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Areca Nut contains both Apoptosis- and Autophagy-inducing Ingredients and its Possible Effects on Cancer Cells

Ching-Yu Yen[1,2,3], **Shyun-Yeu Liu**[1,2,3], **Mei-Huei Lin**[4,5*], **Young-Chau Liu**[5,6*]

1 Department of Dentistry, Taipei Medical University, 250 Wuxing Street, Taipei City 110, Taiwan

2 Department of Dentistry, National Defense Medical Center, No. 161, Sec. 6, Minquan E. Rd., Taipei City 114, Taiwan

3 Oral and Maxillofacial Surgery Section, Chi Mei Medical Center, Tainan City 710, Taiwan

4 Department of Biotechnology, Chia Nan University of Pharmacy, No. 60, Sec. 1, Erren Rd., Rende Dist., Tainan City 71710, Taiwan

5 Department of Medical Research, Chi Mei Medical Center, No. 901, Zhonghua Rd., Yongkang Dist., Tainan City 710, Taiwan

6 Division of Natural Science, College of Liberal Education, Shu-Te University, No. 59, Hengshan Rd., Yanchao Dist., Kaohsiung City 82445, Taiwan

Corresponding author:
Young-Chau Liu
Mei-Huei Lin

god15539@stu.edu.tw
mhlin3224@mail.cnu.edu.tw

Division of Natural Science, College of Liberal Education, Shu-Te University, No. 59, Hengshan Rd., Yanchao Dist., Kaohsiung City 82445, Taiwan

Department of Biotechnology, Chia Nan University of Pharmacy, No. 60, Sec. 1, Erren Rd., Rende Dist., Tainan City 71710, Taiwan

Tel: 886-7-6158000, ext. 4210
886-6-2664911, ext. 2551

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Abstract

Autophagy is an evolutionally-conserved catabolic process that degrades damaged organelles, misfolded proteins, and toxic aggregates, reducing oxidative stress. Malfunction of autophagy causes various diseases, including cancer. Autophagy can be either tumor suppressive or promotive. Thus, autophagy modulation is being considered as a new strategy to improve cancer therapy. Areca nut (AN) is a worldwide popular carcinogen and contains apoptosis-inducing ingredients. However, we recently demonstrated that AN may predominantly induce autophagic responses in various types of cells. Furthermore, chronic exposure of cancer cells to this activity generally resulted in increased tolerance against environmental challenges, such as serum starvation, hypoxia, and anti-cancer drugs, through upregulated autophagy activity. We, therefore, propose that AN may have the potential to modulate tumors into an autophagy-addicted manner, raising the possibility of improving cancer therapy through autophagy inhibition especially in AN-addicted users.

Keywords: Areca nut; Autophagy; Cancer

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Autophagy background

There are three major forms of autophagy:

macroautophagy, microautophagy, and chaperon-mediated autophagy. Among them, macroautophagy (referred to as autophagy hereafter) has received extensive studies in the past decade. This evolutionarily conserved self-eating process delivers intracellular components to the lysosomal compartment for either recycling or degradation. It is thought that damaged proteins and organelles are degraded by basal levels of autophagy to maintain cellular homeostasis, or on the other hand, autophagy can be vigorously triggered for cells to survive nutrient-limited conditions [1]. Impairment of autophagy is now known to be associated with diseases including cancers [2]. Modulation of autophagy may nowadays represent a new direction for improved cancer therapy, which attracts great interest [3].

Tumor suppressive functions of autophagy

Autophagy has been firstly thought to be tumor-suppressive as one of its key mediator, ATG6/BECN1, was demonstrated to be monoallelically lost in significant proportions of various types

of cancers [4,5]. Further studies also showed that heterozygous disruption of beclin 1 increased the frequency of spontaneous malignancies and accelerated the development of hepatitis B virus-induced premalignant lesions [6], and beclin $1^{+/-}$ mutation in mice resulted in a high incidence of spontaneous tumors [7]. However, it is subsequently found that allelic loss of BECN1 promoted p53 activation and reduced tumorigenesis [8], and mutational analysis of BECN1 revealed deletions of both breast and ovarian tumor suppressor breast cancer 1 (BRCA1) and BECN1, and deletions of only BRCA1, indicating BRCA1 to be the driver mutation [9]. Although other large-scale genomic analysis of human cancers also indicated that BECN1 is not a tumor suppressor [10,11], the role of autophagy in tumor suppression is still valid. For examples, autophagy deficiency may trigger oxidative stress, which leads to DNA damage and genome instability and is thought to cause tumor initiation and progression [12-14]. Indeed, autophagy deficient mice are shown to develop multiple benign hepatomas [15]. It is also known that ablation of autophagy brings about the chronic death of hepatocytes and inflammation, the key factors that can cause liver cancers [12,16,17].

Tumor promoting functions of autophagy

Numerous evidence has confirmed that tumor cells may be more rely on autophagy than normal cells because they are frequently challenged by deficiencies in their microenvironment and increased demands of metabolites [18]. For instances, autophagy is upregulated in hypoxic tumor regions to promote the survival of tumor cells and helps melanomas to be more resistant to leucine deprivation [19,20]. In RAS-transformed cells, autophagy is also upregulated to facilitate cell growth, survival, and tumorigenesis, and required for oxidative and glycolytic homeostasis [21-23]. It is thought that mitochondrial metabolic defects and the resulting susceptibility to stress through autophagy inhibition may be the underlying mechanism in RAS-driven cancers. Indeed, it is followingly demonstrated that in RAS-activated mice, ATG7 deletion causes accumulation of dysfunctional mitochondria, p53 activation, and growth arrest of non-small-cell lung cancer (NSCLC) [24]. Furthermore, loss of ATG7 can shift phenotypes from malignant adenomas and carcinomas to benign oncocytomas [25,26], and ATG5 deletion in RAS-activated and NSCLC-bearing mice results in a similar reduction of tumorigenesis as ATG7-deficient mice [27]. These results establish a new concept that RAS-driven cancers may be addicted to autophagy [3].

There is also evidence suggesting that therapy-induced autophagy may function as the tumoral resistance mechanism. Melanomas with mutated BRAF often become resistant to BRAF inhibition (BRAFi). BRAFi-resistant tumors exhibit higher levels of autophagy, and patients with higher levels of therapy-induced autophagy show poor responses to BRAFi and a shorter duration of progression-free survival. In BRAFV600E melanoma cell lines, BRAFi or BRAF/MEK inhibition induced cytoprotective autophagy, and autophagy inhibition enhanced BRAFi-induced cell death [28]. In other settings of research, autophagy inhibition can increase the tumoricidal effect of mTOR inhibitors in both renal cell carcinoma and melanoma [29,30].

As expected, autophagy inhibition has now become a potential method to improve cancer therapy. An important following question is that as autophagy is essential for some normal tissues, whether systemic autophagy inactivation may result in deleterious consequences? The water-soluble anti-malaria drug, hydroxychloroquine (HCQ), known to block lysosome function and the degradation of autophagy cargo, is currently being used in clinical trials against various cancers. It remains elusive whether HCQ is sufficiently selective and effective [31]. Efforts are thus needed in searching for more specific and potent methods to knockdown autophagy activity in tumors.

Areca nut background

Betel quid (BQ) is a psychoactive and addictive carcinogen used by 200-600 million people worldwide. Recipes of BQ are varied in different regions of the world, including areca nut (AN, Areca catechu L.), lime, Piper betle leaf, Piper betle inflorescence, or clove [32,33]. Among these diversified components, AN is the essential and common constituent of BQ, which is also regarded as the human carcinogen [34]. Although tobacco is occasionally included in BQ, it has been found to only marginally increase the cancer risk [35].

Similar to tobacco, the carcinogenic N-nitrosamines can be derived from AN and cause some types of tumors in rats [36,37]. AN extract (ANE) generates reactive oxygen species (ROS) both in solution and saliva and modifies DNA to form 8-hydroxy-20-deoxyguanosine [38,39]. Both cytostatic and cytotoxic effects of ANE on Chinese hamster ovary cells and human oral cancer cells have been described [40-42]. Two AN components, arecoline (the major alkaloid of AN) and oligomeric procyanidins, as well as the hydroxychavicol from the leaf and inflorescence of Piper betle, can induce apoptosis in both oral epithelial KB cells and mouse splenic lymphocytes [42-44]. Thus, chewing AN may conduct apoptotic stimuli to oral cells.

Linkage of AN with autophagy

Unexpectedly, we previously noticed that the partially purified 30-100 kDa fraction of ANE (designated as ANE 30-100K) induced a different death pattern of oral carcinoma cells as that induced by arecoline. Arecoline induces cell shrinkage, caspase-3 activation, peri-nuclear condensation of chromatin, and micronucleation; whereas ANE 30-100K causes swallen morphology, emptiness of cytoplasm, nuclear condensation (without peri-nuclear condensation of chromatin, and micronucleation), accumulation of microtubule-associated protein 1 light chain 3 (LC3)-II (one of the hallmarks of autophagy) and generation of acidic vesicles in oral carcinoma OECM-1 cells [45]. ANE 30-100K, but not arecoline, can also increase LC3-II levels, a phenomenon further enhanced by lysosomal enzyme inhibitors, in both non-tumor oral fibroblasts and esophageal carcinoma CE81T/VGH cells, suggesting ANE 30-100K promotes autophagic flux [46]. In addition to these carcinoma cells and fibroblasts, ANE 30-100K also induce similar autophagic responses in peripheral blood lymphocytes and Jurkat T cells, suggesting ANE 30-100K as an autophagy stimulator in a wide spectrum of distinct cell types [45,46]. We also demonstrate that the autophagy-stimulating activity of ANE

30-100K is sensitive to both cellulase and proteinase K digestion, indicating the autophagy-inducing AN ingredient (AIAI) to be a proteoglycan (or glycoprotein) [47].

We also notice that ANE but not ANE 30-100K activates caspase-3, probably due to the presence of aforementioned ingredients of apoptosis-inducing small molecules like arecoline and oligomeric procyanidins in ANE [45]. Moreover, despite the co-existence of apoptosis- and autophagy-inducing ingredients in ANE, most, if not all, ANE- and ANE 30-100K-treated cells exhibit morphological changes of autophagy rather than apoptosis before their death, suggesting that AN may transmit a dominant autophagic stimulation to oral cells.

Although as introduced earlier, autophagy may suppress cancer initiation and progression through the elimination of dysfunctional mitochondria to prevent the accumulation of detrimental free radical, our previous study reveals that ANE 30-100K itself stimulates an increase of intracellular reactive oxygen species [iROS], which is required for ANE 30-100K-induced autophagy and cytotoxicity [46]. Thus, ANE 30-100K-induced autophagy might be unable to prevent the elevation of iROS effectively. Since AN is a carcinogen with a newly defined autophagy-stimulating activity, it might be reasonable to speculate a positive role of such activity in tumorigenesis.

Analyses of the effects of chronic ANE or ANE 30-100 K treatment on different types of cells have supported our hypothesis. Firstly, both long-term non-cytotoxic ANE- and ANE 30-100K-treated cells generally express higher autophagy activities under glucose deprivation, hypoxia, and serum starvation. Secondly, these treated cells exhibit stronger resistance against hypoxia, anti-cancer drugs, and serum starvation. Finally, upregulated autophagy is illustrated to be responsible for the increased tolerance of chronic ANE- and ANE 30-100K-treated cancer cells against drugs and serum deprivation [48,49]. Additionally, autophagy mediators Beclin 1 and ATG5 are shown to be required for ANE 30-100K-induced autophagy; whereas the role of AMP-activated protein kinase may be cancer-dependent [49]. Collectively, our studies indicate that the autophagy-stimulating activity of AN may be tumor supportive, i.e., as those of RAS-driven cancers, malignant cells developed in AN-addicted users might become autophagy-addicted.

In conclusion, our studies have raised the possibility that inclusion of autophagy inhibition may improve the prognosis of cancer patients with the AN-chewing habit.

References

1 Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. Cell 147: 728-741.

2 Galluzzi L, Pietrocola F, Bravo-San Pedro JM, Amaravadi RK, et al. (2015) Autophagy in malignant transformation and cancer progression. EMBO J 34: 856-880.

3 White E The role for autophagy in cancer. J Clin Invest 125: 42-46.

4 Aita VM, Liang XH, Murty VV, Pincus DL, Yu W, et al. (1999) Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. Genomics 59: 59-65.

5 Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, et al. (1999) Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 402: 672-676.

6 Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, et al. (2003) Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 112: 1809-1820.

7 Yue Z, Jin S, Yang C, Levine AJ, Heintz N (2003) Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc Natl Acad Sci USA 100: 15077-15082.

8 Huo Y, Cai H, Teplova I, Bowman-Colin C, Chen G, et al. (2013) Autophagy opposes p53-mediated tumor barrier to facilitate tumorigenesis in a model of PALB2-associated hereditary breast cancer. Cancer Discov 3: 894-907.

9 Laddha SV, Ganesan S, Chan CS, White E (2014) Mutational landscape of the essential autophagy gene BECN1 in human cancers. Mol Cancer Res 12: 485-490.

10 Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, et al. (2014) Discovery and saturation analysis of cancer genes across 21 tumour types. Nature 505: 495-501.

11 Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, et al. (2013) Cancer genome landscapes. Science 339: 1546-1558.

12 Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, et al. (2009) Autophagy suppresses tumorigenesis through elimination of p62. Cell 137: 1062-1075.

13 Karantza-Wadsworth V, Patel S, Kravchuk O, Chen G, Mathew R, et al. (2007) Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes Dev 21: 1621-1635.

14 Mathew R, Kongara S, Beaudoin B, Karp CM, Bray K, et al. (2007) Autophagy suppresses tumor progression by limiting chromosomal instability. Genes Dev 21: 1367-1381.

15 Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, et al. (2011) Autophagy-deficient mice develop multiple liver tumors. Genes Dev 25: 795-800.

16 Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, et al. (2007) Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell 131: 1149-1163.

17 Sun B, Karin M (2013) Inflammation and liver tumorigenesis. Front Med 7: 242-254.

18 Rabinowitz JD, White E (2010) Autophagy and metabolism. Science 330: 1344-1348.

19 Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, et al. (2006) Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 10: 51-64.

20 Sheen JH, Zoncu R, Kim D, Sabatini DM (2011) Defec-tive regulation of autophagy upon leucine deprivation reveals a targetable liability of human melanoma cells in vitro and in vivo. Cancer Cell 19: 613–628.

21 Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, et al. (2011) Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev 25: 460-470.

22 Lock R, Roy S, Kenific CM, Su JS, Salas E, et al. (2011) Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. Mol Biol Cell 22: 165-178.

23 Yang S, Wang X, Contino G, Liesa M, Sahin E, et al. (2011) Pancreatic cancers require autophagy for tumor growth. Genes Dev 25: 717-729.

24 Guo JY, Karsli-Uzunbas G, Mathew R, Aisner SC, Kamphorst JJ, et al. (2013) Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. Genes Dev 27: 1447-1461.

25 Gasparre G, Romeo G, Rugolo M, Porcelli AM (2011) Learning from oncocytic tumors: why choose inefficient mitochondria? Biochim Biophys Acta 1807: 633-642.

26 Strohecker AM, Guo JY, Karsli-Uzunbas G, Price SM, Chen GJ, et al. (2013) Autophagy sustains mitochondrial glutamine metabolism and growth of BrafV600E-driven lung tumors. Cancer Discov 3: 1272-1285.

27 Rao S, Tortola L, Perlot T, Wirnsberger G, Novatchkova M, et al. (2014) A dual role for autophagy in a murine model of lung cancer. Nat Commun 5: 3056.

28 Ma XH, Piao SF, Dey S, McAfee Q, Karakousis G, et al. (2014) Targeting ER stress-induced autophagy overcomes BRAF inhibitor resistance in melanoma. J Clin Invest 124: 1406-1417.

29 Bray K, Mathew R, Lau A, Kamphorst JJ, Fan J, et al. (2012) Autophagy suppresses RIP kinase-dependent necrosis enabling survival to mTOR inhibition. PLoS One 7: e41831.

30 Xie X, White EP, Mehnert JM (2013) Coordinate autophagy and mTOR pathway inhibition enhances cell death in melanoma. PLoS One 8: e55096.

31 Amaravadi RK, Lippincott-Schwartz J, Yin XM, Weiss WA, Takebe N, et al. (2011) Principles and current strategies for targeting autophagy for cancer treatment. Clin Cancer Res 17: 654-666.

32 Jeng JH, Chang MC, Hahn LJ (2001) Role of areca nut in betel quid-associated chemical carcinogenesis: current awareness and future perspectives. Oral Oncol 37: 477-492.

33 Chu NS (2001) Effects of Betel chewing on the central and autonomic nervous systems. J Biomed Sci 8: 229-236.

34 IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2004) Betel-quid and areca-nut chewing and some areca-nut-derived nitrosamines. IARC Monogr Eval Carcinog Risks Hum 85: 1-334.

35 Sharan RN, Mehrotra R, Choudhury Y, Asotra K (2012) Association of betel nut with carcinogenesis: revisit with a clinical perspective. PLoS One 7: e42759.

36 Rivenson A, Hoffmann D, Prokopczyk B, Amin S, Hecht SS (1988) Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and Areca-derived N-nitrosamines. Cancer Res 48: 6912-6917.

37 Hoffmann D, Brunnemann KD, Prokopczyk B, Djordjevic MV (1994) Tobacco-specific N-nitrosamines and Areca-derived N-nitrosamines:

chemistry, biochemistry, carcinogenicity, and relevance to humans. J Toxicol Environ Health 41: 1-52.

38 Nair UJ, Floyd RA, Nair J, Bussachini V, Friesen M, et al. (1987) Formation of reactive oxygen species and of 8-hydroxydeoxyguanosine in DNA in vitro with betel quid ingredients. Chem Biol Interact 63: 157-169.

39 Chen CL, Chi CW, Liu TY (2002) Hydroxyl radical formation and oxidative DNA damage induced by areca quid in vivo. J Toxicol Environ Health A 65: 327-336.

40 Chang MC, Wu HL, Lee JJ, Lee PH, Chang HH, et al. (2004) The induction of prostaglandin E2 production, interleukin-6 production, cell cycle arrest, and cytotoxicity in primary oral keratinocytes and KB cancer cells by areca nut ingredients is differentially regulated by MEK/ERK activation. J Biol Chem 279: 50676-50683.

41 Lin CC, Chang MC, Chang HH, Wang TM, Tseng WY, et al. (2009) Areca nut-induced micronuclei and cytokinesis failure in Chinese hamster ovary cells is related to reactive oxygen species production and actin filament deregulation. Environ Mol Mutagen 50: 367-374.

42 Lee PH, Chang MC, Chang WH, Wang TM, Wang YJ, et al. (2006) Prolonged exposure to arecoline arrested human KB epithelial cell growth: regulatory mechanisms of cell cycle and apoptosis. Toxicology 220: 81-89.

43 Chang MC, Uang BJ, Wu HL, Lee JJ, Hahn LJ, et al. (2002) Inducing the cell cycle arrest and apoptosis of oral KB carcinoma cells by hydroxychavicol: roles of glutathione and reactive oxygen species. Br J Pharmacol 135: 619-630.

44 Wang CC, Huang PL, Liu TY, Jan TR (2009) Highly oligomeric procyanidins from areca nut induce lymphocyte apoptosis via the depletion of intracellular thiols. Toxicol In Vitro 23: 1234-1241.

45 Liu SY, Lin MH, Hsu YR, Shih YY, Chiang WF, et al. (2008) Arecoline and the 30-100 kDa fraction of areca nut extract differentially regulate mTOR and respectively induce apoptosis and autophagy: a pilot study. J Biomed Sci 15: 823-831.

46 Lin MH, Hsieh WF, Chiang WF, Hong WZ, Hsu YR, et al. (2010) Autophagy induction by the 30-100kDa fraction of areca nut in both normal and malignant cells through reactive oxygen species. Oral Oncol 46: 822-828.

47 Lin MH, Liu SY, Liu YC (2008) Autophagy induction by a natural ingredient of areca nut. Autophagy 4: 967-968.

48 Yen CY, Chiang WF, Liu SY, Cheng PC, Lee SY, et al. (2014) Long-term stimulation of areca nut components results in increased chemoresistance through elevated autophagic activity. J Oral Pathol Med 43: 91-96.

49 Yen CY, Chiang WF, Liu SY, Lin CC, Liao KA, et al. (2015) Impacts of autophagy-inducing ingredient of areca nut on tumor cells. PLoS One 10: e0128011.