Gene expression quantification of anti- and pro-inflammatory genes upon stimulation with Dexamethasone and IL-1β by employing smiFISH

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***Short Abstract* —HeLa Cells imaged under anti-inflammatory stimulation via Dexamethasone (DEX) are compared to basal conditions and under the stimulation of the pro-inflammatory cytokine IL-1β. Custom software along with BigFISH are used for quantitative image analysis of nascent and mature mRNA production of DUSP1, IκBα, and COX-2 under each experimental condition. This methodology combined with mathematical modeling can be used to determine biophysical parameters such as bursting kinetics, as well as degradation rates upon each stimulus, and therefore increase our understanding of the inflammatory response and its resolution through modern experimental techniques.**

Keywords: **Transcriptional bursting, inflammatory response, Dexamethasone, GC, GR, DUSP1, IκBα, COX-2, smiFISH.**

I. Purpose

Glucocorticoids (GC) have a long history in the treatment of proinflammatory cellular responses [1,2,3,4,5]. The gene dual-specificity phosphatase 1 (DUSP1) encodes for the protein product mitogen-activated protein kinase phosphatase 1 (MKP-1), which is a key regulator of the cells anti-inflammatory response through dephosphorylation of the P38 and JUN N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) pathways [2,4,5]. The IκBα protein, on the other hand, is the main inhibitor of the proinflammatory transcription factor NFκB [1]. Transcriptional up-regulation of the counter-inflammatory genes DUSP1 and IκBα occurs through the activation of the glucocorticoid receptor (GR) by GC's [1,2,3,4,5]. This regulatory pathway is thought to be a key mechanism in the suppression of inflammatory mediators such as cyclooxygenase 2 (COX-2) [1,4,5].

Overactivation of these signaling pathways is involved in autoimmune diseases including asthma, atherosclerosis, inflammatory bowel disease, multiple sclerosis, and rheumatoid arthritis [1]. Synthetic GC's such as DEX are standard therapeutics used for the reduction of inflammation [1,2,3]. The mechanism of action of GC's is exerted through the GR. The GR is a cytoplasmic receptor and when bound by either endogenous (cortisol) or exogenous therapeutics translocates to the nucleus where it induces or represses the transcription of thousands of genes by direct/indirect binding to GR DNA response elements (GREs) [3].

Single molecule inexpensive fluorescent *in situ* hybridization (smiFISH) provides a high resolution, high throughput method for the study of endogenous gene expression and builds on bulk averaging experimental techniques which lack the ability to discern genetic heterogeneity.

Both DUSP1 and IκBα have heterogenous basal expression levels in HeLa cells when visualized with smiFISH. Upon continuous stimulation with 100nM DEX there is an increase in mRNA expression starting at approximately 40min for DUSP1 and 60min for IκBα. The expression has been found to reach a peak around 75min for DUSP1 and sustained expression up to 240min for IκBα.

Stimulation with IL-1β has been shown to also increase the expression of DUSP1 and COX-2 mRNA through activation of the transcription factor activator protein 1 (AP-1) via phosphorylation by P38 MAPK, and dissociation of IκBα from NFκB respectively [2,4]. Previous studies have shown that dual stimulation of DEX and IL-1β show amplified DUSP1 expression, however it has also been previously shown that P38 activation phosphorylates and inhibits GR nuclear translocation [5]. We aim to elucidate the impacts of these dynamics by staggering the dual stimulation, allowing for translation of DUSP1 into its protein product, MKP-1. It is hypothesized that pretreatment with IL-1β will decrease the effects of DEX stimulation, while pretreatment with DEX will lead to increased DUSP1 expression following IL-1β stimulation.

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