Comprehensive Methylome Analysis of Breast Cancer Cell Lines

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INTRODUCTION

The epigenomes of breast cancer are plagued by aberrant DNA methylation that is characterized by global hypomethylation and specific hypermethylation in CpG islands (CpGi)[1]. Here we analyze whole genome bisulfite sequencing (WGBS) data from healthy mammary epithelial cells (MECs), hTert immortalized MEC line, and an adenocarcinoma MEC line. Using a forward genetics approach we investigate changes in the methylation of CpGi, particularly those associated with cancer related genes, during the progression from normal to cancerous phenotypes.

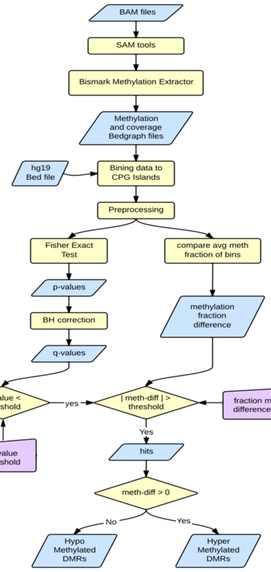
METHODS

Figure 1. (a) Cell types and sequencing reads; **(b)** During bisulfite conversion, unmethylated cytosines are converted to uracils while methylated cytosines remain unchanged; **(c)** Data analysis pipeline for WGBS.

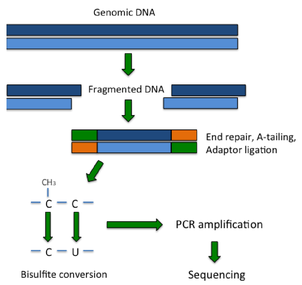
a)

Sample Type	Total number of Reads
Mammary gland adenocarcinoma – MCF10A cells	842,464,512
Mammary gland normal – hTert immortalized cells	902,493,468
Healthy mammary epithelial – primary tissue	871,308,228

b)



c)



RESULTS

Figure 2. Hyper- and hypo methylated CpGi across individual chromosomes. Ideograms depict hypermethylation in CpGi as normal myoepithelial cells transformed into immortal and tumor cells.

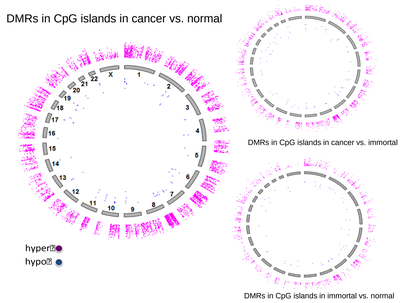


Figure 3. (a) Methylation fraction in regions identified as differentially methylated (DMRs) in tumorigenic MECs when compared to normal MECs. **(b)** Regions recognized as hypermethylated when comparing tumor MECs to normal MECs are hypomethylated or inconsistent in the other sample comparisons. This suggests more similarity between the methylation signatures of immortal and normal MECs compared to normal and cancerous MECs.

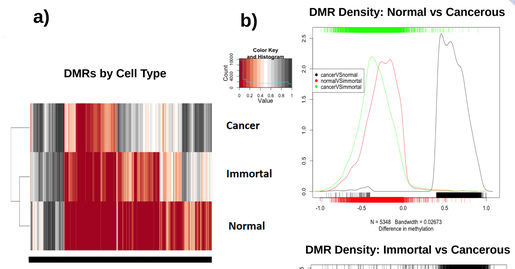


Figure 4. (a) Venn diagrams of the number of unique and overlapping hypermethylated DMRs across all comparisons; **(b)** Distribution of hyper- and hypomethylation across individual chromosomes in tumor (left) and immortal (right) MECs compared to normal MECs.

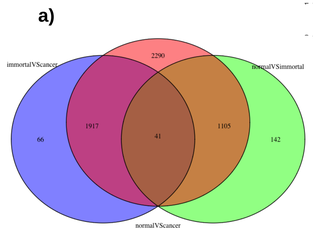


Figure 5. Ideogram of the distribution of hypermethylated CpGi unique to adenocarcinoma on chromosome 11.

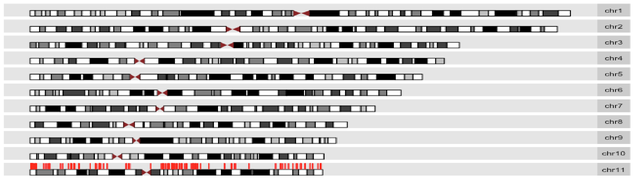


Table 1. Genes previously identified as hypermethylated in ER- breast cancer cell lines[2]. CpGi were found to be hypermethylated in the cancerous cell line relative to normal MECs at q<10e-6; bold red genes were derived from these DMRs from the region-gene association table generated using GREAT..

Genes hits known to be hypermethylated in ER- breast cancer cell lines								
ACOT4	**CACNA1H**	**CXCL12**	**GHR**	**KIFC2**	**NOVA1**	PRKG1	**SAMD11**	**SRMS**
ADAMTS13	**CACNA2D2**	**CXXC5**	**GJD3**	**LMX1B**	**NPNT**	**PRUNE2**	**SDC2**	ST6GALNAC2
ADCY1	**CADM1**	**DNAJA4**	GPR160	**LRRC26**	**NPTXR**	**PSTPIP2**	**SEMA6A**	**STK32B**
AM22	**CASKIN1**	**DSCAML1**	**GRIK3**	**MAPK8IP2**	**P2RX2**	**PTGER3**	SIDT2	STOM
AR	**CELSR1**	ENTPD2	**HS6ST3**	MMP17	**PALM**	**RAPGEFL1**	SLC1646	**SYCP2**
ASCL2	**CLUAP1**	**FGFR4**	**ID2**	MPPED2	PATZ1	**RHBG**	SLC1A2	**TP53I13**
AR	**CNTNAP2**	**FKBP4**	IL17RB	**MPV17L**	**PAX9**	RHOT2	**SLC29A4**	**TP53TG3B**
ATP2A3	**CPLX1**	**FSCN2**	**KCFBP2**	**MSI1**	**PDZRN3**	RICH2	**SLITRK4**	**VPS37D**
BTG2	CRIP2	**GATA3**	**KCNMA1**	**MYRIP**	**PGR**	**RND2**	SPATA7	ZNF512B
C17orf28	CUX2	**GFRA1**	**KIF12**	NKD2	**PIK3CA**	RNF40	SPNS1	**ZNF703**

GATA3, PIK3CA, and genes associated with TP53 (TP53I13, TP53TG3B) identified by the Cancer Genome Atlas project to be altered in >10% of breast cancers showed significant differential methylation between our normal MECs and the cancer cell line[3].

GENE ONTOLOGY

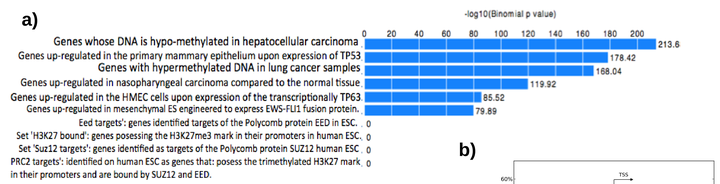


Figure 6. (a) Hypermethylated CpGi unique to cancer MECs were functionally annotated by MSigDB. Embryonic stem cell-like H3K27me3 signatures have been documented in breast and other cancers[4,5]. Polycomb Repressive Complex 2 (PRC2) regulates H3K27me3, a repressive histone modification, to regulate imprinting, X-chromosome inactivation and cell differentiation[4]; **(b)** Region-gene associations binned by distance and orientation to transcription start site.

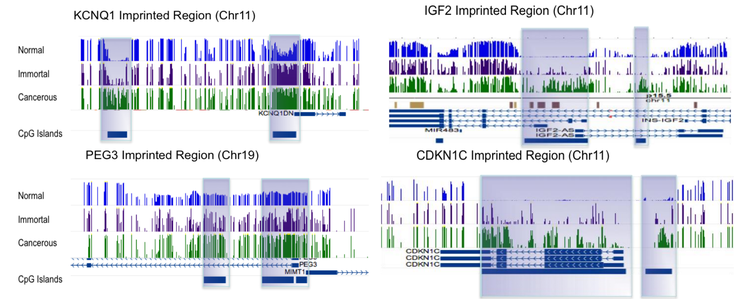


Figure 7. Dysregulation of imprinting is associated with various growth disorders and cancer[5,6]. We discovered aberrations in the IGF2, CDKN1C, KCNQ1 and PEG3 imprinted regions.

CONCLUSIONS

- Hypermethylation at CpGi occurs often as normal cells transform to tumorigenic cells while hypomethylation in CpGi is rare.
- Misregulation of the PRC2 protein complex and H3K27me3 occurs in adenocarcinoma cells, creating an embryonic stem cell-like chromatin signature.
- Perturbation of genetic imprinting was a hallmark of malignancy in MECs.
- Hypermethylation occurs at the CpGi of many tumour suppressor genes during the progression from normal to cancerous cellular states.
- In future studies, incorporating RNA-seq data with WGBS data will strengthen findings regarding the relationship between methylation and gene expression.

REFERENCES

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