BMC Proceedings



Meeting abstract



# Hypermethylated genes as biomarkers of breast cancer

MJ Fackler*[1], V Stearns[1], SA Khan[2] and S Sukumar[1]

Address: [1]Department of Oncology Johns Hopkins University School of Medicine, Baltimore, MD, USA and [2]Department of Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Email: MJ Fackler* - mfackler@jhmi.edu

* Corresponding author

*from* 6th International Symposium on the Intraductal Approach to Breast Cancer
Santa Monica, CA, USA. 19–21 February 2009

Published: 24 July 2009

*BMC Proceedings* 2009, **3**(Suppl 5):S19 doi:10.1186/1753-6561-3-S5-S19

This abstract is available from: http://www.biomedcentral.com/1753-6561/3/S5/S19



Hypermethylated genes are emerging as some of the most promising, practical and powerful biomarkers for breast cancer detection and perhaps even of risk assessment. We have assembled a panel of methylated gene detection markers and tested them by Quantitative Multiplex Methylation-Specific PCR (QM-MSP) in breast cancer. Here we two pilot studies: 1) detection of cancer in DNA from spontaneous nipple discharge (SND) and 2) assessing risk, and its possible reduction in contralateral breast tissue of women undergoing treatment with aromatase inhibitors.

SND is most frequently caused by intraductal papilloma (66%), but in a minority of women (~10%) it is caused by ductal carcinoma in situ (DCIS). Therefore essentially all women with SND are screened for breast cancer. All the current methods used for screening exhibit low sensitivity and specificity for detection of cancer. Mammary endoscopy (ductoscopy) appears to improve localization of lesions in patients with SND and allows retrieval of intraductal cells for diagnostic purposes. Development of a non-surgical method to reliably diagnose cancer would offer the possibility of diagnosis without surgery for the majority of women with SND who have a very low likelihood of significant neoplasia. Further, a reliable diagnostic test of intraductal pathology will enable in-situ ablation of benign lesions with either endoscopic techniques or the intraductal administration of anti-neoplastic agent. In a pilot study we investigated whether quantitative assessment of gene promoter hypermethylation could enhance detection of breast cancer in women with spontaneous nipple discharge (SND) when used in conjunction with ductoscopy. Ducts with significant visualized lesions were surgically resected (36 ducts in 33 women) and those with minimal findings were not (28 ducts in 16 women). QM-MSP data of DNA from cells in ductoscopic washings were compared to ductoscopy findings, cytology, and tissue histology. Cells from ducts with significant ductoscopic findings had higher levels of methylation while cells found in ducts with minimal findings had minimal methylation; methylation was higher in cells from ducts with malignant lesions compared to low methylation in ducts bearing benign lesions such as papilloma. *RASSF1A*, *TWIST1*, and *HIN1* cumulative gene methylation accurately distinguished cells washed from ducts with cancerous vs. benign lesions (100% sensitivity, 72% specificity and AUC of 0.91 according to ROC analyses). Using QM-MSP the positive predictive value of ductoscopy more than doubled because QM-MSP has threefold higher sensitivity than cytology in evaluation of ductal cells. This study demonstrates the potential benefit of targeting surgical ductal excision to ducts that have both high methylation and significant abnormalities on ductoscopy. Future large-scale studies to validate this approach are needed.

Women with a history of breast cancer are at increased risk to develop a contralateral breast (CLB) cancer. Since gene methylation occurs early in tumorigenesis, and is frequently higher in normal tissues adjacent to a breast tumor, we hypothesized that women with a prior breast cancer would harbor higher levels of methylated genes in

the CLB and that treatment with anastrozole, an aromatase inhibitor that reduces the risk of CLB cancer, would decrease methylation levels. We conducted a prospective, single-arm study in 54 postmenopausal women with hormone receptor-positive stage 0–III breast cancer who had completed local therapy, had an intact CLB, and would receive anastrozole as their sole adjuvant therapy. Of those, 33 women underwent an optional CLB biopsy both at baseline and 6 months after initiating Anastrozole. At baseline, 84% of paired samples had measurable cumulative methylation of the 6 gene panel; *TWIST1*, *RASSF1A*, and *RARβ* were most frequently methylated. After 6 months of anastrozole, we observed significant decreases in methylation for *TWIST1*, *RASSF1A*, and *RARβ*, among patients with methylation identified at baseline. These preliminary findings emphasize the need for prospective evaluation of the relationship between changes in methylation and incidence of breast cancer in high-risk women.