Data Article

Dataset of HOXB7, HOXB8 and HOXB9 expression profiles in cell lines representative of the breast cancer molecular subtypes Luminal a (MCF7), Luminal b (BT474), HER2+ (SKBR3) and triple-negative (MDA231, MDA468), compared to a model of normal cells (MCF10A)

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Alterations in HOXB genes expression in breast cancer have been described and related to therapy response and disease progression. However, due to breast cancer complexity and heterogeneity, added to the use of different technical approaches, the observed expression profiles are sometimes contradictory. Here, we provided the analyses of HOXB7, HOXB8 and HOXB9 expression profiles in cell lines extensively used in the literature addressing the putative role of HOXB genes in breast cancer (MCF7, BT474, SKBR3, MDA231 and MDA468) and representative of the clinical breast cancer molecular subtypes (Luminal A, Luminal B, HER2+ and Triple-negatives Claudin-low/Basal), compared to a normal breast model (MCF10A), using quantitative-PCR (qPCR). This
The technique allows a very sensitive quantification of gene expression and was performed using the fluorophore SYBR Green in order to obtain the expression levels relative to a reference gene, GAPDH in this case. We showed that HOXB7 is upregulated in all breast cancer cells analyzed, while HOXB8 and HOXB9 are significantly upregulated in MCF7 (Luminal A), BT474 (Luminal B) and MDA231 cells (Triple-negative Claudin-low). In addition, we found that the magnitude of the upregulation is highly subtype-specific, being the HER2+ cells the model with lowest HOXB7 upregulation, presenting very low or even null expression for HOXB8 and HOXB9, respectively. These results are analyzed in more detail in “HOX genes function in Breast Cancer development” [1] and are potentially relevant for a better understanding of the molecular heterogeneity of breast cancer, in addition to be a valuable tool assisting researchers in the choice of the most suitable cell models to perform functional assays concerning HOXB7, HOXB8 and HOXB9 genes.

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Specifications table

| Subject | Cancer Research |
|---------|----------------|
| Specific subject area | Molecular genetics of breast cancer |
| Type of data | Gene expression profiles of HOXB7, HOXB8 and HOXB9 in breast cancer cell lines |
| How data were acquired | The qPCR runs were made in CFX 96™ Real-Time PCR Detection System (Bio-Rad). The data analyses were performed in CFX Manager 3.1 (Bio-Rad) and Prism 8 (GraphPad) |
| Data format | Raw and analyzed data [2] Histograms |
| Parameters for data collection | The first parameters analyzed were the quality of the duplicate samples' amplification and negative controls, as well as the melting curve pattern for each primer pair in order to guarantee that only the specific PCR product was amplified. Secondly, we made the correct threshold positioning into the exponential phase of the amplification curve. |
| Description of data collection | The raw data were accessed by CFX Manager 3.1 (Bio-Rad) software and a threshold line was put into the exponential phase of the amplification curve generating a Cycle Threshold (CT) number for each sample. The CT numbers were transferred to an Excel file for analyses using the formula: RATIO = E_target / E_GAPDH, in which “E” is the primer pair efficiency and GAPDH is the reference gene. These data are available on Mendeley Data [2]. The statistical analyses were made with Prism 8 (GraphPad) using the unpaired T test with Welch’s correction generating the histograms displayed on Fig. 1A–D. P-values were considered statistically significant when P ≤ 0.05. Data are presented as the mean ± SD of at least three independent experiments. |
| Data source location | This study was conducted at i3S (Institute for Innovation and Health Research) Porto - Portugal 41° 10’ 30.008” N, 8° 36’ 12.488” W |
| Data accessibility | Repository name: Mendeley Data Data identification number: 10.17632/v77kmkzj88.3 Direct URL to data: http://dx.doi.org/10.17632/v77kmkzj88.3 |
| Related research article | S.A. de Bessa Garcia, M. Araújo, T. Pereira, J. Mouta, R. Freitas, HOX genes function in Breast Cancer development. Biochim Biophys Acta Rev Cancer, 2020, doi:10.1016/j.bbcan.2020.188358. Epub ahead of print. |
Value of the data

- The HOXB7, HOXB8 and HOXB9 expression profiles in breast cancer are contradictory in the literature due to disease complexity and technical issues. The data presented here cover these two points by analyzing the expression of these genes in breast cancer cell lines (MCF7, BT474, SKBR3, MDA231 and MDA468) representative of the four molecular subtypes (Luminal A, Luminal B, HER2+, Triple-negatives Claudin-low/Basal) in comparison with a normal cell model (MCF10A), using a very sensitive quantitative technique, the qPCR.
- Researchers interested in the role of HOXB genes in breast cancer can use the data provided to pursue projects aiming to generate knowledge on the molecular networks involved in this highly heterogeneous disease as well as testing their value as therapeutic targets.
- The HOXB7, HOXB8 an HOXB9 expression profiles in different breast cancer cell lines, and in a normal model, using sensitive quantitative techniques, are valuable tools shedding light into the most suitable cellular model to further address the function of these genes in vitro.
- The cell lines analyzed in this work are extensively used in breast cancer research and allow comparisons with the basal expression profiles obtained by Hur et al. using reverse transcriptase PCR [3]. In addition, these cellular models were also used in studies in which HOXB7 expression was manipulated generating effects in the cells SKBR3 [4,7], MCF10A [5], MCF7 [6], BT474 [6], MDA231 [7], supporting their relevance for studies addressing the role of HOXB genes in breast cancer.

1. Data description

The total RNA extracted from one normal breast cell model (MCF10A) and five breast cancer cell lines (MCF7, BT474, SKBR3, MDA231 and MDA468) was reverse transcribed to cDNA and submitted to HOXB7, HOXB8 and HOXB9 relative expression analyses by qPCR. The respective raw and analyzed data are available on Mendley data (doi: 10.17632/v77kmzkj88.3) [2]. The data obtained after analyses show that HOXB7 tends to be upregulated in all breast cancer cell lines analyzed, when compared to the normal cell model (MCF10A) (Fig. 1A and B). However, its upregulation is subtype-dependent being particularly high in Luminal A and B cells (MCF7 and BT474, respectively) and Triple-negative Basal cells (MDA468). It is upregulated in moderate levels in Triple-negative Claudin-low cells (MDA231) and upregulated in low levels in HER2+ cells (SKBR3). In contrast, HOXB8 and HOXB9 are significantly upregulated in Triple-negative Claudin-low (MDA231) and Luminal cells (MCF7 and BT474) (Fig. 1C and D, respectively). Furthermore, these genes do not show expression differences in Triple-negative Basal cells (MDA468) when compared to MCF10A cells and HER2+ cells (SKBR3) present residual (HOXB8) or null expression (HOXB9).

2. Experimental design, materials, and methods

2.1. Cell lines

Five cell lines, representing distinct breast cancer molecular subtypes, were grown in 1 × Dulbecco’s Modified Eagle Medium supplemented with 10% Fetal Bovine Serum and 1 × antibiotic solution penicillin-streptomycin (pen-strep, Gibco™): MCF7 (Luminal A), BT474 (Luminal B), SKBR3 (HER2+), MDA231 (Triple-negative, Claudin-low) and MDA468 (Triple-negative, Basal). Immortalized cells derived from normal human mammary cells, MCF10A, were used as non-malignant control. The base medium for this cell line was DMEM/F12 with the following additives: human EGF (20 ng/mL), human insulin (40 μg/mL), cholera toxin (100 ng/mL), hydrocortisone (500 ng/mL), 5% of horse serum and 1 × penicillin-streptomycin (pen-strep, Gibco™) [8].
Fig. 1. HOXB7, HOXB8 and HOXB9 basal mRNA expression levels analyzed by qPCR in five breast cancer cell lines representing distinct molecular subtypes (blue bars), in comparison with normal breast cells (MCF10A, purple bars). Statistical analyses by unpaired T test with Welch’s correction. Y-axis depicts the ratios of HOXB expression relative to GAPDH expression. *p-value < 0.05, **p-value < 0.01 and ***p-value < 0.001 (A) HOXB7 is significantly upregulated in all breast cancer cell lines analyzed. (B) Higher magnification evidencing HOXB7 upregulation in SKBR3 cells. (C) HOXB8 is significantly upregulated in MCF7, BT474 and MDA231 cells. (D) HOXB9 is significantly upregulated in MCF7, BT474 and MDA231 cells and silenced in SKBR3 cells.
Table 1

|          | Sequence (5′-3′)          | Product bp | Annealing temperature |
|----------|--------------------------|------------|-----------------------|
| HOXB7    | FW: TACCCCTGATGCGAAGCTC  | 171        | 60°C                  |
|          | RV: ATCTCTGATCTGCTTCCGTGA |            |                       |
| HOXB8    | FW: GACAGGTCAAAATCTGGTCC | 111        |                       |
|          | RV: GCTTCTGTTTCTCCAGCTCT |            |                       |
| HOXB9    | FW: CTACGCTCCCTGGTAGGTA  | 198        |                       |
|          | RV: TAACTAAAGACCCGGCTACG |            |                       |
| GAPDH    | FW: ACTGCGGTCTTACCACCAT  | 142        |                       |
|          | RV: TCTTGAGGCTTGTGCACTTC |            |                       |

All cells were maintained in a humidified atmosphere with 5% CO₂ and at 37 °C. Cells were used in experiments when reached 70–80% of confluence. The cell lines are periodically authenticated by the Genomics Core Facility at i3s (University of Porto) using the PowerPlex® 16 HS System (Promega Corporation, #DC2100). Detection of the amplified fragments was made with automated capillary electrophoresis using 3130 Genetic Analyzer (Applied Biosystems) and the assignment of genotypes was performed in GeneMapper software v5.0 (Applied Biosystems).

2.2. cDNA synthesis, quantitative reverse-transcriptase PCR (qPCR) and statistical analyses

Total RNA extraction, from at least three independent experiments, was performed by TRIzol® (Invitrogen) method accordingly to manufacturer’s instructions and adding one more wash with ethanol 75%. For cDNA synthesis, 800 ng of RNA was subjected to reverse transcription, using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, #4368814), following the manufacturer’s instructions. The qPCRs reactions were performed in duplicates and carried out in the real-time thermal cycler CFX96™ (Bio-Rad) using the iTaq™ Universal SYBR® Green Supermix (Bio-rad, #1725121). Each amplification reaction contained 2 μL of the respective cDNA diluted 1:4; 0.5 μL of the forward primer; 0.5 μL of the reverse primer; 5 μL of 2X SYBR Green and 2 μL of H₂O DNase/RNase free, resulting in a 10 μL reaction. For each primer pair we prepared a mix containing all cited components, except the cDNA that was added in the correspondent well of the 96-well plate. The mix was prepared in sufficient quantity to the samples in analysis for a specific primer in the plate and distributed accordingly (8 μL mix/sample). The analyses of the qPCRs were performed using the method described by Schmittgen and Livak [9] and normalized with the glyceraldehyde-3-phosphate dehydrogenate (GAPDH) gene amplification. The raw data were obtained after the run using the CFX Manager 3.1 software (Bio-Rad). The amplification plot for each sample and negative controls, as well as the melting curve for each primer pair that were analyzed in order to guarantee the amplification of only one product and a threshold line was put into the exponential phase of the amplification curve generating a Cycle threshold number (CT) for each sample. The CTs were transferred to an Excel file and the expression differences obtained by the formula: Ratio = E target−(CT sample target gene)/E GAPDH−(CT sample GAPDH), where E refers to primer efficiencies previously determined.

The statistical differences between breast cancer cells and MCF10A normal cells were determined by unpaired T test with Welch’s correction using the Prism8 software (GraphPad). P-values were considered statistically significant when P ≤ 0.05. Data are presented as the mean ± SD of at least three independent experiments. The primers sequences are described in Table 1.
Acknowledgments

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Conflict of Interest

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

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