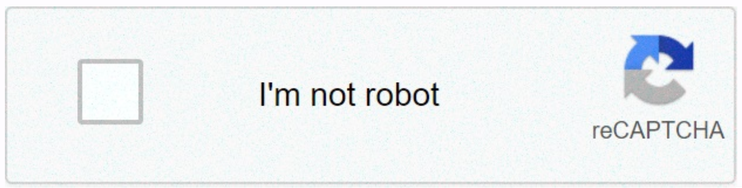

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Apoptosis ppt free

Apoptosis Dr. Shoaib RazaApoptosis - It's a pathway to cell death - Induced by a tightly regulated suicide program - activated enzyme that degrades nuclear DNA, nuclear and cytoplasmic proteins in cells - Formation of apoptotic bodies that contain nuclear materials and cytoplasmic and material organisms - No inflammatory response is obtained. Physiological examples of apoptosis - a normal phenomenon that seems to eliminate cells that are no longer needed - During embryogenesis, metamorphosis, Etc. - Involution of hormone-dependent tissues after hormonal withdrawal - Cell loss in proliferating cell populations - Elimination of potentially harmful self-reactive lymphocytes - Death of host cells that have submitted pathological examples of apoptosis for their useful purposes - Eliminating cells that are injured beyond repair without eliciting a reaction Host Damage - DNA Damage - Accumulation of Misplaced Proteins - Cell Death in Certain Infections (Virals) - Viral Factors (HIV) - Host Factors (HBV) - Pathological atrophy in parenchyrmal organs after obstruction of the ductsMorphology of apoptosis - Cell shrinkage - Smaller size, dense cytoplasm, tight organelles - Chromatin condensation - Peripheral chromatin aggregation, nucleus rupture - Formation of apoptotic bodies - Phagocytosis by macrophagesBiochemistry characteristic of Apoptosis - Activation of caspases Caspases are proteolytic enzymes found in an inactive form in the cytoplasm - cysteine proteases that crack aspartic acid residues (c asp ases) - Caspases are two types - Initiator - Initiate and active other caspases (8 8 esp 9,10) - Bourreau - Performs proteolysis and fragmentation of the nucleus (3.6)Biochemical characteristics of Apoptosis - DEGRADATION of DNA and proteins - Characteristic degradation of large-scale DNA (50,000 to 3,000,000 0) pair Basics - Subsequent DNA cleavage by fragmentation dependent on endonuclease ca or mg in 180-200 base pairs - Membrane alteration and phagocytosis - Membrane expresses more receptors for phagocytic cells - Phagocytosis is mainly by macrophages - No d Inflammatory response is obtainedCaspases Activation - Caspase activation can occur in two ways - Intrinsic way (mitochondrial) - Pro and antipoptotic factors are usually present in cells - An imbalance would lead to apoptosis - The bcl family of proteins are the main regulators - Bcl-2 , Bcl-x are the main antiapoptotic factors - Bax/Bak channels are major proapoptotic mechanisms - The release of Cytochrome-C and other proapoptotic proteins from cytoplasm mitochondria - Cytochrome-C and Apaf-1 (factor 1-activating apoptosis) activates the initiatorsLala - Is activated - Commitment of the plasma membrane death receptor - Fas binds with fasL - Fas associated death domain (FADD) active - Procaspase-8 is converted to caspase-8 - The initiating sequence begins Execution phase - Intrinsic or extrinsic pathway converging towards the activation of the execution route - Caspases important executioner are caspase-3 caspase-6 - They in turn activate Decomposing the Nuclear Matrix - Proteolysis of cytoplasmic proteins Apoptosisexample - Apoptosis after growth factor deprivation: Endometrium, lymphocytes, neurons, etc. - DNA-induced apoptosis: - Apoptosis induced by radiotherapeutic agents - Apoptosis by the TNF receptor family - Fas-FasL Link - Ntological Political Apoptosis: - Foreign Antigen by Perfor Loposis Regulated by Wool and Granzyme BDysregulated - Too Little or Too Much - Defective Apoptosis (Increased Survival) - Cancer (Carcinogenesis) - Autoimmune Disorders - Increased Apoptosis (Excessive Cell Death) - Neurogenerative Disorders - Infarction and Stroke - Death of the virus-infected cell 1 Apoptosis 2 mitochondries-caspase 9Classification of cell death Cell death Caspase-dependent caspase-independent receptor-caspase 8 mitochond caspase 9 3 4 5 Apoptosis Apoptosis is programmed cell death, which prevents damage to nearby cells by controlling how the affected cell dies during apoptosis , the cytoskeleton of the cell is decomposed, causing multiple bulges in the membrane. This is called bleeding. These blebs (aka apoptotic bodies) can separate from the cell, taking part of the cytoplasm with them. Phagocytic cells eventually consume these fragments. Therefore, apoptosis prevents damaged cellular content from spreading and damaging other cells. 6 Two Staurosporin-treated lymphocytes HeLa Healthy Cells Apoptosis Apoptosis Apoptosis Weinberg Fig Different parts of the apoptotic program. Markers for the process. Dna Fragmentation Apoptotic Cell Pyknotic Nuclear Fragments Phagocytosis of Apoptotic Bodies Fragmentation of The Body Golgi Phosphorylation of Histone 2B 7 APOPTOSIS SIGNALS Mitochond artia-dependent apoptosis Death Receptor-Caspase- depending apoptosis Caspase-dependent apoptosis Caspase- independent apoptosis Caspase- necrosis independent Nature Reviews Cancer 2; (2002) 8 Physiological relevance of the regulation of embryonic development of apoptosis,Mbryonic of/by the immune system Negative selection CTL Killing (for example. Immune surveillance, viral infections) Stop the active regulation of the immune response tight regulation of the number of cells (for example. BM, GI, Uterus, Skin) Compensatory response to the intrinsic path of cellular stress (e.g., GF removal, XRT, chemo)) Extrinsic pathway (e.g. FAS. Ag, Activation TNF-R) Senescence (Aging) 9 Necrosis Necrosis is a traumatic cell death caused by injuries. Necrosis of the cells lyses and damages nearby cells by reversing all intracellular content. This causes inflammation of nearby tissues and other traumas. 10 Morphological aspect of necrosisIncreased eosinophilia: RNA in cytoplasm Increased linkage of eosine to denatured cytoplasmic protein More vitreous homogeneous appearance Vacuolated glycogen particle loss and calcification of moth-eaten cytoplasm of necrotic cells 83 11 major damage sites in cell lesion 12 Necrosis vs. Necrosis. Apoptosis Apoptosis - programmed cell deathNecrosis - unscheduled cell death 13 necrotic cells with multiple lesions cell with necrotic cells bleating Necrotic cell with multiple lesions Apoptotic cell with bleeding 14 Death by apoptosis is an orderly and orderly process of apoptosis and necrosis Apoptosis Apoptosis autophagy death is an orderly and orderly process 15 The roles of autophagy and necrosis induced by therapeutic therapy in the treatment of cancerInhibiting autophagy for cancer therapy. A, surgery, chemotherapy, targeted therapies and radiotherapy can all activate autophagy. The treatment of cancer cells with chloroquine derivatives (CQ) leads to the deacidification of lysosomes followed by an accumulation of ineffective autophagic vesicles. In cells dependent on autophagy to survive, chloroquine inhibition of autophagy leads to cell death. B and C, electronic micrographs of untreated (B) or chloroquine-treated (C) prostate cancer cells. Bar, 2 m. 16 The roles of autophagy and necrosis induced by therapy in the treatment of cancerRegulation of autophagy and necrosis. A, autophagy. Multiple stresses, including discontinued growth factor signaling, activate autophagy. Fluctuations in small molecules, such as the increase in ROS, may be the link between signal transduction events and enzymatic activation of autophagy genes. Once initiated, autophagic vesicles form around damaged mitochondria and proteins. This double membrane structure merges with the lysosome causing the content to deteriorate. PI3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin. B, necrosis. Necrosis initiators include NAD-depleting PARP and death receptor adapters RIPK1 and TRAF2. Ripk1 activated is relocated to mitochondria, disrupting the combination of cyclophiline D (Cyc. D) and nucleotide adenosine translocator (ANT). Subsequent exhaustion of NAD and ATP and calcium and ROS accumulation lead to activation of calpain proteases and lipid peroxidation followed by widespread membrane disturbance. Clin Cancer Res 2007;13: The Roles of Therapy-Induced Autophagy and Necrosis in Cancer Treatment 17 Mechanistic Interplay Between Apoptosis and AutophagyResearch in the Lane Lab focuses on the mechanisms of cell death, with a particular interest in the molecular relationship between apoptosis and autophagy. Autophagy is activated during many forms of cellular stress, and is regulated during terminal differentiation. It is generally considered a protective mechanism in cells experiencing starvation and oxidative stress, but has been proposed to move to a full-fledged cell death mechanism when other pathways of death (such as are avoided. The evidence suggests that apoptosis and autophagy are linked by common regulatory factors (see below), in order to identify and characterize the molecules and pathways that couple apoptosis and autophagy can lead to new therapeutic mechanisms for cancer and neurodegeneration - important diseases that present these two cellular processes. Apoptosis Autophagy - Molecules identified todate that play a dual role in apoptosis and autophagy autophagy Beclin-1 (a Bcl-2/Bcl-XL interactor that forms the nucleus of a multi-component, type III PI3-kinase involved in the supply of autophagosome membrane) and Atg5 (a factor required for autophagosmal membrane biogenesis and atg8 recruitment that is split by calpains becoming highly cytotoxic). We reported that Atg4D (a member of the atg4 endopeptidase family needed for Atg8 treatment) is cracked with caspases, on which it gains atg8 treatment activity, but acquires inducing apoptosis abilities. - Autophagy (macroautophagy) is characterized by the formation of double membrane organelles that sequester cytoplasm regions, including protein aggregates and poorly unfolded organelles. The autophagosome membrane is decorated with a protein known as Atg8 (mammalian orthologists include LC3, Gate-16 and GABARAP), allowing the quantitulation of upregulation of Atg8 puncta fluorescence microscopy autoscopy combined with automated microscopy. Em is also commonly used to identify/quantify autophagosomes. Light microscopy - dynamic evaluation electron microscopy - high-resolution evaluation 18 Todate-identified molecules that play a dual role in apoptosis and autophagy include Beclin-1 (a Bcl-2/Bcl-XL interactor that forms the nucleus of a multi-component, pi3-kinase type III involved in the supply of autophagosome membrane) and Atg5 (a factor required for autophagosomal membrane biogenesis and Atg8 recruitment which is split by calpains becoming highly cytotoxic). We reported that Atg4D (a member of the atg4 endopeptidase family needed for Atg8 treatment) is cracked with caspases, on which it gains atg8 treatment activity, but acquires inducing apoptosis abilities. 19 Autophagy and its inhibitors From the following article: Self-feeding and self-slaughter: cross between autophagy and apoptosis M. Chiara Maiuri, Einat Zalckvar, Adi Kimchi and; Guido Kroemer Nature Reviews Molecular Cell Biology 8, (September 2007) Autophagy begins with the gradual engulfment of cytoplasmic materials (cytosol and/or organelles) by the phagophore (also known as the isolation membrane), which sequesters materials in double membrane vesicles called autophagosomes (also known as autophagic vacuoles). In many cellular settings, the first regulatory process (see figure, step 1) involves depressanting the mTOR Ser/Thr kinase, which inhibits autophagy by phosphoryting the protein autophagy-13 (Atg13). This phosphorylation leads to the dissociation of Atg13 from a protein complex that contains atg1 kinase and Atg17, and thus mitigates atg1 kinase activity. When mTOR is inhibited, the re-association of Atg13 dephosphorylateity with Atg1 stimulates its catalytic activity and induces autophagy. Particular atg13 yeast mammal has not yet been identified. Among the first steps of vesicle nucleation is the activation of mammalian Vps34, a class III 3-kinase phosphatidylinositol (PI3K), to generate phosphatidylinositol-3-phosphate (PtdIns3P) (stage 2). Activation Vps34 Vps34 on the formation of a multiprotein complex involving beclin-1 (Becn1; the mammalian orthologist at Atg6), UVRAG (tumor suppressor gene associated with RESISTANCE to UV irradiation) and a myristylated kinase (Vps15, or p150 in humans). Two ubiquitin conjugation systems are part of the vesicle elongation process (stage 3). One pathway involves the covalent conjugation from Atg12 to Atg5, with the help of the enzyme E1-like Atg7 and the enzyme E2-like Atg10. The second pathway involves the conjugation of phosphatidylethanolamine (PE) with LC3/Atg8 (LC3 is one of Atg8's mammalian counterparts) by the sequential action of the Atg4 protease, the enzyme E1-like Atg7 and the enzyme E2-like Atg3. Lipid conjugation leads to the conversion of the soluble form of LC3 (named LC3-I) into an autophagic-vesicle-associated form (LC3-II). LC3-II is used as a marker of autophagy because its lipidization and recruitment specific to autophagosomes provides a transition from diffuse

coloration to punctate of the protein and increases its electrophoretic mobility on gels compared to LC3-I. In addition, green fluorescent proteins-LC3 fusion proteins can be used to visualize autophagosomes by fluorescence videomicroscopy91. The recovery mechanism involving the Atg9 complex is poorly studied (step 4). Autophagosomes mature by fusion with lysosomes to create autolysosomes (stages 5 and 6). In autolysosomes, the internal membrane as well as the luminal content of autophagic vacuoles are degraded by lysosomal enzymes that act optimally in this acidic compartment4, 92. Pharmacological inhibitors and small interfering RNAs capable of inhibiting distinct stages of this process are shown (red blocking arrows). Bcl2 and Bcl-XL are beclin-1 regulators. Lamp2, lysosome-associated membrane glycoprotein-2. 20 Casepase-dependent cell death pathways and caspase From the following article: Self-feeding and self-deprecation: cross between autophagy and apoptosis M. Chiara Maiuri, Einat Zalckvar, Adi Kimchi and Guido Kroemer Nature Reviews Molecular Cell Biology 8, (September 2007) Two main pathways lead to caspase-dependent apoptosis (see figure). The extrinsic pathway involves stimulation of members of the tumor necrosis factor receptor (TNFR) superfamily, such as CD95/Fas, TNFR or TRAILR (death receptors). The intrinsic pathway is characterized by mitochondrial external membrane permeabilisation (MOMP) and release of mitochondrial cytochrome c, which results in the assembly of a caspase-activating complex between caspase-9 and APAF1 (apoptosome). The stimulation of death receptors usually results in the recruitment and activation of caspase-8 by Fas-associated by the domain of death (FADD)/Death domain protein associated with TNFR1 to form a death-inducing signaling complex (DISC) that can further propagate death signals in three ways: BCL2-3 (BH3) homology proteolysis - the only BID protein that causes truncated truncated translocation mitochondria and, therefore, MOMP; direct proteolytic activation of downstream effector caspases; or via the activation of the RIP93 kinase. In the intrinsic pathway, only a range of BH3 proteins act as sentinels for organ cell stress, organelles-specific damage or infection, and can be mobilized by post-translational modification (such as proteolysis or phosphorylation) or subcellular relocation to initiate MOMP. BH3-only proteins stimulate MOMP by triggering BAX and/or bak oligomerization in the outer mitochondrial membrane, therefore forming channels that allow the escape of multiple proteins from mitochondrial intermembrane space26, 56, 57. In the context of DNA damage, stabilization of the p53 tumor suppressor protein may result in transcriptional activation of BH3 proteins only (such as PUMA and NOXA) that can promote MOMP through the BAX/BAK52 channel. Alternatively, DNA damage may activate caspase-2 in a multinuclear complex that involves the p53-induced protein with a death domain (PIDD) and the RIP-associated protein in a death domain (RAIDD) (together known as piddosome)94. Caspase-2 can then induce MOMP and/or direct caspase activation. Several factors among mitochondrial proteins that are released as a result of MOMP (apoptosis-inducing factor (AIF), Omi, EndoG) may promote caspase-independent cell death95, which may also result from stimuli that cause lysosmal membrane permeabilisation (LMP), resulting in the release of cathepsin proteases in cytosol9. These cathepsins can also trigger MOMP, thus stimulating the mitochondrial pathway of apoptosis. JNK, c-Jun N-terminal kinase; LMP, lysosomal membrane permeabilisation; ROS, reactive oxygen species. 21 22 A protein, multiple functions: against molecular reductionism From the following article: Self-feeding and self-slaughter: cross between autophagy and apoptosis M. Chiara Maiuri, Einat Zalckvar, Adi Kimchi and Guido Kroemer Nature Reviews Molecular Cell Biology 8, Many researchers on cell death have assumed that the only function of a whole range of proteins — including BCL2 proteins, caspases and their activators — was the regulation of apoptosis. However, recent work has shown that caspases regulate normal activation and differentiation processes96, 97 and that BCL2 proteins can regulate neuronal plasticity98. Even at Caenorhabditis elegans, it has been found that the only homology BCL2-3 (BH3) protein only EGL-1, which is necessary for developmental cell death, is also necessary for non-lethal autophagy induced by famine58. Similarly, an essential component of the caspase activation complex, ced-4 APAF1 nematode), has a non-apoptotic role in the DNA damage control point99. Some of the Atg proteins, which are necessary for autophagy, may also have unsuspected roles outside the autophagic process. An important example is ATG5, which, under certain circumstances, is proteolyticly activated to become a pro-apoptotic pro-apoptotic molecule transposes into the mitochondria and triggers mitochondrial external membrane permeabilisation (MOMP)51. Another example is beclin-1, which carries a BH358 domain, 62 that could promote apoptosis, at least in certain circumstances. Therefore, it may be an oversimplification to assume that the inhibition of cell death in response to DNA damage or the activation of apoptosis/necrosis during nutrient deprivation (both of which result from kosh or reversal of a single Atg gene) is due to suppression of the autophagic process. Several genes from different autophagy modules should be reversed independently to determine whether the resulting phenotype is similar before the final conclusions are drawn. The same is true for experiments in which apoptotic pathways have been interrupted by knocking out or overthrowing individual genes. 23 A model for the roles of apoptosis and autophagy in tumorigenesis. A common cellular response to metabolic stress is cell death mediated by apoptosis, which limits tumor growth. Tumors can trigger autophagy-based cell survival in certain metabolically stressed tumor regions. In apoptotic-defective and metabolically stressed tumor cells, the activation of autophagy prevents the death of necrosis, while defects of autophagy lead to the accumulation of p62, damaged mitochondria, ROS and protein aggregates, resulting in genome damage and tumorigenesis. For more information, see Refs 68 and 119. 24 The relationship between apoptosis and autophagy Self-feeding and self-slaughter: a cross between autophagy and apoptosis M. Chiara Maiuri, Einat Zalckvar, Adi Kimchi and Guido Kroemer Nature Reviews Molecular Cell Biology 8, (September 2007) Similar stressors can induce either apoptosis or autophagy in a context-dependent manner. It is possible that different thresholds of sensitivity, the exact nature of which have yet to be determined, may dictate whether autophagy or apoptosis will develop. Alternatively, the choice between apoptosis and autophagy is influenced by the fact that both catabolic processes exhibit a certain degree of mutual inhibition. In some cases, a mixed phenotype of apoptosis and autophagy can be detected at the level of a single cell. Although autophagy mainly allows cells to adapt to stress, massive autophagy can also kill cells. 25 Regulation of autophagy and apoptosis by p53 and p19ARF/p14ARFThe oncogene stress can activate two different murin isoforms p19ARF (and human orthologist p14ARF). One isoform corresponds to the full length nucleolar p19ARF/p14ARF and the second corresponds to a shorter shape, smARF, which results from translation. While smARF translocates into mitochondria, reduces the mitochondrial potential of transmembrane (Dm) and stimulates autophagy and caspase-independent cell death, the p19ARF/p14ARF active p53 nucleolar by binding and inhibiting the negative p53 regulator, MDM2. p53 induces caspase-dependent apoptotic cell death by induction of multiple pro-apoptotic, pro-apoptotic proteins, function in the intrinsic way. While the nucleolar pathway is inhibited by Z-VAD, smARF-induced cell death is blocked by reversing beclin-1 (Becn1) or Atg5. In some cellular settings, activated p53 induces a transcriptional program that promotes the accumulation of autophagic vacuoles by induction of the lysososol protein DRAM (damaged-regulated autophagy modulator). P53-obtained DRAM may be essential, both for autophagic vacuolization and for apoptosis. 26 BH3 proteins and mimics act on the beclin-1-BCL2Protein interaction that contains BCL2 homology-3 (BH3) domains or small molecules that mimic BH3 domains may bind to the BH3 receptor domain of BCL2 or BCL-XL and, therefore, competitively disrupt the interaction between BCL2 or BCL XL-and beclin-1. This probably leads to the activation of lipid kinase activity of the 3-kinase 3-kinase Class III tumor suppressor gene (which depends on interaction with UVRAG (tumor suppressor gene resistant to UV irradiation), thus causing the production of phosphatidylinositol-3-phosphate (PtdIns3P). In turn, this leads to vesicle nucleation in a way that probably involves WIPI-1a (WD-repetition protein interacting with phosphoinositides). 27 Molecular Switches Between Apoptosis and Autophagya Double function of protein-5 autophagy (ATG5) in autophagy. The integral ATG5 can participate in the early stages of autophagy using a ubiquitin conjugation system. ATG12 (activated by ATG7 and then transferred to ATG10) is combined with ATG5 to a lysine--amino group, allowing it to link to ATG16. The resulting ATG12-ATG5-ATG16 complex imposes curvature on the phagophore crescent and recruits the LC3 activated to the elongated membrane (LC3 is one of the mammalian counterparts of Atg8, which is conjugated with phosphatidyletamine by the action of ATG4, ATG7 and ATG3) (Box 1). The proteolysis by a calpaine causes a truncated ATG5, which can be translocated into mitochondria and induce momp. b - France BCL2 homology-3 (BH3)-only proteins as inducers of apoptosis and autophagy. Depending on their specificity (for pro- or anti-apoptotic and autophagy-inhibitory proteins that contain BH3 receptors), as well as their preferential subcellular location (mitochondria or endoplasmic reticulum), BH3 proteins only can preferentially activate apoptosis or autophagy. A specific class of bh3-only inactivator proteins interacts with anti-apoptotic and anti-autophagic multidomain proteins of the BCL2 family (red blocking arrows symbolize preferential affinities), thus releasing BH3 activating proteins only that activate allosterically BAX and BAK (for the induction of apoptosis). Alternatively, they release beclin-1 from its inhibitory interaction with multidomain proteins BCL2 family, allowing it to activate the lipid kinase activity of VPS34 and, therefore, activate autophagy. Several stages of this working model still need to be validated experimentally. 28 Scenarios for the between apoptosis and autophagya Schematically, a particular set of insults induces apoptosis (part 1), which, if inhibited, can pass to autophagy. At least in some cellular settings, autophagy serves as a defense mechanism that prevents or delays necrosis (parts 2.3). b - France Some conditions can trigger a fatal autophagic response responsible for cell death, for example, in naïve cells (parts 1.2) or in cells in which the apoptotic pathways have been interrupted. c - France Another set of stimuli (or perhaps just a lower dose of insults) causes a protective autophagic response (part 1), which is necessary for cell adaptation and avoidance of apoptosis (part 2). d - France Frequently, fatal conditions trigger an autophagic response that, regardless of autophagic response, is followed by apoptosis (part 1). In this case, inhibition of apoptosis causes either cell survival (part 2) or necrosis (part 3). In this scenario, the order of events (autophagy, then apoptosis) is chronological, not hierarchical, which means that the inhibition of autophagy does not prevent apoptosis. e - France Autophagy may be essential to maintain the high levels of ATP that are necessary for cells to emit signals to phagocytic cells that engulf apoptotic bodies (part 1). The inhibition of autophagy does not affect apoptotic cell death, but it does abolish heterophagic ablation of apoptotic material (part 2). LPC, lysophosphatidylcholine; PS, phosphatidylserine. 29 30 Eucaryotes have two major systems of protein degradation in cellsUkaryotes have two major systems of protein degradation in cells. One is the ubiquitin-proteasome system, which explains the selective degradation of most short-lived proteins. The other is the lysosomal system. Autophagy (macroautophagy) is the main means of degradation of cytoplasmic constituents in the lysosome. Intracellular Protein Degradation Systems (High) Macroautophagy. A small volume of cytoplasm is enclosed by the autophagic isolation membrane, which eventually results in the formation of an autophagosome. The outer membrane of the autophagosome then merges with the lysosome where the cytoplasm-derived materials are degraded. (Inferior) The ubiquitin-proteasome pathway. Polyubiquitin proteins are degraded by proteasome 26S. This is a selective process. 31 Cellular Events in AutophagyThe cellular events during the digestion of components or intracellular pathogens follow three distinct stages: initiation (phagophore formation), lengthening (growth and closure) and maturation of a double membrane autophagosome in autolysosome. a - | Autophagy sequesters and removes cellular constituents from the cytosol, including excess or damaged cytosol organelles. b - France Autophagy can eliminate bacteria (free in the cytosol or inside a phagosome), viruses and protozoan parasites in a manner similar to the elimination of self-constituents. 32 33 The cellular process of autophagy. Conditions such as starvation, pathogenic infection and other environmental stressors can induce autophagy. Autophagy begins with the isolation of double membrane structures within an intact cell. Previously, these structures were believed to have originated from the ribosome-free region of the rough endoplasmic reticulum82, 83, but recent studies indicate that they may have come from a pre-existing membrane structure called phagophore84, or could be formed from novo80, 85. These membrane structures lengthen and ripen, and the protein 1 light chain 3 (LC3) associated with microtubules is recruited into the membrane. The elongated double membranes form autophagosomes, which sequester cytoplasmic proteins and organelles such as mitochondria. The formation of the pre-autophagosomal structure may be inhibited by the phosphatidylinositol (PI3K) 3-methyladenine (3-MA) 3-phosphate kinase inhibitor. Sequestration requires ATP and is regulated primarily by Class III PI3K46. Autophagosomes mature with acidification by H-ATPase71 and fuse with lysosomes to become autolysosomes (also known as degrading autophagic vacuoles). Microtubules are important mediators of this fusion process. This process is inhibited by the inhibitor H-ATPase bafilomycin A1, or by microtubule inhibitors such as vinblastine and nocodazole86, 87. Finally, the sequestered contents are degraded by lysosomal hydrolases for recycling. A test for autophagic cells is to detect the presence of membrane bound LC3, which accumulates on autophagosomes. 34 Autophagy, which means eating oneself, is one of the main mechanisms for maintaining cellular homeostasis. This pathway is not directly a path of death, rather a path of self-cannibalization. Mediated by lysosomal degradation, autophagy is responsible for the degradation of cellular proteins and is currently the only process known to degrade cell organelles, recycling them to ensure their cellular survival. Autophagy research has been underway for more than 40 years, but has been limited by the lack of knowledge about the molecular mechanisms behind this process. Over the past decade, enormous progress has been made and genetic yeast testing (s. cerevisiae) has identified more than 30 autophagy-related genes (ATG genes), many of which have identified mammalian counterparts (see table below) (1,2,3,4). Autophagy is essential to help maintain the balance between increasing and decreasing the number of a cell population. It is undoubtedly active at the basal level in most cells and contributes to the routine rolling of cytoplasmic components (1, 21). 35 Table of genes associated with identified (ATG genes) 36 37 TABLE 1 Autophagy genes in mammalsProtein function ATG1, ULK1 Atg1 is a serine/threonine protein kinase; it may be involved in the regulation and formation of VEsicles ATG3 Atg3 functions as a ubiquitin-conjugating-like enzyme that covalently attaches Atg/LC3 to phosphatidyletamine ATG4 Atg4 is a split cysteine protease that splits Atg8/LC3 C-terminus to expose a glycine residue for later ATG5 Atg5 conjugation is covalently attached to Atg12, and binds Atg16 as part of a tetrameric complex of unknown function ATG6, Beclin 1-Atg6 is a component of the phosphotididylinositol-3-kinase-class III complex that is required for the autophagia ATG7 Atg7 is a counterpart of the ubiquitin-activator enzyme; it activates both Atg8/LC3 and Atg12 before the ATG8 conjugation, MAP1LC3 Atg8/LC3 has a structural similarity to ubiquitin; it is combined with phosphatidyletamine, and is part of the autophagosome, but its function is not known ATG9 Atg9 is a transmembrane protein that may be involved in the delivery of the membrane to the formation of autophagosome ATG10 Atg10 functions as a ubiquitin-conjugated enzyme like that attaches covalently Atg12 to Atg5 ATG12 has some structural similarity to the ubiquitin; it is combined with an internal Atg5 lysine by its ATG16 Atg16 Atg16 glycine c-terminal binds Atg5 and homo-oligomeric to form a tetrameric complex - In this table, we have listed only certain key genes in which the genetic product has been confirmed to play a role in autophagy in upper eukaryotes. Only the name and number of the autophagy-related gene (ATG) are listed, except when the name is not used in the upper eukaryotes. However, many autophagy genes are present as multiple isoforms, usually indicated as a, b and so on. 38 TABLE 2 Petites molécules connues qui influencent l'autophagieCompound Effet cible Références Rapamycine Cible de rapamycine Induit des niveaux d'autophagie 33,34 lithium, valproate de sodium, enzymes carbamézapine qui affectent finalement les niveaux de myo-inositol-1,4,5-triphosphate, tels que l'inositol monophosphatase Abaisse le myo-inositol-1,4,5-triphosphate niveaux, tels que les niveaux d'inositol monophosphatase Abaisse myo-inositol-1,4,5-triphosphate niveaux, tels que les niveaux d'inositol monophosphatase Abaisse myo-inositol-1,4,5-triphosphate niveaux, tels que les niveaux d'inositol monophosphatase Abaisse myo-inositol-1,4,5-triphosphate niveaux, tels que les niveaux d'inositol monophosphatase Abaisse myo-inositol-1,4,5-triphosphate niveaux, tels que les niveaux d'inositol monophosphatase Abaisse myo-inosi induit l'autophagie 41 3-méthyladène Classe III phosphotidylinositol-3-kinase Inhibe l'autophagie 92 Wortmannin 93 Bafilomycine A1 Vacuolar-ATPase 81,82 Chloroquine 1 PHlysosomal 71 Hydroxychloroquine 83 Tamoxifen Beclin 1 Augmente Beclin 1 , induces autophagy 70 39 Figure 1: Steps in Autophagy and Associated GenesFigure 1: Steps in Autophagy and Associated Genes. The three stages of autophagy accompanied by genes associated with each step are shown. Genes in green have already been evaluated by Ray Choi, the genes listed in red will be the target of evaluation in this article and the genes listed in black remain to be evaluated in the future. 40 The term autophagy covers three processes; chaperone-based microautophagy, macroautophagy and autophagy (1): Microautophagy is the transfer of cytosolic components into the lysosome by direct invagination of the lysosomal membrane and subsequent budding of the vesicles in the lysosomal lumen (17). Macroautophagy involves the formation of a double membrane structure called autophagosome that sequesters cytosolic material and delivers it to lysosome for degradation (18). Although this degradation may be selective (i.e. the specific elimination of damaged mitochondria sparing damaged mitochondria sparing normal mitochondria) (19), (19), (19), soluble cytosolic proteins is not selective. Chaperone-based autophagy (CMA) is characterized by its selectivity to degraded specific substrates (cytosolic proteins). 41 Autophagy - lysosomal system 42 In general terms, autophagy refers to any intracellular process that involves the degradation of cytosodic components by the lysosome. There are at least three distinct autophagic pathways: macroautophagy, microautophagy and chaperone autophagy. The terms autophagy and macroautophagy are used interchangeably. Autophagy is involved in physiological and pathological processes Autophagy is a pathway of lysosomal degradation essential for survival, differentiation, development and homeostasis. In some circumstances, it is considered a non-apoptotic cell death pathway. Autophagy is involved in various pathologies, including infections, cancer, neurodegeneration and aging as well as heart, liver and kidney diseases. The morphological mark of Macroautophagy autophagy is a multi-step process by which parts of cytoplasm, damaged proteins and/or organelles are sequestered in a double or multimembrane structure (autophagosome). When the autophagosome merges with the lysosome (autophagolysome), the sequestered materials are digested by lysosomal hydrolases. Recycling these intracellular constituents serves as an alternative energy source during periods of metabolic stress to maintain homeostasis and viability. 43 Steps in Chaperone-based Macroautophagy and Autophagia (CMA)Steps in Chaperone-based Macroautophagy and Autophagy (CMA). Macroautophagy: 1.) Nucleation. An unidentified membrane source provides lipid bi-layers for phagophore formation. In yeast, this early structure is called pre-autophagosomal structure (PAS), its identity in mammalian cells is uncertain. A Class III PI3K complex consisting of at least BECN1, PIK3C3, PIK3R4, UVRAG and AMBRA1 is required for pasS formation and MAP1LC3 is anchored to the membrane via a phosphoethanolamine (PE) (LC3-II) anchor. 2.) Expansion. The PAS or comparable structure in mammals sequesters cytostolic cargo (either specifically via SQSTM1 [p62] or not specifically) by invagination, forming a double-membranous vesicle. This step is also called an isolation membrane. More membrane and LC3-II is recruited for the vacuole in development. 3.) Maturation. The completed autophagosome undergoes multiple stages of maturation and fusion events with multivehocular bodies (MVB) or endosomes. The exact nature and sequence of this maturation, and whether these steps are still necessary is currently unknown. The lumen becomes more acidified as it matures. 4.) Mooring and merging. During mooring and fusion, the inner membrane compartment and its contents are released into the lysosome/autolysosome and degraded by lysosomal hydrolases. The components of the outer membrane are available for reuse. Reuse. autophagy: 5.) Recognition and fixation. The HSC70 chaperone complex (composed of HSC70, HSP90 and possibly other proteins) recognizes the proteins unfolded with the KFERQ sequence and moves them to the lysosome. 6.) Translocation. LAMP2A and a lysosomal form of HSC70 (l-HSC70) translocate the substrate protein through the lysosomal membrane into the lumen for degradation. The substrates delivered to autophagy degrade inside the lysosomes and their macromolecular components are made available to the cell metabolism by permeases that allow their transport to the cytosol. Jaeger and Wyss-Coray Molecular Neurodegeneration:16 doi: / 44 Macroautophagy Macroautophagy is an almost universal process that eukaryotic cells use to reuse the constituents of cytoplasm and organelles. We used the genetics of Dictyostelium to isolate mutants in genes essential to macroautophagy. These mutants taught us how to design a screen and selection for other genes that regulate macroautophagy and also provide material to study the formation of autophagosome. A collection of mutants has been used to show that macroautophagy, previously considered essential for the replication of the intracellular pathogen Legionella pneumophila, is not necessary for the growth of this parasite. We now wonder how autophagy is linked to development and whether there are other genes essential to autophagy. Macroautophagy: Macroautophagy as a cellular biological and structural phenomenon has been known for many years to manage the bulk degradation of cytoplasm and organelles. One of its interesting features is that it promotes the transfer of material from one topologically distinct compartment to another - from cytosol to vacuole in baker□ yeast or lysosomes in other eukaryotic cells. An initiation structure called pre-autophagosomal structure or PAS has been defined, but its origins, once considered to be derived from the endoplasmic reticulum, are unclear. The favorable vacuolar structure of Saccharomyces cerevisiae was married to the genetics of this organism to understand the biochemical reactions involved in the production of autophagosomes (for reviews see (Abeliovich and Klionsky, 2001; Klionsky and Emr, 2000; Thumm, 2000). S. cerevisiae contains a single large central vacuole, which serves as a lysosome, among other functions. When autophagy is induced, double membrane autophagosomes are produced, and these merge with the vacuole and release single membrane autophagic bodies that are degraded by resident hydrolases and proteases. When vacuolar proteases are inhibited by PMSF, there is an accumulation of small spheres (autophagic bodies) in a larger vacuole. In several screening methods to create mutants in which a build-up of small autophagic bodies is not observed, several laboratories, including those of Ohsumi, Thumm and Klionsky, have isolated autophagic mutants (called atg). The affected genes have been used to a series of phosphorylation and ubiquitination reactions that are necessary for the vesicle transport cytoplasm (CVT) and macroautophagy. CVT summarizes specific targets during growth, while autophagy envelops the bulk cytoplasm and organelles during starvation. Many of the molecules that mediate autophagy reside in the pre-autophagosomal structure (PAS, also known as the perivacuolar compartment (PVC), which, in the nascent yeast, is found next to the vacuole (Suzuki et al., 2001) (Kim et al., 2002) (Noda et al., 2002). Gradient and immunosuper purification studies suggest that PAS does not contain markers from other cell compartments (Kim et al., 2002). The creation of an autophagosome is thought to occur by extension of an enveloping membrane, called an isolation membrane, which then closes around cytoplasmic constituents, as shown in Figure 1 (Kim et al., 2002). A similar situation can occur in mammalian cells (Mizushima et al., 2001). Autophagosomes then merge with late endosomes or lysosomes to create autophagolysomes and provide constituents and energy for the cell. Although there has been progress in understanding this process, particularly in determining the genes involved and mapping known genetic products on physical structures, a number of questions remain. What is the origin of the PAS and what it is composed, beyond the known molecules? Does it assemble de novo or does it derive from pre-existing membranes? How does the PAS transform into an isolation membrane and how does this membrane seal around its cargo? What is the source of the lipid that forms the membrane? How is size determined? Is there selectivity of the cargo? What molecules mediate fusion with the endosomal system? 45 Schematic overview of macroautophagySchematic overview of macroautophagy. Potential therapeutic applications of autophagy David C. Rubinsztein, Jason E. Gestwicki, Leon O. Murphy and Daniel J. Klionsky Nature Reviews Drug Discovery 6, (April 2007) A membrane of unknown origin forms the initial phagophore membrane or isolated membrane. The phagophore expands, sequestering the cytoplasm and at completion forms a double membrane autophagosome. Autophagosome fuses with a lysosome, containing acidic hydrolases (AH), which can now access the internal vesicle, called an autophagic body. The merged compartment where the autophagic body and its contents are degraded is called autophagolysome or autolysosome. After degradation, the resulting macromolecules are released into the cytosol by permeals for reuse in metabolic processes (during starvation). Alternatively, the cargo could be inactivated or killed macroautophagy acts as part of the immune response to eliminate microbial pathogens), or the elimination of cytoplasm could result in cell death. See the main text for more details. 46 This is a conceptually simple, intellectually attractive and experimentally testable hypothesis. Since joining the Faculty of Pharmacology at RWJMS in 2003, 2003, laboratory has conducted extensive research around this fundamental theme. We are working in four interdependent research areas and have made significant progress. First, we are looking for experimental evidence that a defect in autophagy compromises mitochondrial functions, a leading cause of aging. Second, we determine the direct cause of cancer development due to the reduction of autophagy. Third, we are trying to understand the regulation of autophagy. Fourth, we explore the potential to target autophagy as a therapeutic adjuvant for cancer therapy. In addition to the Arnold Levine laboratory, where I received postdoctoral training, we have made several umdnj laboratories our scientific allies along the way, including the laboratories of Eileen White, William Hait and Leroy Liu. These various collaborations have helped to advance science much faster. 300 years ago, most people died before the age of 50. Few had cancer, dementia or type II diabetes. Since the Industrial Revolution, the average lifespan of human beings has increased dramatically. Alas, so is the suffering associated with these age-related diseases. If autophagy is indeed involved in the control of aging and the prevention of these diseases related to aging, understanding autophagy could reveal the secret that prolongs human lifespan without increasing suffering. Are we looking for the Holy Grail? Yes, it's our dream. 47 It has been shown that this strain of mice, which contains a deletion in a gene called beclin1, is much more likely to have cancer when it is old. In other words, this result demonstrates that the normal function of the beclin1 gene is to prevent tumor development in old age. Interestingly, the human version of the beclin1 gene is also suppressed in sporadic human cancers, tumors affecting only the elderly. Approximately 50% of sporadic breast cancers, 75% of sporadic ovarian cancers and 40% of sporadic prostate cancers contain suppression of the human version of the beclin1 gene. What is even more interesting is that the gene is not linked to known processes that cause cancer. Instead, this gene participates in autophagy, an evolutionary conservation process that is involved in the recycling of cytoplasmic components. As shown in Figure 1, autophagy begins with the emergence of a crescent, a double membrane structure, that grows and engulfs part of the cytoplasm, including mitochondria, ribosomes and proteins. The structure then matures to a closed double membrane vesicle with a diameter of between 0.3 and 0.9 micrometers, what is called a vacuole or autophagic. The autophagosome then moves to the lysosome, a garbage depot and a recycling centre in the cell. The outer membrane of the autophagosome is then fused with the lysosome membrane, leading to the release of its cargo, which is wrapped by the internal membrane in the lysosome. The cargoes are then degraded by the different enzymes of the lysosome. Basically, it is is that one of the functions of autophagy is to serve as sanitation workers busy collecting and recycling garbage in cells. Surprisingly, the same autophagy process has been important in regulating the lifespan of the C. elegans flatworm, a common and practical animal model used in the biological laboratory. Beth Levine's lab, MD, at Southwest Medical Center, first cloned the beclin1 gene and independently demonstrated that a strain of mice lacking the beclin1 gene has higher cancer rates, and Leroy Liu, Ph.D., chair of the department of pharmacology at UMDNJ-Robert Wood Johnson Medical School (RWJMS), has demonstrated that inactivation of autophagia genes shortens the life of worms. Meanwhile, other scientists, including Zhenyu Yue, Ph.D., who is now an adjunct professor at Mount Sinai School of Medicine in New York and working on the neurobiological aspects of autophagy, have found that some aging-related neurodegenerative diseases caused by abnormal accumulation of protein aggregates in neurons, such as Huntington's disease, are associated with reduction. Pharmacological induction of autophagy can help eliminate pathogenic aggregates in cells and relieve the symptoms of these diseases. An exciting theme emerged: autophagy is a critical process of collecting and recycling garbage in healthy cells. As we age, the process slows down or becomes less discriminating. As a result, random agents are accumulated in cells, damaging various parts of cells and tissues, leading to certain diseases related to aging. For example, failure to erase protein aggregates in central nervous system neurons causes dementia; the inability to clear ROS-producing mitochondria (reactive oxygen species) leads to nuclear DNA mutations and cancer. Collectively, these pathological conditions contribute to, or even define, the aging process. Figure 1 48 Specific and non-specific Autophagy From the following article: Autophagy: from phenomenology to molecular understanding in less than a decade Daniel J. Klionsky Nature Reviews Molecular Cell Biology 8, (November 2007) During nonspecific autophagy (see figure a), sequestration begins with the formation of a phagophore that develops into a two-membrane autophagosome while surrounding part of the cytoplasm. Autophagosome can fuse with an endosome (the product of endocytosis), which is a form of heterophagia (in a heterophagic process, the cell internalizes and degrades material that originates from outside the cell, unlike autophagy, in which the cell consumes part of itself). The product of endosome-autophagosome fusion is known as a The completed autophagosome or amphisome merges with a lysosome, which provides acidic hydrolases. The resulting enzymes in the compartment, an autolysosome, break down the inner membrane of the autophagosome and degrade the cargo. The resulting macromolecules are released by permeals and recycled into the The yeast cytoplasm-vacuole targeting pathway (Cvt) is an example of specific autophagy (see figure part b), and the only example of a biosynthetic autophagy-related pathway. The overall morphology is identical to non-specific autophagy; however, Cvt's sequestering vesicles are smaller than autophagosomes and they appear to exclude bulk cytoplasm. The phagophore assembly site (PAS) becomes either the sequestered vesicle or generates it. The precursor form of aminopeptidase I (prApe1) forms oligomers in the cytosol, and is targeted by the action of a receptor, Atg19, and the Atg11 adapter or scaffolding protein to enable selective cargo recognition and packaging. The completed vesicle merges with the vacuole, the yeast analog of the mammalian lysosome. The larger size of the vacuole allows the release of the single-brasal inner compartment of the Cvt vesicle, which is now called a Cvt body; during nonspecific autophagy in yeast, this structure is called an autophagic body. Cvt's body is lyse and prApe1 is matured by the proteolytic removal of a propeptide to generate active and resident mApe1 hydrolase. 49 50 Regulation of Autophagy in Mammalian Cells From the following article: Autophagy: from phenomenology to molecular understanding in less than a decade Daniel J. Klionsky Nature Reviews Molecular Cell Biology 8, (November 2007) In the figure, green circles represent components that stimulate autophagy, while purple boxes correspond to inhibitors. 3-methyladenine (3-MA) and wortmannine (Wm) also inhibit class I phosphatidylinositol 3-kinases (PI3K), but the overall effect of these compounds is a block in autophagy (because they inhibit the downstream class III enzyme that produces phosphatidylinositol-3-phosphate (PtdIns(3)P), which is necessary for autophagy). The regulation of autophagy is complex and far from understood. Historically, tor (rapamycin target) has been considered the central regulator of autophagy, because tor inhibition with rapamycin (Rap) induces autophagy. However, it is now clear that there are also types of regulations independent of the TOR. For example, beclin-1 and Atg4 could be regulated by n-terminal kinase c-jun (JNK) and reactive oxygen species (ROS), respectively. Additional information is available in recent reviews (for example, see ref. 74, 75). 51 52 Perspectives Nature Reviews Molecular Cell Biology 8, (November 2007) Autophagy: From Phenomenology to Molecular Understanding in Less than a Decade Daniel J. Klionsky Abstract In 2000, it was suggested to me that autophagy will be the wave of the future; it will become the new apoptosis. Few would have agreed at the time, but this statement proved to be and this process of self-feeding quickly exploded as a field of research, as scientists discovered connections to cancer, neurodegeneration and even extending lifespan. Surprisingly, molecular breakthroughs in autophagy have occurred over the past 53 Autophagy, the process by which invading proteins, organelles and pathogens are sequestered in autophagosomic vesicles and delivered to the lysosome for degradation, is a main route for the turnover of stable, defective and undesirable cellular components. The defects of this cellular system of homeostasis are linked to many human diseases. This process involves a series of dynamic membrane rearrangement reactions mediated by a central set of autophagy proteins (Atg). Although the kinase of preserved proteins, lipid kinase and ubiquitin protein conjugation sub-networks controlling autophagosome formation and cargo recruitment have been identified, several gaps remain in our understanding of autophagy. For example, it has not been clear how the distinct protein complexes involved in autophagy are talking to each other or to other cellular machines involved in membrane formation and membrane trafficking. And how do the key signals that trigger autophagy speak to Atg proteins? Autophagy networks. (Top) Overview of macroautophagy. (Bottom) Main signal transduction systems into autophagy. Recently, we conducted a systematic proteomics analysis of the human autophagy system with the long-term goal of providing a molecular overview of some of the unanswered questions in the field. This effort provided an overview of the overall architecture of the Autophagy Interaction Network (AIN). In short, 65 proteins related to autophagy were proteomically analyzed by LC-MS/MS coupled with intellectual property, revealing a dense network of interaction with numerous cross-talking between functional signaling modules. The current NAI contains many new components related to vesicle trafficking, atg8 conjugation, protein phosphorylation, protein ubiquity and lipid modification, as well as genetic (RNAi) and parallel localization studies, provides a framework for understanding the molecular architecture of the pathway and how individual sub-networks communicate with each other. In addition, we conducted detailed analyses of ATG8 interaction proteins to delimify regulators and cargo of selective autophagy. Together, our work provides a resource for a detailed and systematic mechanistic analysis of this critical pathway of protein homeostasis. 54 Three predominant cellular functions can be assigned to autophagy: autophagy is a response to nutrient starvation. Decreased amino acid levels can induce autophagic response in many cell types and situations, for example. neonatal period, when the supply of nutrients by milk has not yet replaced nutrients with the placenta (6.7) Autophagy is a process of household maintenance by which long-lived proteins and organelles are recycled, for example mitochondria (22) Autophagy has tissue-specific roles, for example during the development of erythrocytes, after expulsion of the nucleus, autophagy is necessary to degrade the remaining organelles. The breakdown of the autophagic vesicle results in the functional biconcave form (23, 24). 55 The Four Steps of Autophagy Autophagy As a result of external/internal stimuli (e.g., nutrient depletion or ischemia), mTOR is inhibited, resulting in the induction of autophagy. Autophagosome formation: Cytosolic proteins and organelles are sequestered by a double membrane vesicle, whose origin is uncertain, but may result from endoplasmic reticulum. The formation of this vesicle is coordinated by Atg protein complexes, which encode for starting codons, which code for methionine to amino acids. Mooring and merging with the lysosome. Degradation of the autophagic vesicle. The molecular mechanism behind fusion with lysosome and subsequent degradation of the autophagic vesicle are poorly understood. 56 Schematic diagram of the steps of the autophagySchematic diagram of the stages of autophagy. Autophagy begins with the formation of the phagophore or isolated membrane (vesicle nucleation stage). It is thought that the concerted action of proteins from the basic autophagy machines at the phagophore assembly site (PAS) leads to the expansion of the phagophore into autophagosome (vesicle lengthening). Autophagosome can engulf bulk cytoplasm not specifically, including whole organelles, or target cargoes specifically. When the outer membrane of the autophagosome merges with an endosome (forming an amphisome before merging with the lysosome) or directly with a lysosome (mooring and fusion stages), it forms an autophagolysosome. Finally, the sequestered material is degraded inside the autophagolyosome (degradation and degradation of the vesicles) and recycled. 57 Human Autophagy 58 Mammal Autophagy Signaling Regulation. In the figure, blue components represent the factors that stimulate autophagy, while reds correspond to inhibitory factors. Autophagy is regulated by a complex signaling network of various stimulating inputs (blue arrows) and inhibitors (red bars). Tor plays a central role in autophagy by integrating class I PtdIns3K signaling and signaling pathways dependent on amino acids. The activation of insulin receptors stimulates the Class I PtdIns3K complex and the small GTPase Ras, leading to the activation of the PtdIns3K-PKB-TOR pathway. PKB phosphorylates and inhibits the complex of tuberous sclerosis 1/2 (TSC1-TSC2), leading to the stabilization of Rheb GTPase, which in turn active TOR, causing the inhibition of autophagy. Amino acids inhibit the Raf-1-MEK1/2-ERK1/2 signaling cascade, leading to the inhibition of autophagy. Energy depletion causes the protein kinase activated by AMP (AMPK) to be phosphorylateated and activated by LKB1. AMPK phosphorylate and active TSC1-TSC2, leading to inactivation of TOR and induction of autophagy. p70S6K kinase is a TOR substrate that can feed negatively on TOR activity, ensuring autophagy that are important for homeostasis. Phosphorylate JNK1 and DAPK and disrupt the association of anti-apoptotic proteins, Bcl-2 and Bcl-XL, with Beclin 1, leading to the activation of the Class III PtdIns3K complex associated with Beclin 1 and Beclin 1 is shown related to the phagophore membrane. 59 Schematic representation of the autophagySchematic representation of autophagy. From Eaten alive: a history of macroautophagy Zhifen Yang1 Daniel J. Klionsky1 Journal name: Nature Cell Biology Volume: 12, Pages: 814-822 (a) (a) In yeast, autophagy and the Cvt way engulf cargoes in separate double membrane vesicles, which are considered to be from the phagophoe assembly site (PAS). PAS is defined as the initial site for the recruitment of autophagy-related proteins (Atg). The Cvt pathway is an example of selective autophagy, and the only example of a biosynthetic pathway related to autophagy. The Cvt vesicle (140-160 nm in diameter) appears to closely envelop the specific cargo — the Cvt complex (composed of the precursor form of aminopeptidase I — prApe1 — and the Atg19 receptor), and exclude the bulk cytoplasm. The autophagosome (300-900 nm in diameter) engulfs the cytoplasm, including organelles, and also the Cvt complex. The completed vesicles then merge with the vacuole, the yeast analog of the mammalian lysosome, and release the singlebrasal internal vesicle (autophagic body or Cvt) into the lumen. The subsequent degradation of the inner vesicles allows the maturation of the prApe1 and the degradation of the cytoplasm, and thus the recycling of macromolecules resulting from vacuolar permeases. (b) Mammal autophagy is initiated by the formation of the phagophore, followed by a series of steps, including the elongation and expansion of the phagophore, closure and completion of a double membrane autophagosome (which surrounds part of the cytoplasm), autophagosome maturation by mooring and fusion with an endosome (the fusion product is known as amphisome) and/or lysosome (the product of fusion is known as autolysosome), degradation and degradation of the internal membrane autophagosome and cargo by acidic hydrolases inside the autolysosome, and recycling of the resulting macromolecules by permeases. So far, there is no evidence of a PAS that exists in mammalian cells, and therefore the mammalian phagophore could be equivalent to yeast NOT, or derived from PAS. Basic molecular machines are also represented, such as the ULK1 and ULK2 complexes that are needed for autophagy induction, Class III PtdIns3K complexes that are involved in the formation of autophagosomes, the mammalian Atg9 (mAtg9) which potentially contributes to the delivery of the membrane to the forming autophagosome and to two conjugation systems, the complex LC3-II and Atg12-Atg5-Atg16L, which are proposed to operate during the elongation and expansion of the phagophore membrane. 60 61 Schematic diagram of suspected role f or C. elegans autophagie proteins involved in the formation of an autophagosome. A) Induction regulation: In yeast, Tor kinase and its effectors regulate the induction of autophagy. UNC-51 is the ortholog C. elegans Atg1, however, it is not clear whether there are regulatory proteins similar to Atg17 or or B) Vesicle nucleation requires a lipid kinase complex, which includes class III 3-kinase (PI3K) phosphatidylinositol phosphatidylinositol (PI3K), Vps34 (in C. elegans LET-512). In yeast, the activation of Vps34 depends on its binding partners, Atg6, Atg14 and Vps15 (Kihara et al., 2001). In C. elegans, the ATG6 ortholog is bec-1, however, orthologists at ATG14 and VPS15 appear to be missing in the genome of C. elegans. The interaction between the antiapoptotic protein CED-9 and BEC-1 is preserved in C. elegans (Takcs-Vellai et al., 2005). A similar interaction between the mammalian proteins Bcl-2 and Beclin 1 inhibits

autophagy (Maiuri et al., 2007a; Maiuri et al., 2007b; Pattingre et al., 2005). 62 C) Two new ubiquitin-like conjugation pathways: the Atg12 conjugation system (Atg5, Atg12, and Atg16), and the Atg8 lipid system (Atg8, Atg3, and Atg7) mediate vesicle expansion, and vesicle completion. Orthologists at all members of these two complexes were found at C. elegans, where they are named ATG-3, ATG-4, ATG-5, ATG-7, ATG-16, LGG-1/Atg8 and LGG-3/Atg12. In yeast, Atg8 underwent two posttranslational treatment events resulting in conjugation with phosphatidylethanolamine (PE) and recruitment to the PAS membrane. ATG-7 is an enzyme in the activation of ubiquitin E1 necessary for the activation of LGG-1. LGG-3-ATG-5 oligomerize with ATG-16 to allow the formation of the multi-memeric complex. ATG-3 and ATG-10 are e2-like ubiquitin combining enzymes and ATG-4 is a cysteine protease. As for its yeast ortholog, LGG-1 seems to remain in the accomplished autophagosome and is therefore an excellent marker for autophagosomal structures early and late. D) Recovery of the ATG-9 integral membrane protein from the phagophore assembly site (PAS) involves ATG-2 and ATG-18, two peripheral proteins interact 63 64 The four stages of autophagy 1) Induction: Following external/internal stimuli (e.g. nutrient depletion or ischemia) mTOR is inhibited, leading to the induction of autophagy. The key yeast genes are Atg1 and Atg13, for which mammalian counterparts have not yet been identified. 2) Autophagosome formation: Cytosolic proteins and organelles are sequestered by a double membrane vesicle, whose origin is uncertain, but may result from endoplasmic reticulum. The formation of this vesicle is coordinated by Atg protein complexes, in particular Atg5 and Atg12, which are combined for the recruitment of LC3 (Atg8). Beclin-1 forms a complex with Atg14. 3) Mooring and fusion with lysosome 4) Autophagic vesicle failure. The molecular mechanism behind the fusion with the lysosome and the subsequent failure of the autophagic vesicle are poorly understood, although lamp-2 is thought to play a key role. Induction - Autophagy can be induced by stimuli external. Autophagy is inhibited under nutrient-rich conditions and therefore simulated by starvation (25, 26). A key regulator or guardian of autophagic induction is Tor/mTOR (14, 27, 28). In nutrient-rich countries mTOR has an inhibitory effect on autophagy, under starvation conditions, mTOR is inactivated - leading to the inhibition of autophagy being released. Tor inactivation leads to downstream dephosphorylation events resulting in transcriptional activation of autophagy genes (1, 28). Autophagosome formation - After induction, a double membrane vesicle forms in the cytosol, sequestering these cytoplasmic components for autophagic degradation. The mechanism of formation of this membrane vesicle is not well defined although it is known that most of the ATG genes identified to date are involved in the formation of this vesicle (29). It has been postulated that endoplasmic reticulum is the origin of this membrane (30, 31) autophagosome fusion - During this phase, autophagosome merges with the lysosome, as a result the contents of the autophagosome are released into the lysosome for degradation by lysosomal proteases (1, 32). Autophagosome failure - Following the fusion of these two vesicle bodies, the autophagosome membrane is decomposition by lysosomal proteases (1, 31). 65 66 Sarkar S, Floto RA, Berger Z, Imarisio S, Cordenier A, Pasco M, Cook LJ and Rubinsztein DC. (2005) Lithium induces autophagy by inhibiting monophosphatase inositol. Journal of Cell Biology 170(7): Access article Additional information Summary: Macroautophagy is a key pathway for the release of aggregate-prone cytosolic proteins. Currently, the only appropriate pharmacological strategy to regulate autophagy in mammalian cells is to use rapamycin, which inhibits the mammalian target of rapamycin (mTOR), a negative regulator of autophagy. Here we describe a new mTOR-independent pathway that regulates autophagy. We show that lithium induces autophagy, and thus improves the release of autophagy substrates, such as mutant huntingtin and alpha-synucleins. This effect is not 24 hours a day by inhibition glycogen synthase kinase 3beta. The autophagy-enhancing properties of lithium were mediated by inhibition of inositol monophosphatase and led to free inositol depletion. This, in turn, decreased levels of myo-inositol-1,4,5-triphosphate (IP3). Our data suggest that the autophagy effect is 24 hours a day at the level (or downstream of) lowered ip3, because it has been abrogated by pharmacological treatments that have increased IP3. This new pharmacological strategy for the induction of autophagy is independent of mTOR, and may help in the treatment of neurodegenerative diseases, such as Huntington's disease, where the toxic protein is an autophagy substrate. Feature: 67 Sci. STKE, 4 October 2005 Vol. 2005, Number 304, p. tw349NEURODEGENERATIVE DISEASE Lithium Decreases IP3 to promote Autophagy Lithium is widely known for its application in the treatment of mood disorders and unipolar. Sarkar et al. provide evidence that lithium may also be effective in treating neurodegenerative disorders resulting from the toxicity of aggregate protein accumulation, such as mutant mutant huntingtin mutant-synuclein, which are involved in Huntington's disease and some forms of Parkinson's disease. Stimulation of autophagy may be a mechanism to prevent the accumulation of these aggegrate-prone proteins, and Sarkar et al. show that lithium promotes autophagy, detected as an increase in the formation of autophagic vesicles and a decrease in the abundance of known autophagic substrates. In addition, lithium decreased the accumulation of mutant synuclein or huntingtin aggregates and the toxicity (cell death) of these proteins in PC12 INductible cell lines or COS-7 cell lines, an effect that was blocked by the 3-methyladenine autophagic inhibitor (3-MA). Lithium is a non-specific inhibitor of several enzymes. The effect on autophagy appeared to be 2met by the inhibition of monophosphatase inositol (IMPase), because autophagy and release of mutant huntingtin and synuclein were also stimulated by inhibition of IMPa with a specific pharmacological agent (L-690,330), while selective inhibition of glycogen synthase kinase-3 (GSK-3, another lithium target) did not promote the release of aggregate-prone proteins. L-690 330 and lithium decreased inositol-1,4,5-trisphosphate (IP3) concentrations. The fact that lithium had its effects on autophagy with reduced levels of IP3 was validated by experiments in which IP3 concentrations were increased prior to lithium treatment by the addition of myo-inositol inhibitor or prolyl 2 endopeptidase (PEI). The increase in ip3 concentration favored the accumulation of mutant huntingtin and cell death and blocked the effects of lithium to reduce these phenomena. Autophagy is also stimulated by the inhibition of mTOR (mammalian target of rapamycin); however, lithium appeared to act independently of the pathway regulated by mTOR, because inhibition of mTOR with rapamycin combined with lithium had an additive effect on the release of aggregate-prone proteins and cell survival. The ability of lithium to stimulate autophagy and the elimination of aggregated proteins has also been observed for other mood-stabilizing drugs that promote inositol depletion. Thus, a pathway involving IP3 and inositol appears to be a mechanism regulating autophagy that can be exploited for therapeutic benefit in certain neurodegenerative diseases. S. Sarkar, A. Floto, Z. Berger, S. Imarisio, A. Cordenier, M. Pasco, L. J. Cook, D.C. Rubinsztein, Lithium induces autophage by inhibiting inositol monophosphate. J. Cell Biol. 170, (2005). [Summary] [Full text] Quote: Lithium decreases IP3 to promote autophagy. Sci. STKE 2005, tw349 (2005). 68 Regulation of membrane traffic by phosphoinositide Drosophila by PI 3-kinases. Autophagy is known to be regulated by both Class I and Class III PI 3-kinases. Class III PI 3-kinase is necessary for autophagy by producing ptdins(3)P via the specific autophagy complex containing Beclin-1, Vps15 and hVps34. (In yeast, an additional accessory protein, extra, is part of the complex.) In contrast, class I PI 3-kinase inhibits autophagy by activating the Akt/PKB pathway. Insect hormone ecdysone acts by nuclear receptors and triggers programmed autophagy in Drosophila's large body by downregulating the class I PI 3-kinase pathway by an unknown mechanism. Overexpression of 3-phosphatase PTEN IP mimics this effect. 69 Sarkar S, Davies JE, Huang Z, Tunnacliffe A and Rubinsztein DCSarkar S, Davies JE, Huang Z, Tunnacliffe A and Rubinsztein DC. (2007) Trehalose, a new autophagy inducer independent of mTOR, accelerates the release of mutant huntingtin and alpha-synuclein. Journal of Biological Chemistry 282(8): Access article Additional information Abstract: Trehalose, a disaccharide found in many non-mammalian species, protects cells from various environmental stresses. While some of the protective effects can be explained by its chemical chaperone properties, its actions are largely unknown. Here we report a new function of trehalosis as a mTOR-independent autophagy activator. Trehalose-induced autophagy improved the release of autophagy substrates such as mutant huntingtin and alpha-synuclein mutants A30P and A53T, associated with Huntington's disease (HD) and Parkinson's disease (PD), respectively. In addition, trehalose and mTOR inhibition by rapamycin together had an additive effect on the release of these aggregate-prone proteins due to increased autophagic activity. By inducing autophagy, we have shown that trehalosis also protects cells from subsequent pro-apoptotic insults through the mitochondrial route. The dual protective properties of trehalosis (as an inducer of autophagy and chemical chaperone) and the combination strategy with rapamycin may be relevant for the treatment of HD and related diseases, where mutant proteins are substrates of autophagy. 70 Autophagosome formation model in mammalian cells Atg12-Atg5 conjugated and Apg16L locate at the isolation membrane throughout its elongation process. LC3 (Homolog Atg8) is recruited to the membrane in a manner dependent on Apg5. Apg12-Apg5 and Apg16L dissociate from the membrane at completion of autophagosome formation, while LC3 (-II) remains on the autophagosome membrane. LC3 dissociates itself from the autolysomal membrane. 71 We have generated embryonic stem cells (ES) atg5-/-mouse and demonstrated that mammalian Atg5 is also necessary for autophagy. Atg5-/- ES cells can grow normally, but bulk protein degradation has been significantly reduced in these cells, suggesting that autophagy is actually a major degradation process. Atg5-/-EScells show a block in the autophagic pathway Cells were grown in Hanks solution for 2 hours to induce autophagy. Bar, 1 uh. (by Dr. Akitsugu Yamamoto at the Nagahama Institute of Bio-Science and Technology) 72 In the mammalian apg12 system, Apg12 is attached to Apg5's Lys130In the mammalian apg12 system, Apg12 is attached to Apg5 Lys130. When the Atg5K130R the Atg5K130R in which Lys130 is replaced by Apg, is expressed in atg5-/- ES cells, Apg5 is no longer conjugated with Apg12. Even in such cells, Apg5K130R is able to locate itself to small autophagosome precursors with Apg16L, suggesting that covalent modification of Apg5 with Apg12 is not necessary for membrane targeting of Apg5 and Apg16L. However, the membrane does not lengthen to form a cup-shaped isolation membrane and autophagosomes. Thus, the Apg12-Apg5-Apg16L complex is essential for lengthening isolation membranes, not for generating precursor structures. The conjugation of Atg12 is not necessary for Atg5 membrane targeting, but it is essential for the maturation of the isolation membrane in autophagosome and the recruitment of LC3 to the membrane. 73 Development of a transgenic mouse model with anAlthic fluorescent autophagosome marker although the possible involvement of autophagy in homeostasis, development, cell death and pathogenesis has been stressed repeatedly, systematic in vivo analysis has not been performed in mammals, mainly due to a limitation of surveillance methods. To understand where and when autophagy occurs in vivo, we generated transgenic mice systemically expressing GFP fused to LC3, which serves as a marker protein for autophagosomes. Microscopic fluorescence analyses have indicated that autophagy is differently induced by nutrient starvation in most tissues. This transgenic mouse model is a useful tool for studying mammalian autophagy. In vivo analysis of autophagy using transgenic mice GFP-LC3 (High) Gastrocnemia Muscle Samples was prepared from transgenic GFP-LC3 mice before (left) or after a 24-hour famine (right). The small dots represent the autophagosomes. (Inferior) GFP-LC3 mouse embryonic fibroblasts were grown in Hanks solution for 2 hours. Bar, 10 uh. 74 Molecular mechanism of autophagyIn yeast, Saccharomyces cerevisiae, at least 16 of the ATG genes have been identified to be necessary for autophagosome formation. The most surprising results are the discoveries of the two new conjugation systems similar to ubiquity: one mediats the conjugation of Atg12 to Atg5, and the other mediats a covalent link between Atg8 (LC3 in mammals) and phosphatidylethanolamine (PE) (Suppl. Fig. 1)B We dissected the autophagic process in mammalian cells using Atg homologs. We proposed a model in which the cup-shaped isolation membrane is developed from a small crescent-shaped compartment. We also examined the location and function of mammalian atg proteins during this process. (A) Punctate signals GFP-Apg5 increase under starvation conditions in ES cells. (B) Hungry ES cells co-expressing CFP-Atg5 and YFP-LC3. 75 76 77 Molecular Mechanism of Autophagy 78 79 80 Autophagy has recently been implicated in various human pathological and physiological conditions, such as neurodegeneration, immunity, cancer, development, myopathies, heart disease, liver disease and longevity. Basal autophagy is essential to eliminate and damaged organelles, and therefore plays a vital role in maintaining cellular homeostasis in all tissues. 81 Autophagy and Disease Autophagic response has been described in various pathophysiological situations, including neurobiology, cancer and more recently cardiovascular disease, but its role has not yet been teased; the node of the problem being, is the response a protective cell, or a mechanism of cell death? 3.1 Studies of neurodegenerative diseases have shown that autophagy plays a role in the elimination of misfolded proteins that accumulate in the cell, such as polyglutamine aggregates formed in Huntington's chorea (33, 34). It could be the case however, that a disproportionate autophagic response could contribute to the pathology seen in such disorders. 3.2 Cancer A number of tumor suppressor proteins control autophagy (e.g. Beclin-1 and PTEN), so it would seem reasonable to assume that a decrease in autophagy would lead to tumor progression (28, 35, 36). Indeed, experimental evidence to support this hypothesis - on the expression of Beclin-1 induces autophagy in cultured breast cancer cells (9) and stimulation of autophagy has been observed in cancer cells following anticanceral treatments (37). Is this regulation of autophagy really a cellular protective mechanism, or does it actually an alternative mechanism of cell death since in cancer cells the pathway of apoptosis is commonly modified (28)? It should also be considered that, during tumor establishment, autophagy may be a mechanism by which targeting tumor cells, once the tumor is established autophagy may actually provide a way for cancer cells to overcome nutrient-limiting conditions especially in the internal mass of the tumor that is poorly vascularized (28, 38, 39) 82 Nature Reviews Drug Discovery 7 , 476-477 (June 2008)Neurodegenerative disease: A new pathway to autophagy Charlotte Harrison Abstract Stimulating autophagy — a major cellular release pathway for intracellular protein aggregates — could be a therapeutic strategy for Huntington's disease. However, the only drug that has been shown to induce autophagy in the brain is rapamycin, a mammalian target of rapamycin (mTOR) kinase inhibitor that modulates several cellular processes in addition to autophagy. 83 Neurodegenerative Disorders: A Help for DigestionSOURCE: Welcome Subscribe A high-throughput screening strategy to identify new modulators of mammalian autophagy can provide avenues for drug development for Huntington's disease. Autophagy, the lysosomal digestion of cytoplasmic proteins and organelles, can a protective role in certain neurodegenerative and infectious diseases. It may also have an inhibitory role in some cancers. Currently, the only small molecule known to regulate autophagy in the mammalian brain is rapamycin. However, due to the involvement of mTOR proteins (mammalian target of rapamycin) in many cellular processes, long-term use of rapamycin is associated with many Writing in Nature Chemical Biology, Sarkar and his colleagues describe a new approach to identifying new modulators of mammalian autophagy, which could provide clues for the development of drugs for Huntington's disease. In order to find safer methods of modulating autophagy, the authors conducted a high-throughput screening of 50,729 compounds in yeast to identify small molecules that increased or suppressed the effects of rapamycin on cell growth. The result was the identification of a structurally non-redundant set of 21 rapamycin inhibitors (SMIRs) and 12 small molecule rapamycin enhancers (SMER). To test the ability of these compounds to modulate mammalian autophagy, regardless of rapamycin, they evaluated their ability to induce the release of the A53T-synuclein autophagy substrate, which is associated with a form of familial Parkinson's disease. Thirteen SMIRs have been shown to slow the release of this autophagy substrate, while four EMRs have increased it. The authors then focused on a subset of autophagy-inducing SMEs that lacked toxicity in various cell lines. These may have therapeutic potential in neurodegenerative disorders such as Huntington's disease, whose underlying cause is the expansion of a polyglutamine tract (polyQ) into the huntingtin protein. In cellular models expressing mutant huntingtin protein — another substrate for autophagy — each of the EMRs improved the release of this substrate, reducing mutant protein aggregation and cell death. In a drosophila model of Huntington's disease, SMEs have protected themselves against neurodegeneration as assessed by reducing the number of rhabdomeres visible in the eye ommatidia over time. Interestingly, unlike rapamycin, these compounds did not reduce the phosphorylation of mTOR kinase substrates, suggesting that their induction of autophagy is 20th by an independent mechanism of mTOR, or by an unknown component of the mTOR autophagy pathway downstream of mTOR. In addition, the treatment of cell models with smers with rapamycin resulted in an additive effect on the release of A53T-synuclein and the reduction of mutant huntingtin aggregation. This study illustrates the use of a high-throughput screening strategy to identify small molecule modulators of mammalian autophagy. The analysis of the structure-activity relationships of the identified SMEs highlighted the functional groups needed for their specific activity and the additional candidates for therapeutic development. Sarah Crunkhorn refers to Sarkar, S. et al. Small molecules improve autophagy and reduce toxicity in Huntington's disease. Nature Chem. Biol. 3, 331-338 (2007). | Article PubMed Huang, J. et al. Find new components of the rapamycin (TOR) signaling network target by chemical genetics and proteome chips. Proc. Natl Acad. Sci. United States 101, 16594-16599 (2004) Article PubMed Rubinzstein, D.C. et al. Potential therapeutics autophagy. Nature Rev. Drug Discov. 6, 304-312 (2007) Article PubMed 84 85 86 Implication of autophagy deregulation in Parkinson's disease (PD)Involvement of autophagy deregulation in Parkinson's disease (PD). There are three types of autophagy: macroautophagy, microautophagy and chaperone-based autophagy (CMA). The deregulation of macroautophagy and CMA is implicated in the pathogenesis of PD. CMA is involved in the degradation of wild-type soluble alpha-synuclein, the main constituent of Lewy bodies. However, once oligomerized or aggregated, alpha-synuclein is probably degraded by macroautophagy instead of CMA. Interestingly, alpha-synuclein mutants A53T and A30P are poorly degraded by CMA, but are rather degraded by macroautophagy. In addition, alpha-synuclein mutants inhibit CMA, reducing the mediated cma-medial degradation of alpha-synuclein and MEF2D survival factor. Along with CMA inhibition, overexpression of alpha-synuclein mutants results in compensatory activation of macroautophagy. The activation of macroautophagy is also evident in cells treated with the neurotoxin MPP, a well-established model for parkinsonism, or following the overexpression of GPR37, another protein that is present in Lewy bodies. Finally, recent studies show that macroautophagy also plays a role in the turnover of fragmented mitochondria. These observations highlight the potential involvement of autophagic pathways in the pathogenesis of PD. 87 Nature Reviews Neurology 4, 60 (February 2008)Justification to treat Huntington's disease with a combination of sirolimus and lithium Abstract Sarkar S et al. (2007) A rational mechanism for the combined treatment of Huntington's disease using lithium and rapamycin. Hum Mol Genet 17: 170-178 PubMed A promising treatment approach for Huntington's disease is the promotion of autophagy to eliminate aggregate proteins such as mutant huntingtin. 88 Sarkar S, Perlstein EO, Imarisio S, Pineau S, Cordenier A, Maglathlin RL, Webster JA, Lewis TA, O'Kane CJ, Schreiber SL and Rubinsztein DC. (2007) Small molecules improve autophagy and reduce toxicity in Huntington's disease models. Nature Chemical Biology 3(6): Access Article Additional Information Additional Information Chemical Compounds Evidenced 'Equal Contribution' Abstract: Target rapamycin proteins regulate various cellular processes, including autophagy, which may play a protective role in certain neurodegenerative and infectious diseases. Here we show that a primary small molecule screen in yeast gives new small molecule modulators of mammalian autophagy. We first identified new small molecules (SMER) and inhibitors (SMIR) cytostatic effects of rapamycin in Saccharomyces cerevisiae. Three SMEs induced autophagy independently of rapamycin in mammalian cells, improving the release of autophagy substrates such as mutant huntingtin and A53T alpha-synuclein, which are associated with Huntington's disease and familial Parkinson's disease, respectively. Respectively. SMEs, which appear to act independently or downstream of the rapamycin target, have mitigated the mutant toxicity of the huntingtin fragment in the Huntington's disease cell and Drosophila melanogaster cell models, suggesting therapeutic potential. We also examined the structural analogs of these SMEs and identified other candidate drugs that improved the clearance of the autophagy substrate. Thus, we have demonstrated the proof of principle for a new approach to the discovery of small molecule modulators of mammalian autophagy. 89 Sarkar S, Krishna G, Imarisio S, Saiki S, O'Kane CJ and Rubinsztein DCSarkar S, Krishna G, Imarisio S, Saiki S, O'Kane CJ and Rubinsztein DC. (2008) A rational mechanism for the combined treatment of Huntington's disease using lithium and rapamycin. Human Molecular Genetics 17(2): Access Article Additional Information Summary: Huntington's disease (HD) is caused by a mutation in the expansion of polyglutamine in the huntingtin protein that confers a toxic function gain and causes the propense to become prone to aggregates. Aggregate-prone proteins are erased by macroautophagy, and the upregulation of this process by rapamycin, which inhibits the mammalian target of rapamycin (mTOR), mitigates their toxicity in various HD models. Recently, we have demonstrated that lithium induces mTOR-independent autophagy by inhibiting levels of monophosphatase inositol (IMPase) and reducing levels of inositol and intellectual property. Here we show that glycogen synthase kinase-3beta (GSK-3beta), another lithium-inhibited enzyme, has opposite effects. Unlike impase inhibition that improves autophagy, GSK-3beta inhibition antlemo mitigates autophagy and mutant huntingtin release by activating mTOR. In order to counteract the inhibitory effects of mTOR activation resulting from lithium treatment, we used the rapamycin mTOR inhibitor in combination with lithium. This combination improves macroautophagy by mTOR-independent (IMPase inhibition by lithium) and mTOR-dependent (mTOR inhibition by rapamycin) pathways. We provide proof of principle for this rational approach to combined treatment in vivo by showing greater protection against neurodegeneration in a HD fly model with tor and lithium inhibition, or in HD flies treated with rapamycin and lithium, compared to either pathway alone. 90 Sarkar S, Ravikumar B, Floto RA and Rubinsztein DCSarkar S, Ravikumar B, Floto RA and Rubinsztein DC. (2009) Independent rapamycin and mTOR autophagy inducers improved the toxicity of polyglutamine-enlarged huntingtin and related proteinopathies. Cell Death and Differentiation 16(1): Access Article Abstract: The Formation of Intra-neuronal mutants is a feature of several human neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease (PD) and polyglutamine disorders, including Huntington's disease (HD). Autophagy is a major release pathway for the elimination of mutant huntingtin associated with HD, and many other disease-causing, cytoplasmic, aggregate-prone aggregates Autophagy is negatively regulated by the mammalian target of rapamycin (mTOR) and can be induced in all types of mammalian cells by the rapamycin mTOR inhibitor. It can also be induced by a recently described mTOR-independent cyclical pathway, which has multiple drug targets, involving links between Ca2-calpain-Gsa and cAMP-Epac-EPc-e-IP3 signaling. Both pathways improve the release of mutant huntingtin fragments and reduce polyglutamine toxicity in cell and animal models. The protective effects of rapamycin in vivo depend on autophagy. In drosophila models of various diseases, the benefits of rapamycin are lost when the expression of different autophagic genes is reduced, implying that its effects are not mediated by processes independent of autophagy (such as a slight suppression of translation). In addition, mTOR-independent autophagy enhancers have no effect on mutant protein release in autophagy-deficient cells. In this review, we describe various drugs and pathways inducing autophagy, which may be potential therapeutic approaches for HD and related conditions. 91 Sarkar S, Korolchuk V, Renna M, Winslow A and Rubinsztein DCSarkar S, Korolchuk V, Renna M, Winslow A and Rubinsztein DC. (2009) Methodological considerations for evaluating autophagy modulators: A study with calcium phosphate precipitates. Autophagy 5(3): Access article Summary: Autophagy has been involved in various physiological conditions and disease in recent years. A number of small molecule modulators have been identified, both as tools and as potential therapeutics. Despite the extensive characterization of autophagy in yeast, mammalian autophagy pathways are not fully understood. Recently, calcium phosphate precipitates (CPP), which are used to transfect DNA in cells, have been reported to induce autophagy, once assayed until 6 h after treatment. Due to the widespread use of this reagent, we have tried to confirm these results. In accordance with the previous study, we showed that CPP induces autophagosome synthesis at the first moments, such as 4 a.m. and 6 a.m. However, at 24 hours after treatment, we were surprised to see that the flow of autophagy was reduced, due to altered autophagosome-lysosome fusion. At that time, there was an accumulation of autophagic substrates and the formation of abnormally large autophagosomes. Thus, one may need to consider appeasing autophagy modulators at different times with a range of analyses in order to understand their effects. Finally, the complex consequences of the CPP on autophagy suggest that it is best avoided as a transfection replenishment in studies aimed at autophagy itself, or processes that are modulated by autophagy, such as apoptosis. 92 Sarkar S, Ravikumar B and Rubinsztein DCSarkar S, Ravikumar B and Rubinsztein DC. (2009) Autophagic release of aggregated proteins associated with neurodegeneration. Methods in Enzymology Klionsky D.J., Editor-in-Chief, Autophagy in disease and clinical applications, Academic Press 453C: Access article article Autophagy has emerged as an area of rapidly growing interest with implications in several disease conditions, such as cancer, infectious diseases, and neurodegenerative diseases. Autophagy is a major pathway of degradation for intracytosolic proteins prone to aggregates causing neurodegenerative disorders, such as Huntington's disease and forms of Parkinson's disease. Autophagy up to regulation can be a table therapeutic intervention to eliminate these pathogenic proteins. The identification of autophagy-enhancing compounds would be beneficial not only in neurodegenerativeautophagy diseases can act as a protective pathway. In addition, modulators of small molecules of autophagy may also be useful in dissecting the pathways governing mammalian autophagy. In this chapter, we highlight analyses that can be used to identify autophagy regulators, such as measuring the elimination of aggregate-prone mutant proteins or autophagic flow with bafilomycin A1. Using these methods, we recently described several autophagy-enhancing compounds independent of mTOR that have protective effects in various models of Huntington's disease. 93 Aguado C, Sarkar S, Korolchuk VI, Criado O, Vernia S, Boya P, Sanz P, de Cordoba SR, Knecht E and Rubinsztein DC. (2010) Laforin, the most common protein mutated in Lafora disease, regulates autophagy. Human Molecular Genetics 19(14): Access Article Additional Information Summary: Lafora disease (LD) is a recessive and progressive autosomal epilepsy of myoclonus, which is characterized by the accumulation of polyglucosan inclusion bodies, called Lafora bodies, in the cytoplasm of central nervous system cells and in many other organs. However, it is not clear at this time whether Lafora's bodies are the cause of the disease, or if they are secondary consequences of primary metabolic alteration. Here we describe that the main genetic lesion that causes LD, loss of function of protein laforin, alters autophagy. This phenomenon is confirmed in the cell lines of human patients, embryonic mouse fibroblasts of mice knock-out laforin and in the tissues of these mice. Conversely, the expression of laforin stimulates autophagy. Laforin regulates autophagy via the mammalian target of the rapamycin kinase-dependent pathway. Changes in laforin-mediated autophagy regulate the accumulation of various autophagy substrates and would be expected to have an impact on Lafora body accumulation and the cellular stress observed in this disease that may eventually contribute to cell death. 94 Autophagy laforine: A possible link between carbohydrates and proteins in maladie de Lafora? Volume 6, Numéro 8 2010 Pages Rajat Puri et Subramaniam Ganesh Voir les affiliations Masquer les affiliations Rajat Puri Department of Biological Sciences and Bioengineering; Institut indien de technologie; Kanpur, Inde Subramaniam Ganesh Department of Biological Sciences and Bioengineering; Institut indien de technologie; Kanpur, Inde L’épilepsie myoclonus progressive de Lafora Lafora (LD) is a fatal form of neurodegenerative disorder associated with progressive intellectual decline and ataxia in addition to epilepsy. The disease may be caused by defects in the EPM2A gene encoding laforin phosphatase or the NHLRC1 gene encoding maline ubiquitin ligase. Laforine and maline work together as a complex in the ubiquitin-proteasome system, and therefore defects in proteolytic processes are thought to underlie some of the symptoms in LD. One of the pathological characteristics of LD is the presence of cytoplasmic inclusions of polyglucosan, the bodies of Lafora. While Lafora bodies are known as a less ram ramed glycogen form with high phosphate content, a physiological basis for their genesis in the cytoplasm has not been well understood. Recently, it has been shown in a mouse model for LD that laforin loss inhibits autophagosome formation, suggesting that laforine plays a critical role in autophagosome biogenesis. Polyglucosan inclusions could be one of the substrates of autophagy, and loss of laforin could affect their sequestration in autophagosomes leading to their aggregation as Lafora's body. Thus, the proposed role of laforin in autophagy suggests a possible link between the proteolytic system and polyglucosan inclusions in LD. 95 THC induces autophagy via ER-evoked p8 stress and upregulation of TRB3. Cannabinoid action induces autophagy cell death- mediated by the stimulation of ER stress in the human cells of glioma Maria Salazar, Arkaitz Carracedo, 'igo J. Salanueva, Sonia Hernandez-Tiedra, Mar Lorente, Ainara Egia, Patricia Vzquez, Cristina Blzquez, Sofàa Torres, Stephane Garcia, Jonathan Nowak, Gian Maria Fimia, Mauro Piacentini, Francesco Cecconi, Pier Paolo Pandolfi, Luis Gonzalez-Feria, Juan L. Iovanna, Manuel Guzm Guillermo Velasco J Clin Invest. 2009; 119(5):1359-1372 THC induces autophagy via stress ER-evoked p8 and TRB3 upregulation. (A and B) Effect of ISP-1 (1 M) on THC-induced eIF2 phosphorylation (A; 3 h; n -3) and immunostaining LC3 (B, left panels; 6 p.m.; percentage of cells with LC3 points relative to the total number of cells, average ± SD; n-3; scale bar: 20 m) in U87MG cells. sipping8, siRNA p8-selective; siTRB3, SiRNA TRB3-selective. (C) Effect of THC on the p8, ATF4, CHOP and TRB3 levels of mSR eIF2-WT and eIF2-S51A, as determined by real-time quantitative pcr (8 h; n-3). The figures show the average increase ± the DS compared to the EIF2-WT CEM processed by the vehicle. (D) Top: Analysis of mNSm p8 and TRB3 levels. The results of a representative RT-PCR experiment are displayed. Figures indicate levels of gene expression determined by real-time quantitative pcr medium fold ± DS compared to siC transfected cells; No. 5). Bottom: Effect of THC on the immunostaining LC3 (green) of U87MG cells transfected with siC, sip8, or siTRB3 (6 p.m.; n-4). The percentage of cells with LC3 points compared to cells cotransfected with a red fluorescent siRNA is shown in each panel (± SD). Scale bar: 20 m. (E) Effect of THC on lipidization in U87MG cells transfected with siC, sip8, or siTRB3 (6 p.m.; n-6). (F) Effect of THC on lipid LC3 (top; 18 h; n 5) and immunostaining LC3 (bottom; 18 h; percentage of cells with LC3 points relative to total number of cells, average ± SD; n - 4; scale bar: 40 m) in p8/p8-//-MEFs. 0.05 and 0.01 compared to U87MG (B), eIF2-WT (C) or P8-/F cells treated with THC and compared to U87MG cells transfected by siC and treated with THC (D). 96 97 The ubiquitin proteasome system in neuropathology by Lehman, Norman LThe ubiquitine proteasome system in neuropathology by Lehman, Norman L. Acta Neuropathologica Vol. 118 Number 3: Ligases E3 of the Cbl family and ligase UBE3A E3 are simple proteins that combine with a Ubc E2 and the target substrate. The ubiquitin ligas of SCF and APC/C E3 are multi-memeric complexes. APC/C has only two known adapter sub-units, Cdc20 and Cdh1 (not to be confused with e-cadherin, which is sometimes also called Cdh1). CFS may be associated with several different substrate adapter proteins known as F-box proteins The functions of neddylation are less understood; however, neddylation of cullin proteins, essential sub-units of some E3 ubiquitin ligase complexes, appears to be required for normal ligase function (fig. 2). The HECT and RING types may have the function of E3 ligase as simple proteins, for example the HECT-type UBE3A ligase and the RING-type Cbl ligase (fig. 2). Examples include ubiquitin ligase Skp/cullin/F (SCF) and anaphase favouring complex E3/cyclosome ubiquitin ligas (APC/C) which have specificity for different substrates depending on the adaptor protein associated with the complex (fig. 2). Familial Juvenile Onset Parkinson's disease is caused by a defect in a CWS ubiquitin ligase known as parkin (PARK2) (fig. 2). The pVHL protein is the substrate recognition component of ubiquitin ligase that ubiquitin HIF-1 [17] (fig. 2). APC/C is associated with two different activating sub-units that confer general substrate specificity, Cdh1 and Cdc20 (fig. 2). 98 99 Disorder Gene produces and functions Parkinson's disease Dominant autosomic (early onset) α-Synuclein (SNCA) (PARK1), aggregates in the bodies of Lewy Autosomal dominant (late onset) Leucine-rich repeat kinase 2 (LRRK2) (PARK8), a ubiquitin ligase substrate CHIP, contains a Roc domain as found in SCF Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) (PARK5), a DUB, acts as an E3 ligase when it is dimer, polymorphisms related to rare forms of familial familial disease Autosomal recessive (juvenile onset) Parkin (PARK2), a sub-unit of a SCF E3 ubiquitine ligase Autosomal recessive (early onset) PINK1 (PARK6), promotes by ubiquitkinin DJ-1 activity (PARK7) chaperone, promotes parkin ubiquitine ligase activity Spinocerebellar ataxias SCA1 Ataxin-1, a ligase UBE3A E3, the mutation blocks its ubiquitination and association with the ubiquitin receptor A1Up and the enzyme DUB USP7 SCA2 Ataxin-2 , associates with c-Cbl E3 ligase and is involved membrane protein endocytosis, and is a substrate ligase parkin E3 SCA3 Ataxin-3, a deubiquitinating enzyme (DUB) 100 prion diseases Prions can block the normal function of proteasome, HECT2D E3 ubiquitin ligase haplotypes are associated with vCJD and Kuru Autosomal recessive ALS ALS2, a protein associated with endosmal membrane involved in endosome membrane fusion and traffic, mutation decreases protein stability ALS2 Angelman syndrome Loss or mutation of ligase Of UBE3A E3 to Angelman/Prader-Willi locus Rett syndrome decreased UBE3A E3 ligase due to mutations MECP2 Autism Alterations in the number of copies of UBE3A and other genes UPS Giant neuropathy axon Mutation gigaxonin, an ibmpfd mutation of the ubiquitin E3 protein containing valosine (VCP) , involved in the ubiquitin-mentté treatment of membrane and cytosolic proteins. Sporadic PCVs and ubiquitin IBM are found in inclusion organisms. von Hippel-Lindau disease pVHL, a sub-unit binding ubiquitin ligase substrate targeting HIF1-α Medulloblastoma Overexpression of several signaling pathway genes that are ubiquitinated by CSF: c-myc, β-catenin, Gli, Gli and β-catenine stabilizing mutations prevent their ubiquitous-dependent proteolysis adamantinomatous craniopharyngioma Stabilizing β-catenine mutations preventing its ubiquitin-dependent proteolysis Gliomas Poor regulation and mutation of cell cycle control proteins regulated by UPS: CDKs, CDK inhibitors, p53, altered expression of ubiquitin ligase regulators APC/C E3 Emi1 and RASSF1A 101 3.3 cardiac myopathies cardiac myocytes are differentiated cells in the terminal phase, so the proper functioning of the autophagic pathway is essential for the maintenance of myocyte homeostasis. Unsurprisingly, therefore, autophagic deficiencies have been associated with a variety of cardiac pathologies (examined in 40). One such example that helped to understand autophagy in the heart system is Danon's disease. Deficiency of the LAMP-2 autophagy gene has been shown to cause this disease. Patients have an increase in autophagic vacuoles leading to cardiomyopathy (15), a phenotype seen in mice with inactivation of the lamp-2 protein (16). Whether the increase in autophagic vacuoles is due to increased regulation of autophagosome formation or due to a decrease in autophagosome - lysosome fusion is currently unclear (reviewed in 36). Autophagic vacuoles have been described in the cardiac tissue of patients with idiopathic dilated cardiomyopathy (41). Studies of models of ischemic heart disease have also shown a role of autophagy. In chronically dizzy swine myocardium, autophagic vacuoles were seen after three episodes of ischemia/reperfusion (I/R) and were after 6 episodes, associated with increasing levels of expression of autophagy-related molecules like Beclin-1. This correlated with decreasing levels of apoptosis between 3-6 I/R episodes, suggesting that the autophagic response seen could be the result of a cardioprotective effect against I/R (42) injury. (42). studies on the role of autophagy in increasing myocyte loss level have studied the role of Beclin-1 after a single I/R episode. These experiments have shown that Beclin-1 levels are regulated following a single I/R episode and that this regulation is correlated with increased autophagy. Indeed, the use of the cardioprotective agent Urocortin reduces levels of Beclin-1 and simultaneously autophagic levels. Suggesting that in order to provide protection against myocyte loss after I/R, therapies that target autophagic response alongside apoptotic and necrotic response should be investigated (43) Obviously, the most fundamental question for autophagy is whether its role is harmful or protective and this remains to be answered. Indeed, it seems that autophagy may have a role during the progression of the disease, moving from a cellular protective mechanism to a mechanism contributing to cellular pathology. The generation of new reagents, in particular, of antibodies with which to study the autophagic pathway will lead to a better understanding of the regulation of this critical homeostatic pathway. Future cellular and clinical studies will be identified to determine whether this pathway can be manipulated to provide therapeutic potential for heart disease. 102 103 Autophagy in Heart DiseaseBy Joseph Hill - Autophagy and Related Antibodies Display Autophagy is an evolutionary conservation pathway for engulfing, degrading and recycling cellular content, including long-lived proteins and organelles. This process promotes cell survival and maintains cellular homeostasis, under conditions of rest and stress. In addition, it plays an essential role in a number of clinical disorders, including heart disease. Autophagy is a complex process that takes place in several stages in the direction of the steps; nucleation, expansion and maturation/recovery. 104 Williams A. , Sarkar S. , Cuddon PWilliams A, Sarkar S, Cuddon P, Ttofi EK, Saiki S, Siddiqi FH, Jahreiss L, Fleming A, Pask D, Goldsmith P, O'Kane CJ, Floto RA and Rubinsztein DC. (2008) New targets for Huntington's disease in an autophagy pathway independent of mTOR. Nature Chemical Biology 4(5): Access Article Additional Information Additional Information Chemical Compounds - Equal Contribution Abstract: Autophagy is a major release route for intracellular proteins prone to aggregates causing diseases such as Huntington's disease. Autophagy induction with mTOR inhibitor rapamycin accelerates the release of these toxic substrates. As rapamycin has non-trivial side effects, we examined FDA-approved drugs to identify new autophagy-inducing pathways. We found that type L-type Ca2-channel antagonists, the aperture minoxidil of the K-ATP channel and the clonidine of the Gi signaling activator induce autophagy. These drugs revealed a cyclical pathway independent of mTOR regulating autophagy, in which AMP regulates IP3 levels, influencing calpaine activity, which completes the cycle by cleaving and activating Gsa, which regulates ampl ampl ampl This pathway has many potential points where autophagy can be induced, and we provide proof of principle for therapeutic relevance in Huntington's disease using models of mammalian cells, flies and zebrafish. Our data also suggest that insults that elevate intracytosolic ca2 (such as excitotoxicity) inhibit autophagy, thus delaying the release of aggregate-prone proteins. 105,106 Therapies targeted by autophagyAutophagy is an important topic in modern cell biology. This is a dynamic process for maintaining cellular and metabolic homeostasis. Scientists' interest in the mechanisms associated with autophagy involved in the pathogenesis of cancer and neurodegenerative diseases (Alzheimer's disease, Huntington's disease) and a range of other disorders is rapidly increasing. Reflecting this interest, the number of publications related to autophagy is increasing exponentially. Prous Institute has launched a research program focused on the conduct mechanisms of autophagy to discover new targets for the design of new small molecule modulators of autophagy as therapeutic agents. Several studies have shown that there may be links between autophagy and apoptosis, a subtype of programmed cell death. For this reason, our Institute has a particular interest in considering apoptosis in its research program 107 108 Autophagy eliminates intracellular microorganisms. Group A Streptococcus captured in an autophagosome. Image kindly provided by Tamotsu Yoshimori, Osaka University, Japan. b - France Mycobacterium bovis bacillus Calmette-Guérin (BCG) present in a mycobacterial autophagosome (MAP) that fuses with a multivesicular body (MVB). Image reproduced with the permission of Referee 42 © (2004) Cell Press. c - France Herpes simplex virus type I (HSV-1) virion (s) in the process of being surrounded by an isolated membrane (left panel), engulfed inside an autophagosome (middle panel) or degraded inside an autolysosome (right panel). Image reproduced with the permission of the ref. 77 © (2006) Landes Bioscience. 109 110 Inhibition of host autophagy by virusAutophagy is a preserved evolutionary mechanism for sequestration and subsequent lysosomal degradation of discrete intracellular parts of eukaryotic cells, facilitating the removal of und degraded materials through the ubiquitin-proteasome route. In addition, autophagy plays an important role in innate and adaptive immune responses to pathogens. Several viruses have developed ways to subvert the pathway for their own benefit in order to avoid the immune response or increase their viral replication. Beclin-1 is an essential autophagy protein and is the main target for the manipulation of autophagy by Virus. For example, HHV-1 ICP34.5 binds to Beclin-1 and inhibits autophagosome formation. HIV and influenza A virus use the same mechanism per nave and M2 respectively, which also interact and inhibit the host Beclin-1 111 Activation of host autophagy by virusAutophagy is a preserved evolutionary mechanism for sequestration and lysosomal degradation of discrete intracellular parts of eukaryotic cells, facilitating the removal of und degraded materials through the ubiquitin-proteasome pathway. In addition, autophagy plays an important role in innate and adaptive immune responses to pathogens Several viruses are capable of activating host autophagy as a mechanism of cellular survival. Viruses can activate programmed cell death during infection that prevents them from

spreading to healthy tissues. By activating autophagy, viruses delay or inhibit apoptosis. For example, the SV40 ST antigen protects glucose-deprived cancer cells by triggering autophagy. KSHV Rta is able to improve the autophagic process to facilitate viral lytic replication. 112 113 Application of autophagy modulation to cancer therapyApplication of autophagy modulation to cancer therapy. From the following article: Role of Autophagy in Cancer Robin Mathew, Vassiliki Karantza-Wadsworth and Eileen White Nature Reviews Cancer 7, (December 2007) a In defective apoptosis tumors that depend on autophagy to survive metabolic stress, autophagy inhibitors can be used to induce acute necrotic cell death that can be facilitated by proteasome inhibition, allowing tumor eradication. b - France In the adjuvant framework, and after the elimination of a large part of the tumor by radiotherapy and chemotherapy, the remaining cells may reside in a disturbed and stressed environment, sensitive to the inhibition of the survival mechanism of autophagy. Tumor cells that are being metastasized may also be vulnerable. c - France Autophagy stimulators may be therapeutically useful for promoting autophagic cell death or for preventing the detrimental effects of autophagy deficiency and mismanagement of metabolic stress leading to DNA damage and tumor progression. By limiting damage to proteins, organelles and ultimately DNA, autophagy stimulators can suppress tumor progression. In breast, ovarian and prostate cancers in men, where allelic loss of BECN1 occurs at high frequency, correcting autophagy deficiency with autophagy stimulators can delay tumor progression by reducing the rate at which tumor-promoting mutations accumulate. 114 115 Role of apoptosis and autophagy in tumorigenesis Tumor-carrying mutational events, such as oncogene activation, promote cell proliferation, but also apoptosis, which limits tumor growth. After acquisition of defects in apoptosis, tumor proliferation is sustained in the absence of apoptotic cell death. B France Tumor growth is initially limited by the absence of a blood supply, which can trigger autophagy-based survival in the most metabolically stressed tumor regions, usually the hypoxic center19, 20, 23. The eventual recruitment of a blood supply cures the tumor of hypoxia and metabolic stress, and tumor cells that once survive through autophagy may emerge to contribute to tumor growth30. c - France In tumors formed by cells with defects in apoptosis and autophagy, autophagy, Cell death is stimulated in metabolically stressed tumor regions and this necrosis is associated with the activation of an inflammatory response, DNA damage and tumor progression19, 20, 23. Analogous to a wound healing response, chronic necrosis and inflammation can stimulate angiogenesis and tumor growth24, 25, 26. 116 Molecular regulation of autophagyThe molecular regulation of autophagy. From the following article: The role of autophagy in the development of cancer and the response to Yasuko Kondo therapy, Takao Kanzawa, Raymond Sawaya and Seiji Kondo Nature Reviews Cancer 5, (September 2005) In the presence of growth factors, signaling of growth factor receptors activates the class I phosphatidylinositol 3-phosphate kinase (PI3K) to the plasma membrane to prevent cells from undergoing autophagy42. PI3K activates the downstream target AKT, leading to the activation of the mammalian target of rapamycin (mTOR), resulting in the inhibition of autophagy. p70S6 kinase (p70S6K) could be a good candidate for controlling autophagy downstream of mTOR. Overexpression of the phosphatase and tensin homologous gene (PTEN), by an inductible promoter, thwarts class I PI3K47 to induce autophagy. RAS has a dual effect on autophagy — when it activates class I PI3K25, autophagy is inhibited, but when it selectively activates RAF1-mitogen activated protein kinase kinase (MEK)-extracellular signal-regulated kinase cascade (ERK), autophagy is stimulated55. Rapamycin, a mTOR inhibitor, induces autophagy39. A Class III PI3K and Beclin 1 (BECN1) complex with a trans-Golgi network acts to induce autophagy49. This pathway is inhibited by 3-methyladenine (3-MA)42. BCL2 downregulation, or upregulation of BCL2-adenovirus E1B 19-kD-interacting protein 3 (BNIP3) or HSPIN1 to mitochondria, also induces autophagy, indicating that BCL2 protects against autophagy52, BNIP3 (Refs 36,53.57) and HSPIN1 (Ref. 54) trigger autophagy. Autophagy is also induced by the protein kinase associated with cell death (DAPK) and the protein kinase 1 (DRP1)51 associated with death. 117,118 Potential Strategies to Treat Cancer by Manipulating Autophagic Processa Cancer cells that have defects in the autophagic pathway could be treated by replacing the autophagic signal with the expression of beclin 1 (BECN1) or the homologous phosphatase and tensin tumor suppressor (PTEN), resulting in the induction of autophagy and cell death, or inhibition of proliferation. b - France Cancer cells that are able to undergo autophagy in response to anticancer therapies could be treated with autophagy inducers, such as rapamycin, to promote autophagy-induced cell death. c - France The cancer patients who undergo autophagy, to protect against the effects of anticancer therapies, could be treated with autophagy inhibitors, such as bafilomycin A1 or short interfering RNAs specific to autophagy-related genes, to induce apoptosis. 119 120 121 A matter of life and death (or both): Understanding autophagy in CancerA, in nutrients (glucose, amino acids and growth factors), protein synthesis is stimulated and autophagy is inhibited. This is 20 thanks to the activation of mTOR (via the activation of PI3K and Akt and the inactivation of the tubercious complex of sclerosis TSC1 and TSC2). mTOR phosphorylates S6 kinase and increases the translation of RNAMs that encode ribosomal proteins and other proteins involved in translation. This initiates translation into 4EBP1 phosphorylant, an initiation inhibitor, causing its dissociation from eIF4E. Active eIF4 promotes cell proliferation by increasing the translation of cycline D1, c-Myc, and endothelial vascular growth factor. mTOR and S6 kinase also release cellular control over peptide elongation in phosphorylating and by inactivating eEF-2 kinase. eEF-2 kinase phosphorylates eEF-2, a 100 kDa protein that mediats the translocation stage in peptiide-chain elongation by inducing the transfer of peptidyl-tRNA from ribosomal site A to P. The phosphorylation of eEF-2 to Thr56 by kinase eEF-2 decreases the affinity of the lengthening factor for ribosomes and ends lengthening. The activation of TOR in yeast inhibits the induction of autophagy by phosphorylation of the APG1-APG-13 complex, a process inhibited by rapamycin in yeast and mammalian cells. B, autophagosome formation. This process requires the coordinated efforts of a series of genetic products that respond to nutrient deprivation (regulatory complex of autophagy), lipid kinase signaling molecules that participate in the formation of vesicles, ubiquitin proteins that complete the formation of vesicles, and a protein complex that mediates the dismantling of autophagosome. The initial step is the wrapping of cytoplasmic materials in a phagophore or insulating membrane. This leads to the sequestration of the cytoplasm in the autophagosome, which are characterized by a double membrane decorated with the microtubule-associated protein 1 light chain 3 (LC3). The fusion of autophagosomes with lysosomes for autolysosome leads to the acidification and degradation of cytoplasmic components for the recycling of amino acids and fatty acids for energy production. Clin Cancer Res 2006;12: William N. Hait, Shengkan Jin and Jin-Ming Yang Autophagy in Cancer A Matter of Life or Death (or Both): Understanding 122 123 ARHI is necessary for autophagy. The ARHI tumor suppressor gene regulates autophagy and tumor dormancy in human ovarian cancer cells Zhen Lu, Robert Z. Luo, Yiling Lu, Xuhui Zhang, Qinghua Yu, Shilpi Khare, Seiji Kondo, Yasuko Kondo, Yinhua Yu, Gordon B. Mills, Warren S.-L. Liao, Robert C. Bast J Clin Invest. 2008; 118(12): 3917-3929 ARHI is required for autophagy. (A-J) ARHI and rapamycin (RM) induce autophagy ovarian cancer cells. (A-D) SKOv3-ARHI cells were transfected with GFP-LC3 and treated with or without DOX to induce ARHI expression or with or without rapamycin to inhibit mTOR activity. Scale bar: 1m. (E-J) The ES2 and OC316 cells were not transfected or were with arhi expression vector and ARHI siRNA or siRNA control for 24 hours before they were transfecated with GFP-LC3. The cells were treated with 50 nM rapamycin at the time of siRNA transfection and examined for autophagy by fluorescence microscopy 48 hours later. Scale bar: 1m. (K) ARHI expression is necessary for rapamycin-induced autophagy in ovarian cancer cells. Ovarian cancer cells ES2 and OC316 were not transfected or were transfected with ARHI or control siRNA for 48 hours. The expression of ARHI and control gapdh were examined by RT-PCR. (L-U) NEZ cells undergo spontaneous autophagy. (L-O) Two nose cell lines were transfected with GFP-LC3 and treated with or without rapamycin. Scale bar: 1m. (P-U) The GFP-LC3 plasmid was transfecated into OSE106 cells alone or was cotransfected with siRNA arhi or siRNA control. Transfected cells were treated with or without rapamycin. Scale bar: 1m. 124 125 126 127 128 129 130 131 132 133 The impaired capacity of apoptosis and autophagy may dictate cellular fate in response to metabolic stress.a Apoptosis is a common response to metabolic stress in which cells activate caspases and die effectively. For immortal epithelial cells, metabolic stress triggers apoptosis within 24 to 48 hours19, 20, 23. The execution of apoptosis occurs in less than an hour and the loss of cellular viability is five to six orders of magnitude19. b - France Defective autophagy (through the loss of BECN1 or ATG5, for example) may increase the death of apoptotic cells in some cells in response to metabolic stress56. In human breast cells in in vitro 3D culture, this accelerated apoptosis manifests itself in increased lumen formation in the breast acini20. Therefore, the preservation of cellular metabolism by autophagy could increase the threshold for the activation of apoptosis. c - France In cells with defects in apoptosis, survival in metabolic stress depends on autophagy and is prolonged for weeks19, 20, 22, 23. During the maintenance phase, activities such as cell division and motility are supported. Prolonged starvation and progressive autophagy cause a gradual decrease in cell size, but restoring nutrients allows recovery. In the preservation phase, cell division and motility decrease, presumably in the form of bioenergenic conservation efforts, creating the minimum cell capable of recovery (MCCR). Finally, restoring nutrients does not allow recovery. In this way, autophagy can be considered an interrupted path to cell death. d - France The with defects in apoptosis and autophagy do not tolerate metabolic stress, suffer metabolic catastrophe and die by necrosis36. 134 135 Molecular events in autophagyMolecular events in autophagy. From the following article: Unveiling the roles of autophagy in the innate and adaptive immunity Beth Levine and Vojo Deretic Nature Reviews Immunology 7, (October 2007) Autophagy is regulated by a set of proteins related to autophagy (ATG proteins). In the absence of amino acids or in response to other stimuli, ATG1 and a Class III PI3K PI3K complex 3-kinase) VPS34 and beclin 1 lead to the activation of downstream ATG factors that are involved in initiation (a), lengthening (b) and maturation (c) of autophagy. a - France Under conditions rich in amino acids, VPS34 contributes to the activation and inhibition of ATG1 and autophagy by mTOR (mammalian target of rapamycin). The membrane sources for autophagosome initiation and elongation may include those containing the only known integral membrane ATG atG9 protein, redistributing between a resting place to autophagosomes in an ATG1- and PI3K-dependent manner. The ATG9 redistribution may depend on ATG18, which binds phosphatidylinositol-3-phosphate (PtdIns3P). b - France The lengthening and shape of the autophagosome are controlled by two protein (and lipid) conjugation systems, similar to ubiquity systems: the ATG12 and LC3 conjugation pathways (also known as ATG8)-phosphatidylethanolamine (PE), which include enzymes conjugated with E1 and E2. ATG12 is first combined with ATG7 (an E1 activating enzyme) and then transferred to the E2 conjugate enzyme ATG10. This intermediary presents ATG12 for conjugation with an ATG5 lysine residue. The ATG5-ATG12 conjugate, stabilized non-covalent by ATG16, triggers oligomerization on the outer membrane of the growing autophagosome, and improves carboxy-terminal lc3 lipidation by the LC3 conjugation system. When the autophagosome is closed, ATG5-ATG12-ATG16 and LC3 (delipided by ATG4) are recycled. c - France LC3 associated with the lemeral membrane remains trapped in the autophagosome and is degraded during maturation in the autolysosome, which involves the fusion of autophagosomes with late endosomes, including endosomal multivehocular bodies and lysosomal organelles, and dissolution of the internal membrane. VPS34 has a role in the formation of late endosmalular multivescular bodies and lysosomaous organelles contributing to the maturation stages of autophagy. 136 Functions of autophagy in innate and adaptive immunity during infection with intracellular pathogens. 137 a Intracellular pathogens (bacteria, parasites and viruses) that are either free inside the cytosol, inside phagosomes or inside pathogen-containing vacuoles, are surrounded by isolated membranes, engulfed in autophagosomes, which fuse with lysosomes and then degrade inside autolysosomes. b - France Viral nucleic acids are transferred by cytoplasm autophagy to intracellular compartments containing the toll-like receptor 7 (TLR7), which signals induction of type I interferon (IFN) production. c - France Viral antigens (and potentially other endogenous and auto-antigen synthesized microbial antigens) are engulfed in autophagosomes that fuse with endorsers containing Class II MHC (MIICs), then loaded onto Class II molecules of the MHC for presentation to CD4 T cells. Cytosolic antigens that contain a KFERQ recognition pattern can also be directly imported into IBDs by chaperone-mediated autophagy. CLIP, invariant chain associated with Class II II 138 139 140 141 Autophagy Regulators Table 1: Some autophagy small molecule inducers. Target Effect Cell Line Starvation Inhibits (mTOR) Active Autophagy HeLa, HepG2, Jurkat Rapamycin HeLa PP242 COMPETITIVE ATP INHIBITOR of mTOR Lithium mTOR independent Trehalose Unknown, MTOR-independent Bafilomycin A1 Inhibits Vacuolar-ATPase Inhibits lysosome Chloroquine Alkalinizes Lysosomal pH Tamoxifen Abolish effect PI3K inhibitor Activates autophagy and inhibits verapamil Hydroxychloroquine Loperamide Clonidine MG-132 Selective proteasome hela inhibitor function, Jurkat Norclomipramine 142 143 144 How to live long and prosper: Autophagy, Mitochondria, and AgingFIGURE 1. Schematic diagram of selective and non-selective autophagy Three fundamentally different modes of autophagy are macroautophagy, microautophagy, and chaperone-media autophagy. Depending on the specificity of the cargoes, autophagy can be a selective or non-selective process. During non-selective autophagy, part of the cytoplasm is sequestered in a double membrane autophagosome, which then merges with the lysosome/vacuole. On the other hand, the specific degradation of peroxisomes under certain conditions can be achieved by a macro- or microautophagy-like mode, called macropexophagy and micropexophagy, respectively. The fragmentary microautophagy of the nucleus allows the degradation of part of the nucleus. The specific degradation of mitochondria, called mitophagy also takes place. A biosynthetic cytoplasm at the vacuole targeting pathway (Cvt) in yeast also shares similar morphological characteristics. Note that this diagram illustrates aspects of autophagy in yeast cells and higher eukaryotes. 145 Schematic diagram of selective and non-selective autophagy physiology, vol. 23, No. 5, , October 2008 Int. Union Physiol. Sci./Am. Physiol. Soc. REVIEW:How to Live Long and Prosper: Autophagy, Mitochondria, and Aging Wei-Lien Yen and Daniel J. Klionsky Schematic diagram of selective and nonselective autophagy Three fundamentally different modes of autophagy are macroautophagy, microautophagy and chaperon-based autophagy. Depending on the specificity of the cargoes, autophagy can be a selective or non-selective process. During non-selective autophagy, part of the cytoplasm is sequestered in a double membrane autophagosome, which then merges with the lysosome/vacuole. On the other hand, the specific degradation of peroxisomes under certain conditions can be achieved by a macro- or microautophagy-like mode, called macropexophagy and micropexophagy, respectively. The fragmentary microautophagy of the nucleus allows the degradation of part of the nucleus. The specific degradation of mitochondria, called mitophagy also takes place. A biosynthetic cytoplasm at vacuole targeting pathway (Cvt) in yeast sharing similar morphological characteristics. Note that this diagram illustrates aspects of autophagy in yeast cells and higher eukaryotes. 146 146 autophagy. Autophagy is a major housekeeping route and under the control of many different signaling cascades. Mammalian Target of rapamycin (mTOR) plays a central role in regulating autophagic activity as it integrates signaling from different sensors of cellular homeostasis. When mTOR is active in yeast, it retains an important ULK1 binding partner (ATG13) phosphorylated, thus inhibiting the induction of autophagy. While signals of abundant nutritional and trophic support activate mTOR (and disable autophagy), starvation signals or other stressors inhibit mTOR (and activate autophagy). Autophagy can be directly stimulated by intracellular debris (such as unfolded proteins and damaged organelles) or by indicators of a submerged ubiquitin-proteasome system (UPS). Some pathogens also activate autophagy. Autophagy can be directly inhibited by the genetic ablation of important Atg genes, inhibitors of the Class III PI3K complex (WM, 3-MA), high nutrient levels and inositol signaling. More recently, screenings of small composite libraries have yielded autophagy inducers and inhibitors, both dependent on mTOR and independent. Finally, transcriptional regulators, such as p53, eIF2, E2F4 or FOXO3, regulate autophagy by controlling the expression levels of many Atg genes. For more details. Jaeger and Wyss-Coray Molecular Neurodegeneration:16 147 The Molecular Machinery of Autophagy: Unanswered QuestionsRegulation of Induction and Vesicular Nucleation. The regulation of autophagy has been characterized in studies on yeast and mammalian cells. (A) In yeast, a Class III PI 3-K is required for autophagic activity and can work at the pre-autophagosomal membrane. A putative complex consisting of Atg1 kinase and several other proteins characterized as being necessary primarily for autophagy (in purple) or the Cvt pathway (in green) may be an effector downstream of Tor kinase to regulate the type of pathway that works, depending on nutritional conditions or other signals. Autophagy in yeast is primarily a famine response; Tor, along with other unstable regulatory components (including pka), responds to nutrient levels. Under nutrient-rich conditions, Atg1 and Atg13 are more strongly phosphorylates and have a lower affinity for each other; during famine, both proteins are partially dephosphoric. The PI 3-K I complex, consisting of Vps15, Vps34, Atg6/Vps30 and Atg14, is required for both the Cvt track and autophagy. (B) In mammalian cells, a Class I 3-K PI is stimulated in response to a ligand binding to a receptor such as insulin (InR). PtdIns (3.4)P2 and PtdIns (3,4.5)P3 generated at the plasma membrane allow the binding and activation of the protein kinase 1 dependent on phosphoinositide 3 (PDK1) and Akt/PKB, while PTEN counteracts this pathway by its activity phosphatase phosphoinositide 3-phosphoinositide. Akt inhibits the TSC1-TSC2 protein complex that activates gtpase, which to activate, resulting in the inhibition of autophagy. Tor and PDK1 stimulate kinase p70S6 (p70S6k). Downregulation of p70S6k activity in starvation conditions (when Tor is inhibited) may prevent excessive autophagy (Scott et al., 2004). It is also possible that p70S6k indirectly inhibits Tor by interfering with the activation of Class I PI 3-K, as suggested by studies in mammalian cells (Um et al., 2004). Under nutrient-rich conditions, the activation of p70S6k should inhibit ft 3-K, allowing a low level of autophagy for homeostatic purposes, while under starvation conditions the possible inactivation of p70S6k should allow the activation of 3-K PI to prevent excessive autophagy. Class III PI 3-K serves a stimulating role perhaps similar to that of the yeast enzyme complex. 148 The regulation of autophagy - unanswered questionsDynamics of Atg1 complexes on autophagy induction in different eukaryotes. (A) In yeast, under nutrient-rich conditions, the active hyperphosphorylates of the TOR 1 complex (TORC1) Atg13 (Kamada et al., 2010). This prevents the association of Atg1 with Atg13, which is related to Atg17, Atg31 and Atg29, leading to the inhibition of autophagy induction. In starvation conditions when TORC1 is inactivated, Atg13 is no longer phosphorused by TORC1, while Atg1 is autophosphorylated, leading to the association of Atg1 with the complex between Atg13, Atg17, Atg31 and Atg29, and subsequent autophagie induction (Cebollero and Reggiori, 2009; Chang and Neufeld, 2010; Kamada et al., 2010; Nakatogawa et al., 2009). (B) Unlike yeast, the mammalian ULK (ULK1 or ULK2, the homologs of the Atg1 yeast) forms a stable complex with the mammal Atg13, FIP200 (a putative counterpart of the yeast Atg17) and Atg101 (an Atg13 binding protein), regardless of the activation of TORC1. Under nutrient-rich conditions, active TORC1 is associated with the ULK complex (or ULK1 (or ULK2)-Atg13-FIP200-Atg101), ulK1 phosphorylates (or ULK2) and atg13 hyperphosphorylates, which inhibits ULK1 kinase activity (or ULK2) and thus blocks the induction of autophagy. In conditions of famine when TORC1 is inactivated, TORC1 dissociates itself from the ULK complex, preventing the phosphorylation of Atg13 and ULK1 (or ULK1) by TORC1 and leading to the induction of autophagy, while ULK1 (or ULK2) still phosphorylate Atg13 and himself, and hyperphosphorylates FIP200 (Chang and Neufeld, 2010; Mizushima, 2010; Yang and Klionsky, 2010). (C) Similar to the situation in mammals, in Drosophila Atg1 forms a complex with Atg13 independently of TORC1 activation (Chang and Neufeld, 2010). Under nutrient-rich conditions, TORC1 active phosphorylates Atg13 and atg1 hyperphosphorylates, leading to inhibition of autophagy induction. In conditions of famine, when TORC1 is Atg1 and Atg13 are no longer phosphorylaté by TORC1, while Atg1 still gets phosphorylate and hyperphosphorylates Atg13, leading to the induction of autophagy. Chang and Neufeld (Chang and 2010) with permission. 149 150 151 Link different treatments extending the lifespan to autophagy.closeSummary from the genetic and pharmacological manipulations of autophagy that cause the extension of lifespan. Pharmacological treatment with spermidine, resveratrol or rapamycin, caloric restriction, depletion of p53 or overexpression of sirtuin 1 prolong lif... 152 Can autophagy promote longevity? Frank Madeo,1 Nektarios Tavernarakis2 and Guido Kroemer3 Affiliations Journal name: Nature Cell Biology Volume: 12, Pages: 842-846 (2010) Abstract Organismal lifespan can be extended by genetic manipulation of cellular processes such as histonety acelation, the insulin/IGF-1 (insulin-like growth factor 1) pathway or the p53 system. Longevity-enhancing diets, including caloric restriction and inhibition of TOR with rapamycin, resveratrol or natural polyamine spermidine, have been associated with autophagy (a cytoprotective auto digestive process) and in some cases have been reported to require autophagy for their effects. We summarize recent developments that describe these links and hypothesize that compensating for cellular damage by autophagy is a common denominator of many manipulations that extend lifespan. 153 Linking the extension of life-life autophagy-mediation to cytoprotection.closePutative mechanisms linking autophagy to the inhibition of cell death (apoptosis or necrosis) and the induction of longevity. Longevity.