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Targeting EGFR-mediated autophagy as a potential strategy for cancer therapy

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Mini Review

Autophagy is a naturally occurring programed cellular catabolic process stimulated by cellular stress for energy homeostasis maintenance and elimination of harmful substances. It mostly works as pro-survival mechanism but on the other hand deregulation of autophagy has been linked to non-apoptotic cell death known as "type II programed cell death." Emerging evidences indicate that EGFR (epidermal growth factor receptor)-mediated RAS/RAF/MEK/ERK signaling pathway plays a critical role in the induction of autophagy in various tumors. It has further been established that this signaling pathway is also involved in several other anti-proliferative events such as apoptosis and senescence. However, the signaling pathway activity and effects are highly dependent on the cell type and the stimulus. It is currently being evident that autophagy induction by RAS/RAF/MEK/ERK pathway through small molecules may be a potential therapeutic strategy for cancer. However, to our best knowledge, the role of EGFR-mediated RAS/RAF/MEK/ERK signaling pathway in autophagy-mediated cell death and survival have not previously been reviewed. In this review, we discuss the current state of knowledge on how RAS/RAF/MEK/ERK signaling pathway regulates autophagy and the role of this EGFR-mediated autophagy in diseases. We further examine the cross-talk between this EGFR-mediated autophagy and apoptosis as well as how this process is currently being utilized for cancer treatment and suggest promoting autophagy-related cell death by small molecules may be exploited to design better therapeutic strategies for early stage and locally advanced tumors.

Introduction

Over the past decade, targeting specific molecular pathways responsible for cancer proliferation and survival has become the most important strategy for cancer treatment. This strategy has a lot more advantage over the non-selective cytotoxic chemotherapies.[1] The epidermal growth factor receptor (EGFR) signaling pathway is one of the major players in the regulation of growth, survival, proliferation and differentiation in mammalian cells.[2,3] Recent researches indicate that EGFR implicates in a wide range of responses, as wide as from cell division to cell death, from motility to adhesion.[4] However, these responses vary depending on the duration, the magnitude and the sub-cellular localization of this receptor and its downstream effector kinases.[5,6] Since EGFR was first implicated in cancer, it remains one of the best investigated signaling pathway and is an important target for cancer treatment.[7] Indeed, accumulating evidences have demonstrated that most epithelial cancers are EGFR-driven.[8,9] Therefore, most of strategies for cancer treatment are focused on this receptor and its tyrosine kinase activity.[10,11] Unfortunately, the therapeutic benefit of these treatment strategies is limited by fast development of resistance.[8] Among other mechanisms by which many tumors with EGFR mutation gain this resistance is autophagy suppression through EGFR-mediated Beclin 1 (BECN 1) phosphorylation.[12] Another established discovery is that all the EGFR downstream signaling pathways are involved in autophagy modulation.[8]

Molecular markers that predict the positive response of patients to EGFR therapy, such as manipulation of autophagy for enhanced treatment response are increasingly being discovered.[13] EGFR have been discovered as one of the determinants of whether autophagy results in cell survival or death.[14] Sustained activation of RAS/RAF/MEK/ERK pathway can result in autophagic cell death.[15] It has been extensively demonstrated that EGFR inhibits BECN 1 so how does the EGFR/RAS/RAF/MEK/ERK induce autophagic cell death?

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Therefore with this question and more, we discuss the role of autophagic cell death mediated by EGFR/RAS/RAF/MEK/ERK signaling in cancer therapy.

Epidermal Growth Factor Receptor (Fig. 1)

The EGFR is a transmembrane receptor tyrosine kinase which belongs to the ErbB family of receptor tyrosine kinases (RTK) including HER2/ErbB2/c-neu, HER3/ErbB3 and HER4/ErbB4.[3] EGFR has also been identified in the nucleus, endosomes and mitochondria and these different sub-localization may exert different functions.[16] The EGFR is activated by EGF, transforming growth factor alpha (TGF-α), heregulin and heparin binding EGF like growth factor (HBEGF).[17,18] Lemmon et al. reported that two EGF molecules additively contribute to stabilize the EGFR dimmer, which lead to trans-auto-phosphorylation of its cytoplasmic domains.[3] The phosphorylated tyrosine residues serve as a docking site for adaptor molecules to promote the activation of many signaling pathways including the MAPK pathway, the PI3K/AKT pathway, the JAK/STAT signaling pathways.[3]

Under normal nutrient-rich conditions, activation of EGFR is involved in the regulation of cell survival, proliferation and differentiation.[19] Overexpression or activating mutations of EGFR leads to poor prognosis of solid tumors due to increased cell proliferation, apoptosis and autophagy inhibition.[10] However, a number of small autophagy inducing molecules have been observed to utilize this overexpressed EGFR against the tumors through induction of RAS/RAF/MEK/ERK-mediated autophagic cell death.[20,21]

Autophagy, Autophagic Flux and Autophagic Cell Death (ACD)

Based on the method of target substance capture and delivery to lysosome, autophagy can be separated into macroautophagy, microautophagy[22] and chaperone-mediated autophagy.[23] Macroautophagy (the focus of this review) is the highly regulated cellular self-digestion, whereby some components of the cell are sequestered and delivered to the lysosome for degradation.[20,24] Autophagic flux refers to the complete process from induction to lysosomal degradation.[25] An understanding of these two obviously interconnected terms has a great impact in the decision of whether to inhibit or enhance autophagy to affect cell death in cancer.[26–28] There are currently approximately 10 identified autophagy-related (ATG) genes in mammals which function at four different steps of autophagy which are extensively discussed in several reviews.[29–37] At excessive levels of autophagy and/or autophagic flux may lead to autophagic cell death.[20] Moreover, there is extensive cross-talk between autophagy and other forms of cell death, such as apoptosis and necrosis.[38,39] Therefore, the effect of autophagy on cell death may also depend on its cross-talk with apoptotic and/or necrotic pathways.[17,21]

Autophagic cell death has been observed in several tumors as a cell death mechanism accompanied by extensive cytoplasmic vacuolization and correlated with increased autophagic flux.[20,24] Therefore, autophagic cell death is restricted to cases where ablation of any autophagy regulating protein (ATG) inhibits cell death. Autophagic cell death is relevant and important as most human tumor cells frequently contain mutations rendering them resistant to apoptosis, and therefore having increased dependency on autophagy for self-distraction.[14,40] Our group has also recently reported that EGFR-mediated autophagy by activation of RAS/RAF1/MAP2K/MAPK1/3 pathway can precede apoptosis-dependent death of gastric cancer cells.[21]

Overview of Autophagy Regulation and the Signaling Pathways (Fig. 2)

The phosphatidylinositol-3-kinase/mammalian target of rapamycin 1 (PI3K/mTOR1) signaling pathway is one of the key pathways in mammalian autophagy.[34] mTOR is a regarded as a master regulator of autophagy due to its energy sensing function.[41] Several mechanisms of autophagy proteins regulation have been well characterized at the transcriptional, post-transcriptional and post-translational level.[42] EGFR and all its downstream signaling pathways have also been demonstrated to modulate autophagy with their effect dependent on their sub-cellular localization.[17,18] Not only the EGFR has been reported to regulate autophagy,[12] but also the AKT/mTOR pathway and STAT3 pathway are involved in the regulation of autophagy.[43] Importantly, recent researches have revealed that RAS/RAF/MEK/ERK signaling pathway is involved in the modulation of autophagy.[15,20] Furthermore, the eukaryotic initiation factor 2α (Eif-2α) kinase Gcn2 and its downstream target Gcn4, a transcriptional transactivator of autophagy genes, also regulates autophagy, turning it on during nutrient depletion periods.[44]

EGFR and Autophagy Process (Fig. 3)

Emerging evidences indicated EGFR as a crucial determinant on whether autophagy results in cell death or survival.[14] It is becoming progressively clear how crucial autophagic cell death is for cancer therapy especially due to increasing resistance of tumors to apoptosis.[45] Indeed, several lines of evidences indicated that EGFR can be found not only on the cell surface membrane but on endosomes, nucleus as well as on the mitochondria.[16,18] This EGFR sub-cellular localization as well as the kinase-active or -independent role gives varied effects on its autophagy modulation.[16]

EGFR subcellular localization effect on autophagy

Following ligand stimulation, EGFR phosphorylation and dimerization, the EGFR is internalized through clathrin-mediated endocytosis (CME) as well as clathrin-independent routes.[46] There is a consensus that this internalization of EGFR is the reason behind the different localization of the receptor. Many studies have further revealed that varied receptor activity is also observed in these different sub-cellular localization.[47,48] Therefore, the roles of EGFR-mediated autophagy are dependent on its location in cells.[18]

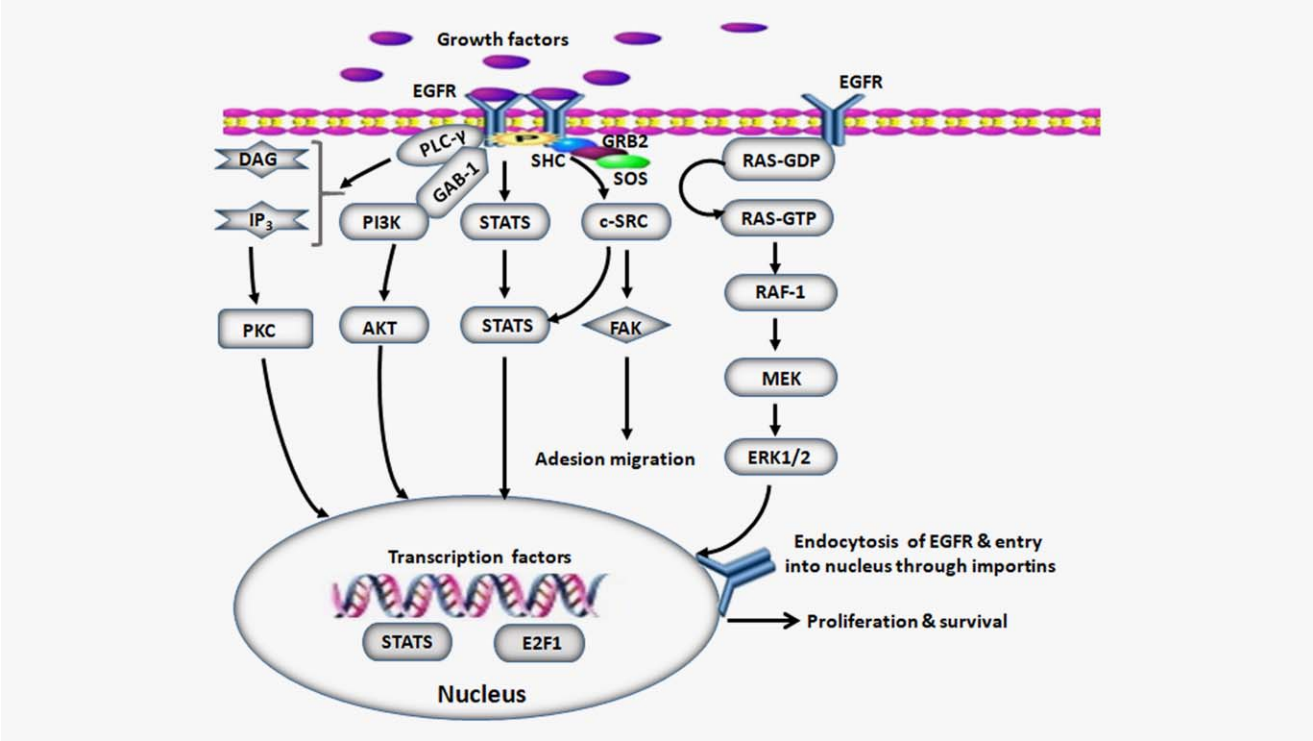

Figure 1. Epidermal growth factor receptor (EGFR) pathways and activity: the EGF,TGF-α and other molecules that stimulate EGFR binds to it, causing phosphorylation of the some tyrosine sides on the intracellular domain thus creating phosphotyrosine docking sides appropriate for small molecules like Shc to bind and results in the RAS/RAF1/MEK/ERK pathway, STAT pathway as well as the PI3K/AKT pathway that ultimately activates the transcription factors in the nucleus and results in proliferation and survival of the cell. [Color figure can be viewed at wileyonlinelibrary.com]

For example, Wei *et al.* have proven that active plasma membrane EGFR (pEGFR) phosphorylates the autophagy protein BECN 1. This phosphorylation enhances the binding of BECN 1 to its inhibitors, the BCL-2 anti apoptotic proteins. BECN 1 interaction with class III phosphatidylinositol 3-kinase (VPS34) kinase to initiate autophagy will then be inhibited.[12] The activated EGFR further suppresses autophagy through activating the AKT-mTORC1 pathway.[24] In ERK-transfected A431vulva squamous carcinoma cells, cetuximab can enhance autophagic flux.[49]

In EGFR activating mutated tumors, the physical interaction of BECN 1 and EGFR apparently without EGF stimulation was observed on the endosomes.[16,18] However, kinase inactive eEGFR interacts with Rubicon (Run domain BECN 1 interacting and cysteine-rich containing protein) which is an autophagy inhibitor through its association with BECN 1. This inactive eEGFR interaction with Rubicon stimulates autophagy as it promotes the protein's dissociation from BECN 1 therefore activating autophagy initiation.[50,51]

One of the worst prognosis of cancer is the EGFR observed in the nucleus.[52] It was in hepatocytes where nuclear EGFR was first observed, and it was seen with its ligands.[16] In cancer cells such as of breast,[53] bladder, ovary, lung,[52] epidermoid, oral cavity, pancreas and malignant glioma, nuclear EGFR (nEGFR) has also been detected.[16] Active nEGFR kinase effect far exceeds that of the conventional RTK moiety.[18] Both the kinase active and inactive nuclear EGFR have been demonstrated to induce autophagy.[18] The receptor interacts with transcription factors such as STAT3 to regulate expression of critical genes such as *CCND1*, *MYC*, *PTGS2*, *HIF1A*. For example, *HIF1A* regulates an important positive regulator of autophagy, a BH3-only protein, BNIP3 (BCL2/adenovirus E1B 19 kDa interacting protein 3).[54] BNIP3 disrupts the interaction between BCL2 (B-cell CLL/lymphoma 2) and BECN 1, thereby inducing autophagy.[55,56]

Stress stimuli as well as EGF induces this EGFR translocation to mitochondria.[57] It has been established that active EGFR in mitochondria inhibits apoptosis and causes enhanced cancer cell motility, through reducing Cox activity and cellular ATP levels by phosphorylating Cytochrome c oxidase subunit II (COXII).[16] However, mitochondrial EGFR (mEGFR) induces cell survival autophagy in tumors with EGFR activating mutation treated with cetuximab.[18] Tumors expressing this cell survival autophagy due to mEGFR have been proven dependent on this process for survival, and this supposedly offers another cancer therapeutic strategy.[18]

EGFR kinase dependent and independent role in autophagy (fold)

Wei *et al.* showed that when cells were treated with EGF, the receptor co-immunoprecipitated with BECN 1 unlike in a serum-free, normal media.[12] This interaction ablated BECN 1

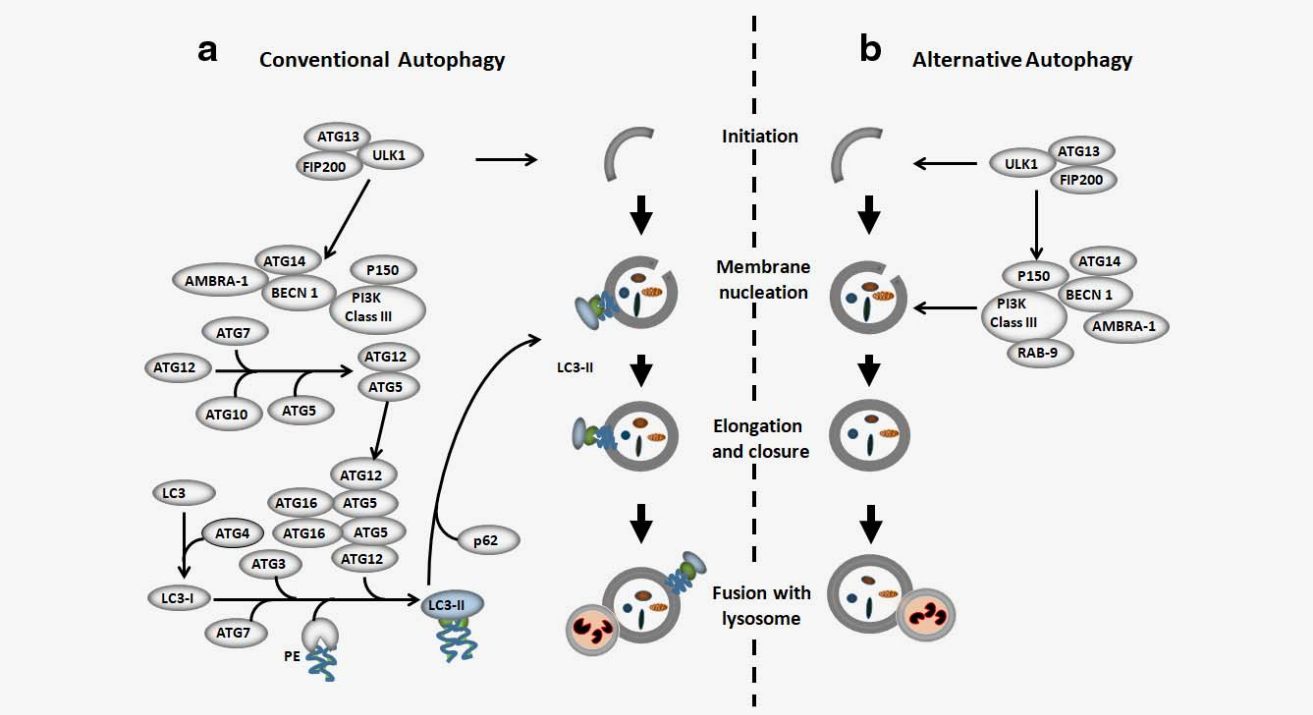

Figure 2. An overview of autophagy (macroautophagy) mechanisms. (*a*) Macroautophagy (Conventional) begins with engulfment of the target cytoplasmic materials by the phagophore, which under the influence of Atg12–Atg5–Atg16 complex and Atg8–phosphatidylethanolamine complex (PE) complete the elongation and enclosure of the phagophore into a double-membrane vesicle, autophagosome. The autophagosome then fuses with the lysosome to form autolysosome, in which degradation of the target cytoplasmic materials occurs. The important macromolecules produced are then released back into the cytoplasm. (*b*) In alternative autophagy, the elongation and completion of the phagophore occurs with the influence of Rab-1 instead of the ATG5/ATG7 or LC3. [Color figure can be viewed at wileyonlinelibrary.com]

availability to induce autophagy. Moreover, Chen *et al.* has reported that under hypoxia, EGF receptor played a critical role in the switch between autophagic cell death and survival in human cancers.[58] They found that in the early hours of hypoxia (4 hr), active EGFR was increasedly binding to BECN 1, which decreased autophagy and lead to cell survival, but after 48 hr cells died and EGFR-BECN 1 binding was greatly decreased.[58] This implies that upregulation of autophagy by inhibition of EGFR could augment cell death under stress. However, our recent work demonstrated that active EGFR-mediated RAS/RAF1/MAP2K/MAPK1/3 signaling pathway could contribute to increase autophagy-dependent apoptosis in gastric cancer cells.[21] Fulda and Kögel also reported that Obatoclax enhanced breast carcinoma cell death induced by lapatinib through suppressing the EGFR/AKT/mTOR signaling and enhanced autophagy.[20]

EGFR has further been demonstrated to exert some of its activity independent of its kinase or ligand activation. Indeed, it is worth noting that the effect of EGFR kinase-death is different from those of a total ablation of EGFR expression.[14,50] In human cancer cells, the function of kinase-independent EGFR is to prevent autophagic cell death.[50] Most lung adenocarcinoma cells with a EGFR activating mutation like HCC827 are an exception to poor initial response of most cancers to EGFR-TKI therapy.[8] However, they speedily develop resistance due to decreased autophagy.[12] Mechanistic studies demonstrated that this autophagy suppression is caused by expression of phosphorylated BECN 1 mutant, which is just the same as one produced by the active EGFR's phosphorylation of BECN 1.[12] This development is independent of the receptor kinase, though it mimics the effect of an activated kinase.

In response to stress, EGFR are internalized and endosomally accumulated. Studies found that inactive EGFR with the aid of lysosomal-associated transmembrane protein 4B (LAPTM4B) and Sec5 at the endosomes dissociated Rubicon from BECN 1, making BECN 1 free to induce autophagy.[51] This inactive EGFR-induced autophagy contributes to make cancer cells resistant to targeting-EGFR therapies.[50] This is speculated to be a mechanism by which these cells make use of their readily available EGFR molecules to survive conditions by activating survival autophagy. However, small autophagy inducing molecules seem to also use this readily available EGFR molecules to effect ACD.[21]

Regulation of autophagy by EGFR signaling pathways

Until now, two well-characterized cascades are responsible for the control of autophagy referred to as mTOR-dependent and mTOR-independent pathways. The mTOR-dependent signaling pathway is the main mechanism that control

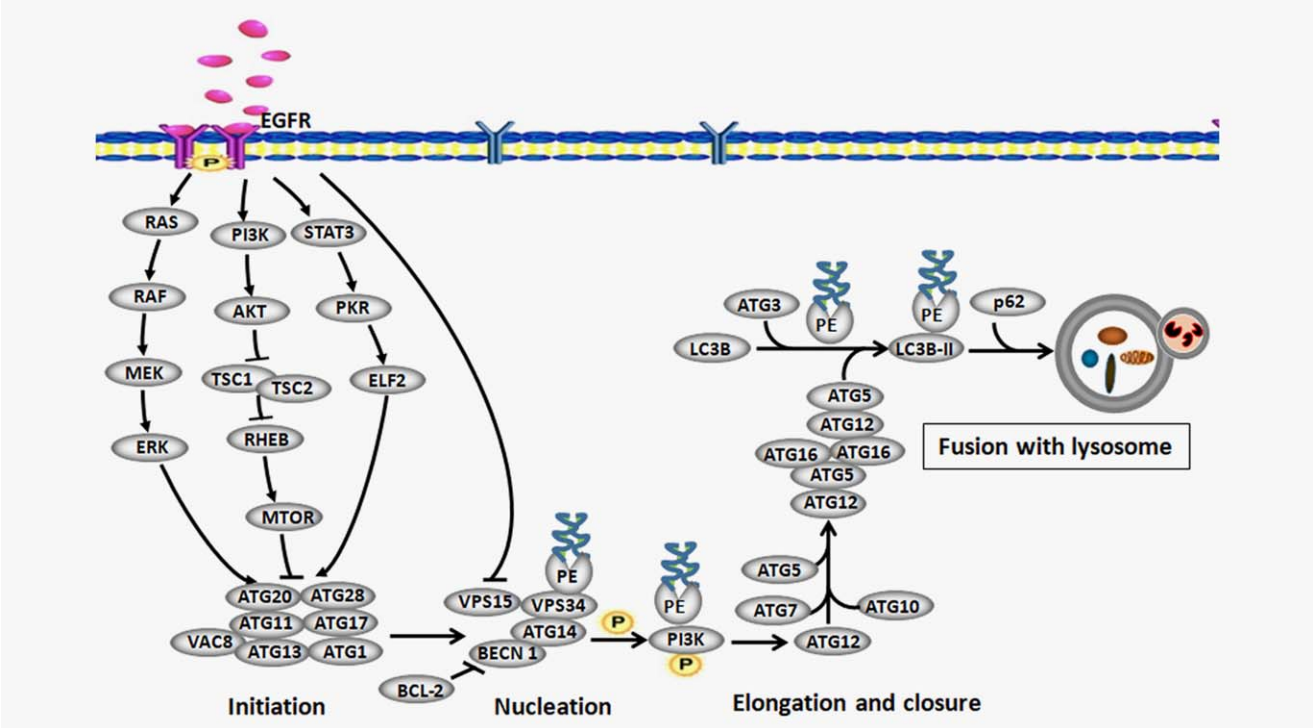

Figure 3. The role of EGFR-mediated signaling pathways in regulating autophagy. Almost all the signaling pathways downstream of the EGF receptor are involved in the regulation of autophagy. Activated EGFR stimulates the PI3K/AKT/mTOR signaling which inhibits eEF-2K and the formation of the ULK1 complex hence inhibiting autophagy. The RAF/MEK/ERK signaling cascade causes activation of autophagy. Phosphorylation of STAT3 by EGFR prevents the binding of STAT3 to PKR to inhibit its activity on eIF2α which enhances autophagy. EGFR phosphorylation of Beclin 1 inhibits autophagy. [Color figure can be viewed at wileyonlinelibrary.com]

autophagy through the amount of insulin or amino acids. Insulin binds to the two insulin receptor substrates (IRS 1 and 2)[42] resulting in their phosphorylation. The phosphorylated form of these substrates binds with PI3K, which catalyses the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate and then further activates AKT by activation of phosphoinositide-dependent kinase (PDK). AKT phosphorylates tuberous sclerosis complex 2 (TSC2) at Ser-989 and Thr-1462 and prevents the formation of the TSC1/2 complex which catalyses the conversion of RAS homolog enriched in brain (Rheb) associated with GTP (GTP-Rheb) to GDP-Rheb. This leads to increased levels GTP-Rheb. GTP-Rheb phosphorylates and activates mTOR which phosphorylates ULK1 and ATG13 suppressing autophagy.[41]

The RAS/RAF/MEK/ERK cascade evidently regulates the activity of many proteins involved in apoptosis[5] and autophagy.[21] Depending on the duration, the magnitude and its subcellular localization, ERK activation controls various cell responses, such as proliferation, migration, differentiation and death.[5,59] This pathway is negatively regulated by protein phosphatases at different levels, including phosphotyrosine phosphatases and phosphoserine/phosphothreonine phosphatases. Importantly, the pathway is also frequently deregulated in most of tumors.[60] Phosphotyrosine residues serve as docking sites[61] for the growth factor receptor binding protein 2 (GRB2) through its Src Homology 2 (SH2) domain. SOS (Son of Sevenless) with a domain rich in proline binds to GRB2. SOS as an adapter protein catalyses the activation of the RAS monomeric protein.[3,5] SOS binding to RAS changes the conformation of RAS and activates it.[3,60] RAS activates RAF. Active RAF phosphorylates the mitogen-activated protein kinase kinase (MAPKK/MEK).[2] MEK is both a tyrosine and Serine/Threonine kinase. After being activated by RAF, MEK can promote the phosphorylation of the extracellular signal-regulated kinase (ERK) which is inactively located in cytoplasm. Active ERK translocates to the nucleus and promotes the cell differentiation and proliferation by activating transcription factors Elk1 and C-myc.[3,60] Furthermore, active ERK had been demonstrated to upregulate BECN 1. When there was moderate upregulation of BECN 1, cyto-protective autophagy was induced while strongly pronounced levels of BECN 1 led to cyto-destructive autophagy.[30]

Amaravadi and Thompson have proposed that identification and inhibition of regulators that downregulate autophagy may be very important in the conversion of the cell survival response to the cell death process thereby enhancing autophagic cell death in cancer treatment.[17] Chen *et al.* also recently reported that targeting inhibition of cathepsin S, which is frequently overexpressed in human cancer, induces autophagy and apoptosis by activation of the EGFR-mediated RAS/RAF/MEK/ERK pathway.[62] These RAS/RAF/MEK/ERK-

mediated autophagic cell death requires prolonged activation of this pathway. Reactive Oxygen Species (ROS) have been speculated to be the main upregulator of ERK, as ERK-sensitive phosphatases are sensitive to ROS.[5] Huang *et al.* demonstrated that autophagy induced by cathepsin S inhibition induces early ROS production, oxidative DNA damage and cell death via xanthine oxidase.[63] Therefore, these studies suggested that ROS may further upregulate ERK activity to induce autophagic cell death.

However, some studies have reported that EGFR-TKIs can also induce autophagy,[38,51] suggesting that this EGFR-TKIs-induced autophagy might be independent of the downstream signaling of EGFR. For example, Tan *et al.* has demonstrated that EGFR might play a critical role in autophagy initiation independent of its kinase.[50] This is also one proposed mechanism by which EGFR dependent cancers gain resistance to EGFR TKIs. Moreover, inhibition of cathepsin S has been demonstrated to delay EGFR degradation process and cause the perinuclear distribution of accumulated EGFR within late endosomes which results in continuous EGFR signaling contributing to sustained downstream AKT and STAT3 but not ERK 1/2 signaling cascade.[62]

However, various recent studies have also reported that the prolonged activation of EGFR downstream effector, ERK can regulate autophagy vacuoles maturation and lead to autophagic cell death.[64] For example, Lindane, a carcinogen, can induce autophagy vacuoles in Sertoli cells by activation of ERK.[15] In addition, activation of ERK-induced autophagy caused death of colon cancer cells treated with soyasaponins,[65] as well as breast cancer cells under capsaicintreatment.[66] Therefore, based on these above reports, two conclusions can be reached that (1) unlike the EGFR downstream effectors, PI3K, AKT and mTOR, which are well-established negative regulators of autophagy,[12,67] the RAS/RAF/MEK/ERK signaling pathway positively regulates autophagy;[29,68] (2) acute, transient ERK pathway activation results in cyto-protective autophagy while sustained ERK activation causes cyto-destructive autophagy, suggesting that this pathway is the main mechanism by which cell survival can switch to cell death. Furthermore, it is also noteworthy that this signaling pathway might mainly promote autophagic-dependent cell survival when either one of the mTOR complexes (mTORC1 or mTORC2) is inhibited causing just a moderate increase in BECN 1. But when both mTOR1 and mTORC2 are inhibited, the ERK pathway effects autophagic cell death and BECN 1 expression is markedly increased.[30,39]

The Crosstalk Between Apoptosis and the EGFR/RAS/RAF/MEK/ERK-Mediated Autophagy

Autophagy (self-eating) and apoptosis (self-killing) are both established cellular degradative processes, which have been observed to share a number of regulating proteins.[12,39] Several autophagy inducing molecules have been demonstrated to cause autophagy-dependent apoptosis. For example, resveratrol treatment of prostate cancer cells activates the RAS/RAF/ERK pathway, which mediates autophagy, and phosphorylates p53 resulting in apoptosis.[69] In these cells, inhibition of the RAS/RAF/MEK/ERK signaling pathway was shown to inhibit both autophagy and apoptosis, proving that apoptosis was dependent on the RAS/RAF/MEK/ERK-mediated autophagy. In T-cell acute lymphoblastic leukemia cells, resveratrol was further proven to induce autophagy through inhibiting AKT/mTOR pathway and activating the RAS/RAF/MEK/ERK pathway with apoptosis.[70] We further identified a 2-amino-nicotinonitrile compound (w09), a novel autophagy inducer which through induction of the RAS/RAF/MEK/ERK pathway effectively induces an autophagy-dependent apoptosis in EGFR-dysregulated gastric cancer cells.[21] Indeed, in autophagic cell death induced in human glioma cells by tetrahydrocannabinol (THC), an active component of cannabinoids, the AKT/mTOR signaling was inhibited and RAS/RAF/MEK/ERK pathway induces with apoptosis shown to be downstream autophagy.[71] These studies shows that autophagy inducing molecules may use the readily available EGFR and RAS/RAF/MEK/ERK signaling in EGFR activating mutated tumors to effect cell death in these tumors. Effective cell death is further achieved due to the fact that autophagy is upstream of apoptosis, degrading the essential cell components. It is further worth noting that in autophagy-dependent apoptosis, aberrant autophagy induction is needed, hence the need for increased levels of the RAS/RAF/MEK/ERK signaling pathway proteins, which is the case in EGFR mutated tumors.

There has further been an interchanging relationship between EGFR-mediated autophagy and apoptosis. A group of triterpenoids known as cucurbitacins which are isolated from an herbal medicine, *Trichosanthes kirilowii* (cucurbitaceae family) have been shown induce ACD through activation of ERK. However, under wortmannin (an autophagy inhibitor) treatment and BECN 1 and ATG5 knockdown, cucurbitacin induced apoptosis dependent on caspase.[72] Furthermore, ERK signaling in colorectal cancer cells inhibited B-cell lymphoma 2-interacting mediator of cell death (BIM) expression.[73] BIM mediates both autophagy and apoptosis, activating them both toward cell death.[54,73]

The Role of RAS/RAF/MEK/ERK Signaling Pathway-Mediated Autophagy in Cancer Treatment (Fig. 4)

The aim of cancer therapy is to disable all the hallmarks of cancer, or at-least inhibit the characteristic in the cancers which these cells are dependent on for survival.[74] Almost all classes of currently used anticancer drugs including microtubule-targeted drugs, DNA damaging agents, death receptor agonists, hormonal agents, kinase inhibitors and many more have been proven to affect autophagy.[75] Most cancers have been reported to contain EGFR activating mutations as well as EGFR overexpression, hence anti-EGFR-based therapy is the most common clinically.[76] However, these therapy is limited by tumor resistance which involves change in autophagy levels.[17] Therefore, there is a further need for even much better therapeutic strategies. Autophagy induced by small molecules through induction of the RAS/RAF/MEK/ERK pathway is increasingly being

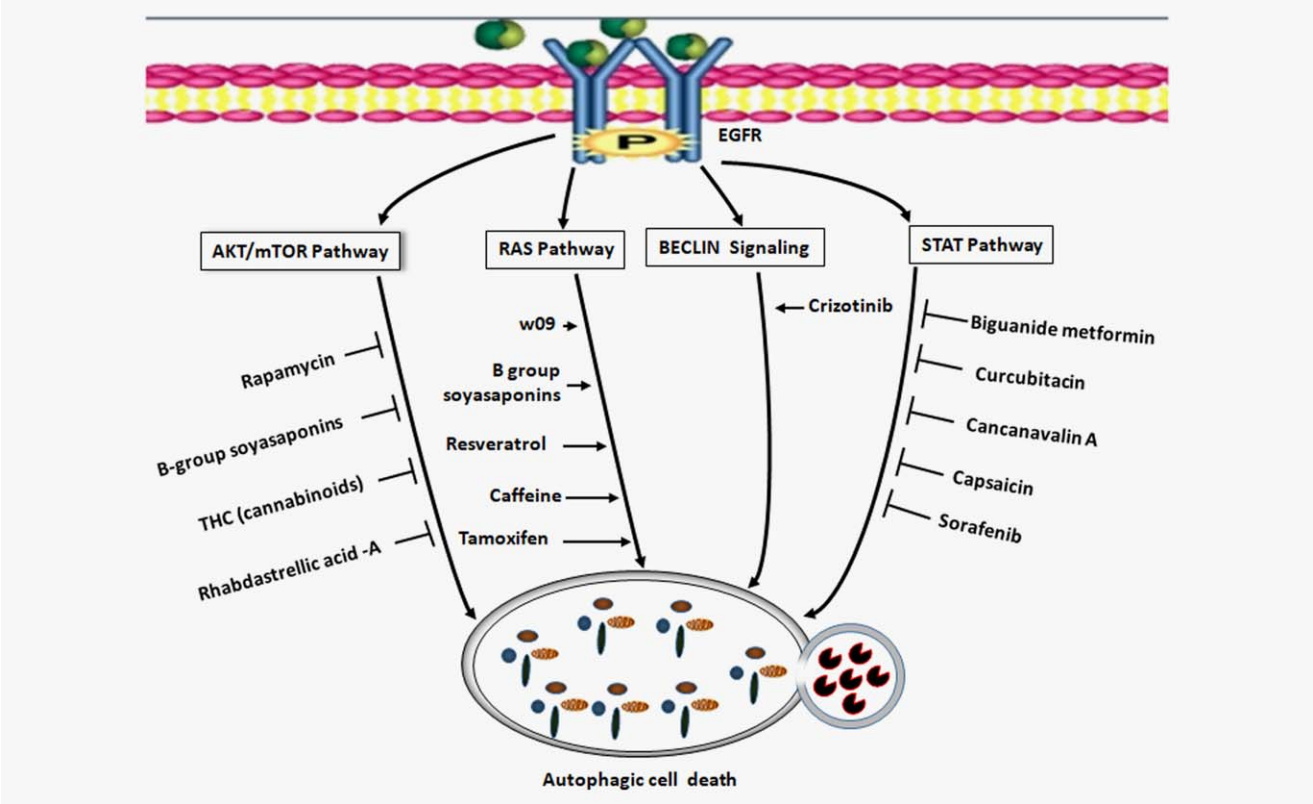

Figure 4. Pharmacological induction of EGFR signaling pathways-mediated ACD. The molecules that control autophagy in different pathways downstream the EGFR tyrosine kinase are shown. To affect ACD, the molecules on the RAS/RAF1/MEK/ERK signaling pathway and BECN 1 pathway are mainly inducers while those on STAT and AKT/mTOR signaling pathway are mainly inhibitors. [Color figure can be viewed at wileyonlinelibrary.com]

proven effective as first line treatment of several cancers especially those with EGFR mutations and overexpression.[20,77] Autophagy is generally a cytoprotective process which when highly induced results in ACD.[24] The overexpression of EGFR and ERK guarantees the high levels of autophagy which will result in ACD. Induction of the ERK signaling pathway and inhibition of the AKT/mTOR pathway has been demonstrated to effectively induce ACD and decrease the viability of colon cancer cells treated with B-group soyasaponins.[65] Indeed, caffeine at high concentrations, imidazo(4,5-c)quinoline derivative NVP-BEZ235 and various other molecules have been shown to affect their antitumor activity through induction of the ERK pathway.[20] Cathepsin S have been shown to be overexpressed in most cancers and its expression correlated with increased metastasis.[74] Inhibition of cathepsin S has been demonstrated to induce ACD and apoptosis through the EGFR/RAS/RAF/MEK/ERK pathway and this inhibits colorectal tumor invasion, metastasis and angiogenesis.[62] Furthermore, in their study on renal and hepatocellular carcinoma, Bareford *et al.* also demonstrated that a multikinase inhibitor, sorafenib promotes autophagy which increases the cytotoxicity of pemetrexed through inhibition of the mTOR signaling cascade and induction of the ERK signaling pathway.[78]

However, with the evidence of EGFR-mediated BECN 1 inhibition and consequent autophagy inhibition, the question is how do these small autophagy-inducing molecules activate EGFR and autophagy at the same time? Indeed, Leng *et al.* demonstrated that ursolic acid can promote autophagic cancer cell death independent of BECN 1 and dependent on ATG5.[79] In another study, McCoy *et al.* also showed that obatoclax induces ATG7 dependent autophagic cell death independent of BECN 1 in NSCLC.[80] It is therefore evident that EGFR-mediated ACD induced by these compounds occurs even when EGFR has phosphorylated and inhibited BECN 1. Inhibition of EGFR results in availability of BECN 1 to induce autophagy but a mutant of phosphorylated BECN 1 has been observed to emerge causing resistance to therapy.[81] Therefore, we propose the use of the readily available EGFR to induce autophagic cancer cell death through the RAS/RAF/MEK/ERK pathway as a therapeutic strategy.

Advantages of the EGFR/RAS/RAF/MEK/ERK Pathway-Mediated Autophagy Over Other Mechanisms of Autophagy Induction in Cancer Therapy

Autophagic cell death can be induced in tumor cells through several mechanisms. An understanding of both the pathophysiology of the tumor and the functional relevance of autophagy within the tumor is central to the development of an effective mechanism to specifically effect death in

tumors.[82] Factors such as the crosstalk between pathways, the cancer genome instability as well as the intra-tumor heterogeneity have to be as well considered in designing an effective anticancer strategy.[1] Autophagy induction for cancer therapeutic purpose is a very versatile strategy that needs deep understanding before execution as in several cancer cases, it has been proven that autophagy may best be inhibited to enhance cytotoxicity.[83] Furthermore, even in cases where autophagy should be activated to induce cell death the mechanism of induction is critical to ensure high levels of autophagy that results in autophagic cell death.[30,39] For example, autophagy inducers like an FDA approved autophagy inducer; Rapamycin, whose effect is through inhibition of mTOR complex have been proven effective against bladder cancers but there is no evidence of its effectiveness in tumors with mutations in the genes of the proteins of the EGFR/RAS/RAF/MEK/ERK pathway.[84] Another discovery is that some autophagy inducing small compounds like resveratrol can affect cell death in various cancer cells with different mechanisms of action depending on the type of the target cancer cells.[84]

There is indeed a general consensus that autophagy acts as a mechanism to prevent tumorigenesis, but once a tumor is formed, it shifts into promoting its growth.[12,85] However, studies shows that persistently high levels of autophagy promote autophagic cell death in established tumors.[5] Therefore a mechanism of autophagy induction should be that which ensures persistently high levels of autophagy in that specific tumor. High expression of proteins in the EGFR/RAS/RAF/MEK/ERK pathway in corresponding activating mutated tumors ensures this high level after exposure to the small molecule that targets this pathway.[21] Development of autophagy inducers specific for the EGFR/RAS/RAF/MEK/ERK pathway mutations is specially critical as activating mutations in this pathway accounts for a large percentage of cancers. For example, RAS mutations are the most common oncogenes, reported present in approximately 30% of human cancers.[86,87] Mutations in RAF alone accounts for 6% of all human cancers.[87] This high statistics therefore warrants the need for greater consideration of this pathway. Furthermore, the existence of p53 mutation has been proven to make autophagy induced in K-RAS mutated pancreatic tumors cytotoxic.[88]

Conclusion and Future Prospects

The evidence that EGFR overexpressing tumors are more resistant to cell death by EGFR TKIs than activating EGFR mutated tumors show the impact of the kinase activity on responsiveness of the tumors to therapy. The synergistic activity of autophagy with apoptotic cell death also impacts treatment tremendously in apoptotic competent cancers. Autophagy has also been shown to be an alternative for cell death in apoptotic deficient cancers. It is evident that in the context where epidermal growth factor receptor plays a significant role in the tumor biogenesis through its kinase activity, autophagy enhancement toward autophagic cell death is crucial otherwise autophagy inhibition is advantageous for tumors independent of EGFR for their progression. This is especially important since the tumor hypoxic microenvironment further enhances this autophagic cell death. We therefore propose that autophagy induction for cancer therapy is the safest and most effective first line treatment for a number of cancer cell types especially the naive, early stage tumors. Though the process had been reported extensively as a cytoprotective mechanism, recent evidence however prove that autophagy's other side of cytotoxicity can as well be induced and utilized for therapy. Autophagy is a safe process which has a lot of advantage for people health living, therefore its inductions both as a preventative mean and as a therapeutic strategy is crucial. There is henceforth an interesting long journey to be undertaken in the study of autophagy to further unveil the exact mechanisms of action by which this drug-induced, EGFR/RAS/RAF/MEK/ERK signaling pathway-mediated autophagy is activated to even better specifically manipulate autophagy, leading to better treatment outcomes.

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