Novel Stable Human Reporter Cell Line for Screening of Anti-Inflammatory Active Pharmaceuticals and Biological Compounds

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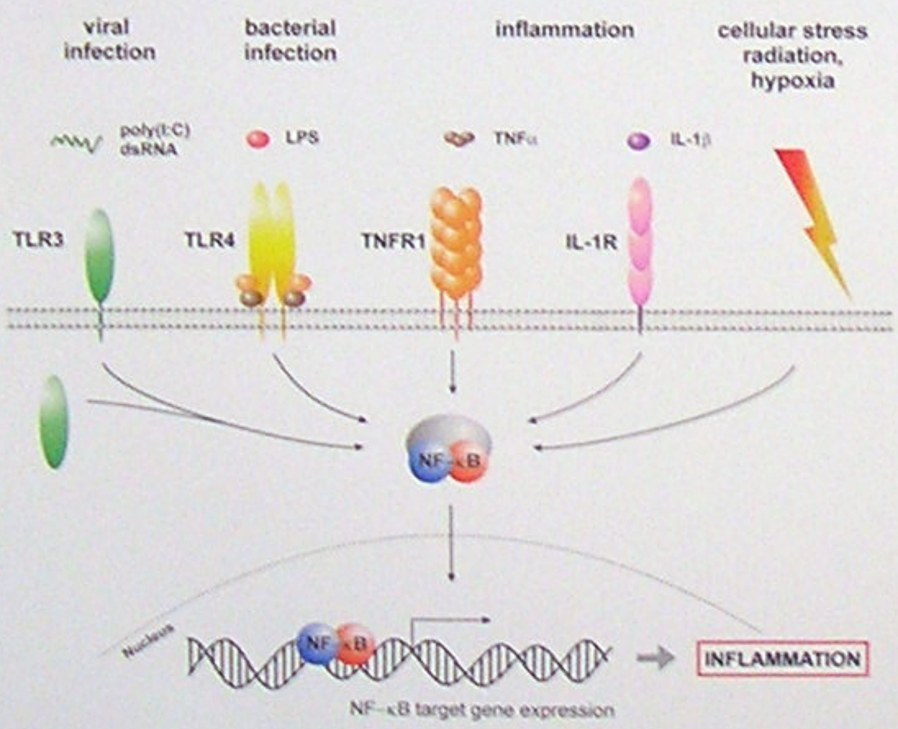
Inflammation is linked to a variety of pathologies including chronic pain, auto-immune diseases, development and progression of cancer as well as to Inflammatory Bowel Disease, Alzeimer`s and Parkinson`s disease. Thus, anti-inflammatory pharmaceutics and biological compounds with anti-inflammatory activity are of high medicinal and commercial interest.

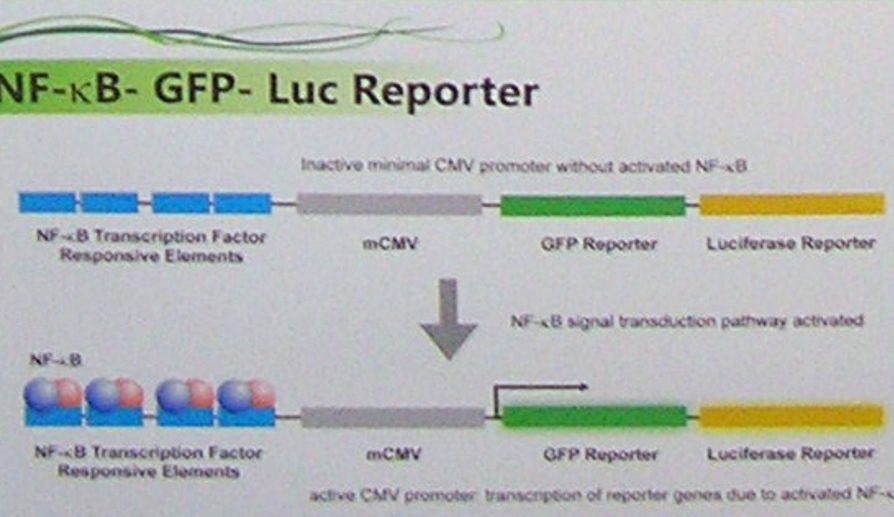
We developed a stable, clonal human cell line to simultaneously test the anti-inflammatory potential and in vitro toxicity of anti-inflammatory drugs and biopharmaceuticals. In particular, a human cell line endogenously expressing inflammation-related receptors (including, but not limited to tumor necrosis factor receptors, interleukin receptors and Toll-like receptors) was stably transduced with gene reporter constructs to monitor activation of an inflammation-related transcription factor. The read-out can be performed in a multi-well format (upscalable) and is based on bioluminescence providing a fast and cheap opportunity to measure the influence of various compound on inflammatory signalling after both bacterial- and viral induced inflammation. Further, using our screening system, a simultaneous fluorescence based toxicity screening can be performed.

Activation of the NF-κB pathway



Activation of the NF-κB pathway
Transcription factors are proteins allowing the cell to adapt its gene expression to a specific condition or stimulus.
Pathogens (bacteria, virus) or trigger of inflammation (TNF, IL) bind to their respective receptors. This leads to activation of NF-κB, an inflammation-related transcription factor. NF-κB than binds to its binding sites on the DNA and enhances the expression of proteins involved in inflammation. NF-κB is also activated in response to cellular stress (radiation, hypoxia).

NF-κB- GFP- Luc Reporter

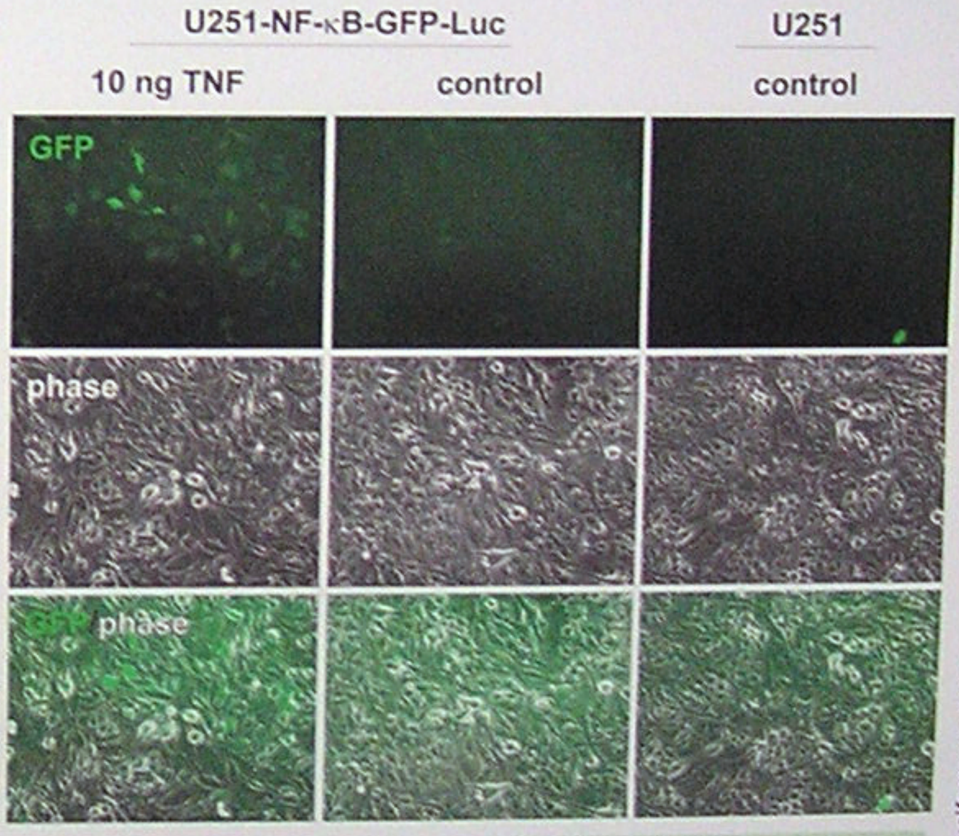


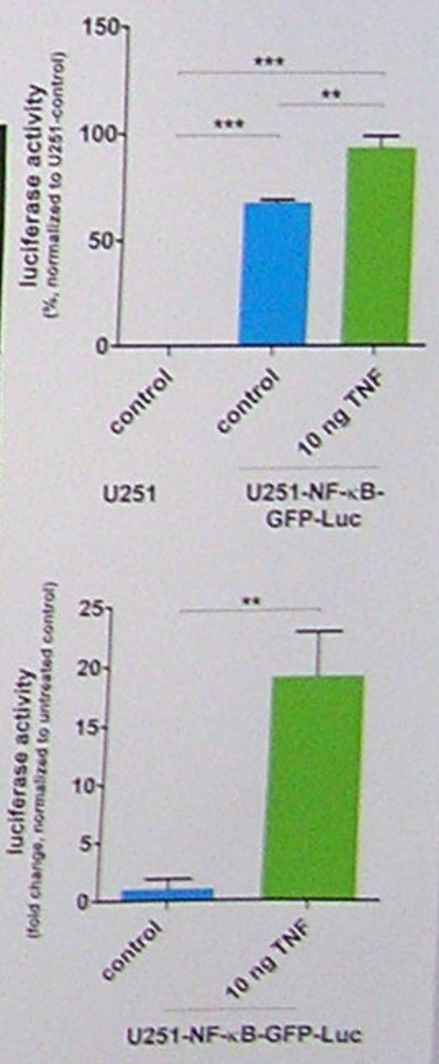
NF-κB- GFP- Luc reporter
NF-κB- GFP-Luc reporter contains GFP (green fluorescent protein) and *firefly* luciferase (bioluminescence) genes. The minimal CMV (mCMV) promoter, which controls expression of the reporters, is only activated by binding of activated NF-κB to the transcription factor response elements.

Applications

- Easy, cheap and fast detection of pro-inflammatory agents by fluorescence
- Upscalable screening of potential activators or inhibitors of inflammation
- Efficiacy of anti-inflammatory active pharmaceuticals and biological compounds can be tested
- simultaneous cytotoxicity test for potential drugs

U251-NF-κB- GFP-Luc cells





U251-NF-κB- GFP- Luc
U251-MG is a human cell line derived from a malignant glioblastoma tumour (brain tumour). It endogenously expresses inflammation-related receptors. NF-κB-GFP-Luc reporter was integrated in U251 cells via lentivirus. Activated NF-κB (e.g. with TNF) results in higher GFP expression and luciferase production.

Advantages

- Easy cultivation
- Endogenous expression of inflammation-related receptors
- Stable expression of inducible NF-κB reporters (GFP and *firefly* luciferase)
- No need of cell transfections
- Fast and simple detection of NF-κB-activation using fluorescence microscopy
- Highly dynamic assessment of NF-κB-activation (*firefly* luciferase)
- No pro-inflammatory trigger needed (basal activity of NF-κB)
- Reduces well-to-well variability (clonal origin)