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Effect of Energy Metabolism on NF-κb activity in Ovarian Cancer

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Abstract

NF-κb is a transcription factor involved in cancer cell growth and survival. The activation of NF-κb can be assessed by monitoring phosphorylation of RelA p65 at Ser-536, which is a surrogate of the NF-κb transcription factor activation. The objective of this study was to determine if the loss of ATP leads to NK-κb deficiency and thus, apoptotic cell death of “bad” cells in ovarian cancer cells. The independent variables were metformin (Met), an anti-diabetic medicine, another compound MinB functionally similar to Met and a glucose transporter inhibitor BAY-876. The dependent variables were the resulting effect of Met and MinB on phosphorylated AMPK at Thr-172 (marker of ATP loss) and RelA p65 at Ser-536 (marker of NF-κb activation). In each experiment, AMPK and RelA phosphorylation were tested by treatment of ovarian cancer cell lines with Met, MinB, BAY-876, Met+BAY-876, MinB+BAY-876. Western blotting was performed to determine the phosphorylation levels of AMPK and RelA p65. For two gels, the process was repeated. In each gel, Met or MinB treatment leads to thicker bands of AMPK- p, indicating decrease in cellular ATP levels following treatments. The effect of Met, MinB, or BAY-876 on RelA p65 was limited, however, co-treatment of Met or MinB with BAY-876 caused strong inhibition of NF-κb, as reflected by reduction in RelA p65- p. These results suggested that ATP deficiency together with inhibition of glucose transport cause inactivation of NK-κb.

Methods

• The research design of this study was experimental as it studied the relationship between ATP production and NF-κb activity.

• Sodium dodecyl sulfate (SDS) was used to denature the protein and provide equal mass to charge ratio so as to allow the protein to move constantly through the gel based on its molecular weight. The SDS caused proteins to denature and separate from each other (excluding covalent cross linking). SDS gel was prepared by first preparing resolving gel and placing that gel into the prepared glass ware. The gel solutions ordered were from National Diagnostics in Atlanta, Georgia. Once membranes were labeled, a black sponge was placed on top of the white paper which is on top of the gel and then the apparatus was closed. Approximately 92 volts was run through the membrane overnight. In the morning, the membranes were placed in primary and then secondary antibodies. All antibodies were ordered from Cell Signaling Technology located in Danvers, MA. For the last step, the membranes were put in a white carrier. The last step is called luminescence. During this step, an ECL reaction takes place in which light induces the signal. The ECL reagent was mixed into a Amershams Hyperfilm film (BioRid, Plymouth, MI). The signal is both bright and time sensitive so too much signal will have a negative effect on the data sample collected. Running of the gel was performed on medical film processor SRX-101A (Konica Minolta Medical & Graphic Inc., Wayne, NJ). The film was then analyzed.

Results

In both ovarian cancer cell lines in all four gels, ATP deficiency plus inhibition of glucose transport lead to a significant decrease in band size. In figures 1 and 2, Metformin and MinB treatment lead to thicker bands of AMPK-p. There wasn’t a significant change in band size when Met, MinB, or BAY-876 was tested on RelA p65. Co-treatment of Met or MinB with BAY-876 showed the thickest bands compared to all other treated bands in figure 1. In figure 2, co-treatment of Met or MinB with BAY-876 showed a significant decrease in band size.

Discussion

• The purpose of this experiment was to see the effect of ATP on NF-κb activity by performing western blotting.

• It was suggested that the decrease in the amount of ATP is shown by the thickening of bands on AMP activated protein kinase at Thr-172. The decrease in ATP results in less apoptosis.

• The thinning of bands in figure 2 by the co-treatment of BAY-876 with MinB and Met indicated strong inhibition of NF-κb. By inhibiting the amount of glucose transport, the cell can’t get to the proper nutrients it needs.

• To summarize, metformin and the metformin like compound had a significant effect on AMPK-p in the two different cell lines. Furthermore, this experiment successfully made use of the symbolic relationship between ATP production and NF-κb.

• When there is ATP deficiency and inhibition of glucose transport, there is an inactivation of NF-κb.

• In regards to the society, a decrease in the amount of ATP, through the symbiotic mechanism between decreased phosphorylated sites on transcription factor and more apoptosis, offers a more palatable way for individuals looking to reduce the number of cancer “bad” cells.

• When future researchers open up to testing different cancer cell lines with different drugs with this same mechanism, there are high hopes that humans and perhaps other species as well, can benefit. Future research can be conducted to measure cytokine production with ELISA assay.

References


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