Characterization of a Direct NF-\(\kappa\)B Ligand

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Five proteins of the NF-κB Family

The two faces of NF-κB in gene regulation

Cancer cells
- Cell survival
- Metastasis
- Proliferation
- Inflammation

Normal cells
- Cell death
- Tumor Suppressors
- Antiproliferative effects
The NF-κB canonical pathway
Small-molecule microarrays (SMMs)

fluorescent features reveal putative binding interactions

compute composite Z-scores

SMM containing 10,800 features and probed with protein

fluorescent-labeled antibody

tag

protein

specificity heat map

...proteins...

...compounds...
AGA is a stronger NF-κB binder as shown by surface plasmon resonance

80% BRD-K92758126

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<thead>
<tr>
<th></th>
<th>80% pure</th>
<th>purified</th>
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<tbody>
<tr>
<td>p50-SPR</td>
<td>$K_D = 226 \text{ nM}$</td>
<td>$K_D = 76 \text{ nM}$</td>
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<tr>
<td>P65-SPR</td>
<td>not tested</td>
<td>$K_D = 2,300 \text{ nM}$</td>
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<td></td>
<td>$K_D = 13,000 \text{ nM}$</td>
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<td>$K_D = 6,340 \text{ nM}$</td>
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AGA inhibits NF-κB translocation

Translocation assay, p50 Ab (green) and Hoechst (blue)

Edgar Ibarra
Rakhee Busanelli
Main goals

• To study the inhibitory effect of AGA on all five NF-\{kappa\}B proteins.

• To determine the effect of AGA on cell viability.
Measuring the effect of AGA in NF-{kappa}B protein translocation

1. Non-stimulated cells

2. TNF-a stimulated

3. TNF-a stimulated + AGA (10nM)
AGA TransAM has a preference for p65

1 hour stimulation
10nM AGA concentration
Normalized A549 TransAM ELISA

Absorbance

NF-kB domains

- p50
- p65
- p52
- c-Rel
- Rel-B

- cells
- TNF-a
- AGA
HEK293 cell viability is not affected upon addition of AGA
Conclusions

• AGA temporarily inhibits NF-{kappa}B translocation to the nucleus.

• The inhibitory effect of AGA appears to vary in different cancer cell lines, but might have a preference for p65.

• AGA does not affect cell viability.
Future directions

• Use of a gene expression database to explain ELISA data.

• Use of higher concentrations of AGA to test for inhibition.

• Synergy studies involving compounds that target other parts of the NF-kB pathway.

• Docking experiments will help theorize sites of interaction.
Acknowledgements

Dr. Angela Koehler
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David Pearlman
Nathan West
Stuart Schreiber
SRPG program
There is no significant increase in absorbance upon 30 minute incubation over 1 hour incubation.

Pulsatile Stimulation Determines Timing and Specificity of NF-kB-Dependent Transcription.
L. Ashall, C. A. Horton. Science 324, 242-246
Varying degree of AGA selectivity among different cancer cells

NF-κB p50/p65 TransAM ELISA for additional cell lines

3T3L1    HeLa    C2C12