Statins, autophagy, and cancer metastasis

Jing Zhang, Zuozhang Yang, Lei Xu, Jinlei Zhang, Da Xu, Xuefeng Liu

Department of Orthopedics, The Third Affiliated Hospital of Kunming Medical University, Tumor Hospital of Yunnan Province, Kunming 650118, P.R.China.

Corresponding author: Zuozhang Yang, M.D, Ph.D. Department of Orthopedics, The Third Affiliated Hospital of Kunming Medical University, Tumor Hospital of Yunnan Province, Kunming 650118, P.R.China.

E-mail: yangzuozhang@163.com

Tel: 86-13987135820

Abstract

Statins are inhibitors of 3-hydroxy-methylglutaryl (HMG) CoA reductase. They are traditionally considered to be cholesterol-lowering agents, but recent years more and more functions of stains were revealed, including anti-inflammation, immunomodulation, neuroprotection, improvement on bone metabolism, and antitumor effect. Recently, there has been growing interest in the mechanisms of “pleiotropic” effects beyond cholesterol reduction. In the past few years, extensive studies have shown that statins can induce autophagy in tumor cells as well as in some normal cells. Further studies show that activation of AMPK-TOR signaling pathway
may be a major mechanism of statins-induced autophagy. Depleting cellular geranylgeranyl diphosphate activates AMPK and inactivates TOR, leading to autophagic responses. It has been shown that autophagy, a strategy of self-adaption, acts as a double-edged sword in tumor metastasis. On one hand, autophagy contributes to anti-metastasis activity by, for example, restricting tumor necrosis and inflammatory cells infiltration to tumors, and promoting release of high-mobility group box protein 1 (HMGB1) that triggers strong antitumor immune responses. On the other hand, it also exhibits a pro-metastasis activity sometimes. In any case, autophagy is probable novel mechanism responsible for statins-induced anti-metastasis effect beyond the conventional view that Rho GTPases inhibition by statins prevents tumor metastasis.

1 Introduction

Statins are inhibitors of the first key enzyme of mevalonate pathway, 3-hydroxy-methylglutaryl (HMG) CoA reductase [1; 2]. They are structural analogs of HMG-CoA reductase, which inhibit the conversion of HMG-CoA to L-mevalonic acid and further inhibit following cholesterol biosynthesis and isoprenoid metabolites such as geranylgeranyl pyrophosphate (GGPP1) and farnesyl pyrophosphate (FPP) [1; 2; 3]. GGPP and FPP involve in the post-translational event (isoprenylation) of several cell signaling proteins, including the small GTPase family members Ras, Rac, and Rho [4; 5; 6] (Fig.1). Isoprenylation is quite necessary for the activation and intracellular transport of these proteins that control multiple pathways and cell functions including cell proliferation, differentiation, cytokines expression and cell shape.

The statin family consists of many members, including lovastatin, simvastatin, mevastatin, fluvastatin, pravastatin, atorvastatin, rosuvastatin and cerivastatin [7]. Of these drugs, cerivastatin, simvastatin, lovastatin, mevastatin, fluvastatin and atorvastatin are lipophilic, and pravastatin and rosuvastatin are hydrophilic. Statins
were traditionally considered to be cholesterol-lowering agents, but recent years more and more functions of stains were revealed, including anti-inflammation, immunomodulation, neuroprotection, improvement on bone metabolism, and antitumor effect [8; 9; 10; 11; 12].

The molecular mechanisms of antitumor role of statins are divided into two branches: HMG-CoA reductase-dependent processes and HMG-CoA-independent processes [13]. HMG-CoA reductase-dependent processes lead to activation of small GTPase family members Ras, Rac, and Rho. Emerging studies demonstrate the importance of Rho proteins in carcinogenesis [14; 15; 16; 17; 18]. High levels of RhoA and/or RhoC indicate poor prognosis in colorectal cancer, pancreas, breast, bladder, etc [13]. Moreover, RhoA serves an function in epithelial-to-mesenchymal transition, an important event in tumor progression [14; 16], and RhoC involves in stimulating tumor invasion. RAS might also contribute to the effects of statins, for example, by crosstalk with Rho-mediated signaling pathways [14; 16; 19]. The typical HMG-CoA-independent process involves interaction with integrin LFA1. Lovastatin directly binds to the L-site of the I (inserted) domain of the integrin LFA1 and induces a conformational change in LFA1 and interferes the interaction of LFA1 with intercellular-adhesion molecule 1 (ICAM1) [20]. Mevastatin and simvastatin can also bind to L-site of LFA1. Blocking the LFA1-ICAM1 interaction may attenuate the tumor cell adhesion, invasion and inflammation [20]. In addition, statins also play a role in protein degradation, especially the proteasome pathway [21]. Inhibition of the proteasome could account for the effects of statins on p27 and p21 [22].

In this present study, we review another HMG-CoA-dependent process, statins-induced autophagy and its role in cancer especially in metastasis.

Statins inhibit the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate and further interfere the biosynthesis of farnesyl pyrophosphate and geranylgeranyl pyrophosphate. In this pathway, farnesylation can be converted into various other products, including cholesterol. In addition, farnesylation pyrophosphate and geranylgeranyl pyrophosphate involve in the farnesylation and geranylgeranylation of some small GTPases superfamily members
such as Ras and Rho.

2 Statins and autophagy

Autophagy is a lysosomal catabolic pathway whereby cells recycle macromolecules and organelles. Cells employ autophagy to maintain cellular metabolism under starvation conditions and to remove damaged organelles under stress conditions, thus improving the survival of cells (Fig.2). In recent years, many researchers have reported that statins can induce autophagy in tumors cells, stem cells, vascular endothelial cells, cardiac cells, osteoblasts, etc.

In short, starvation or drugs (e.g. bredeldin A, statins) activates AMPK and further represses mTOR activity, leading to autophagy. Inhibition of mTOR activity promotes activation of Atgs and initiates autophagy processes including membrane isolation, autophagosome formation, and autolysosome formation. Class I/III PI3K involves in regulation of autophagy and exerts antagonistic roles. In addition, ER stress also leads to autophagy by activation of AMPK or direct activation of Atgs. AMPK, adenosine monophosphate-activated protein kinase; Atg, autophagy genes; mTOR, mammalian target of rapamycine; PI3K, phosphatidylinositol 3-kinase. ER, endoplasmic reticulum.

2.1 Statins induce autophagy in tumor cells

It has been reported that hydrophobic statins induce autophagy and cell death in human rhabdomyosarcoma cells by depleting geranylgeranyl diphosphate. The extent of inhibition of HMG-CoA reductase in the cell is positively related with the ability of a statin to induce autophagy. Further study implies that statins induce autophagy by depleting cellular geranylgeranyl diphosphate via an unknown pathway that does not involve two major small G proteins, Ras and Rheb [23; 24]. Another study shows that statins, such as atorvastatin, lovastatin, fluvastatin, and simvastatin, induce autophagy and autophagy-associated cell death in PC3 cells through inhibition of geranylgeranylation, and suggests that autophagic response to statins may partially explain the protective effects of statins on prostate cancer progression [25; 26].
Further study demonstrates that atorvastatin-induced autophagy in prostate cancer PC3 cells through activation of LC3 transcription, and Erk and Jnk may mediate the atorvastatin-induced autophagy [27].

Research shows that several kinds of statins induce autophagy in hepatocellular carcinoma Huh7 cells and colorectal carcinoma HCT116 cells. The mechanism is probably due to statins-induced AMPK/p21 signaling activation. That AMPK/p21 signaling causes endoplasmic reticulum (ER) stress response leading to the induction of autophagy [28; 29]. Similarly, Misirkic group reported that simvastatin induced autophagy in antiglioma via AMPK-dependent pathway [30]. Wojtkowiak et al found that cotreatment of the human malignant peripheral nerve sheath tumor cell line STS-26T cells with lovastatin and farnesyl transferase inhibitor (FTI)-1 induced an abortive autophagic program and nonapoptotic cell death, which was correlated with loss of lysosome-associated membrane protein (LAMP)-2 expression [31]. Their group also showed that GGTIs (a new found geranylgeranyl transferase inhibitor) in combination with lovastatin inhibited proliferation and induced autophagy in STS-26T MPNST Cells. The combination treatment also induced autophagy in the MCF10.DCIS model of human breast ductal carcinoma in situ and in 1c1c7 murine hepatoma cells [32].

2.2 Statins induce autophagy in normal cells

It is reported that autophagy plays a protective role in hypoxia-serum deprivation-induced apoptosis of mesenchymal stem cells (MSC). Atorvastatin can effectively activate autophagy via AMPK/mTOR pathway to enhance MSC survival during hypoxia-serum deprivation [33; 34]. Halayko’s group demonstrated that statins induces apoptosis and autophagy in human lung mesenchymal cells and they further showed that statins simultaneously induces activation of the apoptosis, autophagy and the unfolded protein response (UPR) in primary human atrial fibroblasts (hATF) [35]. A recent study shows that simvastain alleviates the progression of apical periodontitis by promoting autophagy to protect osteoblasts from turning apoptotic[36]. Another study indicates that simvastatin is able to induce autophagy both in cardiac cell lines and in the in vivo mouse heart. Cells treated with simvastatin display slight
mitochondrial depolarization as compared to controls and increased expression of PTEN. PTEN is correlated with mitochondrial quality control through the PTEN-induced putative kinase 1 (PINK1), which recruits the E3 ubiquitin ligase Parkin to mitochondrial membranes in response to depolarization. Parkin further primes the mitochondria for degradation. This demonstrates that simvastatin is able to induce mitochondrial loss and PTEN-mediated autophagy, which is linked to cardioprotection [37].

On the contrary, a study shows that statins may inhibit autophagy sometimes. Atorvastatin can inhibit vascular endothelial cells autophagy, which may be related to the role of atorvastatin in improvement on endothelial function. However, using atorvastatin, prior to the occurrence of induced autophagy, can not effectively inhibit the occurrence of autophagy [38; 39].

2.3 The signaling pathways of statins-induced autophagy

Autophagy shares a very complicated molecular machinery and regulatory pathways[30]. For now, a lot of signaling pathways have been shown to regulate autophagy under various conditions, which may cross talk and regulate at different steps in the autophagic processes, including induction and expansion of isolation membrane, autophagosome formation, and fusion with lysosome [40]. For statins-induced autophagy, activation of AMPK/mTOR pathway seems to be a common mechanism [23; 24; 25; 33].

TOR kinase is a main regulator of autophagy that blocks autophagy in cells growing in nutrients-rich environment [41; 42; 43]. As an autophagy inhibitor, TOR activity inhibits the formation of Atg complexes including the Atg1-Atg13-Atg17 serine/threonine protein kinase complexes and Vps34-Atg6-Vps15 lipid kinase complex [44; 45; 46], as well as two ubiquitin-like conjugation systems of autophagy, which prevents the induction and expansion of the isolation membrane. On the contrary, TOR is inactivated and when cells are in starvation, turning on the switch of autophagy. Thus, TOR may play a central role in autophagic signaling pathways [47; 48], although it remains unknown whether TOR inhibition is a universal mechanism.
for autophagy under different kinds of cellular stresses.

AMP-dependent protein kinase (AMPK) is sensitive to the cytosolic AMP-to-ATP ratio and responds to metabolic stresses affecting this ratio, such as glucose deprivation, hypoxia, ischemia, heat shock, and oxidative stress [49]. In yeast, AMPK is required for autophagy induction during metabolic stress [50]; however, whether AMPK is necessary in mammalian autophagy is controversial. A major mechanism for AMPK to induce autophagy is to suppress the TOR signaling. AMPK phosphorates mTOR and directly inhibits its activity [51; 52]. Furthermore, AMPK can activate tuberous sclerosis complex (TSC), leading to the inhibition of TOR. Statins, inhibitors of the HMG-CoA reductase, interfere the biosynthesis of mevalonic acid and the following biosynthesis of geranylgeranyl diphosphate. Depleting cellular levels of geranylgeranyl diphosphate activates AMPK and inactivates TOR, leading to autophagic responses [23; 24; 25; 33].

In addition, statins-induced AMPK/p21 signal activation causes endoplasmic reticulum (ER) stress response and induces the autophagy in some cell lines [28]. Statins induce p21 expression by inhibition of HDAC (histone deacetylase) activity, which probably results from direct interplay or AMPK-mediated phosphorylation [53]. Nuclear p21 is responsible for cell cycle arrest and apoptosis. Phosphorylation of p21 by AMPK or Akt makes its cytosolic localization. Cytosolic p21 induces ER stress and autophagy which, together with Akt, protects cells from apoptosis [54].

P53 also involves in statins-induced autophagy [55]. It is reported that statins can prevent skeletal metastasis of breast cancer by upregulation of p53 and downregulation of CD44 [56]. Accumulating evidences show that p53 plays a dual role in the control of autophagy. On the one hand, nuclear p53 can induce autophagy by transactivating autophagy-inducing genes. On the other hand, cytoplasmic p53 may act as a repressor of autophagy [57; 58]. Moreover, simvastatin triggers mitochondrial loss and PTEN-mediated autophagy in cardiac cells [37].

3 Autophagy and metastasis
Metastasis is the major cause of lethality in cancer patients and comprises multiple discrete steps, including: invasion of tumor cells from the primary tumor site; intravasation into the blood or lymphatic circulation and survival in the circulation; extravasation of tumor cells at the target organ site; the colonization of tumor cells at the new site [59; 60; 61; 62]. Autophagy, a strategy of self-adaption, acts as a double-edged sword in tumor metastasis [63; 64; 65; 66].

3.1 The anti-metastasis role of autophagy

Inflammatory cells, especially macrophages, infiltrate tumor sites in response to necrosis resulting from hypoxia and metabolic stress, both of which commonly promote the metastasis of solid tumors [67; 68; 69; 70; 71]. By promoting survival during metabolic stress and hypoxia, autophagy improves tumor cell necrosis and consequent macrophage infiltration of the primary tumor. It has been demonstrated that the ability of autophagy to restrict necrosis and preclude macrophage associated tumor inflammation serves a function in suppressing primary tumor growth and metastasis [72].

In addition, autophagy can directly modulate tumor-associated inflammatory responses by affecting the release of immunoregulatory factors such as high-mobility group box protein 1 (HMGB1) from tumor cells [60]. For example, glioblastoma cells treated with a targeted toxin exhibit high levels of autophagy during cell death, followed by HMGB1 release from dying cells [73]. When released, HMGB1 activates dendritic cells by targeting Toll-like receptor 4, which triggers a strong antitumor immune response and restrict metastasis [74].

The prophylactic application of the TLR4 and TLR9 agonist complex triggers antimetastatic immunity, leading to the autophagy-associated death of melanoma cells via IFNγ/STAT1 activation and attenuated tumor metastasis. Activation of autophagy by rapamycin after tumor inoculation, with or without the TLR4/9 agonist complex, could inhibit metastasis [75]. In another case, depletion of tissue factor suppresses hepatic metastasis and tumor growth in colorectal cancer via the downregulation of MMPs and the induction of autophagy and apoptosis [76].

3.2 The pro-metastasis role of autophagy
In order to metastasize, cancer cells must acquire the ability to survive and expand in the absence of ECM contact while circulating in blood or lymphatic systems and invading a foreign microenvironment at distant sites [59; 60; 77; 78]. Otherwise, cancer cells will be executed by anoikis. We have known that the over-activation of growth factor pathways is a common mechanism utilized by cancer cells to escape anoikis [79]; in addition, oncogenes involved in activating vital growth factor signals, such as the Ras/MAPK and PI3K/Akt pathways, protect tumor cells from death [80]. However, recent studies demonstrate that autophagy is another mechanism protecting matrix-detached cells from anoikis [60; 81; 82; 83]. Detachment-induced autophagy was first observed during luminal clearance in mammary epithelial acini grown in three-dimensional cultures [84]. Further experiments show that autophagy is induced upon either substratum detachment or β1 integrin blocking [82]. The possible mechanism may be related to a smart self-modulation: autophagy in ECM-detached cells may compensate for the loss of extrinsic signals promoting nutrient and energy metabolism [81]. In the context of a rapidly growing tumor with high energy and biosynthetic demands, detachment-induced autophagy improves the fitness of cells deprived of ECM contact [85].

Interestingly, disseminated tumor cells unable to form strong and firm ECM contacts with a new microenvironment have been transformed to tumor dormancy [86]. Dormancy describes the considerable ability of disseminated tumor cells to survive for years to decades at distant sites without developing secondary tumors. The dormant cell population may comprise only a small cluster of cells that disseminate from the primary tumor and keep the ability to metastasize at proper time. Suppression of β1 integrin signaling induces autophagy and facilitates dormancy in the MMTV–PyMT model of breast cancer [82; 87]. Therefore, it is probable that since disseminated tumor cells cannot successfully engage a foreign ECM, impaired integrin signal transduction may initiate autophagy for survival and form dormancy. Tumor dormancy likely reflects a mechanism of evolutionary fitness that disseminated tumor cells use to survive when exposed to an unfavorable microenvironment [86].
3.3 Statins, autophagy and cancer metastasis

Several lines of evidence suggest that statins inhibit cancer cells invasion and metastasis. Lovastatin inhibits tumor growth and lung metastasis in mouse mammary carcinoma model [88]. Simvastatin prevents skeletal metastasis of breast cancer by inhibiting the expression of CD44 and enhancing the expression of p53 [56]. Pravastatin reduces lung metastasis of rat hepatocellular carcinoma via a coordinated decrease of MMP expression and activity [89]. Fluvastatin can be used for prophylaxis of renal cancer metastasis [90]. Atorvastatin inhibits Rho activation and reverts the metastatic phenotype of human melanoma cells in vitro, as well as inhibits metastasis of melanoma cells overexpressing RhoC in vivo [91]. Lovastatin exhibits antimetastatic properties by affecting the organization of cytoskeleton and the modulation of adhesion, motility and proteolysis [92].

Traditionally, Rho GTPases are considered as the key signaling molecules that drive metastases. Statins may prevent this process by interfering the RhoA/ FAK/Akt pathway in highly malignant breast cancer cells [90]. Statins inhibit Rho proteins geranylgeranylation and further affect the subcellular localization and activity of them, which attenuates the metastatic ability of melanoma cells [91]. In addition, statins can block TNF-α-induced upregulation of E-selectin on endothelial cells by downregulating the activities of RhoA and RhoB. E-selectins are adhesive molecules that are necessary for the attachment of tumor cells to the endothelium, which is required for metastasis [93].

However, this is not the whole mechanism of stains-induced anti-metastasis activity. We have reviewed above that statins are definitive inducers of autophagy, and autophagy may contribute to anti-metastasis effect by, for example, restricting tumor necrosis and inflammatory cells infiltration to tumors, and promoting release of HMGB1[66]. Therefore, autophagy may be another modulator of the anti-metastasis activity of statins [55; 94]. This point should be further validated by more direct studies surrounding these three factors.

4 Conclusions
Statins are inhibitors of HMG-CoA reductase that lower cholesterol and prevent cardiovascular disease. They are one of the most widely prescribed drugs. Recently, there has been growing interest in their mechanisms of “pleiotropic” effects beyond cholesterol reduction. In the past few years, extensive studies have shown that statins can induce autophagy in tumor cells as well as in stem cells, vascular endothelial cells, cardiac cells, osteoblasts, etc. Autophagy, a strategy of self-adaption, acts as a double-edged sword in tumor metastasis. Autophagy contributes to anti-metastasis activity by, for example, restricting tumor necrosis and inflammatory cells infiltration to tumors, and promoting release of HMGB1. In some circumstances, it also exhibits a pro-metastasis activity by protecting matrix-detached cells from anoikis and facilitating dormancy of disseminated tumor cells that cannot engage a foreign ECM. In any case, autophagy is probable novel mechanism responsible for statins-related anti-metastasis effect in addition to Rho GTPases inhibition by statins (Fig.3).

Statins inhibits HMG-CoA reductase and prevents the biosynthesis of mevalonate and the downstream geranylgeranyl pyrophosphate. Depleting cellular geranylgeranyl pyrophosphate activates AMPK and inactivates TOR, leading to autophagic responses. Phosphorylation of p21 by AMP K or Akt leads to its cytosolic localization. Cytosolic p21 causes ER stress and autophagy. P53 also involves in regulation of autophagy. Autophagy exhibits anti-metastasis activity by, for example, restricting tumor necrosis and inflammatory cells infiltration to tumors, and promoting release of HMGB1 that can trigger strong antitumor immune responses. However, autophagy also has a pro-metastasis effect in some circumstances. In summary, autophagy is probable novel mechanism responsible for statins-induced anti-metastasis effect in addition to the Rho GTPases inhibition by statins.
References


[34] L. Vucicevic, M. Misirkic, K. Janjetovic, U. Vilimanovich, E. Sudar, E. Isenovic, M. Prica, L. Harhaji-Trajkovic, T. Kravic-Stevovic, V. Bumbasirevic, Compound C induces protective autophagy in cancer cells through AMPK inhibition-independent blockade of Akt/mTOR


[71] C. Guruvayoorappan, Tumor versus tumor-associated macrophages: how hot is the link?


**Figure legends**

Fig.1 Mevalonate pathway.

Fig.2 A diagram of major processes of autophagy.

Fig. 3 A diagram of the relationship among statins, autophagy, and cancer metastasis.
Figure 1

HMG-CoA → HMG-CoA reductase → Mevalonate
+ 2 ATP → 5-Pyrophosphomevalonate
+ 1 ATP → Isopentenyl pyrophosphate

---

Dimethylallyl pyrophosphate

---

Geranyl pyrophosphate

---

Geranylgeranyl pyrophosphate

---

Farnesyl pyrophosphate

---

Isopentenyl pyrophosphate

---

Ras, Rho, Rac, Cdc42

---

Cholesterol, Ubiquinone...

Statins