

RIP2 enhances survival through NF- κ B in Triple Negative Breast Cancer

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Abstract

RIP2 is receptor interacting protein kinase well known for its function in immunity and inflammation by activation of the NF- κ B pathway. It was also reported to be involved in cell proliferation and cancer development and metastasis. Here we show role of RIP2 in triple negative breast cancer. RIP2 increased cell count and resistance to death induced by cytotoxic drugs in MDA-MB-231 cells through NF- κ B activation in these cells.

1. Introduction

ER-negative and basal breast cancers (BC) are known to have high NF- κ B activity (1,2). McCarthy et al showed that receptor-interacting protein kinase 2, RIP2, activates NF- κ B signaling (3), and according to Singel et al RIP2 is significantly over-expressed in triple-negative BC(4). Here we show the relevance of RIP2 in BC cells and its impact on NF κ B signaling and survival.

2. Methods

Online BC databases were analyzed for RIP2 impact on patient survival and correlation with NF κ B. MDA-MB-231 BC cells were transfected with plasmids encoding RIP2, various RIP2 mutants and shRNA against RIP2. The cells were then treated with cytotoxic and genotoxic drugs. Cell death and apoptosis was measured by various assays. MDA-MB-231 luciferase-reporter cells were used to measure NF- κ B activity.

3. Results

Kaplan-Meier survival plots showed that RIP2 over-expression in BC patients significantly decreases overall patient survival and progression-free survival. In addition, RIP2 expression is positively correlated with NF- κ B (p52) expression in patients with basal BC ($P < 0.001$). In MDA-MB-231 cells we observed that RIP2 overexpression increased NF- κ B activity, and enhanced cell resistance to ceramide and taxol-induced cell death (Figure 1). In addition, RIP2 mutants and knockdown decreased NF- κ B activity, increased sensitivity of these cells to cytotoxins (Figure 1), increased caspase-3 activity and altered the cell cycle

profile, such that more cells were observed in the subG1 phase which is indicative of apoptosis.

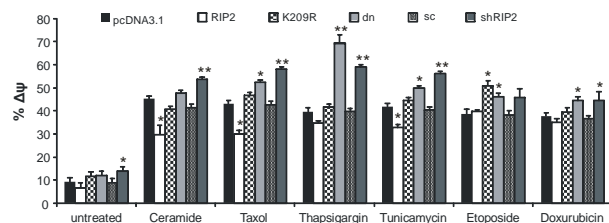


Figure 1: RIP2 expression affects cell response to treatments. TMRE analysis upon treatment of MDA-MB-231 cells with drugs (ceramide 30 μ M, taxol 1 μ M, thapsigargin 3 μ M, tunicamycin 10 μ g/ml, etoposide 100 μ M and doxorubicin 1 mM). Results were average data of 3 separate experiments \pm SE. Statistical significance is expressed in comparison to treated control (* $P < 0.05$, ** $P < 0.01$).

Targeting NF- κ B by peptide inhibition (SN50) or NF- κ B super-repressor plasmid reversed the RIP2 cell survival effect.

4. Conclusion

RIP2 enhances survival and proliferation in BC and protects it from apoptotic death induced by cytotoxic drugs. By targeting RIP2, it might be possible to interfere with the NF- κ B pro-survival signaling pathway and sensitize BC cells to induction of apoptosis by cyto/genotoxic drugs and may improve outcomes in advanced BC patients.

8. References

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