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A direct comparative study of methylation-specific PCR in ductal lavage fluid, breast cancer tissue, normal breast parenchyma and plasma in women with early breast cancer

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Introduction

Breast duct lavage for analysis by methylation-specific PCR is a novel but established method for detecting the presence of cancer and may contribute additional information about prognosis and response to treatment. The aim of this consecutive series of breast cancer patients was a case-control study to evaluate qualitative methylation of five published tumour suppressor genes[1] in breast cancer tissue, adjacent normal breast parenchyma, duct lavage fluid and plasma.

Methods

Breast cancer tissue and adjacent normal parenchymal tissue was obtained at the time of surgery in 24 women. Normal breast tissue from 22 patients undergoing breast surgery without breast cancer were used as controls. In all patients, tissue cores, matched plasma, ipsilateral and contralateral duct lavage fluid were obtained for methylation-specific PCR. DNA was purified from microdissected tissue, plasma and breast duct biofluids using the QUIAGEN DNeasy kit. Purified DNA was then modified (EZ Zymo methylation kit) for detection of methylated regions in five genes: HIN-1, RIL, RASSF1A, CDH13 and RARβ2, by optimised specific-PCR. Proportional data with binomial errors were used to compare cancer vs control samples.

Results

Methylated DNA products were qualitatively scored and a positive correlation identified between tumour tissue and ipsilateral duct lavage in the breast cancer group (n = 24): HIN-1 (58 and 50%), RIL (63 and 42%), RASSF1A (71 and 54%), CDH13 (42 and 33%) and RARβ2 (38 and 25%. Methylation was significantly higher in tumour and ipsilateral duct lavage fluid when compared with adjacent normal tissue and contralateral duct lavage. (See Table 1.)

Methylation was less when scored as accumulated methylation events in benign breast tissue and duct lavage of 22 non-cancer patients: HIN (4% and 4%), RIL (8% and 0%), RASSF1A (13 and 0%), CDH13 (13 and 4%), RARβ2 (0%). No difference was found in plasma methylation of cancer patients versus controls. ($a_{1\text{-sided}}$ = 5%; power = 90%)

Conclusion

Our data demonstrates the feasibility to modify purified DNA from cellular breast duct fluid for detection of methylated products by methylation-specific PCR, with a significant difference in rates of DNA methylation in duct lavage of cancer patients versus controls. These differences were unrelated to plasma methylation profiles, limiting blood testing as a biomarker of breast cancer progression. The inclusion of other tumour suppressor genes in future

Table 1:

	CANCER (n = 24)					CONTROLS (n = 22)		
	Tumour tissue	Normal tissue	Ipsilat DL	Contra DL	Plasma	Normal tissue	Ipsilat DL	Plasma
HIN-1	14	5	12	0	0	1	1	0
RIL	15	4	10	1	2	2	0	1
RASSF1A	17	7	13	0	0	3	0	0
CDH13	10	5	8	2	0	3	1	0
RARβ2	9	4	6	0	1	0	0	0

Ipsilat = Ipsilateral; Contra = Contralateral; DL = ductal lavage.

studies may increase the sensitivity and specificity of breast-specific biofluids in subclinical cancer.

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