TISSUE-SPECIFICITY OF APOPTOSIS IN HEPATOMA-DERIVED CELL LINES

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ABSTRACT

Apoptosis is known to a critical role in development and homeostasis in metazoans. Although apoptotic responses vary widely among cell types, the underlying mechanisms responsible for these differences are not known. In order to understand the molecular basis for these differences, we have studied a cell culture model comparing hepatoma cells to dedifferentiated cell lines derived from them. The dedifferentitated cells, unlike hepatoma cells, undergo apoptosis in response to multiple compounds, including lipopolysaccharide .(LPS). LPS-mediated cell death requires inhibition of protein synthesis. Surprisingly, we found that NF-κB signaling does not appear to play a role in preventing LPS-mediated apoptosis. We are currently examining other signaling pathways that may be involved in protecting the dedifferentiated cells, including p38, ERK and JNK pathways. These results suggest that pathways dictating hepatic phenotype also affect general cellular survival mechanisms in response to multiple agents.

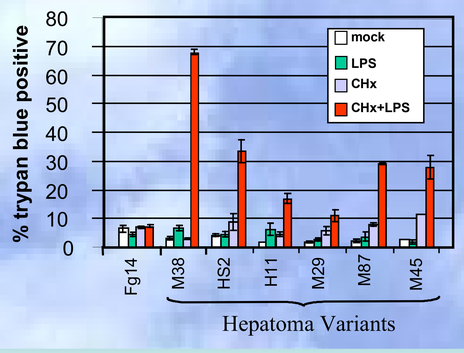
INTRODUCTION

A central question to understanding the role of apoptosis in development and disease is to determine how distinct cell types respond differently to the same signals. We previously reported that the hepatoma variant cell lines, but not a panel of hepatoma cells lines derived from human, rat and mouse, undergo apoptosis in response to LPS in the absence of protein synthesis (1). Remarkably, rescue of the hepatic phenotype in the hepatoma variant cells by chromosome transfer also rendered the hepatoma cells resistant to LPS-mediated apoptosis, suggesting a link between liver-specific gene expression and cellular response to apoptotic signals (2).

To determine the mechanisms responsible for increased apoptosis of the hepatoma variants, we studied the response of the hepatoma variants to multiple stimuli. We report here that, unlike the hepatoma parental cells, the hepatoma variant M38 cells preferentially undergo apoptosis when exposed to several compounds, but that LPS-induced apoptosis is independent of NF-κB signalling.

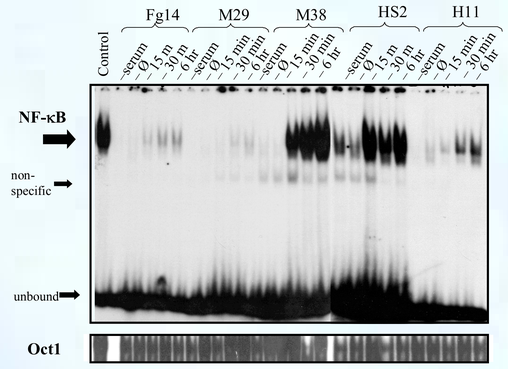
RESULTS

Fig 1. Mutant hepatoma cells undergo apoptosis when exposed to LPS plus the protein synthesis inhibitor cycloheximide



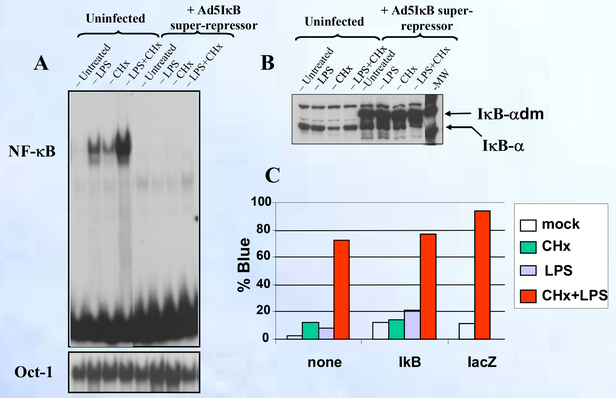
Cells were induced with 1 mg/ml LPS alone, 10mM CHx alone or LPS +CHx for 14 hrs. Cells were trypsinized and dead cells scored by trypan blue exclusion Fg14 is the hepatoma parental cell line. Fg14 = parental hepatoma cells; CHx= cycloheximide

Fig 2. NF-κB induction correlates with the degree of apoptosis in LPS treated dedifferentiated hepatoma cells



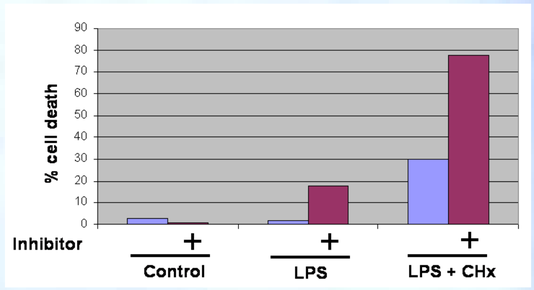
Cells were induced with 1 mg/ml LPS alone, 10mM CHx alone or LPS +CHx for 14 hrs. Treated cells were harvested and nuclear extracts prepared. 10 mg of each extract was incubated with an oligonucleotide previously shown to bind NF-κB. Complexes were resolved on a 4% non-denaturing polyacrylamide gel (lover panel). Fg14 is the hepatoma parental cell line. Fg14 = parental hepatoma cells; CHx= cycloheximide

Fig 3. NF-κB induction is not required for protection from LPS-mediated apoptosis



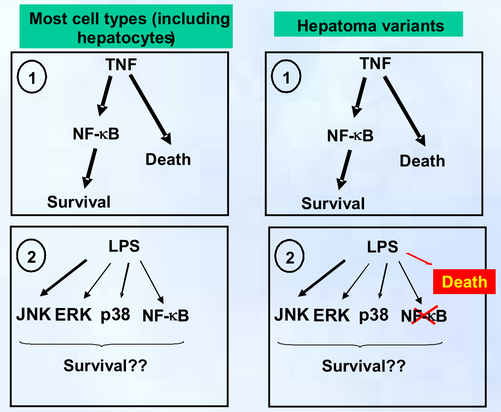
M38 cells were mock-infected or infected with adenoviral constructs Ad-LacZ or Ad-IκBadm at a multiplicity of infection of 250pfu/cell. At 24 hours post-infection, cells were treated with LPS (100ng/ml), CHx (10ug/ml) or LPS + CHx for 24 hours. Nuclear extracts were prepared and assayed for (A) NF-κB by EMSA (using Oct1 binding as a control) and (B) IκBα (name antibody used) expression by Western analysis. (C) M38 cells treated as describe above were scored for apoptosis using trypan blue exclusion. As a control for infection efficiency, cells were infected with a AD-lacZ virus. Nearly 100 % of cells stained blue (results not shown). LPS = lipopolysaccharide; CHx= cycloheximide.

Fig 4. JNK signaling provides protection from LPS-mediated apoptosis



M38 cells were induced with 1 mg/ml LPS alone or LPS + CHx for 14 hrs in the presence or absence of JNK inhibitor SP600125. Cells were scored for apoptosis using trypan blue exclusion. LPS = lipopolysaccharide; CHx= cycloheximide.

CONCLUSIONS



Loss of hepatic gene expression in the hepatoma variant cell lines results in cells that are sensitive to drug-induced apoptosis. Although NF-κB induction has been shown to drive pro-survival pathways in response to TNFα, NF-κB induction does not appear play a role in survival from LPS-mediated cell death in these cells. Thus, other pathways, such as p38, JNK, or ERk pathways likely induce expression of genes that prevent LPS-mediated apoptosis. These results suggest a direct link between cellular differentiation state and the ability of cells to survive apoptotic signals.

1. Bulla GA, Givens E, Brown S, Oladiran B, Kraus D. Protection of dedifferentiated hepatoma variant cells from LPS-induced apoptosis by restoration of hepatic gene expression. J. Cell Science, 114:1205-1212, 2001.
2. Kraus DA, Bulla GA. Defective NF-κB signaling in dedifferentiated hepatoma cells. Somat. Cell. Molec. Genet., 27:275-286, 2002.

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