Unravelling the biological characteristics of MammaPrint extreme risk subgroups

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Background

MammaPrint® (MP) is a 70-gene based prognostic test that stratifies early-stage breast cancer patients into low- and high-risk of relapse (van 't Veer et al. 2002, Cardoso et al. 2016). BluePrint® (BP) is a 80-gene signature test that performs breast cancer molecular subtyping (Basal, Luminal or HER2) and identifies sub-populations with potentially distinct treatment response (Krijgsman et al. 2012, Whitworth et al. 2017). Recently, further stratification of the 70-gene risk results identified extreme low- and high-risk subgroups with specific clinical outcomes (Delahaye et al. 2017, Esserman et al. 2017) and treatment response characteristics (Wolf et al. 2017). However, the biological profiles of these extreme MP subgroups are not fully investigated. In this study, we aim to gain more insight into their biological significance using differentially expressed genes (DEGs) analysis.

Methods

We selected 400 samples from the whole MP range and defined 4 subgroups (Ultra high [UH], High risk [HR], Low risk [LR], Ultra low [UL]), for which FFPE microarray full-transcriptome data were available at Agendia. DEGs analysis was performed with limma and genes with absolute $\log_2$ fold change ($\log_2$FLC) >=1 and FDR <= 0.05 were considered to be significant. Gene set enrichment analysis (GSEA) was performed using GSEA 3.0 with a pre-ranked gene list. Significant gene set was filtered based on FDR q-values (< 0.05). Hierarchical clustering on the top 1000 most variable genes across all samples based on Euclidean distance metric was used to classify the subgroups.

Results

Based on clustering, two distinct clusters were observed **(Figure 1**). The UH subgroup, all classified as BP Basal type, clearly separates from the other subgroups indicating its functional heterogeneity that may uncovers unanticipated biological principles dictating poor outcomes.

Two separate comparative analyses were carried out to unravel biological processes associated with extreme risk subgroups: UL vs. LR and UH vs. HR. In UL vs. LR comparison, 22 genes were found to be differentially expressed (**Figure 2**) and among them 5 genes were overlapping with 70-gene MP signature (**Figure 3**). Similarly in UH vs. HR comparison, 942 genes were differentially expressed (**Figure 2**) and among them 22 genes were overlapping with 70-gene MP signature (**Figure 3**).

Based on GSEA (**Figure 4**), the UL subgroup was more homogeneous, with enrichment in pathways reflecting low proliferative and metastatic features. This is in line with the favorable long-term outcome characteristics of the UL group. Conversely UH exhibited higher heterogeneity, with the enrichment of more diverse pathways including immune response, cell cycle, proliferation and genomic instability. This would support the recent finding of UH samples being more sensitive to veliparib/carboplatin combination therapy compared to HR samples (Wolf et al. 2017).

Figure 1

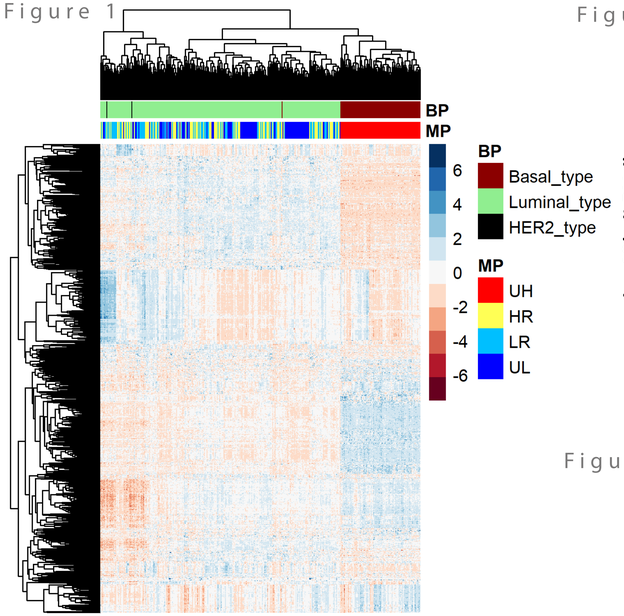


Figure 2

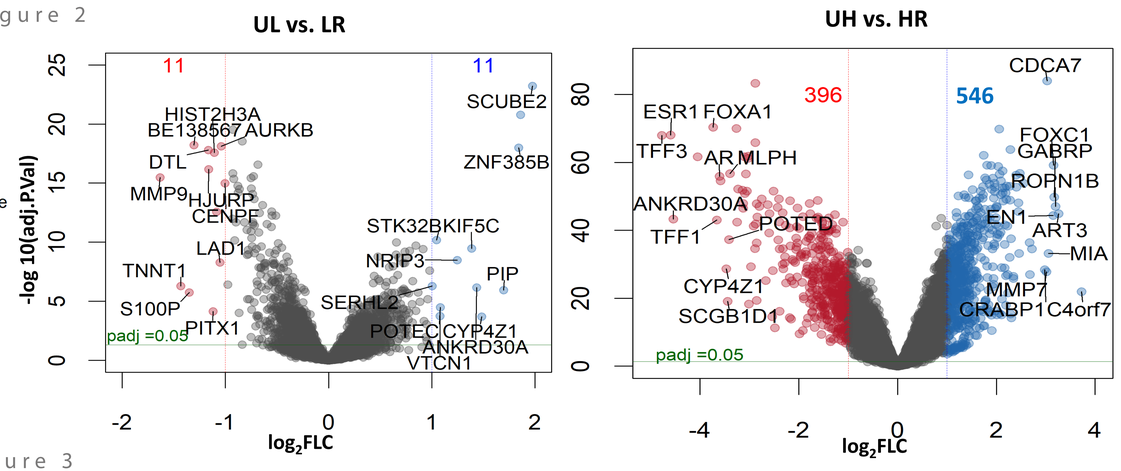
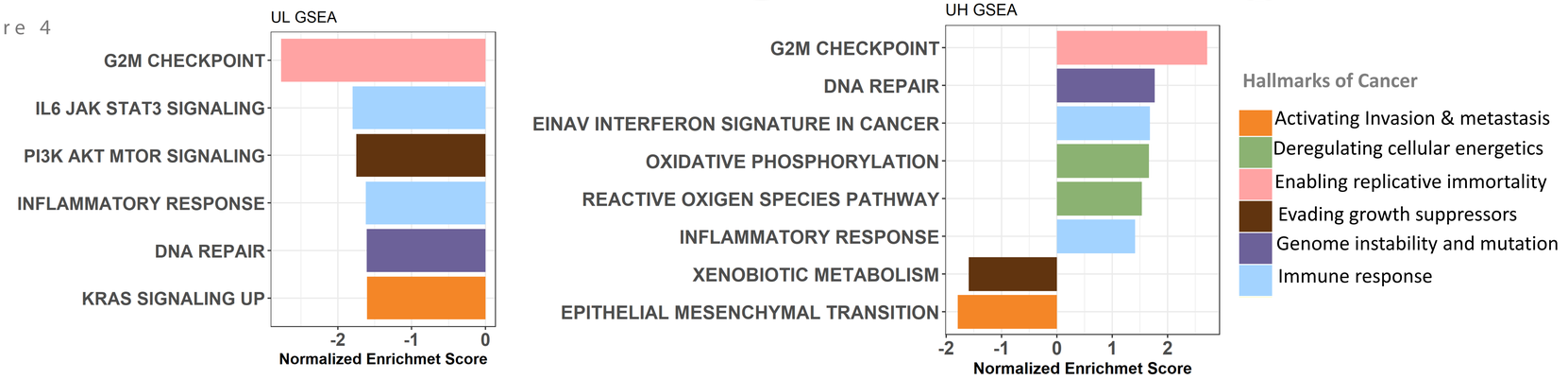


Figure 3

Figure 4



Conclusion

Our preliminary findings give additional insights into the biological processes associated with extreme MP groups, which might open new avenues for therapeutic intervention in breast cancer.

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