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Data Article

Data on 2D culture characterisation of potential markers in human HER2-positive breast cancer cell lines

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ABSTRACT

To obtain this dataset, two human HER2-positive breast cancer cell lines (SKBR3 and MDA-MB-453 cell lines) were cultured in basal growth media to 80% confluence. Cells were passaged and total RNA extracted, RNA converted to cDNA and diluted to a working concentration of 40 ng/µL. Gene expression panels of cancer markers including Fibroblast growth factors (*FGF*), FGF receptors (*FGFRs*), cyclin-dependent kinases, cytokeratins, and WNT pathway components were then examined using Q-PCR. Gene expression was normalised against the expression of the endogenous gene *18S*. This article describes the data used in the research article "Syndecan-4 regulates the HER2-positive breast cancer cell proliferation cells via CK19/AKT signaling" [1]. The data presented demonstrates the range of gene expression profiles of these cells and aims to provide more detail of all gene expression changes observed in these cell lines.

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Specifications Table

Subject	Biological sciences
Specific subject area	Human genetics Breast Cancer research Molecular Biology
Type of data	Graphs
How the data were acquired	SKBR3 and MDA-MB-453 cell lines were obtained from ATCC. SKBR3 (1 × 1e4 cells/cm^2) and MDA-MB-453 (2 × 1e4 cells/cm^2) cells were grown as a monolayer under basal growth conditions (80% confluence). Total RNA was extracted by Direct-zol™ RNA MiniPrep kit (Zymo Research Corp., USA), then converted to complementary DNA (cDNA) using iScript (BioRad). The cDNA template was the utilised to perform Q-PCR using the QuantStudio 7 Flex Real-Time PCR system (Thermo Fisher Scientific).
Data format	Raw
Description of data collection	RNA was extracted from SKBR3 and MDA-MB-453 cell lines following culture in basal conditions. Following extraction, total RNA was converted to cDNA prior to Q-PCR analysis. The gene expression of the hallmarks of cancer panel was examined on the QuantStudio 7 Flex Real-Time PCR system (Thermo Fisher Scientific). Lists of primer sequences are presented in Table 1. Gene expression data was normalised against the endogenous control gene *18S* and analysed using $2^{-\Delta\Delta Ct}$. Relative gene expression is presented on graphs as ddCt x 1 × 1e6 generated using the Gaphpad Prism 9 software. Each gene expression experiment was performed in biological triplicate and technical quadruplicate ($n = 4$).
Data source location	• Institution: Queensland University of Technology • City/Region: Brisbane, Queensland • Country: Australia
Data accessibility	Repository name: Mendeley Data Data identification number: 10.17632/fkfh69gnty.2 Link: https://data.mendeley.com/datasets/fkfh69gnty/2
Related research article	For a published article: Pham, S.H., et al., Syndecan-4 regulates the HER2-positive breast cancer cell proliferation cells via CK19/AKT signaling. Biochimie, 2022. https://doi.org/10.1016/j.biochi.2022.11.010

Value of the Data

- These data provide basal expression profiles in two HER-2 positive BC cell lines of additional markers including cadherins, cytokeratins and Wnt pathway markers, providing researchers with an extended set of related gene expression data in HER2-positive BC cells.
- Researchers in cellular biology and oncology will benefit from these data.
- The dataset can be a reference for identifying the hallmarks of HER2-positive breast cancer cells in basal conditions for further investigations. The hallmarks of cancer panel of genes have been identified across numerous studies in breast and other cancers to help predict the survival risks of cancer patients.

1. Objective

Despite definition as a HER2-positive breast cancer subtype, the non-uniformity in HER2 expression remains challenging for therapeutic decision-making [2,3]. In addition, after diagno-

sis, the expression of HER2 may change in time [4,5], impacting high-quality treatment options when initial decisions have been made based mainly on HER2 status. In this dataset, a broad panel of essential genes was examined under normal growth conditions to identify the more prominent biomarker(s) regulating HER2-positive breast cancer cells *in vitro* [6]. This dataset found the cytokeratin family genes [7,8] to be significantly more highly expressed than other gene families, such as the cadherin [9], signaling pathway [10]. This gene expression pattern directed us to examine Cytokeratin 19 in more detail for its role in HER2 positive breast cancers [11–14].

2. Data Description

2.1. Cell marker panel in HER2-positive BC cell lines under basal culture conditions

We examined the hallmarks of cancer gene panel in HER2-positive BC cell lines (SKBR3 and MDA-MB-453) under basal culture conditions. The gene expression level of cytokeratin (*CK*) family members was determined to be highly expressed in both cell lines [15]. The raw q-PCR data for Fig. 1 is the standard output from the QuantStudio 7 Flex Real-Time PCR system and consists of the RNA threshold cycle (CT) values detected in each sample. For the negative control (NCT), a volume of 3 uL of distilled water was replaced with the same volume for cDNA, displaying only the gene name in the raw CT file. Therefore, no sample name was displayed in the table for the NCT wells. Genes that were not detected (undetermined) were shown as blank cells in the Ct mean columns due to these values could not be calculated by the machine.

Data summarised in Fig. 1 demonstrates that *CK18* and *CK19* (also known as *KRT19*) gene expression levels were highest when compared to other markers in the tested panel, with Cadherin 1,3 (*CDH1, CDH3*) and *CK4* gene was lowly expressed in the HER2-positive cells. Gene expression of *CK8* showed the opposite pattern of gene expression in the two cell lines where it was found to be highly expressed in SKBR3 cells and lowly expressed in MDA-MB-453 cells. The gene expression level of cyclin-dependent kinase 2 (*CDK2*) were determined to be higher than other cyclin dependant kinase and inhibitor genes in the panel in both SKBR3 (Fig. 1A) and MDA-MB-453 (Fig. 1B) cell lines. Interestingly, Nuclear Catenin beta-1 (*CTNNB1*) gene expression were highly expressed in SKBR3 cultures (Fig. 1A), but not expressed in MDA-MB-453 cultures (Fig. 1B). Gene expression of cyclin dependent kinase inhibitor 1A, 2A (*CDKN1A, 2A*) was not found in SKBR3 and MDA-MB-453 cells, except for *CDKN1A* which was shown to be lowly expressed in MDA-MB-453 Cells. Gene expression of fibroblast growth factor receptor 1 (*FGFR1*), Wnt family members 1,4 (*WNT1* and *4*), smooth muscle actin alpha 2 (*ACTA2*), protein phosphatase 1,2 (*AXIN1* and *2*), apoptosis signal-regulating kinase 1 (*ASK1*) were observed to have low levels of expression in both HER2-positive BC cell lines examined under basal culture conditions (Fig. 1).

2.2. HER2-positve BC cell line dose responses with Heparin

Heparin is an heparan sulfate (HS) agonist, which is known to increase the cell proliferation of the highly invasive BC [16]. Cell number in both HER2-positive BC cell lines showed a comparable increase between day 3 and day 5 (D3/D5). Higher doses of heparin treatment (25, 50 µg/ml) resulted in no significant difference in cell proliferation observed between D3/D5; in addition, no significant change in cell number was observed between the 25 and 50 µg/ml heparin dose at D7 in either cell line. Analysis of cell numbers in the 10 µg/ml heparin culture condition identified comparably increased SKBR3 and MDA-MB-453 cell numbers between the day 1 (D1)/D3 and the D3/D5 timepoints (Fig. 2). This result suggested 10 µg/ml heparin as an appropriate dose with which to monitor the effects of increased cell proliferation including gene

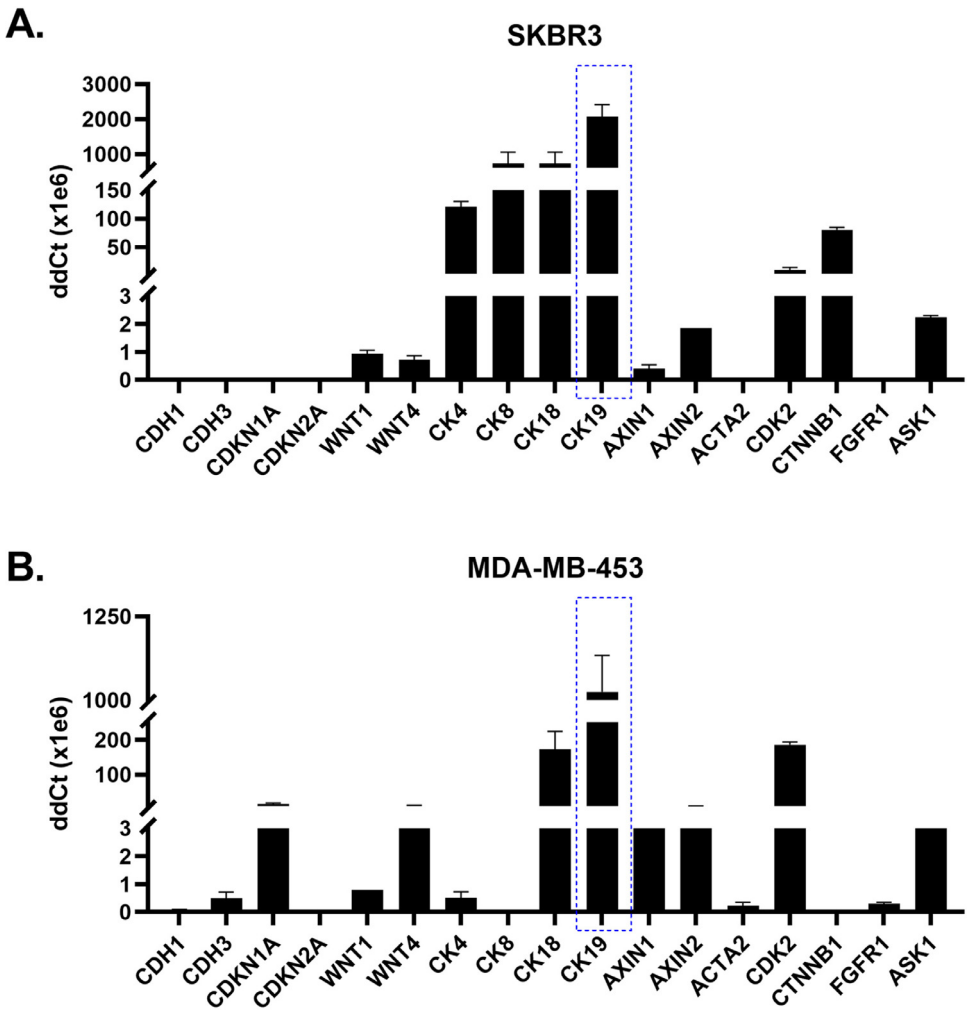

Fig. 1. Gene expression profile of marker panel in basal condition (80% confluence) in two HER2-positive BC cell lines. The marker panel included genes in SKBR3 cultures (A) and in MDA-MB-453 cultures (B): E-Cadherin (*CDH1*), P-Cadherin (*CDH3*), p21-cyclin dependent kinase inhibitor 1A (*CDKN1A*), p16-cyclin dependent kinase inhibitor (*CDKN2A*), Wingless-related integration site 1 (*WNT1*), Wingless-related integration site 4 (*WNT4*), Cytokeratin 4 (*CK4*),Cytokeratin 8 (*CK8*), Cytokeratin 18 (*CK18*), Cytokeratin 19 (*CK19*), Axin-1 (*AXIN1*), Axin-2 (*AXIN2*), Smooth muscle Actin Alpha 2 (*ACTA2*), Cyclin-dependent kinase 2 (*CDK2*), Nuclear Catenin B1 (*CTNNB1*), Fibroblast growth factor receptor 1 (*FRFR1*), MAP3K5 Mitogen-activated protein kinase 5 (*ASK1*). Graph bars = relative gene expression, all error bars are presented as standard deviation (SD).

expression of panel markers. At D7, no significant difference in cell number was observed between the different heparin concentrations for either cell line. Thus, in subsequent experiments, time points at D1, D3 and D5 were identified to be examined in more detail the effect of heparin on these cell cultures.

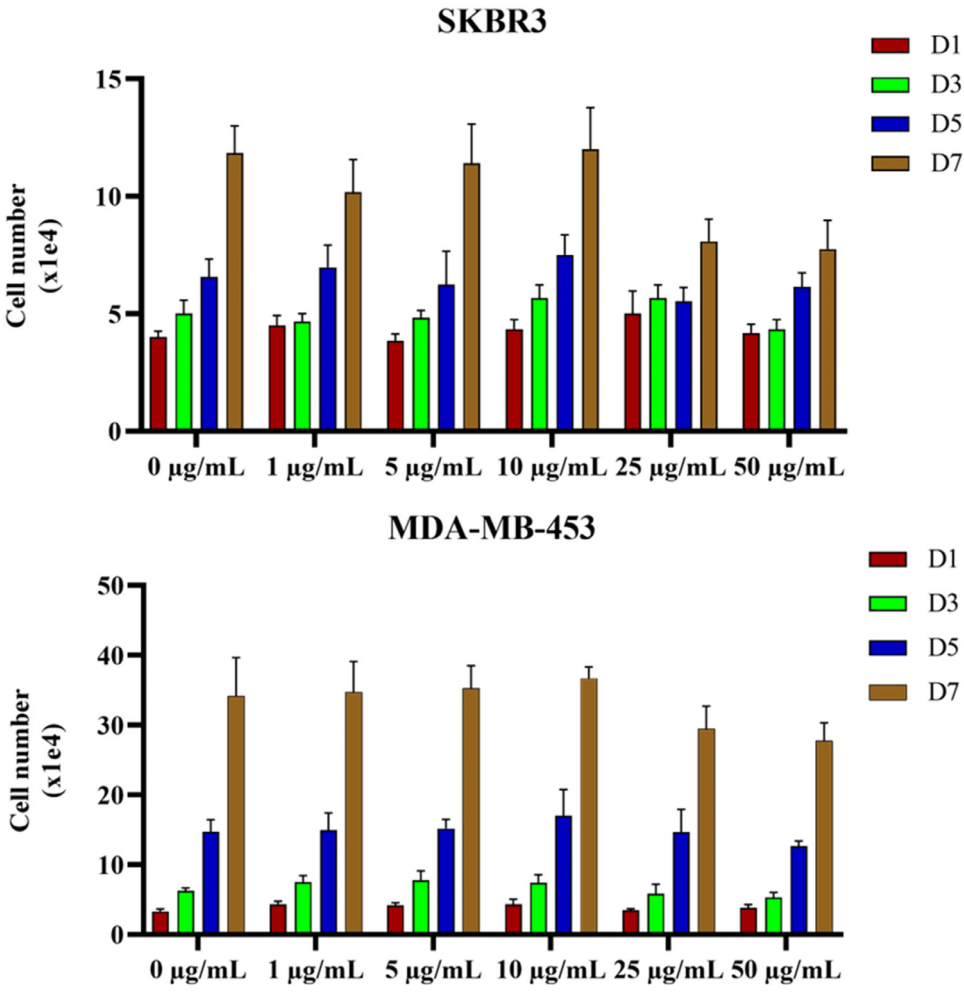

Fig. 2. Average cell number of HER2-positive breast cancer cells treated with varying concentrations (0–50 µg/ml) of heparin from day 1 (D1) to day 7 (D7). Cells were plated at 3000 cells/well for SKBR3, and 1 × 1e4 cells/well for MDA-MB-453, and in 24 well-plates. Total live cell count was assessed in cells collected at D1, D3, D5 and D7 of SKBR3 and MDA-MB-453 cultures treated with 0 µg/ml, 1 µg/ml, 5 µg/ml, 10 µg/ml, 25 µg/ml, and 50 µg/ml. Error bar = Standard deviation (SD).

3. Experimental Design, Materials and Methods

3.1. Cell Growth and Maintenance

Two HER2-positive BC cell lines SKBR-3 and MDA-MB-453 were obtained from the ATCC and grown in Roswell Park Memorial Institute Medium (RPMI) consisting of 10% FBS and 1% Penicillin/Streptomycin. Cells were used at early passages (SKBR3 cells: passages 7–11, MDA-MB-453 cells: passages 6–10) to avoid changes in gene expression affected by trypsinisations. The cells obtained from ATCC were certified as mycoplasma free. Cells were continuously grown and routinely passaged at 70–85% confluence by removing growth media, washing cell with 5 mL of 1 X Phosphate Buffered Saline (PBS), and adding 5 mL of trypsin/T75 before incubating at 37 °C for 5 min to allow detachment of the cell monolayer [17]. Trypsin was neutralised with RPMI before cell counting using a haemocytometer. Briefly, 10 µL of cell suspension was taken for cell counting using the Trypan Blue exclusion method. For basal growth conditions, cells were resuspended in appropriate volumes of growth media and replated at $1 \times 1e4$ cells/cm^2.

For the heparin dose response, each well in a 24-well plate was washed with 100 µL 1 X PBS, then dissociated with 100 µL trypsin and neutralised with 100 µL RPMI. Aliquots (10 µL) were taken from all replicates mixing with 10 µL of trypan blue for cell counting using a haemocytometer as described above.

3.2. RNA Extraction and Reverse Transcription

Cells were centrifuged at 3000 x g for 5 min. After the supernatant was discarded, the pellets were resuspended in 1 X PBS prior to being centrifuged at 5000 x g for 5 min. Then, PBS supernatant was discarded and the cell pellet resuspended in 300 µL of TRIzol (*Invitrogen*) for basal conditions or 100 µL for dose response. RNA extraction was performed with TRIzol using a Direct-zol RNA MiniPrep Kit (Zymo Research Corp., USA) according to the manufacturer's protocol.

BioRad iScript^{TM} Reverse Transcription kit was utilised to convert RNA to complementary DNA (cDNA). The reverse transcriptase included RNA (150 ng) was added with 300 ng of Random Hexamer Primers (*Invitrogen*) into a reaction volume of 19.5 μL at 65 °C for 10 min and 4 °C for 5 min. After incubation, 20 U of RNaseOUT (*Invitrogen*), 1 mM dNTPs (BioLab) and 10 U of Transcriptor Reverse Transcriptase (BioRad) was added resulting in a final volume of 30 μL in 1 X Reverse Transcriptase buffer (BioRad). This was incubated at 25 °C for 5 min then 46 °C for 20 min and finally 95 °C for 1 min. Following incubation, the collected cDNA was further assessed for concentration and purity using the NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Australia) with a 260/280 nm ratio of 1.8 used to specify pure cDNA. The cDNA was then diluted in RNAase-free water to a working concentration of 40 ng/μL and stored at −20 °C until use.

3.3. Q-PCR

Genes in the marker panel were examined using the QuantStudio 7 Flex Real-Time PCR system (Thermo Fisher Scientific). Lists of primer sequences are presented in Table 1. All Q-PCR was conducted in 384-well plates with each biological triplicate run in quadruplicate. The cDNA (120 ng) was amplified in a 10 μL reaction volume, containing 5 μL of 1 X SYBR Green PCR Master Mix (Promega), forward (200 ng) and reverse primer (200 ng) and 0.1 μM CXR reference dye (Promega). The standard cycling conditions were applied (for the hold stage: 50 °C for 2 min, 95 °C for 3 min, and the PCR stage: 50 cycles of 95 °C for 3 s and 60 °C for 30 s). The data for gene expression was normalised against *18S* expression (endogenous control) and analysed using $2^{-\Delta\Delta Ct}$. Biological triplicates and technical quadruplicates ($n = 4$) were performed

Table 1
Primer sequence used for Q-PCR.

	Markers	Gene Symbol	Forward Primer	Reverse Primer	Amplicon Size	Ref Seq
1	Fibroblast growth factor receptor 1	FGFR1	CCGTATGTCCAGATCCTGAAGA	GATAGAGTTACCCGCCAAGCA	126	NM_001354368.1
2	Nuclear Catenin B1	CTNNB1	GAAACAGCTCGTTGTACCGC	ATCCACTGGTGAACCAAGCA	123	NM_001904.3
3	Smooth muscle Actin, alpha 2	ACTA2	TAAGACGGGAATCCTGTGAAGC	TACAGAGCCCAGAGCCATTG	90	NM_001141945.1
4	p16 (cyclin dependent kinase inhibitor)	p16(CDKN2A)	TTCGGCTGACTGGCTGGC	GCCCATCATCATGACCTGGAT	133	NM_000077.4
5	p21 (cyclin dependent kinase inhibitor 1A)	CDKN1A	GCCGAAGTCAGTTCCTTGTG	TTCTGACATGGCGCCTCCT	84	NM_000389.5
6	Cytokeratin 8	CK8 (KRT8)	CCTGCAGGAAGGGATCTCCG	CCTGATGGACATGGTAGAGGC	70	NM_001256282.2
7	Cytokeratin 4	CK4 (KRT4)	TCCTTCATCGACAAGGTGCAG	GGGCTCAAGGTTTTTGCTGG	111	NM_002272.4
8	Cytokeratin 18	CK18	TCAGCAGATTGAGGAGAGCAC	TTCTCCAAGCTGGCCTTCAG	150	NM_000224.2
9	Cytokeratin 19	CK19	ACAGCCACTACTACACGACC	CGTCTCAAACTTGGTTCGGA	134	NM_002276.4
10	Cyclin-dependent kinase 2	CDK2	CGAGCTCCTGAAATCCTCCTG	GGCGAGTCACCATCTCAGCAA	94	NM_001798.5
11	Wingless-related integration site 1	WNT-1	CCGATGGTGGGGTATTGTGA	GATCCCCGGATTTTGGCGTA	139	NM_005430
12	Wingless-related integration site 4	WNT-4	GAGCAACTGGCTGTACCTGG	TGAGTTTCTCGCACGTCTCC	74	NM_030761.5
13	E-Cadherin	CDH1	AATCCCACCACGTACAAGGG	ATTGGGGGCATCAGCATCAG	90	NM_004360.5
14	P-Cadherin	CDH3	CTTCTCCAGGTTTGCTGGCT	GAATACTTTCCCCAGCGCCT	129	NM_001793.6
15	Axin-1	AXIN1	ACAGGATCCGTAAGCAGCAC	GGTACGTGCGGGGAATGT	90	NM_003502.3
16	Axin-2	AXIN2	TAACCCCTCAGAGCGATGGA	CCTCCTCTCTTTTACAGCAGGG	80	NM_004655.3
17	MAP3K5 Mitogen-activated protein kinase 5	ASK1	AGAGAGCCTGTGCTAACGAC	GATCCAGCTGAAAGAGCTGA	100	NM_005923.4

for each gene expression experiment. Relative expression was further presented on graphs at ddCt x 1e6.

Ethics Statements

All human BC cell lines and human primary mammary epithelial cells utilised in this study were obtained commercially from American Type Culture Collection (ATCC) and Thermo Fisher Scientific and considered of negligible-low risk. Ethical approval for the use of these cell lines was obtained from the QUT Human Research Ethics Committee with approval number: 1,800,000,179.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data on 2D culture characterisation of potential markers in human HER2-positive breast cancer cell lines (Original data) (Mendeley Data).

CRediT Author Statement

Son H. Pham: Methodology, Writing – original draft, Conceptualization, Data curation, Visualization, Investigation, Writing – review & editing; **Lyn R. Griffiths:** Supervision, Writing – review & editing; **Rachel K. Okolicsanyi:** Conceptualization, Methodology, Data curation, Visualization, Investigation, Supervision, Writing – review & editing; **Larisa M. Haupt:** Conceptualization, Methodology, Data curation, Visualization, Investigation, Supervision, Writing – review & editing.

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