A Drop in the Ocean of the Fight against Cancer

Investigation of two non-canonical NF-kB pathway inhibitors as treatments for Glioblastoma Multiforme

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1. Background

- My project is based on a protein complex called NF-kB, responsible for inflammation and cancer development. Specifically, it involves the role of the less-studied non-canonical NF-kB pathway in GBM (glioblastoma multiforme), a type of brain cancer. GBM was modeled in this project using two cell lines: U87 and U118
- Studies [1][2] have identified mechanisms by which the non-canonical NF-kB pathway can promote growth and proliferation of GBM **(Fig. 1)**
- Prof. Perkins' collaborators have recently produced a series of novel drugs, NIK and IKK-alpha inhibitors, that have therapeutic potential for the treatment of cancer

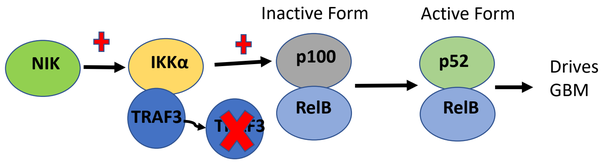


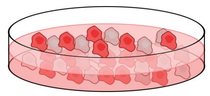
Figure 1. Mechanism by which NF-kB pathway stimulates GBM development.

2. Aims

- Investigate whether these drugs will stop the pathway
- Investigate whether these drugs promote programmed cell death (apoptosis)

3. Methods

Drug treated culture cells left at 37° C for 4h/24h intervals



Separated proteins mixed with **negatively charged** agent

Proteins get separated by **size**

Individual proteins detected via luminiscent antibodies

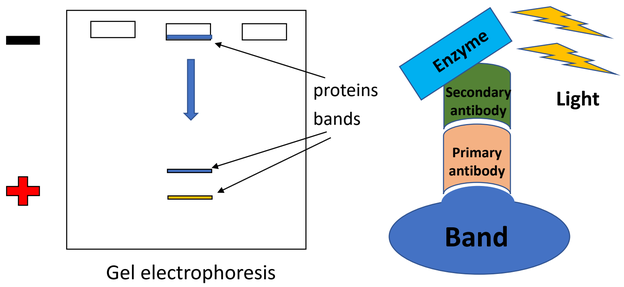


Figure 2. NF-kB can be stimulated with a chemical activator

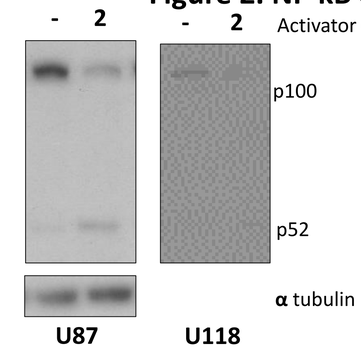


Figure 1. Protein levels of p100/p52 and α-tubulin control in U87 and U118 cell lines treated with NF-kB activator for 4h.

p100 to p52 conversion indicates NF-kB pathway activation.

α-tubulin shows the total amount of protein loaded is equal.

Upon adding the activator, p100 levels drop and p52 levels rise. This indicates the activator stimulates the NF-kB pathway.

Figure 3. NIK inhibition stops NF-kB activation and induces apoptosis in U87 cells

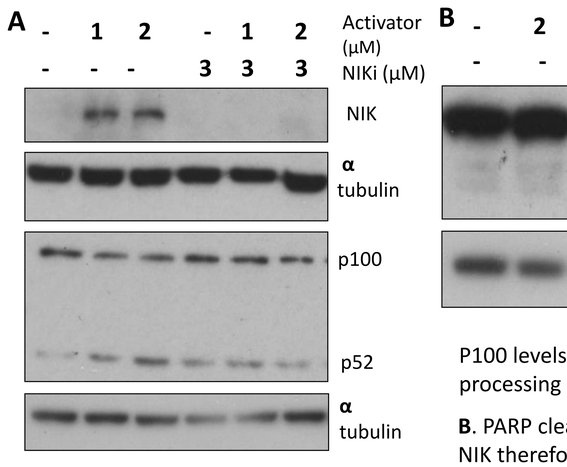


Figure 2. Protein levels of p100/p52, NIK (A) and cleaved PARP (B) treated with NIK inhibitor for 4h.

A. NIK protein acummulates when NF-kB is stimulated by the activator. However, NIK levels drop significantly when NIKi is added with activator. This suggests it hits the intended target.

P100 levels rise and p52 levels drop with the NIKi, meaning processing stops.

B. PARP cleavage is an indirect measure of apoptosis. Inhibition of NIK therefore increases apoptosis in cells with an active NF-kB.

Figure 4. NIK inhibition stops NF-kB activation and induces apoptosis in U118 cells

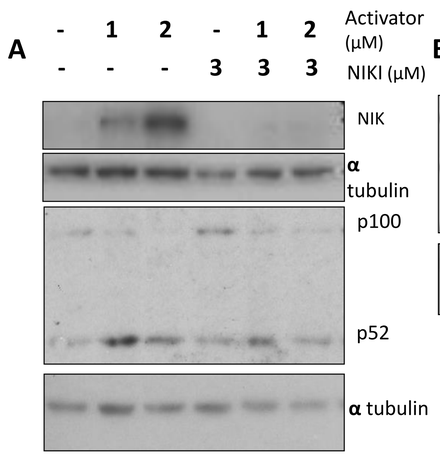


Figure 4. Protein levels of p100/p52, NIK (A) and cleaved PARP (B) treated with NIK inhibitor for 4h.

Further, NIKi effect was investigated in U118 cells.

A. Again, NIK levels drop significantly when the NIK inhibitor is added. This suggests it hits the intended target.

P100 levels rise and p52 levels drop with the NIKi.

B. PARP cleavage occurs, indicating induction of apoptosis in the presence of NIKi.

3. Discussion

The NIK inhibitor affects both cell lines, but especially U87

- A chemical inhibitor of NF-kB can be used to study the pathway in GBM **(Fig. 2)**
- Measuring p100/p52 levels allows effects on the non canonical NF-kB pathway to be observed
- The NIK inhibitor is potently inhibiting this pathway because the levels of p100 processing to p52 fall in both cell lines **(Fig. 3A & 4A)**
- The U87 cell line exhibits a more accentuated decrease in p100 to p52 conversion than U118 cells. This indicates NIKi affects U87 cells more efficiently
- Consistent with this, more apoptosis was observed in U87 cells treated with the NIK inhibitor **(Fig 3B & 4B)**. This was detected because U87 cells showed more PARP cleavage relative to the controls than U118 cells

Off-target effects

- These findings were confirmed with two more NIK inhibitors and an IKK α inhibitor that targets a different protein in the NF-kB pathway **(Fig. 5.)**. They also showed an effect on pathway inhibition and apoptosis (data not shown)
- One of the NIK inhibitors tested was intended to be structurally similar to the potent NIK inhibitors but unable to act on NIK. However this inhibitor also affected NF-kB activity and apoptosis suggesting the effects observed could be off-target

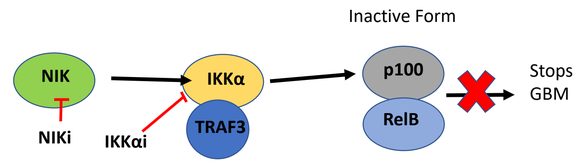


Figure 5. Mechanism of action of the two NF-kB pathway inhibitors tested.

4. Conclusion

- NIK inhibitor shows more effective pathway inhibition in U87 compared to U118 cells
- NIK inhibitor induces apoptosis more effectively in U87 compared to U118 cells
- By targeting different pathway proteins, the same beneficial effects arise in inhibiting the cancer development

References:
[1] Tchoghandjian A et al. Smac mimetic promotes glioblastoma cancer stem-like cell differentiation by activating NF-κB. Cell Death Differ. 2014 21(5):735-47
[2] Li Y et al. Non-canonical NF-κB signalling and ETS1/2 cooperatively drive C250T mutant TERT promoter activation. Nat Cell Biol. 2015 17(10):1327-38