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Oestrogen metabolism by steroid sulphatase and 17beta-hydroxysteroid dehydrogenases promotes colorectal cancer proliferation via the G-protein coupled oestrogen receptor

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BACKGROUND

Colorectal cancer (CRC) is the third most common cancer worldwide(1). Although not traditionally viewed as a hormonal cancer, evidence suggests that peripheral synthesis of active oestrogens in CRC worsens prognosis(2). Oestrogen metabolising enzymes include steroid sulphatase (STS), which de-sulphates oestrogens into their active forms and 17β-hydroxysteroid dehydrogenases (17βHSD) which are oestrogen oxidoreductases. We previously demonstrated TNFα and IL-6 can increase STS activity *in vitro* and STS activity is raised in human CRC, but further assessment of the oestrogen pathway in CRC is still needed; such as their impact on proliferation. 17βHSD-1, 7 and 12 all reduce oestrone (E_1) to the most potent oestrogen, oestradiol (E_2), and have not been characterised in CRC. Also, although ERα and β have previously been examined in the colon(3), the G-protein coupled oestrogen receptor (GPER) has not been explored and is a potential target for oestrogen action.

OBJECTIVES

1. To determine whether E_2 synthesis pathways, through STS and 17β-HSDs, are elevated in human CRC?
2. To examine whether CRC cell lines proliferate in response to oestrogen?
3. To determine if oestrogens stimulate GPER to affect proliferation in CRC cell lines?

METHODS

1. Characterised 17βHSD-1,7 and 12 and GPER expression in human CRC tissue and cell lines using qPCR and Western Blotting.
2. Proliferation response in CRC cell lines oestrogen treatment, stable overexpression of STS, GPER agonist (G1), or antagonist (G15) using BrdU assays. Treatments were for 48 hours.
3. Quantified oestrogen and their sulphates in CRC cell lines using a novel mass spectrometry method developed at The University of Birmingham.
4. Statistics were calculated using Student's two-tailed t-test with $P<0.05$ deemed significant. Results are expressed as mean SEM.

REFERENCES

(1).World Cancer Research Fund International. *Colorectal Cancer Statistics*. 2012 [cited 2015 26 Jan]; Available from http://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/colorectal-cancer-statistics

(2).Sato, R., et al., *Steroid sulfatase and estrogen sulfotransferase in colon carcinoma: regulators of intratumoral estrogen concentrations and potent prognostic factors.* Cancer Res, 2009. **69**(3): p. 914-22.

(3) .Campbell-Thompson M, Lynch IJ, Bhardwaj. *Expression of estrogen receptor (ER) subtypes and ERbeta isoforms in colon cancer*. Cancer Res. 2001; 61: p 632-640.

Human Colorectal Cancer Up-regulates E_2 Synthesis Pathways

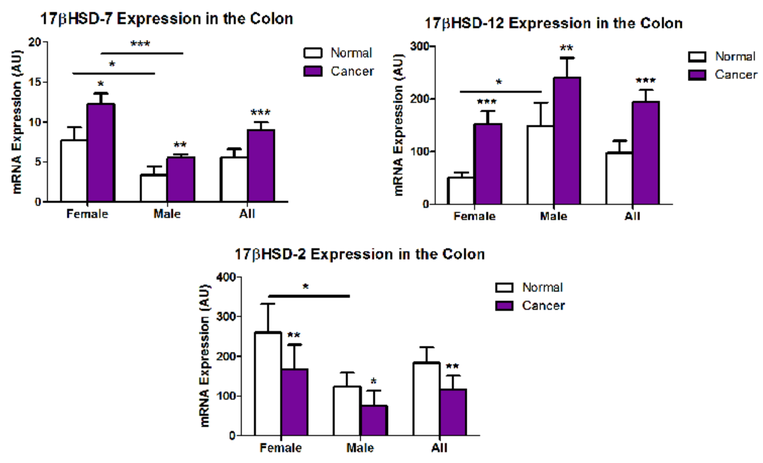

Figure 1. mRNA expression of 17βHSD oestrogen oxidoreductases. 17βHSD-1 was not expressed in the colon (data not shown). (A) 17βHSD-7 and 12 mRNA is increased in CRC suggesting an increase in E_1 to E_2 metabolism. (B) 17βHSD-2 mRNA is reduced in CRC, implying reduced E_2 to E_1 metabolism.

G-Protein Coupled Oestrogen Receptor is Expressed in Human Colon

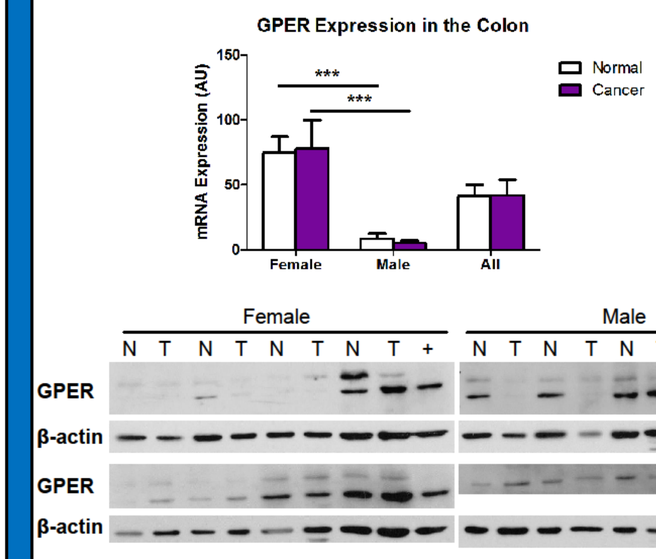

Figure 2. (A) GPER mRNA is expressed in males and females in both normal and cancerous colon, but at a higher level in women. (B) Western blots demonstrating GPER expression at a protein level in normal (N) and matched tumour (T) colon with MCF7 used as a positive control (+).

Oestrogen Augments Proliferation in CRC Cell Lines

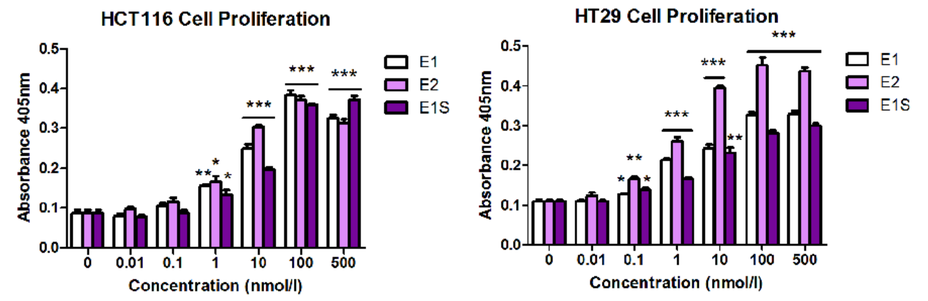

Figure 3. Oestrogen treated HCT116 (A) and HT29 cells (B), but not Caco-2 cells (not shown) resulted in an increase in proliferation, especially with E_2 treatment.

Overexpressing STS and GPER Stimulation increases Proliferation in CRC Cell Lines

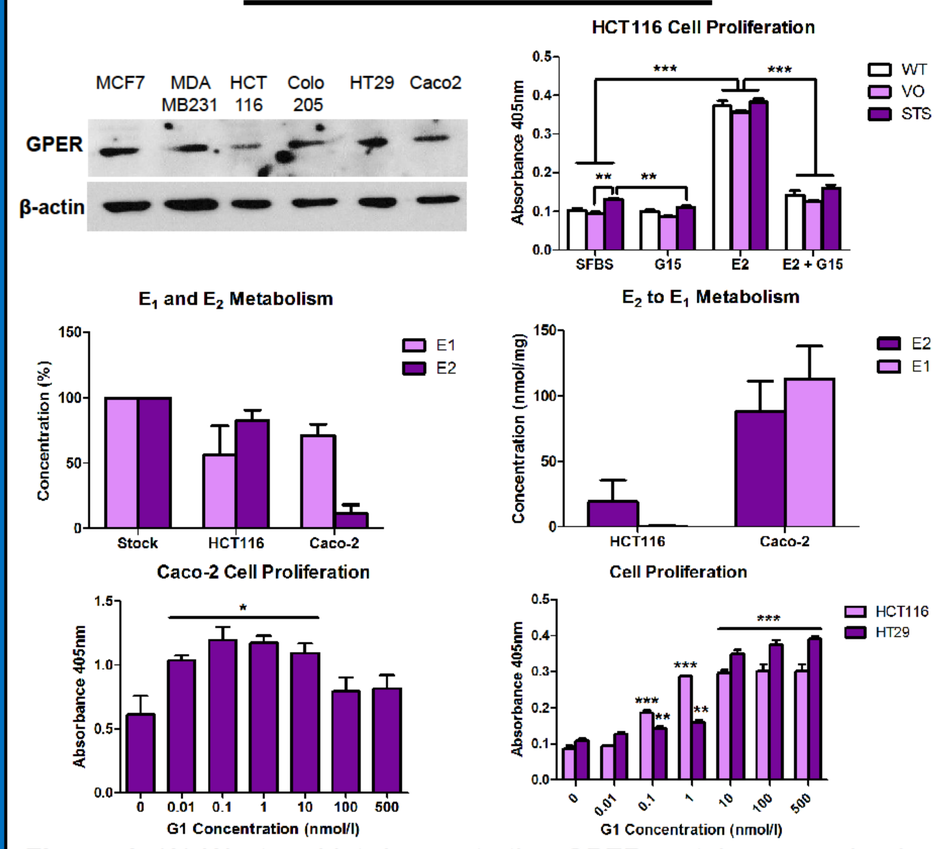

Figure 4. (A) Western blot demonstrating GPER protein expression in all CRC cell lines. (B) Stably overexpressing STS and E_2 (100nmol/l) treatment in HCT116 cells enhanced proliferation, which was inhibited by G15 (1μM) , a GPER antagonist. (C) and (D) HCT116 and Caco-2 cells were treated with 100nM E_1 or E_2 for 24 hours and media subjected to LC/MS analysis for oestrogen metabolites. (C) Percentage of E_1 or E_2 metabolised by each cell line in 24 hours. (D) E_2 metabolism corrected for protein concentration (BCA assay). E_2 is a GPER ligand, but Caco-2 cells oxidise E_2 to E_1, which would not activate GPER. GPER agonist, G1, increased proliferation in (E) Caco-2, (F) HCT116 and HT29 cells.

CONCLUSION

- Findings suggest the majority of human CRC escalate intratumoural E_2 concentrations through 17βHSD-7 and 12 and STS (Figure 5). This local oestrogen rise likely acts through GPER to augment tumour proliferation.
- Therefore, inhibiting STS and 17βHSD-7 and 12 together with GPER antagonists may benefit some CRC patients.

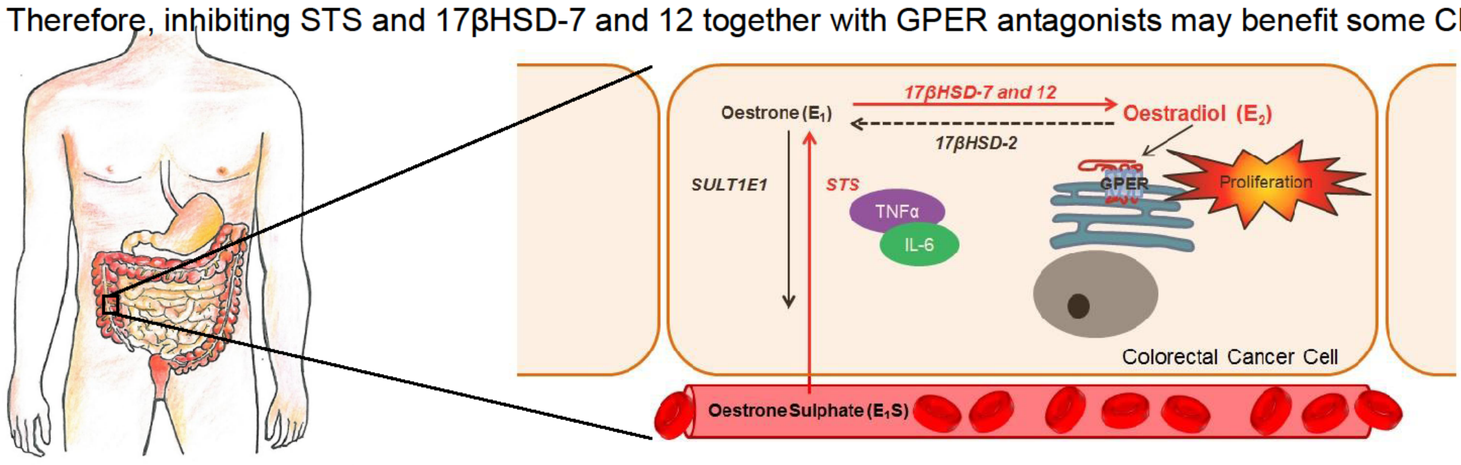

Figure 5. Oestrogen pathway in colorectal cancer. Circulating E_1S is taken up by tumour cells and desulphated by STS to active E_1. E_1 is then reduced by 17βHSD-7 and 12 to E_2, the most potent oestrogen. IL-6 and TNFα in the tumour microenvironment increase STS activity.

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