A new 5-genes Signature predictive of Risk of Relapse in Early Breast Cancer

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Abstract

Background

Gene Signatures can be used to assist in making treatment decisions in breast cancer. They are expensive and time consuming. We were interested in looking for the "core" genes of published signatures in order to find a less expensive tool.

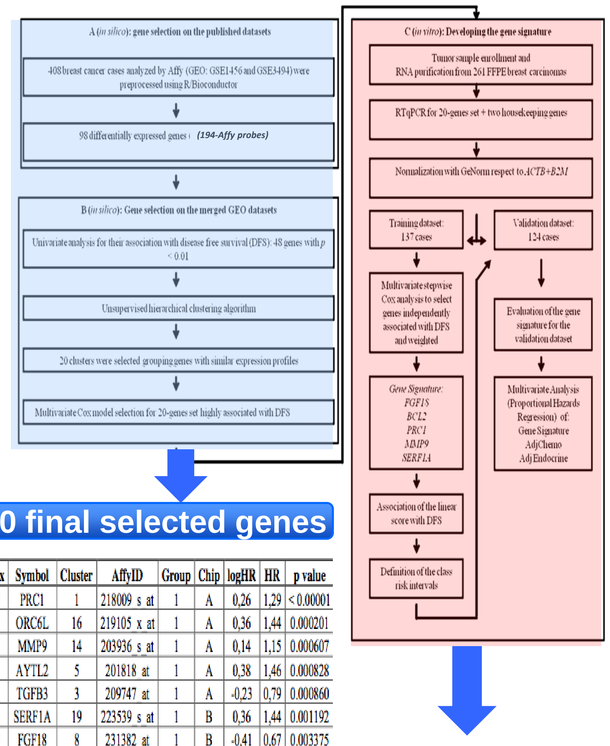
Materials & Methods

To select the candidate genes we used data of NCBI Gene Expression Omnibus including 408 breast cancers. Quantitative reverse transcriptase PCR was done on a set of 261 consecutive breast cancer cases with minimum follow up of 5 years (paraffin embedded tissue). Raw intensity data of Affymetrix HU133A and HU133B arrays of the two datasets (GSE1456 and GSE3494) were preprocessed using R/Bioconductor , using the supercomputer Michelangelo. The candidate genes were selected from the "70-gene signature"[1,2], the "recurrence-score"[3], the "two-gene–ratio model" [4] and the "Insuline Resistence" signature[5], for a total of 98 genes.We evaluated the 20 mRNA more significantly related to DFS by embedded sections, split into a training (n 137) and a validation set (n 124).

Results

The signature was developed on the training set and a multivariate stepwise Cox analysis selected 5 genes independently associated with DFS: *FGF18* (HR=1.13, p=0.05) , *BCL2* (HR=0.57, p=0.001), *PRC1* (HR=1.51, p=0.001), *MMP9* (HR=1.11, p=0.08), *SERF1a* (HR=0.83, p=0.007). These five genes were combined into a linear score weighted according to the coefficients of the Cox model, as: *0.125 FGF18 - 0.560 BCL2 + 0.409 PRC1 + 0.104 MMP9 - 0.188 SERF1A.* The linear score was highly associated with DFS in the training set (HR=2.7, 95%CI=1.9-4.0, p<0.001). The signature was then evaluated on the validation set assessing the discrimination ability by a Kaplan Meier analysis, using the same cut offs classifying patients at low, medium or high risk of relapse as defined on the training set. The score resulted highly associated with DFS also in the validation set (p<0.001).

Materials & Methods: Flow Chart



20 final selected genes

Index	Symbol	Cluster	AffyID	Group	Chip	logHR	HR	p value
114	PRC1	1	218009 s at	1	A	0,26	1,29	< 0.00001
120	ORC6L	16	219105 x at	1	A	0,36	1,44	0.000201
38	MMP9	14	203936 s at	1	A	0,14	1,15	0.000607
11	AYTL2	5	201818 at	1	A	0,38	1,46	0.000828
69	TGFB3	3	209747 at	1	A	-0,23	0,79	0.000860
145	SERF1A	19	223539 s at	1	B	0,36	1,44	0.001192
163	FGF18	8	231382 at	1	B	-0,41	0,67	0.003375
156	QSOX2	18	227146 at	1	B	0,51	1,66	0.003409
143	MS4A7	15	223344 s at	1	B	-0,16	0,85	0.004351
126	FBXO31	7	219785 s at	1	A	0,31	1,36	0.004459
164	GPR180	9	231871 at	1	B	0,33	1,39	0.005603
54	PITRM1	17	205273 s at	1	A	0,26	1,30	0.007143
33	BCL2	6	203685 at	2	A	-0,16	0,85	0.003310
68	IGF1	2	209540 at	3	A	-0,22	0,80	0.000001
35	IGFBP6	2	203851 at	3	A	-0,40	0,67	0.000002
47	IL6ST	12	204863 s at	3	A	-0,19	0,83	0.000028
45	IRS1	13	204686 at	3	A	-0,19	0,82	0.001258
7	IGFBP7	4	201163 s at	3	A	-0,41	0,66	0.001529
102	TNFSF10	20	214329 x at	3	A	-0,20	0,82	0.004448
26	IDE	11	203328 x at	3	A	0,52	1,68	0.005188

5- Gene Signature Formula

0.125□*FGF18*

0.560□*BCL2*

0.409□*PRC1*

0.104□*MMP9*

0.188□*SERF1*

Patients Characteristics

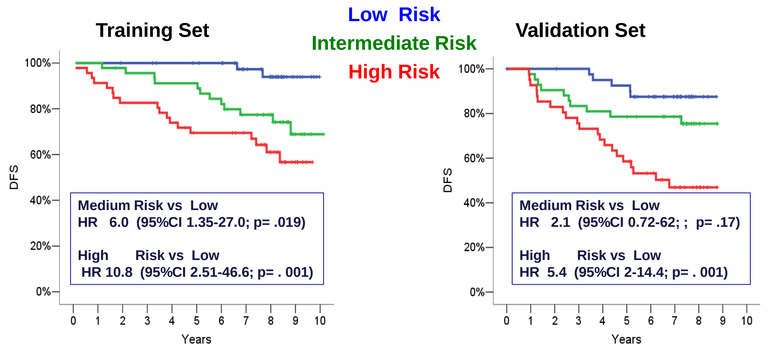
	Training Set		Validation Set		
Nr of Patients	137		124		
Mean Age (range)	62.3 (35-87)		61.1 (33-87)		
Mean Follow up (months) (range)	100.7 (59-123)		89.2 (61-121)		
	n	**%**	**n**	**%**	**p value**
Histology					ns
Ductal	86	62.8	83	66.9	
Lobular	26	19	16	12.9	
Tubular-Lobular	12	8.8	10	8.5	
Medullary/Apocrine	1/1	1.4	2/1	2.4	
Other	11	8.02	12	9.6	
T Size					ns
T1	78	56.9	82	66.1	
T2	53	38.7	37	29.8	
T3	3	2.2	3	2.4	
Tx	3	2.2	2	1.6	
N Status					ns
pN0	89	65.0	75	60.5	
pN1a	26	19.0	26	21.0	
pN+ 4-10	11	8.1	7	5.6	
pN+ >10	10	7.3	14	11.3	
NX					
ER/PgR pos	123	85.4	97	76.38	ns
HER2 NA	125	91.2	79	73.7	p=0.05*
Grading					
G1	33	24.1	20	16.1	ns
G2	51	37.2	57	46.0	ns
G3	27	19.7	38	30.6	p=0.04
G NA	26	19.0	9	7.3	ns
Ki67					
High (>14%)	60	43.8	60	48.4	ns
Low (<15%)	77	56.2	60	48.4	
Adjuvant Chemo	49	35.8	57	46.0	ns
Antra-based	22	16.0	40	32.2	p= 0.01
Adjuvant Endocrine (any)	110	80.3	96	77.4	ns
Relapses	33	24.0	38	30.6	ns
Mean DFS, months	51.4		47.2		ns
Deaths	33	24.0	39	31.4	ns

Multivariate Cox proportional-hazards analysis HR

Variable	Regr Coeff (B)	SE	Exp (B)	Mean	Z Value	Prob Level
T Size	.565	.145	1.760	1.448	3.88	.000103
Nodal Status	.558	.104	1.748	.659	5.32	.000000
Ki67 (High/Med/Low)	9.727E-02	.136	1.102	1.773	.71	.475607
AdjChemo (Yes/No)	.168	.278	1.183	1.597	.61	.544261
Adj Endocrine (Yes/No)	5.159E-02	.291	1.052	1.210	.18	.859396
5 gene Sign (High/Med/Low)	0.628	.179	1.874	1.984	3.51	.000448

*: Ki67 cut offs: Low: <15 ; Med: >15<25; High : >25

% DFS according to 5-gene Signature



Discussion

5 gene-Signature: possible role in Breast Cancer

FGF18: overexpressed in tumours; affects tumour and microenvironment.
"Self-sufficiency in growth signal"

BCL2: overexpressed also in BC, negative prognostic role (OncotypeDx, Mammaprint)
"Evading apoptosis"

PRC1: associate with the mitotic spindle, crucial role in the completion of cytokinesis, downregulated by p53, overexpressed in p53 defective cells
"Limitless replication potential"

MMP9: often upregulated in tumor microenvironment, influences extracellular matrix, cell adhesion, cell surface receptors, regulates bioavailability of growth factors and chemokines
"Tissue invasion and metastasis"

SERF1a: function still unknown but reported to be related to a worse prognosis

Conclusions

Overexpression of "BCL2" and "SERF1a"
Is related to an higher risk of relapse.
Overexpression of "FGF18"" PRC1" and "MMP9"
is related to a better prognosis.

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