Data in Brief

Genome-wide miRNA, gene and methylation analysis of triple negative breast cancer to identify changes associated with lymph node metastases

Kelly A. Avery-Kiejda, Andrea Mathe, Rodney J. Scott

Centre for Information Based Medicine, Hunter Medical Research Institute, NSW 2305, Australia
Priority Research Centre for Cancer Research, Innovation and Translation, School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, NSW 2308, Australia
Pathology North, John Hunter Hospital, Newcastle, NSW 2305, Australia

ABSTRACT

Triple negative breast cancer (TNBC) is a particularly important breast cancer subtype with an aggressive clinical phenotype that is associated with a higher likelihood of metastasis. This subtype is characterized by an absence of the estrogen (ER) and progesterone (PR) receptors, as well as the human epidermal growth factor receptor 2 (HER2/HER neu). The absence of the three receptors significantly reduces targeted treatment options for patients with TNBC and as such, there is an urgent need to identify novel treatment targets. Here, we provide detailed information regarding the design of a multi-platform dataset that describes genome-wide assessment of miRNA (assessed by microarray, GSE38167) and gene expression (assessed by microarray, GSE61723), as well as methylation (assessed by Illumina HM450K BeadChip, GSE78751) in TNBCs, matched normal adjacent tissues and matched lymph node metastases. The use of this multi-platform dataset is likely to uncover novel markers and key pathways involved in progression to lymph node metastasis in TNBC.

Specifications

| Specifications          | Homo sapiens/breast cancer specimens (triple negative subtype) |
|------------------------|---------------------------------------------------------------|
| Organism/cell line/tissue | Female                                                        |
| Sex                     | Agilent Human miRNA microarrays (Sanger Release 14.0)         |
| Sequeaker or array type  | Affymetrix Human Gene 2.0 ST Array (HuGene-2.0-st)            |
|                         | Illumina HumanMethylation450 BeadChip (HumanMethylation450_15017482) |

Data format

| Data format | Raw (.tar) and processed (.txt) |

Experimental factors

| Experimental factors | Matched normal adjacent tissue, tumour tissue, matched lymph node metastases |

Experimental features

This study has investigated miRNA, gene and methylation profiles in primary TNBC cases (miRNA (n = 31), gene (n = 33), methylation (n = 23)) and matched lymph node metastases (miRNA (n = 13), gene (n = 15), methylation (n = 12)) compared with matched normal breast tissues (miRNA (n = 23), gene (n = 17), methylation (n = 11, 3 pooled samples and 1 single sample)).

Consent

This study complies with the Helsinki Declaration with ethical approval from the Hunter New England Human Research Ethics Committee (Approval number: 09/05/20/5.02). In accordance with the National Statement on Ethical Conduct in Research Involving Humans, a waiver of consent was granted for this study.

Sample source location

Newcastle, NSW, Australia

1. Direct link to deposited data

- miRNA arrays (GSE38167): https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38167.
- Gene expression arrays (GSE61723): https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61723.
- Illumina HM450K BeadChip (GSE78751): https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE78751.
Table 1
Labelling of patient samples in individual studies of whole genome gene, miRNA and methylation analysis. Cases shaded in grey are lymph node negative and unshaded cases are lymph node positive.

| Gene expression analysis (GSE61723) | miRNA analysis (GSE38167) | Methylation analysis (GSE78751) |
|-------------------------------------|---------------------------|-------------------------------|
| Normal adjacent tissues (NAT)       |                           |                               |
| Patient 3 NAT                       | Patient 26_NAT            |                               |
| Patient 5 NAT                       | Patient 20_NAT            |                               |
| Patient 6 NAT                       | Patient 34_NAT            |                               |
| Patient 7 NAT                       | Patient 5_NAT             |                               |
| Patient 8 NAT                       | Patient 27_NAT            |                               |
| Patient 9 NAT                       | Patient 11_NAT            | Patient 7 NAT                 |
| Patient 10 NAT                      | Patient 13_NAT            |                               |
| Patient 11 NAT                      | Patient 18_NAT            |                               |
| Patient 12 NAT                      | Patient 16_NAT            |                               |
| Patient 18 NAT                      | Patient 25_NAT            |                               |
| Patient 19 NAT                      | Patient 17_NAT            |                               |
| Patient 23 NAT                      | Patient 6_NAT             |                               |
| Patient 26 NAT                      | Patient 14_NAT            |                               |
| Patient 28 NAT                      | Patient 33_NAT            | Patient 10_NAT                |
| Patient 30 NAT                      | Patient 12_NAT            |                               |
| Patient 32 NAT                      | Patient 21_NAT            |                               |
| Patient 33 NAT                      | Patient 3_NAT             |                               |
| NAT pool 1: Patient 21, 30, 31      |                           |                               |
| NAT pool 2: Patient 1, 3, 32        |                           |                               |
| NAT pool 3: Patient 5, 7, 14, 33    |                           |                               |
| Invasive ductal carcinomas (IDC)    |                           |                               |
| Patient 1 IDC                       | Patient 23_IDC            | Patient 17 IDC                |
| Patient 2 IDC                       | Patient 4_IDC             | Patient 6 IDC                 |
| Patient 3 IDC                       | Patient 16 IDC            |                               |
| Patient 4 IDC                       | Patient 1_IDC             |                               |
| Patient 5 IDC                       | Patient 20_IDC            | Patient 3 IDC                 |
| Patient 6 IDC                       | Patient 34_IDC            | Patient 18 IDC                |
| Patient 7 IDC                       | Patient 5_IDC             |                               |
| Patient 8 IDC                       | Patient 27_IDC            | Patient 14 IDC                |
| Patient 9 IDC                       | Patient 11_IDC            | Patient 7 IDC                 |
| Patient 10 IDC                      | Patient 13_IDC            | Patient 4 IDC                 |
| Patient 11 IDC                      | Patient 16_IDC            |                               |
| Patient 12 IDC                      | Patient 16_IDC            |                               |
| Patient 13 IDC                      | Patient 22_IDC            |                               |
| Patient 14 IDC                      | Patient 31_IDC            |                               |
| Patient 15 IDC                      | Patient 9 IDC             |                               |
| Patient 16 IDC                      | Patient 15_IDC            | Patient 23 IDC                |
| Patient 17 IDC                      | Patient 18 IDC            |                               |
| Patient 18 IDC                      | Patient 17_IDC            |                               |
| Patient 19 IDC                      | Patient 19_IDC            | Patient 5 IDC                 |
| Patient 20 IDC                      | Patient 19_IDC            | Patient 2 IDC                 |
| Patient 21 IDC                      | Patient 2_IDC             | Patient 2 IDC                 |
| Patient 22 IDC                      | Patient 8_IDC             |                               |
| Patient 23 IDC                      | Patient 6_IDC             | Patient 21 IDC                |
| Patient 24 IDC                      | Patient 9_IDC             |                               |
| Patient 25 IDC                      | Patient 32_IDC            |                               |
| Patient 26 IDC                      | Patient 14_IDC            | Patient 1 IDC                 |
| Patient 27 IDC                      | Patient 7_IDC             | Patient 19                    |
| Patient 28 IDC                      | Patient 33_IDC            |                               |
| Patient 29 IDC                      | Patient 10_IDC            | Patient 20 IDC                |
| Patient 30 IDC                      | Patient 12_IDC            | Patient 8 IDC                 |
| Patient 29_IDC                      | Patient 12_IDC            | Patient 12 IDC                |
| Patient 30_IDC                      | Patient 12_IDC            | Patient 12 IDC                |
| Patient 30_IDC                      | Patient 29_IDC            | Patient 22 IDC                |
| Patient 32 IDC                      | Patient 21_IDC            |                               |
| Patient 33 IDC                      | Patient 5_IDC             |                               |
| Patient 34 IDC                      | Patient 24_IDC            | Patient 15 IDC                |
| Patient 34 IDC                      | Patient 28_IDC            | Patient 13 IDC                |
| Patient 34 IDC                      | Patient 28_IDC            | Patient 13 IDC                |
2. Experimental design, materials and methods

2.1. Study cohort

A total of 38 grade 3, triple negative, invasive ductal carcinomas (IDCs), 23 matched normal adjacent tissues (NAT), and 15 lymph node metastases (LNMet) were used for this analysis. All samples were formalin-fixed, paraffin-embedded (FFPE) and obtained from the archives of Pathology North, John Hunter Hospital, Newcastle, Australia. This cohort has been described previously [1–3]. Due to a lack of material not all samples were included in all studies and the number of samples in each of the studies is as follows: TNBC cases (miRNA (n = 31), gene (n = 33), methylation (n = 23)), matched lymph node metastases (miRNA (n = 13), gene (n = 15), methylation (n = 12)) and matched normal breast tissues (miRNA (n = 23), gene (n = 17), methylation (n = 11.3 pooled samples and 1 single sample)). Because the analysis of miRNA, gene and methylation was performed at distinct times, the patient identifiers do not match between the individual studies described in Gene Expression Omnibus and Table 1 shows the corresponding patient identifiers between the studies so that miRNA, gene and methylation data can be linked to an individual patient.

2.2. RNA extraction

Total RNA was extracted using the miRNeasy FFPE kit (Qiagen, Doncaster, VIC, Australia). RNA was quantified using the Quant-it Ribogreen RNA Assay kit (Invitrogen, Mulgrave, VIC, Australia) and purity assessed by A260/A280 and A260/230 ratios (> 1.8) using the RiboGreen RNA Assay kit (Invitrogen, Mulgrave, VIC, Australia). The RNA integrity of selected samples was analysed using the 2100 Bioanalyzer and the RNA 6000 Nano kit (Agilent Technologies, Mulgrave, VIC, Australia).

2.3. DNA extraction

The Gentra Puregene Tissue Kit (Qiagen, Venlo, Limburg, Netherlands) was used to isolate DNA from FFPE tissue following the manufacturers’ instructions with minor modifications as previously described [2]. DNA was quantitated using the Qubit dsDNA BR Assay Kit according to the manufacturers’ instructions (Life Technologies, Carlsbad, CA, United States of America).

2.4. miRNA profiling

miRNA profiling was performed as previously described [1]. Briefly, 100 ng of total RNA was dephosphorylated and labelled with Cy3 using the miRNA Complete Labelling and Hyb Kit (Agilent Technologies). Labelled RNA was hybridised to Human miRNA microarrays (Sanger Release 14.0) according to the manufacturers’ instructions (Agilent Technologies) and scanned on an Agilent High-resolution C scanner. Data from 15,000 probe features representing 904 unique miRNAs was extracted using Agilent Feature Extraction software (v10.7.3.1) and converted to background subtracted signal intensities. The extracted data was imported into Genespring GX (Agilent Technologies) where it was log2 transformed and median normalised. 570 miRNA transcripts had a signal intensity threshold above background in at least one sample. Unpaired t-tests identified miRNAs with significantly altered expression (> 2-fold, p < 0.05, FDR < 5%).

2.5. Gene expression profiling

Gene expression profiling was performed as previously described [3]. Briefly, 100 ng total RNA was amplified (Ovation FFPE WTA kit) and biotinylated (Encore Biotin module) according to the manufacturers’ instructions (Nugen, San Carlos, California, USA). The samples were hybridised to HuGene 2.0 arrays (Affymetrix, Santa Clara, California, USA) and 17 h later washed and stained. The HumanGene 2.0 arrays (Affymetrix) contain probe features representing 11,000 lncRNAs, 24,000 genes, and 30,000 coding transcripts. The arrays were scanned on a GeneChip Scanner 3000 7G (Affymetrix). The data was imported to Genomic Suite 6.6 (Partek, St Louis, Missouri, USA) and a robust multi-array analysis (RMA) was performed, which included log2 transformation, background correction, quantile normalisation and summarisation of the probe features resulting in a set of expression signal intensities. Unpaired t-tests identified genes that were found to be significantly different between samples (p-value < 0.05; fold change > 1.5 or < -1.5). Correction for multiple testing was performed using Benjamini – Hochberg procedure.

2.6. DNA methylation profiling

DNA methylation profiling was performed as previously described [2]. The Infinium HD FFPE quality control (QC) Assay (Illumina, San Diego, CA, USA) was used to assess the integrity of the DNA used for the analysis according to the manufacturers’ instructions. Bisulfite conversion was performed using the EZ-96 DNA Methylation Kit (Zymo Research, Irvine, CA, USA). FFPE Restoration was undertaken following the Infinium HD FFPE Restore Protocol (Illumina). The Infinium HD FFPE Methylation Assay (Illumina) with the hybridisation, washing and staining of the arrays as well as the scanning (iScan) of the HumanMethylation 450 K BeadChip arrays was performed using the manufacturers’ instructions. The data from all samples was imported in form of the idat files into Genomic Suite 6.6 (Partek) and Illumina normalisation was performed. ANOVA analysis was performed to detect differentially methylated loci between groups (e.g. IDC versus NAT, LNMet versus NAT, and IDC versus LNMet). Significance was granted if p < 0.05 and the estimated difference between groups (Δp) was <

| Lymph node (LN) metastases | Patient 4 LN | Patient 5 LN | Patient 7 LN | Patient 9 LN | Patient 11 LN | Patient 14 LN | Patient 16 LN | Patient 18 LN | Patient 20 LN | Patient 25 LN | Patient 30 LN | Patient 31 LN | Patient 32 LN | Patient 33 LN | Patient 35 LN |
|---------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Patient 1 LN | Patient 20 LN | Patient 25 LN | Patient 11 LN | Patient 7 LN | Patient 18 LN | Patient 31 LN | Patient 30 LN | Patient 26 LN | Patient 19 LN | Patient 32 LN | Patient 12 LN | Patient 8 LN | Patient 21LN | Patient 28 LN | Patient 28 LN |
| Patient 28 LN | Patient 29 LN | Patient 30 LN | Patient 24 LN | Patient 26 LN | Patient 5 LN | Patient 8 LN | Patient 24 LN | Patient 28 LN | Patient 28 LN | Patient 24 LN | Patient 28 LN | Patient 13 LN | Patient 28 LN | Patient 28 LN | Patient 13 LN |
− 0.1 or > 0.1, signifying a methylation change of at least 10%. These analyses were performed on single loci and on differentially methylated regions (DMR) − a minimum of three significant consecutive probes. Pathway enrichment analysis was performed using Genomic Suite 6.6 (Partek). All significant loci were filtered to include loci that are within enhancer and/or promoter regions. These were then used for pathway enrichment analysis, a tool within Genomic Suite 6.6 (Partek). The enrichment score is the negative natural log of the enrichment p-value derived from the Fisher’s exact test of the pathway enrichment analysis.

3. Discussion

Here we have described a dataset involving miRNA, gene and epigenome analysis of TNBC primary breast cancers, their matched normal adjacent tissues and matched lymph node metastases. This dataset also includes breast cancers of the triple negative breast cancer subtype with no known lymph node involvement, hence, providing a unique opportunity to dissect clinically relevant changes associated with lymph node metastasis in triple negative breast cancer. To the best of our knowledge, no other multi-platform dataset exists that includes matched lymph node metastases in this subtype. In breast cancer, the number of positive lymph nodes (LN) is known to have an inverse linear correlation with prognosis and survival [4]. This is not the case with TNBC, where it has been shown that any LN involvement is associated with worse disease-free and overall survival [5]. Hence, the use of this multi-platform dataset is likely to uncover novel markers and key pathways involved in progression to lymph node metastasis in TNBC.

Conflict of interest

The authors declare that there are no competing interests.

Acknowledgements

The authors would like to thank Dr. Ricardo Vilain for pathological review of all tumour and normal tissue specimens used in this analysis as well as Ms. Tina Hope and Ms. Sarah Nielsen for assistance with archival specimens. This work was supported by funding from the National Breast Cancer Foundation (CG-12-07) and the Hunter Medical Research Institute (HMRI 10-22).

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