

CLA on p53, p21WAF1 and bcl-2 expression in addition to the cytotoxic effects of CLA on breast tumour growth.

Methods: Oestrogen receptor positive and negative breast cancer cells (MCF7, MDA-MBA-231) were grown in supplemented RPMI media, containing CLA at concentrations (0 μ M to 200 μ M) for 24 hours. The effects on cell growth were assessed using MTT assay. Following treatment with CLA, northern blotting and ELISA were performed to determine the effects on the expression of p53, p21WAF1 and bcl-2.

Results: Exposure to CLA resulted in a dose dependent reduction in growth of MCF-7 cells - 20% at 6.25 μ M, 50% at 100 μ M and 65% at 200 μ M ($p < 0.001$). Similar results were obtained for MDA-MBA-231 cells. Northern blot analysis showed that CLA treatment caused a dose dependent increase in wild-type p53 expression (MCF-7 cells) by 284% at 12.5 μ M, 347% at 100 μ M, and 523% at 200 μ M of CLA ($p < 0.01$). There was no change in bcl-2 expression. The expression of p21WAF1, a key downstream regulator of p53, was raised to 203%. CLA did not change the expression of mutant p53 (present in MDA-MBA-231 cells) or p21WAF1, but did increase the expression of bcl-2 by 103% at 12.5 μ M, 201% at 50 μ M, and 207% at 100 μ M of CLA ($p < 0.01$). Similar over-expression of the corresponding proteins were noted by ELISA.

Conclusions: This is the first demonstration which shows that CLA exerts its anti-tumour effects by increasing the expression of the wild-type p53 and the p21WAF1 gene. However, in cells with mutant p53, CLA inhibits cell growth, through a p53-independent pathway.

O-83. HYPERSENSITIVE K303R OESTROGEN RECEPTOR VARIANT NOT FOUND IN DUCTAL CARCINOMA *IN SITU*

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Non-atypical hyperplasia of the breast (hyperplasia of usual type) is believed to be a non-obligate precursor of breast cancer. As such, genetic abnormalities or mutations in such lesions may play a role in progression toward malignancy. One recently described mutation, occurring in about one third of hyperplasia tested is an A908G (K303R) change in the oestrogen receptor a gene that creates a hypersensitivity to oestradiol (Fuqua *et al.*, Cancer Res 2000, 60: 4026–4029).

We have examined a significant number of DCIS, by sequencing PCR products from microdissected samples.

No evidence of the A908G mutation was found, either individually or together with the wild-type allele. Enough cases of DCIS (44) were studied to make this results statistically significant ($P < 0.001$; Fisher Exact Test).

Retention of the A908G mutation in more advanced lesions, such as ductal carcinoma *in situ* (DCIS), would provide evidence that this mutation is involved in breast cancer progression. That the mutation was not found, leads us to believe that either the

mutation is not retained during progression, that it may be involved only in the progression of lower grade DCIS, or that this mutation is limited to HUT that fail to progress via DCIS.

O-84. OESTROGEN WITHDRAWAL REDUCES EPITHELIAL CELL PROLIFERATION IN OESTROGEN RECEPTOR (ER) POSITIVE BUT NOT NEGATIVE DUCTAL CARCINOMA *IN-SITU* (DCIS)

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Aims: Over 50% of DCIS is ER negative which will not respond to hormone therapy. To investigate the effect of hormone manipulation on epithelial proliferation we studied 100 women who had undergone diagnostic core biopsy followed by surgery for DCIS 14–21 days later. ER status and ki67 (a measure of epithelial cell proliferation) was determined by counting 1,000 cells after immuno-histochemical staining on paired sections of the core biopsy and operative specimens for each woman. In ER negative DCIS epithelial proliferation did not change between diagnosis and treatment. Only in ER positive patients who stopped HRT was a fall in epithelial proliferation observed (see table).

Group	ER (No)	Median core Ki67 (IQR)	Operative Ki67 (IQR)	P value*
1. Never taken HRT (control)	– (24) + (32)	9.8 (5.6–18.5) 8.2 (4.9–14.9)	11.3 (7.5–15.5) 7.7 (4.4–11.8)	0.92 0.49
2. Continued HRT	– (4) + (17)	11.4 (6.1–28.8) 8.8 (3.8–16.8)	16.0 (6.9–28.8) 8.8 (2.0–15.4)	1.00 0.76
3. Stopped HRT	– (6) + (15)	15.9 (10.5–24.4) 9.3 (2.3–17.1)	16.4 (10.7–23.7) 3.3 (0.8–8.9)	0.94 0.04

*Mann-Whitney 2-tailed test

Conclusions: Oestrogen withdrawal reduces proliferation in ER positive but not in ER negative DCIS. Therefore adjuvant anti-oestrogen therapy is likely only to benefit ER positive patients.

O-85. CHROMOSOMAL ALTERATIONS ASSOCIATED WITH TRANSFORMATION OF A TAMOXIFEN-SENSITIVE CELL LINE INTO ITS DRUG RESISTANT CLONE

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Introduction: Tamoxifen resistance is a serious problem in the management of breast cancer. Knowledge of the genetic pathways leading to tamoxifen resistance may allow the development of novel therapeutic strategies.

Aims: To determine the genetic changes between MCF-7 a tamoxifen sensitive human breast cancer cell line and its resistant clone CL-9 using comparative genomic hybridisation (CGH).