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Addendum

Regulation of Autophagy by NFκB Transcription Factor and Reactives Oxygen Species

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Addendum to:

NF-κB Activation Represses TNF-α-Induced Autophagy

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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 cancer[3] 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 stimulation 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 function[10] 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

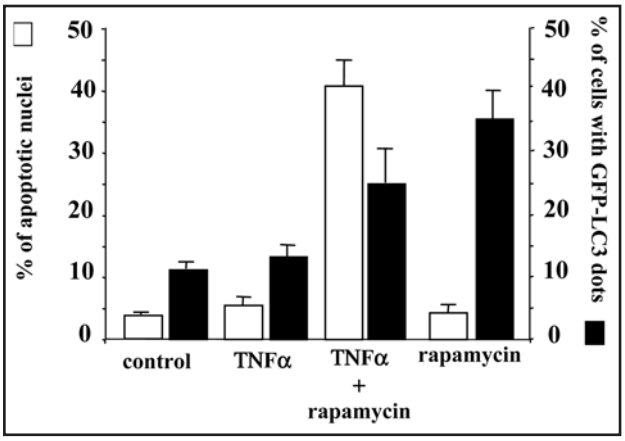

Figure 1. Stimulation of autophagy by rapamycin sensitizes Ewing sarcoma-derived cell lines to the cytotoxic effect of TNFα. Ewing sarcoma cells were transfected with green fluorescent protein (GFP) fused to LC3 protein, a well-established marker of autophagosomes. Twenty-four hours later, cells were 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.

of ROS is responsible of the induction of autophagy in TNFα-treated cells carrying a repressor of NFκB activation. In addition, we provide evidence that direct addition of exogenous ROS is also able to induce autophagy in these cells.Consistent with these observations, both TNFα and ROS induce an increase in Beclin 1 expression. Moreover, rapamycin-induced autophagy is accompanied by ROS accumulation and lipid peroxidation in yeast[13] and ROS accumulation is required for starvation-induced autophagy.[14] Inversely, it has been shown that autophagy can regulate ROS metabolism: Z-VAD-FMK (benzyloxycarbonyl-val-Ala-Asp(Ome)-fluoromethylketone), a broad spectrum caspase inhibitor causes selective autophagic degradation of catalase, one of the major antioxidants, leading to intracellular ROS accumulation.[15] Also, in cells carrying a repressor of NFκB activation, we have observed that autophagy contributes to ROS accumulation induced by TNFα (data not shown). These findings demonstrate that autophagy and ROS metabolism regulate each other.

AUTOPHAGY 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 exogeneous ROS. These results indicate that autophagy participates in the apoptotic signaling pathway induced by these compounds.

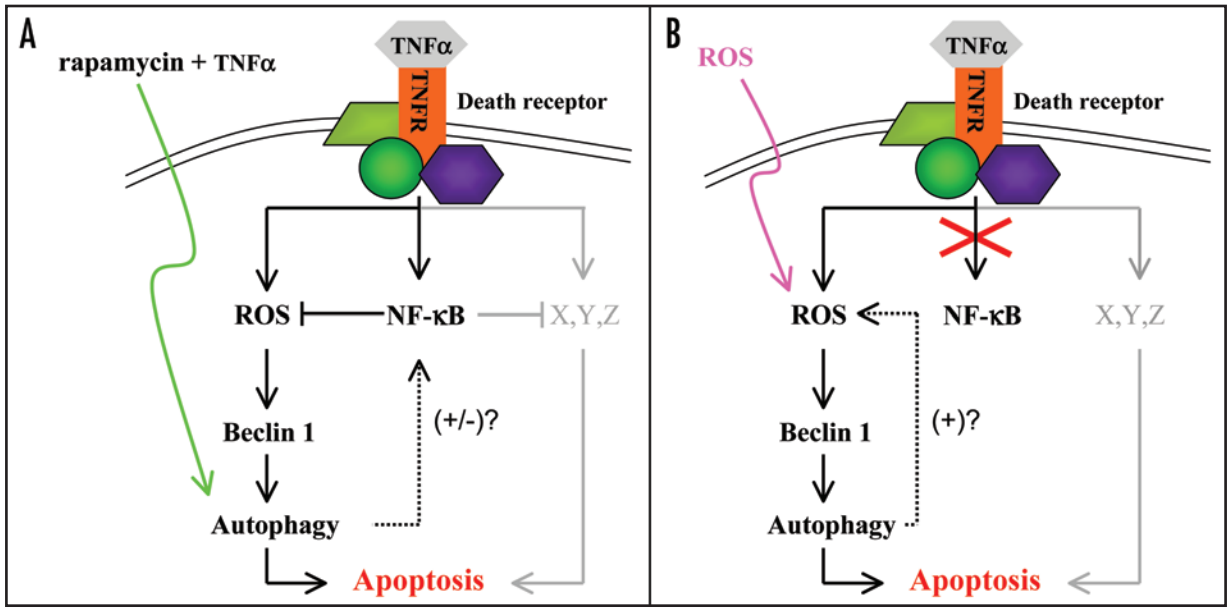

Figure 2. Proposed models for the cross-talk between autophagy and the NFκB- and ROS-activated pathways. (A) TNFα signaling pathway causes NFκB activation that subsequently induces several anti-apoptotic responses, including the repression of autophagy. This inhibition of autophagy is probably due to 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.

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.[4] It is worth nothing 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.

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