Toxicology in Vitro 32 (2016) 166–180

Contents lists available at ScienceDirect

Toxicology in Vitro

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

Autophagic activity in BC3H1 cells exposed to yessotoxin

Mónica Suárez Korsnes [a,*], Hilde Kolstad [b], Charlotte Ramstad Kleiveland [a,e], Reinert Korsnes [c,d], Elin Ørmen [b]

[a] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU) — Campus Ås, P.O. Box 5003, NO-1432 Ås, Norway
[b] Imaging Centre, Norwegian University of Life Sciences (NMBU) — Campus Ås, P.O. Box 5003, NO-1432 Ås, Norway
[c] Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway
[d] Norwegian Defense Research Establishment (FFI), Kjeller, Norway
[e] Smerud Medical Research, Oslo, Norway

ARTICLE INFO

Article history:
Received 20 August 2015
Received in revised form 1 December 2015
Accepted 15 December 2015
Available online 30 December 2015

Keywords:
Autophagy
Yessotoxin
LC3-II
Atg proteins
Lamellar bodies
Autophagosomes
BC3H1 cells

ABSTRACT

The marine toxin yessotoxin (YTX) can induce programmed cell death through both caspase-dependent and -independent pathways in various cellular systems. It appears to stimulate different forms of cellular stress causing instability among cell death mechanisms and making them overlap and cross-talk. Autophagy is one of the key pathways that can be stimulated by multiple forms of cellular stress which may determine cell survival or death. The present work evaluates a plausible link between ribotoxic stress and autophagic activity in BC3H1 cells treated with YTX. Such treatment produces massive cytoplasmic compartments as well as double-membrane vesicles termed autophagosomes which are typically observed in cells undergoing autophagy. The observed autophagosomes contain a large amount of ribosomes associated with the endoplasmic reticulum (ER). Western blotting analysis of Atg proteins and detection of the autophagic markers LC3-II and SQSTM1/p62 by flow cytometry and immunofluorescence verified autophagic activity during YTX-treatment. The present work supports the idea that autophagic activity upon YTX exposure may represent a response to ribotoxic stress.

1. Introduction

Autophagy is a general term for cellular processes by which unnecessary or dysfunctional components including organelles are delivered to the lysosomes for degradation or for recycling (Levine and Kroemer, 2008; Mizushima et al., 2010). Normal autophagy is tightly regulated at a basal level to maintain cellular homeostasis whereas unregulated degradation of cytoplasmic contents can be lethal (Levine, 2005; Mizushima et al., 2008).

Autophagy has typically been considered in a context of cell survival (Levine and Kroemer, 2009). It may also play a role in programmed cell death. However, this role is hard to identify since autophagic activity in dying cells can be an attempt to survive (Tsujimoto and Shimizu, 2005; Kroemer et al., 2007).

The concept of autophagic cell death stems from observations of formation of autophagosomes and lysosomes in regions of cells where programmed cell death tend to take place (Tsujimoto and Shimizu, 2005). Autophagic cell death may also result from excessive levels of cellular self digestion (Levine, 2005).

It seems that some cytotoxic drugs can activate autophagic death in cells that are resistant to apoptosis such as those expressing BCl-2 anti-apoptotic proteins or those lacking Bax and Bak (Shimizu et al., 2004; Yu et al., 2004). Such cells can contain various autophagosomes, but their presence can be abolished by treatment of autophagy inhibitors or by silencing Atg5 and Atg6 proteins (Tsujimoto and Shimizu, 2005).

The role of autophagy as a programmed cell death mechanism is unclear. However, it can provide nutrients to the cells to survive or recycle components necessary for organ morphogenesis and tissue remodelling. The autophagic process might in this case have an advantage over apoptotic cell death reducing the workload of phagocytes (Tsujimoto and Shimizu, 2005; Kroemer et al., 2007). Elimination of cancer cells by activation of autophagic activity has been reported (Liang et al., 1999; Yue et al., 2003; Qu et al., 2003). Autophagy plays a role in cellular immune responses, especially when it precedes cell death (Ma et al., 2013). It can take part within ciliogenesis and control of cilia length to sense various extracellular changes (Orhon et al., 2014).

Mammalian autophagy involves many molecular components (Kroemer et al., 2010). Two of these components are ubiquitin-like proteins called Atg12 and Atg8/LC3 conjugation systems. They are essential for formation and maturation of autophagosomes and signalling to regulate autophagy (Yang and Klionsky, 2010a). The core pathway of mammalian autophagy begins with the formation of the isolation membrane (phagophore) which can be in close proximity to subcellular structures like mitochondria and endoplasmic reticulum (ER), where MAMS (mitochondria-associated ER membranes) facilitates the recruitment of the Atg5–Atg12–Atg16 complex indispensable for the autophagosome

* Corresponding author.
E-mail address: monica.suarez.korsnes@nmbu.no (M.S. Korsnes).

http://dx.doi.org/10.1016/j.tiv.2015.12.010

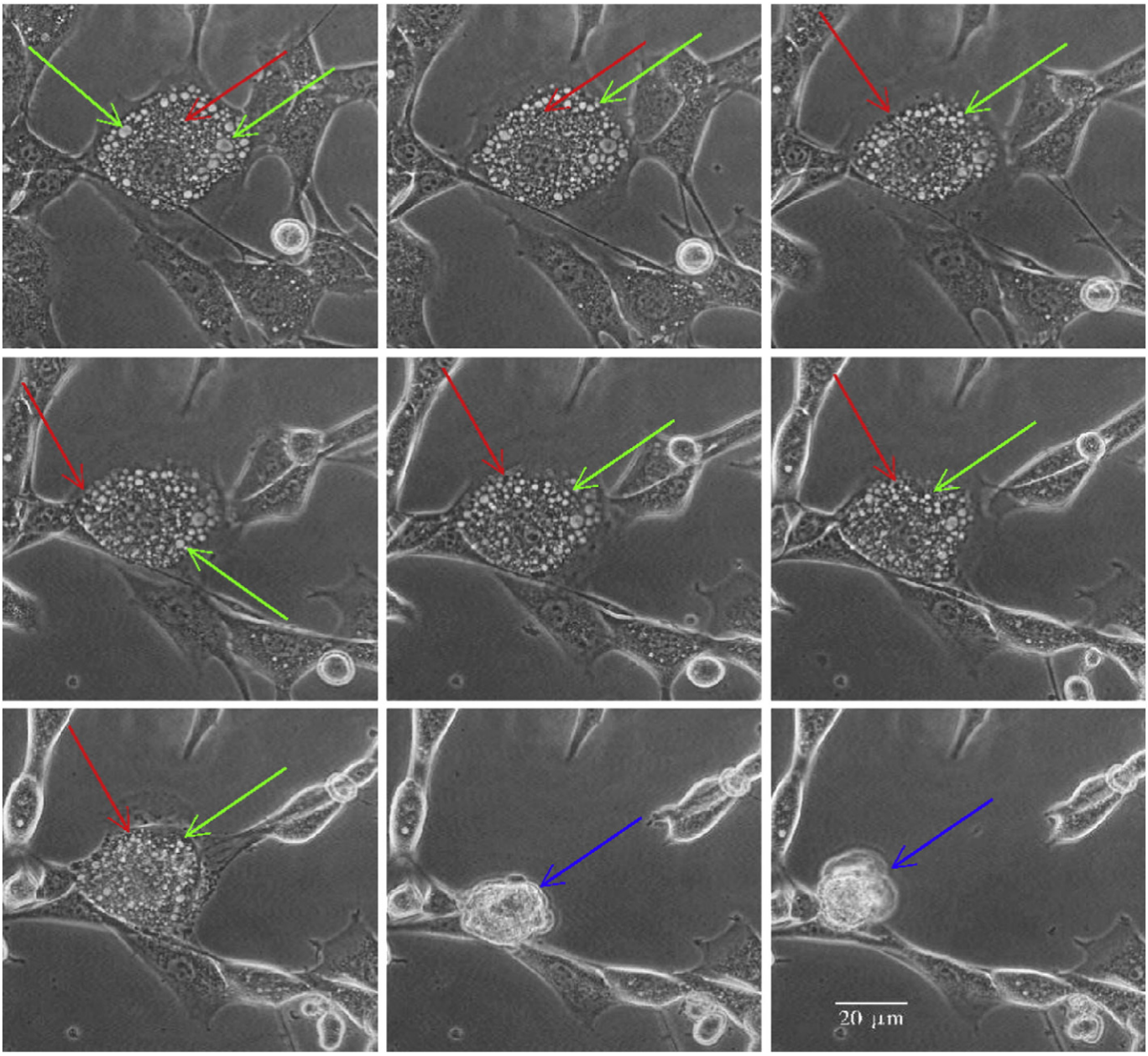

Fig. 1. Subsequent phase contrast images, 25 min apart, showing cytoplasmic vacuolisation in BC3H1 cells upon 100 nM YTX treatment. Green arrows indicate membrane-enclosed vacuoles, and red arrows indicate membranous dense bodies. Blue arrow marks sign of cell death.

formation (Ma et al., 2013). The proximity of the phagophore to the MAMS may facilitate a connection between autophagy and activation of stress response pathways (Hamasaki et al., 2013).

The marine toxin yessotoxin (YTX) is a polyether compound produced by the dinoflagellates *Protoceratium reticulatum* and *Gonyaulax grindleyi* (Satake et al., 1997; Satake et al., 1999; Draisci et al., 1999). The toxin was initially isolated from the digestive gland of scallops *Pactinopecten yessoensis* (Murata et al., 1987). It can trigger a broad spectrum of cellular effects of possible medical interest (Korsnes et al., 2006; López et al., 2008; L.M.B. López et al., 2011; Korsnes, 2012; A.M. López et al., 2011; Alonso et al., 2013; Korsnes et al., 2014). YTX mechanisms of action vary among cells and they appear to be cell-specific and concentration-dependent (De la Rosa et al., 2001; Alfonso et al., 2003; Franchini et al., 2004; Malagoli et al., 2006; Callegari and Rossini, 2008; Ronzitti and Rossini, 2008; Young et al., 2009; Orsi et al., 2010; Martn-López et al., 2012; Fernández-Araujo et al., 2015).

YTX can trigger diverse signalling pathways involving stress responses such as endoplasmic reticulum and ribotoxic stress (Rubiolo et al., 2014; Korsnes et al., 2014). Autophagy is one of the key pathways mediating diverse stress responses (Kroemer et al., 2010). Cellular stress response may determine whether cells can adapt their metabolism and protect themselves against damage. Autophagy constitutes therefore a protective mechanism in response to stress and eliminate damaged components through metabolism and recycling to maintain nutrient and cellular homeostasis (Kroemer et al., 2010). Autophagy may also target specific organelles such as ER, mitochondria, peroxisomes and ribosomes. Such types of autophagy are respectively referred to as reticulophagy, mitophagy, pexophagy and ribophagy (Ma et al.,