

“Mecanismos de invasión del cáncer de mama: Diferencias génicas CDIS vs CDI”



Siglo XX

Enrique Lerma
Hospital de la Santa Creu i Sant Pau
Barcelona.

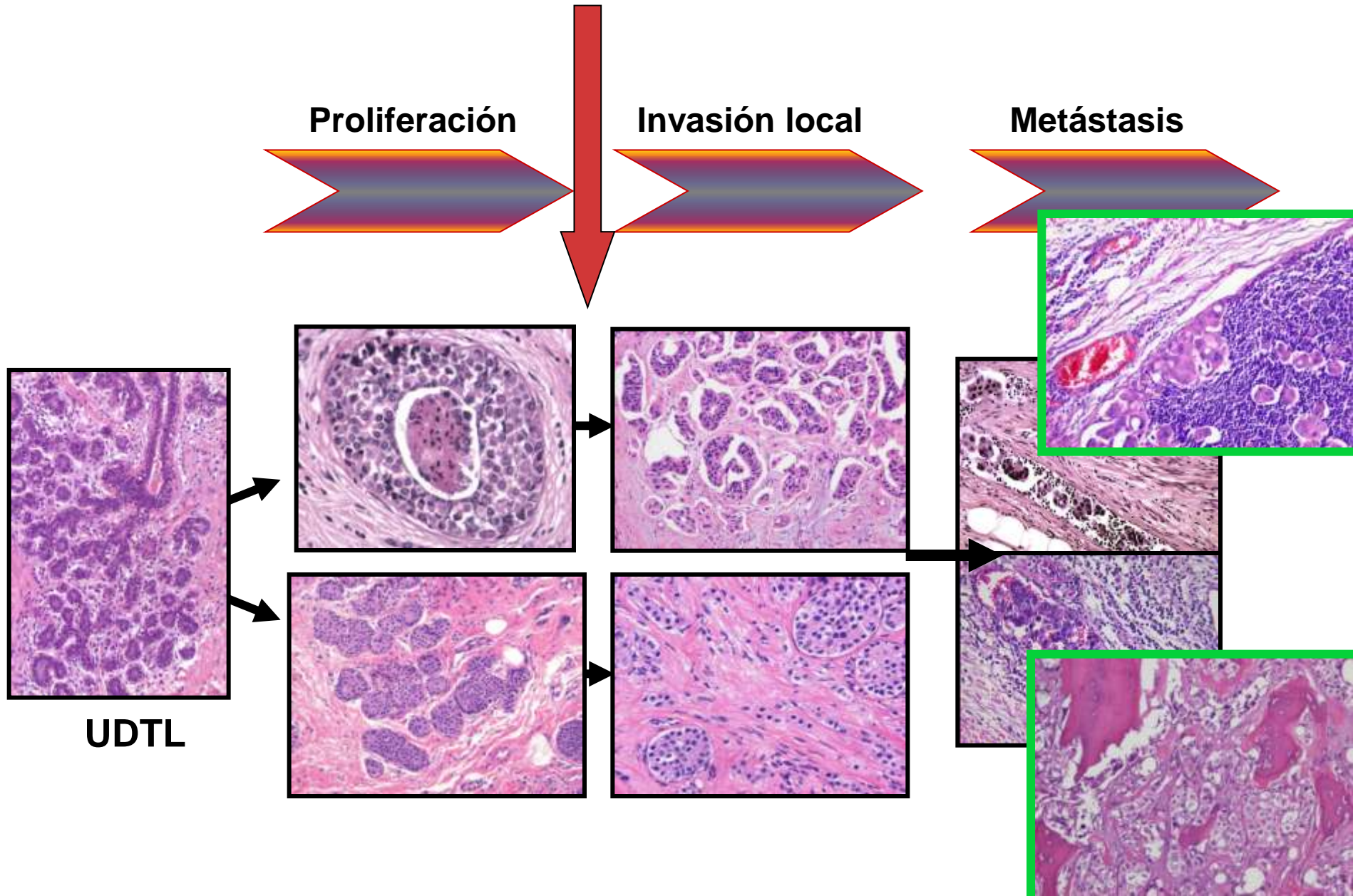


Siglo XXI

Índice

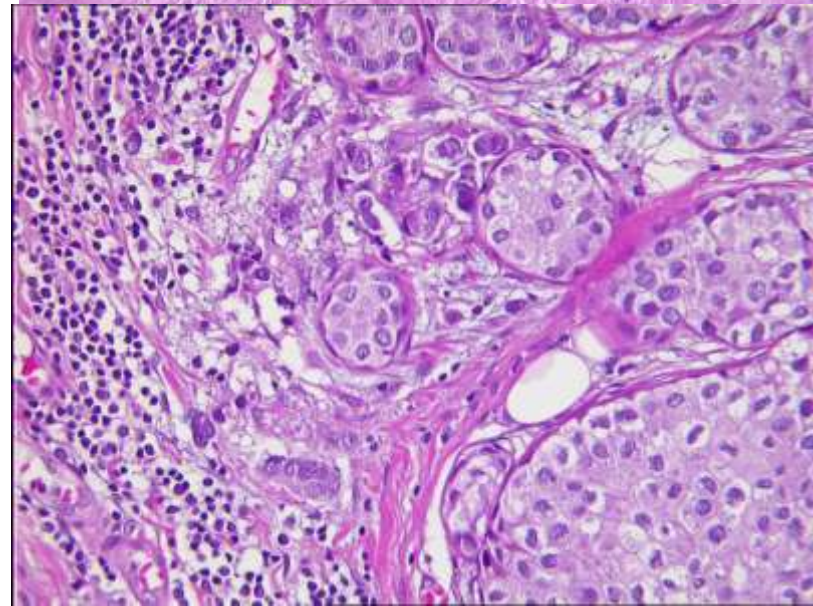
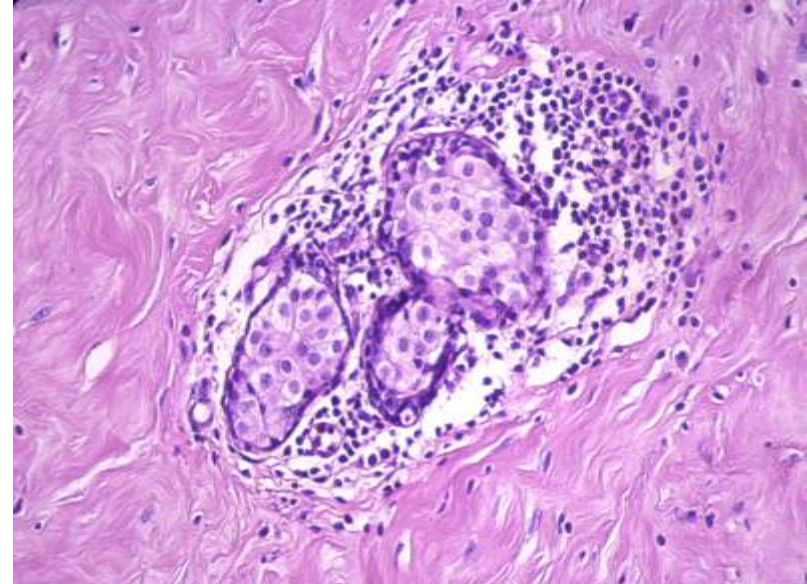
- Progresión del cáncer de mama
- Carcinoma ductal in situ. Significado de la microinvasión
- Factores relacionados con la invasión en CDI
- Diferentes mecanismos de invasión de CLI
- Conclusiones

Progresión neoplásica mamaria



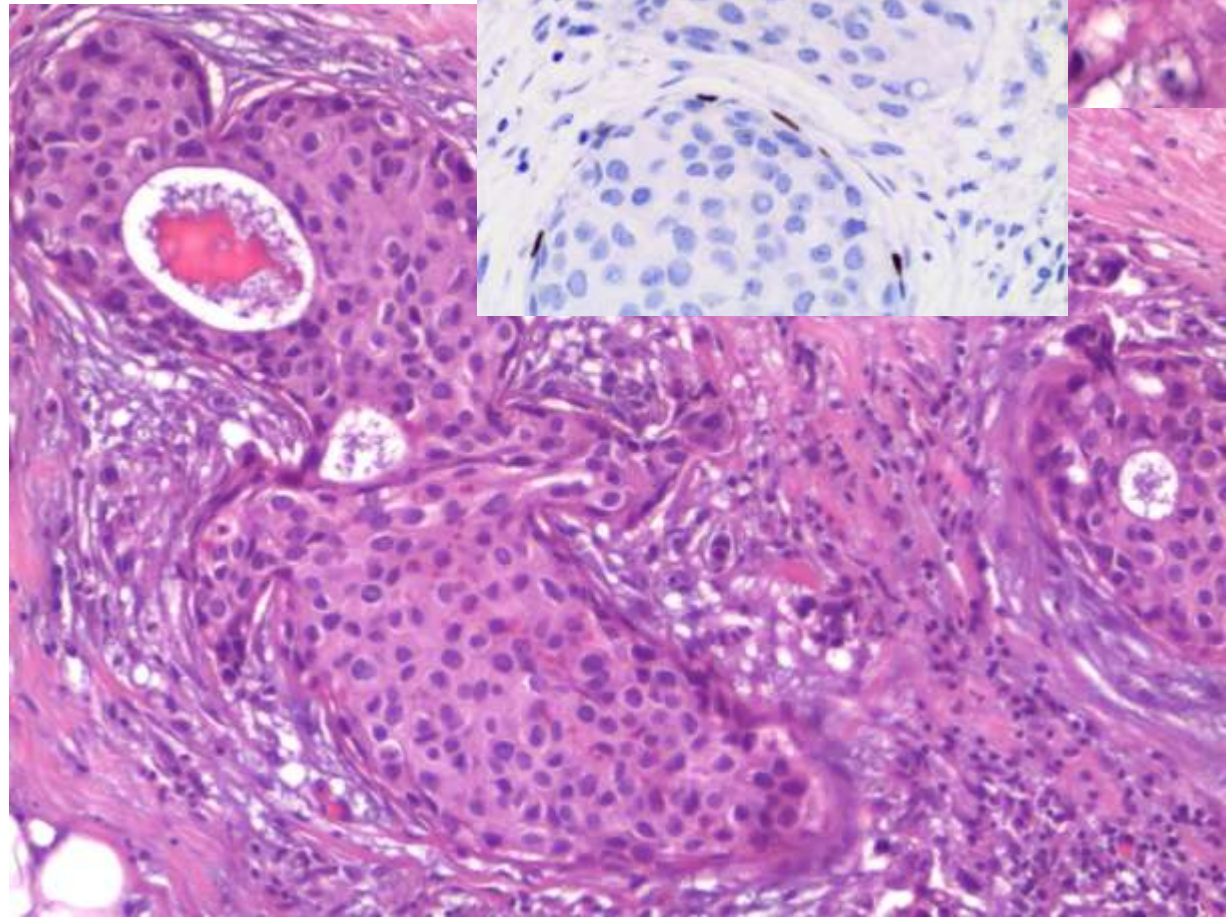
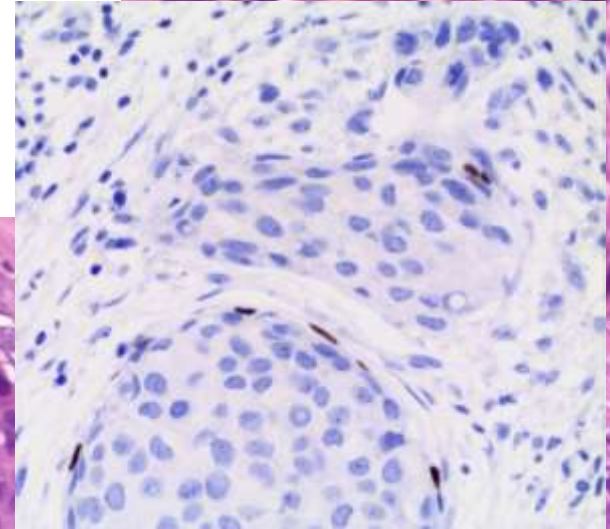
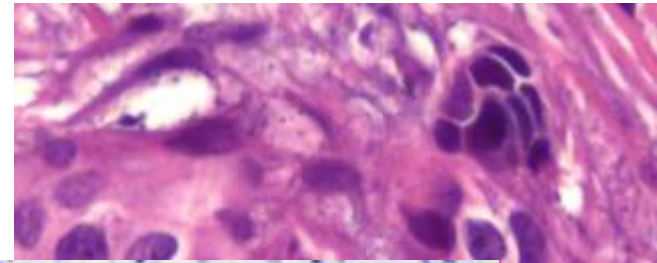
Carcinoma Ductal in Situ

- Menos del 40% de tumores tienen un patrón puro
- Invasión ganglionar <1%
- Recidivas: 75-80% en lecho tumoral
- Recidivas como ca invasor (50%)
- Metástasis ganglionares en recidivas: 15-20%
- Mastectomía: 0,7% recidivas.
- Radioterapia disminuye recidiva (7,5%)
- Microinvasión
- CDI con DCIS extenso



Microinvasión

- “CDIS con uno/varios focos <1mm.”
- Nidos o células sueltas
- Mayor frecuencia en:
 - comedo
 - T>25mm.
 - T multicéntricos

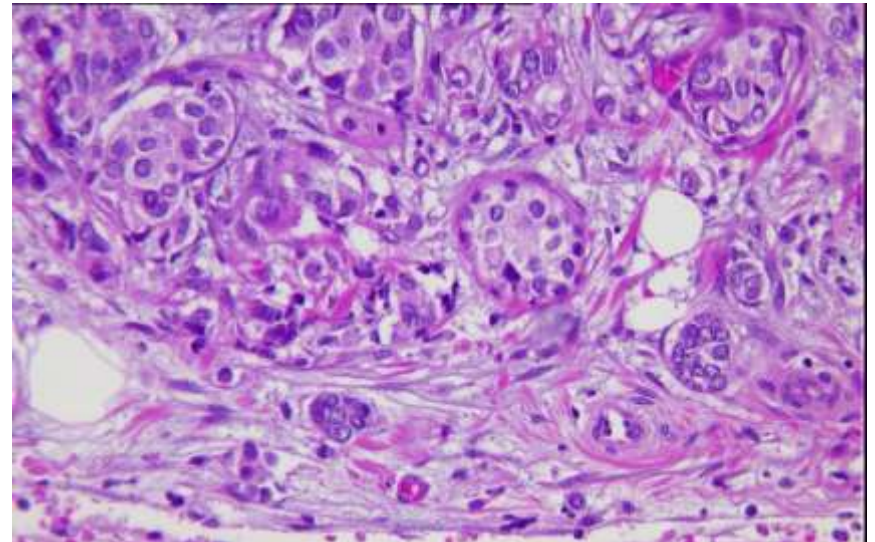
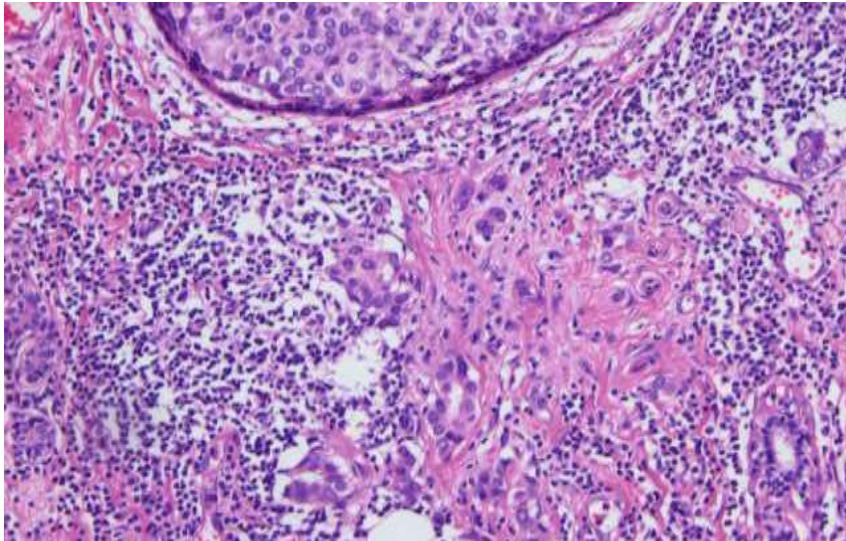


**Inmunohistoquímica
(Células mioepiteliales
y membrana basal):**
S-100, p63, actina,
miosina, colágeno IV,
CD10, laminína...

Ca Ductal con Microinvasión

(Mascarel et al. Cancer 2002; 94:2134)

	<u>Gang +</u>	<u>Mortalidad</u>
• Ca ductal in situ	•1,4%	1%
• CDM, células aisladas	•0%	•1,4%
• CDM, grupos de células (nidos o ductos)	•10%	•5,8%
• CD invasor con DCIS > 90%	•27,6%	•15,2%

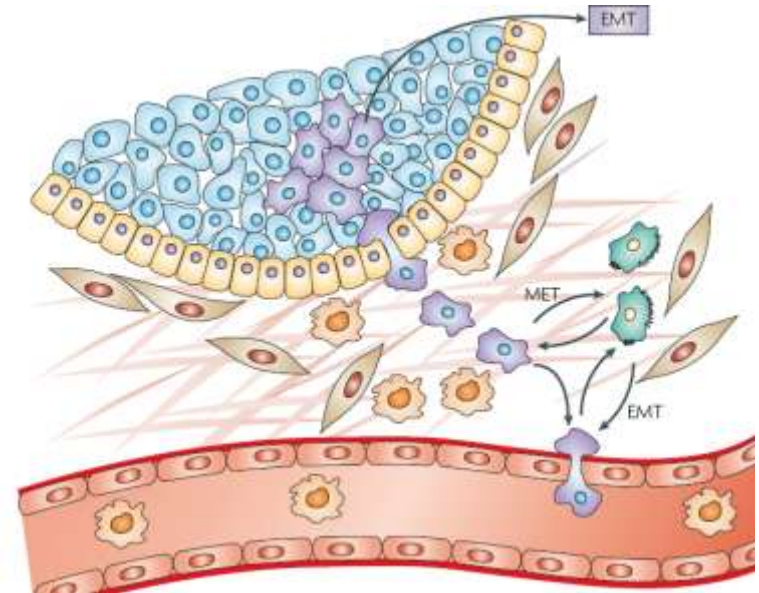


Factores relacionados con invasión en cáncer de mama

- Célula tumoral
 - Proliferación celular
 - Transición epitelio-mesénquima
- Pérdida de células mioepiteliales
 - “supresores de tumores”
- Microambiente
 - Fibroblastos
 - Células endoteliales
 - Reacción inflamatoria
 - Matriz extracelular (MMPs)
 - Interacciones enzimáticas

Invasión en cáncer de mama (célula tumoral)

- Mecanismos de proliferación....
- Chemoquinas, VEGF (paracrinos)
- **Transición epitelio-mesénquima****
 - (sin cambios fenotípicos)
 - Pérdida de Cadherina-E
 - Snail, Zeb, Twist, etc
 - TGF- β
 - Estadios iniciales: supresor tumoral
 - Facilita metástasis
 - Otros: Cripto-1, factores de transcripción (familia Six, Sim2), Wnt/ β -catenina,



Cheng CW, et al. Oncogene 2001;20: 3814–23.
Blanco MJ, et al. Oncogene 2002; 21:3241–6.
Moody SE, et al.. Cancer Cell 2005; 8: 197–209.
Elloul S, et al. Cancer 2005; 103:1631–1643.
Côme CH, et al. Clin Cancer Res 2006; 12:5395-5402.
Peinado H, et al. Nature review (Cancer) 2007;7:415-428.

Immunohistochemical heterogeneity of breast carcinomas negative for estrogen receptors, progesterone receptors and Her2/neu (basal-like breast carcinomas)

Esther Latorre¹, Gloria Pizarro¹, Susana Barreira¹, Susa Fernández¹, Daniel Martínez¹, Carolina Díaz¹, Fina Vázquez¹, Jorge M. Sobrido¹, Carmen Álvarez¹, Stefan Uppin¹, Javier Paez¹ and Agusti Barbañán¹

	Ductal (NOS) %	TN %
Basal genotype	15-20	60-90
Grade III	47	75-100
RE	75-80	0
RPg	57-60	0
HER2	15-20	0
Cytokeratin 5/6	21	60
EGFR IHC	20-40	70-80
EGFR amplif	6	25
P-53 >10%	22	56
Vimentin	15-19	88
SMA	14.5	47
s-100	3	69
C-kit	10	38-72
P63	0	25
P-cadherin	44	99
E-cad partial loss	18	65

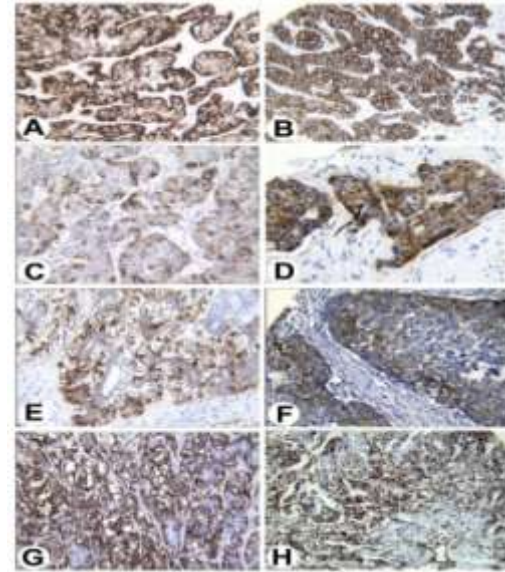
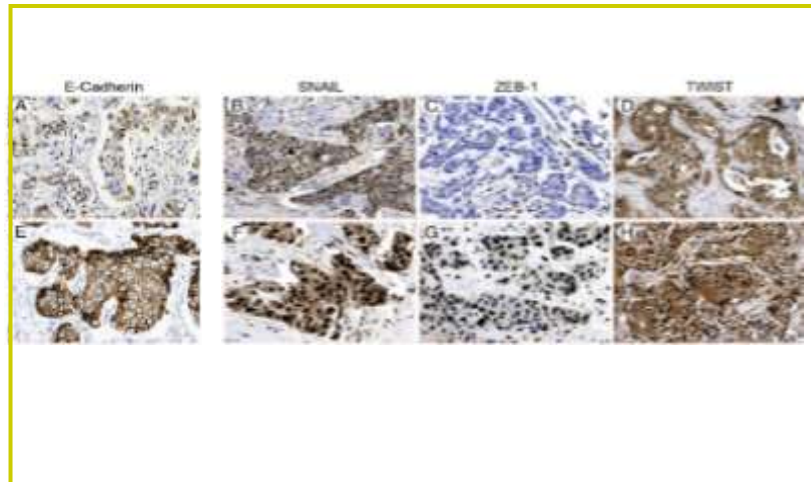
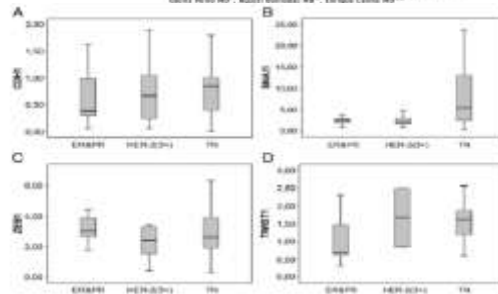


Figure 1

Repression of E-cadherin by SNAIL, ZEB1, and TWIST in invasive ductal carcinomas of the breast: a cooperative effort?

Walter Martínez Pardo^{1,2}, Silvana Salazar Pardo^{1,2}, Daniel Susa Pardo¹, Susa Salazar Pardo¹, Jorge Paez Pardo¹, Susana Barreira Pardo¹, Susa Fernández Pardo¹, Daniel Martínez Pardo¹, Carolina Díaz Pardo¹, Fina Vázquez Pardo¹, Estrella Barbañán Pardo¹, Javier Paez Pardo¹



Células mioepiteliales

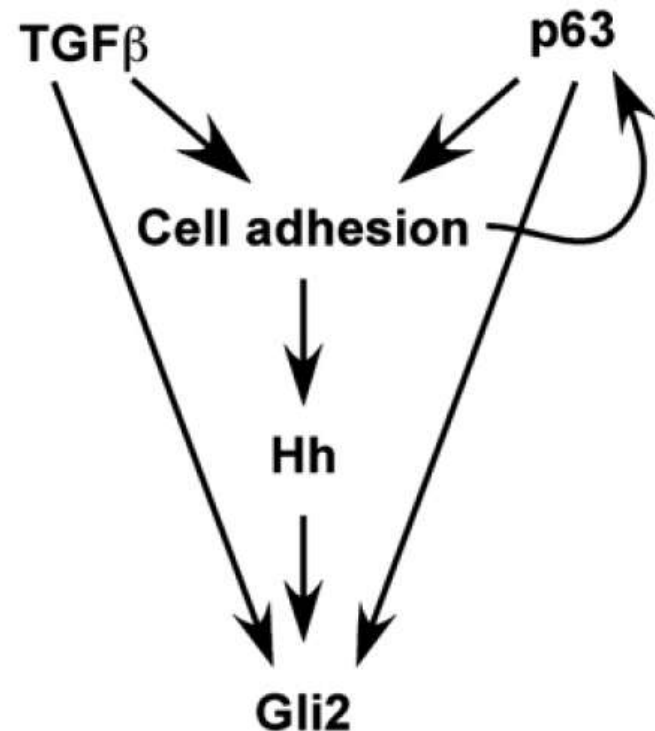
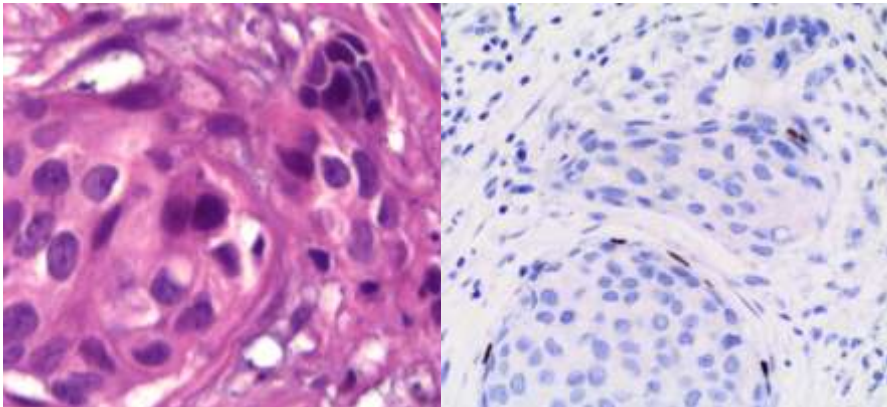
- “supresores de tumores”
- Interacción con fibroblastos y miofibroblastos

Regulation of In Situ to Invasive Breast Carcinoma Transition

Min-Hu,^{1,4} Jun Yao,^{1,4} Danielle K. Carroll,² Stanisława Winiarska,^{3,4} Haiyan Chen,^{5,6} Daniel Carrasco,¹ Andrea Richardson,^{1,4} Sheila Violette,³ Tatiana Nikolekaya,⁷ Yuri Nikolov,⁷ Erica L. Sauerlein,^{1,4} William C. Hahn,^{1,4} Rebecca S. Gelman,^{2,4} Craig Allred,⁴ Mira J. Bissell,^{1,6} Stuart Schmitz,^{5,6} and Komola Polyak^{1,4}

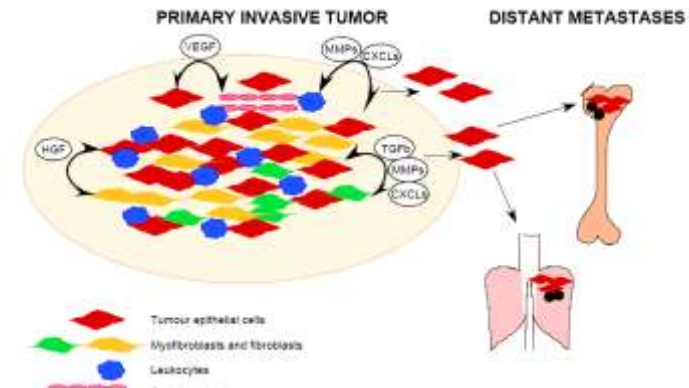
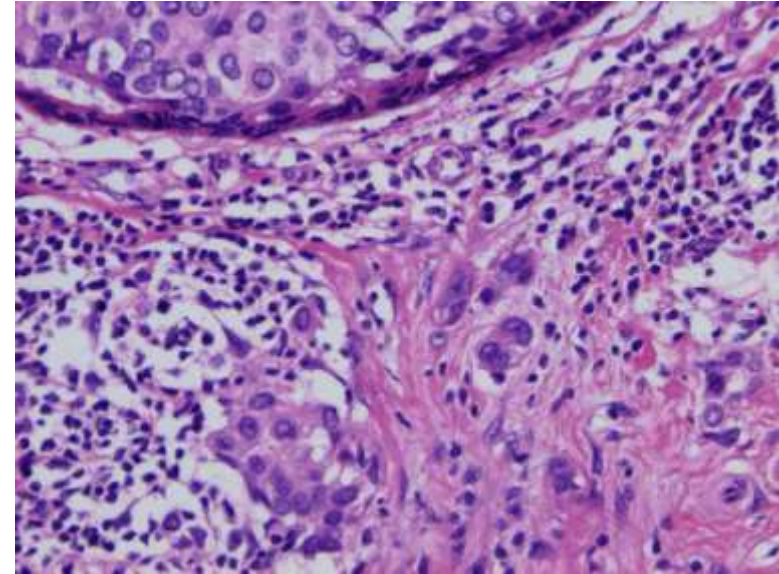
SUMMARY

The transition of ductal carcinoma in situ (DCIS) to invasive carcinoma is a poorly understood key event in breast tumor progression. Here, we analyzed the role of myoepithelial cells and fibroblasts in the progression of in situ carcinomas using a model of human DCIS and primary breast tumors. Progression to invasion was promoted by fibroblasts and inhibited by normal myoepithelial cells. Molecular profiles of isolated luminal epithelial and myoepithelial cells identified an intricate interaction network involving TGF β , Hedgehog, cell adhesion, and p63 required for myoepithelial cell differentiation, the elimination of which resulted in loss of myoepithelial cells and progression to invasion.



Microambiente en cáncer de mama

- Fibroblastos
 - Alteraciones genéticas mal definidas
 - Alteraciones epigenéticas: Sobre expresión de **CXCL12 y CXCL14**
- Células endoteliales
 - VEGF tumoral
- Reacción inflamatoria
 - Cox2 (otras neoplasias)
 - AINEs reducen prostaglandinas y riesgo de cáncer
 - Producción de quemoquinas, y CXCL5 y CXCL12 por células tumorales
 - **Inhibición de TGF- β en fibroblastos aumenta producción de TGF- α , MSP y HGF.**
 - Macrófagos facilitan invasión
 - Células NK : aumento de MMP
- Matriz extracelular (MMPs)
 - Facilitan invasión tumoral.

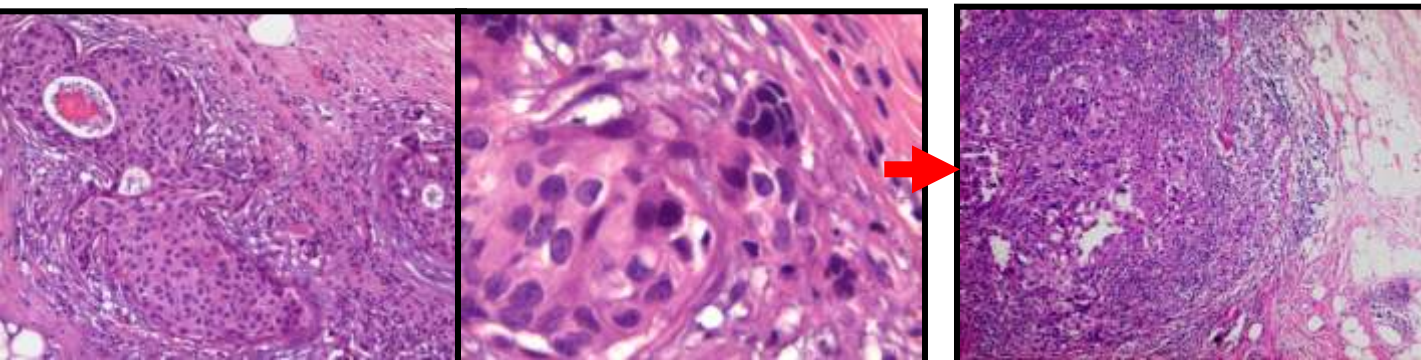
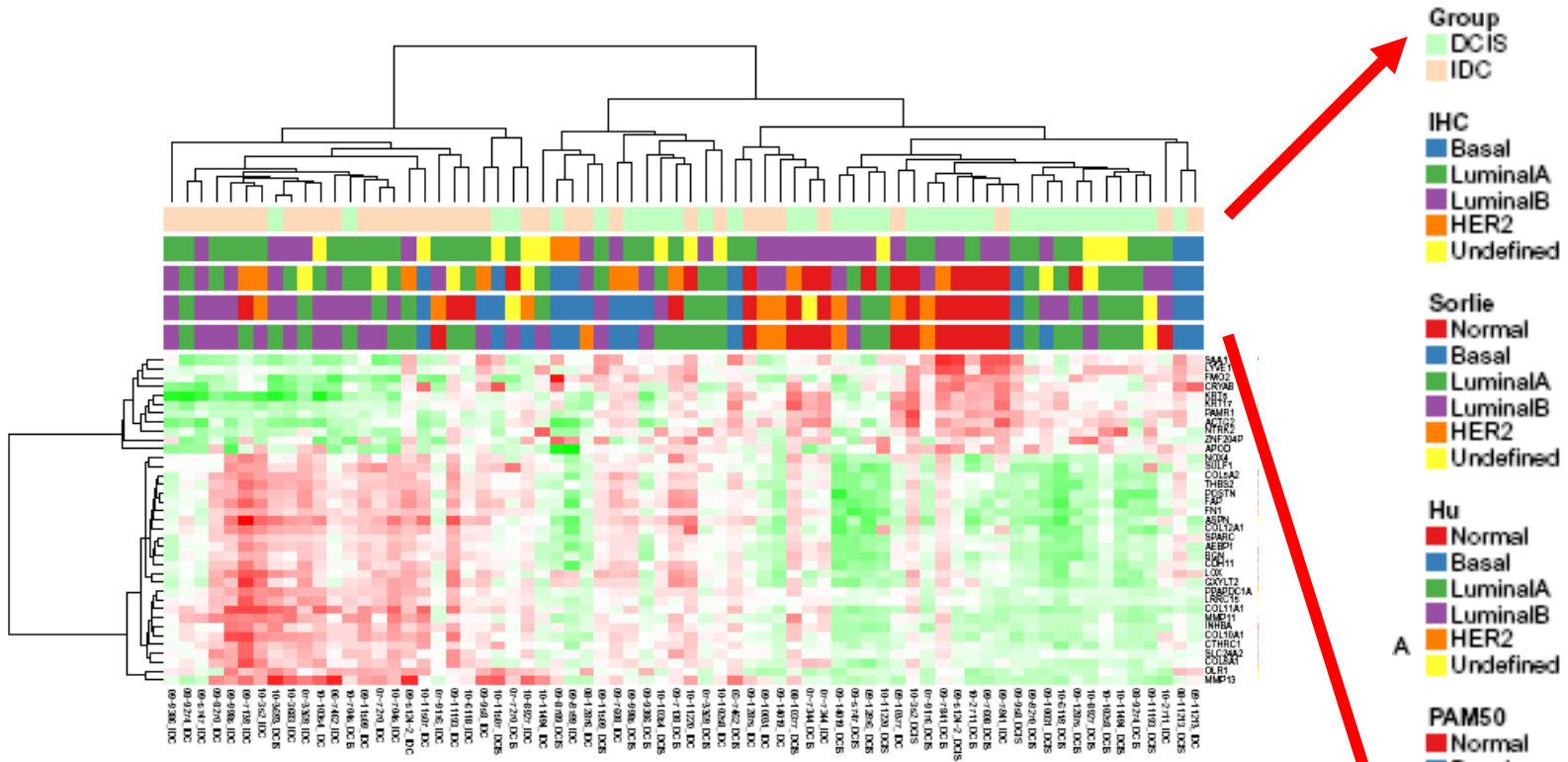


Clasificación molecular de 35 casos con CDI y CDIS sincrónicos

	Sorlie et al, 2003	Hu et al, 2006	Parker et al, 2009
Concordantes	18	18	19
Discordantes	12	15	15
No definidos	5	3	1

Clasificación basada en 3 SSP (single sample predictor) de **centroides** de los subtipos moleculares

Marcadores de invasión mediante microarrays de ARN



Sobre-expresión de genes de TEM e interacción con matriz extracelular en CDI



EM interaction and focal adhesion EMT

MMP11, MMP14

FN1

THBS2

SPARC

PLAUR, PLAU

ASPN

BGN

GPC6

Cell to cell signaling cell adhesion

GJB2

Cell growth

ST6GAL2

INHBA



Cell adhesion

TNXA, TNXB

F8

Cell motility

SERPINB5

ENPP2

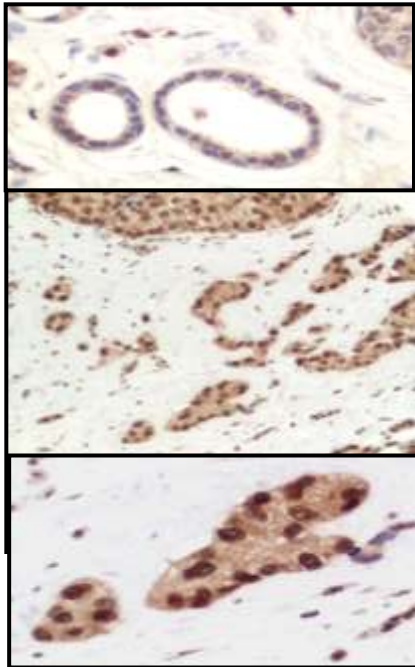
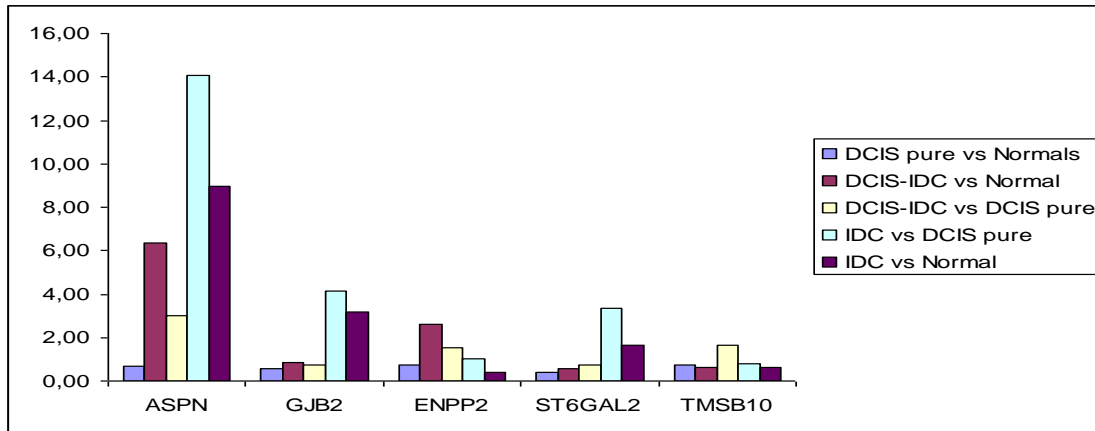
Intermediaty filament proteins

KRT5

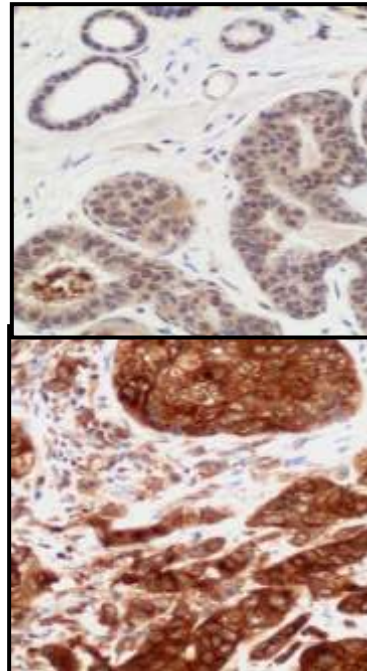
Signal transducing

RHOU

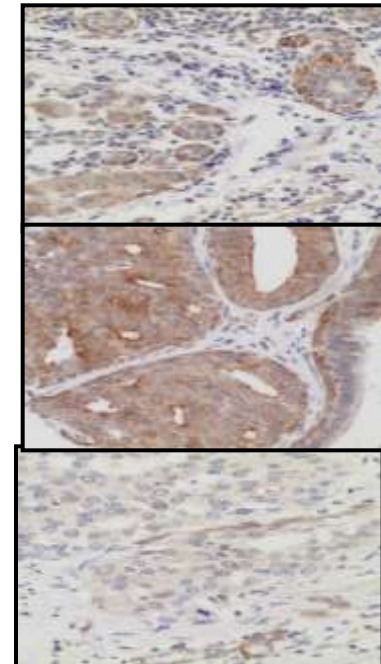
ENPP2



Asporina

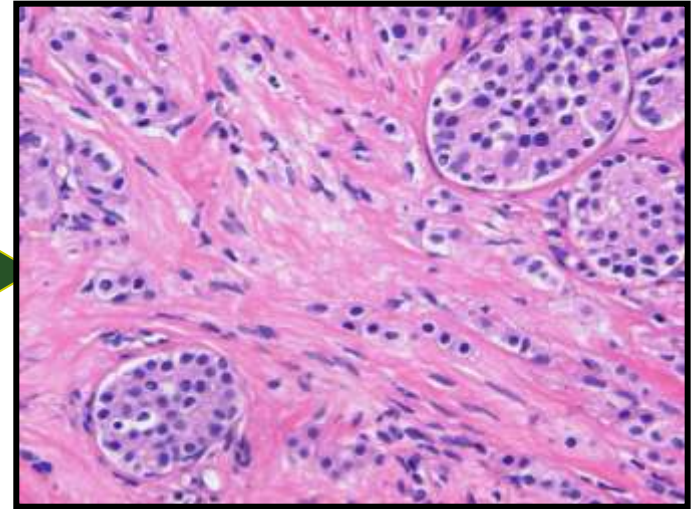
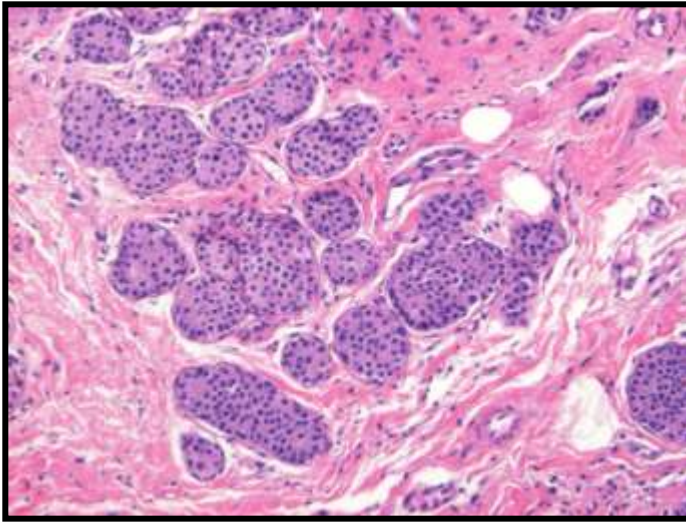


GJB2



ST6GAL2

10 CLI vs 20 CDI



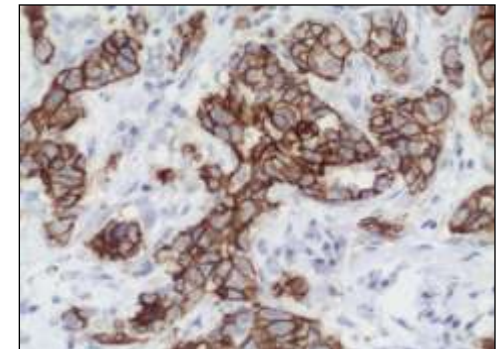
**253 genes intrinsicos
en CM**

PLEKHA7 and PRDX4

SERPINB5 and TMSB10

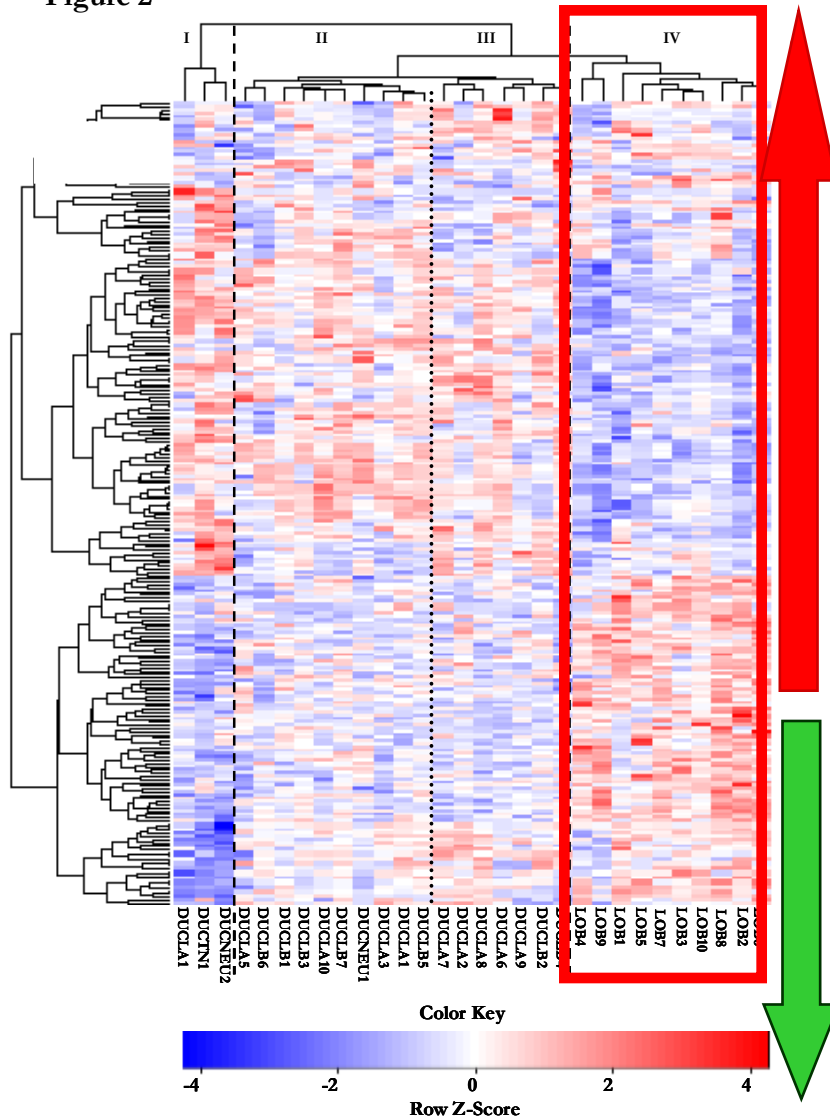
Castellana B et al. (In press)

Pérdida de E-cadherina



Expresión génica diferencial entre CLI y CDI

Figure 2



Cell migration associated genes (*FUT8*, *CRIPAK*)

Actin, calcium and ion binding and transport (*SHOORM1*, *MARCH8*)

Regulation of transcription (*FOXP1*, *DACH1*, *NBPF4*)

Signal transducing (*PER*, *TSC2*, *GARNL3*)

Lipid/fatty acid transport and metabolism (*PLA2G4B*, *PNPLA7*)

Protein binding (*NOSTRIN*, *PLEKHA7*)

Modification-dependent protein catabolic processes (*UBA7*, *CSAD*)

Cytoskeleton reorganization (*TMSB10*)

ATP metabolism (*ATP5I*, *ATP5J2*)

Cell adhesion (*CASK*, *ANXA2P1*)

Cell proliferation (*AREG*)

Regulation of translation (*RPL19*, *RPS27*)

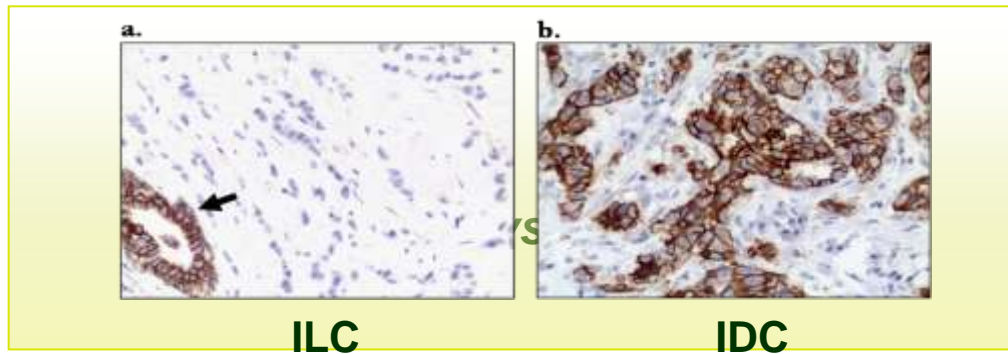
Oxidoreductase activity (*PRDX4*, *TMX2*)

Validacion por qPCR de microarray

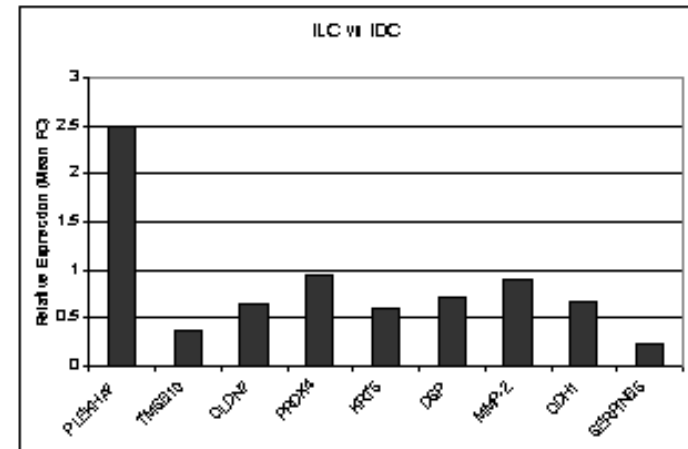
Table 4. Q-PCR findings in invasive lobular carcinomas (ILCs) versus invasive ductal carcinomas (IDCs). # of cases and mean values

Gene Symbol (Gene name)	ILC vs IDC		ILC vs. IDC ER+/PR+		ILC vs. IDC (ER+/PR+ HER2+)	
	Mean FC	<i>P</i> value	Mean Fold Change	<i>P</i> value	Mean Fold Change	<i>P</i> value
PLEKHA7	2,497	0,002	4,529	0,000	2,676	0,015
TMSB10 (Thymosin beta 10)	0,366	0,000	1,976	0,037	0,590	0,040
CLDN7 (Claudin 7)	0,641	N.S.	0,601	N.S.	1,048	N.S.
PRDX4 (Peroxisredoxin 4)	0,948	0,049	0,516	N.S.	1,338	0,035
KRT5 (Keratin 5)	0,595	0,012	0,233	N.S.	0,500	N.S.
DSP (Desmoplakin)	0,704	N.S.	1,153	N.S.	0,531	N.S.
MMP-2 (Metalloproteinase 2)	0,887	N.S.	1,097	N.S.	0,778	N.S.
CDH1 (E-cadherin)	0,676	N.S.	0,469	N.S.	0,503	N.S.
SERPINB5 (Maspin)	0,233	0,001	0,484	N.S.	0,352	0,046

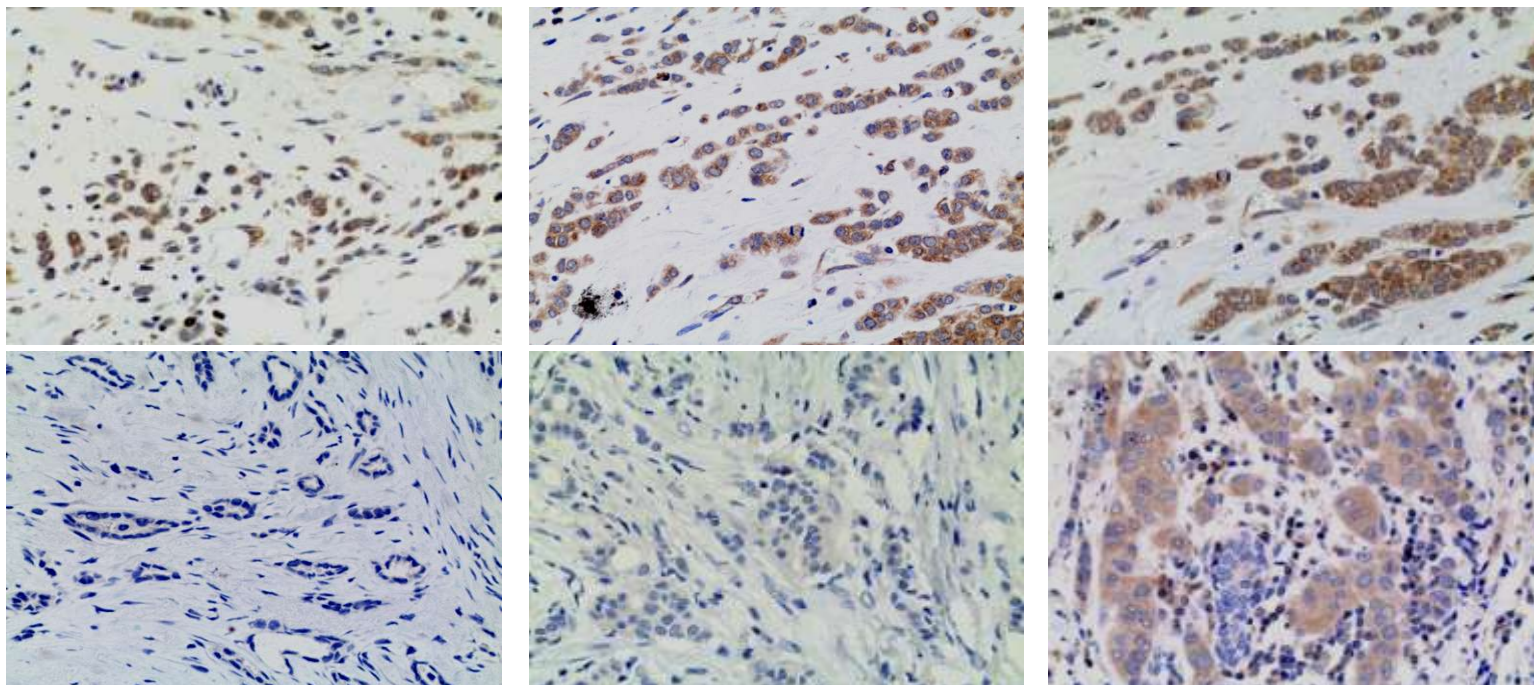
Abbreviations: N.S., non-significant



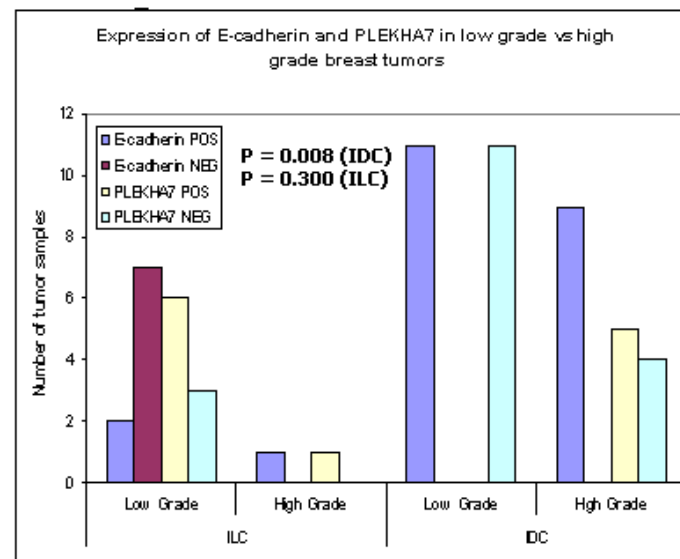
E-Cadherina



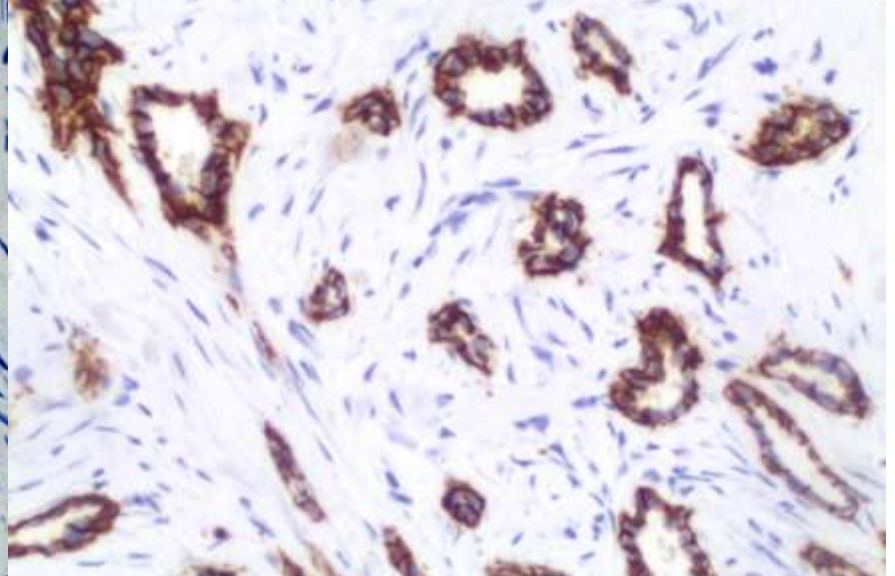
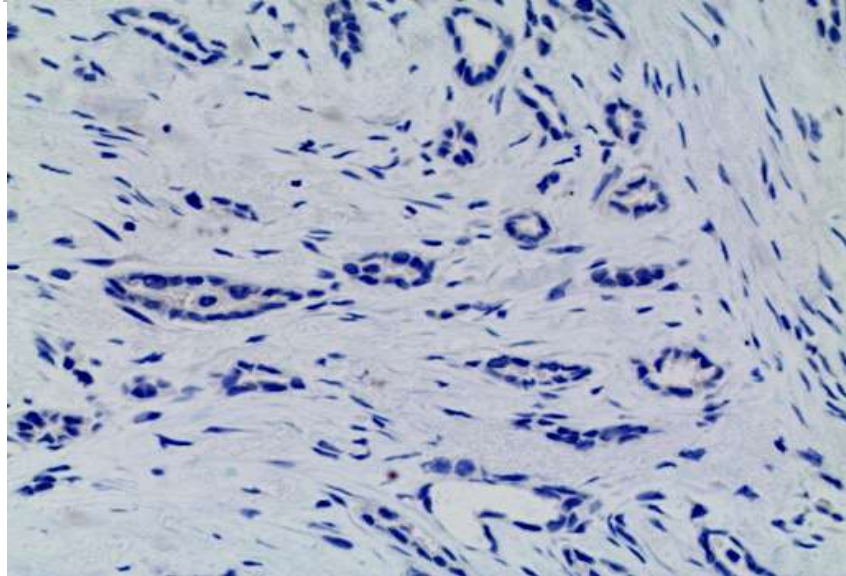
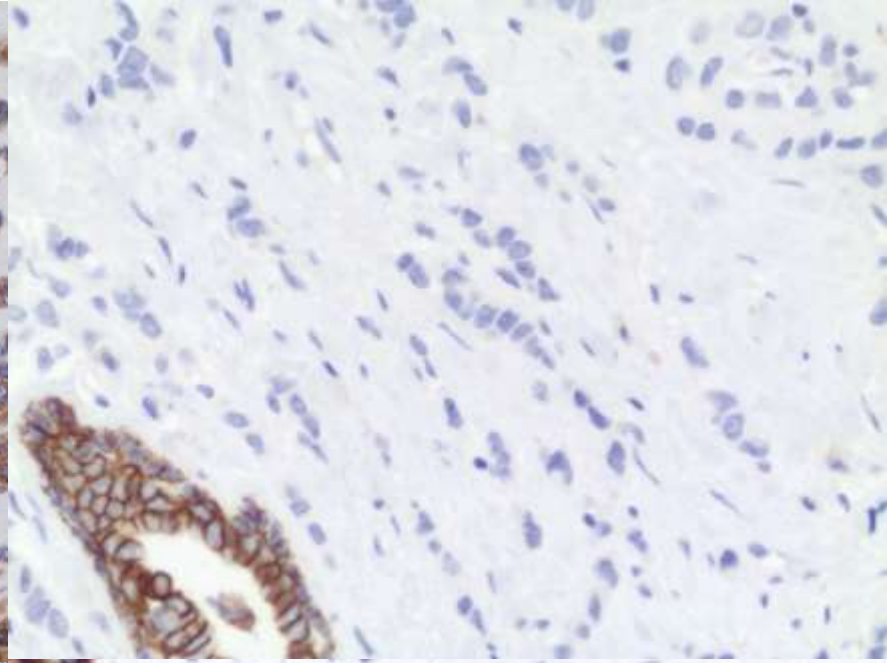
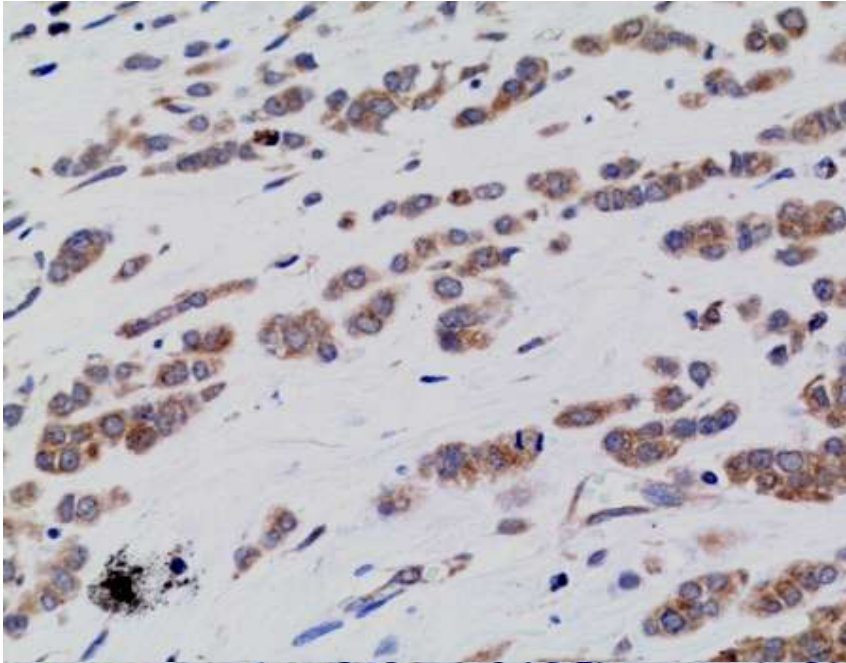
La expresión de PLEKHA7 es inversa a la de E-Cadherina



		N	E-cadherin		PLEKHA7	
			POS	NEG	POS	NEG
ILC	Low Grade	9	2	7	6	3
	High Grade	1	1	0	1	0
IDC	Low Grade	11	11	0	0	11
	High Grade	9	9	0	5	4

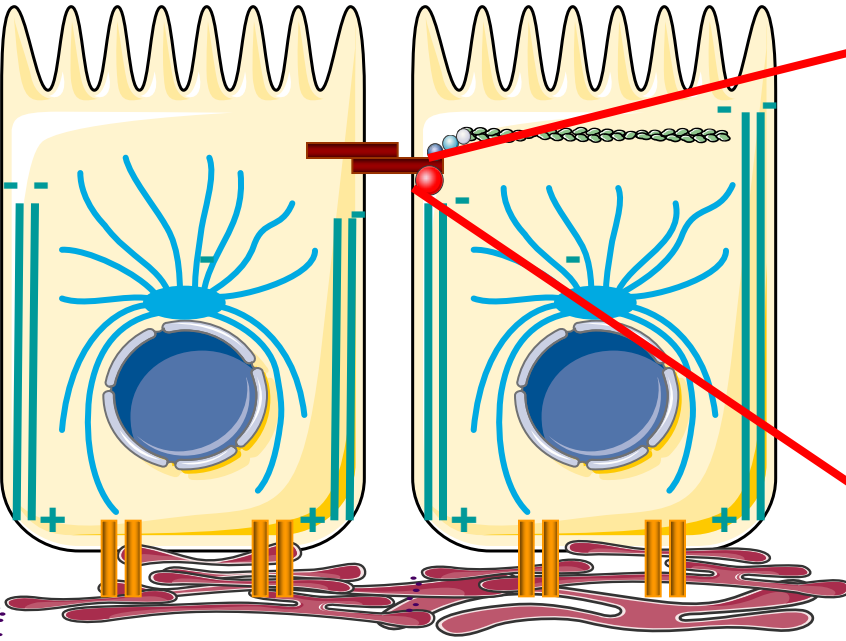


La expresión de PLEKHA7 es inversa a la de E-Cadherina

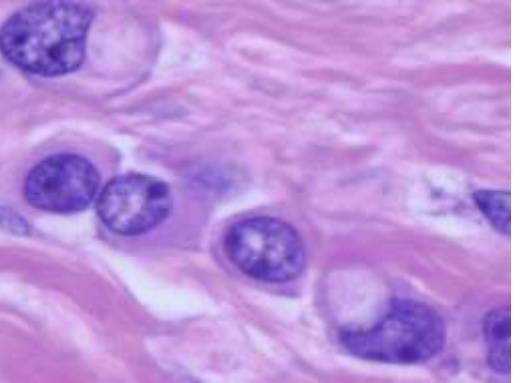
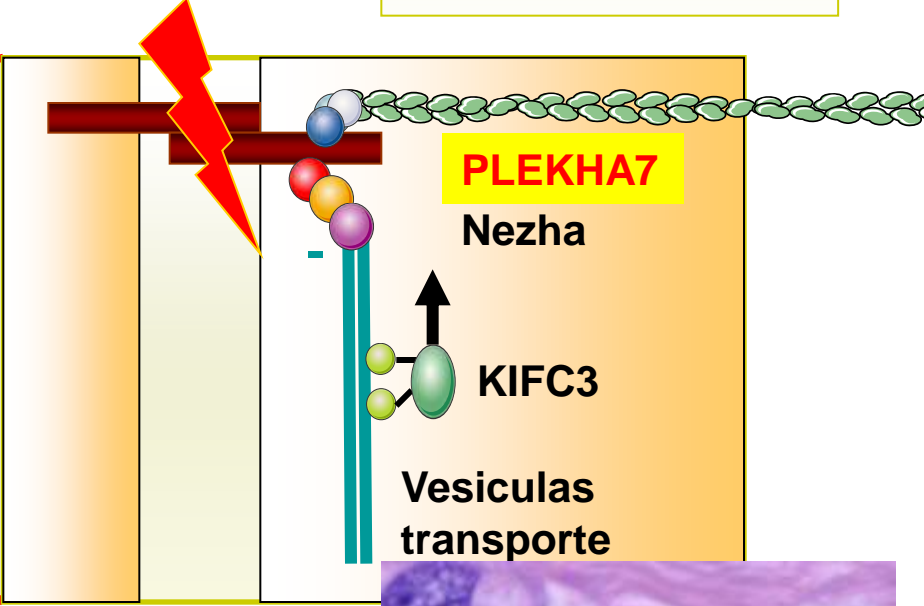


PLEKHA7 y E-Cadherina tienen un patrón de tinción inverso

- EPLIN
- α Catenin
- β Catenin
- P120



Extracellular matrix



Conclusiones

- La invasión del cáncer de mama es un proceso muy complejo, dinámico y difícil de estudiar en el participan:
- Células tumorales
 - ASPN, GJB2 y ST6GAL2
 - Transición epitelio-mesénquima: Cadherina E y PLEKHA7
- Pérdida de células mioepiteliales
 - “supresores de tumores” ?
- Microambiente
 - Fibroblastos, células endoteliales y reacción inflamatoria



Muchas gracias