Analysis of the breast cancer methylome using formalin-fixed paraffin-embedded tumour.

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Abstract

Aberrant DNA methylation occurs frequently in breast carcinogenesis. Tools for translational epigenetic studies of breast cancer involving formalin-fixed paraffin-embedded (FFPE) human tissues have now been developed. Few studies have measured genome-wide methylation in DNA derived from paraffin-embedded tumour tissues and compared the DNA methylation in corresponding adjacent non-tumour ductal epithelium (ADJ_{NT}). These studies are technically challenging due to; the spectrum of breast cancer pathologies, the variable suitability of DNA extracted from FFPE material and the difficulties in identifying ADJ_{NT}. We assessed the suitability of FFPE breast cancer material for genome-wide DNA methylation assessment of tumour and ADJ_{NT}. Twenty-one archival breast tumour tissues with paired ADJ_{NT} obtained from separate blocks and at least 2 cm from the tumour were sourced from The Melbourne Collaborative Cohort Study (MCCS). DNA was prepared from macrodissected tissue samples and assessed for genome-wide methylation using the Infinium HumanMethylation450 Beadchip (HM450K) array. The 1,000 most differentially methylated probes between tumour and ADJ_{NT} tissues in this FFPE-derived dataset differentiated tumour and ADJ_{NT} tissues in The Cancer Genome Atlas Network data (TCGA; derived from high molecular weight DNA using the same HM450K array). Large-scale studies of genome-wide DNA methylation using FFPE breast cancer specimens offer the opportunity to further refine the pathological classification of tumours, to include subtypes that are underrepresented in the TCGA data and provide the capacity to further explore intra-tumoural heterogeneity.

Keywords Breast cancer, FFPE, methylome.

Abbreviations

ADJ _{NT}	adjacent non-tumour ductal epithelium
β	Beta
μg	microgram
μl	microliter
μm	micron
Cq	quantitation cycle
DMP	differentially methylated probe
FCD	fibrocystic disease
FFPE	formalin-fixed paraffin-embedded
H&E	haematoxylin and eosin
<i>HIN-1</i>	<i>high in normal-1</i>
HM450K	Infinium HumanMethylation450 Beadchip
IDC	infiltrating ductal carcinoma
MCCS	Melbourne Collaborative Cohort Study
ng	nanogram
<i>NKX6-2</i>	<i>NK6 Homeobox 2</i>
NOS	not otherwise specified
QC	quality control
qPCR	quantitative polymerase chain reaction
<i>RASSF1A</i>	<i>RAS association domain family protein 1A</i>
SWAN	Subset-quantile Within Array Normalisation
TCGA	The Genome Cancer Genomic Atlas Network

Introduction

DNA methylation plays a critical role in cancer. Virtually all tumour cells feature genomic instability induced through global loss of DNA methylation and transcriptional silencing of tumour suppressor genes from promoter hypermethylation [1]. DNA methylation aberrations at several tumour suppressor genes have been measured in breast tumours and some of these methylation marks may explain specific clinicopathological characteristics [2]. Importantly, DNA methylation profiles may enable stratification over and beyond gene expression data. DNA methylation is more stable than gene transcripts and therefore more feasible for clinical use. For example, Dedeurwaerder et al (2011) have identified novel DNA methylation profiles that suggest existence of additional breast cancer subtypes above those classified by current expression subtypes [3].

Recent data from TCGA has dramatically expanded the number of genes identified as aberrantly methylated in breast cancers and several studies have found that DNA methylation might be responsible for at least some of the observed tumour heterogeneity [4,5]. Further, DNA methylation marks are dynamic and change throughout tumour progression [2]. Hoesel et al (2013) reported DNA hypermethylation of selected genes such as *RASSF1A* and *RAR β 2* occur early in breast carcinogenesis suggesting the potential of their use as biomarkers for early detection [6]. Taken together, the potential for DNA methylation marks to be utilised as diagnostic and therapeutic targets in breast cancer is well established.

Recent advances in technology have enabled research in this area to expand from single or few molecularly-targeted assays to platforms that conduct genome-wide assessment of the methylome. The capacity of these platforms to scan the genome in one assay and their demonstrated utility for the analysis of FFPE material offer great promise to further refine our

understanding of DNA methylation in cancer, including its role in neoplastic change and tumorigenic pathways of cancer progression [7]. Fulfilling this promise could enable the resources of large epidemiological studies, that commonly collect and store diagnostic FFPE material, to underpin ongoing research and identify new diagnostic and therapeutic agents and provide the evidence base for translation.

For some research questions there is great importance and reliance on assessing methylation in both tumour and adjacent non-tumour ductal epithelium (ADJ_{NT}) for alterations in the tumorigenic process. The utility of this approach has been demonstrated by several studies that have primarily used loci-specific assessment of DNA extracted from fresh frozen material [8-11]. For example, Bardowell et al (2013) assessed the methylation levels of 70 candidate genes (across 1200 CpG dinucleotides) in fresh frozen tumour and matched ADJ_{NT} breast tissues and identified six distinct methylation patterns in these breast tumours, with some displaying correlated methylation-gene expression patterns. Li and colleagues measured DNA methylation in 48 genes from 150 paired fresh frozen breast tumour and ADJ_{NT} tissue samples and reported a methylation pattern which could be used to predict the onset of breast cancer with specific clinicopathological features. Using pyrosequencing technology, Rauscher et al. (2015) showed that large field effects were present in breast cancer when they pyrosequenced 16 gene loci in more than 100 FFPE breast tumours, ADJ_{NT} breast tissue and unaffected control reduction mammoplasty samples. This observation was confirmed by the most comprehensive study of this type to date by Teschendorff et al (2016) who measured genome-wide methylation using the Infinium HumanMethylation450 Beadchip (HM450K) array from DNA extracted from snap-frozen breast cancer tissues, normal/benign tissues and matched breast and ADJ_{NT} tumour tissues (total n=569).

Selecting tissue for analysis is challenging as it is complicated by several aspects related to the histological variation of breast cancers that result in: i) tumour-enriched DNA samples being prepared for analysis (variably contaminated with non-tumour material); ii) contamination of ADJ_{NT} tissue with tumour cells; and iii) availability (identification) of histopathologically normal tissue from the adjacent fields. Work involving the use of FFPE material offers increased capacity for precision in regard to tissue selection, but the DNA extracted from this material is a more complex template (in terms of quality) for the current, high density molecular platforms.

In contrast to the work described above, we sought to evaluate the capacity of diagnostic FFPE material to provide DNA samples representing the tumour and ADJ_{NT} breast tissue suitable for assessment on the HM450K array. We utilised resources from the MCCS and measured genome-wide methylation in DNA extracted from 21 pairs of breast tumour and ADJ_{NT} tissue.

Materials and Methods

Melbourne Collaborative Cohort Study (MCCS)

The MCCS is a prospective study of more than 40,000 men and women who were recruited in the years 1990-1994 [12]. The vast majority of participants (>99%) were aged 40-69 at recruitment (baseline), when a wide range of epidemiological data was collected from the participants as well as blood samples and physical measurements. Pathology materials related to breast cancers diagnosed in female participants were retrieved from the diagnostic service laboratory and reviewed by qualified pathologists. Representative haematoxylin and eosin (H&E) stained sections and unstained sections (3 µm) were prepared and stored desiccated at 4 °C for up to 15 years. Immunohistochemical staining and breast cancer subtyping were

conducted as described by Blows et al (2010) [13]. Written informed consent was obtained from each participant and the study was approved by the Human Research Ethics Committee of Cancer Council Victoria.

DNA extraction from FFPE breast tumour and ADJ_{NT} tissues

DNA was extracted as previously described from FFPE breast tumour and ADJ_{NT} material that had been fixed in separate tissue blocks [7]. Briefly, the tumour area and the ADJ_{NT} tissue for 21 individuals (42 FFPE tissue samples in total) most suitable for macrodissection was identified by a single pathologist (CAM) using the WHO Classification of Tumours of the Breast criteria [14]. FFPE tissue was macrodissected from at least two 3 µm methylGreen-stained sections using a 21-guage needle followed by protease digestion using Proteinase K for 48 hours. Macrodissection for all FFPE tissue samples was performed on the same day by the same individual (EMW) to reduce potential batch effects. DNA was extracted using the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany) as per manufacturer's protocol except that DNA was eluted twice to obtain a final volume of 25 µl and stored at 4 °C.

Sodium bisulfite conversion

Sodium bisulfite conversion was performed using the EZ-DNA Methylation-Gold kit (Zymo, Irvine, CA, USA). FFPE-derived DNA was bisulfite converted (24 - 750 ng input DNA) and eluted in a final volume of 8 µl elution buffer. Bisulfite converted DNA was stored long-term at -20 °C.

Quality control (QC) assessments

The following QC assessments were applied to extracted FFPE DNA samples prior to the application on the HM450K array: 1) QC1: confirm the availability of DNA after extraction using the Qubit dsDNA BR assay (Life Technologies, Carlsbad, CA, USA); 2) QC2: evaluate the quality of archival tissue DNA using the Infinium HD FFPE QC assay (Illumina, San Diego, CA, USA); and 3) QC3: confirm the availability of DNA after sodium bisulfite conversion and FFPE restoration using an in-house bisulfite-specific qPCR assay [7].

Infinium HumanMethylation450 Beadchip (HM450K) assay

The HM450K assay (Illumina, San Diego, CA, USA) was performed as per manufacturer's instructions. One FFPE tumour and ADJ_{NT} DNA pair were run as replicates to ensure the reproducibility of data and one high molecular weight DNA extracted from a U266 multiple myeloma cell line was included to further control the assay.

Data processing and statistical analysis

Raw data was imported into R environment, normalised and processed using the *minfi* package as previously described [15,16]. HM450K probes or individual samples that did not meet the following criteria were removed from further analysis: 1) average detection p-value across all probes/sample ≤ 0.01 ; 2) individual probe detection p-value of ≤ 0.05 . β and M-values were calculated from 480,136 CpG probes in 36 samples. Three-dimensional principal component analysis (3D-PCA) plots were generated using the *scatterplot3d* R package. Differentially methylated probes were identified using the *limma* Bioconductor package [17], taking the pairwise design into account. All graphics were generated using *ggplot2* unless stated otherwise.

Analysis of TCGA data

The HM450K data of matched tumour and ADJ_{NT} tissues (> 2 cm from tumour) from 90 women was downloaded from the National Cancer Institute webpage (<https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp>). The data was background corrected and normalised according to the Illumina and Subset-quantile Within Array Normalization (SWAN) methods [18]. The clinical information was also downloaded from the NCI website.

Comparison with other tumour-ADJ_{NT} studies

To test for the age-associated methylation in normal breast tissue as reported by Johnson et al (2014) [19], linear regression was performed using M-values from the 18 ADJ_{NT} tissues and patient age, which ranged from 51.7 to 77.6 years [17]. Differentially Methylated Regions (DMRs) were identified between the 18 tumour and ADJ_{NT} pairs using the *DMRcate* R package [20] and individually compared with the 37 differentially methylated gene promoters reported by Li et al (2015) [9].

Results

Histological examination of the FFPE breast material collected from 21 breast cancer cases participating in the MCCS identified tumour enriched and ADJ_{NT} areas for macrodissection. The ADJ_{NT} areas included nine cases with histologically normal duct epithelium (N), six cases with atrophic duct tissue and three cases with fibrocystic disease (FCD) (Table 1). The estimated percentage of normal ductal epithelium on a background of stroma and adipose components ranged from 3 - 10 %. The tumours comprised > 99.5 % infiltrating tumour in all cases. Ductal Carcinoma In Situ constituted < 0.5 % of macrodissected material.

The amount of DNA extracted from these 42 tumour and ADJ_{NT} FFPE tissue samples (21 pairs) ranged from 24 ng to 1.5 ug. Due to insufficient DNA, one ADJ_{NT} sample and its matched tumour DNA were not progressed further. The remaining 40 tumour and ADJ_{NT} DNA samples passed the Infinium HD QC assay threshold cutoff of $\Delta Cq \leq 6$. After sodium bisulfite conversion [and FFPE restoration](#), two ADJ_{NT} tissue DNA samples did not meet the final bisulfite-specific qPCR threshold cutoff ($\Delta Cq \geq 4$) and together with their matched tumour DNA were not assayed on the HM450K array [7]. A total of 36 FFPE tumour and ADJ_{NT} DNA samples from 18 individuals remained for genome-wide methylation profiling on the HM450K array.

Genome-wide methylation profiles

After removing probes that did not pass the HM450K data quality criteria, 480,136 Infinium probes (98.9%) remained for further analysis. Three-dimensional PCA was performed to illustrate overall similarities based on DNA methylation levels detected by all remaining CpG probes. Figure 1 shows the relationship between all tumour and ADJ_{NT} samples and also the relationship between each tumour and its matching ADJ_{NT} sample from each of the 18 women. [Supplementary Figure 1 shows the scatterplots between all 18 tumour and ADJ_{NT} pairs and their respective correlation coefficients.](#) Figure 2 further illustrates sample 3 ($R = 0.984$) and sample 7 ($R = 0.853$).

Comparison of FFPE genome-wide methylation with TCGA

We compared the FFPE HM450K dataset with the publicly available TCGA HM450K dataset. A total of 105,545 marks were differentially methylated ($FDR_{adj} \text{ p-value} \leq 0.01$) between the archival FFPE tumour and ADJ_{NT} DNAs. Of these, 101,672 (> 96 %) marks were also differentially methylated in the TCGA dataset (data not shown). To test whether

the methylation status of DMPs identified from this FFPE dataset could also separate tumour samples from matched ADJ_{NT} tissues in TCGA dataset (generated from fresh frozen material), we plotted a methylation heatmap using the 1000 most significantly DMPs identified in the FFPE dataset. We found that the DMPs were effective for differentiating tumour and ADJ_{NT} tissues within the TCGA dataset (Figure 3).

We further compared the DNA methylation levels between the FFPE-derived samples and fresh frozen samples from TCGA in selected genes. The *RAS association domain family protein 1A (RASSF1A)* and *high in normal-1 (HIN-1)* genes were assessed as these tumour suppressor genes have been shown to often be deregulated through methylation aberration in breast cancer. The *NK6 Homeobox 2 (NKX6-2)* gene was assessed as it was identified to be one of most significant differentially methylated regions (clusters of CpG sites) in the FFPE-derived samples. For all three genes, methylation profiles observed in both the FFPE and TCGA datasets were highly correlated (Supplementary Figure 2).

Comparison of FFPE genome-wide methylation with other tumour-ADJ_{NT} studies

Johnson et al (2014) reported 24 HM27K probes with age-related methylation in normal breast tissue were also significantly differentially methylated between normal and breast tumour tissues [19]. While cg15465321 probe was not available in the HM450K array, the FFPE data demonstrated statistically significant ($FDR_{adj.} \text{ p-value} < 0.05$) differential methylation at 17 of the remaining 23 probes reported by Johnson et al (Supplementary Figure 3). Although we did not identify any significant probes associated with age in the ADJ_{NT} tissues, this was likely to be due to the lack of power.

Using microfluidic PCR-based target enrichment and next-generation bisulfite sequencing technology, Li et al (2015) analyzed methylation status of 48 candidate genes in paired tumour and normal tissues from 180 Chinese breast cancer patients [9]. The promoter regions of 31 of the 37 genes reported by Li et al. were also differentially methylated in the FFPE-derived dataset (Supplementary table 1). Interestingly, *SOX17* that had been identified as one of the most significant candidate biomarker for cancer prediction had one of the smallest Stouffer-transformed FDR adjusted p-value in the FFPE dataset ($\text{FDR}_{\text{adj. p-value}} = 4.17 \times 10^{-39}$).

Discussion

There is an increasing need to conduct large-scale studies examining genome-wide DNA methylation of FFPE breast cancer specimens as it offers opportunities to increase histological precision, to study subtypes that are underrepresented in the TCGA data and to provide capacity to further explore intra-tumoural heterogeneity. This report demonstrates the potential of FFPE material to be used in this setting. Although TCGA has comprehensive and systematic analyses of molecular changes such as DNA methylation for 707 breast tumours [4], there are several limitations for its clinical use: 1) there is missing molecular subtype information on 37% of TCGA breast cancer cases; 2) family history was not collected in TCGA, and there is incomplete clinical information on many of the cases making it impossible to stratify the results for clinical counseling based on underlying risk of breast cancer; and 3) only 13% of samples have methylation profiles in the corresponding non-tumour tissues. As the epigenomes vary according to characteristics of the study sample including the distribution of age, race/ethnicity and underlying risk, it is essential to select a well characterized sample for the identification of markers so the results will be useful for

integration into clinical care [21]. Thus it is critical to know how viable FFPE methods are when applying them to large epidemiologic cohorts.

The comparison of this FFPE DNA-derived HM450K data with TCGA HM450K data demonstrated consistencies between the two datasets despite the differences in sample size and DNA source. By applying the 1000 most DMPs in the FFPE dataset to TCGA data from fresh frozen material, we found that the same DMPs were able to differentiate ADJ_{NT} from tumour tissue in TCGA dataset (Figure 3). [Further demonstration of the reliability of the FFPE methylation data was achieved by comparison of the data with the studies of Johnson et al \(2014\) and Li et al \(2015\) \[9,19\].](#)

We additionally performed a simple methylation assessment of several genes widely reported to be aberrantly methylated in cancer. Both *RASSF1A* and *HIN-1* are tumour suppressor genes that are frequently methylated in breast cancer [6,22-24]. In *RASSF1A*, only one of the two CpG islands in the promoter region is methylated in breast cancer tissue [24,25]. Evaluation of the Infinium probes across the *RASSF1A* promoter confirmed that the probes overlapping this CpG island (chromosome 3: 50,377,815 - 50,378,540) had high levels of methylation in the FFPE-derived breast tumours (Supplementary Figure 2). Evaluation of the FFPE data found the *NKX6-2* gene to be differentially methylated in tumour compared with ADJ_{NT} tissues across all probes specific to the gene (Individual probe $\Delta\beta = 0.02 - 0.45$). *NKX6-2* is a homeobox gene that has previously been demonstrated to be methylated in renal cell and bladder cancer [26,21]. Methylation of this gene in breast cancer has not been reported previously.

This work provides evidence that the resources of large collections of FFPE material could be used in studies examining the role of methylation in breast cancer tumourigenesis. This could allow epidemiological studies to underpin ongoing research aiming to identify new diagnostic markers and therapeutic agents and at the same time provide the evidence base for translation of such information.

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Author's Contributions

EMW performed all the laboratory experiments described in this manuscript. EMW and JEJ performed the analysis of the experimental data. CAM contributed histopathological expertise. LB, DRE, MCS, GS and JLH made prior contribution to the MCCS resource utilised in this report. LB, MCS and DRE provided grant support for this study. RM contributed to the MCCS resource and logistical aspects of the project. GGG is the principal investigator of the Melbourne Collaborative Cohort Study. MCS provided additional grant support, the initial idea of the research described and overall supervision of the laboratory and analytical approaches. EMW, JEJ and MCS wrote the first draft of the manuscript to

which all authors contributed. MBT and HCW contributed to manuscript preparation. The final manuscript was read and approved by all the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval: “All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all participants in this research study.

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Fig 1 Genome-wide DNA methylation profiles tumour DNA (black) and ADJ_{NT} (green) samples presented as a 3D-PCA plot. Tumour and ADJ_{NT} samples are numbered as per Table 1. Dotted lines connect each matched tumour and ADJ_{NT} pair.

Fig 2 Comparison between matched tumour and ADJ_{NT} tissues for samples 3 and 7. a) Scatterplot of 480, 136 methylation probes between tumour (x-axis) and ADJ_{NT} (y-axis) DNA. b) Tumour tissue (H&E, X10) c) ADJ_{NT} tissue (H&E, X5).

Fig 3 Unsupervised clustering analysis of the 1000 most differentially methylated probes between tumour (T) and ADJ_{NT} (N) DNA in the a) FFPE HM450K dataset and b) TCGA HM450K dataset.

Sup Fig 1 Scatterplots and correlation coefficients for ADJ_{NT} (x-axis) and tumour (y-axis) pairs based on 480, 136 methylation probes.

Sup Fig 2 Comparison of Illumina probe methylation levels between the FFPE and TCGA datasets for a) *HIN-1* and b) *NKX6-2* and c) *RASSF1A*.

Sup Fig 3 Scatterplots of 23 HM450K probes reported by Johnson et al (2014) to be differentially methylated between tumour and normal samples. * denotes probes that are significantly differentially methylated in the FFPE dataset between tumour (black) and ADJ_{NT} (red) samples ($FDR_{adj.} p\text{-value} < 0.05$).

Sup Table 1 Thirty-one of the 37 promoter regions reported to be differentially methylated between tumour and ADJ_{NT} samples in the FFPE dataset.

Fig 1

Fig 2

Fig 3

Table 1: Histological and molecular classification of tumour and ADJ_{NT} tissue.

Sample ID	ADJ _{NT} tissue classification	Tumour tissue histological classification	Tumour tissue molecular subtype	Tumour grade	Estrogen receptor status
1	N	IDC, NOS	Luminal A	2	Negative
2	Atrophic duct	IDC, NOS	Luminal A	1	Positive
3	N	IDC, NOS	Luminal A	1	Positive

4	N	IDC, NOS	Luminal A	3	Positive
5	Atrophic duct	IDC, NOS	Luminal A	3	Positive
6	N	IDC, NOS	Luminal A	3	Positive
7	FCD	IDC, NOS	Luminal A	1	Positive
8	Atrophic duct	IDC, NOS	Luminal A	2	Positive
9	N	IDC, NOS	HER2-enriched	3	Negative
10	FCD	IDC, NOS	Luminal A	2	Positive
11	N	IDC, NOS	Luminal A	1	Positive
12	N	IDC, NOS	Luminal A	2	Positive
13	Atrophic duct	IDC, NOS	Luminal A	2	Positive
14	N	Tubular	Luminal A	1	Positive
15	N	IDC, NOS	Luminal A	2	Negative
16	Atrophic duct	IDC, NOS	TN	2	Negative
17	Atrophic duct	Lobular, classical	Luminal A	2	Positive
18	FCD	Mucinous, colloid	Luminal A	1	Positive

FCD: Fibrocystic disease; IDC: Infiltrating ductal; NOS: Not Otherwise Specified;

TN: Triple negative (ER-/PR-/HER2-); N: histologically normal breast epithelium.

Sup Table 1 Thirty-one of the 37 promoter regions reported to be differentially methylated between region, ³ Minimum FDR adj. p-value of the region, ⁴ Stouffer transformation of FDR adj p-val of in

Chromosome	Start coordinate	Stop coordinate	# CpGs ¹	Promoters ²
chr2	211089678	211090487	10	ACADL-001, ACADL-003, ACA
chr9	18473902	18474243	2	ADAMTSL1-005, ADAMTSL1-0
chr5	112073686	112074043	3	APC-001, APC-004, APC-003,
chr6	7728676	7729794	5	BMP6-001
chr11	115371789	115373119	6	CADM1-007, CADM1-008
chr11	115088647	115088907	4	CADM1-016
chr7	116164108	116165941	21	CAV1-001, CAV1-003, CAV1-0
chr12	4378267	4384890	61	CCND2-001, CCND2-004, CCN
chr7	94023308	94024254	11	COL1A2-001
chr5	149492480	149493814	8	CSF1R-001, CSF1R-004, PDG
chr1	68515209	68517691	24	DIRAS3-001, DIRAS3-201, AR
chr7	55086091	55088104	8	EGFR-001, EGFR-003, EGFR-
chr7	55177182	55177623	2	EGFR-009, EGFR-202
chr6	152125861	152130332	44	ESR1-001, ESR1-002, ESR1-0
chr6	152011103	152011666	7	ESR1-004, ESR1-201
chr6	152200760	152201611	4	ESR1-010
chr11	67349309	67352041	19	GSTP1-004, GSTP1-001, GST
chr17	1957529	1958237	25	HIC1-005, HIC1-006, HIC1-002
chr11	534232	534632	4	HRAS-002, HRAS-004, HRAS-
chr19	10397031	10399157	10	ICAM5-001, ICAM4-001, ICAM
chr11	536758	537737	13	LRRC56-001, HRAS-002, HRA
chr7	24323128	24325371	15	NPY-001, NPY-002, NPY-003
chr5	149535646	149535765	4	PDGFRB-001, PDGFRB-004, I
chr5	149512117	149513303	4	PDGFRB-009
chr17	56832804	56833425	13	PPM1E-001
chr11	6340283	6342510	17	PRKCDBP-001, PRKCDBP-00
chr1	186649153	186650479	15	PTGS2-001, PTGS2-002, PTG
chr9	36035988	36037454	6	RECK-001, RECK-003, RECK-
chr1	27188505	27190639	13	SFN-001
chr8	41164679	41168336	23	SFRP1-001, SFRP1-003
chr4	154709441	154714852	55	SFRP2-001
chr8	55370192	55372569	18	SOX17-001
chr5	1282357	1283350	7	TERT-003
chr5	1266145	1266595	3	TERT-007
chr5	1293871	1296007	10	TERT-201, TERT-001, TERT-0
chr3	30646532	30648407	16	TGFBR2-001, TGFBR2-002
chr22	33195343	33197394	16	TIMP3-001
chr8	23020966	23022403	9	TNFRSF10D-001
chr12	65514473	65516002	9	WIF1-001, WIF1-002
chr8	30892276	30892490	4	WRN-001, PURG-001, PURG-
chr8	30888801	30890632	15	WRN-001, PURG-001, PURG-

Sup Fig 1

Sup Fig 2

Sup Fig 3