was assessed with electron microscopy (EM)-based scoring to evaluate the number of autophagic vacuoles on serially collected peripheral blood mononuclear cells. This revealed that the dosage of HCQ used in the phase II trial was unreliable at achieving autophagy inhibition in all patients, which is a major limitation. However, it suggests that there is a call for further research to find a suitable dosage regime to achieve autophagy inhibition in humans.

To date, there have been two randomised controlled trials assessing radiotherapy with the addition of HCQ in GBM. Despite only acquiring

small sample sizes, both trials found significantly higher survival in patients who received CQ (average 24 vs 11 months and 33 vs 11 months, respectively) [94,95]. Smaller non-randomised clinical trials, giving patients with brain metastasis, CQ or HCQ in combination with radiotherapy has also yielded positive results [88,96]. Rojas-Puentes *et al.* randomised seventy-tree patients to whole brain radiotherapy with concomitant CQ (150 mg) for four weeks or placebo [88]. The progression-free survival of brain metastases rates at one year were 83.9% (95% CI 69.4–98.4) for the CQ arm and 55.1% (95% CI 33.6–77.6) for the control arm. These results warrant further clinical trials of CQ and HCQ to improve radiotherapy success.

At present, clinical trials focusing on the modulation of autophagy to enhance radiotherapy efficacy do not consider the intricate interplay between autophagy, hypoxia, and radiotherapy. However, if this can be further understood, there exists several existing licensed drugs that are known to modulate autophagy which could be repurposed for use in combination with radiotherapy. A list of autophagy inducing and autophagy inhibiting drugs are outlined in Table 1 and Table 2, respectively (covered more extensively in [97]). The most investigated drugs for autophagy modulation combined with radiotherapy are CQ and HCQ because they are readily available, FDA approved and relatively cheap (discussed above). However, there are active lines of research investigating metformin [98], brigatinib [99], and nelfinavir [100]. These investigations although promising are still very much in their infancy and at a preclinical stage. Repurposing drugs for combination therapy with radiotherapy allows for the exploration of novel treatment strategies without compromising patient safety. Indeed, having already been approved for other indications, drug safety profiles and potential side effects are well-documented. This knowledge can guide researchers in determining appropriate dosage, timing, and potential interactions with radiotherapy, ultimately improving patient outcomes and minimizing risks. Finally, it could potentially provide more cost-effective treatment options, considering identifying or developing new drugs can be an extremely lengthy and expensive process, involving significant research, preclinical studies, and clinical trials.

Conclusions

Autophagy is a highly co-ordinated and complex process which is poorly understood in the context of cancer development and treatment. It is likely that the role autophagy plays in cancer is multifactorial and may evolve during the natural history of a cancer. This corresponds with the fact that all tumours will undergo a degree of autophagy, however not all are resistant to IR. Certain factors that influence the process of autophagy and how this responds to radiotherapy treatment include, stage of cancer (early vs late), degree of hypoxia, type of cancer radiation type and dose/dose rate. In late-stage hypoxic cancers, autophagy seems to correspond negatively to treatment outcomes. Future research will need to explore whether autophagy inhibitors can be personalized based on the specific role autophagy plays in individual cancers. Additionally, the specific mechanisms linking autophagy, hypoxia, and radiotherapy require further investigation, and there are exciting avenues for future preclinical exploration in this area. Thus, gaining a deeper understanding of the role of autophagy in cancer and the relationship to the radiotherapy response could have significant implications for improving treatment outcomes and ultimately improving patient survival rates.

CRediT authorship contribution statement

Rhianna M. Hill: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Matthew Fok:** Investigation, Writing – original draft, Writing – review & editing. **Gabrielle Grundy:** Writing – review & editing, Supervision. **Jason L. Parsons:** Writing – review & editing, Supervision, Funding acquisition. **Sonia Rocha:** Conceptualization, Writing – review & editing, Supervision, Project administration.

Table 1
FDA approved drugs with autophagy inducing properties.

Drug	Current use	Mechanism	References
Artesunate	Anti-malarial treatment	Modulates mTOR pathway	[101]
Brigatinib	Non-small cell lung cancer treatment	Increasing endoplasmic reticulum stress	[102,103]
Chlorporthixene	Antipsychotic medication	Upregulate levels of ATG12 and BECLIN1	[104]
Loperamide	Antipsychotic medication	Modulates mTOR pathway and ATG5 and ATG7 proteins	[105]
Metformin	Diabetes	Modulates mTOR pathway	[106]
Nelfinavir	HIV treatment	Increasing endoplasmic reticulum stress and modulates mTOR pathway	[107,108]
Niclosamide	Antihelminthic treatment	Induces autophagosome formation and modulates mTOR pathway	[109,110]
Prazosin	High blood pressure treatment	Modulates mTOR pathway	[111]

Table 2
FDA approved drugs with autophagy inhibiting properties.

Drug	Current use	Mechanism	References
Amlexanox	Mouth ulcers	Decreased LC3B-II production	[112]
Benproperine phosphate	Cough suppressant	Autophagic flux suppression	[113]
Chloroquine (CQ and HCQ)	Prevention and treatment of malaria	Prevents autophagosome and lysosome fusion	[114,115]
Chlorpromazine	Antipsychotic medication	Inhibits mTOR pathway and prevents autophagosome lysosome fusion	[116,117]
Ferroquine	Anti-malarial treatment	Prevents autophagosome and lysosome fusion	[118]
Fingolimod	Disease modifying therapy for multiple sclerosis	Autophagic flux suppression	[119]
Thioridazine	Antipsychotic medication	Prevents autophagosome and lysosome fusion	[120,121]
Trifluoperazine	Antipsychotic medication	Prevents autophagosome and lysosome fusion	[122,123]

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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