UGT Enzymes as Novel Modulators of Lipid Biosynthesis in Breast Cancer

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Introduction

- Lipid metabolism is frequently perturbed in steroid-dependent breast cancers[1].
- The activity of the sterol regulatory binding protein (SREBP) transcription factors that regulate lipid biosynthesis are frequently elevated in these cancers. Elevated SREBP activity provides cancerous cells with lipids to fuel proliferation and promotes membrane lipid saturation to protect against oxidative stress[1,2].
- UDP-glycosyltransferases (UGTs) are a superfamily of enzymes that conjugate sugars to small lipophilic molecules. The expression of the UGT genes, UGT2B11 and UGT2B28, have been linked with pathogenic features of breast cancer, however, their biological functions remain poorly understood[3,4,5].
- Analysis of the Cancer Genome Atlas Breast Cancer RNAseq (TCGA-BRCA) dataset correlates expression of these UGTs with genes involved in SREBP-mediated lipogenesis[6]. Guided by this finding, we investigated the functions of these UGTs in breast cancer cell lines.

SREBP Signalling Overview

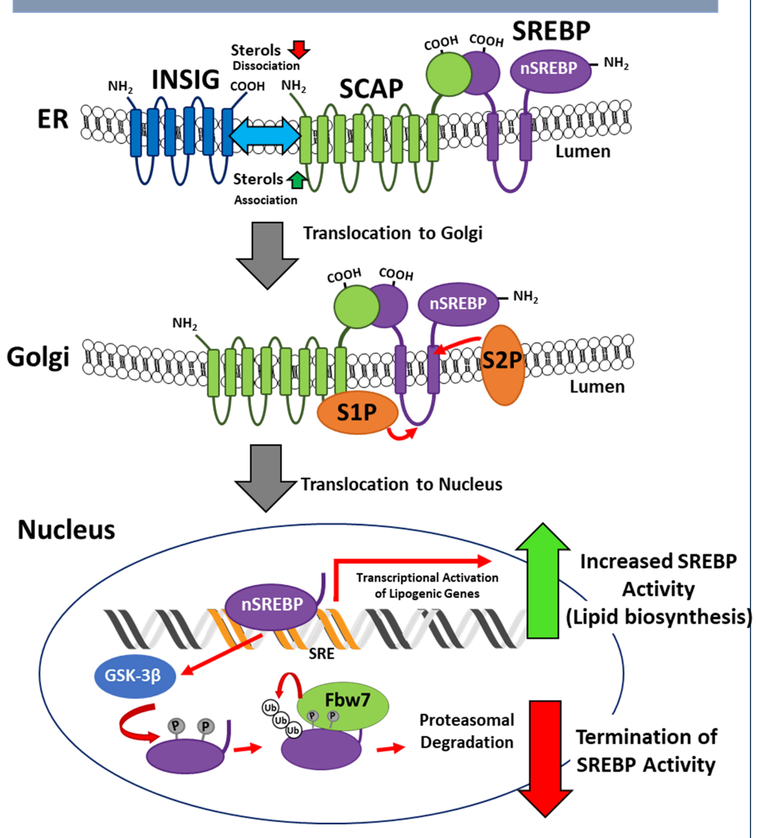


Figure 1: Schematic of SREBP activation and termination pathways. *Activation:* Sterol sensing by SCAP in the ER causes dissociation of SCAP/SREBP from INSIG, allowing the SCAP/SREBP complex to traffic to the Golgi where it is proteolytically activated to allow the active nSREBP transcriptional element to translocate to the nucleus and promote SREBP target gene expression. *Termination:* In the nucleus active nSREBPs are phosphorylated by GSK-3β, leading to the recruitment of the FBW7 ubiquitin ligase, ubiquitination, proteasomal degradation and termination of SREBP signalling

Aims

The objectives of this project are to determine the function of UGT2B11 and UGT2B28 in breast cancer lipid biosynthetic pathways, specifically the following aims are being investigated.

Aim 1: Characterise how UGT2B11 & UGT2B28 promote activation of SREBP Signaling

Aim 2: Characterise how UGT2B11 & UGT2B28 promote termination of SREBP Signaling

Results

UGT2B11 and UGT2B28 promote breast cancer cell growth

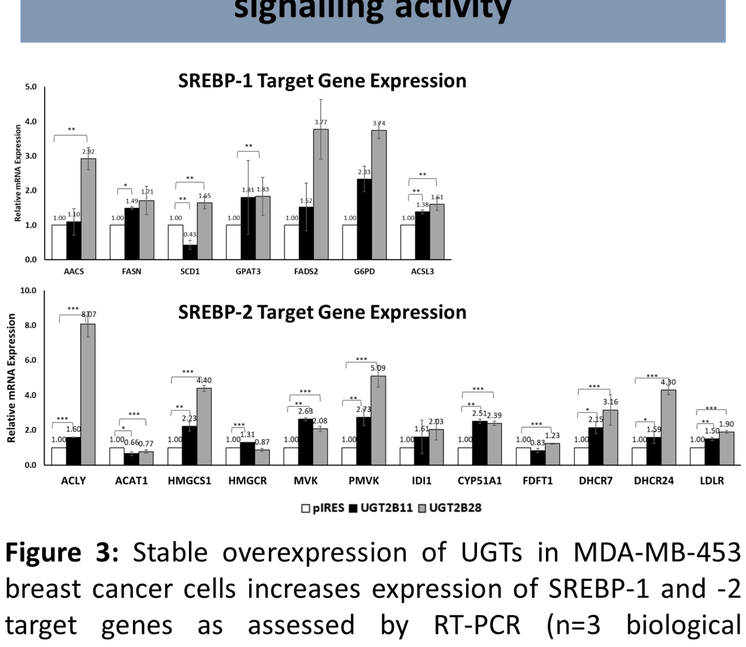


Figure 2: (A) Topology of Wildtype (WT) UGTs and domain structure of WT and naturally occurring inactive truncated (Tr) variants. (**B)** Stable overexpression of any UGT2B11 or UGT2B28 variant in MDA-MB-453 breast cancer cells promotes proliferation (n=3 biological replicates)

UGT2B11 and UGT2B28 promote SREBP signalling activity

Figure 3: Stable overexpression of UGTs in MDA-MB-453 breast cancer cells increases expression of SREBP-1 and -2 target genes as assessed by RT-PCR (n=3 biological replicates)

UGT Mediated SREBP Activation Working Model

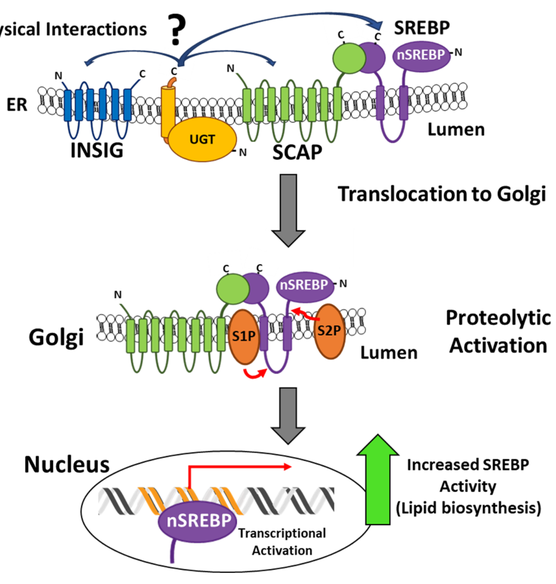


Figure 4: Schematic of UGT mediated SREBP Activation. As UGTs promote SREBP activation in what is predicted to be through non-catalytic activity (as overexpression of inactive or active variants shows a similar biological effect) , they are hypothesised to interact with components of the INSIG/SCAP/SREBP sterol sensing complex to promote SREBP signalling.

UGT2B11 and UGT2B28 promote turnover of nuclear SREBPs

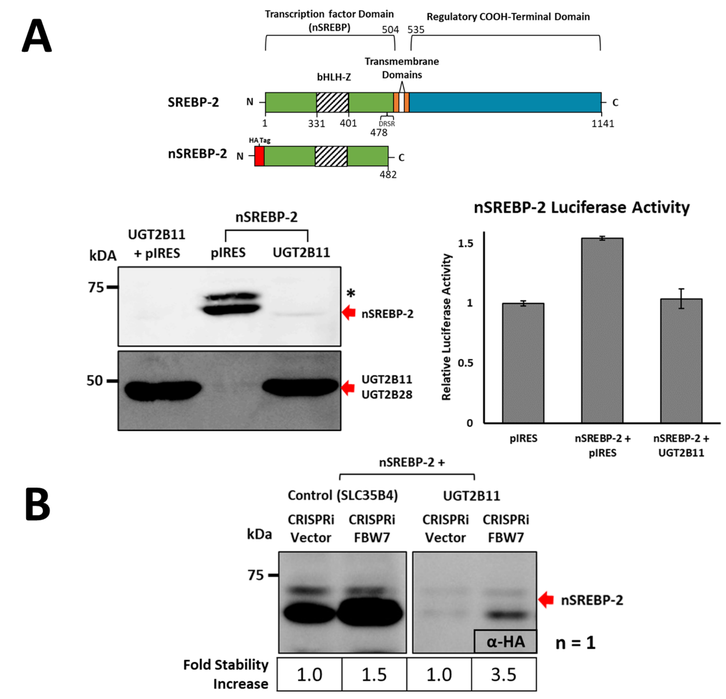


Figure 5: (A) Co-transfection of nSREBP-2 with UGT2B11 results in almost complete loss of nSREBP-2 protein (n=7) and ~30% reduction in SREBP activity (assessed via luciferase, n=2) in HEK-293T cells. **(B)** Depletion of the FBW7 ubiquitin ligase with CRISPRi stabilised nSREBP-2 protein in control and UGT2B11 co-transfection conditions. This suggests that UGTs promote turnover on nSREBPs via a process upstream of FBW7 recruitment.

UGT mediated nSREBP Turnover Working model

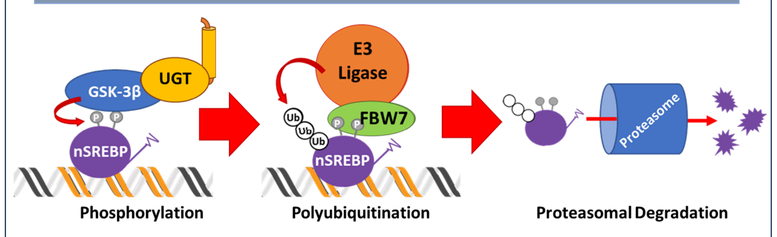
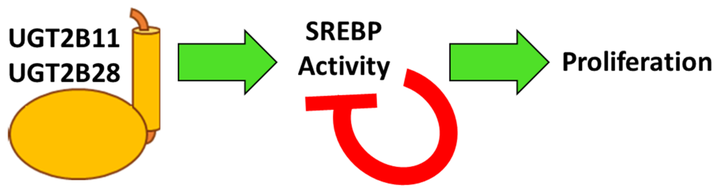


Figure 6: Schematic of UGT mediated nSREBP degradation. UGTs are postulated to be involved in GSK-3β or FBW7 recruitment to transcriptionally active nSREBPS. This hypothesised activity would lead to increased phosphorylation or ubiquitination of nSREBPS, causing increased degradation by the proteasome and termination of SREBP activity.

Summary



We have shown that the overexpression of UGT2B11 and UGT2B28 provides a proliferative advantage to breast cancer cells and increased the expression of SREBP target genes, suggesting a possible role in SREBP activation (Figs 2-4). Additionally, we have shown that UGTs can promote proteolytic turnover of nSREBPs and may terminate nSREBP activity (Figs 5-6).

Together, our data support a model where UGTs control the balance between SREBP activation and termination. The finding that UGTs may be novel regulators of lipid biosynthesis may help explain their association with poor breast cancer outcomes and suggests that they require further investigation as novel therapeutic targets

References
[1] Horton et al. (2002) *J Clin Investig;* [2] Eberle et al. (2004) *Biochimie;* [3] Belledant et al. (2016) *Euro Urol;* [4] Wang et al. (2017) Int J Cancer; [5] Curtis et al. (2012) Nature; [6] Tang et al. (2017) *Nucleic Acid Res*

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