NF-κB is required for hydrogen peroxide induced caspase independent cell death

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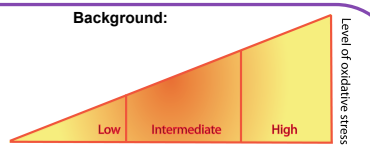
Abstract:

Reactive oxygen species (ROS) are key second messengers in many intracellular signal transduction pathways, eliciting diverse biological responses such as inflammation and proliferation, as well as cell death. ROS act through the NF-kappaB (NF-κB) and JNK signal transduction pathways to mediate cell survival and death, respectively. However, it is yet unknown whether the NF-κB pathway is involved in regulating the JNK pathway in response to hydrogen peroxide (H_2O_2) induced cell death. Therefore, we set out to characterize the molecular mechanisms by which H_2O_2 induced cell death occurs in MEF cells, and address whether cross-talk between the NF-κB and JNK pathways occurs. Surprisingly, we have found that treatment of MEF cells with a chronic level of H_2O_2 results in caspase- independent cell death and that NF-κB is required for this cellular response. Moreover, NF-κB is activated via the canonical activation pathway, and the canonical pathway is required in mediating the pro-cell death function of NF-κB. Interestingly, although JNK activation is delayed in cells lacking NF-κB, we were unable to relate the delay in JNK activation to the delayed rate in cell death. Thus, the precise mechanism of the pro-cell survival function of NF-κB in response to chronic levels of H_2O_2 remains to be identified.

Background:



Signaling Pathway:	Nrf-2	NF-κB AP-1	NF-κB? JNK?
Outcome:	Anti-oxidant response	Inflammatory response	Cell Death

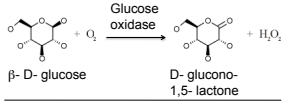
Adapted from Veal, 2007

Project Goals:

Characterize the molecular mechanisms by which hydrogen peroxide induced cell death occurs in murine 3T3 cells

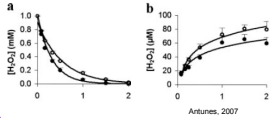
- NFκB pathway
- JNK pathway
- cross- talk?

How does glucose oxidase (G.O.) work?



1mM bolus addition of H_2O_2

4mU/ml glucose oxidase



Upon addition of 25mU/ml G.O., H_2O_2 is produced throughout the duration of the treatment.

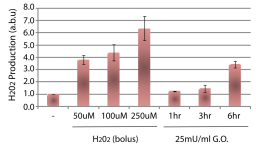


Figure1: H_2O_2 production was measured using DCFH-DA, a membrane permeable fluorescent dye, which is converted to DCF upon interaction with ROS. Based on the standard H_2O_2 curve, it appears as if <50uM H_2O_2 is being produced within the first 3 hrs and roughly 50-100uM H_2O_2 within the last 3 hrs of treatment with G.O..

Glucose oxidase induced MEF cell death occurs independently of caspase activation

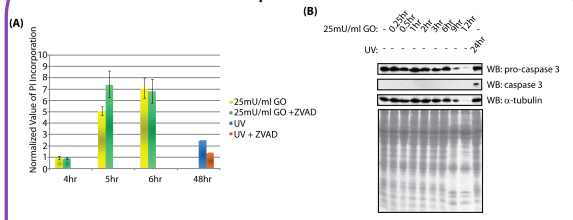


Figure 2: **(A)** Wild type (wt) MEFs were treated for the indicated times with either 25mU/ml glucose oxidase (G.O.) or UV (200 J/m^2). Cell viability was determined as the normalized value of propidium iodide incorporation. To determine whether caspase activation is required for cell death, cells were pretreated with caspase inhibitor VI (ZVAD) for one hour prior to treatment with GO.**(B)** Following the indicated treatment,the cells were lysed and the cell lysate was analyzed by western blotting with anti- pro-caspase 3 (top panel), anti- caspase 3 (second panel from top), and anti- a tubulin (third panel from top). The cell lysates were also stained by Coomassie Brilliant Blue to demonstrate equal loading (bottom panel).

NFκB augments cell death in oxidative stress induced cell death

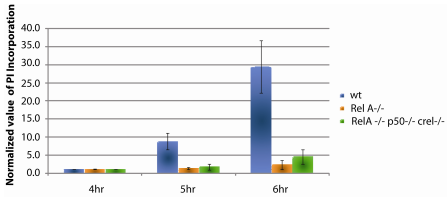


Figure 3: WT and *RelA-/-* and *RelA-/- p50-/- crel-/-* MEFs were treated with 25mU/ml G.O. for the indicated periods of time. Cell viability was determined as the normalized value of propidium iodide incorporation.

NFκB is activated via the canonical activation pathway in response to oxidative stress.

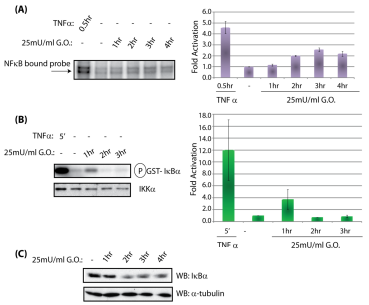


Figure 4: (A) Nuclear extracts were obtained from MEFs either treated with TNFα or 25mU/ml G.O. and were then used to detect NFκB DNA binding by EMSA. **(B)** Kinase assays were performed using the cytoplasmic extracts of glucose oxidase treated cells. Quantification of both EMSA and IKK kinase assay experiments are shown in the right hand panels. **(C)** Following treatment with 25mU/ml GO for the indicated times, the cell lysate was analyzed by immuno-blotting against IκBα (upper panel) and α-tubulin (lower panel).

The canonical NF-κB activation pathway is required for the pro-cell death function of NF-κB in response to oxidative stress.

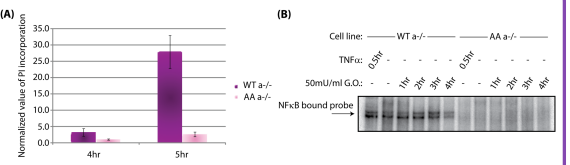


Figure 5: (A) IkBa-/- MEFs reconstituted with either WT IκBα, named WT a-/-, or the non-IKK phosphorylatable mutant of IκBα, named AA a-/-, were treated with 50mU/ml G.O. for the indicated periods and cell viability was measured by determining the normalized value of PI incorporation. (B) Following treatment with either 50mU/ml G.O. or 1ng/ml TNFα, the nuclear extracts were prepared and used to determine NF-κB binding by EMSA.

Activation of JNK does not appear to mediate the delay in cell death seen in cells lacking NF-κB

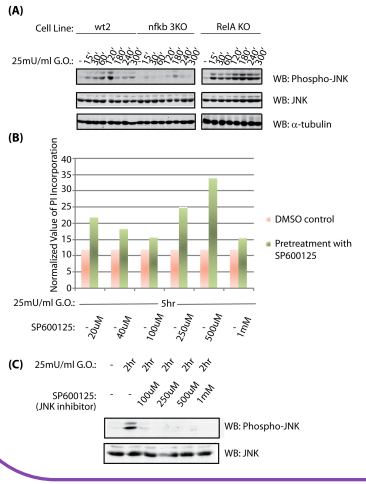


Figure 6: (A) Cell lysates of WT MEFs, *rela-/-p50-/-crel-/-*MEFs (named nfkb 3KO), and *rela-/-* MEFs (named RelAKO) treated with 25mU/ml G.O. were immuno-blotted against phospho-JNK (top panel), JNK (middle panel), and α- tubulin (bottom panel). (B) Following pre-treatment with SP600125, a specific JNK inhibitor, cell death in response to 25mU/ml GO was determined as the normalized value of PI incorporation. (C) To determine whether the inhibitor was functional, cell lysates of cells treated with both SP600125 and 25mU/ml GO were analyzed by immuno-blotting against phospho-JNK (top panel) and JNK (bottom panel).

Remaining Questions:

- How does NF-κB mediate caspase -independent cell death in response to H_2O_2?
- Does activated or basal NF-κB mediate the difference cell death?
- What role does JNK play in mediating cell death?

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