


MINI REVIEW

The role of FOXOs and autophagy in cancer and metastasis—Implications in therapeutic development

Mohd Farhan¹ | Marta Silva¹ | Shuai Li¹ | Fengxia Yan² |
 Jiankang Fang¹ | Tangming Peng¹ | Jim Hu³ | Ming-Sound Tsao³ |
 Peter Little⁴ | Wenhua Zheng¹ 

¹Faculty of Health Sciences, Centre of Reproduction, Development and Aging, Institute of Translational Medicine, University of Macau, Taipa, Macau SAR, China

²Department of Medicine, Jinan University, Guangzhou, China

³Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

⁴School of Pharmacy, Pharmacy Australia Centre of Excellence, The University of Queensland, Woolloongabba, Queensland, Australia

Correspondence

Wenhua Zheng, Faculty of Health Sciences,
 University of Macau, Room 3057, Building E12,
 Taipa, Macau 999078, China.
 Email: wenhuazheng@umac.mo

Funding information

Universidade de Macau, Grant/Award Number:
 MYRG2018-00134-FHS; Fundo para o
 Desenvolvimento das Ciências e da Tecnologia,
 Grant/Award Numbers: 0044/2019/AGJ,
 0113/2018/A3, 0127/2019/A3; National
 Natural Science Foundation of China,
 Grant/Award Number: 31771128

Abstract

Autophagy is a highly conserved intracellular degradation process that plays a crucial role in cell survival and stress reactions as well as in cancer development and metastasis. Autophagy process involves several steps including sequestration, fusion of autophagosomes with lysosomes and degradation. Forkhead box O (FOXO) transcription factors regulate the expression of genes involved in cellular metabolic activity and signaling pathways of cancer growth and

Abbreviations: AML, acute myeloid leukemia; AMPK, AMP-activated protein kinase; BECN1, beclin1; CBP, CREB-binding protein; CML, chronic myelogenous leukemia; CQ, chloroquine; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; FOXO, forkhead box O; G6Pase, glucose-6-phosphatase; HCQ, hydroxychloroquine; HDAC, histone deacetylase; HMGB1, high-mobility group box 1; JNK, c-Jun N-terminal kinase; LC3, microtubule-associated protein 1 light chain 3 alpha; LIC, leukemia-initiating cells; MDR, multidrug resistance; MMP, matrix metalloproteinase; MST1, mammalian Ste20-like kinase; mTOR, mammalian target of rapamycin; NRF2, nuclear factor erythroid 2-related factor 2; PARP1, poly(ADP-ribose) polymerase 1; PCK1, phosphoenolpyruvate carboxykinase; PEPCCK, phosphoenolpyruvate carboxykinase 2; PI3K-AKT, phosphatidylinositol 3-kinase-protein kinase B; PRMT1, protein arginine methyltransferase 1; PUMA, p53-upregulated modulator of apoptosis; SIRT2, silent information regulator 2; SREBP, sterol regulatory element-binding protein; TLR4, Toll-like receptor 4; VPS34, vacuolar-sorting protein 34; XBP1, X-box-binding protein 1.

Mohd Farhan, Marta Silva, and Shuai Li contributed equally to this study.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2020 The Authors. *Medicinal Research Reviews* Published by Wiley Periodicals LLC

metastasis. Recent evidence suggests that FOXO proteins are also involved in autophagy regulation. The relationship among FOXOs, autophagy, and cancer has been drawing attention of many who work in the field. This study summarizes the role of FOXO proteins and autophagy in cancer growth and metastasis and analyzes their potential roles in cancer disease management.

KEYWORDS

autophagy, cancer, FOXO proteins, metastasis

1 | INTRODUCTION

Cancer is generally characterized by a set of features that includes sustained chronic proliferation, insensitivity to growth suppressors, unlimited replicative potential, resistance to apoptosis, induction and sustained angiogenesis, tissue invasion, and metastatic formation.¹ Metastases are accountable for ~90% of all cancer deaths.² Their formation is quite complex requiring primary tumor cells to detach and enter the blood circulatory system to reach distant tissue sites, where they adapt to survive and thrive forming secondary tumors.³⁻⁵ Despite accumulating evidence for the occurrence of genetic and epigenetic alterations, mechanisms underlying cancer progression and metastatic formation are still poorly understood.⁴

Forkhead box O (FOXO) transcription factors have been reported to influence the cellular responses to external energy changes, growth factors stimulation, and nutritional status. Evidence also suggest their involvement in several cellular processes and their dysfunction has been implicated in aging, diabetes, and cancer.⁶⁻¹⁰ In fact, FOXOs expression and transcriptional activity have been reported to be altered in many cancers. Due to their antiproliferative and proapoptotic functions, FOXOs are generally viewed as tumor suppressors.¹¹ They have also been implicated in autophagy modulation in different cancer cells.¹²⁻¹⁴ Autophagy or macroautophagy is a biologically ancient and highly conserved process present in all cells.¹⁵ It involves the intracellular formation of specialized membranous vesicles, which engulf target proteins and organelles for transport to lysosomes for degradation.¹⁶ Many genes involved in autophagy have been identified and the basic process of autophagy is only partially understood.^{17,18} There is still a significant gap between our understanding of the signaling pathways that control autophagy and the impact of these pathways in cancer cell biology.^{19,20} Emerging evidence support a connection between FOXO proteins and autophagy in cancer suggesting their potential as leading targets for the development of new therapeutic approaches. This review summarizes the role of FOXO proteins and autophagy in cancer growth and metastasis and analyzes the most recent findings regarding the potential therapeutic opportunities related to FOXO's-autophagy regulation.

2 | FOXO TRANSCRIPTION FACTORS

From FOXA to FOXS, forkhead genes encode proteins that contain a conserved DNA-binding domain (the forkhead box), often referred as a "winged-helix" due to its butterfly-like appearance.²¹⁻²³ Mammalian FOXOs including FOXO1, FOXO3, FOXO4, and FOXO6 gene products are evolutionarily conserved and function as transcription factors by binding to target DNA through the forkhead domain leading to either inhibition or activation of downstream target genes.²⁴ Accordingly, FOXO proteins participate in several crucial processes that range from cell proliferation to cell apoptosis including the regulation of autophagy, metabolism, inflammation, and differentiation (Table 1).

TABLE 1 FOXOs family members

Gene name	Human chromosomal location	Subcellular localization	Total amino acids	Expression pattern	Cellular Function	References
FOXO1	13	Nucleus/ cytoplasm	655	Ubiquitous with higher expression in adipose tissue	Proliferation (↓/↑) Metabolism (↑) Apoptosis (↑) Inflammation (↑) Autophagy (↑)	25-31
FOXO3	6	Nucleus/ cytoplasm	673	Ubiquitous with higher expression in brain, heart, kidney and spleen	Proliferation (↓/↑) Metabolism (↑) Apoptosis (↑) Inflammation (↑) Differentiation (↓/↑) Autophagy (↓/↑)	30-40
FOXO4	X	Nucleus/ cytoplasm	505	Ubiquitous with higher expression in skeletal muscle	Proliferation (↑) Apoptosis (↑) Inflammation (↑)	31,41-43
FOXO6	1	Nucleus	492	Brain	Proliferation (↑) Metabolism (↑)	31,44,45

Abbreviation: FOXO, forkhead box O.

FOXOs transcriptional regulation is controlled by a variety of posttranslational modifications (PTMs), such as phosphorylation, ubiquitination, acetylation, methylation, glycosylation, and poly(ADP-ribosyl)ation (PARylation) that dictate FOXOs subcellular localization.⁴⁶⁻⁵⁰

FOXOs regulation by insulin or insulin-like growth factor-1 occurs via the ubiquitous phosphatidylinositol 3-kinase-protein kinase B (PI3K-AKT) pathway.⁵¹⁻⁵³ AKT phosphorylates nuclear FOXOs at three conserved serine/threonine residues (Thr32, Ser253, and Ser315 in FOXO3) leading to the binding of 14-3-3 proteins.⁵⁴ With the exception of FOXO6, all FOXOs have these three evolutionarily conserved phosphorylation sites and binding of 14-3-3 promotes the translocation of FOXO proteins to the cytoplasm blocking their transcriptional activity.^{55,56} While phosphorylation of Thr32, FOXO3 residue is involved in binding of 14-3-3 chaperone proteins, phosphorylation of Ser253, which is a part of the nuclear localization sequence, causes signal disruption and prevents the reentry of FOXOs into the nucleus due to the negatively charged phosphor group attached to the basic sequence.⁵⁶ FOXOs phosphorylation induced by other kinases such as serum/glucocorticoid regulated kinase 1, casein kinase 1 alpha 1 (CK1), dual-specificity tyrosine phosphorylation-regulated kinase 1A, extracellular signal-regulated kinase (ERK), and I kappa B kinase also inhibits FOXOs transcriptional activity.⁵⁷ Conversely, FOXOs phosphorylation by upstream c-Jun N-terminal kinase (JNK), mammalian sterile 20-like kinase (MST1), and AMP-activated protein kinase (AMPK) promotes FOXOs transcriptional activity.^{11,24} Under oxidative stress conditions, JNK phosphorylates FOXO4 at Thr447 and Thr451 residues promoting its translocation from the cytoplasm to the nucleus.⁵⁸ JNK can also phosphorylate FOXO3 at Ser574, counteracting AKT signaling, and thereby promoting FOXO3 nuclear translocation and transcriptional activity.⁵⁹ JNK-mediated activation of FOXOs transcriptional activity also involves JNK phosphorylation of 14-3-3 chaperone proteins, thereby promoting their dissociation from FOXO3,

counteracting AKT signaling.⁶⁰ Oxidative stress also promotes MST1-induced phosphorylation of FOXO3 at Ser207 leading to the disruption of 14-3-3 binding and to FOXOs nuclear relocalization.⁶¹ Similarly, under conditions of nutrient stress, AMPK was shown to phosphorylates FOXO3 at six residues (Thr179, Ser399, Ser413, Ser555, Ser588, and Ser626), triggering its transcriptional activity without interfering with its subcellular localization.⁶²

FOXOs ubiquitination may involve mono- and polyubiquitination, both having different roles on their transcriptional activity regulation. Oxidative stress conditions induce the monoubiquitination of FOXO4 at K199 and K211 residues, triggering its relocalization into the cell nucleus and increasing its transcriptional activity.⁶³ Accumulated FOXOs in the cytosol, as a result of AKT-mediated phosphorylation, are degraded by polyubiquitination through the ubiquitin-proteasome pathway.⁶⁴

FOXO proteins function is also regulated by several acetylases and deacetylases.⁶⁵ In response to oxidative stress, CREB-binding protein (CBP) and its paralog p300 (CBP/p300), acetylate FOXO proteins inhibiting their activity. CBP-induced acetylation of FOXO1 and FOXO4 leads to the reduction of their transcriptional activity.^{66,67} Silent information regulator 2 (SIRT2)-induced deacetylation of FOXO1 reverses the acetylation effect increasing FOXO1 transcriptional activity.⁶⁷ FOXOs activity regulation may also result from the interplay of their PTMs. For example, acetylation decreases FOXO1 DNA-binding activity and consequently enhances its availability to AKT-mediated phosphorylation.⁶⁸

Other PTMs such as methylation by protein arginine methyltransferase 1 (PRMT1), N- and O-linked glycosylation and PARYlation by poly(ADP-ribose) polymerase 1 (PARP1), also regulate FOXOs transcriptional activity. Specifically, PRMT1 methylates FOXO1 inhibiting AKT-induced phosphorylation and thereby preventing its nuclear translocation.⁴⁷ Regarding FOXO1 glycosylation, in spite of not influencing its subcellular localization, it also enhances its transcriptional activity.⁴⁸ Concerning PARP1-induced modifications, these have been shown to negatively regulate FOXOs transcriptional activity. Specifically, phosphorylation of FOXO3 by PARP1 prompts its nuclear export impairing its transcriptional activity.^{49,50}

3 | FOXOs IN CANCER AND METASTASIS

FOXO1, FOXO3, and FOXO4 were initially discovered because chromosomal translocations were detected in some forms of leukemia supporting their association with cancer development. While FOXO1 was found in alveolar rhabdomyosarcoma as a fusion protein involving DNA-binding domain of PAX3 or PAX7,⁶⁹⁻⁷¹ FOXO3 and FOXO4 were discovered in acute myeloid leukemias (AMLs) as fusion proteins with mixed-lineage leukemia (MLL) gene.^{72,73} Nonetheless, the underlying mechanisms tying these genetic alterations with FOXOs role in carcinogenesis are still unclear.

The increased metabolic demands associated with cancer progression causes tumor cells to reprogram their metabolism to grow and proliferate. Some of the metabolic alterations promoted by cancer cells affect both glucose and lipid metabolisms, which regulation has also been shown to be modulated by FOXOs transcriptional activity.⁷⁴ To generate ATP, cancer cells resort to glycolysis, a mechanism promoted by the proto-oncogene Myc that is only used by healthy cells in hypoxic conditions.^{75,76} Several studies have reported that FOXOs antagonize Myc function, which is reported to be upregulated in many cancers, thus inhibiting glycolysis and, therefore, impairing glucose uptake.⁷⁴ In fact, acetylated FOXOs were shown to promote the increase of Myc levels in glioblastoma and, upon energy stress conditions, FOXOs were shown to induce the upregulation of FOXO-induced long noncoding RNA 1 promoting the decrease of Myc protein levels in renal cancer cells.^{77,78} In contrast, FOXOs were shown to have a key role in the regulation of hepatic gluconeogenesis enhancing the glucose output via the activation of phosphoenolpyruvate carboxykinase 1 (PCK1) and glucose-6-phosphatase (G6Pase), thereby upregulating phosphoenolpyruvate carboxykinase 2 (PEPCK) levels.^{79,80} FOXO1 nuclear exclusion mediated by the tumor suppressor p53 was shown to impair the expression of both PCK1 and G6Pase and, therefore, to compromise gluconeogenesis in different cancer cell lines.⁸¹ Congruently, a different study reported that AKT loss mediated by the mammalian

target of rapamycin (mTOR) inhibition promoted FOXO1 nuclear retention and the upregulation of PEPCK levels, thus increasing the glucose output by cancer cells.⁸² Despite these evidence, FOXOs involvement in the regulation of gluconeogenesis in cancer cells is still unclear. Lipid metabolism rewiring by tumor cells is usually achieved by enhancing fatty acid synthesis, a process regulated by sterol regulatory element-binding proteins (SREBPs).^{83,84} Evidence suggest that FOXO1 is able to impair lipogenesis by reducing the transcriptional activity of SREBPs and thereby downregulating the expression of the enzyme fatty acid synthase.⁸⁵ In contrast, FOXOs were shown to induce the expression of the enzyme adipose triacylglycerol lipase, consequently upregulating lipolysis in adipocytes.⁸⁶ As the influence of FOXO proteins in cells metabolism has been extensively reported, more studies are still necessary to unravel their regulatory role in cancer cells metabolism.

3.1 | FOXOs as tumor suppressors

FOXOs are generally considered to suppress cancer. This view is sustained by the tumor-prone phenotype of FOXOs knockout mice. Studies from knockout mice with up to five deleted FOXO alleles resulted in a mild tumor phenotype.⁸⁷ Nonetheless, the simultaneous deletion of all alleles lead to the development of thymic lymphomas and hemangiomas, indicating a redundant tumor suppressive function for these transcription factors in cancer.⁸⁷ FOXOs suppressive role is further reinforced by studies reporting their inactivation in multiple human cancers by the PI3-AKT signaling pathway, which is commonly hyperactivated during cancer due to the occurrence of mutations in RAS, suppressor proteins PTEN or PI3K.⁸⁸ FOXOs inactivation promotes the downregulation of genes implicated in cell cycle arrest, such as retinoblastoma-related protein p130 and cell cycle kinase inhibitor p27^{kip1}, and enhances cell survival decreasing the expression of the proapoptotic tumor necrosis factor-related apoptosis-inducing ligand.⁸⁹ Downregulation of FOXO1 and increased levels of FOXO1 phosphorylation is reported to be correlated with poor prognosis in patients with soft tissue sarcoma,⁹⁰ AML,⁹¹ prostate,⁹² and breast cancers.⁹³ Likewise, the downregulation of FOXO3 correlated with poor survival in patients with neuroblastoma,⁹⁴ breast,⁹⁵⁻⁹⁷ ovarian,⁹⁸ and colorectal cancers.⁹⁹ Similarly, the upregulation of FOXO3 phosphorylation levels were identified as adverse prognostic biomarkers in ovarian cancer¹⁰⁰ and AML.¹⁰¹ FOXOs influence in metastasis formation and progression has also been reported. Specifically, the downregulation of FOXO3 was shown to be important for tumor metastasis progression in colorectal cancer⁹⁹ and renal cell carcinoma.¹⁰² Moreover, FOXO3 nuclear localization was correlated with better prognosis and less frequent metastasis formation in estrogen receptor-positive breast cancer.⁹⁷ Downregulation of FOXO4 in patients with prostate cancer was also correlated with earlier metastatic formation.¹⁰³

3.2 | FOXOs as tumor promoters

Despite being considered tumor suppressors by most studies, new evidence regarding FOXOs role in the promotion of cancer progression and in the resistance to cancer therapy in certain contexts has come to light. In fact, occurrences of FOXO1 single-nucleotide somatic mutations that promote its nuclear localization were correlated to a poor prognosis in large B-cell lymphomas.¹⁰⁴ Likewise, increased levels of FOXO3 were associated with poor outcomes in hepatocellular carcinoma (HCC) and glioblastoma.^{105,106} Evidence also suggests the involvement of FOXO proteins in the regulation of leukemia-initiating cells (LIC) maintenance in chronic myelogenous leukemia (CML) and AML.¹⁰⁷ Specifically, LICs were shown to present an increased nuclear localization of FOXO3 due to a decrease of AKT activation and these cells' ability to cause CML was significantly reduced by FOXO3a deficiency.¹⁰⁸ Likewise, active FOXOs were found in patients with AML and, either activation of AKT or FOXOs deletion, impaired leukemic cell growth and LIC function promoting the survival of an AML mice model.¹⁰⁹ In line with these findings, high levels of FOXO3 correlated to a lower overall survival and recurrence free survival of

patients with AML.¹¹⁰ Similar roles for FOXO proteins in other cancer stem-like cells have also been reported.^{32,96,111} Despite the abovementioned evidence, linking FOXOs increased levels to reduced metastasis formation, several studies also suggest that FOXO proteins can facilitate metastasis. In fact, the nuclear localization of both FOXO3 and elevated β -catenin contents correlated with the formation of metastases and with a shorter survival in colorectal cancer.¹¹² Likewise, FOXO3 overexpression correlated with lymph node metastasis formation and with a poor disease free survival of patients with triple-negative breast cancer.¹¹³ FOXOs' facilitating role in metastasis has also been observed in breast cancer cell lines. Specifically, nuclear retention and activation of FOXO3, due to serum deprivation resultant from increased mass tumor, were shown to upregulate matrix metalloproteinase-9 (MMP-9) and MMP-13 levels which further promote invasive cellular migration.¹¹⁴ Likewise, FOXO1 was reported to directly regulate MMP-1 expression enhancing metastasis formation in breast cancer cells.¹¹⁵ FOXO3 knockdown was also reported to significantly suppress tumor formation and metastasis in pancreatic ductal adenocarcinoma.¹¹⁶ In summary, FOXOs present a paradoxical role in cancer and metastasis in which they are able to act as tumor suppressors, by inducing apoptosis and cell-cycle arrest but may also promote cancer progression (Figure 1).

4 | AUTOPHAGY

Autophagy is a highly conserved cellular process that involves the intracellular degradation and removal of damaged proteins and organelles supporting cellular renovation and homeostasis.^{117,118} This process may be induced by starvation or nuclear stress, initiating with the formation of autophagosomes that engulf the intracellular cargo and carry them to lysosomes for degradation and recycling.^{117,118} Autophagy initiation is controlled by the serine/

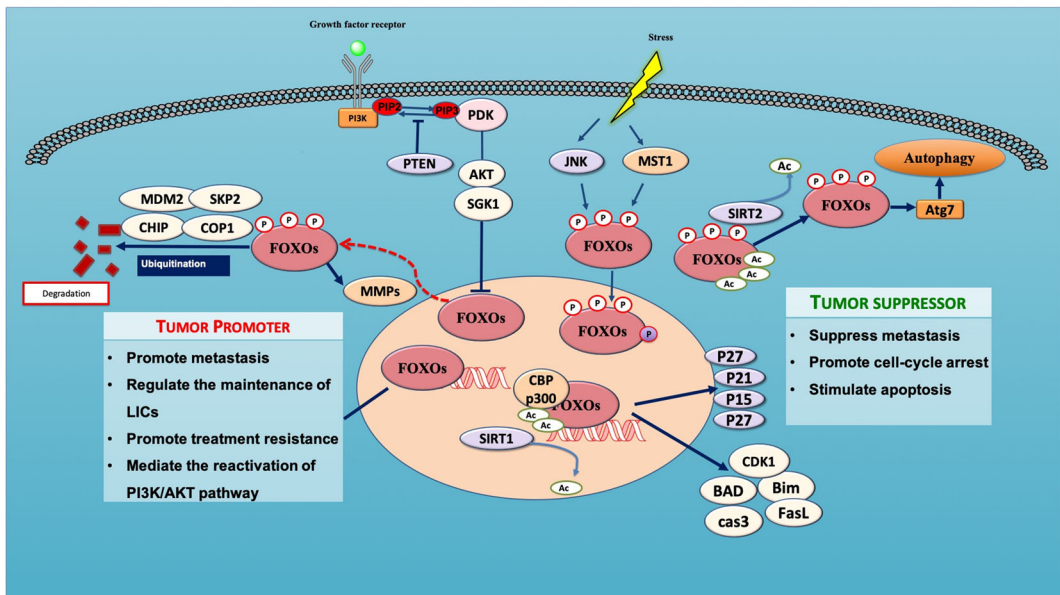


FIGURE 1 The role of FOXOs in cancer and its related pathways. FOXOs are generally considered as tumor suppressors but they can also promote the progression of cancer. Modulating FOXOs could be a promising therapeutic approach for the treatment of cancer. AKT, protein kinase B; CBP, CREB binding protein; FOXO, forkhead box O; JNK, c-Jun N-terminal kinase; LIC, leukemia-initiating cells; MMP, matrix metalloproteinase; MST1, mammalian Ste20-like kinase; PI3K, phosphatidylinositol 3-kinase; SGK1, serum/glucocorticoid-regulated kinase 1; SIRT2, silent information regulator 2 [Color figure can be viewed at wileyonlinelibrary.com]

threonine kinase ULK1 complex, which is a direct target of mTOR pathway and AMP-activated kinase (AMPK).¹¹⁹ The mTOR pathway constitutes a major negative regulator of autophagy¹²⁰ being activated downstream of PI3K-AKT signaling pathway which is frequently deregulated in cancer.^{121,122} Autophagy can also be initiated by AMPK activation, which inhibits mTOR.^{123,124} Once mTORC1 kinase activity is inhibited, autophagy initiates the formation of autophagosomes by activating ULK1 complex.^{119,123} This step is followed by the expansion of the autophagosomal membrane promoted by the activation of a second protein complex composed of vacuolar sorting protein 34 (VPS34), a class III PI3K, and beclin 1 (BECN1).^{119,125} Continued expansion of the autophagosomal membrane is regulated by the conjugation of essential autophagy genes Atg5 to Atg12 and of microtubule-associated protein 1 light chain 3 alpha (LC3) to phosphatylethanolamine (PE), forming LC3-PE conjugate (also known as LC3-II).^{119,123,126} The engulfment of the cytoplasmic constituents then occurs both in a selectively, through the action of scaffolding proteins such as the adaptor protein p62/sequestosome 1, and nonselectively manner.^{119,127,128} This is followed by sealing of the two ends of the membrane to form a completed autophagosome which then fuses with lysosomes through a mechanism mediated by small GTPases such as RAB7 and SNARE-like proteins.^{119,129-131} This process is finalized by the degradation and recycling of autophagosome contents.¹¹⁹

Multiple pieces of evidence suggest that autophagy is involved in tumor initiation and development, cancer progression, and metastasis. However, the precise role of autophagy in cancer is not clear due to the fact that it can either inhibit tumor cell initiation or promote tumor cell survival (Figure 2) depending on cancer type, stage of progression, and genetic context.^{118,132,133}

4.1 | Autophagy as tumor suppressor

The suppressive role of autophagy in cancer progression was initially suggested by studies showing that one allele of Atg6/BECN1 was depleted in 40% to 75% of human ovarian, breast and prostate cancers, that BECN1 upregulation inhibited the growth of human breast cancer cell lines and that BECN1 heterozygous mutant mice

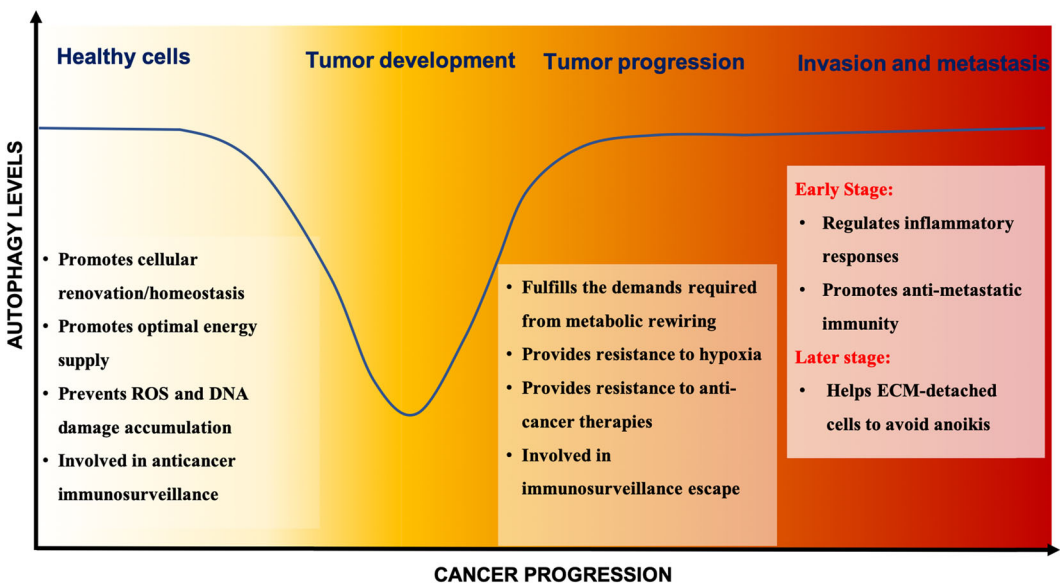


FIGURE 2 The role of autophagy in cancer. Autophagy plays a dual role in cancer since it can either inhibit tumor cell initiation and early metastasis or promote tumor cell survival and invasion. ECM, extracellular matrix; ROS, reactive oxygen species [Color figure can be viewed at wileyonlinelibrary.com]

suffered from a high incidence of spontaneous tumor formation.¹³⁴⁻¹³⁸ BECN1, an important protein involved in the autophagosome formation step is regulated by UV radiation resistance-associated gene and Bax-interacting factor-1 whose impairment has been shown to promote tumor cell proliferation in several cancers.¹³⁹⁻¹⁴¹ In addition, the deletion of vital autophagy genes Atg5 and Atg7 in mice induced the development of benign liver tumors.¹⁴² Possible mechanisms behind the autophagy tumor suppressive role have been already identified.^{132,143} The PI3K/AKT/mTOR signaling pathway has been shown to be commonly deregulated during cancer. Specifically, the constitutive activation of PI3K/AKT, which may be due to the occurrence to PI3K mutations, loss of PTEN or even overexpression of AKT, and subsequent stimulation of mTOR, inhibits the autophagic activity supporting its suppressive role in cancer progression.¹⁴⁴⁻¹⁴⁶ Failure in the control of damaged proteins and organelles due to autophagy inhibition leads to the accumulation of reactive oxygen species, increased DNA damage, and mitochondrial defects increasing the susceptibility for tumor development.¹⁴³ Oxidative stress upregulation promotes the transcription of genes involved in the antioxidant defense by nuclear factor erythroid 2-related factor 2 (NRF2) which is activated by p62.^{143,147,148} In fact, defective autophagy was shown to increase p62 levels which promotes NRF2 activation to fight against oxidative stress and therefore enabling cell survival.^{143,149} Conversely, p62 ablation was shown to suppress tumorigenesis in mouse models with defective autophagy.¹⁴⁷ Since p62 was also shown to regulate other oncogenic pathways involving nuclear factor- κ B and mTOR as a signaling adaptor,¹⁵⁰ it would be of great interest to ascertain its involvement in autophagy impairment and tumor promotion. Autophagy cancer suppressive function has also been associated to its aptitude to control early infiltration of regulatory T cells.¹⁵¹ In addition, in later stages of cancer, autophagy may have a protective role in tumor cells immunity, as its inhibition was shown to restrict cancer progression.¹⁵¹

4.2 | Autophagy as tumor promoter

Despite being generally considered as a tumor suppressor in the initial stage of tumor development, in more-established cancers, autophagy promotes tumorigenesis since it tries to fulfill the increased biosynthetic and metabolic needs of tumor cells.^{135,152} Autophagy has been shown to be essential in the promotion of tumor cell survival undergoing hypoxia and that are under pressure from therapeutic interventions.^{135,153} In fact, hypoxic conditions promote hypoxia-inducible factor 1 α -dependent and independent (via AMPK activation and mTOR inhibition) autophagy induction, thus enabling cell survival.^{154,155} Likewise, hypoxic activation of autophagy was shown to induce p62 downregulation which further promoted an increased Ras/ERK activity that contributed to cell survival and proliferation.¹⁵⁶ Autophagy tumor promoter role was further established by studies performed in conditional autophagy gene FIP200 knockout mice showing that lack of FIP200 reduced the development of mammary tumors.¹⁵⁷ Moreover, in cells transformed with oncogenic RAS, autophagy was shown to have an essential role in promoting tumor cells survival, growth, invasion, and metastasis.¹⁵⁸⁻¹⁶¹ Therefore, by conferring tumor cells a survival advantage, autophagy may also be seen as a mechanism that facilitates cancer progression.

4.3 | Autophagy in cancer metastasis

As autophagy activation shares many of the environmental triggers known to promote metastatic formation, its activity was shown to be upregulated during metastasis. In fact, increased staining of the autophagy-associated protein LC3 was associated with lymph node metastasis and decreased survival rate in human breast cancer patient samples.^{162,163} Moreover, studies performed in melanoma metastasis samples revealed an increased expression of LC3 and BECN1.¹⁶⁴ Increased LC3 expression also correlated with metastasis in HCC.^{165,166} As with primary cancer cells, autophagy has demonstrated both anti- and prometastatic effects.^{167,168} By supporting the survival of tumor cell under hypoxic and metabolic stress conditions, which results in the reduction of tumor cell necrosis and

subsequent infiltration of inflammatory cells, autophagy inhibits early -stage metastasis.^{153,167-169} Moreover, by directly mediating the secretion of immunomodulatory factors by the dying tumor cells, such as high-mobility group box 1 (HMGB1), autophagy is able to directly regulate the cancer-triggered inflammatory responses.¹⁷⁰ In fact, released HMGB1 was reported to bind to dendritic cells Toll-like receptor 4 (TLR4) thereby triggering an antitumor immune response that restricts metastasis.¹⁷¹ In a mouse model of melanoma metastasis, the prophylactic treatment with TLR4/TLR9 agonist complex promoted antimetastatic immunity by inducing autophagy-associated cell death via interferon- γ /signal transducer and activator of transcription 1 activation and impairing tumor metastasis.¹⁷² In another study, AKT was actively phosphorylated by CXCL12 and several cytokines, and inhibition of the CXCR4/mTOR pathway induced autophagic cell death and inhibited metastasis of peritoneally disseminated gastric cancer cells.¹⁷³ Loss of PTEN and subsequent activation of the AKT pathway in prostate cancer have also been pointed as an early prognostic marker for metastasis. The upregulation of PI3K/AKT/mTOR signaling pathway decreases the autophagic flux, thus promoting cancer progression.¹⁷⁴ Accordingly, the inhibition of AKT pathway was shown to promote a decrease in metastasis incidence.¹⁷⁵

During advanced stages of metastasis progression, autophagy may promote metastasis by helping the tumor cells that detach from the extracellular matrix (ECM) to avoid anoikis thereby enhancing their survival and colonization in secondary sites.^{168,176,177} In spite of the precise mechanisms of autophagy protection towards anoikis remain unclear, recent evidence suggests that protein kinase R-like endoplasmic reticulum kinase promotes the survival of ECM-detached cells by inducing the autophagic activity and a proper antioxidant response.¹⁷⁸ Autophagy inhibition in an animal model of HCC lung metastasis resulted in the decrease of metastasis formation by promoting anoikis resistance.¹⁶⁵ Since tumor cells detachment is associated with a loss of extrinsic signaling that support proper nutrient and energy uptake, autophagy may work as a compensatory mechanism, as suggested by a study showing that autophagy is induced by either ECM detachment or β 1 integrin inhibition.¹⁷⁹ Thus, autophagy might postpone anoikis initiation by giving cells more time to connect with a suitable ECM.¹⁶⁸ Disseminated cells that are unable to reconnect with ECM in the secondary sites may enter a dormant state promoted by autophagy.¹⁸⁰ Understanding the contexts that dictate autophagy role in cancer progression and metastasis will be pivotal to the development of more effective therapies.

5 | FOXOs-AUTOPHAGY REGULATION IN CANCER

Several lines of evidence support a connection between FOXO proteins and autophagy. FOXOs have been shown to regulate autophagy by both transcriptional-dependent and transcriptional-independent mechanisms. While located in the nucleus, FOXOs are able to promote the transcription of several autophagy genes involved in the different steps of the autophagic process, but when translocated to the cytosol, FOXOs also regulate autophagy by interacting directly with cytosolic autophagy proteins.¹⁸¹ During muscle atrophy, FOXO3 was reported to upregulate several autophagy-associated genes, like LC3, BNIP3, Gabarapl1, VPS34, ULK2, and Atg12.^{33,182} Moreover, FOXO1 deacetylation was shown to occur in mouse cardiomyocytes with the assistance of NAD-dependent deacetylase SIRT1 (sirtuins) which induces the expression of RAB7A, GTP-binding protein. This protein aids in the fusion of lysosomes and mature autophagic vesicles.¹⁸³ Study of PI3K-AKT-FOXO pathway revealed that FOXOs upregulation of glutamine synthetase results in mTOR inhibition and consequently promotes autophagy. This is indicative that autophagy induction by FOXOs-mediated increase of glutamine metabolism might allow cellular survival during starvation by protecting cells from damage accumulation.¹⁸⁴ FOXOs induction of Sestrin3 and RICTOR was also reported to enhance the autophagic flux by inhibiting mTOR1.¹⁸⁵ In addition, studies performed in mouse embryonic fibroblast and embryonic kidney (HEK293T) cell lines showed that FOXO3 coordinately regulates FOXO1 to induce transcription-dependent autophagy.³⁴ FOXO proteins were also shown to control the autophagic flux by epigenetic mechanisms and more recently autophagy was reported as being able to degrade FOXO proteins (reviewed in detail by Cheng¹⁸¹).

The exact role of FOXOs regulation of autophagy in tumor biology is still unclear. The hyperactivation of PI3K/AKT signaling pathway in cancers promotes FOXOs phosphorylation thereby promoting their nuclear exclusion. Upon stress conditions, cytosolic FOXO1 was shown to induce autophagy in human colon cancer cells by interacting with Atg7. In this study, FOXO1-mediated autophagy induction resulted in an increase of cell apoptosis, which is associated with FOXO1 antineoplastic effect.¹³ Epigenetic modulation of FOXO proteins by histone deacetylase inhibition was reported to induce autophagy and apoptosis through FOXO1-mTOR signaling and FOXO1 transactivation of autophagy genes in human cancer cells.²⁵ Conversely, FOXO3 was shown to inhibit the cytosolic accumulation and expression of FOXO1 in multiple cancer cell lines, resulting in autophagy suppression.³⁵ FOXO3 was also reported to trigger autophagy in neuroblastoma cells by upregulating the decidual protein induced by progesterone (C10ORF10/DEPP) expression.¹⁴ In this study, FOXO3-induced autophagy partly protected neuroblastoma cells from apoptosis, and pharmacological inhibition of autophagy enhanced chemotherapeutic intervention outcomes.¹⁴ Some studies have also been reporting that autophagy deregulation in cancer may promote FOXO proteins turnover mediated by AKT induced phosphorylation.^{186,187} More recently, FOXO3 was shown to be degraded by basal autophagy by acting as its cargo. In fact, autophagy inhibition in cancer cells was shown to upregulate FOXO3 activity consequently increasing the proapoptotic protein p53 upregulated modulator of apoptosis (PUMA) levels conferring the cells a higher sensitivity to an apoptosis inducer. This can explain how autophagy inhibition can increase apoptosis upon the administration of anticancer drugs.^{188,189}

6 | CANCER THERAPY

6.1 | Targeting FOXOs for cancer therapy

Cancer therapies based in restoring the expression and activation of FOXOs may be effective in tumor suppression. In fact, FOXOs are involved in the mediation of cancer cell apoptosis promoted by the use of chemotherapeutic drugs such as 5-fluorouracil, paclitaxel, and resveratrol, and inhibitors of BCR-ABL, PI3K, or AKT.¹⁹⁰⁻¹⁹⁴ Nonetheless, the effectiveness of the treatments is often vulnerable to the development of multidrug resistance (MDR) as demonstrated by studies showing that FOXO1 and FOXO3 are key regulators of the MDR1 in adriamycin-resistant breast cancer cells and in doxorubicin chemotherapy, respectively.^{195,196} Therapeutic strategies designed to target FOXO proteins may also be jeopardized by feedback mechanisms that promote cell survival. Upon treatment with inhibitors of PI3K or AKT, cancer cells have the ability to readjust to the loss of oncogenic signaling by FOXOs-mediated reactivation of PI3K-AKT pathway. In fact, the activation of FOXO3 by doxorubicin promoted K562 CML cell survival by upregulating the expression of PI3K catalytic subunit p110 α and thereby activating PI3K/AKT pathway.¹⁹⁷ Also, the inhibition of the PI3K-AKT pathway in renal cell carcinoma increased FOXOs activity, which enhanced the expression of TORC2 component RICTOR promoting AKT activation.¹⁹⁸ Altogether, these findings reinforce the paradoxical function of FOXOs in cancer.

6.2 | Targeting autophagy for cancer therapy

Treatments aiming at targeting autophagy in tumor cells have been focusing on either its induction and/or inhibition. While the induction of cytoprotective autophagy in premalignant lesions may prevent cancer progression, in more established cancers autophagy inhibition may prevent tumors from growing and spreading.^{199,200} Autophagy-inducing drugs include mTOR1 inhibitors like rapamycin and rapamycin derivatives temsirolimus (CCI-779) and everolimus (RAD001), which have been used in the treatment of different cancers.²⁰¹⁻²⁰⁴ Other anticancer and autophagy stimulatory compounds have also been identified, including metformin that upregulates AMPK^{205,206}; resveratrol that activates SIRT1²⁰⁷; and perifosine that inhibits AKT and mTOR.^{208,209} Despite the

induction of cytoprotective autophagy in early-stage tumors may suppress cancer progression, its potentiation by chemotherapy and radiation therapy has been identified as a resistance mechanism in several cancers.^{200,210,211} Together with the fact that tumor cells from advanced cancers benefit from autophagic activity to survive the augmented metabolic demands, current anticancer therapies have been mainly focused on autophagy inhibition combined with different types of anticancer approaches. The rationale behind the employment of combination therapies is to render cells the sensitivity to the treatments. Different autophagy inhibitors actuate on the different phases of the autophagic process. Inhibitors that actuate at the initiation stage, by blocking autophagosome formation through class III PI3K targeting, include wortmannin, 3-methyladenine, and LY294002.²¹²⁻²¹⁵ Other inhibitors such as chloroquine (CQ), hydroxychloroquine (HCQ), monensin, and Bafilomycin A1, the latter being a specific inhibitor of vacuolar-ATPase, inhibit the autophagosome-lysosome fusion.^{123,216} By being able to cross the lysosomal membrane, these drugs inhibit autophagy by preventing lysosomes acidification and blocking the fusion of autophagosomes with lysosomes.²¹⁷ Curiously, CQ antitumor actions and ability to sensitize cancer cells to chemotherapy have also been described to occur through autophagy-independent mechanisms.²¹⁸⁻²²⁰ At the moment, CQ and HCQ are the only clinically available inhibitors and they have been evaluated alone or in combination with other anticancer therapies in different cancers (Table 2).^{210,221,222} Past clinical trials demonstrated that CQ and HCQ are safe autophagy inhibitors and despite presenting diverging clinical outcomes its administration still holds encouraging therapeutic benefits. More importantly, current research suggests the need to design new delivery approaches able to improve the drugs penetration into cancer cells, and to develop more potent and specific autophagy inhibitors.²¹⁰ In this sense, new promising autophagy inhibitors have been developed envisioning their application in forthcoming clinical trials and include ULK1, VPS34, ATG4B, palmitoyl-protein thioesterase 1, and phosphoinositide kinase-type finger-containing kinase (PIKFYVE) inhibitors²²³ (Table 3) (reviewed in detail by Amaravadi et al²¹⁰). At the moment, an ongoing phase 1 clinical trial is testing the safety and pharmacokinetics of the PIKFYVE inhibitor apilimod in patients with non-Hodgkin's lymphoma (NCT02594384). The discovery of more reliable biomarkers to screen the tumors before treatment may also result in better therapeutic outcomes.^{199,210}

6.3 | Therapeutic opportunities related to FOXOs-autophagy regulation

Evidence regarding FOXOs-autophagy regulation in cancer may allow the emergence of trustworthy biomarkers and novel therapeutic strategies aiming to halt cancer progression. In fact, the subcellular localization of FOXO3 upon PI3K inhibition was reported to function as a biomarker to predict the outcome of therapies combining PI3K and autophagy inhibitors in cervical cancer cells with PIK3CA mutations.²⁴⁶ As the use of PI3K inhibitors is often accompanied by the development of drug resistance, combined treatment with HCQ was shown to enhance PI3K inhibitor efficacy in cells that exhibited FOXO3 nuclear translocation.²⁴⁶ In a different study, FOXO1 turnover was shown to be promoted by ERK-induced interaction of FOXO1 with X-box-binding protein 1 (XBP1). XBP1 was found to be inversely proportional to FOXO1 and autophagy as shown by XBP1 upregulation and FOXO1 downregulation in human colon cancer samples.²⁴⁷ Therefore, ERK inhibition may impair cancer progression by inducing FOXO1 expression and autophagy. However, in RAS- or BRAF-mutant cancers, autophagy induction by MAPK inhibition was recently associated with the development of treatment resistance. Therefore, the combination of MAPK inhibitors with autophagy inhibitors may offer promising results.²⁴⁸⁻²⁵⁰ treatment, a natural isothiocyanate, was shown to inhibit PI3K/AKT and mitogen-activated protein kinase/ERK signaling pathways thereby promoting FOXOs transcriptional activity in pancreatic cancer cells.¹⁹³ In another study, benzyl isothiocyanate treatment promoted FOXO1 acetylation thus inducing the upregulation of autophagy and inhibiting the growth of human breast-cancer cells.²⁵¹ Interferon regulatory factor-4 binding protein, whose expression has been highly correlated with the malignancy and invasiveness of human breast cancer cells, was shown to have a suppressive effect on these cells autophagic activity that relied on the activation of mTOR2/AKT/FOXO3

TABLE 2 Published chloroquine and hydroxychloroquine trials in cancer

Cancer	Inhibitor	Clinical trial phase	Combination treatment	Biomarkers	Reference
Breast cancer, NSCLC, and brain metastases	CQ	Phase 2	Radiation	Not analyzed	224
Benign (solid) tumor	HCQ	Phase 1	Vorinostat	EM analysis of PBMCs for AVs IHC detection of LC3II	225
Glioblastoma	CQ	Phase 3	Radiation and TMZ	Not analyzed	226
Benign tumor and melanoma	HCQ	Phase 1	Tenisirolimus	EM analysis of PBMCs for AVs	227
Relapsed glioblastoma	CQ	Five patients case series	Radiation	Not analyzed	228
Benign tumor	HCQ	Phase 1	Docetaxel, rapamycin with metronomic Cyproterone	Not analyzed	229
Pancreatic tumor	HCQ	Phase 2	None	Analysis of LC3II in peripheral lymphocyte from metastatic pancreatic cancer patient	230
Advanced pancreatic cancer	HCQ	Phase 2	Gemcitabine and nab-paclitaxel	Not analyzed	223
Renal cell carcinoma	HCQ	Phase 1/phase 2	Everolimus	Not analyzed	231

Abbreviations: AVs, autophagic vesicles; CQ, chloroquine; EM, electron microscopy; HCQ, hydroxychloroquine; IHC, immunohistochemistry; LC3, microtubule-associated protein 1 light chain 3 alpha; NSCLC, non-small cell lung cancer; PBMC, peripheral blood mononuclear cell.

TABLE 3 Novel autophagy inhibitors for cancer therapy

Inhibitor	Targets	Inhibition point
ULK101 ²³² SBI-0206965 ²³³	ULK1	Initiation of autophagic vesicle nucleation
SAR405 ²³⁴ Compound 13 ²³⁵ SB02024 ²³⁶ Autophinib ²³⁷	VPS34	Initiation of autophagic vesicle nucleation
S130 ²³⁸ FMK-9a ²³⁹ NSC185058 ²⁴⁰ LV-320 ²⁴¹	Atg4B	Autophagosome formation and/or autophagosome fusion with the lysosome
Lys05 ^{242,243} DQ661 ²⁴⁴ DC661 ²⁴³	Palmitoyl-protein thioesterase 1	Autophagosome fusion with the lysosome
Apilimod ²⁴⁵	PIKFYVE	Lysosomal degradation

pathway.²⁵² The development and induced innate immunity of natural killer (NK) cells were also shown to be dependent of FOXOs-mediated autophagy.²⁵³ The efficacy of anticancer immunotherapies resorting to NK could therefore be enhanced by upregulating FOXOs transcriptional activity and autophagy. The recent discovery that autophagy modulates FOXO3 turnover is another example of how the synergy between them may pave the way to the development of more effective therapies, as the inhibition of autophagy caused cancer cells to become more sensitive to anticancer drugs due to the upregulation of FOXO3 activity and consequent increase of PUMA levels.^{188,189}

7 | CONCLUSION AND FUTURE DIRECTIONS

Emerging evidence has shown that FOXO proteins and autophagy are promising therapeutic targets for cancer treatments. However, despite their great potential, it is still necessary to fully comprehend their role in cancer progression. Identifying the contexts where FOXOs support or suppress cancer progression is paramount for the development of more effective therapeutic approaches. The supportive role for FOXO proteins in cancer progression complicates treatment strategies aiming the reactivation of FOXOs activity. Therefore, alternative strategies may include a combination of FOXO protein inhibitors and drugs able to target PI3K to avoid the acquisition of drug resistance. Improvement of drug specificity and development of suitable methods to measure FOXOs activity before and after treatment also pose as valuable tools to enhance therapeutic efficacy.

Autophagy studies addressing whether it should be induced or inhibited, and which patients would benefit from autophagy manipulation will also result in better therapeutic outcomes. Current clinical trials involving autophagy modulation were designed to evaluate autophagy inhibition alone or in combination with other treatments. Nonetheless, determining which is the better therapy combination and which tumors are more susceptible

to autophagy modulation is still a challenge. The development of more reliable biomarkers to measure the autophagic flux in vivo will facilitate the patients that could benefit from treatments envisioning autophagy inhibition.

Efforts have been made to understand the mechanisms underlying FOXOs-mediated autophagy in cancer and its therapeutic potential. A great body of evidence suggest that FOXOs are key autophagy inducers promoting both health and illness depending on the different contexts.¹⁸¹ In fact, autophagy regulation by FOXO proteins in tumor cells is different from its regulation in healthy conditions and their established paradoxical role in carcinogenesis further complicates the understanding of the mechanisms underlying the regulation of this axis during cancer. The modulation of FOXOs transcriptional activity through its upstream regulators, such as PI3K or ERK, could offer a potential approach to regulate the autophagic activity of tumor cells. Nonetheless, the establishment of potential treatment resistance mechanisms would also need to be addressed. The recent discovery that autophagy inhibition upregulates FOXO3 proteins rendering cells a higher responsiveness to chemotherapy opens new doors to searching of new approaches. Many questions still need to be addressed to fully comprehend how to benefit from FOXOs-autophagy regulation during cancer.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (File No. 31771128), the Science and Technology Development Fund, Macau SAR (File Nos. 0127/2019/A3, 0044/2019/AGJ, and 0113/2018/A3), and the University of Macau (File No. MYRG2018-00134-FHS).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Wenhua Zheng  <http://orcid.org/0000-0001-9767-4295>

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674. <https://doi.org/10.1016/j.cell.2011.02.013>
2. Lambert AW, Pattabiraman DR, Weinberg RA. Emerging biological principles of metastasis. *Cell*. 2017;168(4):670-691. <https://doi.org/10.1016/j.cell.2016.11.037>
3. Fidler IJ. The pathogenesis of cancer metastasis: the "seed and soil" hypothesis revisited. *Nat Rev Cancer*. 2003;3(6):453-458.
4. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. *Cell*. 2011;147(2):275-292. <https://doi.org/10.1016/j.cell.2011.09.024>
5. Leber MF, Efferth T. Molecular principles of cancer invasion and metastasis (review). *Int J Oncol*. 2009;34(4):881-895. https://doi.org/10.3892/ijo_00000214
6. Zhang X, Tang N, Hadden TJ, Rishi AK. Akt, FoxO and regulation of apoptosis. *Biochim Biophys Acta, Mol Cell Res*. 2011;1813(11):1978-1986. <https://doi.org/10.1016/j.bbamcr.2011.03.010>
7. Brucker DP, Maurer GD, Harter PN, Rieger J, Steinbach JP. FOXO3a orchestrates glioma cell responses to starvation conditions and promotes hypoxia-induced cell death. *Int J Oncol*. 2016;49(6):2399-2410. <https://doi.org/10.3892/ijo.2016.3760>
8. Hori YS, Kuno A, Hosoda R, Horio Y. Regulation of FOXOs and p53 by SIRT1 modulators under oxidative stress. *PLoS One*. 2013;8(9):e73875. <https://doi.org/10.1371/journal.pone.0073875.g007>
9. Charitou P, Burgering BMT. Forkhead box(O) in control of reactive oxygen species and genomic stability to ensure healthy lifespan. *Antioxid Redox Signal*. 2013;19(12):1400-1419. <https://doi.org/10.1089/ars.2012.4921>
10. Burgering BMT. A brief introduction to FOXOlogy. *Oncogene*. 2008;27(16):2258-2262. <https://doi.org/10.1074/jbc.M106091200>
11. de Brachène AC, Demoulin J-B. FOXO transcription factors in cancer development and therapy. *Cell Mol Life Sci*. 2016;73:1159-1172. <https://doi.org/10.1007/s00018-015-2112-y>
12. Kim CG, Lee H, Gupta N, et al. Role of forkhead box class O proteins in cancer progression and metastasis. *Semin Cancer Biol*. 2018;50:142-151. <https://doi.org/10.1016/j.semcancer.2017.07.007>

13. Zhao Y, Yang J, Liao W, et al. Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity. *Nat Cell Biol.* 2010;12(7):665-675. <https://doi.org/10.1038/ncb2069>
14. Salcher S, Hermann M, Kiechl-Kohlendorfer U, Ausserlechner MJ, Obexer P. C10ORF10/DEPP-mediated ROS accumulation is a critical modulator of FOXO3-induced autophagy. *Mol Cancer.* 2017;16:95.
15. Tanida I, Tanida-Miyake E, Komatsu M, Ueno T, Kominami E. Posttranslational modification of autophagy-related proteins in macroautophagy. *Cell Death Differ.* 2014;11(1):28-45. <https://doi.org/10.1074/jbc.M200385200>
16. Bento CF, Renna M, Ghislat G, et al. Mammalian autophagy: how does it work? *Annu Rev Biochem.* 2016;85(1):685-713. <https://doi.org/10.1146/annurev-biochem-060815-014556>
17. Hurlley JH, Young LN. Mechanisms of autophagy initiation. *Annu Rev Biochem.* 2017;86(1):225-244. <https://doi.org/10.1146/annurev-biochem-061516-044820>
18. Vlahakis A, Debnath J. Triggering selective autophagy at the right place and the right time. *Mol Cell.* 2016;64(2):215-216. <https://doi.org/10.1016/j.molcel.2016.10.004>
19. Qian X, Li X, Cai Q, et al. Phosphoglycerate kinase 1 phosphorylates beclin1 to induce autophagy. *Mol Cell.* 2017;65(5):917-931.e6. <https://doi.org/10.1016/j.molcel.2017.01.027>
20. Baek SH, Kim IK. Epigenetic control of autophagy: nuclear events gain more attention. *Mol Cell.* 2017;65(5):781-785. <https://doi.org/10.1016/j.molcel.2016.12.027>
21. Clark KL, Halay ED, Lai E, Burley SK. Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. *Nature.* 1993;364(6436):412-420. <https://doi.org/10.1038/364412a0>
22. Weigel D, Jurgens G, Kuttner F, Seifert E, Jackie H. The homeotic gene fork head encodes a nuclear protein and is expressed in the terminal regions of the drosophila embryo. *Cell.* 1989;57:645-658.
23. Jin C, Marsden I, Chen X, Liao X. Sequence specific collective motions in a winged helix DNA binding domain detected by 15N relaxation NMR. *Biochemistry.* 1998;37(17):6179-6187.
24. Wang Y, Zhou Y, Graves DT. FOXO transcription factors: their clinical significance and regulation. *BioMed Res Int.* 2014;2014(3):925350. <https://doi.org/10.2337/db11-0548>
25. Zhang J, Ng S, Wang J, et al. Histone deacetylase inhibitors induce autophagy through FOXO1-dependent pathways. *Cell Death Differ.* 2015;11(4):629-642. <https://doi.org/10.1080/15548627.2015.1023981>
26. Prasad SB, Yadav SS, Das M, et al. Down regulation of FOXO1 promotes cell proliferation in cervical cancer. *J Cancer.* 2014;5(8):655-662.
27. Ai J, Duan J, Lv X, et al. Overexpression of FoxO1 causes proliferation of cultured pancreatic beta cells exposed to low nutrition. *Biochemistry.* 2010;49(1):218-225. <https://doi.org/10.1021/bi901414g>
28. Gross DN, van den Heuvel APJ, Birnbaum MJ. The role of FoxO in the regulation of metabolism. *Oncogene.* 2008;27(16):2320-2336. <https://doi.org/10.1038/onc.2008.25>
29. Martinez SC, Tanabe K, Cras-Meneur C, Abumrad NA, Bernal-Mizrachi E, Permutt MA. Inhibition of Foxo1 protects pancreatic islet beta-cells against fatty acid and endoplasmic reticulum stress-induced apoptosis. *Diabetes.* 2008;57(4):846-859. <https://doi.org/10.2337/db07-0595>
30. Seiler F, Hellberg J, Lepper PM, et al. FOXO transcription factors regulate innate immune mechanisms in respiratory epithelial cells. *J Immunol.* 2013;190(4):1603-1613. <https://doi.org/10.4049/jimmunol.1200596>
31. Fu Z, Tindall DJ. FOXOs, cancer and regulation of apoptosis. *Oncogene.* 2008;27(16):2312-2319. <https://doi.org/10.1074/jbc.M205424200>
32. Sunayama J, Sato A, Matsuda K, et al. FoxO3a functions as a key integrator of cellular signals that control glioblastoma stem-like cell differentiation and tumorigenicity. *Stem Cells.* 2011;29(9):1327-1337. <https://doi.org/10.1002/stem.696>
33. Zhao J, Brault JJ, Schild A, et al. FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. *Cell Metab.* 2007;6(6):472-483. <https://doi.org/10.1016/j.cmet.2007.11.004>
34. Puig O. FOXO3 induces FOXO1-dependent autophagy by activating the AKT1 signaling pathway. *Cell Death Differ.* 2012;8(12):1712-1723. <https://doi.org/10.1101/gad.1098703>
35. Zhu WL, Tong H, Teh JT, Wang M. Forkhead box protein O3 transcription factor negatively regulates autophagy in human cancer cells by inhibiting forkhead box protein O1 expression and cytosolic accumulation. *PLoS One.* 2014;9(12):e115087. <https://doi.org/10.1371/journal.pone.0115087.g006>
36. McClelland Descalzo DL, Satoorian TS, Walker LM, Sparks NRL, Pulyanina PY, Nieden Zur NI. Glucose-induced oxidative stress reduces proliferation in embryonic stem cells via FOXO3A/beta-catenin-dependent transcription of p21(cip1). *Stem Cell Rep.* 2016;7(1):55-68. <https://doi.org/10.1016/j.stemcr.2016.06.006>
37. Marlow LA, von Roemeling CA, Cooper SJ, et al. Foxo3a drives proliferation in anaplastic thyroid carcinoma through transcriptional regulation of cyclin A1: a paradigm shift that impacts current therapeutic strategies. *J Cell Sci.* 2012;125(Pt 18):4253-4263. <https://doi.org/10.1242/jcs.097428>
38. Jensen KS, Binderup T, Jensen KT, et al. FoxO3A promotes metabolic adaptation to hypoxia by antagonizing Myc function. *EMBO J.* 2011;30(22):4554-4570. <https://doi.org/10.1038/emboj.2011.323>

39. Chen Y-F, Pandey S, Day CH, et al. Synergistic effect of HIF-1 α and FoxO3a trigger cardiomyocyte apoptosis under hyperglycemic ischemia condition. *J Cell Physiol*. 2018;233(4):3660-3671. <https://doi.org/10.1002/jcp.26235>
40. Gopinath SD, Webb AE, Brunet A, Rando TA. FOXO3 promotes quiescence in adult muscle stem cells during the process of self-renewal. *Stem Cell Rep*. 2014;2(4):414-426.
41. Zhu M, Goetsch SC, Wang Z, et al. FoxO4 promotes early inflammatory response upon myocardial infarction via endothelial Arg1. *Circ Res*. 2015;117(11):967-977. <https://doi.org/10.1161/CIRCRESAHA.115.306919>
42. Su L, Liu X, Chai N, et al. The transcription factor FOXO4 is down-regulated and inhibits tumor proliferation and metastasis in gastric cancer. *BMC Cancer*. 2014;14(1):378. <https://doi.org/10.1186/1471-2407-14-378>
43. Qiao Y, Wang B, Zhang H, Yan Y, Niu L. FOXO4 overexpression suppresses hypoxia-induced-MCF-7 cell survival and promotes apoptosis through the HIF-2 α /Bnip3 signal pathway. *RSC Adv*. 2019;9(44):25912-25918. <https://doi.org/10.1039/C9RA04380B>
44. Kim DH, Perdomo G, Zhang T, et al. FoxO6 integrates insulin signaling with gluconeogenesis in the liver. *Diabetes*. 2011;60(11):2763-2774. <https://doi.org/10.2337/db11-0548>
45. Qinyu L, Long C, Zhen-dong D, et al. FOXO6 promotes gastric cancer cell tumorigenicity via upregulation of C-myc. *FEBS Lett*. 2013;587(14):2105-2111. <https://doi.org/10.1016/j.febslet.2013.05.027>
46. Calnan DR, Brunet A. The FoxO code. *Oncogene*. 2008;27(16):2276-2288. <https://doi.org/10.1046/j.0022-3042.2002.00783.x>
47. Yamagata K, Daitoku H, Takahashi Y, et al. Arginine methylation of FOXO transcription factors inhibits their phosphorylation by Akt. *Mol Cell*. 2008;32(2):221-231. <https://doi.org/10.1016/j.molcel.2008.09.013>
48. Kuo M, Zilberfarb V, Gangneux N, Christeff N, Issad T. O-glycosylation of FoxO1 increases its transcriptional activity towards the glucose 6-phosphatase gene. *FEBS Lett*. 2008;582(5):829-834. <https://doi.org/10.1016/j.febslet.2008.02.010>
49. Lu J, Zhang R, Hong H, et al. The poly(ADP-ribosyl)ation of FoxO3 mediated by PARP1 participates in isoproterenol-induced cardiac hypertrophy. *Biochim Biophys Acta*. 2016;1863(12):3027-3039. <https://doi.org/10.1016/j.bbamcr.2016.09.019>
50. Wang C, Xu W, Zhang Y, Zhang F, Huang K. PARP1 promote autophagy in cardiomyocytes via modulating FoxO3a transcription. *Cell Death Dis*. 2018;9(11):1047. <https://doi.org/10.1038/s41419-018-1108-6>
51. Wang H, Duan X, Ren Y, et al. FoxO3a negatively regulates nerve growth factor-induced neuronal differentiation through inhibiting the expression of neurochondrin in PC12 cells. *Mol Neurobiol*. 2013;47(1):24-36. <https://doi.org/10.1007/s12035-012-8357-7>
52. Liao R, Yan F, Zeng Z, et al. Amiodarone-induced retinal neuronal cell apoptosis attenuated by IGF-1 via counter regulation of the PI3k/Akt/FoxO3a pathway. *Mol Neurobiol*. 2017;54(9):6931-6943. <https://doi.org/10.1007/s12035-016-0211-x>
53. Zeng Z, Wang X, Bhardwaj SK, et al. The atypical antipsychotic agent, clozapine, protects against corticosterone-induced death of PC12 cells by regulating the Akt/FoxO3a signaling pathway. *Mol Neurobiol*. 2017;54(5):3395-3406. <https://doi.org/10.1007/s12035-016-9904-4>
54. Brunet A, Bonni A, Zigmund MJ, et al. Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. *Cell*. 1999;96:857-868.
55. Jacobs FMJ, Van der Heide LP, Wijchers PJEC, Burbach JPH, Hoekman MFM, Smidt MP. FoxO6, a novel member of the FoxO class of transcription factors with distinct shuttling dynamics. *J Biol Chem*. 2003;278(38):35959-35967. <https://doi.org/10.1093/emboj/19.5.989>
56. Brunet A, Kanai F, Stehn J, et al. 14-3-3 transits to the nucleus and participates in dynamic nucleocytoplasmic transport. *J Cell Biol*. 2002;156(5):817-828. <https://doi.org/10.1074/jbc.272.21.13717>
57. Van der Heide LP, Hoekman MFM, Smidt MP. The ins and outs of FoxO shuttling: mechanisms of FoxO translocation and transcriptional regulation. *Biochem J*. 2004;380:297-309.
58. Essers MAG, Weijzen S, de Vries-Smits AMM, et al. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J*. 2004;23(24):4802-4812. <https://doi.org/10.1038/sj.emboj.7600476>
59. Wang X, Hu S, Liu L. Phosphorylation and acetylation modifications of FOXO3a: independently or synergistically? *Oncol Lett*. 2017;13(5):2867-2872.
60. Sunayama J, Tsuruta F, Masuyama N, Gotoh Y. JNK antagonizes Akt-mediated survival signals by phosphorylating 14-3-3. *J Cell Biol*. 2005;170(2):295-304. <https://doi.org/10.1083/jcb.200409117>
61. Lehtinen MK, Yuan Z, Boag PR, et al. A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. *Cell*. 2006;125(5):987-1001. <https://doi.org/10.1016/j.cell.2006.03.046>
62. Greer EL, Oskoui PR, Banko MR, et al. The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J Biol Chem*. 2007;282(41):30107-30119. <https://doi.org/10.1074/jbc.M705325200>
63. van der Horst A, de Vries-Smits AMM, Brenkman AB, et al. FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. *Nat Cell Biol*. 2006;8(10):1064-1073. <https://doi.org/10.1038/ncb1469>

64. Huang H, Regan KM, Wang F, Smith DI, van Deursen JM, Tindall DJ. Skp2 inhibits FOXO1 in tumor suppression through ubiquitin-mediated degradation. *Proc Natl Acad Sci U S A*. 2005;102:1649-1654.
65. Van der Heide LP, Smidt MP. Regulation of FoxO activity by CBP/p300-mediated acetylation. *Trends Biochem Sci*. 2005;30(2):81-86. <https://doi.org/10.1016/j.tibs.2004.12.002>
66. Fukuoka M, Daitoku H, Hatta M, Matsuzaki H, Uemura S, Fukamizu A. Negative regulation of forkhead transcription factor AFX (Foxo4) by CBP-induced acetylation. *Int J Mol Med*. 2003;12(4):503-508.
67. Daitoku H, Hatta M, Matsuzaki H, et al. Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. *Proc Natl Acad Sci U S A*. 2004;101:10042-10047.
68. Matsuzaki H, Daitoku H, Hatta M, Aoyama H, Yoshimoshi K, Fukamizu A. Acetylation of Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. *PNAS*. 2005;102:11278-11283.
69. Galili N, Davis RJ, Fredericks WJ, et al. Fusion of a fork head domain gene to PAX3 in the solid tumour alveolar rhabdomyosarcoma. *Nat Genet*. 1993;5(3):230-235. <https://doi.org/10.1038/ng1193-230>
70. Schmitt-Ney M, Camussi G. The PAX3-FOXO1 fusion protein present in rhabdomyosarcoma interferes with normal FOXO activity and the TGF-beta pathway. *PLoS One*. 2015;10(3):e0121474.
71. Davis RJ, D'Cruz CM, Lovell MA, Biegel JA, Barr FG. Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. *Cancer Res*. 1994;54(11):2869-2872.
72. Borkhardt A, Repp R, Haas OA, et al. Cloning and characterization of AFX, the gene that fuses to MLL in acute leukemias with a t(X;11)(q13;q23). *Oncogene*. 1997;14(2):195-202. <https://doi.org/10.1038/sj.onc.1200814>
73. Hillion J, Le Coniat M, Jonveaux P, Berger R, Bernard OA. AF6q21, a novel partner of the MLL gene in t(6;11)(q21;q23), defines a forkhead transcriptional factor subfamily. *Blood*. 1997;90(9):3714-3719.
74. Yadav RK, Chauhan AS, Zhuang L, Gan B. FoxO transcription factors in cancer metabolism. *Semin Cancer Biol*. 2018;50:65-76. <https://doi.org/10.1016/j.semcancer.2018.01.004>
75. Hay N. Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy? *Nat Rev Cancer*. 2016;16(10):635-649. <https://doi.org/10.1038/nrc.2016.77>
76. Zheng J. Energy metabolism of cancer: glycolysis versus oxidative phosphorylation (review). *Oncol Lett*. 2012;4(6):1151-1157.
77. Masui K, Tanaka K, Akhavan D, et al. mTOR complex 2 controls glycolytic metabolism in glioblastoma through FoxO acetylation and upregulation of c-Myc. *Cell Metab*. 2013;18(5):726-739. <https://doi.org/10.1016/j.cmet.2013.09.013>
78. Xiao Z-D, Han L, Lee H, et al. Energy stress-induced lncRNA FILNC1 represses c-Myc-mediated energy metabolism and inhibits renal tumor development. *Nat Commun*. 2017;8(1):783. <https://doi.org/10.1038/s41467-017-00902-z>
79. Matsumoto M, Poci A, Rossetti L, DePinho RA, Accili D. Impaired regulation of hepatic glucose production in mice lacking the forkhead transcription factor Foxo1 in liver. *Cell Metab*. 2007;6(3):208-216. <https://doi.org/10.1016/j.cmet.2007.08.006>
80. O-Sullivan I, Zhang W, Wasserman DH, et al. FoxO1 integrates direct and indirect effects of insulin on hepatic glucose production and glucose utilization. *Nat Commun*. 2015;6:7079. <https://doi.org/10.1038/ncomms8079>
81. Zhang P, Tu B, Wang H, et al. Tumor suppressor p53 cooperates with SIRT6 to regulate gluconeogenesis by promoting FoxO1 nuclear exclusion. *Proc Natl Acad Sci U S A*. 2014;111(29):10684-10689. <https://doi.org/10.1073/pnas.1411026111>
82. Khan MW, Chakrabarti P. Gluconeogenesis combats cancer: opening new doors in cancer biology. *Cell Death Dis*. 2015;6:e1872. <https://doi.org/10.1038/cddis.2015.245>
83. Santos CR, Schulze A. Lipid metabolism in cancer. *FEBS J*. 2012;279(15):2610-2623. <https://doi.org/10.1111/j.1742-4658.2012.08644.x>
84. Munir R, Lisek J, Swinnen JV, Zaidi N. Lipid metabolism in cancer cells under metabolic stress. *Br J Cancer*. 2019;120(12):1090-1098. <https://doi.org/10.1038/s41416-019-0451-4>
85. Deng X, Zhang W, O-Sullivan I, et al. FoxO1 inhibits sterol regulatory element-binding protein-1c (SREBP-1c) gene expression via transcription factors Sp1 and SREBP-1c. *J Biol Chem*. 2012;287(24):20132-20143. <https://doi.org/10.1074/jbc.M112.347211>
86. Chakrabarti P, Kandror KV. FoxO1 controls insulin-dependent adipose triglyceride lipase (ATGL) expression and lipolysis in adipocytes. *J Biol Chem*. 2009;284(20):13296-13300. <https://doi.org/10.1074/jbc.C800241200>
87. Paik J-H, Kollipara R, Chu G, et al. FoxOs are lineage-restricted redundant tumor suppressors and critical regulators of endothelial cell homeostasis. *Cell*. 2008;182:309-323.
88. Dansen TB, Burgering BMT. Unravelling the tumor-suppressive functions of FOXO proteins. *Trends Cell Biol*. 2008;18(9):421-429. <https://doi.org/10.1016/j.tcb.2008.07.004>
89. Modur V, Nagarajan R, Evers BM, Milbrandt J. FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. *J Biol Chem*. 2002;277(49):47928-47937. <https://doi.org/10.1038/sj.cgt.7700333>
90. Zhang B, Tomita Y, Ch'ng E, et al. Prognostic significance of phosphorylated FOXO1 expression in soft tissue sarcoma. *Ann Surg Oncol*. 2009;16(7):1925-1937. <https://doi.org/10.1245/s10434-009-0481-x>

91. Cheong J-W, Eom JI, Maeng H-Y, et al. Constitutive phosphorylation of FKHR transcription factor as a prognostic variable in acute myeloid leukemia. *Leuk Res.* 2003;27(12):1159-1162.
92. Zhang H, Pan Y, Zheng L, et al. FOXO1 inhibits Runx2 transcriptional activity and prostate cancer cell migration and invasion. *Cancer Res.* 2011;71(9):3257-3267. <https://doi.org/10.1158/0008-5472.CAN-10-2603>
93. Wu Y, Elshimali Y, Sarkissyan M, Mohamed H, Clayton S, Vadgama JV. Expression of FOXO1 is associated with GATA3 and Annexin-1 and predicts disease-free survival in breast cancer. *Am J Cancer Res.* 2012;2:104-115.
94. Santo EE, Stroeken P, Sluis PV, Koster J, Versteeg R, Westerhout EM. FOXO3a is a major target of inactivation by PI3K/AKT signaling in aggressive neuroblastoma. *Cancer Res.* 2013;73(7):2189-2198. <https://doi.org/10.1158/0008-5472.CAN-12-3767>
95. Hu MCT, Lee D-F, Xia W, et al. IkappaB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. *Cell.* 2004;117:225-237.
96. Smit L, Berns K, Spence K, et al. An integrated genomic approach identifies that the PI3K/AKT/FOXO pathway is involved in breast cancer tumor initiation. *Oncotarget.* 2016;7:2596-2610.
97. Habashy HO, Rakha EA, Aleskandarany M, et al. FOXO3a nuclear localisation is associated with good prognosis in luminal-like breast cancer. *Breast Cancer Res Treat.* 2011;129(1):11-21. <https://doi.org/10.1128/MCB.01789-06>
98. Fei M, Zhao Y, Wang Y, et al. Low expression of Foxo3a is associated with poor prognosis in ovarian cancer patients. *Cancer Invest.* 2009;27(1):52-59. <https://doi.org/10.1080/07357900802146204>
99. Bullock MD, Bruce A, Sreekumar R, et al. FOXO3 expression during colorectal cancer progression: biomarker potential reflects a tumour suppressor role. *Br J Cancer.* 2013;109(2):387-394. <https://doi.org/10.1038/bjc.2013.355>
100. Lu M, Xiang J, Xu F, Wang Y, Yin Y, Chen D. The expression and significance of pThr32-FOXO3a in human ovarian cancer. *Med Oncol.* 2012;29(2):1258-1264. <https://doi.org/10.1007/s12032-011-9919-7>
101. Kornblau SM, Singh N, Qiu Y, Chen W, Zhang N, Coombes KR. Highly phosphorylated FOXO3A is an adverse prognostic factor in acute myeloid leukemia. *Clin Cancer Res.* 2010;16(6):1865-1874. <https://doi.org/10.1158/1078-0432.CCR-09-2551>
102. Ni D, Ma X, Li HZ, et al. Downregulation of FOXO3a promotes tumor metastasis and is associated with metastasis-free survival of patients with clear cell renal cell carcinoma. *Clin Cancer Res.* 2014;20(7):1779-1790. <https://doi.org/10.1158/1078-0432.CCR-13-1687>
103. Su B, Gao L, Baranowski C, et al. A genome-wide RNAi screen identifies FOXO4 as a metastasis-suppressor through counteracting PI3K/AKT signal pathway in prostate cancer. *PLoS One.* 2014;9:e101411. <https://doi.org/10.1371/journal.pone.0101411.s007>
104. Trinh DL, Scott DW, Morin RD, et al. Analysis of FOXO1 mutations in diffuse large B-cell lymphoma. *Blood.* 2013; 121(18):3666-3674. <https://doi.org/10.1182/blood-2013-01-479865>
105. Ahn H, Kim H, Abdul R, et al. Overexpression of forkhead box O3a and its association with aggressive phenotypes and poor prognosis in human hepatocellular carcinoma. *Am J Clin Pathol.* 2018;149(2):117-127. <https://doi.org/10.1093/ajcp/aqx132>
106. Qian Z, Ren L, Wu D, et al. Overexpression of FoxO3a is associated with glioblastoma progression and predicts poor patient prognosis. *Int J Cancer.* 2017;140(12):2792-2804. <https://doi.org/10.1002/ijc.30690>
107. Zhang X, Rielland M, Yalcin S, Ghaffari S. Regulation and function of FoxO transcription factors in normal and cancer stem cells: what have we learned? *Curr Drug Targets.* 2011;12:1267-1283.
108. Naka K, Hoshii T, Muraguchi T, et al. TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature.* 2010;463(7281):676-680. <https://doi.org/10.1038/nature08734>
109. Sykes SM, Lane SW, Bullinger L, et al. AKT/FOXO signaling enforces reversible differentiation blockade in myeloid leukemias. *Cell.* 2011;146(5):697-708. <https://doi.org/10.1016/j.cell.2011.07.032>
110. Santamaría CM, Chillón MC, García-Sanz R, et al. High FOXO3a expression is associated with a poorer prognosis in AML with normal cytogenetics. *Leuk Res.* 2009;33(12):1706-1709. <https://doi.org/10.1016/j.leukres.2009.04.024>
111. Gargini R, Cerliani JP, Escoll M, Anton IM, Wandosell F. Cancer stem cell-like phenotype and survival are coordinately regulated by Akt/FoxO/Bim pathway. *Stem Cells.* 2015;33(3):646-660. <https://doi.org/10.1002/stem.1904>
112. Tenbaum SP, Ordóñez-Morán P, Puig I, et al. β -catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. *Nature Med.* 2012;18(6):892-901. <https://doi.org/10.1038/nm.2772>
113. Rehman A, Kim Y, Kim H. FOXO3a expression is associated with lymph node metastasis and poor disease-free survival in triple-negative breast cancer. *J Clin Pathol.* 2018;71:806-813. <https://doi.org/10.1136/2017.01.161/cc.26421>
114. Storz P, Doppler H, Copland JA, Simpson KJ, Tokar A. FOXO3a promotes tumor cell invasion through the induction of matrix metalloproteinases. *Mol Cell Biol.* 2009;29(18):4906-4917. <https://doi.org/10.1128/MCB.00077-09>
115. Feng X, Wu Z, Wu Y, et al. Cdc25A regulates matrix metalloprotease 1 through Foxo1 and mediates metastasis of breast cancer cells. *Mol Cell Biol.* 2011;31(16):3457-3471. <https://doi.org/10.1128/MCB.21.14.4818-4828.2001>
116. Kumazoe M, Takai M, Bae J, et al. FOXO3 is essential for CD44 expression in pancreatic cancer cells. *Oncogene.* 2017; 36:2643-2654. <https://doi.org/10.1038/ncr.2016.426>

117. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell*. 2011;147(4):728-741. <https://doi.org/10.1016/j.cell.2011.10.026>
118. Amaravadi R, Kimmelman AC, White E. Recent insights into the function of autophagy in cancer. *Genes Dev*. 2016;30(17):1913-1930. <https://doi.org/10.1016/j.molcel.2016.02.011>
119. Thorburn A, Thamm DH, Gustafson DL. Autophagy and cancer therapy. *Mol Pharmacol*. 2014;85(6):830-838. <https://doi.org/10.1124/mol.114.091850>
120. Morselli E, Galluzzi L, Kepp O, et al. Anti- and pro-tumor functions of autophagy. *Biochim Biophys Acta, Mol Cell Res*. 2009;1793(9):1524-1532. <https://doi.org/10.1016/j.bbamcr.2009.01.006>
121. Sekulić A, Hudson CC, Homme JL, et al. A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res*. 2000;60:3504-3513.
122. Vogt PK. PI 3-kinase, mTOR, protein synthesis and cancer. *Trends Mol Med*. 2001;7:482-484.
123. Yang ZJ, Chee CE, Huang S, Sinicrope FA. The role of autophagy in cancer: therapeutic implications. *Mol Cancer Ther*. 2011;10(9):1533-1541. <https://doi.org/10.1158/1535-7163.MCT-11-0047>
124. Marinković M, Šprung M, Buljubašić M, Novak I. Autophagy modulation in cancer: current knowledge on action and therapy. *Oxid Med Cell Longevity*. 2018;2018(5):8023821. <https://doi.org/10.18632/oncotarget.6908>
125. Pattingre S, Espert L, Biard-Piechaczyk M, Codogno P. Regulation of macroautophagy by mTOR and Beclin 1 complexes. *Biochimie*. 2008;90(2):313-323. <https://doi.org/10.1016/j.biochi.2007.08.014>
126. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. *Nat Rev Mol Cell Biol*. 2001;2:211-216.
127. Pankiv S, Clausen TH, Lamark T, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem*. 2007;282:24131-24145.
128. Birgisdottir AB, Lamark T, Johansen T. The LIR motif—crucial for selective autophagy. *J Cell Sci*. 2013;128:3237-3247. <https://doi.org/10.1242/jcs.126128>
129. Gutierrez MG. Rab7 is required for the normal progression of the autophagic pathway in mammalian cells. *J Cell Sci*. 2004;117(13):2687-2697. <https://doi.org/10.1242/jcs.01114>
130. Diao J, Liu R, Rong Y, et al. ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. *Nature*. 2015;520(7548):563-566. <https://doi.org/10.1006/jmbi.1996.0399>
131. Itakura E, Kishi-Itakura C, Mizushima N. The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. *Cell*. 2012;151(6):1256-1269. <https://doi.org/10.1016/j.cell.2012.11.001>
132. Galluzzi L, Pietrocola F, Bravo-San Pedro JM, et al. Autophagy in malignant transformation and cancer progression. *EMBO J*. 2015;34(7):856-880. <https://doi.org/10.15252/embj.201490784>
133. Eisenberg-Lerner A, Kimchi A. The paradox of autophagy and its implication in cancer etiology and therapy. *Apoptosis*. 2009;14(4):376-391. <https://doi.org/10.1007/s10495-008-0307-5>
134. Aita VM, Liang XH, Murty VVVS, et al. Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. *Genomics*. 1999;59(1):59-65.
135. Choi AMK, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med*. 2013;368(7):651-662. <https://doi.org/10.1056/NEJMra1205406>
136. Liang XH, Jackson S, Seaman M, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature*. 1999;402(6762):672-676. <https://doi.org/10.1038/45257>
137. Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci U S A*. 2003;100:15077-15082.
138. Qu X. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest*. 2003;112(12):1809-1820. <https://doi.org/10.1172/JCI200320039>
139. Takahashi Y, Coppola D, Matsushita N, et al. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol*. 2007;9(10):1142-1151. <https://doi.org/10.1038/ncb1634>
140. Coppola D, Khalil F, Eschrich SA, Boulware D, Yeatman T, Wang H-G. Down-regulation of Bax-interacting factor-1 in colorectal adenocarcinoma. *Cancer*. 2008;113(10):2665-2670. <https://doi.org/10.1002/cncr.23892>
141. Kim MS, Jeong EG, Ahn CH, Kim SS, Lee SH, Yoo NJ. Frameshift mutation of UVRAG, an autophagy-related gene, in gastric carcinomas with microsatellite instability. *Hum Pathol*. 2008;39(7):1059-1063. <https://doi.org/10.1016/j.humpath.2007.11.013>
142. Takamura A, Komatsu M, Hara T, et al. Autophagy-deficient mice develop multiple liver tumors. *Genes Dev*. 2011;25(8):795-800. <https://doi.org/10.1101/gad.2016211>
143. White E. Deconvoluting the context-dependent role for autophagy in cancer. *Nat Rev Cancer*. 2012;12(6):401-410. <https://doi.org/10.1073/pnas.1107969108>
144. Maehama T. PTEN: its deregulation and tumorigenesis. *Biol Pharm Bull*. 2007;30(9):1624-1627. <https://doi.org/10.1248/bpb.30.1624>

145. Shaw RJ, Cantley LC. PI(3)K and mTOR signalling controls tumour cell growth. *Nature*. 2006;441(7092):424-430. <https://doi.org/10.1038/nature04869>
146. Chen N, Karantza-Wadsworth V. Role and regulation of autophagy in cancer. *Biochim Biophys Acta*. 2009;1793(9):1516-1523. <https://doi.org/10.1016/j.bbamcr.2008.12.013>
147. Komatsu M, Waguri S, Koike M, et al. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell*. 2007;131(6):1149-1163. <https://doi.org/10.1016/j.cell.2007.10.035>
148. Lau A, Wang X-J, Zhao F, et al. A noncanonical mechanism of Nrf2 activation by autophagy deficiency: direct interaction between Keap1 and p62. *Mol Cell Biol*. 2010;30(13):3275-3285. <https://doi.org/10.1128/MCB.00248-10>
149. Mathew R, Karp CM, Beaudoin B, et al. Autophagy suppresses tumorigenesis through elimination of p62. *Cell*. 2009;137(6):1062-1075. <https://doi.org/10.1016/j.cell.2009.03.048>
150. Moscat J, Diaz-Meco MT. p62: a versatile multitasker takes on cancer. *Trends Biochem Sci*. 2012;37(6):230-236. <https://doi.org/10.1016/j.tibs.2012.02.008>
151. Rao S, Tortola L, Perlot T, et al. A dual role for autophagy in a murine model of lung cancer. *Nat Commun*. 2014;5:3056. <https://doi.org/10.1038/ncomms4056>
152. Singh SS, Vats S, Chia AY-Q, et al. Dual role of autophagy in hallmarks of cancer. *Oncogene*. February 2018;37:1-17. <https://doi.org/10.1038/s41388-017-0046-6>
153. Degenhardt K, Mathew R, Beaudoin B, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell*. 2006;10(1):51-64. <https://doi.org/10.1016/j.ccr.2006.06.001>
154. Masoud GN, Li W. HIF-1 α pathway_ role, regulation and intervention for cancer therapy. *Acta Pharm Sin B*. 2015;5(5):378-389. <https://doi.org/10.1016/j.apsb.2015.05.007>
155. Mazure NM, Pouyssegur J. Hypoxia-induced autophagy: cell death or cell survival? *Curr Opin Cell Biol*. 2010;22(2):177-180. <https://doi.org/10.1016/j.ccb.2009.11.015>
156. Pursiheimo J-P, Rantanen K, Heikkinen PT, Johansen T, Jaakkola PM. Hypoxia-activated autophagy accelerates degradation of SQSTM1/p62. *Oncogene*. 2009;28(3):334-344. <https://doi.org/10.1038/onc.2008.392>
157. Wei H, Wei S, Gan B, Peng X, Zou W, Guan JL. Suppression of autophagy by FIP200 deletion inhibits mammary tumorigenesis. *Genes Dev*. 2011;25(14):1510-1527. <https://doi.org/10.1101/gad.2051011>
158. Komatsu M, Waguri S, Ueno T, et al. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *Mol Biol Cell*. 2011;169(2):165-178. <https://doi.org/10.1073/pnas.2436255100>
159. Lock R, Kenific CM, Leidal AM, Salas E, Debnath J. Autophagy-dependent production of secreted factors facilitates oncogenic RAS-driven invasion. *Cancer Discov*. 2014;4(4):466-479. <https://doi.org/10.1158/2159-8290.CD-13-0841>
160. Guo JY, Chen HY, Mathew R, et al. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev*. 2011;25(5):460-470. <https://doi.org/10.1101/gad.2016311>
161. Yang S, Wang X, Contino G, et al. Pancreatic cancers require autophagy for tumor growth. *Genes Dev*. 2011;25(7):717-729. <https://doi.org/10.1101/gad.2016111>
162. Zhao H, Yang M, Zhao J, Wang J, Zhang Y, Zhang Q. High expression of LC3B is associated with progression and poor outcome in triple-negative breast cancer. *Med Oncol*. 2013;30(1):475. <https://doi.org/10.1007/s12032-013-0475-1>
163. Lazova R, Camp RL, Klump V, Siddiqui SF, Amaravadi RK, Pawelek JM. Punctate LC3B expression is a common feature of solid tumors and associated with proliferation, metastasis, and poor outcome. *Clin Cancer Res*. 2012;18(2):370-379. <https://doi.org/10.1158/1078-0432.CCR-11-1282>
164. Han C, Sun B, Wang W, et al. Overexpression of microtubule-associated protein-1 light chain 3 is associated with melanoma metastasis and vasculogenic mimicry. *Tohoku J Exp Med*. 2011;223(4):243-251.
165. Peng Y-F, Shi Y-H, Ding Z-B, et al. Autophagy inhibition suppresses pulmonary metastasis of HCC in mice via impairing anoikis resistance and colonization of HCC cells. *Cell Death Differ*. 2013;9(12):2056-2068. <https://doi.org/10.4161/auto.26398>
166. Peng Y-F, Shi Y-H, Shen Y-H, et al. Promoting colonization in metastatic HCC cells by modulation of autophagy. *PLoS One*. 2013;8(9):e74407.
167. Kenific CM, Thorburn A, Debnath J. Autophagy and metastasis: another double-edged sword. *Curr Opin Cell Biol*. 2010;22(2):241-245. <https://doi.org/10.1016/j.ccb.2009.10.008>
168. Su Z, Yang Z, Xu Y, Chen Y, Yu Q. Apoptosis, autophagy, necroptosis, and cancer metastasis. *Mol Cancer*. 2015;14:48. <https://doi.org/10.1186/s12943-015-0321-5>
169. DeNardo DG, Johansson M, Coussens LM. Immune cells as mediators of solid tumor metastasis. *Cancer Metastasis Rev*. 2008;27(1):11-18. <https://doi.org/10.1007/s10555-007-9100-0>
170. Thorburn J, Horita H, Redzic J, Hansen K, Frankel AE, Thorburn A. Autophagy regulates selective HMGB1 release in tumor cells that are destined to die. *Cell Death Differ*. 2008;16(1):175-183. <https://doi.org/10.1074/jbc.M401680200>
171. Apetoh L, Ghiringhelli F, Tesniere A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature Med*. 2007;13(9):1050-1059. <https://doi.org/10.1038/nm1622>

172. Yan J, Wang Z-Y, Yang H-Z, et al. Timing is critical for an effective anti-metastatic immunotherapy: the decisive role of IFN γ /STAT1-mediated activation of autophagy. *PLoS One*. 2011;6(9):e24705. <https://doi.org/10.1371/journal.pone.0024705.s004>
173. Hashimoto I, Koizumi K, Tatematsu M, et al. Blocking on the CXCR4/mTOR signalling pathway induces the anti-metastatic properties and autophagic cell death in peritoneal disseminated gastric cancer cells. *Eur J Cancer*. 2008;44(7):1022-1029. <https://doi.org/10.1016/j.ejca.2008.02.043>
174. Schmitz M, Grignard G, Margue C, et al. Complete loss of PTEN expression as a possible early prognostic marker for prostate cancer metastasis. *Int J Cancer*. 2007;120(6):1284-1292. <https://doi.org/10.1002/ijc.22359>
175. Zhang H, Gordon R, Li W, et al. Genistein treatment duration effects biomarkers of cell motility in human prostate. *PLoS One*. 2019;14(3):e0214078.
176. Debnath J. Detachment-induced autophagy during anoikis and lumen formation in epithelial acini. *Cell Death Differ*. 2008;4(3):351-353.
177. Lock R, Debnath J. Extracellular matrix regulation of autophagy. *Curr Opin Cell Biol*. 2008;20(5):583-588. <https://doi.org/10.1016/j.ceb.2008.05.002>
178. Avivar-Valderas A, Salas E, Bobrovnikova-Marjon E, et al. PERK integrates autophagy and oxidative stress responses to promote survival during extracellular matrix detachment. *Mol Cell Biol*. 2011;31(17):3616-3629. <https://doi.org/10.1128/MCB.05164-11>
179. Fung C, Lock R, Gao S, Salas E, Debnath J. Induction of autophagy during extracellular matrix detachment promotes cell survival. *Mol Biol Cell*. 2008;19:797-806. <https://doi.org/10.1091/mbc.E07-10>
180. Mowers EE, Sharifi MN, Macleod KF. Autophagy in cancer metastasis. *Oncogene*. 2017;36:1619-1630. <https://doi.org/10.1038/onc.2016.333>
181. Cheng Z. The FoxO-autophagy axis in health and disease. *Trends Endocrinol Metab*. 2019;30(9):658-671. <https://doi.org/10.1016/j.tem.2019.07.009>
182. Mammucari C, Milan G, Romanello V, et al. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab*. 2007;6(6):458-471. <https://doi.org/10.1016/j.cmet.2007.11.001>
183. Hariharan N, Maejima Y, Nakae J, Paik J, DePinho RA, Sadoshima J. Deacetylation of FoxO by Sirt1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. *Circ Res*. 2010;107(12):1470-1482. <https://doi.org/10.1038/nature03029>
184. van der Vos KE, Eliasson P, Proikas-Cezanne T, et al. Modulation of glutamine metabolism by the PI(3)K-PKB-FOXO network regulates autophagy. *Nat Cell Biol*. 2012;14(8):829-837. <https://doi.org/10.1038/ncb2536>
185. Chen C-C, Jeon S-M, Bhaskar PT, et al. FoxOs inhibit mTORC1 and activate Akt by inducing the expression of Sestrin3 and Rictor. *Dev Cell*. 2010;18(4):592-604. <https://doi.org/10.1016/j.devcel.2010.03.008>
186. Cheong JK, Zhang F, Chua PJ, Bay BH, Thorburn A, Virshup DM. Casein kinase 1 α -dependent feedback loop controls autophagy in RAS-driven cancers. *J Clin Invest*. 2015;125(4):1401-1418. <https://doi.org/10.1172/JCI78018>
187. Zhang F, Virshup DM, Cheong JK. Oncogenic RAS-induced CK1 α drives nuclear FOXO proteolysis. *Oncogene*. 2018;37(3):363-376. <https://doi.org/10.1038/onc.2017.334>
188. Fitzwalter BE, Towers CG, Sullivan KD, et al. Autophagy inhibition mediates apoptosis sensitization in cancer therapy by relieving FOXO3a turnover. *Dev Cell*. 2018;44(5):555-565.e3. <https://doi.org/10.1016/j.devcel.2018.02.014>
189. Fitzwalter BE, Thorburn A. Autophagy inhibition improves anti-cancer drugs via FOXO3a activation. *Oncotarget*. 2018;9:25384-25385.
190. Sunters A, Madureira PA, Pomeranz KM, et al. Paclitaxel-induced nuclear translocation of FOXO3a in breast cancer cells is mediated by c-Jun NH2-terminal kinase and Akt. *Cancer Res*. 2006;66(1):212-220. <https://doi.org/10.1158/0008-5472.CAN-05-1997>
191. Essafi A, Fernández de Mattos S, Hassen YAM, et al. Direct transcriptional regulation of Bim by FoxO3a mediates STI571-induced apoptosis in Bcr-Abl-expressing cells. *Oncogene*. 2005;24(14):2317-2329. <https://doi.org/10.1038/sj.onc.1208421>
192. Chen Q, Ganapathy S, Singh KP, Shankar S, Srivastava RK. Resveratrol induces growth arrest and apoptosis through activation of FOXO transcription factors in prostate cancer cells. *PLoS One*. 2010;5(12):e15288. <https://doi.org/10.1371/journal.pone.0015288>
193. Roy SK, Srivastava RK, Shankar S. Inhibition of PI3K/AKT and MAPK/ERK pathways causes activation of FOXO transcription factor, leading to cell cycle arrest and apoptosis in pancreatic cancer. *J Mol Signal*. 2010;5:10. <https://doi.org/10.1186/1750-2187-5-10>
194. Li Y, Yu J, Du D, et al. Involvement of post-transcriptional regulation of FOXO1 by HuR in 5-FU-induced apoptosis in breast cancer cells. *Oncol Lett*. 2013;6(1):156-160.
195. Han C-Y, Cho K-B, Choi H-S, Han H-K, Kang K-W. Role of FoxO1 activation in MDR1 expression in adriamycin-resistant breast cancer cells. *Carcinogenesis*. 2008;29(9):1837-1844. <https://doi.org/10.1093/carcin/bgn092>

196. Hui RC-Y, Francis RE, Guest SK, et al. Doxorubicin activates FOXO3a to induce the expression of multidrug resistance gene ABCB1 (MDR1) in K562 leukemic cells. *Mol Cancer Ther.* 2008;7(3):670-678. <https://doi.org/10.1158/1535-7163.MCT-07-0397>
197. Hui RC-Y, Gomes AR, Constantinidou D, et al. The forkhead transcription factor FOXO3a increases phosphoinositide-3 kinase/Akt activity in drug-resistant leukemic cells through induction of PI3KCA expression. *Mol Cell Biol.* 2008;28(19):5886-5898. <https://doi.org/10.1128/MCB.01265-07>
198. Lin A, Piao H-L, Zhuang L, Sarbassov DD, Ma L, Gan B. FoxO transcription factors promote AKT Ser473 phosphorylation and renal tumor growth in response to pharmacologic inhibition of the PI3K-AKT pathway. *Cancer Res.* 2014;74(6):1682-1693. <https://doi.org/10.1158/0008-5472.CAN-13-1729>
199. Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. *Nat Rev Cancer.* 2017;17(9):528-542. <https://doi.org/10.1038/nrc.2017.53>
200. Duffy A, Le J, Sausville E, Emadi A. Autophagy modulation: a target for cancer treatment development. *Cancer Chemother Pharmacol.* 2015;75(3):439-447. <https://doi.org/10.1007/s00280-014-2637-z>
201. Witzig TE, Geyer SM, Ghobrial I, et al. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. *J Clin Oncol.* 2005;23(23):5347-5356. <https://doi.org/10.1200/JCO.2005.13.466>
202. Atkins MB, Hidalgo M, Stadler WM, et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J Clin Oncol.* 2004;22(5):909-918.
203. Amato RJ, Jac J, Giessinger S, Saxena S, Willis JP. A phase 2 study with a daily regimen of the oral mTOR inhibitor RAD001 (everolimus) in patients with metastatic clear cell renal cell cancer. *Cancer.* 2009;115(11):2438-2446. <https://doi.org/10.1002/cncr.24280>
204. Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet.* 2008;372(9637):449-456. [https://doi.org/10.1016/S0140-6736\(08\)61039-9](https://doi.org/10.1016/S0140-6736(08)61039-9)
205. Saha A, Blando J, Tremmel L, DiGiovanni J. Effect of metformin, rapamycin, and their combination on growth and progression of prostate tumors in HiMyc mice. *Cancer Prev Res (Phila).* 2015;8(7):597-606. <https://doi.org/10.1158/1940-6207.CAPR-15-0014>
206. Sesen J, Dahan P, Scotland SJ, et al. Metformin inhibits growth of human glioblastoma cells and enhances therapeutic response. *PLoS One.* 2015;10(4):e0123721.
207. Wang J, Li J, Cao N, Li Z, Han J, Li L. Resveratrol, an activator of SIRT1, induces protective autophagy in non-small-cell lung cancer via inhibiting Akt/mTOR and activating p38-MAPK. *Oncol Targets Ther.* 2018;11:7777-7786.
208. Richardson PG, Eng C, Kolesar J, Hideshima T, Anderson KC. Perifosine, an oral, anti-cancer agent and inhibitor of the Akt pathway: mechanistic actions, pharmacodynamics, pharmacokinetics, and clinical activity. *Expert Opin Drug Metab Toxicol.* 2012;8(5):623-633. <https://doi.org/10.1517/17425255.2012.681376>
209. Fu L, Kim Y-A, Wang X, et al. Perifosine inhibits mammalian target of rapamycin signaling through facilitating degradation of major components in the mTOR axis and induces autophagy. *Cancer Res.* 2009;69(23):8967-8976. <https://doi.org/10.1158/0008-5472.CAN-09-2190>
210. Amaravadi RK, Kimmelman AC, Debnath J. Targeting autophagy in cancer: recent advances and future directions. *Cancer Discov.* 2019;9(9):1167-1181. <https://doi.org/10.1158/2159-8290.CD-19-0292>
211. Rebecca VW, Amaravadi RK. Emerging strategies to effectively target autophagy in cancer. *Oncogene.* 2016;35(1):1-11. <https://doi.org/10.1038/ncr.2015.99>
212. Bhat P, Kriel J, Priya BS, Basappa, Shivananju NS, Loos B. Modulating autophagy in cancer therapy: advancements and challenges for cancer cell death sensitization. *Biochem Pharmacol.* 2018;147:170-182. <https://doi.org/10.1016/j.bcp.2017.11.021>
213. Guo X, Li D, Hu F, et al. Targeting autophagy potentiates chemotherapy-induced apoptosis and proliferation inhibition in hepatocarcinoma cells. *Cancer Lett.* 2012;320(2):171-179. <https://doi.org/10.1016/j.canlet.2012.03.002>
214. Liu B, Bao J-K, Yang J-M, Cheng Y. Targeting autophagic pathways for cancer drug discovery. *Chin J Cancer.* 2013;32(3):113-120.
215. Abedin MJ, Wang D, McDonnell MA, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. *Cell Death Differ.* 2007;14(3):500-510. <https://doi.org/10.1038/sj.cdd.4402039>
216. Boya P, González-Polo R-A, Casares N, et al. Inhibition of macroautophagy triggers apoptosis. *Mol Cell Biol.* 2005;25(3):1025-1040. <https://doi.org/10.1128/MCB.25.3.1025-1040.2005>
217. Yang Y, Hu L, Zheng H, et al. Application and interpretation of current autophagy inhibitors and activators. *Acta Pharmacol Sin.* 2013;34(5):625-635. <https://doi.org/10.1038/aps.2013.5>
218. Eng CH, Wang Z, Tkach D, et al. Macroautophagy is dispensable for growth of KRAS mutant tumors and chloroquine efficacy. *Proc Natl Acad Sci U S A.* 2016;113(1):182-187. <https://doi.org/10.1073/pnas.1515617113>

219. Maes H, Kuchnio A, Peric A, et al. Tumor vessel normalization by chloroquine independent of autophagy. *Cancer Cell*. 2014;26(2):190-206. <https://doi.org/10.1016/j.ccr.2014.06.025>
220. Maycotte P, Aryal S, Cummings CT, Thorburn J, Morgan MJ, Thorburn A. Chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy. *Cell Death Differ*. 2012;8(2):200-212. <https://doi.org/10.4161/auto.8.2.18554>
221. Chude Cynthia, Amaravadi Ravi. Targeting autophagy in cancer: update on clinical trials and novel inhibitors. *Int J Mol Sci*. 2017;18(6):1279. <https://doi.org/10.4137/JEN.S25475>
222. Xu R, Ji Z, Xu C, Zhu J. The clinical value of using chloroquine or hydroxychloroquine as autophagy inhibitors in the treatment of cancers. *Medicine*. 2018;97(46):e12912. <https://doi.org/10.1097/MD.00000000000012912>
223. Karasic TB, O'Hara MH, Loaiza-Bonilla A, et al. Effect of gemcitabine and nab-paclitaxel with or without hydroxychloroquine on patients with advanced pancreatic cancer: a phase 2 randomized clinical trial. *JAMA Oncol*. 2019;5(7):993-998. <https://doi.org/10.1001/jamaoncol.2019.0684>
224. Rojas-Puentes LL, Gonzalez-Pinedo M, Crismatt A, et al. Phase II randomized, double-blind, placebo-controlled study of whole-brain irradiation with concomitant chloroquine for brain metastases. *Radiat Oncol*. 2013;8:209. <https://doi.org/10.1186/1748-717X-8-209>
225. Mahalingam D, Mita M, Sarantopoulos J, et al. Combined autophagy and HDAC inhibition: a phase I safety, tolerability, pharmacokinetic, and pharmacodynamic analysis of hydroxychloroquine in combination with the HDAC inhibitor vorinostat in patients with advanced solid tumors. *Cell Death Differ*. 2014;10(8):1403-1414. <https://doi.org/10.4161/auto.29231>
226. Sotelo J, Briceno E, Lopez-Gonzalez MA. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 2006;144(5):337-343.
227. Rangwala R, Chang YC, Hu J, et al. Combined MTOR and autophagy inhibition: phase I trial of hydroxychloroquine and temsirolimus in patients with advanced solid tumors and melanoma. *Cell Death Differ*. 2014;10(8):1391-1402. <https://doi.org/10.4161/auto.29119>
228. Bilger A, Bittner M-I, Grosu A-L, et al. FET-PET-based reirradiation and chloroquine in patients with recurrent glioblastoma: first tolerability and feasibility results. *Strahlenther Onkol*. 2014;190(10):957-961. <https://doi.org/10.1007/s00066-014-0693-2>
229. Chi K-H, Ko H-L, Yang K-L, Lee C-Y, Chi M-S, Kao S-J. Addition of rapamycin and hydroxychloroquine to metronomic chemotherapy as a second line treatment results in high salvage rates for refractory metastatic solid tumors: a pilot safety and effectiveness analysis in a small patient cohort. *Oncotarget*. 2015;6(18):16735-16745. <https://doi.org/10.18632/oncotarget.3793>
230. Wolpin BM, Rubinson DA, Wang X, et al. Phase II and pharmacodynamic study of autophagy inhibition using hydroxychloroquine in patients with metastatic pancreatic adenocarcinoma. *Oncologist*. 2014;19(6):637-638. <https://doi.org/10.1634/theoncologist.2014-0086>
231. Haas NB, Appleman LJ, Stein M, et al. Autophagy inhibition to augment mTOR inhibition: a phase I/II Trial of Everolimus and Hydroxychloroquine in Patients with Previously Treated Renal Cell Carcinoma. *Clin Cancer Res*. 2019;25(7):2080-2087. <https://doi.org/10.1158/1078-0432.CCR-18-2204>
232. Martin KR, Celano SL, Solitro AR, et al. A potent and selective ULK1 inhibitor suppresses autophagy and sensitizes cancer cells to nutrient stress. *iScience*. 2018;8:74-84. <https://doi.org/10.1016/j.isci.2018.09.012>
233. Egan DF, Chun MGH, Vamos M, et al. Small molecule inhibition of the autophagy kinase ULK1 and identification of ULK1 substrates. *Mol Cell*. 2015;59(2):285-297. <https://doi.org/10.1016/j.molcel.2015.05.031>
234. Ronan B, Flamand O, Vescovi L, et al. A highly potent and selective Vps34 inhibitor alters vesicle trafficking and autophagy. *Nat Chem Biol*. 2014;10(12):1013-1019. <https://doi.org/10.1038/nchembio.1681>
235. Pasquier B, El-Ahmad Y, Filoche-Rommé B, et al. Discovery of (2S)-8-[(3R)-3-methylmorpholin-4-yl]-1-(3-methyl-2-oxobutyl)-2-(trifluoromethyl)-3,4-dihydro-2H-pyrimido[1,2-a]pyrimidin-6-one: a novel potent and selective inhibitor of Vps34 for the treatment of solid tumors. *J Med Chem*. 2015;58(1):376-400. <https://doi.org/10.1021/jm5013352>
236. Dyczynski M, Yu Y, Otrocka M, et al. Targeting autophagy by small molecule inhibitors of vacuolar protein sorting 34 (Vps34) improves the sensitivity of breast cancer cells to sunitinib. *Cancer Lett*. 2018;435:32-43. <https://doi.org/10.1016/j.canlet.2018.07.028>
237. Robke L, Laraia L, Carnero Corrales MA, et al. Phenotypic identification of a novel autophagy inhibitor chemotype targeting lipid kinase VPS34. *Angew Chem Int Ed Engl*. 2017;56(28):8153-8157. <https://doi.org/10.1002/anie.201703738>
238. Fu Y, Hong L, Xu J, et al. Discovery of a small molecule targeting autophagy via ATG4B inhibition and cell death of colorectal cancer cells in vitro and in vivo. *Cell Death Differ*. 2019;15(2):295-311. <https://doi.org/10.1080/15548627.2018.1517073>
239. Chu J, Fu Y, Xu J, et al. ATG4B inhibitor FMK-9a induces autophagy independent on its enzyme inhibition. *Arch Biochem Biophys*. 2018;644:29-36. <https://doi.org/10.1016/j.abb.2018.03.001>

240. Akin D, Wang SK, Habibzadegah-Tari P, et al. A novel ATG4B antagonist inhibits autophagy and has a negative impact on osteosarcoma tumors. *Cell Death Differ.* 2014;10(11):2021-2035. <https://doi.org/10.4161/auto.32229>
241. Bosc D, Vezekov L, Bortnik S, et al. A new quinoline-based chemical probe inhibits the autophagy-related cysteine protease ATG4B. *Sci Rep.* 2018;8(1):11653. <https://doi.org/10.1038/s41598-018-29900-x>
242. McAfee Q, Zhang Z, Samanta A, et al. Autophagy inhibitor Lys05 has single-agent antitumor activity and reproduces the phenotype of a genetic autophagy deficiency. *Proc Natl Acad Sci U S A.* 2012;109(21):8253-8258. <https://doi.org/10.1073/pnas.1118193109>
243. Rebecca VW, Nicastrì MC, Fennelly C, et al. PPT1 promotes tumor growth and is the molecular target of chloroquine derivatives in cancer. *Cancer Discov.* 2019;9(2):220-229. <https://doi.org/10.1158/2159-8290.CD-18-0706>
244. Rebecca VW, Nicastrì MC, McLaughlin N, et al. A unified approach to targeting the Lysosome's degradative and growth signaling roles. *Cancer Discov.* 2017;7(11):1266-1283. <https://doi.org/10.1158/2159-8290.CD-17-0741>
245. Gayle S, Landrette S, Beeharry N, et al. Identification of apilimod as a first-in-class PIKfyve kinase inhibitor for treatment of B-cell non-Hodgkin lymphoma. *Blood.* 2017;129(13):1768-1778. <https://doi.org/10.1182/blood-2016-09-736892>
246. Kim H-J, Lee SY, Kim CY, Kim YH, Ju W, Kim SC. Subcellular localization of FOXO3a as a potential biomarker of response to combined treatment with inhibitors of PI3K and autophagy in PIK3CA-mutant cancer cells. *Oncotarget.* 2017;8:6608-6622.
247. Zhao Y, Li X, Cai M-Y, et al. XBP-1u suppresses autophagy by promoting the degradation of FoxO1 in cancer cells. *Cell Res.* 2013;23:491-507. <https://doi.org/10.1038/cr.2013.2>
248. Levy JMM, Thompson JC, Griesinger AM, et al. Autophagy inhibition improves chemosensitivity in BRAF(V600E) brain tumors. *Cancer Discov.* 2014;4(7):773-780. <https://doi.org/10.1158/2159-8290.CD-14-0049>
249. Bryant KL, Stalneckner CA, Zeitouni D, et al. Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer. *Nature Med.* 2019;25(4):628-640. <https://doi.org/10.1038/s41591-019-0368-8>
250. Kinsey CG, Camolotto SA, Boespflug AM, et al. Protective autophagy elicited by RAF->MEK->ERK inhibition suggests a treatment strategy for RAS-driven cancers. *Nature Med.* 2019;25(4):620-627. <https://doi.org/10.1038/s41591-019-0367-9>
251. Xiao D, Bommarreddy A, Kim S-H, Sehrawat A, Hahn E-R, Singh SV. Benzyl isothiocyanate causes FoxO1-mediated autophagic death in human breast cancer cells. *PLoS One.* 2012;7(3):e32597.
252. Chen S, Han Q, Wang X, et al. IBP-mediated suppression of autophagy promotes growth and metastasis of breast cancer cells via activating mTORC2/Akt/FOXO3a signaling pathway. *Cell Death Dis.* 2013;4:e842. <https://doi.org/10.1038/cddis.2013.380>
253. Wang S, Xia P, Huang G, et al. FoxO1-mediated autophagy is required for NK cell development and innate immunity. *Nat Commun.* 2016;7:11023. <https://doi.org/10.1038/ncomms11023>

AUTHOR BIOGRAPHIES



Mohd Farhan obtained his Bachelor's Degree in Biotechnology from Integral University, India, in 2011. He completed his Master's degree in biomedical genetics from Vellore Institute of Technology, Vellore, India, in 2013. He is currently pursuing a PhD degree from the University of Macau in the area of cancer biology under the supervision of Dr Wenhua Zheng. He is working on novel drug targeting and drug development for the cancer and its metastasis.



Marta Silva obtained her PhD degree in neuroscience from the Faculty of Medicine of the University of Porto, Portugal, in 2016. She completed her Master's degree in biomedical engineering from the Faculty of Engineering of the University of Porto in 2008 and her degree in teacher education in physics and chemistry from the Faculty of Sciences of the University of Porto in 2005. She has great interest in chronic pain, specifically in the alterations occurring in key brainstem areas devoted to pain modulation during diabetic neuropathic pain; endovanilloid signaling; neuropharmacology; and biomaterials development and characterization. Recently she obtained a position at the University of Macau under the UM Macao Post-Doctoral Fellowship of the UM Macao Talent Program. Her postdoctoral scholarship project focuses on the study of the therapeutic effect of active components of Chinese medicines, like artemisinin, on neurodegenerative diseases and cancer.



Wenhua Zheng obtained his Medical degree and MSc degree in pharmacology from Zhongshan (Sun Yat-sen) University of Medical Science, Guangzhou China, and a PhD degree from the Department of Pharmacology at McGill University, Montreal, Canada. He was awarded several postdoctoral research training positions in prestigious research institutes and universities around the world (Visiting Fellow the NIH 1993-1996, a Visiting Scientist at the University of Rochester, NY, in 1992 and a Post-Doctoral Fellow at McGill University in 1996). He joined as a Professor at Sun Yat-sen University in 2006. In 2015, he joined the Faculty of Health Science at the University of Macau. He has a keen interest in cancer, mechanism of cancer metastasis, neuropharmacology; and degenerative retinal diseases, molecular mechanisms of neuronal death and survival and new drug developments in cancer and neurodegenerative disease. Some of the specific current research projects going on in his lab are the alteration of signaling pathways (new biomarkers) in cancer, aging, and degenerative disorders, such as Alzheimer's disease and degenerative retinal diseases and identification of new biomarkers.

How to cite this article: Farhan M, Silva M, Li S, et al. The role of FOXOs and autophagy in cancer and metastasis—Implications in therapeutic development. *Med Res Rev.* 2020;40:2089–2113.

<https://doi.org/10.1002/med.21695>