Regulation of autophagy by NFκB transcription factor and reactivies oxygen species.
Mojgan Djavaheri-Mergny, Manuella Amelotti, Julie Mathieu, Francoise Besançon, Chantal Bauvy, Patrice Codogno

To cite this version:

HAL Id: insertm-00175265
http://www.hal.inserm.fr/inserm-00175265
Submitted on 19 Mar 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Addendum

Regulation of Autophagy by NFκB Transcription Factor and Reactives Oxygen Species

Mojgan Djavaheri-Mergny1,2,*  
Manuella Amelotti1-3  
Julie Mathieu4  
Francoise Besançon4  
Chantal Baudy1,2  
Patrice Codogno1,2

1INSERM U756, Châtenay-Malabry, France  
2Université Paris-Sud, Faculté de Pharmacie, Châtenay-Malabry, France  
3Laboratory of Biochemistry and Molecular Biology, San Paolo Medical School, Milan, Italy  
4INSERM U685, Centre Hayem, Hôpital Saint-Louis, Paris, France  
*Correspondence to: Mojgan Djavaheri-Mergny; INSERM U756, Faculté de Pharmacie, Université Paris-Sud 11, 5, Rue Jean-Baptiste Clément, Châtenay-Malabry 92296 France; Tel.: 33.1.46.83.55.28; Fax: 33.1.46.83.58.44; Email: mojgan.mergny@u-psud.fr

Original manuscript submitted: 04/02/07  
Manuscript accepted: 04/09/07  
Previously published online as an E-publication:  
http://www.landesbioscience.com/journals/autophagy/article.php?id=4248

ABSTRACT

The NFκB transcription factor is an important anti-apoptotic factor, which is frequently deregulated in cancer cells. We have recently demonstrated that NFκB activation mediates the repression of autophagy in response to TNFα in three models of cancer cell lines. In contrast, in the absence of NFκB activation, TNFα induces macroautophagy (autophagy), which requires reactive oxygen species (ROS) production and participates in the TNFα-induced apoptotic signaling pathway. Autophagy-dependent apoptosis was also observed following direct addition of ROS to cells. Moreover, addition of rapamycin to TNFα renders these cells susceptible to the cytotoxic effect of this cytokine. These findings highlight the regulation of autophagy by oxidative stress and support the idea that repression of autophagy by NFκB may constitute a novel anti-apoptotic function of this transcription factor. We also bring evidence that direct stimulation of autophagy may represent a new therapeutic strategy for overcoming the NFκB-dependent chemoresistance of cancer cells.

INACTIVATION OF NFκB ACTIVATES AUTOPHAGY IN TNFα-TREATED CELLS

NFκB is now widely recognized as a major culprit in cancer.1 This transcription factor activates genes whose products are involved in several tumor-promoting signals including those that favor cell survival, metastasis and pro-inflammatory responses.2 Autophagy is another mechanism involved in the control of cancer3 but little is known about its modulation by apoptosis-regulatory factors.

We investigated whether NFκB, which is a key regulator of apoptosis, modulates autophagy. For this purpose, we compared autophagic activity in response to TNFα, an efficient stimulator of NFκB activation, in NFκB-competent cells versus cells carrying a repressor of NFκB activity. We observed that, in cell lines derived from three types of cancer (Ewing sarcoma, breast cancer and acute promyelocytic leukemia), TNFα-induced NFκB activation causes repression of autophagy.4 In accordance with our results, it has been recently reported that inhibition of NFκB results in an enhancement of starvation-induced autophagy.5 Nevertheless, the mechanism involved in such NFκB-mediated repression of autophagy remains largely unknown. One possibility is an NFκB-dependent modulation of the mTOR pathway which is known to negatively regulate autophagy.6 This hypothesis is based on our observation that mTOR activity is induced by TNFα but only in NFκB-competent cells.4 Another possibility is a modulation of the Beclin 1/Bcl-2 balance by NFκB since Bcl-2 can control autophagy by interacting with Beclin 1,7 and NFκB can regulate Bcl-2 levels.8

Interestingly, recent studies provided evidence that, in turn, NFκB activity can be modulated by autophagy-related signaling. Indeed, NFκB activity is positively regulated by an inhibitor of the mTOR pathway, TSC2.9 Inversely, the IκB kinase, an upstream activator of NFκB, was shown to be degraded by autophagy following loss of Hsp90 function10 supporting that, in certain conditions, NFκB activity can be negatively regulated by autophagy. Such a repression of NFκB activity by autophagy may constitute an amplifying loop of cell death since both apoptosis and autophagy are inhibited by this transcription factor.

REGULATION OF AUTOPHAGY BY NFκB IS A REDOX-SENSITIVE MECHANISM

We and others have previously shown that inhibition of NFκB activation results in increased ROS production in TNFα-treated cells.11,12 We found that this accumulation...
NFκB, ROS and Autophagy

AUTOPIHAGY CONTRIBUTES TO THE INDUCTION OF APOPTOSIS IN TNFα-TREATED CELLS

The involvement of autophagy in the apoptotic pathway has recently been a subject of debate. Under certain stress conditions, autophagy displays an anti-apoptotic function, whereas recent findings support a pro-apoptotic role for this process.3 We found that knockdown of autophagy effectors with small interfering RNAs reduced TNFα-induced apoptosis in cells carrying a repressor of NFκB activation. A similar effect was observed in both NFκB-competent and NFκB-incompetent cells treated with exogenous ROS. These results indicate that autophagy participates in the apoptotic signaling pathway induced by these compounds.

Figure 1. Stimulation of autophagy by rapamycin sensitizes Ewing sarcoma-derived cell lines to the cytotoxic effect of TNFα. Ewing sarcoma cells were transfected with green fluorescent protein (GFP) fused to LC3 protein, a well-established marker of autophagosomes. Twenty-four hours later, cells were incubated with rapamycin (400 nM) for 2 h prior treatment with or without TNFα (2000 units/ml). The extent of autophagy was determined by the analysis of GFP-LC3 distribution in cells by fluorescence microscopy. The percentage of cells with GFP-LC3 fluorescent dots (indicative of the redistribution of GFP-LC3 into autophagosome) per total GFP-LC3 cells was scored 16 h after the beginning of TNFα treatment (black bar). For each condition, cells were also subjected to Hoechst staining and the percentage of apoptotic cells was determined (white bar). Results shown are the mean ± SD of three independent experiments.

Figure 2. Proposed models for the cross-talk between autophagy and the NFκB- and ROS-activated pathways. (A) TNFα signaling pathway causes NFκB activation that subsequently induces several anti-apoptotic responses, including the repression of autophagy. This inhibition of autophagy is probably due to the NFκB-dependent inhibition of ROS accumulation. Rapamycin (an inducer of autophagy) associated with TNFα treatment sensitizes cells to TNFα-induced apoptosis. In turn, some observations argue for the regulation of NFκB activity by autophagy. (B) Conversely, in the situation where NFκB activity is impaired, TNFα upregulates the expression of the autophagy-promoting protein Beclin 1 and subsequently induces autophagy through a ROS-dependent mechanism. Similar responses have been observed following direct addition of ROS. In both cases, the stimulation of autophagy contributes to apoptotic signaling pathways. X, Y and Z represent other pro-apoptotic pathways that are stimulated in response to TNFα.

www.landesbioscience.com

Autophagy
These observations prompted us to investigate whether direct induction of autophagy could sensitize NFκB-competent cells to TNFα-induced apoptosis. For this purpose, cells were pretreated with rapamycin (a well-known activator of autophagy) prior to addition of TNFα. As shown in Figure 1, although NFκB-competent cells are completely resistant to the cytotoxic effect of TNFα, the addition of rapamycin induced an accumulation of autophagic vacuoles and rendered these cells susceptible to the cytotoxic effect of TNFα. Of note, TNFα treatment reduced the stimulation of autophagy induced by rapamycin which is in accordance with our result showing that NFκB activation by TNFα inhibited autophagy. It is worth noting that rapamycin, when used alone, did not increase the percentage of apoptotic cells. These results suggest that autophagy amplifies apoptosis when associated with a death receptor signaling pathway. One possible mechanism involved in such a pro-apoptotic effect of autophagy induced by rapamycin may be mediated, at least in part, by the inhibition of NFκB activity by this agent.

CONCLUSION

Altogether, these findings delineate the crosstalk between autophagy, NFκB and ROS (Fig. 2) and raise the question as to whether inducers of autophagy (rapamycin and its analogues) can be used in combination with anti-cancer therapies that activate NFκB in order to improve their effectiveness.

References