Supplementary Table 1. Current epigenetic modifications in TNBC and basal-like tumors

No.	Findings	Samples	Methods	Ref.
	DNA methylation			
1	- 3 Methylation clusters, with the most hypomethylated associated with better prognosis and the medium methylated with worst prognosis - 17 Potential prognostic regions—lower methylation in low-risk groups - Hypermethylation of genes in axon guidance pathway - Methylation of gene bodies *WT1* and *WT1-AS* vs. promoter methylation	19 Formalin-fixed paraffin-embedded TNBC tissue and 6 matched normal samples	- Whole genome methylation capture sequencing (MBDCap-Seq)	[23]
2	- Methylation of 5 genes (*CDKN2B*, *CD44*, *MGMT*, *RB*, and *p73*) and non-methylation of 11 genes (*GSTP1*, *PMS2*, *MSH2*, *MLH1*, *MSH3*, *MSH6*, *DLC1*, *CACNA1A*, *CACNA1G*, *TWIST1*, and *ID4*) are specific to TNBC	- 61 Breast cancer tissue samples, including 28 TNBC	- Methylation profile of 110 CpGI located within 69 cancer-involved genes (MS-MLPA)	[27]
3	- 27%–37% of TNBC samples show *BRCA1* promoter methylation	377 TNBC samples	- Array Comparative Genomic Hybridisation (aCGH) - *BRCA1* promoter methylation	[31]
4	- *BRCA1* and *ESR1* methylation in TNBC compared to non-TNBC - miR-4417, miR-590-5p higher expression in TNBC	278 Formalin-fixed paraffin-embedded breast cancers containing 79 TNBC	- Promoter methylation (MS-MLPA) of 24 tumor suppressor genes - qRT-PCR for miR expression	[32]
5	- *EGR1* downregulation inversely correlated to methylation - 16 TNBC specific genes show altered DNA methylation, including *IGF1* and *IL6ST* - Higher methylation of *SPRY2*, *EGR1*, *GREB1*, *ITIH5*, *LRRC17* and lower methylation of *AMIGO* are associated with better survival	23 Primary TNBC samples, 12 matched lymph node metastases, 11 matched normal adjacent tissues	- 450 K DNA methylation BeadChip array analysis (Illumina)	[37]
6	- *BRMS1* downregulated by DNA methylation in TNBC cell lines and breast cancer samples - Inverse correlation with lymph node metastasis	- TNBC cell lines MDA-MB-231, HCC-1937, MDA-MB-435 and normal breast tissue MCF-10A - 42 Paired normal and TNBC tissue samples	- RT-PCR - Methylation specific PCR	[38]
7	- Cancer stem cells are regulated by hypomethylation of specific CpG sites of genes associated with stem cell properties *CD44*, *CD133*, and *Musashi-1* (*MSI*), promoter methylation being lower in TNBC	- 4 TNBC cell lines (MDA-MB-231, BT-549, BT-20, and HCC1937) and 5 non-TNBC cell lines (MCF-7, T47D, ZR-75-1, ZR-75-30, and SK-BR-3) - 91 Invasive ductal carcinomas, including 32 TNBC	- Methylation analysis (MassARRAY EpiTYPER sequencing)	[39]
8	- 5 Distinct DNA methylation groups - Group 5–most hypomethylated–associated with basal-like tumors - 80% TP53 mutations in basal-like tumors - Loss of *RB1*, *BRCA1* in basal-like tumors	- Primary breast tumor samples and germline DNA from 825 patients (802 samples for DNA methylation)	- DNA methylation - Exome sequencing - mRNA arrays - miRNA sequencing - Reverse-phase protein arrays	[40]
9	-Specific methylation patterns corresponding to luminal A, B and basal-like subtypes, the most hypomethylated being basal-like and most hypermethylated luminal B - *BRCA2* carriers tumors more methylated than *BRCA1* - *RASSF1*, *GSTP1* unmethylated in basal-like tumors - *ARHGDIB*, *GRB7*, *SEMA3B* methylated in basal-like tumors	- 189 Fresh frozen primary breast tumors and 4 normal breast tissue samples	Array based methylation assay for 1505 CpG loci corresponding to 807 cancer related genes	[41]

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Supplementary Table 1. Continued

No.	Findings	Samples	Methods	Ref.
10	- Methylation of 6 genes (*CDH1*, *CEACAM6*, *CST6*, *ESR1*, *LCN2*, and *SCNN1A*) in basal-like cell lines - Aberrant DNMT3b expression - Elevated total DNA methyltransferase activity	12 Breast cancer cell lines (BT20, BT549, Hs578T, MCF7, MDA-MB-231, MDA-MB-415, MDA-MB-435S, MDA-MB-436, MDA-MB-453, MDA-MB-468, SKBR3, and ZR-75-1) and normal breast epithelial cell line MCF12A	- Gene expression (RT-PCR), promoter methylation of 64 genes - DNA methyltransferase machinery assessment (total DNMT activity and expression of DNMT1, DNMT3a, and DNMT3b proteins)	[44]
11	- Enhanced effect of doxorubicin, paclitaxel and 5-fluorouracil after DNMT3b inhibition - Re-expression of methylated genes, including *ESR1*	MDA-MB-453, BT549, Hs578T cell lines	- Treatment with 5-aza - RNAi-mediated DNMT3b mediated knockdown and treatment with doxorubicin, paclitaxel and 5-fluorouracil	[45]
	Noncoding RNAs			
12	- TNBC classification by mRNA and lncRNA profiling - 4 clusters: immunomodulatory (IM), luminal androgen receptor (LAR), mesenchymal-like (MES) and basal-like and immune suppressed (BLIS)	165 TNBC samples	- Transcriptome profiling (human transcriptome microarrays)	[46]
13	- lncRNAs with differential expression were found in TNBC, with no functional correlations so far	3 Pairs of TNBC and adjacent non-tumor tissues plus 12 paired samples for validation	- lncRNA expression microarray - qRT-PCR validation	[47]
14	- lncRNAs with differential expression were found in TNBC - Possible association between ER, *ANKRD30A* and lnc RNA *LINC0099*	3 Pairs of TNBC and adjacent non-tumor tissues plus 48 paired samples for validation	- lncRNA expression microarray - qRT-PCR validation - Bioinformatics analysis for lncRNA functions (gene ontology)	[48]
15	- lncRNA *MALAT1* promotes metastasis of TNBC and may be useful as a prognostic marker in lymph-node negative patients	- TCGA microarray data set (493 breast cancer samples) - Normal breast cell line MCF10A and breast cancer cell lines -TNBC subtype: MDA-MB-231, Hs578T, HCC1806; HER2+ subtype: SKBR3; luminal subtype: MCF7, T-47D for interrogating functional roles	- MALAT1 expression (qRT-PCR)	[49]
16	- *HOTAIRM1* is upregulated in basal-like tumors	658 Infiltrating breast ductal carcinomas, including 126 basal-like samples (from the TCGA breast cancer RNA-Seq data)	- Bioinformatic analysis	[50]
17	- lncRNA *FOXCUT* is overexpressed in basal-like tumors	- 55 Primary breast cancer samples, including 25 basal-like - MDA-MB-231 and MDA-MB-468 cell lines	- Expression profile (RT-qPCR and siRNA transfection)	[51]
18	- lncRNA *HOTAIR* is up-regulated in MCF-7-TNR cells (basal-like derivative of the luminal-like MCF-7), BT-549 and MDA-MB-157 and plays a role in maintaining the basal-like phenotype	MCF-7-7TNR, MC-7, BT-549 and MDA-MB-157 cell lines	- *HOTAIR* expression and siRNA inhibition	[53]
19	- lncRNA *HOTAIR* expression is repressed by combined treatment of lapatinib plus imatinib through β-catenin downregulation	- MCF-7, T47D, BT474, MDA-MB-468, MDA-MB-231, ZR-75–1, SK-BR3, SUM159 and HCC1806 cell lines - 21 Formalin-fixed paraffin-embedded primary breast tumor tissue, including 11 TNBC	- Lapatinib+imatinib treatment of TNBC cell lines (MDA-MB-231, MDA-MB-468, HCC1806, and SUM159) - *HOTAIR* expression	[54]

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Supplementary Table 1. Continued

No.	Findings	Samples	Methods	Ref.
20	- Upregulation of miR-493 and miR-155 correlate with better outcome - Downregulation of miR-30e and miR27a correlate with poor outcome	173 Paraffin-embedded TNBC samples	- miRNA expression profiling	[55]
21	- miR-10b and miR-26a can downregulate *BRCA1* expression in MDA-MB-231 (TN) and MCF7 (luminal) cell lines	9 Sporadic human breast cancer cell lines of which 7 TNBC and 1 normal breast tissue sample	- miRNA expression profiling	[56]
22	- BRCA1 expression positively correlates with miR-146a and leads to downregulation of *EGFR*	Breast cancer cell lines including 3 TNBC, SKOV3 ovarian cancer cell line and HMLE, MCF10A mammary epithelial cell lines	- miRNA profiling - miR knockdown - Protein expression studies - Mammosphere formation assay	[57]
23	- miR-146a and miR-146b-5p downregulate *BRCA1* in TNBC	- 3 Normal mammary cell lines and 15 breast cancer cell lines, including 3 TNBC - 76 Primary breast tumor tissues - 167 Breast tumor tissues	- miRNA target prediction algorithms - miR-146a/b-5p expression and inhibition studies	[58]
24	- miR-4417, miR-590-5p higher expression in TNBC	278 Formalin-fixed paraffin-embedded breast cancers containing 79 TNBC	- qRT-PCR for miR expression	[32]
25	- miR-200c downregulation correlates with locus methylation and is associated with lymph node metastasis in TNBC - Low levels of miR-200c are associated with high levels of ZEB1 transcription factor which promotes EMT - miR-200c/ZEB1 axis as target for metastatic TNBC	- 51 TNBC samples - TCGA data set - MDA-MB-231 and MDA-MB-157 cell lines for functional analysis	- qRT-PCR and methylation analysis	[59]
26	- miR-200b suppresses TNBC migration and metastasis by inhibiting protein kinase Cα	- MCF-7, T-47D, BT-474, MDA-MB-453, SKBR-3, MDA-MB-468, BT-20, Hs578T and BT-549 cell lines - Mouse mammary xenograft tumor model	- miR-200b expression and knockdown studies - *In vitro* and *in vivo* migration and metastasis studies respectively	[60]
27	- miR-200a modulates TNBC migration through regulating the EPHA2 oncogene	- Breast cancer dataset for mRNA levels of EPHA2 and corresponding patient survival - HC11, MDA-MB-231, SUM159 cell lines	- miR200a transfection study - miRNA expression analysis - Proliferation and migration assays	[61]
28	- Overexpression of miR-200b-3p and miR-429-5p inhibits the proliferation, migration, and invasion of TNBC cell lines	MDA-MB-231 and HCC1937 TNBC cells	- miR transfection - Proliferation, migration and invasion assays	[62]
	Histone modifications			
29	- Distinct H3K36me3 patterns in the TNBC cell lines - AR pathway genes active especially in claudin-low TNBC cell lines, while AR pathway regulators had lower expression levels in basal-like - AFAP1-AS1 found as TNBC specific gene marked by the active H3K4me3 and H3K79me2 modifications	- 2 Normal immortalized cell lines, 76NF2V and MCF10A - 2 Luminal A lines, MCF7, ZR751 - 2 Luminal B lines, MB361, UACC812 - 2 HER2 lines SKBR3, AU565, HCC1954 - 2 Basal TNBC cell lines, MB468 and HCC1937 - 2 Claudin low TNBC cell lines, MB231 and MB436	- ChIP-Seq, GRO-Seq and RNA-Seq analysis - siRNA mediated depletion of AFAP1-AS1 in MDA-MB-231 and HCC1937	[64]

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Supplementary Table 1. Continued

No.	Findings	Samples	Methods	Ref.
30	- BCL11A interacts with histone methyltransferase (PRC2) and histone deacetylase (NuRD and SIN3A) complexes and contributes to maintenance of a chemoresistant breast cancer stem cell population in TNBC including basal-like	- Microarray data sets - Immortalized non-tumourigenic mouse EpH4 and human HMLE cell lines - TNBC cell lines 4T1 (mouse), MDA231, SUM159 and HMLER (human) - Immune compromised mice - BCL11A conditional knockout and knock-in mice	- BCL11A overexpression studies and mammosphere assay - shRNA knockdown of BCL11A - Modified cells' injection to assess tumor formation - TNBC-like tumor promotion by DMBA staining	[65]
31	- *KAT5* (histone acetylase inhibitor), *DOT1L* (H3K79 methylator) and *G9a* (histone methyltransferase) downregulation induce E-CAD expression to promote an epithelial phenotype	- MDA-MB-231 cell line	- siRNA library screening for EMT regulators (729 chromatin modifying targets)	[74]
32	- Overexpression of macroH2A1.1 correlates with claudin-low subtype and TNBC poor outcome	- GEO, EMBL-EBI and publisher databases - MCF-7, MDA-MB231, ZR-75, MDA-MB436 and Hs578T cell lines	- Biostatistical correlation studies on intrinsic molecular subclasses of breast cancer and molecular characteristics of EMT - Protein quantification, qRT-PCR	[75]
33	- 3 Groups of histone modification patterns - Hypomodified cluster, characterized by moderate to low levels of lysine acetylation (H3K9ac, H3K18ac, and H4K12ac), lysine (H3K4me2 and H4K20me3), and arginine methylation (H4R3me2) associated with basal-like and HER2+ subtypes	- 880 Invasive breast carcinomas	- Tissue microarray, immunohistochemistry - Immunofluorescence and western blotting for validation	[21]
34	- Differential H3K4me2 & H3K27me3 methylation between CSC and non-CSC suggest Wnt & GnRH signaling pathways are responsible for aggressiveness in TNBC	- MDA-MB-231 cell line - BALB/c nude+ mice	- Invasion and xenotransplantation assays - RN-seq, WGBS and CHIP-seq analysis	[77]
35	- HDACi suberoylanilide hydroxamic acid (vorinostat) and sodium butyrate inhibit cell proliferation, induce apoptosis and downregulate transcription of mutant p53 in TNBC cell lines	- TNBC cell lines MDA-MB-231 and BT-549	- Transfection studies - Cell cycle and apoptosis assays	[79]
36	- HDACi panabinostat induces hyperacetylation of histones H3 and H4, decreases proliferation and survival, and induces apoptosis in TNBC cell lines and decreases tumor size *in vivo*	- TNBC cell lines MDA-MB-157, MDA-MB-231, MDA-MB-468, BT-549 - Orthotopic MDA-MB-231 and BT-549 mouse xenograft models	- Histone acetylation assays - Proliferation assay and cell cycle analysis - Protein expression studies	[83]
37	- HDACi vorinostat enhances the growth inhibitory ability of PARP inhibitor olaparib in TNBC cells with overexpression of PTEN and *in vivo* in an MDA-MB-231 mouse model	- Human breast cancer cells (MDA-MB-157, -231, -453, -468, BT-549, MCF7, T47D, SK-BR-3, HCC70, HCC1143, and Hs578T) - Breast cancer xenograft mouse model	- Cytotoxic assay and cell cycle analysis - *PTEN* transfection - Proliferation, apoptosis and autophagy analysis	[84]
38	- Combination of vorinostat and immune checkpoint inhibitors (PD-1 and CTLA-4 blockade) on mice models of TNBC lead to decreased tumor growth and prolonged survival	- Human breast cancer cell lines, including 1 TNBC - Mouse breast cancer cells - *In vivo* model of mouse breast cancer cell line similar to TNBC	- PD-L1 expression analysis - Co-culture with peripheral blood mononuclear cells - *In vivo* therapy of mouse model with vorinostat, anti-PD-1 blockade or both drugs	[86]
39	- HDACi entinostat decreases the ability of TNBC to form lung metastasis in an *in vivo* mouse model and reduces tumor formation from patient derived xenografts	- Breast cancer cell lines including 1 TNBC - MDA-MB-231 mouse xenograft - Patient derived xenograft	- Protein and miRNA expression analysis - Mammosphere formation assay - Tumor formation and metastasis development *in vivo*	[87]

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Supplementary Table 1. Continued

No.	Findings	Samples	Methods	Ref.
40	- Vorinostat has the ability to prevent brain metastasis of TNBC *in vivo*	- Mouse model of 231-BR brain trophic subline of the MDA-MB-231 TNBC cell line	- Pharmacokinetic and pharmacodynamic studies of vorinostat uptake in the brain - Histone acetylation, cell cycle and apoptosis analysis *in vitro* and *in vivo*	[88]
41	- Histone methyltransferase hSETD1A positivity correlated with worse outcome	- 159 TNBC samples	- Protein expression studies—immunohistochemistry, qRT-PCR	[90]

TNBC=triple-negative breast cancer; RT-PCR=reverse transcription polymerase chain reaction; qRT-PCR=quantitative RT-PCR; mRNA=messenger RNA; miRNA=microRNA; RNAi=RNA interference; lncRNA=long noncoding RNA; ER=estrogen receptor; TCGA=The Cancer Genome Atlas; siRNA=small interfering RNA; EMT=epithelial-to-mesenchymal transition; AR=androgen receptor; HER2=human epidermal growth factor receptor 2; shRNA=small hairpin RNA; DMBA=7,12-dimethylbenz(a)anthracene – a potent carcinogen; CSC=cancer stem cells; HDACi=histone deacetylase inhibitors; PARP=poly (ADP-ribose) polymerase.

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