Whole transcriptome analysis comparing HR+ HER2- breast cancer tumors from patients ≤ 50 years and > 50 years

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BACKGROUND

- Recent prospective clinical trials have demonstrated a differential chemotherapy effect based on age (≤ 50 vs. > 50 years) or menopausal status (pre- vs. post-) in a genomic low risk group[1,2]. However, it is unclear whether these differences are a direct anti-tumor effect of chemotherapy or a secondary ovarian suppression effect caused by chemotherapy.
- In the current study, we aimed to compare the biological characteristics of breast cancer tumors from patients aged ≤ 50 years and from patients aged > 50 years using whole transcriptome analysis to provide insights into this differential chemotherapy response.

METHODS

FLEX Study: The FLEX study (NCT03053193) is an ongoing, prospective study of stage I-III breast cancer patients that receive the MammaPrint (MP) 70-gene signature test with or without the BluePrint (BP) 80-gene signature test and consent to clinically annotated gene expression data collection.

Patient Cohort: 3868 patients with HR+ HER2- tumors were evaluated, of whom 808 were aged ≤ 50 years and 3060 were aged > 50 years. Clinical risk was assessed based on the MINDACT algorithm[3]. MP classified tumors as Low Risk (LR) or High Risk (HR). HR was stratified to H1 or H2; H2 exhibits a greater chemotherapy response[4,5]. BP and MP classified tumors as a Luminal A-, Luminal B-, HER2-, or Basal-type.

Gene Expression Analysis: Differential gene expression analysis of microarray data was performed with the R package 'limma'. Older patients were randomly selected to obtain an equal sample size as younger patients. Differentially expressed genes (DEGs) were compared in five iterations in all samples, and in subgroups based on clinical risk, MP risk, and BP subtype. DEGs were considered significant if FDR < 0.05 and fold change ≥ 2.

Statistical Analysis: Differences in MP, BP, and clinical features were assessed by Chi-Squared, Fishers' exact test[‡], or *t* test.

RESULTS

Table 1. Patient-Tumor Characteristics

	≤ 50	> 50	Significance
Total	808	3060	
Menopausal status			
Pre or Peri	566 (81%)	159 (5%)	p <0.001
Post	137 (19%)	2771 (95%)	
AOL Clinical Risk			
High Risk	312 (54%)	801 (39%)	p <0.0001
Low Risk	267 (46%)	1264 (61%)	
cT			
Multi	4 (0.5%)	15 (0.5%)	p < 0.001[†]
cT1	282 (35%)	1297 (42%)	
cT2	204 (25%)	476 (16%)	
cT3	33 (4.1%)	67 (2.2%)	
cT4	8 (1%)	14 (0.5%)	
cTX	277 (34%)	1191 (39%)	
cN			
Multi	6 (1.1%)	1 (0.1%)	p <0.001[†]
cN0	386 (74%)	1500 (81%)	
cN1	104 (20%)	244 (13%)	
cN2	8 (1.5%)	16 (0.9%)	
cN3	1 (0.2%)	6 (0.3%)	
cNX	20 (3.8%)	86 (4.6%)	
MammaPrint			
Low Risk	383 (47%)	1724 (56%)	p < 0.001
High Risk	425 (53%)	1336 (44%)	
MammaPrint HR			
High 1, H1	319 (75%)	1101 (82%)	p < 0.01
High 2, H2	106 (25%)	235 (18%)	
BluePrint			
Luminal A-type	383 (47%)	1724 (56%)	p <0.001[†]
Luminal B-type	373 (46%)	1218 (40%)	
HER2-type	0 (0%)	5 (0.2%)	
Basal-type	52 (6%)	113 (3.7%)	

Figure 1. Percentage of MammaPrint results and BluePrint subtypes by age group. (A) MP results as Low Risk versus High Risk. Within the High Risk group, women were categorized as H1 or H2 (B). (C) BP tumor subtypes in women ≤ 50 (left) and > 50 years (right).

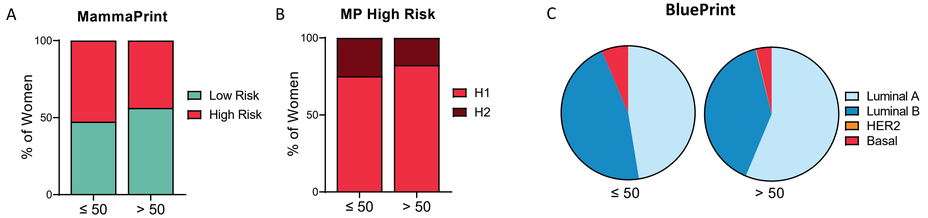

Figure 2. Principal component analysis of the top 500 genes with the highest variance.

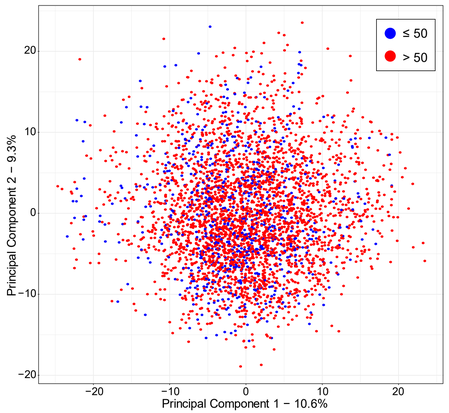

Table 2. DEGs between ≤ 50 versus > 50 in all samples, and in subgroups based on clinical risk, MP risk, and BP subtype.

	Up/Down	Genes
Overall	Upregulated	AREG, CLIC6
	Downregulated	CLEC3A, CYP4Z1, ESR1
Clinical Low Risk	Upregulated	CXCL13, AREG, CLIC6, PEG10
	Downregulated	CLEC3A, ESR1
Clinical high risk	Upregulated	CLIC6
	Downregulated	CLEC3A, CYP4Z1, ESR1
MP Low Risk	Upregulated	AREG, CLIC6, CXCL13,
	Downregulated	CLEC3A, ESR1
MP High Risk – H1 (no DEGs detected in H2)	Upregulated	AREG
	Downregulated	CLEC3A
BP Luminal-type	Upregulated	AREG, CLIC6
	Downregulated	CLEC3A
BP Basal-type	Upregulated	
	Downregulated	PDLIM3

Table 3. Gene function for the five DEGs comparing tumors in women ≤ 50 versus > 50 years.

Gene	Function
AREG	An autocrine growth factor that interacts with EGF/TGF-α receptors to promote growth of normal epithelial cells. It is enriched in invasive breast carcinomas.
CLIC6	A member of the chloride intracellular channel protein family, which regulates chloride ion transport.
CLEC3A	May play a role in cell adhesion, and promotes tumor progression and poor prognosis in breast invasive ductal cancer.
CYP4Z1	Part of the cluster of cytochrome P450 genes which are related to drug metabolism. It is overexpressed in breast cancer cells, which is regulated by glucocorticoid and progesterone receptors.
ESR1	Encodes estrogen receptor alpha (ERα), a transcription factor that is overexpressed in breast cancer.

RESULTS

- Approximately 81% of patients aged ≤ 50 were pre- or peri-menopausal, whereas 95% of patients aged > 50 were post-menopausal (**Table 1**).
- A higher proportion of patients aged ≤ 50 had tumors of high clinical risk (54%) compared to patients aged > 50 (39%) (p < 0.0001) (**Table 1**).
- Approximately 53% of patients aged ≤ 50 had a HR tumor, while patients aged > 50 had a lower frequency (44%) of HR tumors (p < 0.001) (**Table 1, Figure 1A**). A higher frequency of younger patients were classified as H2 (25%) compared to those > 50 (18%) (**Table 1, Figure 1B**).
- Younger patients had a higher proportion of tumors that classified as BP Luminal B- and Basal-type than older patients (p < 0.001) (**Table 1, Figure 1C**).
- Principle component analysis of the top 500 genes with the highest variance revealed no distinct clustering by age group (**Figure 2**).
- Five DEGs were detected in tumors from patients aged ≤ 50, and fewer were detected when adjusted for MP risk and BP subtype (**Table 2**). **Table 3** lists functions for the respective five DEGs.

CONCLUSIONS

- Whole transcriptome analysis identified no substantial differences in gene expression between tumors, including Low Risk Luminal-type tumors, from women aged ≤ 50 (mostly pre- or peri-menopausal) and women aged > 50 (mostly post-menopausal).
- No clear biological distinction between age groups suggests that MP and BP provide consistent results and are not influenced by patient age.
- These data support the likely explanation that the observed age-dependent difference in chemotherapy benefit is not due to intrinsic biological differences in breast cancers due to age, but rather to differences in the effect of chemotherapy on the host.

References

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