Title: EPIGENETIC SILENCING OF E-CADHERIN (*CDH1*) IN NON-NEOPLASTIC EPITHELIA PREDISPOSES TO LOBULAR BREAST NEOPLASIA AND IDENTIFIES AS A TARGET FOR CHEMOPREVENTION

Names: Zou D, Yoon H-S, Perez D, Weeks RJ, Guilford P and Humar B

Institution: University of Otago, Dunedin, New Zealand

Abstract:

Background:
Invasive lobular carcinoma (ILC) of the breast is believed to develop from *in-situ* lesions such as atypical lobular hyperplasia (ALH) and lobular carcinoma *in-situ* (LCIS). Down-regulation of the cell–cell adhesion protein E-cadherin is a defining feature of lobular breast cancer (LBC) and already occurs in ALH and LCIS. Apart from mutational mechanisms, epigenetic silencing of the E-cadherin gene (*CDH1*) is thought to be involved in E-cadherin downregulation and has been observed at a high frequency in ILC. Whether *CDH1* promoter methylation is already present in *in-situ* lesions and thus contributes to the initiation of LBC is not established.

Design:
We thus examined microdissected archived tissue from 20 LBCs using an Arcturus LM200 laser-capture microdissection facility to determine the *CDH1* methylation status of lobular lesions. Methylation status was measured by methylation specific PCR using the bisulphate converted DNA from microdissected tissue. The PCR products were cloned and sequenced using an ABI 3730 Genetic Analyser. For assessment of *CDH1* expression, RNA was isolated from microdissected tissue and cDNA was prepared. Wild-type *CDH1* mRNA was amplified and detected using an NWG probe on an ABI 7900HT Fast Real-time PCR system.

Immunohistochemistry and immunofluorescence were used for assessment of expression of E-catherin, *β*-catena, lin-7, p120 and Ki-67.

Results:
Nineteen of the 20 LBCs had a hypermethylated *CDH1* promoter, including 13/14 ILCs and 13/13 ALHs or LCIS. Bisulphite sequencing indicated that methylation was complete within the investigated promoter fragment. Intriguingly, *CDH1* methylation was likewise present in 8/8

adjacent non-neoplastic epithelia, but not in 6/6 mammary epithelia from healthy control subjects. E-cadherin protein and mRNA were down-regulated in *in-situ* lesions relative to adjacent epithelia. Together, these results indicate that *CDH1* promoter methylation occurs in LBC prior to E-cadherin down-regulation and neoplastic development.

Conclusions:
We thus propose that epigenetic silencing represents the first of the two hits required to silence both *CDH1* alleles for LBC to develop. The presence of *CDH1* methylation in pre-neoplastic epithelia suggests the existence of mammary regions with increased disease susceptibility, providing an explanation for the frequent multifocal presentation of LBC. Furthermore, because promoter methylation is in principle reversible, our findings suggest that chemoprevention of LBC by epigenetic drugs should be feasible.