Differential Gene Expression Analysis of Culture Architecture Effects in JIMT1 Breast Cancer Cells
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1. Introduction

Tumor cell behavior is strongly influenced by the surrounding microenvironment. Although two-dimensional (2D) monolayer cultures remain the standard in vitro platform for mechanistic and pharmacological studies, they provide a simplified and often artificial representation of tumor architecture observed in vivo (Weigelt et al., 2014). In 2D systems, cells grow on rigid plastic substrates with limited spatial organization and reduced cell–cell and cell–extracellular matrix (ECM) interactions, conditions that can substantially alter gene expression patterns, signaling pathways, metabolism, and drug responsiveness (Kenny et al., 2007; Hongisto et al., 2013).

Three-dimensional (3D) culture systems and xenograft models more closely mimic physiological tumor environments (Tentler et al., 2012; Imamura et al., 2015). Matrigel-based 3D cultures provide an ECM-rich basement membrane-like context that promotes integrin signaling and organized spheroid formation, whereas polyHEMA-coated surfaces prevent cell attachment, generating anchorage-independent growth driven primarily by cell–cell interactions (Hongisto et al., 2013). Xenograft models introduce additional biological complexity, including stromal interactions, vascularization, and hypoxic gradients (Hidalgo et al., 2014). These distinct environmental contexts are expected to induce substantial transcriptomic reprogramming in cancer cells.

Previous studies have demonstrated that breast cancer cells exhibit markedly different transcriptional profiles in 2D versus 3D conditions, with 3D cultures often resembling in vivo tumors more closely (Kenny et al., 2007; Hongisto et al., 2013). In JIMT1 HER2-positive breast cancer cells, hierarchical clustering revealed that Matrigel 3D cultures clustered with xenograft tumors, whereas 2D and polyHEMA cultures formed a separate branch (Hongisto et al., 2013). Moreover, alterations in key oncogenic pathways, including HER2 and PI3K/Akt signaling, have been observed across culture models (Hongisto et al., 2013).

The publicly available dataset GSE42529 provides gene expression profiles of JIMT1 cells grown in 2D monolayer, xenograft tumors, Matrigel-based 3D culture, and polyHEMA-based 3D culture (Hongisto et al., 2013). Given the role of JIMT1 as a trastuzumab-resistant HER2-positive model, this dataset offers a valuable framework to examine how culture architecture influences transcriptional programs relevant to drug resistance and tumor biology.

In the present study, we performed differential gene expression analysis using GEO2R to compare (i) 2D monolayer versus xenograft, representing in vitro versus in vivo conditions, and (ii) Matrigel versus polyHEMA, representing distinct 3D microenvironmental contexts. By integrating statistical evaluation and exploratory transcriptomic analyses, we aim to characterize the magnitude and biological implications of culture-dependent transcriptional divergence in breast cancer cells.

2. Materials and Methods

2.1 Dataset Description

The transcriptomic dataset analyzed in this study was obtained from the NCBI Gene Expression Omnibus (GEO) under accession number GSE42529 (Hongisto et al., 2013). The dataset includes gene expression profiles of the human HER2-positive breast cancer cell line JIMT1 cultured under four conditions: 2D monolayer (n = 2), xenograft tumors (n = 2), Matrigel-based 3D culture (n = 4), and polyHEMA-based anchorage-independent 3D culture (n = 4). Expression profiling was performed using the Illumina HumanHT-12 v4.0 microarray platform, comprising 47,323 probes. These models represent increasing microenvironmental complexity from simplified in vitro systems to in vivo tumor growth.

2.2 Group Definition and Experimental Contrasts

Samples were grouped within the GEO2R interface according to GEO metadata annotations. Two primary contrasts were analyzed: (i) 2D monolayer versus xenograft (2 vs 2), representing in vitro versus in vivo conditions, and (ii) Matrigel versus polyHEMA (4 vs 4), representing two distinct 3D systems differing in extracellular matrix composition and adhesion context. All biological replicates were retained to preserve statistical power. The study design therefore includes both a smaller balanced comparison (2 vs 2) and a larger balanced comparison (4 vs 4).

2.3 Differential Expression Analysis

Differential gene expression analysis was performed using GEO2R, which implements the limma statistical framework (Smyth, 2004; Ritchie et al., 2015). Linear models were fitted for each probe, and moderated t-statistics were calculated using empirical Bayes variance shrinkage. Multiple testing was controlled using the Benjamini–Hochberg false discovery rate (FDR), and genes with adjusted p-values (Padj) < 0.05 were considered significant. No additional fold-change cutoff was applied.

2.4 Quality Control and Reproducibility

Data quality was assessed using boxplots, density plots, histograms of adjusted p-values, QQ plots, and UMAP for dimensionality reduction. Volcano and MA plots were used to visualize contrast-specific differential expression, and Venn diagrams summarized overlap across contrasts. To ensure reproducibility, the GEO2R workflow was executed three times with identical parameters, yielding consistent results across runs. While the 2 vs 2 comparison may have limited statistical power, the moderated variance approach implemented in limma enhances robustness under small sample conditions.

3. Results and Interpretation

3.1 Data Quality Assessment

Quality control analysis showed that the data were consistent across all 12 samples. The boxplots had similar median values and spread, which suggests that the samples were properly normalized (Figure 1A). The density plots also showed strong overlap between samples, indicating that there was no major technical bias affecting specific groups (Figure 1B). The mean–variance trend plot showed that variance changes depending on expression level, which is common in microarray data (Figure 1C). The limma method models this relationship using empirical Bayes moderation. This suggests that the statistical analysis is reliable, even though some groups have small sample sizes.
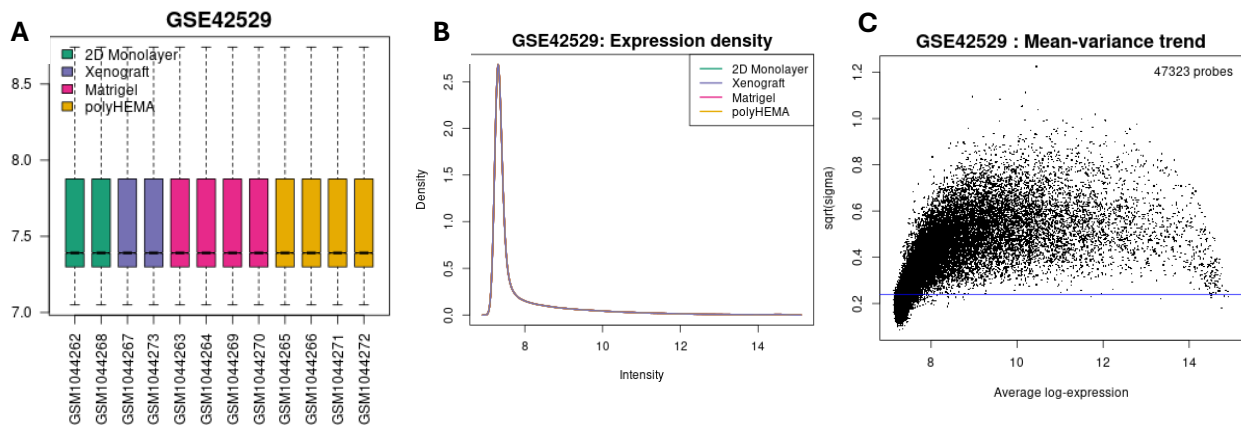

Figure 1. Data quality assessment of the GSE42529 dataset. (A) Boxplot showing similar expression distributions across all 12 samples. (B) Density plot showing strong overlap between samples, indicating consistent normalization. (C) Mean–variance trend plot showing the relationship between expression level and variance across 47,323 probes, modeled using the limma framework.

3.2 Global Transcriptomic Separation

UMAP analysis showed clear clustering of samples based on culture condition. Samples grown in 2D, xenograft, Matrigel, and polyHEMA formed separate groups. This means that the culture environment strongly affects global gene expression patterns. In particular, 2D samples were clearly separated from xenograft samples, indicating major transcriptional differences between in vitro and in vivo conditions. The two 3D systems (Matrigel and polyHEMA) also formed distinct clusters, suggesting that different 3D environments can lead to different transcriptional states.

3.2.1 2D Monolayer vs Xenograft

The comparison between 2D monolayer and xenograft samples showed many differentially expressed genes (Figure 2). The volcano plot showed significant genes in both directions, meaning some genes were upregulated and others were downregulated. The MA plot showed that these changes occurred across different expression levels, not only in low-expression genes. This suggests broad transcriptional changes between in vitro and in vivo conditions. Biologically, this is reasonable because xenograft tumors grow in a complex environment with stromal cells, extracellular matrix, and different oxygen levels. These conditions are not present in 2D culture, which likely explains the large differences observed.
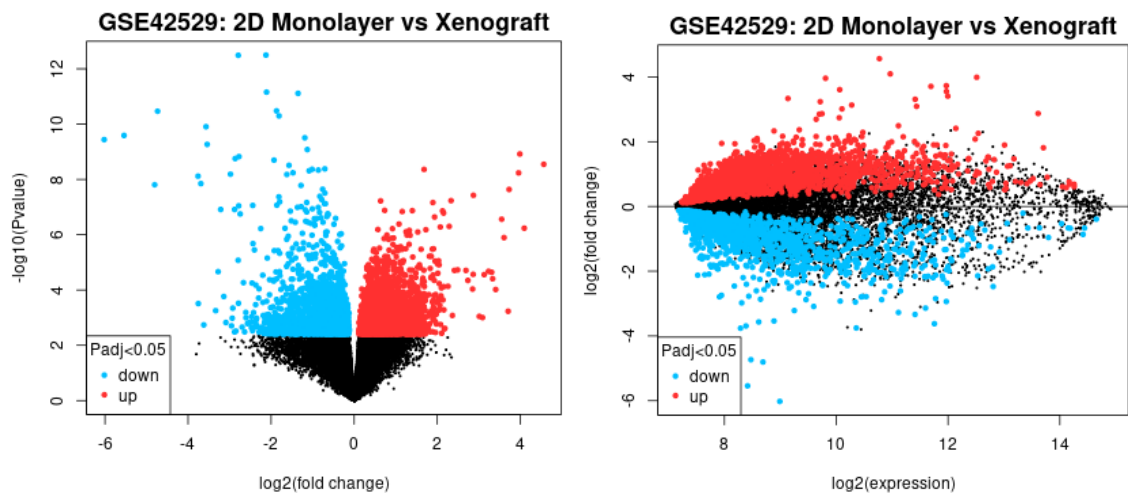
Figure 2. Differential expression analysis of 2D monolayer vs xenograft. Left: Volcano plot showing significantly upregulated (red) and downregulated (blue) genes at adjusted p-value < 0.05. Right: MA plot displaying log2 fold change across different expression levels, indicating widespread transcriptional differences between in vitro and in vivo conditions.

3.2.2 Matrigel vs polyHEMA

The comparison between Matrigel and polyHEMA also showed many differentially expressed genes, even though both are 3D culture systems (Figure 3). The volcano and MA plots showed significant changes in both directions. This indicates that not all 3D systems behave the same. Matrigel provides an extracellular matrix-rich environment that supports cell–matrix interactions, while polyHEMA prevents cells from attaching to the surface and promotes anchorage-independent growth. These differences in adhesion and microenvironment likely affect gene regulation.
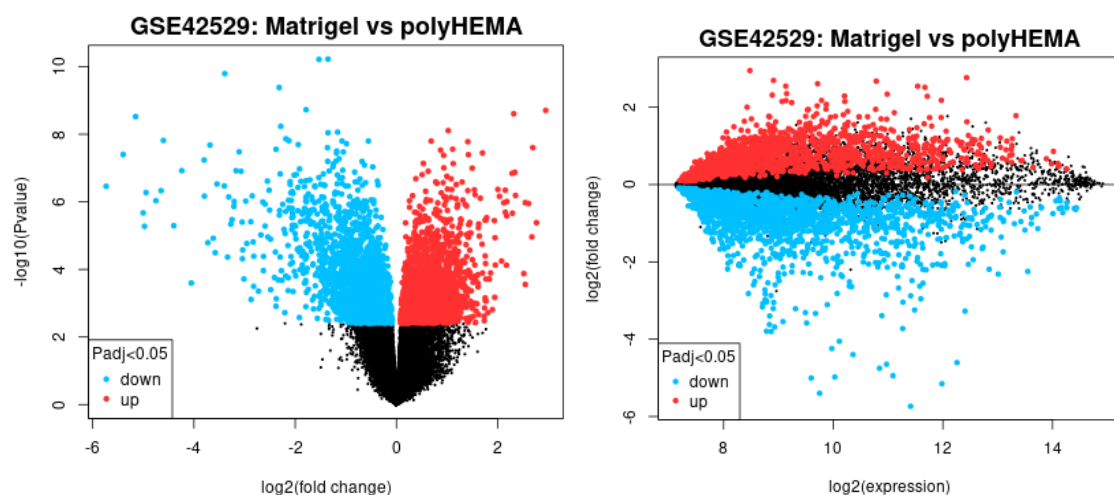

Figure 3. Differential expression analysis of Matrigel vs polyHEMA. Left: Volcano plot showing significantly upregulated (red) and downregulated (blue) genes at adjusted p-value < 0.05. Right: MA plot illustrating log2 fold changes across expression levels, indicating transcriptional differences between the two 3D culture systems.

3.3 Overlap of Differentially Expressed Genes

The Venn diagram showed that many significant genes were specific to each contrast (Figure 4). In particular, the 2D versus xenograft comparison had many unique genes, suggesting a strong transcriptional shift between these two conditions. The overlap between contrasts was limited, which means that gene expression changes depend on the specific comparison being made. This suggests that transcriptional responses are context-dependent rather than universal across all culture systems.
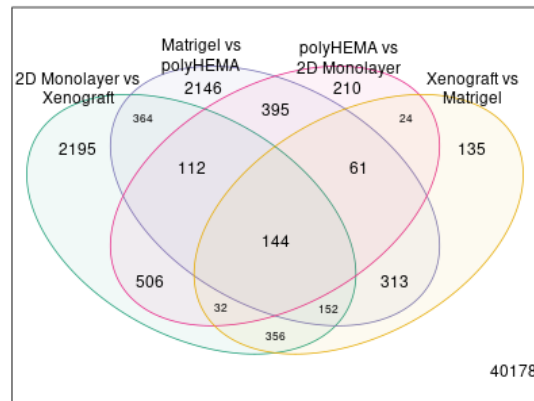

Figure 4. Overlap of differentially expressed genes across contrasts (Padj < 0.05). Venn diagram showing the number of significant genes shared and unique among different pairwise comparisons, indicating that most transcriptional changes are contrast-specific.

3.4 Comparison of Statistical Patterns Between Contrasts

The histogram of adjusted p-values and QQ plot patterns were different between contrasts. The 2D versus xenograft comparison showed stronger deviation from the null distribution, indicating stronger overall transcriptional differences (Figure 5). In contrast, the Matrigel versus polyHEMA comparison showed a more moderate pattern (Figure 6). This suggests that while differences exist between the two 3D systems, the magnitude of change is smaller compared to the shift from 2D to xenograft.
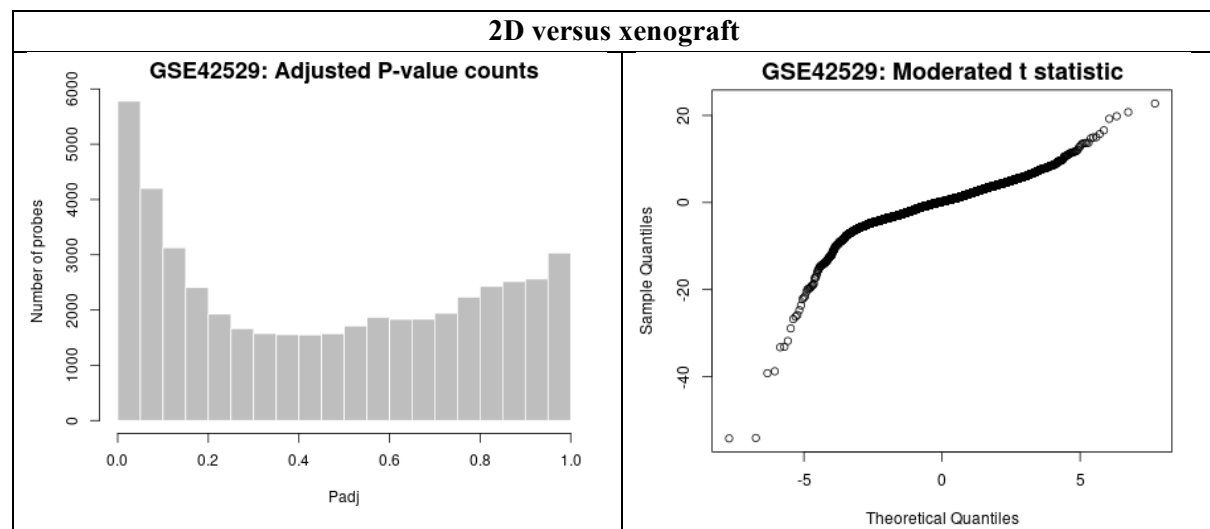

Figure 5. Statistical evaluation of differential expression (2D vs xenograft). Left: Histogram of adjusted p-values showing enrichment at lower values. Right: QQ plot of moderated t-statistics indicating deviation from the null distribution, supporting the presence of significant transcriptional differences.
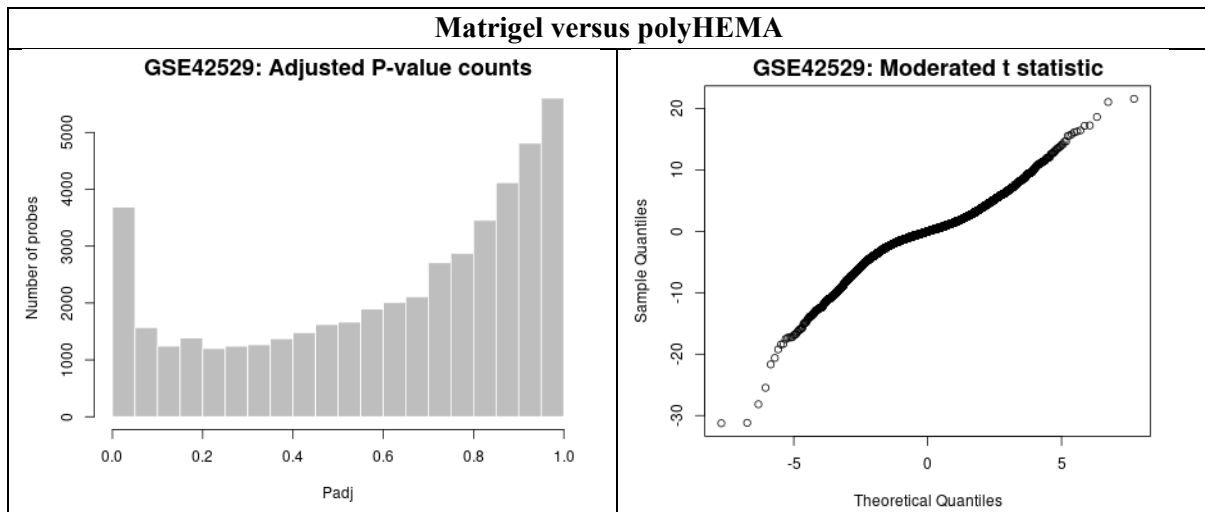
Figure 6. Statistical evaluation of differential expression (Matrigel vs polyHEMA). Left: Histogram of adjusted p-values showing the distribution of significant probes. Right: QQ plot of moderated t-statistics illustrating deviation from the null distribution, indicating significant gene expression differences between the two 3D culture systems.

3.5 Integrated Interpretation

Overall, the results show that the way cancer cells are grown strongly affects their gene expression patterns. Quality control analyses confirmed that the data were consistent and reliable, and UMAP clustering clearly showed that samples grouped according to culture condition. This indicates that differences in gene expression are driven by biological factors rather than technical variation.

The largest difference was observed between 2D monolayer and xenograft samples. This suggests that growing cells in a simple in vitro environment is very different from growing them in a living organism. Xenograft tumors are influenced by interactions with surrounding tissues, extracellular matrix, oxygen levels, and other in vivo factors, which likely explain the broad transcriptional changes observed.

Although Matrigel and polyHEMA are both 3D culture systems, they also showed clear differences in gene expression. This means that not all 3D models behave the same way. The presence or absence of extracellular matrix components and differences in cell adhesion conditions can shape how genes are regulated. The limited overlap of significant genes between comparisons further supports that gene expression changes depend on the specific microenvironment.

In summary, culture conditions play a major role in determining the transcriptional state of JIMT1 cells. These findings highlight the importance of selecting appropriate experimental models when studying cancer biology and interpreting transcriptomic results.

4. Conclusion

This study shows that culture conditions significantly affect the gene expression profile of JIMT1 breast cancer cells. The largest differences were observed between 2D monolayer and xenograft samples, highlighting the strong impact of the in vivo tumor microenvironment. Differences were also detected between the two 3D systems (Matrigel and polyHEMA), indicating that extracellular matrix and adhesion conditions influence transcriptional regulation. Overall, the results emphasize that culture architecture plays an important role in shaping cellular behavior and should be carefully considered in cancer research and drug response studies.

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