13-gene signature to predict rapid development of brain metastases in HER2-positive advanced breast cancer patients

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Background and Study Aims

- Brain metastases of breast cancer constitute an important part of therapeutic failures and are associated with severe morbidity and mortality
- The risk of brain metastases is particularly high in HER2-positive advanced breast cancer patients
- Using RNA-microarray technology, we earlier developed in this group a 13-gene signature strongly predicting for rapid development of brain metastases (J Clin Oncol 2008; 26: 45s)
- Now, using qRT-PCR technology we investigated the clinical outcome of genes constituting this signature in an independent group of patients, and employed a culture model system

Discovery (DASL) Series: HER2-Positive Advanced Breast Cancer Patients (n=87)

Age	Mean, years (range)	49 (29-75)
Menopausal status	Pre/Post/Unknown	47/39/1
Pathology type	Ductal/Lobular/Other	69/4/14
Dominant site of disease	Viscera/Soft tissue/ Bones	78/4/5
ER status	(+)/(-)/Unknown	29/55/3
Grade	2/3/Unknown	26/48/13
Trastuzumab therapy	Yes/No	72/15
Brain metastases (BM)	Yes/No	47/40
Median time to BM development	55 months	

DASL Analysis

- RNA (200 ng) extracted from FFPE primary tumor samples using HighPure RNA Paraffin Kit (Roche Applied Bioscience)
- RNA pre-qualified using iScript (Bio-Rad) to reverse transcribe and SYBR Green Master Mix (Applied Biosystems) to perform quantitative RT-PCR
- DASL assay of 502 known cancer genes performed using the Sentrix Universal Array (Illumina Corp)
- Microarray data normalized through the sample median
- Biostatistics data analysis using t-test with unequal variances, significant analysis of microarray, and predictive analysis of microarray

13-gene Signature

HER2 related

- *CDK4* (Cyclin dependent kinase 4)
- *CCNC* (Cyclin C)
- *PTK2* (FAK, Focal adhesion kinase)
- *MYC* (Myc)

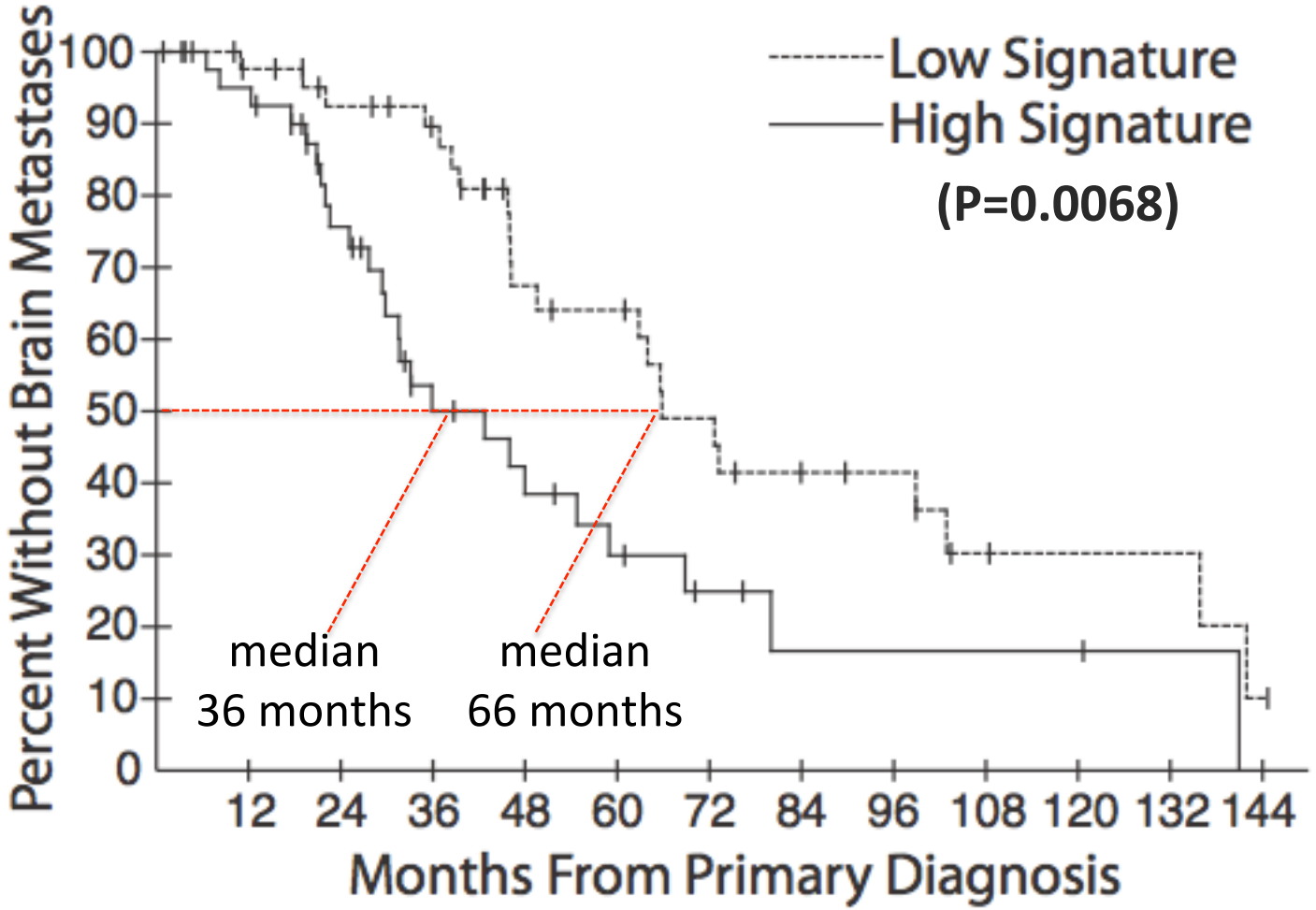
DNA double strand break repair

- *BARD1*
- *RAD 51* (Rad 51)
- *FANCG* (Fanconia anemia group G)

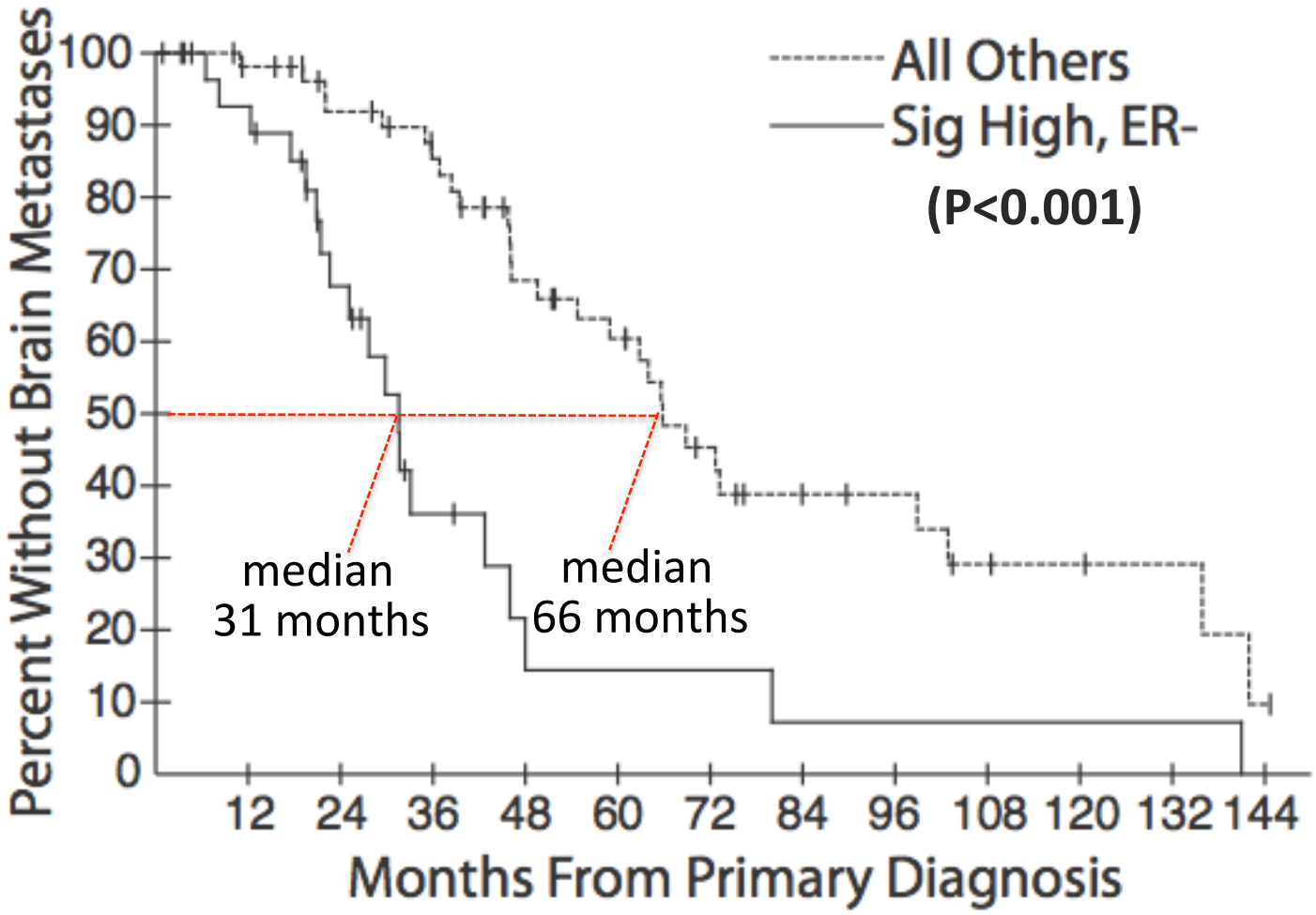
Others

- *PCNA* (Proliferating cell nuclear antigen)
- *PRCC* (Papillary renal cell carcinoma-translocation associated)
- *TPR* (Translocated promoter region)
- *EMS1* (Cortactin)
- *DSP* (Desmoplakin)
- *HDGF* (Hepatocyte derived growth factor)

Time from Diagnosis to Brain Relapse by 13-Gene Signature (DASL Series)



Time from Diagnosis to Brain Relapse by 13-Gene Signature in ER-, HER2+ primary tumors (DASL Series)



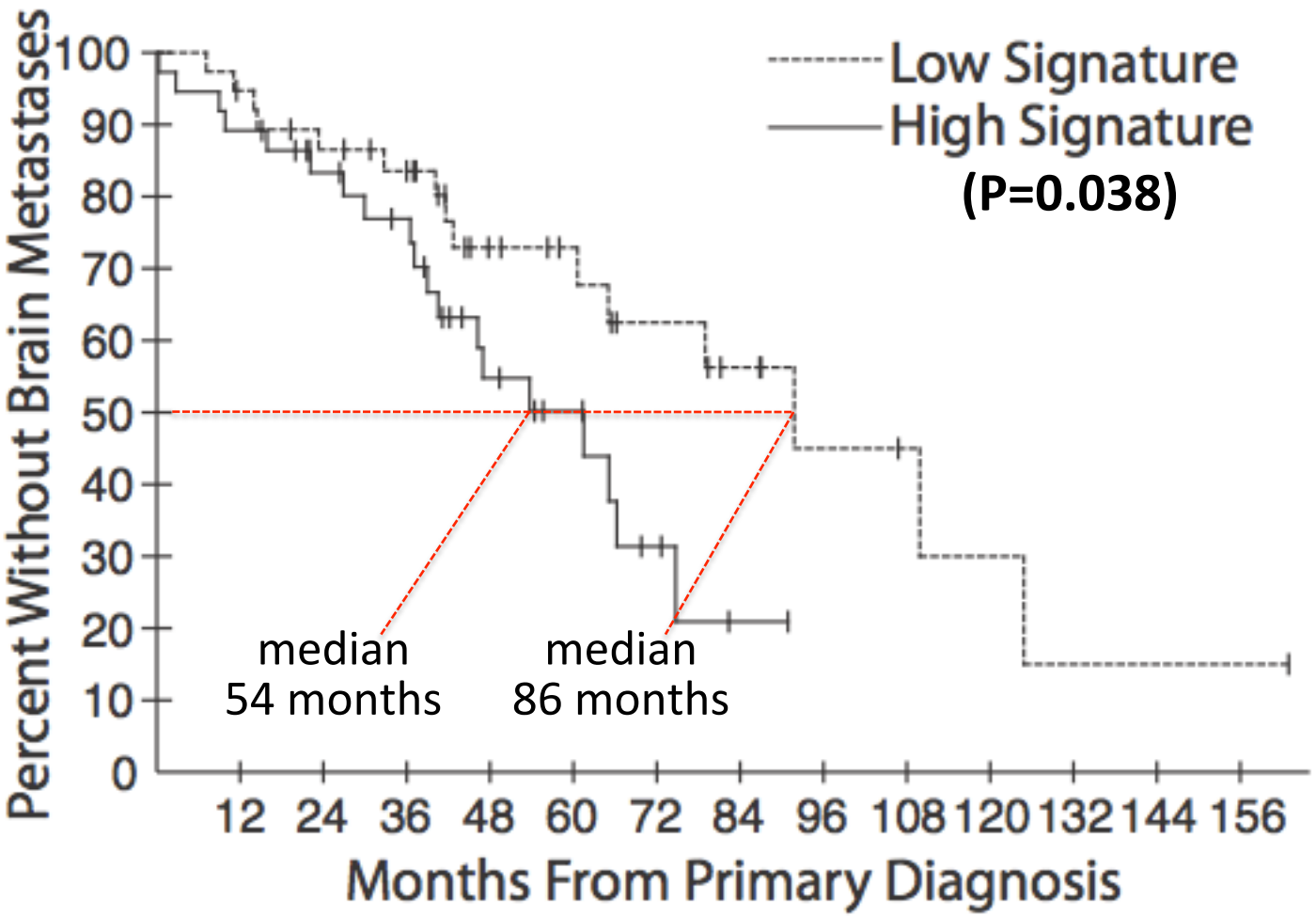
Independent qRT-PCR Series: HER2-Positive Advanced Breast Cancer Patients (n=75)

Age	Mean, years (range)	52 (28-71)
Menopausal status	Pre/Post/Unknown	47/28/0
Pathology type	Ductal/Lobular/Other+Unkn.	58/5/12
Dominant site of disease	Viscera/Soft tissue/Bones	52/15/7
ER status	(+)/(-)/Unknown	32/42/1
Grade	2/3/Unknown	32/33/10
Trastuzumab therapy	Yes/No	63/12
Brain metastases (BM)	Yes/No	34/41
Median time to BM development	65 months	

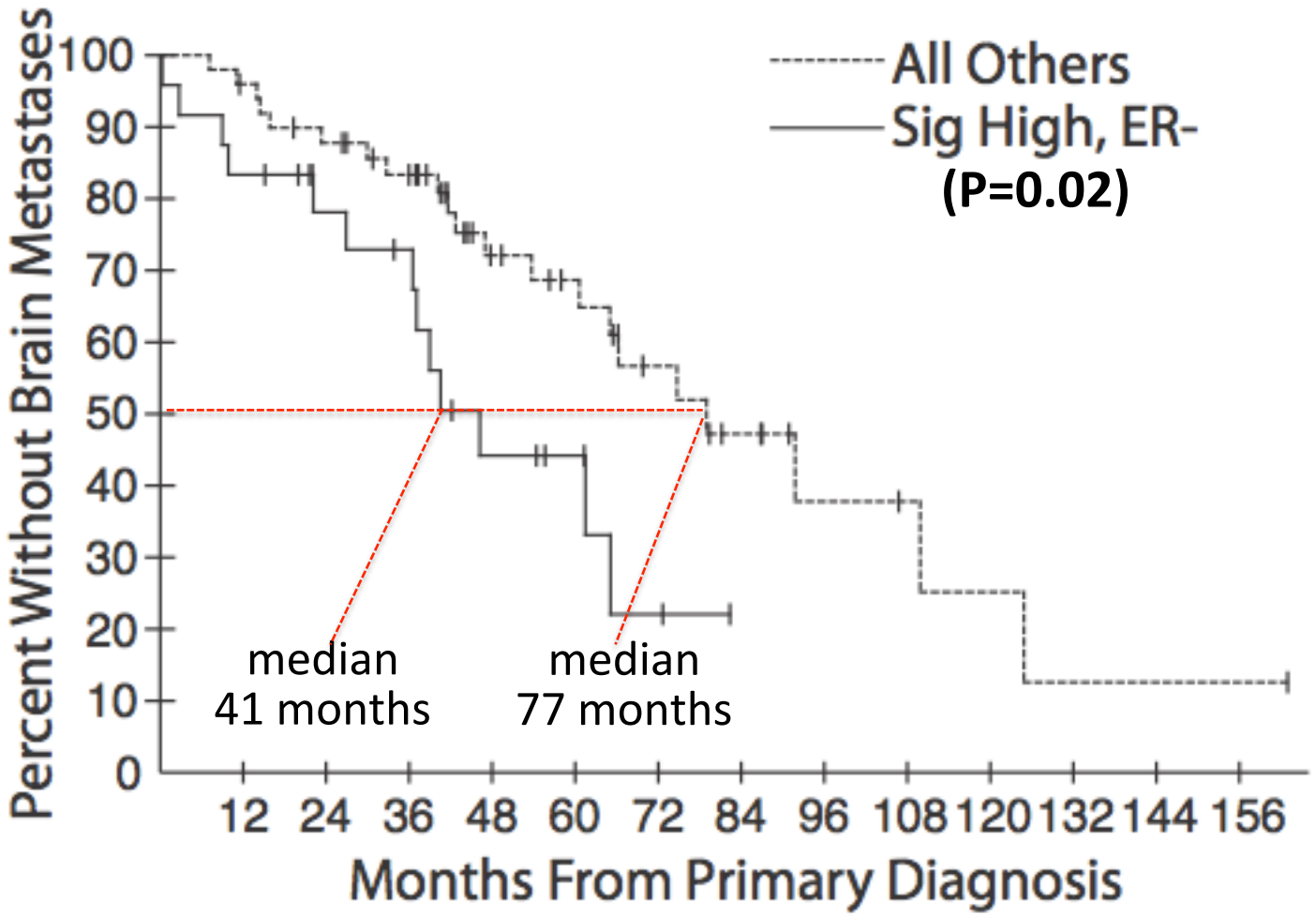
qRT-PCR Cohort Analysis
Statistical Methods

- qRT-PCR was performed for 13 genes plus two control genes (*GAPDH, ACTB*)
- Delta cycle threshold (ΔCt) values were generated by ABI Biosystems Data Assist Software and normalized to control genes
- ΔCt values were standardized for use in univariate Cox regressions by subtraction of mean, division by SD for each gene
- ΔCt values were weighted by individual Cox PH coefficients
- Expression score = sum of weighted ΔCt values for the 13 genes
- Median of data used to divide into high vs. low expression groups
- Cox regression used to test association of expression score with time to brain metastasis

Time from Diagnosis to Brain Relapse by 13-Gene Signature (qRT-PCR Series)



Time from Diagnosis to Brain Relapse by 13-Gene Signature in ER-, HER2+ Primary Tumors (qRT-PCR Series)



Association Between 13-Gene Signature and Standard Patient Traits

	Number (%) of patients with 13-gene signature					
	DASL cohort			PCR cohort		
	Low	High	P	Low	High	P
ER Negative	24 (28%)	30 (35%)		18 (24%)	24 (32%)	
Positive	18 (21%)	13 (15%)	0.26	19 (26%)	13 (18%)	0.24
PR Negative	31 (36%)	30 (35%)		25 (34%)	28 (38%)	
Positive	11 (13%)	13 (15%)	0.81	12 (16%)	9 (12%)	0.61
Menopausal status Pre	25 (29%)	20 (23%)		15 (20%)	13 (17%)	
Post	17 (20%)	24 (28%)	0.20	23 (31%)	24 (32%)	0.81
Primary tumor grade 2	20 (25%)	15 (19%)		19 (29%)	13 (20%)	
3	18 (23%)	26 (33%)	0.18	12 (19%)	21 (32%)	0.08
Patient age (median (years)	50	48.5	0.94	53	51	0.55

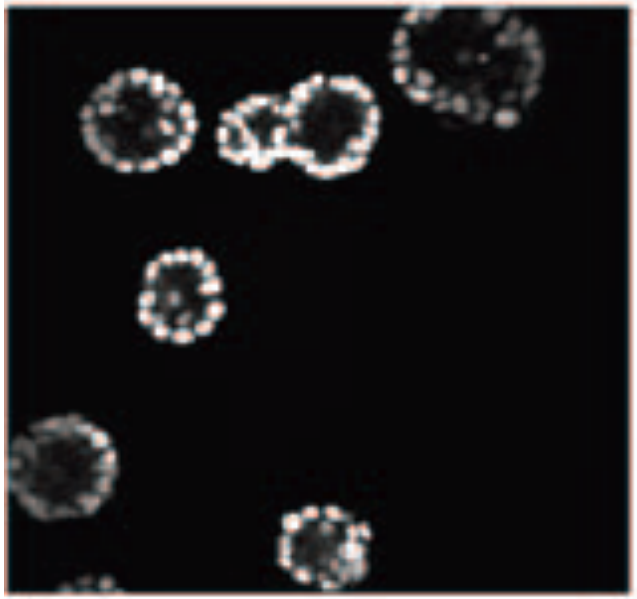
DNA Double Strand Break Repair Genes in the 13-Gene Signature (Repair by Homologous Recombination)

- *RAD51*; serves to recombine the broken single stranded DNA with the homologous, intact strand
- *BARD1*; an obligate binding partner for *BRCA1*
- *FANCG*; a part of the Fanconi Anemia complex

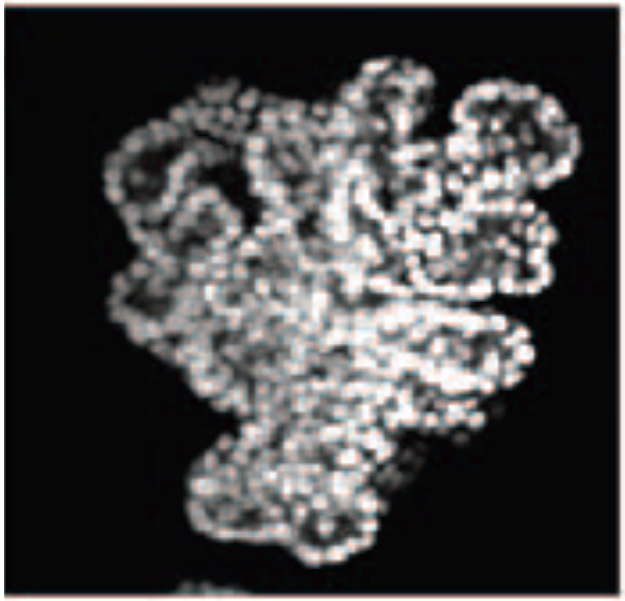
MCF-10A Three Dimensional Culture Model

- Does overexpression of *RAD51* and/or *BARD1* exert a phenotypic effect on breast tumorigenesis?

MCF-10A three dimensional culture model



Normal breast acinus

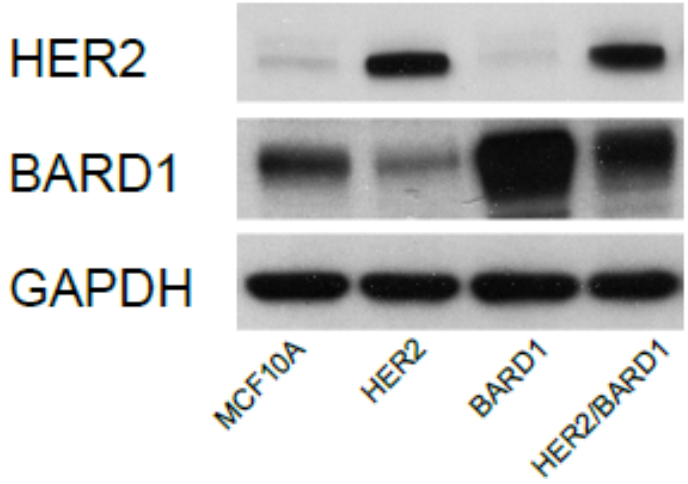


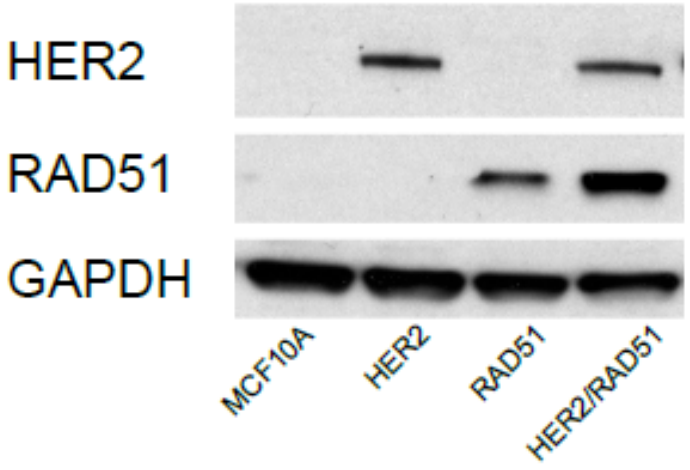
HER2 overexpression

Nature Rev. Cancer 5:675,2005

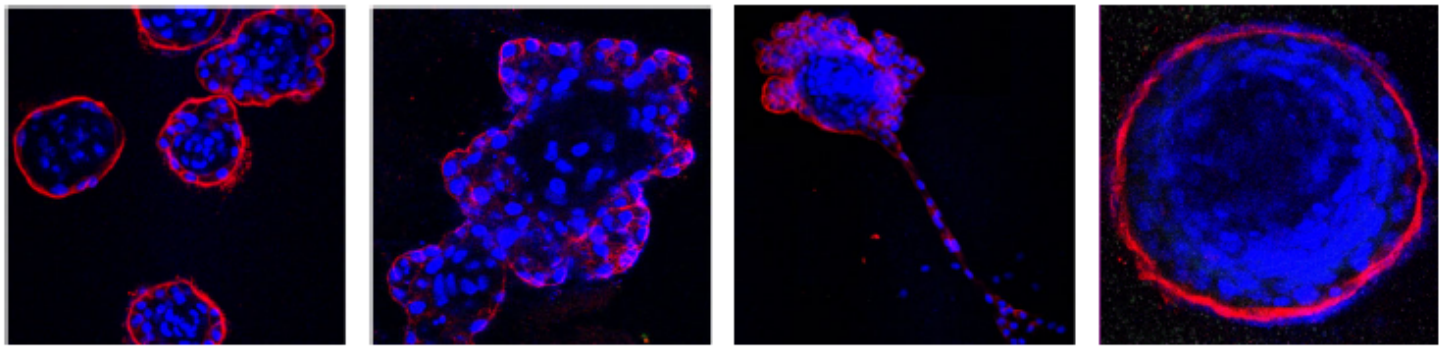
- Does overexpression of *RAD51* or *BARD1* promote brain metastasis? Experimental brain metastasis assays ongoing

Transfection of MCF-10A Immortal Breast Cells With *RAD51*, *BARD1* and/or HER2

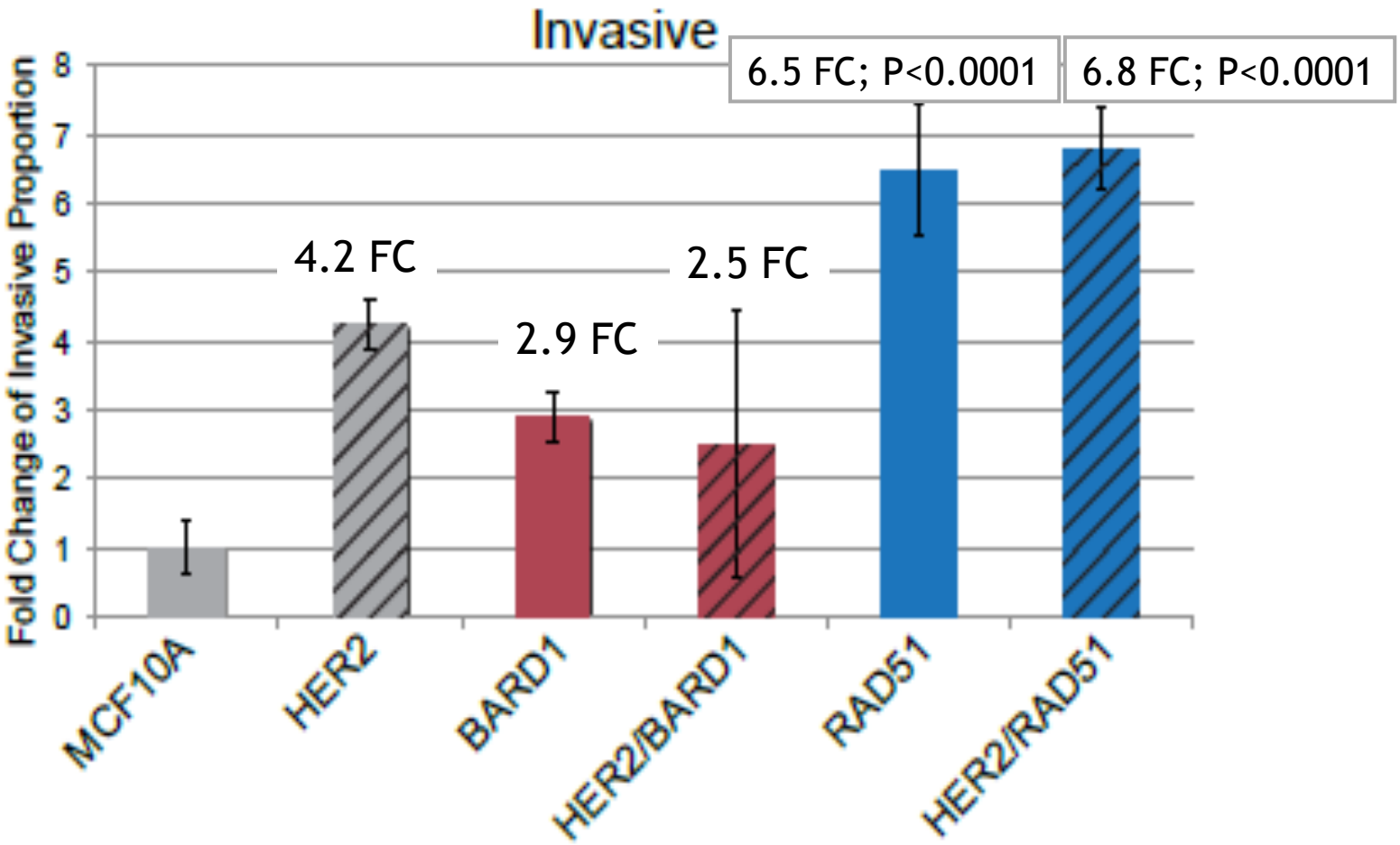




In three-dimensional culture, the transfectants developed disctinct morphologies:



RAD51 Overexpression in Immortal MCF-10A Cells Increased Invasion In Vitro



Fold change (FC) of % invasive acini growth for each transfected gene in MCF10A compared with MCF10A control transfectants

Conclusions

- 13-gene signature and ER-negativity predict rapid development of brain metastases in HER2-positive advanced breast cancer patients
- *RAD51* may promote aggressiveness in breast epithelial cells
- These data may be useful in the design of brain metastasis preventive trials and may prompt new treatment strategies

Acknowledgments

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