Active autophagy in the tumor microenvironment: A novel mechanism for cancer metastasis (Review)

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Abstract. Autophagy is a lysosomal degradation process which is key for the regulation of the turnover of long-lived or damaged proteins and organelles and which promotes cell survival during nutrient deprivation or other microenvironmental stresses. Current evidence supports the hypothesis that autophagy suppresses tumorigenesis, particularly during the early stages of tumor initiation. However, in established tumors, autophagy promotes survival under stressful conditions during cancer progression and in response to chemotherapy; however, the mechanism by which autophagy influences cancer metastasis remains unknown. In this review, we discuss the capacity of an abnormal tumor environment to induce autophagy and consider how this relates to tumor metastasis and the attractive prospect of manipulating autphagic signaling pathways as potential targets for the treatment of cancer metastasis.

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1. Introduction

Autophagy is an evolutionarily conserved catabolic process in which intracellular membrane structures sequester proteins and organelles to degrade and turn over these cytoplasmic constituents; thus, it is essential for growth regulation and the maintenance of homeostasis (1-3). Autophagy is a multi-step process characterized by nucleation, elongation and autophagosome and autolysosome formation, and is tightly regulated by a limited number of highly conserved genes called autophagy regulators (ATGs) (4,5). Defective autophagy is correlated with diverse pathologies, including neurodegeneration, liver, heart and muscle diseases, ageing, inflammation and cancer (6).

Autophagy is activated in response to multiple stresses during cancer progression, including hypoxia, nutrient deprivation, extracellular matrix (ECM) detachment, endoplasmic reticulum (ER) stress and other diverse stresses (7,8). Autonomous proliferating cancer cells are often exposed to conditions such as hypoxia or/and nutrient deprivation, so there must be an alternative metabolic pathway to protect tumor cells from these environmental stresses (9). Moreover, in order to metastasize, tumor cells must adapt to a stressful microenvironment as they disseminate into the systemic circulation and colonize distant organ sites (10). Therefore, when environmental stresses emerge, tumor cells are able to catabolize existing cytoplasmic components to provide essential ingredients to maintain survival by autophagy (11).

Autophagy facilitates cellular survival by enabling cancer cells to grow under stressful conditions. The enhancement of autophagy leads to degradation of proteins and organelles to provide amino acids, fatty acids and nucleotides for reuse (12). It is increasingly appreciated that autophagy provides cancer cells with certain selective advantages in response to various stresses in the primary tumor microenvironment as well as the...

Abbreviations: ATGs, autophagy regulators; AMPK, AMP-responsive protein kinase; ATF4, activating transcription factor 4; BNIP3, Bel-2/adenovirus E1B 19 kDa-interacting protein; BNIP3L, BNIP3-like protein; ECM, extracellular matrix; HIFs, hypoxia-inducible factors; mTOR, mammalian target of rapamycin; SLS, ‘stone-like’ intracellular structures; UPR, unfolded protein response

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of autophagy (Fig. 2) (27,28). Although hypoxia-driven tumor signaling pathway and activation of AMP-responsive protein kinase mTOR, which are all associated with the induction of autophagy (Fig. 2) (27,28). Although hypoxia-driven tumor metabolism and autophagy have been demonstrated, a more detailed mechanism of the interaction between autophagy and a hypoxic tumor microenvironment remains to be determined.

4. Nutrient deprivation

Proliferating cancer cells require continuous access to resources that sustain intracellular energy and nutrient levels, but the tumor microenvironment is not sufficient to supply these essential ingredients for cancer cell survival (29). Under these conditions, cancer cells are likely to encounter a shortage of nutrients; therefore, cancer cells must seek alternative metabolic processes to cope with this stress and maintain their survival. Studies have shown that autophagy plays a critical role in protecting cells against a shortage of nutrients by removing damaged substrates for recycling, but the exact mechanism by which cancer cells obtain energy sources under conditions in which their external nutrient supply is extremely limited remains unclear (30,31).

Nutrient (including amino acids and glucose) depletion is the most potent known physiological inducer of autophagy. Ammonia, generated from glutamine deamination in mitochondria, was found to function as an autocrine- and/or paracrine-acting stimulator of autophagic flux (32). Autophagosomes were actively induced and promptly consumed in colorectal cancer cells under amino acid- and glucose-deprived conditions, which may contribute to the survival of the cancer cells in their microenvironment (29). Glucose deprivation may cause oxidative stress and stimulate autophagy (33). mTOR and AMPK have been best characterized as critical signaling pathways regulating nutrient deprivation-induced autophagy (Fig. 2) (25,34). Autophagy is also triggered to protect cancer cells from nutrient deprivation by activation of AMPK (35). A previous study has suggested that ubiquilins also accelerate autophagosome maturation and promote cell survival during nutrient starvation (36). The cellular amino acids, especially branched chain amino acids, are a crucial upstream component for the functional activation of mTORC1. The absence of amino acids induces autophagy through the regulation of mTOR activity (Fig. 2) (37). In addition to amino acids, cells must also be supplied with glucose to maintain a constant supply of ATP; during a lack of glucose, autophagy is often activated to maintain intracellular energy homeostasis (38,39). Moreover, it has been reported that the receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis by inhibiting mTOR, resulting in the promotion of pancreatic tumor cell survival (40). Overall, autophagy constitutes a major protective mechanism that allows cells to survive nutrient deprivation.

5. ECM detachment

Integrin-mediated attachment of epithelial cells to the ECM is vital for cell growth and survival (41). The loss of ECM attachment leads to apoptosis, termed anoikis (42). However, previous studies have shown that a lack of appropriate matrix contact also robustly induces autophagy to promote cell survival, either during early carcinoma formation or in the later stages of dissemination and metastasis (43,44). Moreover, ECM components modulate autophagy and mitigate its role
in cell survival. In HeLa cells, the mechanism by which this occurs has been shown to be dependent on the adhesion of the cells to collagen I or IV (45). In a three-dimensional (3D) culture system using MCF10A mammary epithelial cells grown in low ECM attachment conditions, autophagy was rapidly induced to enhance cell survival during anoikis (46). Although the intracellular signals linking ECM detachment to autophagy remain unclear, the results suggest that autophagy may be a previously unrecognized mechanism which enhances the survival of tumor cells lacking proper ECM contact.
6. ER stress

The ER is an organelle responsible for crucial biosynthetic and signaling functions in eukaryotic cells (47). Dysfunction of ER or ER stress may result from various disturbances, including hypoxia and oxidative stress, which elicit a cellular stress response known as the UPR (48). The UPR initially serves as an adaptive mechanism to maintain ER homeostasis. However, severe or prolonged ER stress also switches the cytoprotective functions of UPR and autophagy into cell death, usually by activating intrinsic apoptosis (49).

It has been recognized that in order to clear the accumulation of terminally misfolded protein aggregates that cannot be degraded by the proteasome, the UPR may upregulate the autophagy machinery (50). Activating transcription factor 4 (ATF4) has been shown to facilitate autophagy through direct binding to a cyclic AMP response element binding site in response to ER stress (51). Activation of AMPK by atorvastatin enhances p21 expression and ER stress response, leading to autophagy, which promotes the survival of cancer cells (52). Autophagy may also eliminate a specific type of misfolded procollagen and play a protective role in cell survival against ER stress (53). By contrast, persistent ER stress also induces cell death by activating apoptosis. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells (54). Moreover, the ER stress activates radiation-induced autophagy by PERK-eIF2α in caspase-3/7-deficient cells, which promotes radiosensitivity in vitro and in vivo (55). It has been demonstrated that ER stress-induced cell death was mediated by autophagy (56), which was partly attributed to the inactivation of AKT/TSC/mTOR (Fig. 2). As discussed above, it is clear that ER stress and autophagy are capable of activating prosurvival mechanisms as well as lethal programs, but the specific mechanisms linking UPR to autophagy during ER stress remain poorly understood.

7. Autophagy induced by tumor microenvironmental stresses and tumor metastasis

Tumor microenvironmental stresses have recently gained much attention as a critical determinant of tumor progression since autophagy is often induced as a major protective mechanism that allows cells to survive in response to these stresses. In addition, some clinical evidence suggests that autophagy is used as a survival strategy by established tumors to promote tumor progression.

Autophagy may promote metastasis by enhancing tumor cell fitness in response to microenvironmental stresses. Pancreatic cancer remains a devastating and poorly understood malignant cancer and hypoxia in pancreatic cancers is known to increase malignant potential. In the peripheral area of pancreatic cancer tissue, high expression of LC3, a key component of autophagy, is correlated with poor overall survival and a shorter disease-free period (57). Recent study has also suggested that high expression of the autophagy-related Beclin 1 protein predicts poorer overall survival, progression-free survival and distant metastasis-free survival for nasopharyngeal carcinoma patients (58). The microtubule-associated protein 1 light chain 3 (LC3A) is an essential component of the autophagic vacuoles and LC3A immunohistochemistry renders three patterns of autophagic expression in breast carcinomas: diffuse cytoplasmic, perinuclear and ‘stone-like’ intracellular structures (SLS). Perinuclear LC3A accumulation in colorectal tumour cells is a marker of good prognosis, while high SLS counts were associated with metastases and poor prognosis (59). Phospho-enriched protein in astrocytes (PEA-15) is a 15-kDa phosphoprotein that induces autophagy in human ovarian cancer cells and is associated with prolonged overall survival (60). γ-aminobutyric acid type A (GABAA) receptor-associated protein (GABARAP), the mammalian homolog of yeast Atg8, is involved in autophagosome formation during autophagy and is a new independent prognostic marker for colorectal carcinoma and the overexpression of this protein is associated with poor differentiation as well as shortened overall survival in colorectal cancers (61).

Conversely, autophagy may also inhibit metastasis. Beclin 1 and LC3, crucial genes for autophagy, are altered in several types of human cancer. A higher level of Beclin 1 expression is strongly associated with longer survival of colon cancer patients with stage IIIIB disease (62). Autophagy-active Beclin 1 has also been shown to be significantly correlated with the survival of non-Hodgkin lymphoma patients (63). Moreover, Beclin 1 and LC3 significantly decrease with melanoma progression (64). Beclin 1 may play a role in the inhibition of the development of breast cancer and this inhibition may be due to an interaction with Bcl-2 protein and inactivation of PI3K/PKB signaling pathway (65,66). The high expression level of Beclin 1 protein has been demonstrated to be positively correlated with apoptosis and negatively with cell proliferation in gliomas (67). Beclin 1 defects caused by the overexpression of Bcl-XL may facilitate tumor malignant differentiation, which results in a more aggressive cancer cell phenotype and poor prognosis of hepatocellular carcinoma (68). Low Beclin 1 expression is associated with worse overall survival and progression-free survival in extranodal natural killer T-cell lymphoma (69).

Although these proteins have been used to detect and measure levels of autophagy in human tumor samples, few may be universally and accurately applied for autophagy detection in clinical samples. Consequently, there is a rapidly growing need for exploiting ‘gold standard’ for methods and better markers to monitor autophagic activity (70).

8. Manipulating autophagy induced by tumor microenvironmental stresses for cancer therapy

As discussed above, cancer cells gain survival and proliferation advantages by autophagy to cope with microenvironmental stresses. Despite the determination of the survival-promoting role of autophagy, it is also well recognized that elevated and/or prolonged autophagy may result in cell death. Therefore, inhibiting autophagy induced by tumor microenvironmental stresses or enhancing excessive microenvironmental stresses to give rise to autophagic cell death may be a promising strategy for cancer therapy. Based on the correlation between microenvironmental stresses and autophagy, certain chemotherapeutic agents and antineoplastic therapies have been reported as an adjuvant therapy for cancer, including acid sphingomyelinase (71), thiazolidinediones (72), tetraspanin (73), bortezomib (74), δ(9)-tetrahydrocannabinol (54), etformin (75), 2-deoxyglucose (76) and the arginine...
deimination ADI-PEG20 (77). However, this therapy has not been further explored for clinical application. In order to accelerate this clinical application, large-scale and multicenter collaboration are necessary.

9. Conclusions/perspectives

Autophagy is a catabolic adaptive process usually activated in response to adverse microenvironmental stresses which may have either a beneficial or detrimental cellular effect, depending on the response to environmental stresses (78,79). Currently, it is becoming clear that autophagy is a survival pathway that enables tumor cells to survive under stressful conditions, including hypoxia, nutrient deprivation, ECM detachment and ER stress. By contrast, prolonged activation of autophagy may lead to cell death by cellular self-degradation (80-82).

The tumor environment is a complex and highly dynamic environment, playing a central role in controlling tumor cell behavior and metastasis formation (83). Reduced levels of oxygen and nutrients and malfunction of ECM and ER are critical parameters modulating the tumor microenvironment. As discussed above, abnormality in the tumor microenvironment induces autophagy to aid the maintenance of cancer cell viability and promote cancer cell metastasis under these stressful conditions. However, in certain cases autophagy also contributes to cancer cell death and inhibits metastasis. Based on the functional correlation between microenvironmental stresses and autophagy, a number of new cancer therapeutics have been explored, but certain limitations prevent widespread clinical application. First, the question of whether we should try to enhance or inhibit autophagy in cancer treatment is not straightforward since it is unclear how autophagic cell death is distinguished from autophagy during cell survival. The engulfment receptor Draper was found to be the first factor that distinguishes autophagy associated with cell death from that associated with cell survival (84). This finding is especially critical since numerous current cancer therapeutics activate or inhibit autophagy, although Draper has not been applied to cancer research. Second, to maximize the potential to be applied for more stringent clinical study, characteristics of methods and better markers to monitor autophagic activity may need to be examined. Third, published studies concerning antineoplastic therapies based on the correlation between the autophagy and tumor microenvironment are short of high-level clinical evidence. Large-scale and multicenter collaborations are necessary in the future. Finally, the molecular mechanisms that underlie autophagy induced by multiple tumor microenvironmental stresses and cancer metastasis remain to be determined.

References

1. Klionsky DJ: Autophagy: from phenomenology to molecular understanding in less than a decade. Nat Rev Mol Cell Biol 8: 931-937, 2007.
2. Hippi PM, O’Toole PS and Thorburn A: Autophagy in cancer: good, bad, or both? Cancer Res 66: 9349-9351, 2006.
3. Hoyer-Hansen M and Jäättelä M: Autophagy: an emerging target for cancer therapy. Autophagy 4: 574-580, 2008.
4. Chen N and Debnath J: Autophagy and tumorigenesis. FEBS Lett 584: 1427-1435, 2010.
5. Tsuichiha K, Fuji s S and Esumi H: Autophagy and cancer: dynamism of the metabolism of tumor cells and tissues. Cancer Lett 278: 130-138, 2009.
6. Bao XH, Naomi Y, Hao HF, Watanabe N, Sakurama K, Noma K, et al: Autophagy: Can it become a potential therapeutic target? Int J Mol Med 25: 493-503, 2010.
7. Kondo Y, Kanazawa T, Sawaya R and Kondo S: The role of autophagy in cancer development and response to therapy. Nat Rev Cancer 5: 726-734, 2005.
8. Yang Z and Klionsky DJ: An overview of the molecular mechanism of autophagy. Curr Top Microbiol Immunol 335: 1-32, 2009.
9. Vousden KH and Ryan KM: p53 and metabolism. Nat Rev Cancer 9: 691-700, 2009.
10. Kenific CM, Thorburn A and Debnath J: Autophagy and metastasis: another double-edged sword. Curr Opin Cell Biol 22: 241-245, 2010.
11. Lum JJ, DeBerardinis RJ and Thompson CB: Autophagy in malignancy: cell survival in the land of plenty. Nat Rev Mol Cell Biol 6: 439-448, 2005.
12. Apel A, Zentgraf H, Büchler MW and Herr I: Autophagy-A double-edged sword in oncology. Int J Cancer 125: 991-995, 2009.
13. Roy S and Debnath J: Autophagy and tumorigenesis. Semin Immunopathol 32: 383-396, 2010.
14. Brech A, Ahlquist T, Lothe RA and Stenmark H: Autophagy in tumour suppression and promotion. Mol Oncol 3: 366-375, 2009.
15. Wang RC and Levine B: Autophagy in cellular growth control. FEBS Lett 584: 1417-1426, 2010.
16. Altman BJ and Rathmell JC: Autophagy: not good OR bad, but good AND bad. Autophagy 5: 569-570, 2009.
17. Rosenfeld MT and Ryan KM: The role of autophagy in tumour development and cancer therapy. Expert Rev Mol Med 11: e36, 2009.
18. Kroener G, Mariño G and Levine B: Autophagy and the integrated stress response. Mol Cell 40: 280-293, 2010.
19. Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, et al: Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 10: 51-64, 2006.
20. Bertout JA, Patel SA and Simon MC: The impact of O, availability on human cancer. Nat Rev Cancer 8: 967-975, 2008.
21. Mazure NM and Pouysségur J: Hypoxia-induced autophagy: cell death or cell survival. Curr Opin Cell Biol 22: 177-180, 2010.
22. Mazure NM and Pouysségur J: Atypical BH3-domains of Bnip3 and Bnip3L lead to autophagy in hypoxia. Autophagy 5: 868-869, 2009.
23. Sandovál H, Thiągarañaj P, Dasgupta SK, Schumacher A, Prchal JT, Chen M and Wang J: Essential role for Nix in autophagic maturation of erythroid cells. Nature 454: 232-235, 2008.
24. Martinez-Outschoorn UE, Trimmer C, Lin Z, Whitaker-Menezes D, Chiaravità B, Zhou J, et al: Autophagy in cancer associated fibroblasts promotes tumor cell survival: Role of hypoxia, HIF1 induction and NFκB activation in the tumor stromal microenvironment. Cell Cycle 9: 3515-3533, 2010.
25. Srinivas V, Bohensky J, Zahm AM and Shapiro IM: Autophagy in mineralizing tissue and microenvironmental perspectives. Cell Cycle 8: 391-393, 2009.
26. Li X and Fan Z: The epidermal growth factor receptor antibody cetuximab induces autophagy in cancer cells by downregulating HIF-1alpha and Bcl-2 and activating the beclin 1/hVps34 complex. Cancer Res 70: 5942-5952, 2010.
27. Rouschop KM and Wouters BG: Regulation of autophagy through multiple independent hypoxic signaling pathways. Curr Mol Med 9: 417-424, 2009.
28. Pouysségur J, Dayan F and Mazure NM: Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 441: 457-463, 2006.
29. Sato K, Tsuichiha K, Fuji s S, Sugiyama M, Goya T, Atomi Y, et al: Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation. Cancer Res 69: 9677-9684, 2009.
30. Moreau K, Luo S and Rubinsztein DC: Cytoprotective roles for autophagy. Curr Opin Cell Biol 22: 206-211, 2010.
31. Jin S and White E: Role of autophagy in cancer: management of metabolic stress. Autophagy 3: 28-31, 2007.
32. Eng CH and Abraham KY: Glutaminolysis yields a metabolic hydroxyl that sustains autophagy. Autophagy 6: 968-970, 2010.
33. Marambio P, Toro B, Sanhueza C, Troncoso R, Parra V, Verdejo H, et al: Glucose deprivation causes oxidative stress and stimulates aggresome formation and autophagy in cultured cardiac myocytes. Biochim Biophys Acta 1802: 509-518, 2010.
34. Neufeld TP: TOR-dependent control of autophagy: biting the hand that feeds. Curr Opin Cell Biol 22: 157-168, 2010.
35. Lu T, DeBerardinis RJ and Thompson CB: Autophagy in metazoans: cell survival in the land of plenty. Nat Rev Mol Cell Biol 6: 439-448, 2005.

36. ND Duran GN, Debnath J and Brown EF: Ubiquilins accelerate autophagosome maturation and promote cell survival during nutrient starvation. Autophagy 5: 573-575, 2009.

37. Liu LX, Majithia A, Huang X and Kimmel AR: Growth control via TOR kinase signaling, an intracellular sensor of amino acid and energy availability, with crosstalk potential to proline metabolism. Cell 135: 761-771, 2008.

38. Kumar SH and Ranjaraan A: Simian virus 40 small T antigen activates AMPK and triggers autophagy to protect cancer cells from nutrient deprivation. J Virol 83: 8565-8574, 2009.

39. Harde DG: ATF4-activated/stress-induced transcription factors: stakeholders in protein homeostasis and cell fate decisions. Genes & Development 15: 387-407, 2001.

40. Kang R, Tang D, Schapira NE, Livesey FM, Karras A, Loughran P, et al: The receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis, promoting pancreatic tumor cell survival. Cell Death Differ 17: 666-676, 2010.

41. Miranti CK and Brugge JS: Sensing the environment: a historical perspective on integrin signal transduction. Nat Cell Biol 4: E83-E90, 2002.

42. Gilmore AP: Anoikis. Cell Death Differ 12 (Suppl 2): 1473-1477, 2005.

43. Debnath J: Detachment-induced autophagy during anoikis and lumen formation in epithelial acini. Autophagy 4: 351-353, 2008.

44. Lock R and Debnath J: Extracellular matrix regulation of autophagy. Curr Opin Cell Biol 20: 583-588, 2008.

45. Tuloup-Minguez V, Greffard A, Codogno P and Botti J: Regulation of autophagy by extracellular matrix glycoproteins in human mammary acini. Autophagy 7: 77-78, 2011.

46. Debnath J, Mills KR, Collins NL, Reginato MJ, Muthuswamy SK, et al: Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol 26: 9220-9231, 2006.

47. Inagi R: Endoplasmic reticulum stress as a progression factor for kidney injury. Curr Opin Pharmacol 10: 156-165, 2010.

48. Kaushik S, Singh R and Cuervo AM: Autophagic pathways and the reticularum stress. Mol Cell Biol 26: 9220-9231, 2006.

49. N'Diaye EN, Debnath J and Brown EJ: Ubiquilins accelerate autophagy by promoting the opening of the endoplasmic reticulum. Autophagy 4: 351-353, 2008.

50. Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, et al: Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol 26: 9220-9231, 2006.

51. Ryzinski T, Milani M, Pike L, Buffa F, Moller HR, Winchester L, et al: Regulation of autophagy by Akt in response to severe hypoxia. Oncogene 29: 4424-4435, 2010.

52. Yang PM, Liu YL, Lin YC, Shun CT, Wu MS and Chen CC: Activation of autophagy during cell death requires the engulfment of extracellular matrix and immune killer cells. Crit Rev Immunol 30: 529-545, 2010.

53. Miracco C, Ceccarini G, Franchi A, Luzi P, Cesi E, Mourmouras V, et al: Beclin 1 and LC3 autophagic gene expression in cutaneous melanocytic lesions. Hum Pathol 41: 503-512, 2010.

54. Miracco C, Ceccarini G, Franchi A, Luzi P, Cesi E, Mourmouras V, et al: Beclin 1 and LC3 autophagic gene expression in cutaneous melanocytic lesions. Hum Pathol 41: 503-512, 2010.

55. Miao Y, Zhang Y, Chen Y, Chen L and Wang F: GABARAP is overexpressed in colorectal carcinoma and correlates with shortened patient survival. Hepatogastroenterology 57: 257-261, 2010.

56. Li BX, Li CY, Peng RQ, Wu XJ, Wang HY, Wan DS, et al: The expression of beclin 1 is associated with favorable prognosis in stage IIIB colon cancers. Autophagy 5: 303-306, 2009.

57. Nicotra G, Mercalli F, Peracchio C, Castino R, Follo C, Valenste G and Isidoro C: Autophagy-active beclin-1 correlates with favourable prognosis in Ewing sarcoma in non-Hodgkin lymphomas. Mod Pathol 23: 937-950, 2010.

58. Miracco C, Ceccarini G, Franchi A, Luzi P, Cesi E, Mourmouras V, et al: Beclin 1 and LC3 autophagic gene expression in cutaneous melanocytic lesions. Hum Pathol 41: 503-512, 2010.

59. Duan ZL, Peng ZL and Wang ZH: Expression and involved signal transduction pathway of autophagy gene Beclin 1 in epithelial ovarian cancer. Sichuan Da Xue Xue Bao Yi Xue Ban 38: 239-242, 2007 (In Chinese).

60. Pirtoli T, Ceccarini G, Tini P, Vannini M, Oliveri G, Marsili S, et al: The prognostic role of Beclin 1 protein expression in high-grade gliomas. Autophagy 5: 930-936, 2009.

61. Ding ZB, Shi YH, Zhou J, Qiu SJ, Xu Y, Dai Z, et al: Association of autophagy defect with high malignant phase and poor prognosis of hepatocellular carcinoma. Cancer Res 68: 9176-9175, 2008.

62. Huang JJ, Li HR, Huang Y, Jiang WQ, Xu RH, Huang HQ, et al: Beclin 1 expression: a predictor of prognosis in patients with extranodal natural killer T-cell lymphoma, nasal type. Cytometry 77: 777-92, 2010.

63. Mizushima N, Yoshimori T and Levine B: Methods in mammalian autophagy research. Cell 140: 313-326, 2010.

64. SL Elman and Schuchman EH: Acid sphingomyelinase overexpression enhances the antineoplastic effects of irradiation in vitro and in vivo. Mol Ther 16: 1565-1571, 2008.

65. Wei S, Kulp SK and Chen CS: Energy restriction as an antitumor target of thiazolidinediones. J Biol Chem 285: 9780-9791, 2010.

66. Zismanov V, Lishner M, Tartakover-Matalon S, Radnay J, Shapiro H and Drucker J: Tetraspanin-induced death of myeloma cell lines is autophagic and involves increased UPR signaling. Br J Cancer 101: 1402-1409, 2009.

67. Fels DR, Ye J, Segan AT, Kridel SJ, Spiotto M, Olson et al: Preferential cytotoxicity of bortezomib toward hypoxic tumor cells via overactivation of endoplasmic reticulum stress pathways. Cancer Res 68: 9323-9330, 2008.

68. Buzza M, Jones RG, Amgouni RK, Lum JJ, DeBerardinis RJ, Zhao F, et al: Systemic treatment with the antiangiogenic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res 67: 6745-6752, 2007.

69. Ben-Sahra I, Lauret K, Giuliano S, Larbet F, Ponzi G, Guuron P, et al: Metformin reactivates a specific species of misfolded procalcogen and plays a protective role in cell survival against ER stress. Autophagy 5: 1217-1219, 2009.

70. Salazar M, Carracedo A, Salanueva JJ, Hernandez-Tiedra S, Lorente M, Egia A, et al: Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. J Clin Invest 119: 1359-1372, 2009.

71. Kim KW, Moretti L, Mitchell LR, Jung DK and Lu B: Endoplasmic reticulum stress mediates radiation-induced autophagy by perf-e121Phalpa in caspase-3/7-deficient cells. Oncogene 29: 3241-3251, 2010.

72. Qin L, Wang Z, Tao L and Wang Y: ER stress negatively regulates AKT/TSC/mTOR pathway to enhance autophagy. Autophagy 6: 239-247, 2010.

73. Fuji S, Mitsunaga S, Yamazaki M, Hasebe T, Ishii G, Kojima M, et al: Autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. Cancer Sci 99: 1813-1819, 2008.

74. Shan WB, Fan XJ, Chen MY, Xiang J, Huang PY, Guo L, et al: Elevated Beclin 1 expression is correlated with HIF-lalpha in predicting poor prognosis of nasopharyngeal carcinoma. Autophagy 6: 395-404, 2010.

75. GirotCamon MG, Kouchourou MI, Harris AL, Polychronidou A, Gatter KC and Sivridis E: Prognostic relevance of light chain 3 (LC3A) autophagy patterns in colorectal adenocarcinomas. J Clin Pathol 63: 867-872, 2010.

76. Bartholomeusz C, Rosen D, Wei C, Kazanskys A, Yamafuki F, Takahashi K, et al: A15 induces autophagy in human ovarian cancer cells and is associated with prolonged overall survival. Cancer Res 68: 9302-9310, 2008.