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Autophagy and apoptotic genes implicated in Alzheimer's disease are modulated following infection of neuronal cells with Chlamydia pneumoniae

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Autophagy and apoptotic genes implicated in Alzheimer's disease are modulated following infection of neuronal cells with *Chlamydia pneumoniae*

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Abstract

Background: The focus of the current studies was to determine the relationship between the molecular mechanisms interconnecting autophagy and apoptosis following *Chlamydia pneumoniae* infection in neuronal cells. Dysfunctions in apoptosis and autophagy have been implicated in the neurodegeneration associated with Alzheimer's disease (AD). Autophagy in AD pathogenesis has been shown to play a role in amyloid processing through the endosomal-lysosomal system. Apoptosis may contribute to the neuronal cell loss observed in AD; however, there is limited evidence of the apoptotic process proceeding to terminal completion. Although Aβ1-42 has been shown to induce apoptosis in neurons and may be an early factor in AD, our previous investigations demonstrated that neurons infected with *Chlamydia pneumoniae* are resistant to apoptosis, and that Aβ1-42 is induced following this infection. Thus, these studies address infection as an initiator/trigger or inhibitor for the processes of autophagy and apoptosis observed in Alzheimer's disease.

Methods: SKNMC neuronal cells obtained from ATCC were infected with the AR39 strain of *Chlamydia pneumoniae* at an MOI=1 for 24, 48, and 72hrs and were analyzed using Real-time PCR arrays from SABiosciences specific for autophagy and apoptosis genetic markers.

Results: Some major genes associated with apoptosis such as BID, DAPK1, TP53, TP73 were down regulated by 72hrs post-infection. Genes associated with the regulation of autophagic vacuole formation such as ATG3, ATG4B, ATG4C, ATG9A, ATG9B, ATG12, IRGM, and BECN1 were up-regulated within 72hrs post-infection. With regards to genes involved with co-regulation of autophagy and apoptosis, BNIP3 was significantly up-regulated within 48-72hrs post-infection. Of the genes linking autophagosomes to lysosomes, FAM176A was up-regulated throughout 24-72hrs post-infection.

Conclusions: Modulation of autophagy and apoptosis genes occurs in neuronal cells at 24, 48, and 72hrs post- infection with *Chlamydia pneumoniae*. These genetic changes lead to dysfunction in these basic cellular processes; dysfunction in these processes has been shown to contribute to the neuropathology of late-onset Alzheimer's disease. This work will allow future studies to further focus on the apoptotic and autophagic pathways to better understand how a pathogen such as *Chlamydia pneumoniae* plays a role in the development of late-onset Alzheimer's disease.

Introduction

Neurodegeneration has been well documented in the CNS of Alzheimer individuals and a continuum of abnormalities has been identified within the AD brain. Strong evidence suggests that abnormalities of the autophagy and apoptosis pathways are contributing factors in the pathogenesis of Alzheimer's disease. Our laboratory has focused on studying the role of infection with *C. pneumoniae* as a causative agent in late onset AD. In separate studies, polymerase chain reaction detected *C. pneumoniae* DNA in 80 to 90% of postmortem sporadic AD brain samples [1,2], but only 5-11% of postmortem brain samples from age-matched, non-AD, control individuals. Furthermore, a murine model has been developed in which mice infected with *C. pneumoniae* demonstrate deposits of amyloid in brain areas typically affected in AD [3].

Recently, we have demonstrated that *C. pneumoniae* is capable of inhibiting apoptosis in neuronal cells thereby prolonging the viability of the infected neuronal cell [4]. Other laboratories have demonstrated that chlamydiae-infected host cells were resistant to proapoptotic stimuli such as TNFα, Fas antibody, staurosporine, and UV-light [5]. *C. pneumoniae* infection has also been shown to modulate pro-apoptotic cytoplasmic proteins, such as caspase-3 and cytochrome c, as well as the anti-apoptotic mitochondrial protein Bcl-2 and the anti-apoptotic nuclear protein NF-κB[6].

In some instances, cells may activate the autophagy pathway instead of, or before, initiating apoptosis. Induction of the autophagic process may lead to modulation of the apoptotic process. Autophagy is associated with the endosomal-lysosomal system, in which contents of an autophagosome are degraded as a result of the autophagosome fusing with the lysosome[7,8]. An increase in the number of autophagic vacuoles (AVs) was identified in neurons from AD brains implicating autophagy as a pathological process in AD [9]. The endosomal pathway is linked to the lysomal system because early endosomes fuse with late endosomes or lysosomes. Neurons from AD brains have been found to exhibit enlarged early endosomes. This is significant in AD because early endosomes take in proteins such as apolipoprotein E and APP, and it has been determined that Aβ is formed in early endosomes [10].

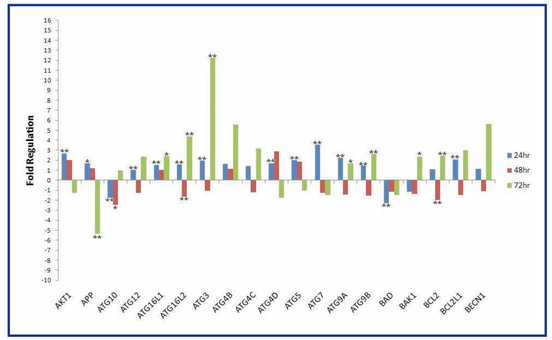
Typically, autophagy and apoptosis are common pathways by which infected cells attempt to rid themselves of the infectious agent and cells incapable of eliminating the infectious agent, undergo cell death. Intriguingly, these pathways appear to be altered in *C. pneumoniae* infected neuronal cells. These studies are an attempt to identify the defects in the regulation of genes associated with apoptosis and autophagy in neuronal cells as observed in AD following an infection by *C. pneumoniae*

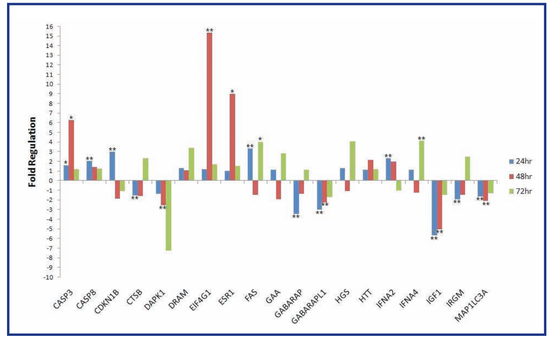
Material and Methods

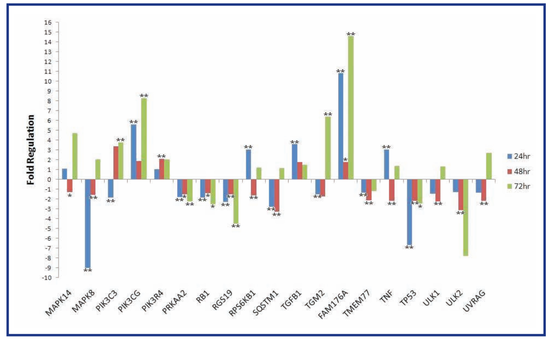
The SKNMC (ATCC) human neuroblastoma cell line were infected with ATCC's AR39 strain of *Chlamydia pneumoniae* at an MOI = 1 for 24, 48, and 72 hrs. Cells were immunolabled with FITC- chlamydia antibody 61C75 (Fitzgerald) for verification of infection. The Human Autophagy RT² Profiler™ PCR Array from SAbiosciences was used to analyze the expression of a panel of genes involved in autophagy and apoptosis. All data was derived from experiments performed in triplicate.

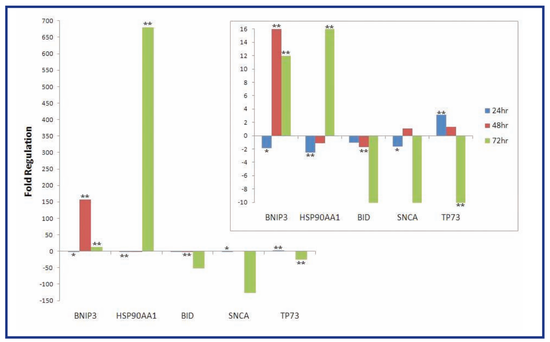
Results

RT-PCR of SKNMC neuronal cells infected with *C. pneumoniae* for 24, 48, and 72hrs



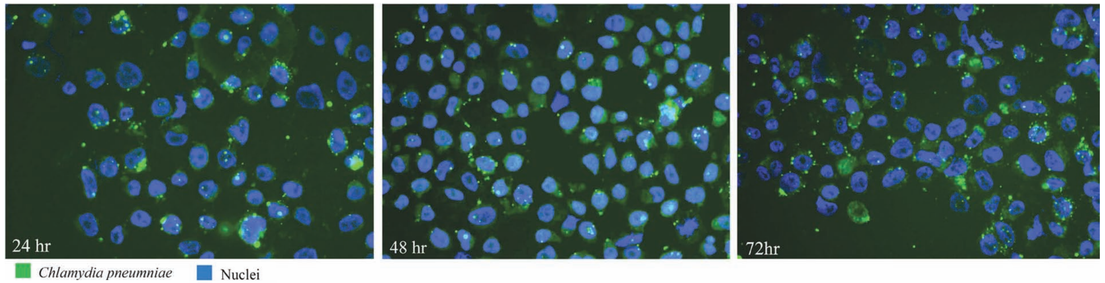






Varification of *C. pneumoniae* infection for 24, 48, and 72hrs by immunocytochemistry.

Varification of *C. pneumoniae* infection in neuronal cells used in RT-PCR gene arrays



	Function	Gene Symbol Gene Name
Autophagy Machinery Components		
•Autophagic Vacuole Formation		
	AMBRA1	Autophagy/beclin-1 regulator 1
	ATG4A	ATG4 autophagy related 4 homolog A (S. cerevisiae)
	ATG4C	ATG4 autophagy related 4 homolog C (S. cerevisiae)
	ATG9A	ATG9 autophagy related 9 homolog A (S. cerevisiae)
	BECN1	Beclin 1, autophagy related
	MAP1LC3B	Microtubule-associated protein 1 light chain 3 beta
	ATG4D	ATG4 autophagy related 4 homolog D (S. cerevisiae)
•Genes Linking Autophagosome to Lysosome		
	DRAM	Damage-regulated autophagy modulator
	FAM176A	Family with sequence similarity 176, member A
• Protein Transport		
	ATG10	ATG10 autophagy related 10 homolog (S. cerevisiae)
	ATG16L1	ATG16 autophagy related 16-like 1 (S. cerevisiae)
	RAB24	RAB24, member RAS oncogene family
Co-Regulators of Autophagy and Apoptosis		
	BAK1	BCL2-antagonist/killer 1
	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3
	CASP3	Caspase 3, apoptosis-related cysteine peptidase
	FAS	Fas (TNF receptor superfamily, member 6)
	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
Autophagy Induction by Intracellular Pathogens		
	IFNA2	Interferon, alpha 2
	IFNA4	Interferon, alpha 4
	IFNG	Interferon, gamma
Autophagy in Response to Other Intracellular Signals		
	ARSA	Arylsulfatase A
	EIF4G1	Eukaryotic translation initiation factor 4 gamma, 1
	ESR1	Estrogen receptor 1
	PIK3C3	Phosphoinositide-3-kinase, class 3
	PIK3R4	Phosphoinositide-3-kinase, regulatory subunit 4
	PRKAA2	Protein kinase, AMP-activated, alpha 2 catalytic subunit
	TMEM74	Transmembrane protein 74
	TMEM77	Transmembrane protein 77
	ULK2	Unc-51-like kinase 2 (C. elegans)
	UVRAG	UV radiation resistance associated gene

Conclusions

Modulation of genes associated with the autophagy and apoptosis pathways is observed in neuronal cells after 24, 48, and 72hrs of infection with *C. pneumoniae*. These genetic changes lead to dysfunction in the basic cellular processes of autophagy and apoptosis; consequently dysfunction in these processes has been shown to contribute to the neuropathology of late onset Alzheimer's disease. This work will allow future studies to further focus on the analysis of genes involved in the apoptotic and autophagic pathways at to better understand how a pathogen such as *C. pneumoniae* plays a role in the development of AD.

Funding

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