Biomolecular Discovery in Nutrigenomics: a high throughput TNFα promoter assay for food components active against inflammatory bowel disease

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Introduction

Nutrigenomics New Zealand (NuNZ) is a collaborative research programme using a variety of approaches to study the interaction of diet and the genome, focusing on genes associated with inflammatory bowel disease.

Tumour necrosis factor alpha (TNFα) is a pro-inflammatory cytokine important in the pathogenesis of Crohn's disease and ulcerative colitis. A polymorphism (G to A) at the -308 position of the TNFα promoter is associated with higher gene expression levels[1], and has been associated with ulcerative colitis disease activity and progression in a New Zealand study[2].

We describe here a luciferase gene reporter assay for both -308 G/A variants of the TNFα promoter to screen food extracts, fractions and food-based compounds for anti-inflammatory activity directed preferentially at the -308A variant (Fig. 1).

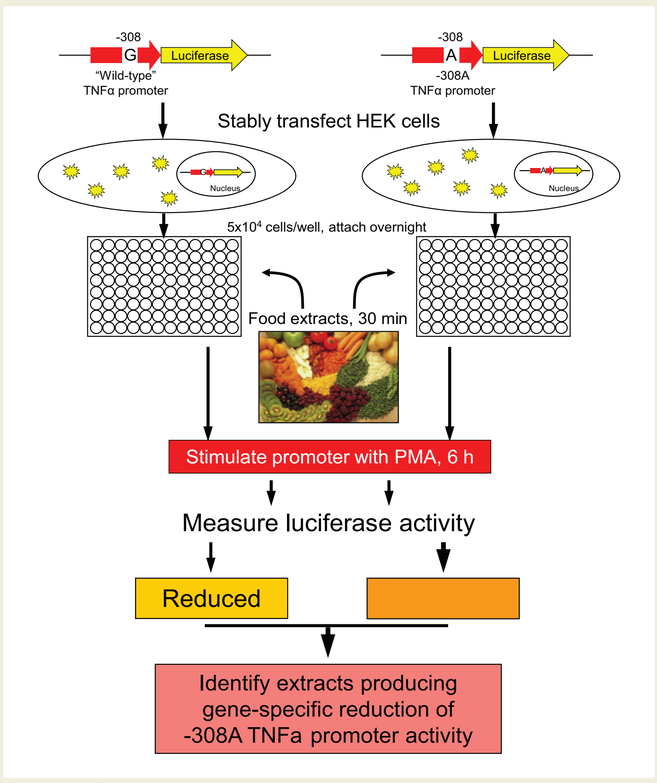


Figure 1. TNFα promoter assay overview.

Materials and Methods

TNFα promoter stable transfection

A 1420 bp wild-type human TNFα promoter fragment was isolated by polymerase chain reaction, subcloned into luciferase reporter vector pGL4.14 (Promega), and the 308G/A variant subsequently generated by site-directed mutagenesis (QuikChange® II, Stratagene). Plasmids were linearised and stably transfected into Flp-In™-293 cells (Invitrogen) by electroporation. Transfected cells were then maintained under selection with 150 µg/mL hygromycin and 100 µg/mL zeocin to permit isolation of stably transfected clones.

Food extract and fraction preparation

Freeze-dried, ground food samples were extracted with ethanol at 10 mL/g dry weight. Crude extracts were dried, dissolved in DMSO or 25% DMSO in water, diluted in media and assayed up to a maximum concentration of 400 µg dry weight equivalent/mL. Extracts were reversed-phase (RP) fractionated on solid phase extraction cartridges to provide 12 fractions based on polarity, with an effective 10-fold concentration of material over the crude extracts.

References

1. Wilson AG, et al. 1997. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 94: 3195-9.
2. Ferguson LR, et al. 2008. Single nucleotide polymorphism in the tumor necrosis factor-alpha gene affects inflammatory bowel diseases risk. World J Gastroenterol 14: 4652-61.
3. Matta R, et al. 2009. Triptolide induces anti-inflammatory cellular responses. Am J Transl Res 1: 267-82.
4. Klaas CA, et al. 2002. Studies on the anti-inflammatory activity of phytopharmaceuticals prepared from Arnica flowers. Planta Med 68: 385-91.
5. Perry NB, et al. 2009. Sesquiterpene lactones in Arnica montana: helenalin and dihydrohelenalin chemotypes in Spain. Planta Med 75: 660-6.

Results and Discussion

- In stable transfectants, the TNFα promoter was induced by PMA (Fig. 2A) or by TNFα (not shown). Triptolide, an anti-inflammatory diterpene triepoxide from the Chinese medicinal herb *Tripterygium wilfordii*[3], was used as an inhibitory control (Fig. 2B).
- Anti-inflammatory activity was detected in extracts of the medicinal herb Arnica montana (Fig. 3). This activity concentrated in three RP fractions (Fig. 4), which contained the known anti-inflammatory sesquiterpene lactones[4] methacryloyl helenalin and tigloyl helenalin[5] (Fig. 5).
- The assay has been used to screen over 1500 samples, with other active extracts and compounds identified, including green tea (EGCG), turmeric (curcumin), and root ginger (6-gingerol, 6-shogaol).

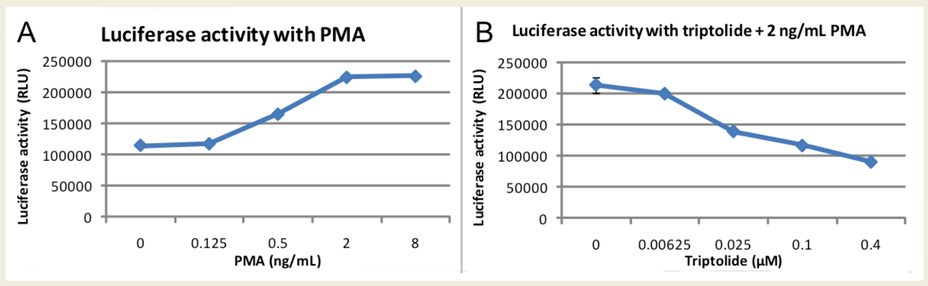
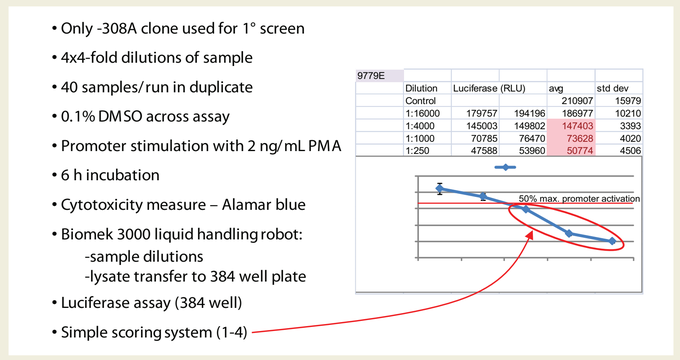


Figure 2. Induction and inhibitory controls for TNFα promoter assay. A) Promoter induction with phorbol 12-myristate 13-acetate (PMA) treatment (6 h). B) Inhibition of PMA-induced TNFα promoter activity by 30 min pre-treatment with triptolide. Data shown are taken from an assay run for the -308A promoter variant.



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Dilution	Luciferase (RLU)		avg	std dev
Control			210907	15979
1:16000	179757	194196	186977	10210
1:4000	145003	149802	147403	3393
1:1000	70785	76470	73628	4020
1:250	47588	53960	50774	4506

Figure 3. The TNFα promoter assay at a glance, showing example of graphical readout for *Arnica montana* extract.

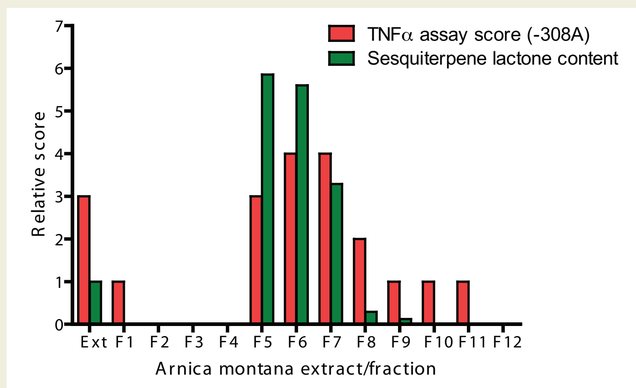

Figure 4. TNFα promoter assay activity corresponds to relative sesquiterpene lactone content of *Arnica montana* extract and fractions.

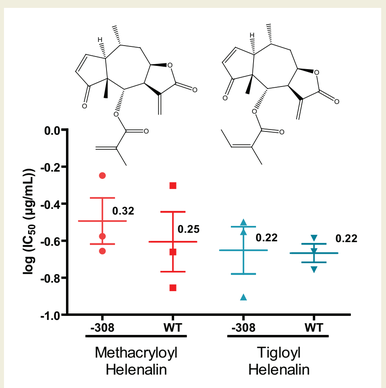

Figure 5. IC_{50} data for purified major sesquiterpene lactones from *Arnica montana*. Data shown are mean ± SEM (n=3). Mean IC_{50} values are indicated (in µg/mL) alongside each graph. There were no significant differences observed.

Conclusions

- We have developed a TNFα promoter assay to screen food extracts and fractions for anti-inflammatory activity directed preferentially against the 308A isoform of the TNFα promoter, associated with inflammatory bowel disease.
- Proof of principle for the TNFα assay has been demonstrated with the medicinal herb *Arnica montana*, for which anti-inflammatory activity of an extract was tracked through bioactivity-directed isolation to the purified major sesquiterpene lactones methacryloyl helenalin and tigloyl helenalin.