Identification of transcriptional changes with MammaPrint and BluePrint in early-stage breast cancer after neoadjuvant chemotherapy

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Background

The use of neoadjuvant chemotherapy (**NAC**) in patients with early-stage breast cancer (**EBC**) increases the opportunity for genomic testing which can help predict treatment response and optimize outcomes.

MammaPrint (**MP**) classifies tumors as having a Low Risk (**LR**) or High Risk (**HR**) of distant recurrence[a]. MP with BluePrint (**BP**), a molecular subtyping assay, categorize tumors as Luminal A (MP LR), Luminal B (MP HR), HER2, or Basal-Type[b].

Our recent analysis comparing matched pre- and post-NAC tumors found 25% of pre-NAC Luminal B tumors changed to Luminal A post-NAC, which corresponded with improved 5-year outcomes compared with patients who remained Luminal B post-NAC[c].

Here, we report differential gene expression (**DGE**) and pathway analyses in these matched tumors that may distinguish the different responses.

Methods

Patients: Among the 128 patients with EBC who received NAC at Cedars Sinai Medical Center between 2007-2016, 38 with residual disease (**RD**) had paired pre- and post-NAC tissues.

Molecular Classification: In patients with Luminal tumors, 8 were Luminal B pre- and post-NAC (HR/HR), and 7 were Luminal B pre-NAC but changed to Luminal A post-NAC (HR/LR).

Whole Transcriptome Analysis: Limma R package was used for quantile normalization and DGE analyses. Differentially expressed genes (**DEGs**) with < 0.05 false discovery rate and > 2-fold change were considered significant. Functional pathway enrichment was performed using Metascape.

Results

Figure 1. Transcriptional changes in tumors that (A) changed to MammaPrint Low Risk after NAC and (B) tumors that stayed MammaPrint High Risk. Significant DEGs are indicated by color: upregulated genes are blue and downregulated genes are red.

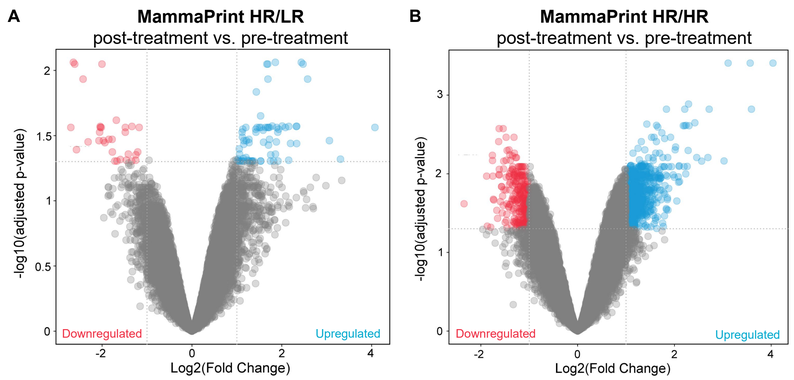


Figure 2. Number of unique and shared DEGs between MammaPrint HR/LR and HR/HR tumors.

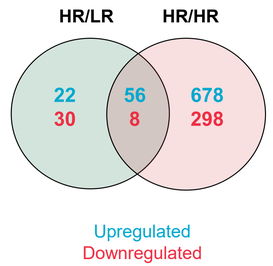


Figure 3. Functional enrichment analysis comparing post-NAC with pre-NAC tumors. The top 20 pathways for MammaPrint/BluePrint Luminal tumors (HR/LR and HR/HR) are shown.

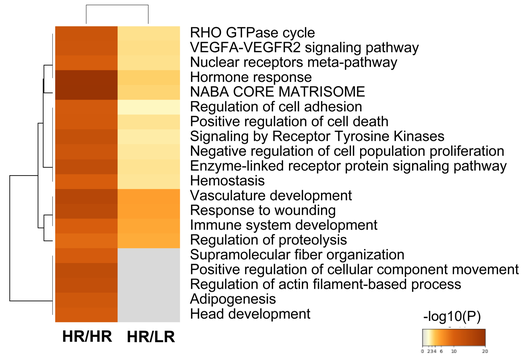
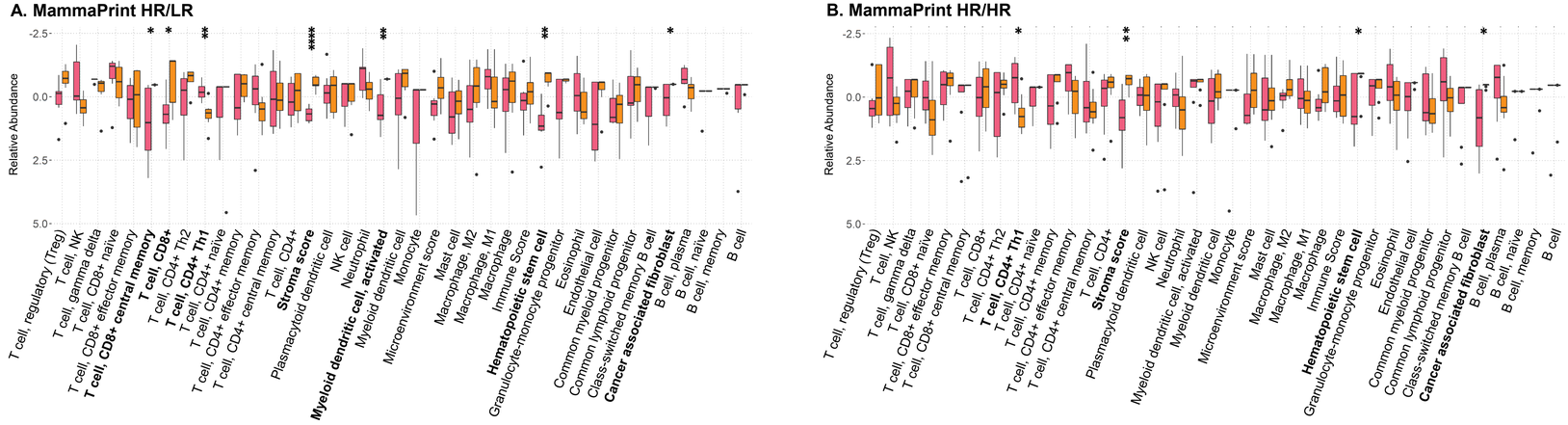


Figure 4. Change in relative abundance of immune cells by immune deconvolution (xcell) following NAC in (A) HR/LR and (B) HR/HR Luminal breast cancers. Abundance of immune cells in pre-NAC (**orange**) and post-NAC (**pink**) samples are shown**.** Cell types with significant differences between pre- and post-NAC are in **bold.**



Within HR/LR tumors, a DGE analysis identified 31 downregulated and 73 upregulated DEGs in post-NAC tissues relative to pre-NAC (**Figure 1A**). Interestingly, there was a more robust transcriptional change in HR/HR tumors, with 956 DEGs between post- and pre-NAC samples (281 downregulated and 675 upregulated; **Figure 1B**).

Among the differentially expressed gene transcripts, 22 upregulated and 30 downregulated transcripts are unique to HR/LR tumors. In contrast, 678 upregulated and 298 downregulated transcripts are unique to HR/HR tumors (**Figure 2**).

Functional enrichment analysis of post-NAC versus pre-NAC tumors revealed changes in cell cycle/proliferation pathways in HR/LR tumors, and enrichment of RHO and VEGF signaling, angiogenesis, and wound healing pathways in HR/HR tumors (**Figure 3**).

Notably, immune pathway enrichment was in both HR/LR and HR/HR groups, although the nature of enrichment differed. Immune deconvolution identified significant increases in activated myeloid dendritic cells (DC) and CD8+ T cells in HR/LR but not in HR/HR post-NAC tumors (**Figure 4**), suggestive of a host immune response.

Conclusion

Although post-NAC RD correlates with poor prognosis, even in Luminal tumors, these data suggest gene expression profiling may distinguish a subset with good prognosis. Using matched samples, we assessed the transcriptional differences in tumors that changed MP risk (HR/LR) with tumors that stayed MP HR post-NAC (HR/HR). Overall, HR/HR tumors had a larger transcriptional response with metastatic-related pathway enrichment. Given these patients with HR/HR tumors displayed worse outcomes[c], pathway changes may indicate resistance and patients may need additional therapy. Differential changes in immune cells between HR/HR and HR/LR tumors were also observed. The activated immune response in HR/LR tumors may be a biomarker for treatment response and improved outcome and will be the focus of further evaluation.

References

a. Cardoso et al. NEJM, 2016.
b. Krijgsman et al. BCRT, 2012.
c. Chung et al. SABCS, 2021.

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