Therapeutic Targeting of Endoplasmic Reticulum Stress in Inflammatory Bowel Disease

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Abstract

Endoplasmic reticulum (ER) stress occurs when proteins misfold during biosynthesis in the ER. ER stress in intestinal secretory cells has been implicated in the aetiology of inflammatory bowel diseases (IBD) and intestinal inflammation in mice. Intestinal secretory cells are susceptible to ER stress due to high rates of protein synthesis, and ER stress in these cells results in reduced production of cell surface and secreted proteins leading to thinner mucus with a lower anti-microbial content, allowing penetration by luminal microbes, leading to inflammation. Cells experiencing ER stress attempt to restore homeostasis via the unfolded protein response (UPR), which enables the cells to increase the protein folding capacity of the ER.

The primary focus of this thesis is to examine whether the drugs that are efficacious in IBD treatment modulate goblet cell function and ER homeostasis, and whether drugs that modulate ER stress can suppress intestinal inflammation and restore intestinal homeostasis. In order to explore the ER stress-inflammation nexus I utilized well established IBD anti-inflammatory agents, 5-Aminosalicylate (5-ASA), 6-thioguanine (6-TG), the anti-TNF antibody, infliximab, and the glucocorticoid dexamethasone (DEX). Chemical chaperones (TUDCA and sodium 4-PBA) and UPR modulators (guanabenz, salubrinal and 4µ8C) were used to investigate how modulation of ER stress affects goblet cell function and intestinal inflammation. In vivo studies were carried out in Winnie mice, which have ER stress in goblet cells due to a Muc2 misfolding mutation resulting in colitis involving both innate and adaptive immunity. To understand the direct effect of these drugs on ER stress in goblet cells in the absence of confounding inflammatory factors, in vitro experiments were performed using the human colonic adenocarcinoma LS174T cell line, a cell line containing cells with goblet cell differentiation, where ER stress was induced either by inhibition of N-glycosylation by tunicamycin or by depletion of Ca^{2+} by thapsigargin.

Accumulation of terminally misfolded Muc2 precursor in Winnie mice increased clinical and histological inflammation and initiated the ER stress response with upregulation of all the arms of the UPR and decreased Muc2 biosynthesis. Therapeutic targeting with 5-ASA and 6-TG inhibited inflammation, restored
Muc2 biosynthesis and suppressed ER stress in Winnie mice. However, these drugs failed to modulate ER stress in LS174T cells suggesting that their role in modulating intestinal homeostasis and goblet cell functioning is secondary to their suppression of inflammation. Infliximab, failed to inhibit inflammation and ER stress both in vivo and in vitro, although these experiments may be influenced by the lower affinity of infliximab for murine TNF. Endogenous intestinal glucocorticoids are important for homeostasis and glucocorticoid drugs are efficacious in IBD. In Winnie the glucocorticoid dexamethasone (DEX) suppressed ER stress and activation of the UPR and inflammation, substantially restoring goblet cell production of Muc2. In cultured human intestinal secretory cells, in a glucocorticoid receptor-dependent manner, DEX suppressed multiple forms of ER stress and UPR activation induced by blocking N-glycosylation, reducing ER Ca\(^{2+}\) or depleting glucose. DEX upregulated mRNA expression of genes encoding chaperones and elements of ER associated degradation (ERAD), including EDEM1. siRNA knockdown of EDEM1 partially blocked the DEX suppression of misfolding-induced ER stress showing that DEX enhances ERAD. DEX inhibited tunicamycin-induced accumulation of MUC2 precursor and promoted production of mature mucin, and restored ER exit and secretion of Winnie mutant recombinant Muc2 domains, consistent with enhanced protein folding.

TUDCA and 4-PBA suppressed ER stress in LS174T cells but failed to inhibit ER stress or suppress inflammation in Winnie mice. Guanabenz, which selectively inhibits eIF2 dephosphorylation, partially ameliorated ER stress and restored mucin biosynthesis in Winnie mice, and inhibited ER stress in LS174T cells. Salubrinal, which blocks translation of proteins by inhibiting eIF2 dephosphorylation, also suppressed ER stress in LS174T cells. The IRE1-binding molecule, 4µ8C, selectively inhibited tunicamycin- and thapsigargin-induced XBP1 splicing in LS174T cells but failed to modulate other arms of the UPR. Therapeutic intervention with salubrinal and 4µ8C was not possible in Winnie mice due to their pleiotropic side effects.

The ER protein disulphide isomerase family member, AGR2, is tightly co-expressed with MUC2 in the intestine and deficiency in Agr2 in mice leads to cessation of Muc2 biosynthesis. In LS174T cells I demonstrated that ER stress and upregulation of mucin biosynthesis both increase AGR2 transcription, and
siRNA silencing of AGR2 induces ER stress, suggesting that AGR2 is critical for mucin folding and biosynthesis. I have also demonstrated that haplo-insufficiency of Agr2 by itself does not perturb intestinal homeostasis but enhances mucin misfolding and misfolding induced ER stress in vivo in mice carrying one Winnie mutant allele. Better understanding of the role of AGR2 will help in gaining insights in disease susceptibility.

Overall this thesis demonstrates that therapeutic inhibition of local intestinal inflammation suppresses ER stress and restores mucin biosynthesis in the intestinal goblet cells. I show that glucocorticoids are more efficacious than drugs which modulate just inflammation or ER stress by altering both pathways. Although components of ER stress pathways are important in maintaining homeostasis, my results indicate that therapeutic intervention with ER stress modulators will not be efficacious if inflammation is already established. Outcomes from my research will provide insights in determining the efficacy of therapeutic manipulation of the inflammatory-ER stress nexus in order to devise new strategies to alleviate or prevent IBD, and have wider ramifications for chronic inflammatory diseases involving ER stress.
Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed)_____________________________

Indrajit Das
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<tr>
<td>4-PBA</td>
<td>4-phenylbutyric acid</td>
</tr>
<tr>
<td>AMPK</td>
<td>5/-AMP-activated protein kinase</td>
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<tr>
<td>HPRT</td>
<td>Hypoxanthine phosphoribosyltransferase</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IEC</td>
<td>Intestinal epithelial cells</td>
</tr>
<tr>
<td>IRGM</td>
<td>IRG protein control intracellular pathogens</td>
</tr>
<tr>
<td>JIK</td>
<td>c-Jun-N-terminal inhibitory kinase</td>
</tr>
<tr>
<td>MAPKKK</td>
<td>Mitogen-activated protein kinase kinase kinase</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mammalian target of rapamycin</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemotactic peptide</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen activated protein kinase</td>
</tr>
<tr>
<td>NO</td>
<td>Nitrogen oxide</td>
</tr>
<tr>
<td>NOD2</td>
<td>Nucleotide-binding oligomerization domain-containing protein 2</td>
</tr>
<tr>
<td>NLR</td>
<td>NOD-like receptors</td>
</tr>
<tr>
<td>ENU</td>
<td>N-ethyl-N-nitrosourea</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptors</td>
</tr>
<tr>
<td>PPRE</td>
<td>PPARγ response element</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PAS</td>
<td>Periodic Acid Schiff’s staining</td>
</tr>
<tr>
<td>RAC1</td>
<td>Ras-related C3 botulinum toxin substrate 1</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RANTES</td>
<td>Regulated upon Activation, Normal T-cell Expressed, and Secreted</td>
</tr>
<tr>
<td>Abbr</td>
<td>Full Name</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------------------------------------</td>
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<tr>
<td>RXR</td>
<td>Retinoid X receptor</td>
</tr>
<tr>
<td>SPDEF</td>
<td>SAM pointed domain containing Ets transcription factor</td>
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<tr>
<td>STAT3, 4</td>
<td>Signal transducer and activator of transcription 3,4</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>TUDCA</td>
<td>Taurine-conjugated ursodeoxycholic acid</td>
</tr>
<tr>
<td>Tg</td>
<td>Thapsigargine</td>
</tr>
<tr>
<td>TPMT</td>
<td>Thiopurine-S-methyltransferase</td>
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<tr>
<td>TLR2</td>
<td>Toll like receptor 2</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
</tr>
<tr>
<td>TNBS</td>
<td>Trinitrobenzene sulfonic acid</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>TL1A</td>
<td>Tumour necrosis factor–like ligand 1</td>
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<tr>
<td>TRAIL 1</td>
<td>Tumour necrosis factor-related apoptosis-inducing ligand</td>
</tr>
<tr>
<td>TNFRS7</td>
<td>Tumour necrosis factor receptor superfamily member 7</td>
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<tr>
<td>TACE</td>
<td>TNFα converting enzyme</td>
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<tr>
<td>TNFR  1,2</td>
<td>TNF receptor type 1,2</td>
</tr>
<tr>
<td>TRADD</td>
<td>TNFR1–associated death domain</td>
</tr>
<tr>
<td>Tm</td>
<td>Tunicamycin</td>
</tr>
<tr>
<td>UC</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td>vWf</td>
<td>von Willebrand factor</td>
</tr>
<tr>
<td>XO</td>
<td>Xanthine oxidase</td>
</tr>
</tbody>
</table>
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For any errors or inadequacies that may remain in this work, of course, the responsibility is entirely my own.
List of Publications

**Published manuscripts**


**Manuscripts under review/revision**


**Manuscripts under preparation**

1. Das I, Hasnain S Z, Oancea I, Chen A, Florin T H and McGuckin M A. **Therapeutic effect of anti-inflammatory drugs and ER stress modulators on protein misfolding induced ER stress and inflammation.** (Targeted journal *Journal of Inflammation*).


