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Cholesterol side-chain hydroxylation is associated with expression of P-glycoprotein and disease-free survival in triple negative breast cancer patients

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Triple negative breast cancers (TNBCs) lack cellular protein receptors that can be targeted by the most effective anti-cancer drugs. Instead these patients are treated with non-specific, systemic, cytotoxic chemotherapy agents, which cause severe side effects. Furthermore, chemotherapy resistance is common, rendering many treatments ineffective. P-glycoprotein (Pgp) is a chemotherapy efflux pump commonly elevated in chemoresistant tumours. Direct Pgp inhibitors have typically failed owing to its crucial role in other tissues; tumour specific mechanisms of Pgp regulation should instead be targeted to induce chemosensitization. Pgp is regulated by cholesterol side chain hydroxylation products (scOHC) and liver x receptor (LXR) in the blood brain barrier [1], potentially linking nutrition and cholesterol metabolism to cellular drug efflux. Interestingly, breast cancer relapse is associated with diets that drive elevated levels of LXR ligands via high circulating cholesterol [2]. Our aim was to determine if expression of LXR and synthesis of its scOHC ligands was associated and/or causative of *i*) elevated P-gp expression in TNBC, and *ii*) disease free survival of TNBC patients.

A tissue micro-array was generated with 148 TNBC tumours from TNBC patients treated at Leeds Teaching Hospitals Trust (LTHT) between 2008 and 2012 (06/Q1206/180). Pgp and scOHC synthesising enzyme expression was assessed with IHC and histoscore (as previously [3]). In a separate cohort of 31 fresh/frozen TNBC tumours obtained from the Leeds Breast Research Tissue Bank (LBRTB; 15/HY/0025) we determined: scOHC concentrations for 24OHC, 25OHC, and 26OHC using LC-MS/MS [4]; and P-gp mRNA expression using TaqMan-qPCR ($\Delta\Delta$cT). *In vitro*, TNBC cell lines (MDA.MBA.231 and MBA.MB.468) were treated with scOHCs and changes to Pgp mRNA expression assessed by TaqMan. Epirubicin efficacy after scOHC exposure was determined with colony forming assays and MTT. Spearman rank correlation, log-rank Kaplan Meier plots, and one-way ANOVA were used for statistical analysis.

In the LTHT cohort (n=148) Pgp expression in cancer cells was correlated with CYP46A1 (R^2=0.3; p<0.0001), CH25H (R^2=0.53; p<0.0001), and CYP27A1 (R^2=0.07; p<0.01). High levels of P-gp, CYP46A1 and CH25H (log-rank test: p<0.01 for all) were all associated with reduced disease-free survival. In the LBRTB cohort (n=31), 24OHC correlated with P-gp mRNA (R^2=0.5; p=0.04), but only elevated 25OHC and 26OHC associated with reduced disease-free survival (p<0.05). *In vitro*, addition of scOHC elevated P-gp mRNA and abrogated epirubicin mediated cytotoxicity in TNBC cells (p<0.001).

These data suggest that intratumour oxysterol content is linked to reduced disease-free survival. Nutritional and pharmacological reduction of oxysterol levels should be explored as routes to reversing chemoresistance in TNBC.

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