Biochimica et Biophysica Acta 1793 (2009) 1516–1523

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Role and regulation of autophagy in cancer

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ARTICLE INFO

Article history:
Received 26 November 2008
Received in revised form 19 December 2008
Accepted 20 December 2008
Available online 2 January 2009

Keywords:
Autophagy
Tumorigenesis
Tumor suppression
Autophagy regulation
Cancer therapy
Autophagy modulation

ABSTRACT

Autophagy is an evolutionarily conserved process whereby cytoplasm and cellular organelles are degraded in lysosomes for amino acid and energy recycling. Autophagy is a survival pathway activated in response to nutrient deprivation and other stressful stimuli, such as metabolic stress and exposure to anticancer drugs. However, autophagy may also result in cell death, if it proceeds to completion. Defective autophagy is implicated in tumorigenesis, as the essential autophagy regulator *beclin 1* is monoallelically deleted in human breast, ovarian and prostate cancers, and *beclin* $1^{+/-}$ mice are tumor-prone. How autophagy suppresses tumorigenesis is under intense investigation. Cell-autonomous mechanisms, involving protection of genome integrity and stability, and a non-cell-autonomous mechanism, involving suppression of necrosis and inflammation, have been discovered so far. The role of autophagy in treatment responsiveness is also complex. Autophagy inhibition concurrently with chemotherapy or radiotherapy has emerged as a novel approach in cancer treatment, as autophagy-competent tumor cells depend on autophagy for survival under drug- and radiation-induced stress. Alternatively, autophagy stimulation and preservation of cellular fitness by maintenance of protein and organelle quality control, suppression of DNA damage and genomic instability, and limitation of necrosis-associated inflammation may play a critical role in cancer prevention.

1. Introduction

Macroautophagy, hereafter referred to as autophagy, is a cellular self-consumption process characterized by sequestration of bulk cytoplasm, long-lived proteins and cellular organelles in double-membrane vesicles called autophagosomes, which are delivered to and degraded in lysosomes [1]. Basal autophagy plays an important role in cellular homeostasis by degrading excessive, damaged and/or aged proteins and organelles, and thus maintaining quality control of essential cellular components [2,3]. Defective autophagy has been implicated in the pathogenesis of diverse disease states, such as myopathy [4], neuronal degeneration [5], microbial infection [6], inflammatory bowel disease [7,8], aging [9] and cancer [10]. In addition to its basal function, autophagy is readily induced in response to nutrient deprivation [11–14], metabolic stress [15–17], endoplasmic reticulum (ER)-stress [18,19], radiation [20] and anticancer drugs [21–24]. The role of autophagy as an alternate energy source, and thus as a temporary survival mechanism, under stressful conditions is well recognized [25]. The presence of autophagosomes in dying cells raises the possibility that autophagy may also play an active role in cell death [26]. However, in most occasions, it is unclear whether this is the case or autophagy is just a bystander merely representing the cell's desperate attempt to sustain survival upon severe stress and/or injury.

The mechanism by which defective autophagy contributes to tumorigenesis is under intense investigation. Inactivation of apoptosis, and thus deregulation of cell death, is a frequent occurrence in tumor cells [27], indicating that aberrant cell survival and cell death drive cancer progression. Loss of a survival pathway, such as autophagy, might have then been expected to undermine tumorigenesis; however, the recognition of the essential autophagy regulator *beclin 1* as a haploinsufficient tumor suppressor [28,29] argues against this simplistic scenario.

In this publication, the role of autophagy in tumorigenesis and the possible implications of the functional status of autophagy on treatment responsiveness will be reviewed. What is currently known on the regulation of autophagy in tumor cells will also be presented, together with a discussion on how pharmacologic modulation of autophagy may lead to improved combinatorial anticancer regimens.

2. *beclin 1* as a haploinsufficient tumor suppressor

The autophagy-related (*atg*) genes play essential roles at different stages of the autophagic process, including induction, vesicle formation, and autophagosome degradation, and were first identified and characterized in yeast [30]. Autophagy is evolutionarily conserved, and many yeast Atg proteins have homologues in higher eukaryotes.

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doi:10.1016/j.bbamcr.2008.12.013

beclin 1 is the mammalian ortholog of the *Saccharomyces cerevisiae atg6/VPS30* gene, which is required for both autophagy and sorting of the vacuole resident hydrolase carboxypeptidase Y through the Vps pathway [31]. The possibility that defective autophagy may play a role in cancer was first recognized through studies focused on Beclin 1, as summarized below.

beclin 1 was originally discovered during the positional cloning of *BRCA1* and was entered in GenBank as a gene of unknown function [32]. Beclin 1 was independently rediscovered in a yeast two-hybrid screen of an adult mouse brain library as a Bcl-2 interacting protein [33]. *beclin 1* maps to a centromeric region of *BRCA1* on chromosome 17q21 that is commonly deleted in 75, 50 and 40% of ovarian, breast, and prostate cancers, respectively [34–38]. In addition to *beclin 1*, this commonly deleted region contains at least 11 more genes, 6 genes of known function and not considered cancer-related, and 5 completely novel genes. FISH analysis of human breast cancer cell lines using the *beclin 1*-containing PAC 452O8 as a probe revealed that 9 out of 22 cell lines had allelic *beclin 1* deletions [39]. The cell lines examined were mostly aneuploid with 3–7 copies of chromosome 17 and showed only a "partial" *beclin 1* deletion, still retaining 2–3 copies of *beclin 1*. Sequencing of the retained *beclin 1* alleles did not reveal any mutations, and, interestingly, Beclin 1 mRNA levels were comparable in all cell lines, independently of *beclin 1* copy number [39]. The autophagy potential of these cell lines has been only marginally characterized so far [40,41].

Ectopic expression of Beclin 1 restores full autophagy potential in MCF-7 cells, which are tetraploid, but have three *beclin 1* copies, and slows cell proliferation *in vitro* and in xenograft tumors *in vivo* [41]. This finding together with the frequent allelic deletion of *beclin 1* in human breast cancer cell lines raised the possibility that *beclin 1* may be a tumor suppressor, a hypothesis subsequently confirmed by knockout mouse technology. *beclin 1* heterozygous mice develop lymphomas, liver and lung cancers as they age, as well as hyperproliferative, preneoplastic mammary lesions [29,42]. The second *beclin 1* allele is retained in all tumors developing in *beclin* $1^{+/-}$ mice, and is neither mutated nor silenced [28], indicating that *beclin 1* is a haploinsufficient tumor suppressor. Monoallelic *beclin 1* loss also accelerates the development of hepatitis B virus-induced premalignant liver lesions [28]. Furthermore, *beclin* $1^{+/-}$ immortalized baby mouse kidney (iBMK) cells [15,17] and immortalized mouse mammary epithelial cells (iMMECs) [16], which exhibit compromised autophagy under metabolic stress, display accelerated tumorigenesis in nude mouse allografts.

Lower Beclin 1 protein expression, as compared to Beclin 1 levels in normal adjacent breast tissue, was confirmed in a small series of human breast tumors [41], but any correlation between allelic *beclin 1* loss, and thus defective autophagy, and clinical outcome in breast cancer remains to be investigated. Decreased Beclin 1 mRNA and protein expression was also demonstrated in glioblastoma multiforme (GBM) and other high-grade brain tumors [43]. In contrast, higher expression of Beclin 1 was detected in the majority of colorectal (95%) and gastric (83%) carcinomas examined as compared to the normal stomach and colon mucosa, which show very low or undetectable Beclin 1 levels [44]. In this case, Beclin 1 expression did not show any correlation with pathological and clinical characteristics, such as stage, invasion, and metastasis [44]. It is, therefore, conceivable that the tumor suppressive function of Beclin 1 may be tissue-specific, which is certainly worthy of further investigation.

3. The Beclin 1 protein network

As mentioned earlier, Beclin 1 was originally identified as a Bcl-2 interacting protein in a yeast two-hybrid system [33]. This interaction is not very surprising, given that Beclin 1 possesses a putative Bcl-2-homology-3 (BH3) amphipathic alpha-helix (amino acids 112–123), as demonstrated by X-ray crystallography [45], NMR spectroscopy [46], and mutational analysis [47], which can interact with the BH3 receptor domain (hydrophobic grove) of the anti-apoptotic proteins Bcl-2, Bcl-xL, Bcl-w, and to a lesser extent Mcl-1 [48]. Binding of Beclin 1 to Bcl-2 or Bcl-xL is much weaker than that of proapoptotic BH3-only proteins, such as Bid, Bad, Bak, and Bim [46]. Upon nutrient deprivation, which is the most potent autophagy inducer, BH3-only proteins induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2 or Bcl-xL [49]. At this point, it is unclear whether the autophagy-inhibitory Beclin 1–Bcl-2 or Beclin 1–Bcl-xL interaction plays any role in the tumor promoting function of the anti-apoptotic Bcl-proteins, especially since only endoplasmic reticulum (ER)-targeted Bcl-2 binds Beclin 1 and the interaction between these two proteins is disrupted under conditions of metabolic stress, which is a frequent occurrence in tumors *in vivo*.

The role of autophagy in tumor suppression has further been established by the identification and characterization of Beclin 1-interacting proteins. Co-immunoprecipitation studies have identified class III phosphoinositide-3-kinase (PI3KIII)/Vps34 as a major physiological partner of Beclin 1 in a complex required for autophagy initiation [50] and tumor suppression [51]. The UV irradiation resistance-associated gene protein, UVRAG, interacts with Beclin 1 and PI3KIII to promote autophagosome formation, autophagy activation and inhibition of human colon cancer cell proliferation and tumorigenicity [52]. UVRAG was recently shown to disrupt an *in vitro* and *in vivo* observed Beclin 1-dimer interface, normally stabilized by Bcl-2-like proteins, to induce autophagy [53]. Similarly to *beclin 1*, *UVRAG* is monoallelically deleted in human colon cancers [52]. Furthermore, a polyadenine tract in the *UVRAG* gene (A10 in exon 8) is a target for frameshift mutations decreasing the autophagy potential of colon [54] and gastric [55] cancers with microsatellite instability (MSI).

Bif-1, also known as SH3GLB1 or Endophilin B1, was originally discovered as a Bax-binding protein [56,57], and was subsequently shown to associate with membranes of intracellular organelles, such as the Golgi apparatus [58,59] and mitochondria [60,61], and participate in vesicle formation and membrane dynamics. More recently, Bif-1 was identified as a Beclin 1-interacting protein through UVRAG, a PI3KIII activator, and thus a regulator of autophagosome formation, and a novel tumor suppressor, as *bif-*$1^{-/-}$ mice develop lymphomas and solid tumors at about 12 months of age [62].

4. Other autophagy-related genes as tumor suppressors

Whereas *beclin* $1^{-/-}$ mice die early in embryogenesis [28,29], *atg5*$^{-/-}$ and *atg7*$^{-/-}$ mice are born normally, but die soon after birth [11,12]. Also, in contrast to aging *beclin* $1^{+/-}$ mice which are tumor-prone, older *atg5*$^{+/-}$ and *atg7*$^{+/-}$ mice do not develop malignancies, and neither tumorigenesis nor enhanced cell proliferation is observed in atg7-deficient liver, which is abnormally large mostly due to hepatocyte swelling [11]. These studies indicate that Beclin 1 may play a more important role in embryonic development and tumor suppression than Atg5 and Atg7 or, alternatively, that autophagy-independent properties of Beclin 1 may be responsible for these functions. More recently, *atg5*$^{-/-}$ iBMK cells were found to be more tumorigenic than *atg5*$^{+/-}$ and *atg5*$^{+/+}$ iBMK cells in nude mouse allografts [17], suggesting that autophagy defects indeed play a role in tumorigenesis. This was further confirmed by the finding that mice deficient in atg4C/autophagin-3, a cysteine protease involved in Atg8/LC3 processing required for autophagy execution [63], show increased susceptibility to chemical carcinogen-induced fibrosarcoma development [64].

5. How does autophagy suppress tumorigenesis?

The mechanism by which autophagy defects lead to accelerated tumorigenesis is not readily apparent, especially given the well-