No ATG is an island—the connection of autophagy with diverse pathways and functions

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The sixth International Symposium on Autophagy took place in October 2012 in Okinawa, Japan. It brought together scientists from all over the world to cultivate a better understanding of cutting-edge autophagy research from molecular mechanisms to disease states.

The International Symposium on Autophagy (ISA) is an important meeting with a long history devoted solely to the topic of autophagy. Yoshimori Ohsumi (Tokyo Institute of Technology, Japan), who identified a series of autophagy-related genes (ATG genes) in yeast [1], proposed and organized the first ISA in Okazaki, Japan, in 1997. Over the years, the ISA symposia have witnessed the explosive growth of autophagy research in the following specific areas: molecular dissection of core ATG gene products; genetic studies of the role of ATGs in multiple species; and pathophysiological aspects of autophagy in higher eukaryotes, including neurodegeneration, cancer and inflammation. The ISA, together with the prestigious Gordon Research Conferences (2003–), Keystone Symposia (2007–) and EMBO Courses and Workshops (2009–), has always provided an international forum for rosters of excellent speakers to present new discoveries that advance our global understanding of autophagy.

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The sixth ISA was no exception. It took place between 28 October and 1 November 2012, in Okinawa, an island with a unique history and subtropical climate, surrounded by beautiful beaches and the Pacific Ocean. It was organized by Tamotsu Yoshimori (Osaka U., Japan)

and was devoted to six topics: autophagy machinery, selective autophagy, regulation of autophagy, autophagy and diseases, autophagosome formation, and emerging autophagy. Here, we focus on newly identified players that participate in autophagosome biogenesis and fusion, selective autophagy, and tumorigenesis associated with autophagy suppression—topics that were discussed at this conference.

Autophagy is highly inducible—starvation and other stresses increase the number of autophagosomes. Autophagosomes are generated on or in close proximity to the endoplasmic reticulum. However, fundamental questions remain as to whether the endoplasmic reticulum membrane is the sole source for the autophagosome and whether it is involved directly in autophagosome formation. Studies suggest that additional membranes derived from the Golgi complex, mitochondria, the plasma membrane and recycling endosomes also contribute to autophagosome formation [2]. Thus, it is probable that autophagosome formation involves multiple and complicated processes. Elucidation of the origin of autophagosomal membranes, as well as the molecular mechanisms underlying autophagosome formation, are two of the most intriguing issues in the autophagy field.

Yoshimori Ohsumi and Hayashi Yamamoto (Tokyo Institute of Technology, Japan) identified small cytoplasmic vesicles containing Atg9 (designated Atg9 vesicles) and showed that only a few Atg9 vesicles are required for a single round of

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autophagosome formation. They further identified Atg27, Trs85 (a specific subunit of the transport protein particle III) and Ypt1 (a Rab GTPase) as components of Atg9 vesicles. Although Atg9-containing structures were thought to be a source of the lipids required for autophagosome formation, their data suggest that only a small quantity of the lipids is supplied through these vesicles. Rather, Atg9 vesicles might be important to transport the TRAPPIII complex and Ypt1 to the pre-autophagosomal structure and phagophore assembly site (PAS), allowing them to function in the process of autophagosome formation.

Sharon Tooze (Cancer Research UK) demonstrated that vesicular transport from recycling endosomes also contributes to autophagosome formation. Her group showed that Rab11- and Unc-51-like kinase 1 (ULK1)-positive vesicles are derived from recycling endosomes—a process that is negatively regulated by a Rab GAP, TBC1D14—and are required for autophagosome formation. Interestingly, Rab11 has been proposed to be a mammalian homologue of Ypt32 regulated by Ypt1. Further studies should provide new insights into the function of TRAPPIII complex, Rab11 and Ypt1 in autophagosome formation.

Once autophagosome formation is complete, the autophagosome fuses with the lysosome and any late endosomes. The molecular mechanism(s) of the fusion event have not been fully characterized. It has been suggested that Vam3, Vam7, Ykt6, and Vti1 participate in autophagosome–vacuole fusion in yeast, and VAMP7, VAMP8 and VTI1 in mammals. However, specific soluble N-ethylmaleimide-sensitive fusion (NSF) attachment protein receptor (SNARE) protein(s) on the autophagosomal membrane remain to be identified. Noboru Mizushima (U. Tokyo, Japan) and his colleagues identified syntaxin 17 (Stx17) as an autophagosomal SNARE. They found that Stx17 localizes to the outer membrane of the completed autophagosome on autophagy induction, although the location from which Stx17 is derived is not clear. Stx17 interacts with both synaptosomal-associated protein 29 (SNAP29) and the endosomal and lysosomal SNARE, VAMP8, which mediates fusion between autophagosomes and lysosomes. The isolation membranes and phagophores do not have Stx17, preventing premature fusion with the lysosome. Further experiments are needed to clarify how Stx17 localizes only to completed autophagosomes but not to the isolation membrane.

Besides the fundamental role of starvation-induced autophagy—in other words, the supply of building blocks for new macromolecule synthesis and for energy production—mounting evidence points to the importance of selective autophagy, which degrades aggregated proteins, unnecessary or damaged mitochondria and invading bacteria through ubiquitin signalling [3]. Such selective autophagy occurs both constitutively and in response to cellular stresses to maintain cellular homeostasis.

...PINK1 is autophosphorylated [...] [This] is required for parkin recruitment to mitochondria

Studies have described the molecular mechanism by which damaged mitochondria are selectively targeted to autophagy (mitophagy), and these studies also suggest that defects in this process underlie familial Parkinson disease [4]. Specifically, when the mitochondrial membrane potential is dissipated, the E3 ubiquitin ligase parkin translocates from the cytosol to mitochondria and ubiquitinates multiple substrates, ultimately leading to the sequestration and degradation

of mitochondria by autophagy. This process depends on the serine/threonine kinase (PTEN-induced putative kinase 1) PINK1, which is imported and degraded in healthy mitochondria, but targeted and accumulated on the surface of dysfunctional mitochondria. Keiji Tanaka (Tokyo Metropolitan Institute of Medical Science, Japan) and colleagues have demonstrated that PINK1 is autophosphorylated after membrane potential dissipation. Importantly, this post-translational modification, which is disrupted by most of the pathogenic PINK1 mutations, is required for parkin recruitment to mitochondria. How autophosphorylation activates PINK1 to localize parkin on damaged mitochondria remains to be elucidated. Regarding the physiological relevance of the parkin–PINK1-mediated pathway, Richard Youle (NIH, USA) reported that loss of parkin causes synthetic, stronger neurodegeneration phenotypes in a mouse model expressing proofreading-defective mitochondrial DNA (mtDNA) polymerase (mtDNA mutator mouse). One scenario could be that mitochondria rapidly accumulate mtDNA mutations in the absence of quality control through mitophagy.

...a phosphorylation-defective beclin 1 could suppress Akt-dependent tumorigenesis both *in vitro* and *in vivo*

When bacteria invade host cells through endocytosis and phagocytosis, a selective type of autophagy termed ‘xenophagy’, engulfs the pathogens to restrict their growth [5]. Although neither the target proteins nor the E3 ubiquitin ligases have been identified, invading bacteria such as *Salmonella enterica*, *Listeria monocytogenes* and *Shigella flexneri* become positive for ubiquitin when they access the cytosol by rupturing the endosome- and phagosome-limiting membrane. For example, the ubiquitin coat surrounding cytosolic *Salmonella* is bound by the autophagy receptors p62 and nuclear dot protein 52 (NDP52) that also interact with microtubule-associated proteins 1A/1B light chain 3 (LC3). Ivan Dikic (Goethe U. Frankfurt, Germany) and colleagues have shown that the protein kinase TANK-binding kinase 1 (TBK1) phosphorylates optineurin (OPTN)—a new autophagy receptor—at Ser177, enhancing LC3-binding and autophagic degradation of cytosolic *Salmonella*. Dikic demonstrated that Ser473 and Ser403 in the OPTN- and

p62-ubiquitin-binding domains (UBAN and UBA), respectively, are also phosphorylated by TBK1, and that these modifications are crucial for *Salmonella* autophagy. Interestingly, Nobuyuki Nukina (RIKEN Brain Science Institute, Japan) and colleagues have discovered that casein kinase 2 (CK2) phosphorylates p62 Ser403, increasing the affinity between UBA and Lys63-linked polyubiquitin chains, which then facilitates the targeting of polyubiquitinated substrates for autophagic clearance. Clearly, diverse signalling pathways regulate their downstream kinases to activate selective autophagy through both ubiquitin- and LC3-binding. Looking at the upstream events, Ramnik Xavier (Harvard Medical School, USA) showed that leucine-rich repeat and sterile alpha motif containing 1 (LRSAM1), a previously identified interacting partner for the Atg8 family member gamma-aminobutyric acid receptor-associated protein-like 2 (GABARAPL2), is an E3 ubiquitin ligase required for *Salmonella* autophagy. Further investigations are needed to address the issue of how LRSAM1 is targeted to the ruptured bacteria-containing membrane structures and whether it mediates ubiquitination of the specific substrate(s). Paolo Manzanillo from Jeffery Cox’s laboratory (U. California San Francisco, USA) provided surprising data suggesting that *parkin*-deficient mice and flies are susceptible to bacterial infections, and that parkin marks cytosolic bacteria with Lys63-linked ubiquitin chains. It seems plausible that multiple protein kinases and E3 ubiquitin ligases cooperate efficiently to recognize the invading pathogens and target them to xenophagy through multiple autophagy receptors.

In normal cells, autophagy prevents tumorigenesis through selective clean-up of damaged organelles and specific proteins such as p62. Furthermore, cells with defective autophagy undergo necrotic cell death followed by inflammation, which enhances the incidence of tumorigenesis. However, it is uncertain whether autophagic activity is truly attenuated during tumorigenesis in humans, and the molecular mechanisms also remain unclear. In 1999, *becclin 1* was identified as a tumour suppressor gene, suggesting for the first time a tumour-suppressive role for autophagy [6]. *Beclin 1*^{+/−} mice have significantly reduced autophagic activity and increased cancer risk; these mice develop spontaneous tumours, including lymphomas, lung

...K-Ras-driven tumorigenesis was suppressed by loss of autophagy [...] ablation of autophagy in lung tumour cells harbouring activated K-Ras resulted in more benign tumours

adenocarcinoma and hepatocellular carcinoma. Beth Levine (U. Texas Southwestern Medical Center, USA) and colleagues have discovered that a serine/threonine kinase, Akt, the activation of which is often associated with human malignancies, phosphorylates beclin 1 at Ser295 and Ser234. This tethers beclin 1 to intermediate filament proteins such as vimentin through direct interaction with an adaptor protein, 14-3-3. It inhibits autophagy in a mammalian target of rapamycin (mTOR)-independent manner and, more importantly, a phosphorylation-defective beclin 1 could suppress Akt-dependent tumorigenesis both *in vitro* and *in vivo*. Their results imply that suppression of autophagy is a predisposing factor for tumorigenesis in Akt-activated cells. David Rubinsztein (Cambridge Institute for Medical Research, UK) showed that a beclin 1-interacting protein, Bim, translocates beclin 1 to microtubules through interaction with the dynein light chain 1 (DLC1/LC8), which also inhibits autophagy. Starvation induces phosphorylation of Thr116 of Bim through c-Jun N-terminal kinase (JNK) and then causes dissociation of the interaction between Bim and beclin 1. Although the released beclin 1 induces autophagy, the free Bim might promote apoptosis. These presentations indicate that beclin 1 localization through phosphorylation by Akt or JNK controls autophagy, the dysregulation of which might be involved in tumorigenesis in humans.

Zhenyu Yue (Mount Sinai School of Medicine, USA) used *beclin 1* conditional knockout mice to show that beclin 1 might be involved in multiple membrane transport pathways. However, it remains to be clarified whether impaired autophagy contributes solely to neoplasia in humans.

On the other hand, autophagy provides tumour cells—which require enormous quantities of nutrients—with a supply of amino acids, fatty acids and glucose, and has therefore been considered necessary for the efficient growth of malignant tumours. As such, autophagy satisfies the metabolic demands of tumours and enables their resistance to the microenvironment once they become constantly proliferative, implying that autophagy is a double-edged sword when it comes to cancer [7]. Cancer cells, particularly those with Ras mutations such as pancreatic cancer, rely heavily on autophagy to the extent of ‘addiction’. Although the precise molecular mechanism remains unclear, the blockade of autophagy is sufficient to inhibit the proliferation of pancreatic cancer cells. Further, loss of autophagy in pancreatic cancer cells is accompanied by impaired oxidative phosphorylation, probably due to the decreased supply of intermediates from the tricarboxylic acid cycle. Hence, it is plausible that cancer cells are ‘addicted’ to autophagy, as it is crucial to both the quality control of organelles, such as mitochondria and peroxisomes, and the supply of amino acids to support the survival and proliferation of cancer cells under metabolic stress conditions. Eileen White (The Cancer Institute of New Jersey at Rutgers U., USA) provided *in vivo* evidence of an essential role for autophagy in the malignant progression of oncogenic K-Ras-induced lung cancer. White’s group has developed a genetically engineered mouse model for non-small-cell lung cancer driven by oncogenic K-ras with and without tumour-specific *Atg7*-knockout. White revealed that K-Ras-driven tumorigenesis was suppressed by loss of autophagy. Interestingly, the ablation of autophagy in lung tumour cells harbouring activated K-Ras resulted in more benign tumours. This observation is in good agreement with reports that spontaneous benign tumorigenesis is observed in the livers of mice with systemic mosaic deletion of *Atg5*, or with *Atg7* disrupted specifically in hepatocytes. By contrast, Kevin M. Ryan (The Beatson Institute for Cancer Research, UK) and colleagues

demonstrated that loss of *Atg7* in pancreatic ductal adenocarcinoma affects metabolic reprogramming, which affects tumour development. Thus, autophagy might have different roles in tumour development depending on cellular context, cancer stage and genetic background.

In conclusion, the past decade has seen the elucidation of diverse aspects of the autophagic process through the dissection of core Atg products. This symposium marked a new phase in which old players and newcomers in the autophagy family are discovered to connect to diverse pathways and functions. Fifty years after the coining of the phrase ‘autophagy’ by Christian de Duve, we have come to understand the necessity and importance of comprehensive studies in the next step of autophagy-targeted therapy for neurodegeneration, infectious diseases and cancer.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Tsukada M, Ohsumi Y (1993) *FEBS Lett* **333**: 169–174
2. Tooze SA (2010) *Semin Cell Dev Biol* **21**: 677–682
3. Johansen T, Lamark T (2011) *Autophagy* **7**: 279–296
4. Youle RJ, Narendra DP (2011) *Nat Rev Mol Cell Biol* **12**: 9–14
5. Fujita N, Yoshimori T (2011) *Curr Opin Cell Biol* **23**: 492–497
6. Liang XH *et al* (1999) *Nature* **402**: 672–676
7. White E (2012) *Nat Rev Cancer* **12**: 401–410

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