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Title: High-throughput DNA methylation profiling using promoter microarrays

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Background: Pancreatic cancer is a genetic and epigenetic disease characterized by widespread and profound alterations in DNA methylation. Effective high-throughput genome-wide methods for identifying DNA methylation patterns are needed accelerate the discovery abnormal DNA methylation patterns. We have developed a novel microarray-based method for detecting differential methylation patterns based on a methylated CpG island amplification (MCA) strategy using SmaI methylation sensitive restriction enzyme. Sequentially MCA fractions are interrogated on high-resolution promoter microarrays with competitive hybridization.

Methods: Replicate DNA was extracted from the pancreatic cancer cell line, Panc-1 and HPDE, a normal pancreatic epithelial line. Amplicons from Panc-1 and HPDE were hybridized to Agilent human 44K promoter array to profile differential methylation. Candidate methylated genes identified by microarray were further evaluated using conventional bisulfite- modified sequencing (BMS).

Results: After normalization and analysis with CGH analytics 3.4[®], we identified 589 probes with a significant higher signal in the pancreatic cancer line relative to the normal line (p-value<0.001 and log₂ ratio>1.5). A further 493 probes were hypomethylated in Panc-1 relative to HPDE. Replicate analysis of array data showed high correlation value (R²=0.92~0.93). Several well-known genes as a cancer specific hypermethylation were identified, including ppENK and HOXA5. 21 of 22 novel genes predicted to be methylated at the CpGs by microarray data were methylated by BMS.

Conclusion: Our microarray based method is an accurate and powerful method for detecting methylated DNA profiles in human samples.