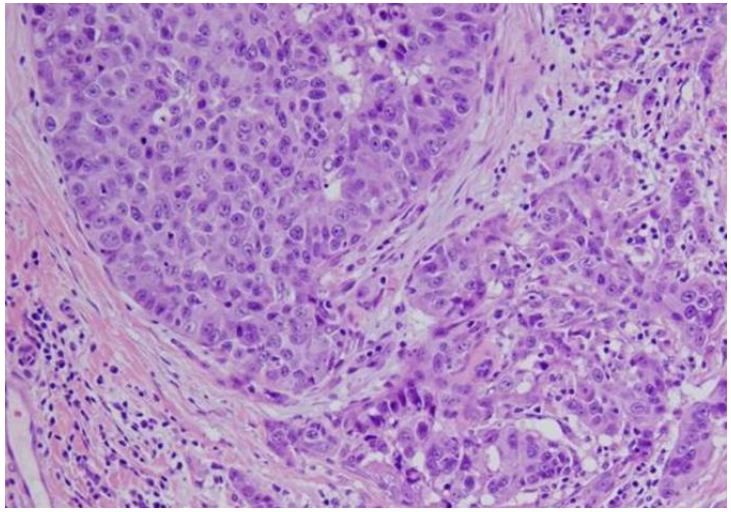
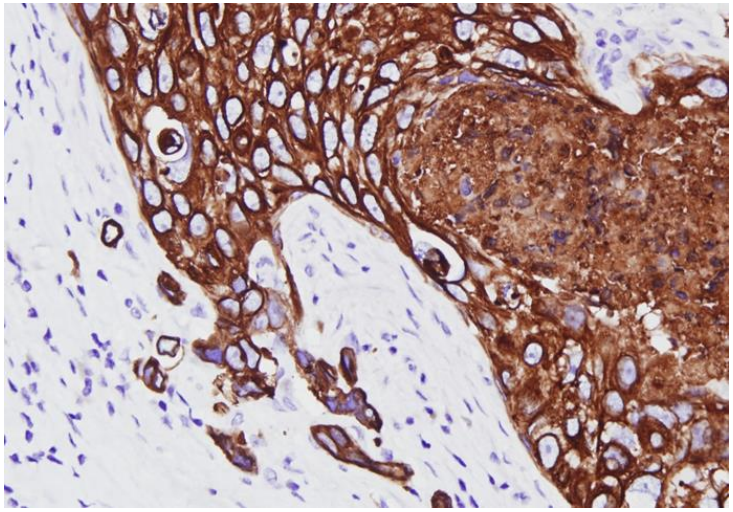
Molecular and Pathological Changes During Progression from in situ to Invasive Cancer





So Yeon Park, M.D., Ph.D.

Department of Pathology

Seoul National University College of Medicine

Seoul National University Bundang Hospital

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- ***Pathologic characteristics of ductal carcinoma in situ***
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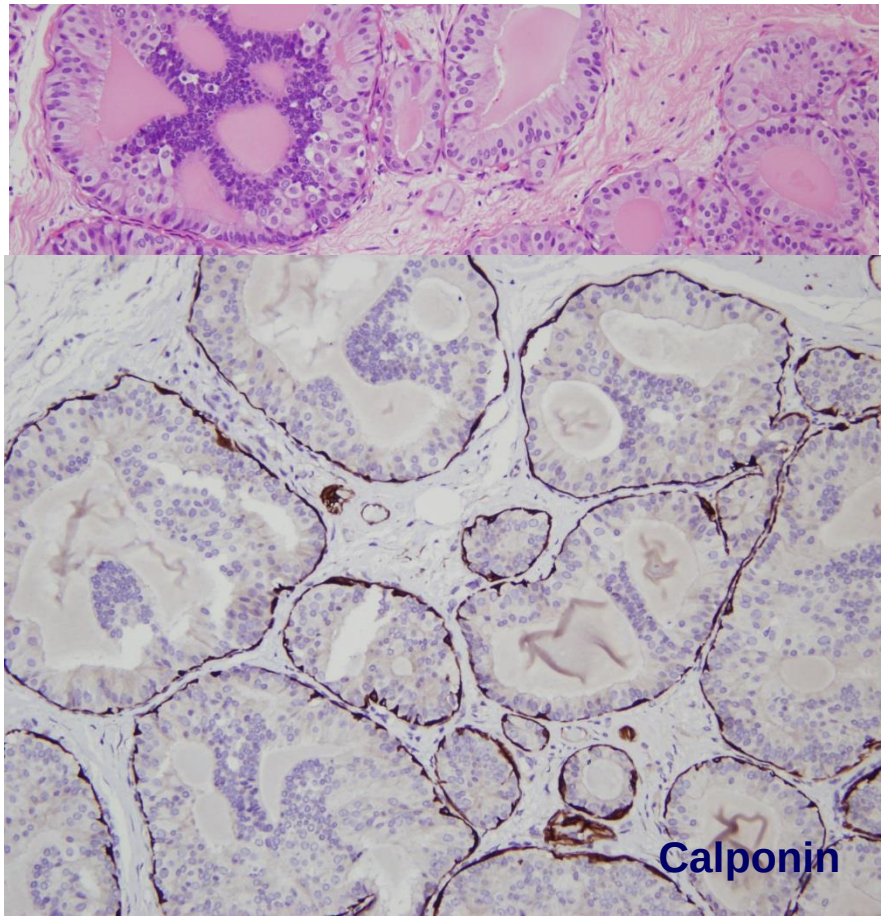
Ductal carcinoma in situ

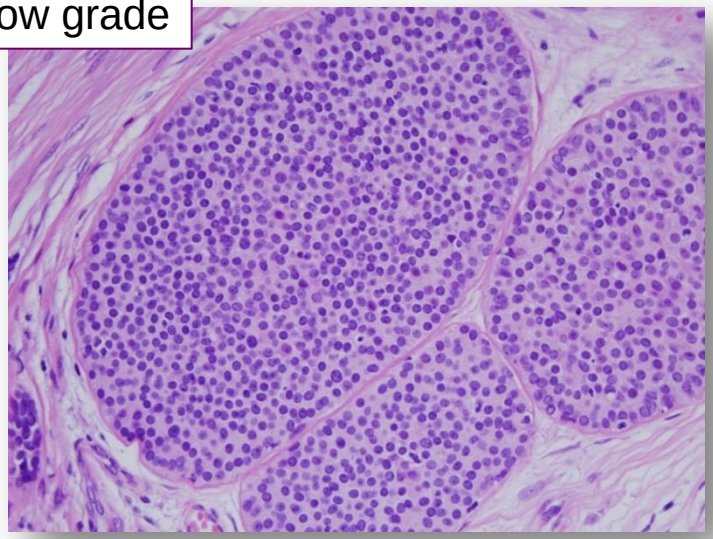


- DCIS is an early pathologic stage of breast cancer characterized by proliferation of tumor cells within the ductal-lobular system.

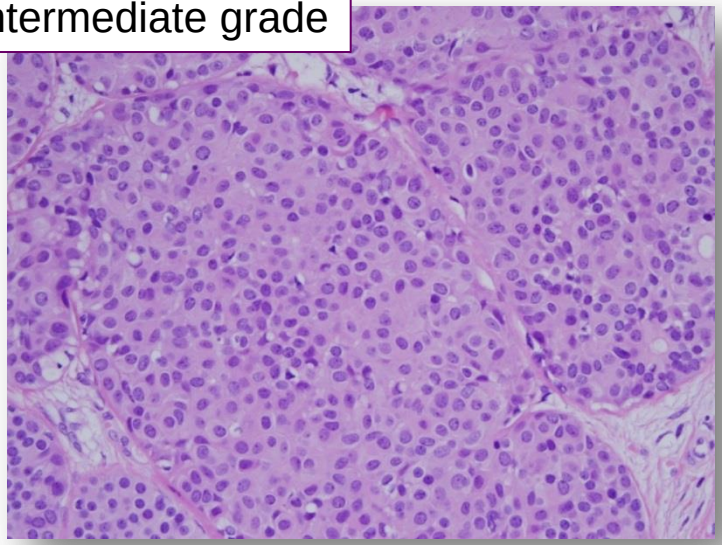
- It is surrounded by a continuous myoepithelial cell layer and a basement membrane, not extending through them.

Histologic diversity of ductal carcinoma in situ

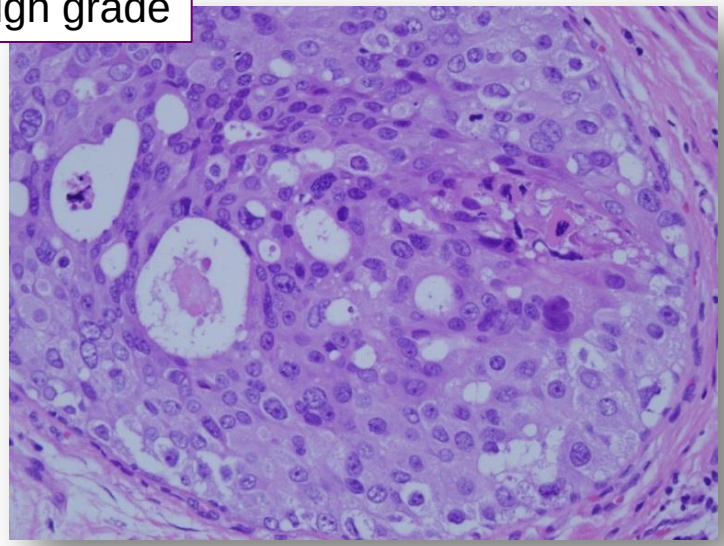
Low grade



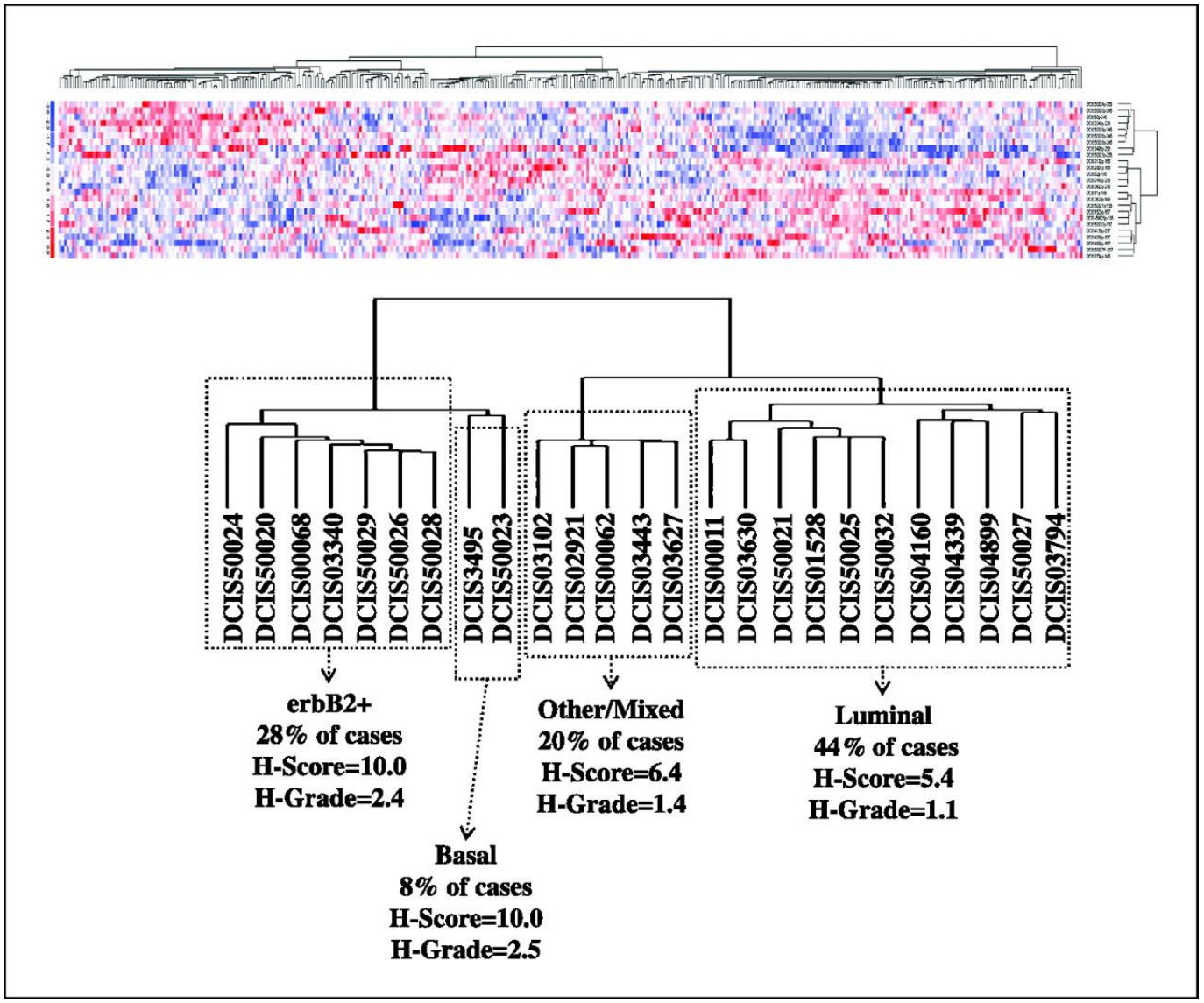
Intermediate grade



High grade



Intrinsic subtypes in ductal carcinoma in situ



Allred et al. Clin Cancer Res 2008;14:370-378

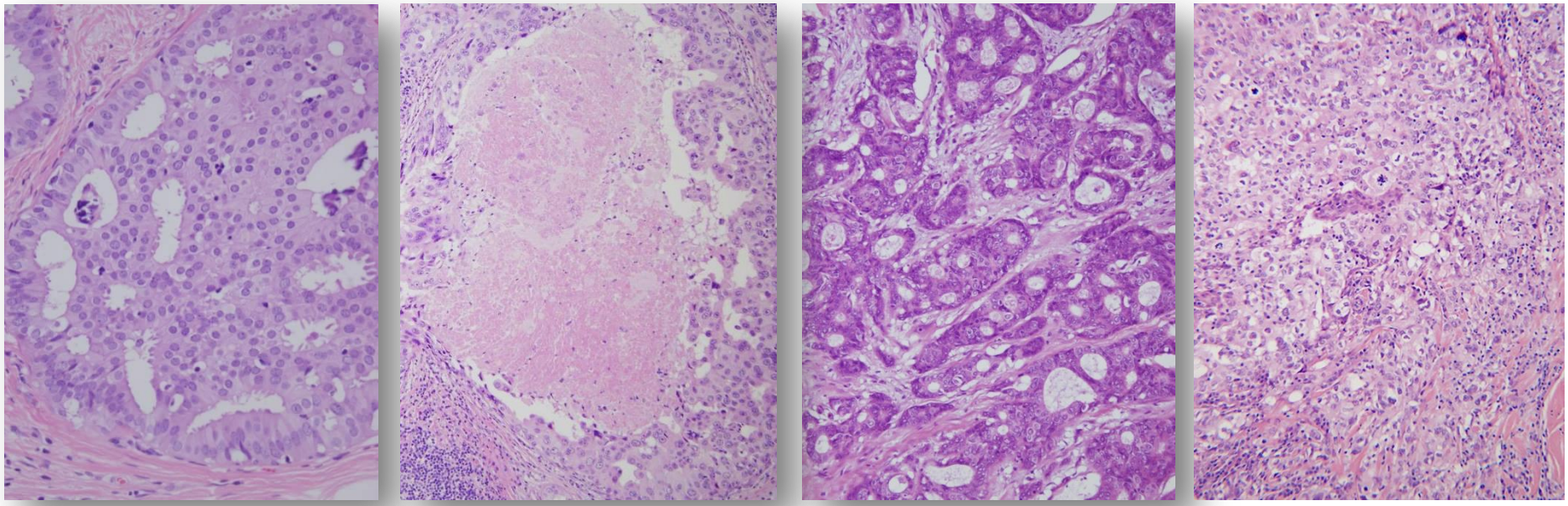
Natural history of DCIS

Type of evidence	Conclusions	Limitations
Studies of DCIS initially misdiagnosed as benign lesions	Suggest 14~53% may progress to invasive cancer over 10-15 years	• Higher grade lesions less likely to have been misdiagnosed. • Follow-up likely to be more complete for women subsequently diagnosed with cancer.
Recurrence of DCIS as invasive cancer	Overall recurrence rate between 1.5% and 22.5%	• May not reflect situation in absence of surgery. • Recurrence strongly depends on excision margins.
Autopsy studies	Larger reservoir of undetected DCIS in the population, thus not all DCIS progress to invasive cancer.	• Modelling predicts such a reservoir would be expected due to differing growth rated of tumors.
Epidemiology	Risk factors similar between DCIS and invasive cancer	• Does not give estimate of progression rates, only that DCIS is likely to be a precursor for invasive cancer.

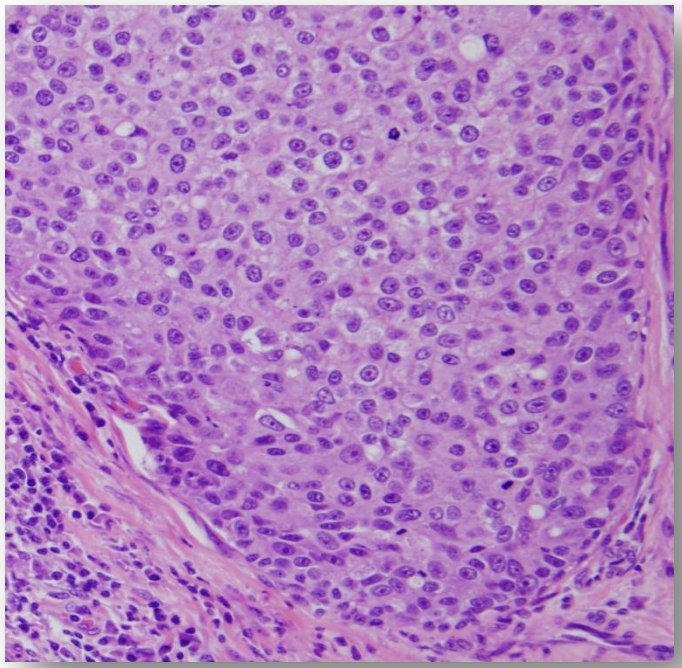
DCIS is a non-obligate precursor of invasive breast caner

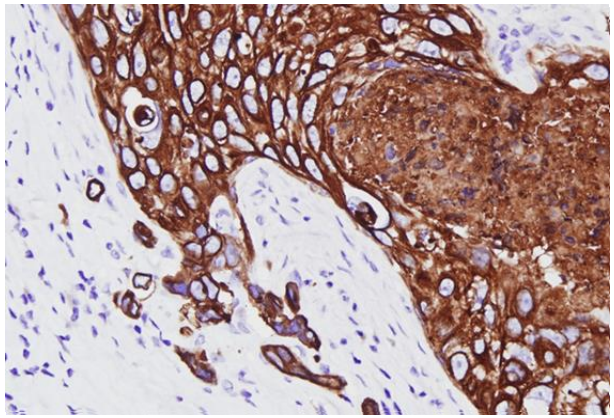
Erbas B et al., Breast Cancer Res Treat 2006;97:135-144

Ductal carcinoma in situ vs. Invasive carcinoma

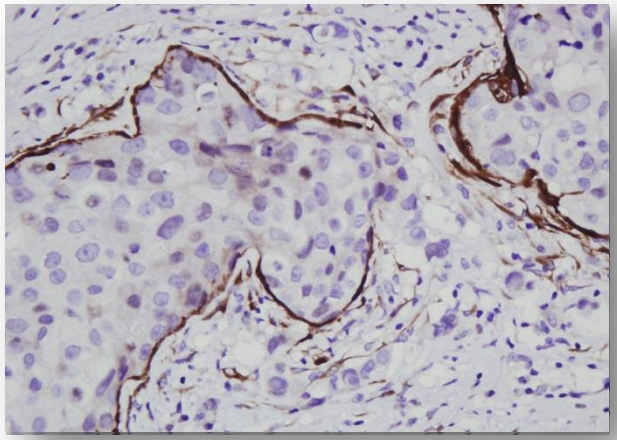


Ductal carcinoma in situ	Invasive carcinoma
Round configuration	Irregular and angulated configuration
Pushing border	Invasive growth
Presence of myoepithelial cells	***Absence of myoepithelial cells***
Rare reactive fibroblastic stroma *(except for high grade DCIS)*	***Reactive fibroblastic stroma***
Rare inflammatory cell infiltration *(except for high grade DCIS)*	***Variable inflammatory cell infiltration***

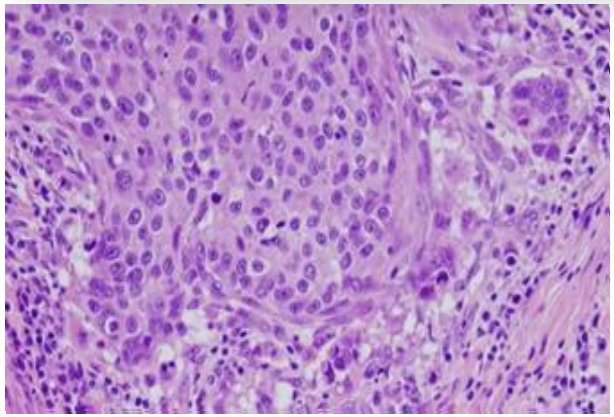




Invasive property of tumor cells



Loss of myoepithelial cells



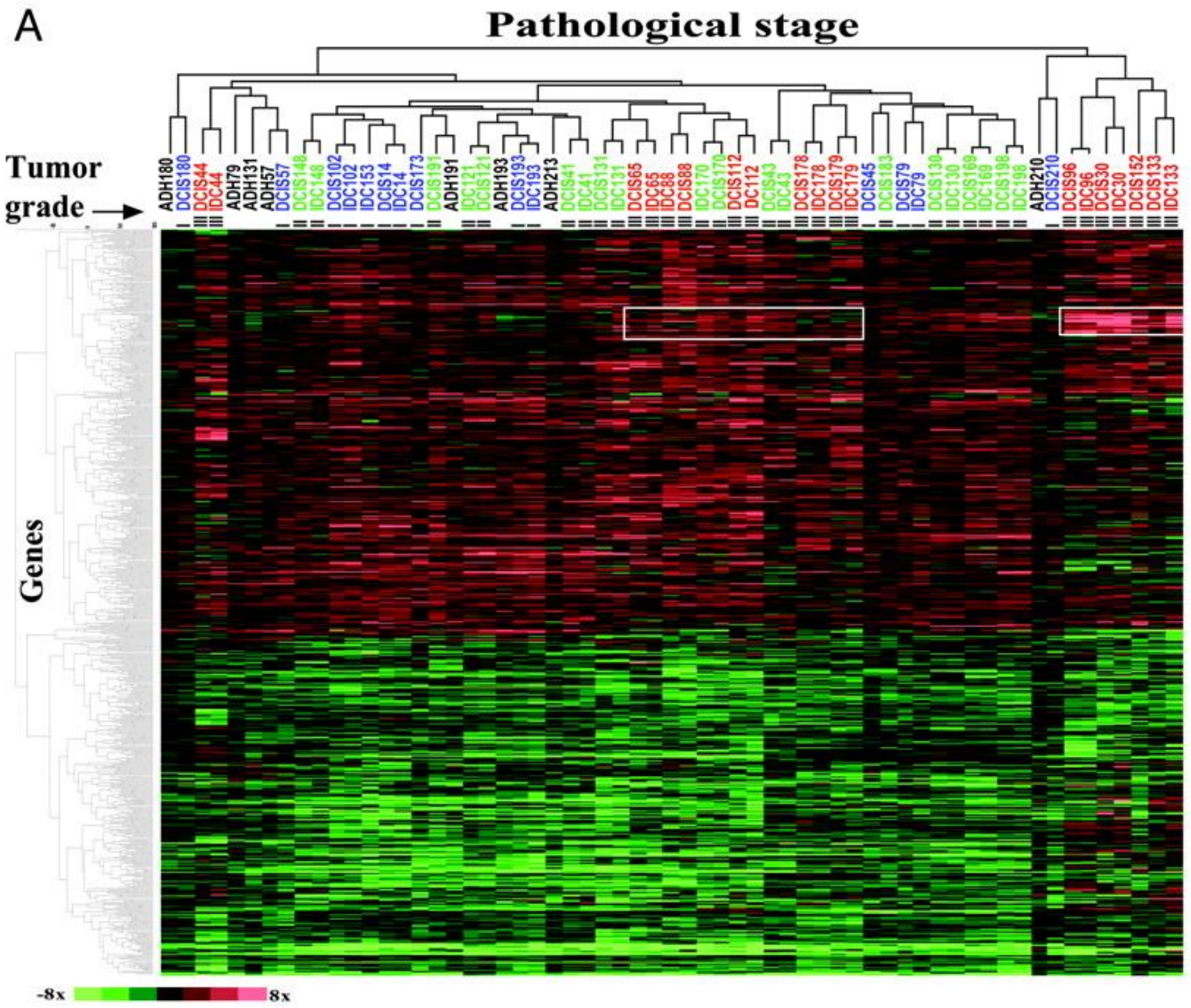
Changes in stromal cells

Mechanism of progression from in situ to invasive breast cancer

- **Tumor progression by genetic aberrations or altered expression in critical genes for invasion in tumor cells**
  - **Gene amplification**
  - **Epithelial-mesenchymal transition**

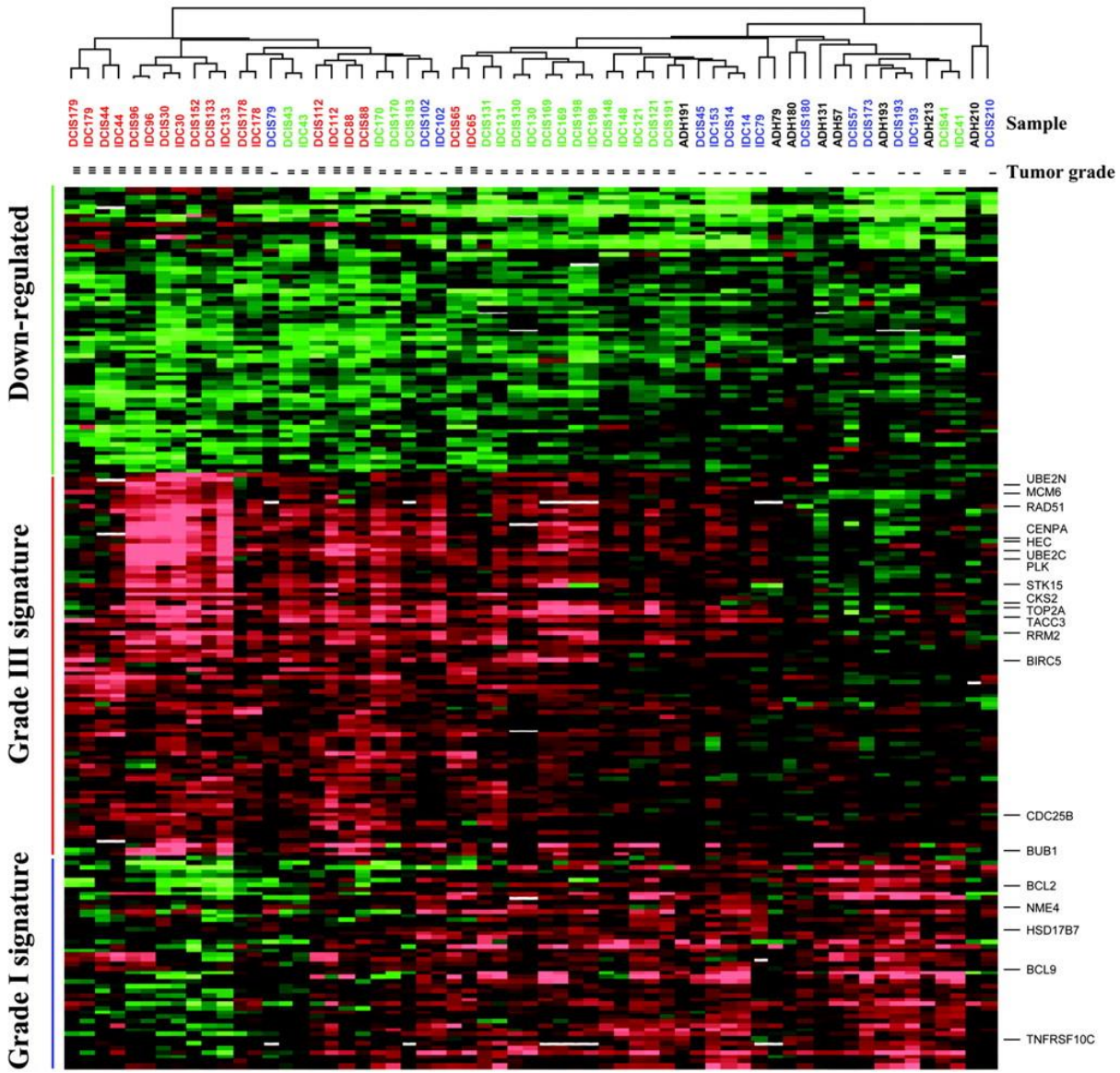
- **Tumor progression driven by tumor microenvironment**

Gene Expression Profiles of Breast Cancer Progression Stages



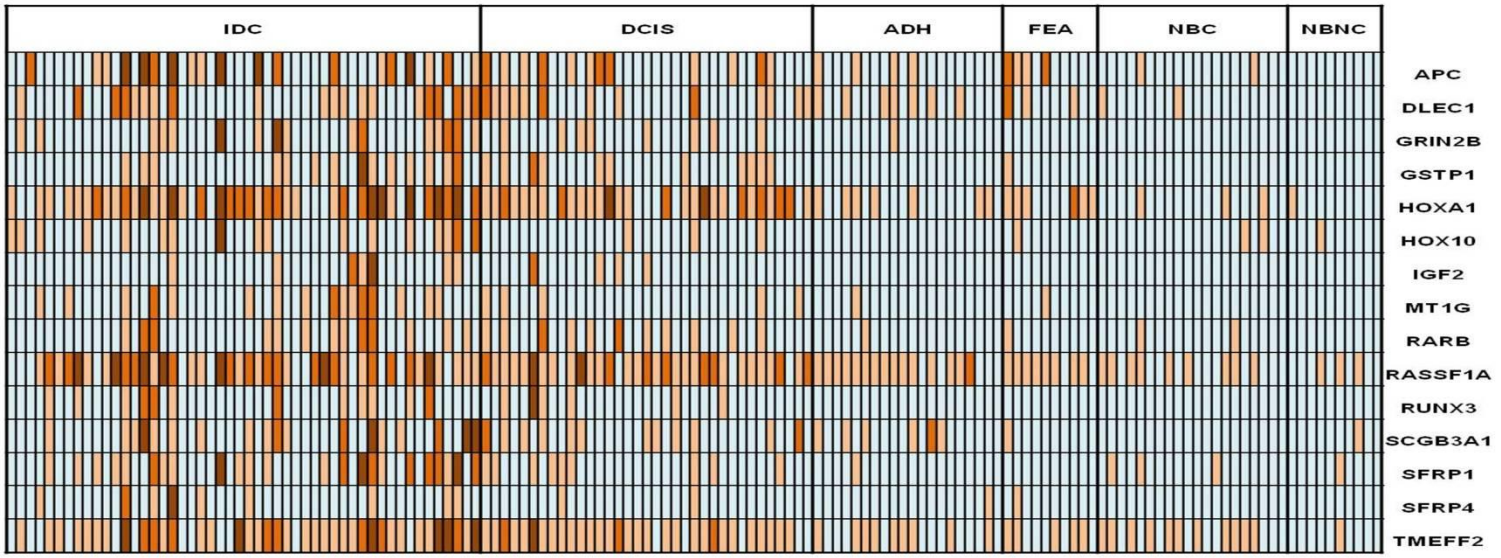
Ma X et al., PNAS 2003;100:5974-5979

Gene Expression Profiles of Breast Tumor Grades

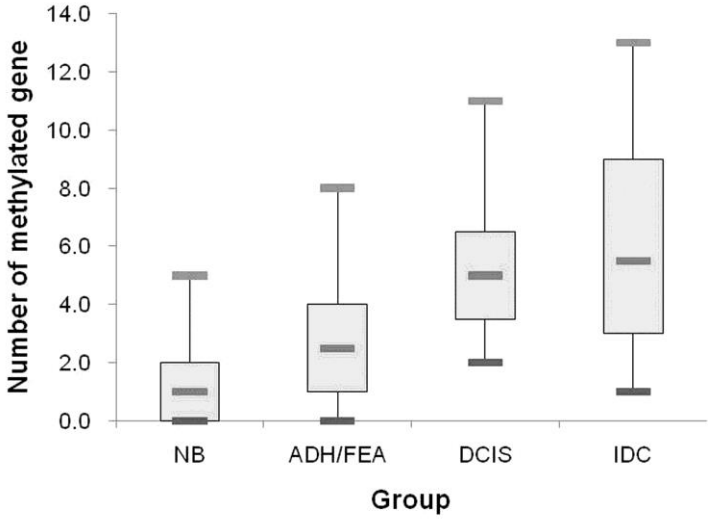


Ma X et al., PNAS 2003;100:5974-5979

Promoter CpG island methylation during breast cancer progression

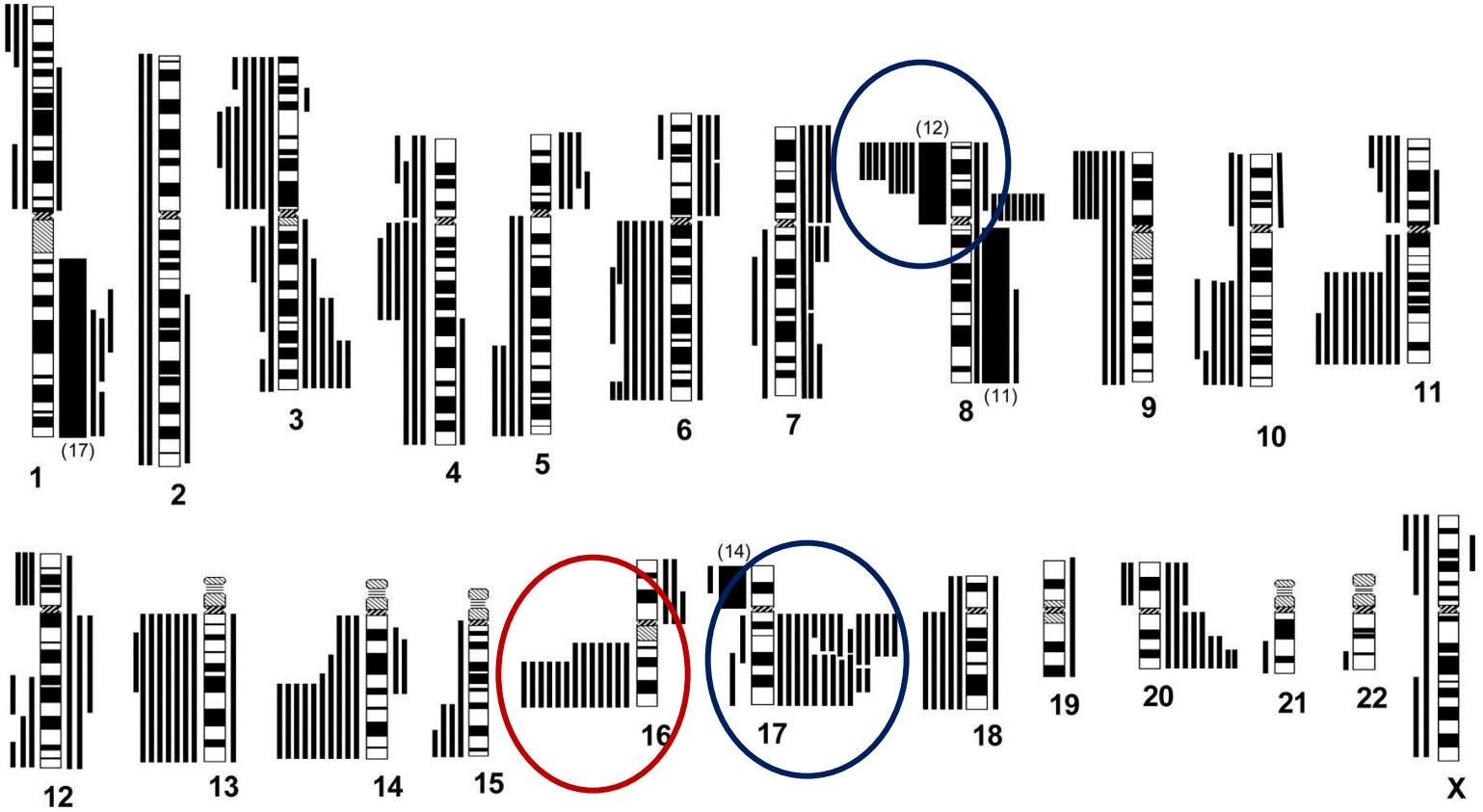


[0<PMR<4 (light blue), 4≤PMR<20 (light orange), 20≤PMR <50 (orange), PMR≥50 (brown)]



Park SY et al., Virchows Arch 2011;458:73-84

Patterns of chromosomal alterations in pure ductal carcinoma in situ



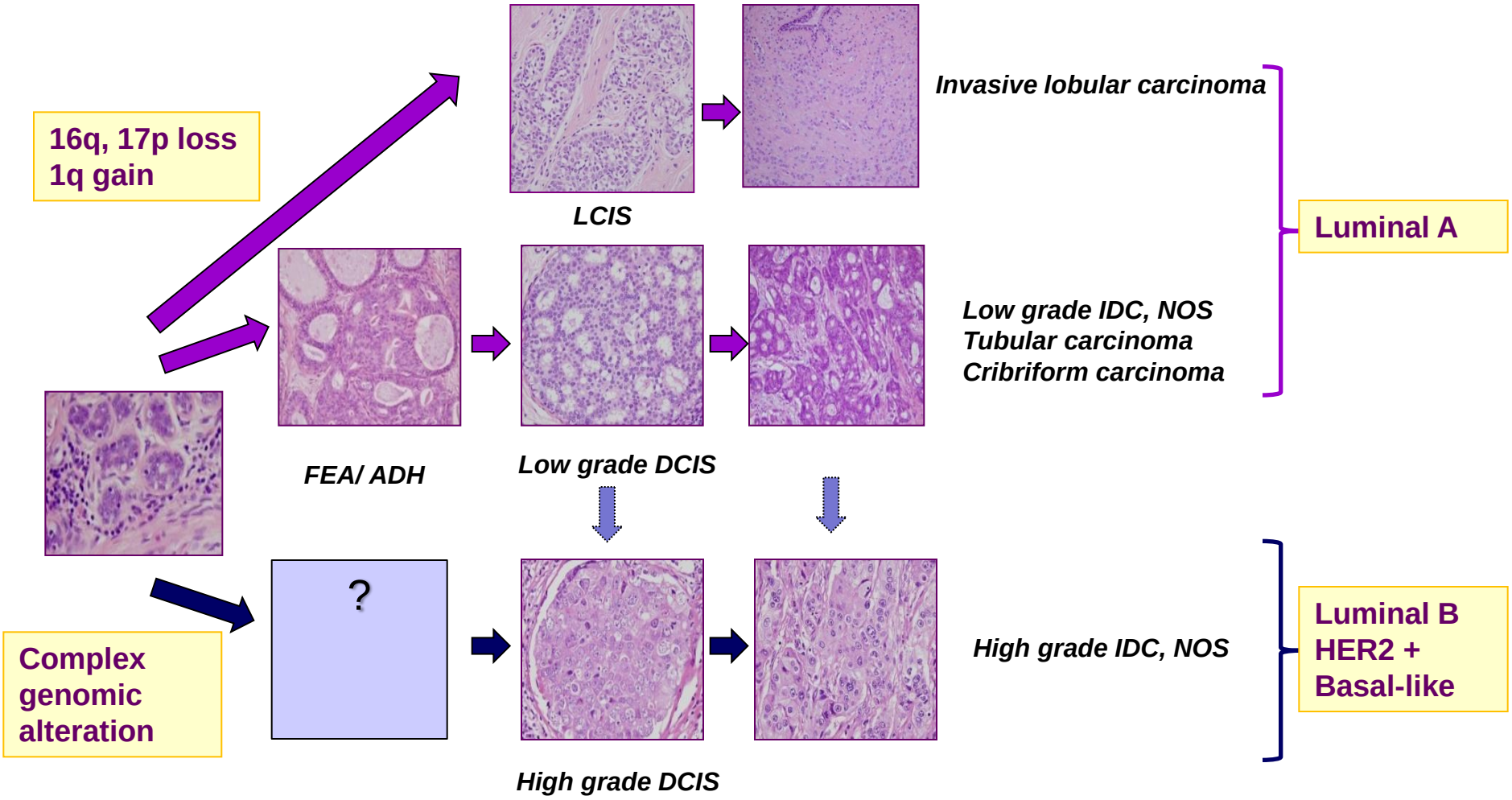
Hwang ES et al., Clin Cancer Res 2004;10:5160-5167

Comparison of chromosomal alterations in synchronous DCIS and invasive ductal carcinoma

Case no.	Changes in common	Changes only in DCIS	Changes only in IDC	% concurrence	CGH changes in common	CGH changes only in DCIS	CGH changes only in IDC
17	14	0	0	100	3q24-qter+; 5p+; 5q31+; 5q32-qter-; 6q25-qter-; 7p+; 8p-; 8q+; 9q22-qter-; 16q23-qter+; 17q11-q21+; 20q13+; Xp-; Xq11-q26-	None	none
30	2	0	0	100	1q+; 16q-	None	none
10	4	0	0	100	1p31-pter-; 1p12-p22+; 1q+; 2p23-p16-		
6	17	1	1	94	1q41-qtr+; 2q21-q24-; 2q32-q36+; 4p15-ptr-; 6q15-q22+; 7p+; 8p-; 11p11-p14+; 11q11-q12+; 12q21-qtr-; 13q12-q21-; 14q11-q23+; 14q24-qtr-; 15q25-qtr+; 16q-; 17q23-qtr+; 18q12-q21-	6q23-qter+	12p+
13	5	0	1	91	1q+; 7q11-q21+; 7q22-q32-; 8p-; 16q21-qtr-	None	X-
31	9	2	0	90	6p+; 6q12-q16-; 6q21-q22+; 6q23-; 6q24+; 6q25-qtr-; 8p11-p12+; 8p21-ptr-; 8q+; 11q14-qtr-; 17p-; 19+	7+; 17q+	none
29	4	0	1	89	1q+; 4q33-qtr-; 6q22-q23-; 8q22-qtr-	None	14q22-qter-
7	11	1	5	79	8p21-ptr-; 8p11-p12+; 8q+; 11q13+; 11q14-qter-; 12q15+; 12q21-qtr-; 16q-; 17p11+; 17q11-q21+; 22q+	7+	6p+; 6q-; 13q-; 14q-; 17q22-qter+
8	6	2	2	75	1q+; 4p-; 5p+; 14q22-qtr-; 17p-; 17q11-q24+	8q21-qter+; 14q12-q21+	8p-; 11p-
16	2	3	0	57	17p-; 17q21-qtr+	6p21-ptr+; 6q16-q22+; 20q13+	none
12	2	4	5	31	3p11-p23-; 20q+	1+; 13q+; 14q21-q31-; 22q13+	6p22+; 9q13-qtr-; 10q24-qtr-; 16q24-; 17p-
9	1	8	2	17	16q-	1p-; 1q+; 3-; 5-; 6+; 16p+; 20+; 22q-	8p-; 13q21-qter-

Hwang ES et al., Clin Cancer Res 2004;10:5160-5167

Progression of breast cancer:
Low grade vs. high grade pathway



Gene amplification in the progression of in situ to invasive breast cancer

- *The role of gene amplification in the progression of DCIS to invasive breast cancer is uncertain.*
- *Some workers found no difference in gene amplification frequencies between DCIS and invasive carcinomas.*
- *Others have suggested that C-MYC amplification plays an important role in the transition. However, this finding was not confirmed in other studies.*
- *We compared the gene amplification frequencies of HER2, C-MYC, CCND1 and FGFR1 in a relatively large series of pure DCIS, DCIS associated with invasive carcinoma, and invasive carcinomas, to investigate the role of gene amplification in the progression of DCIS to invasive carcinomas.*

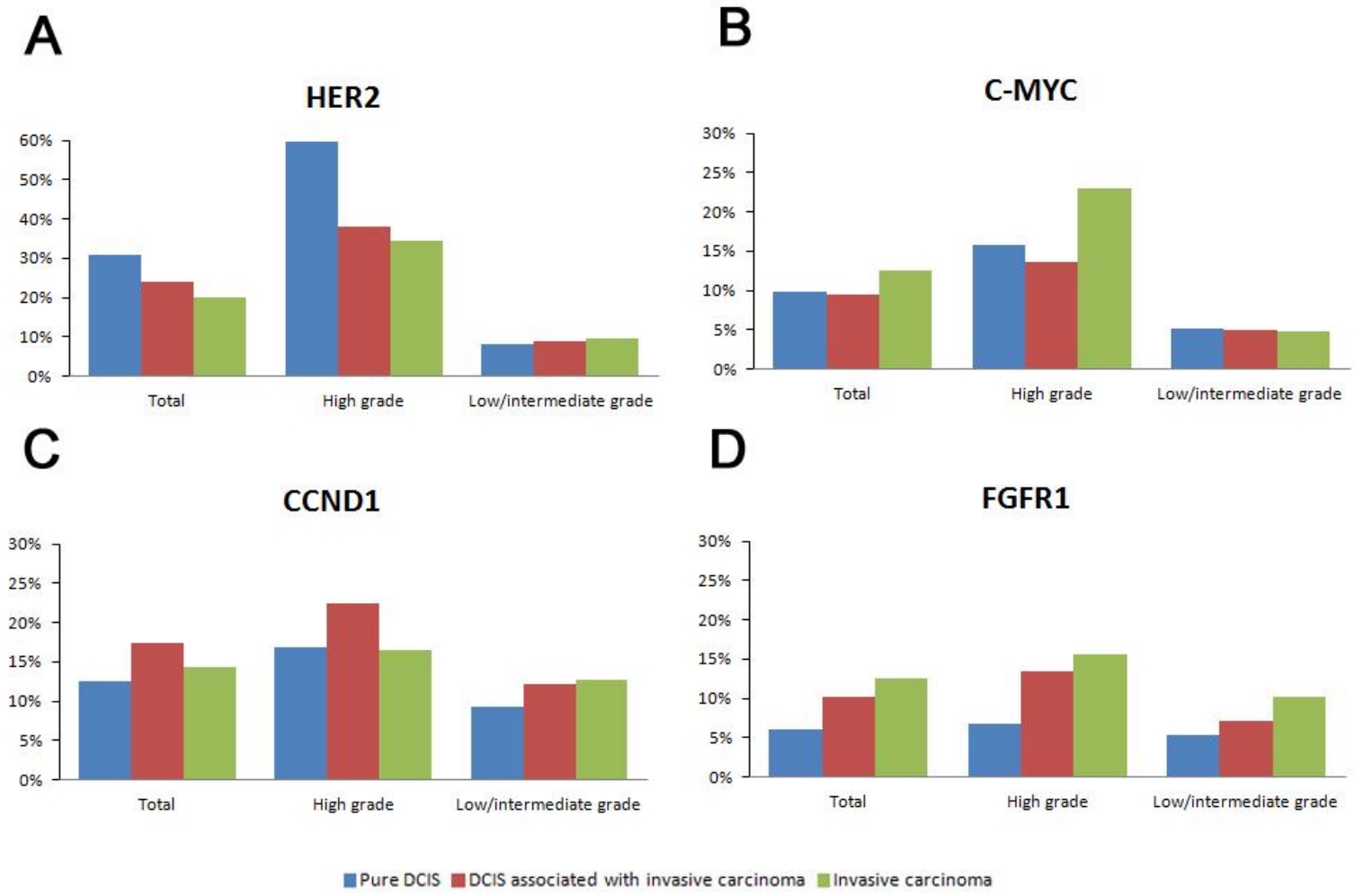
Jang M et al. Breast Cancer Res 2012;14:R155

The relationship between gene amplification and molecular subtype

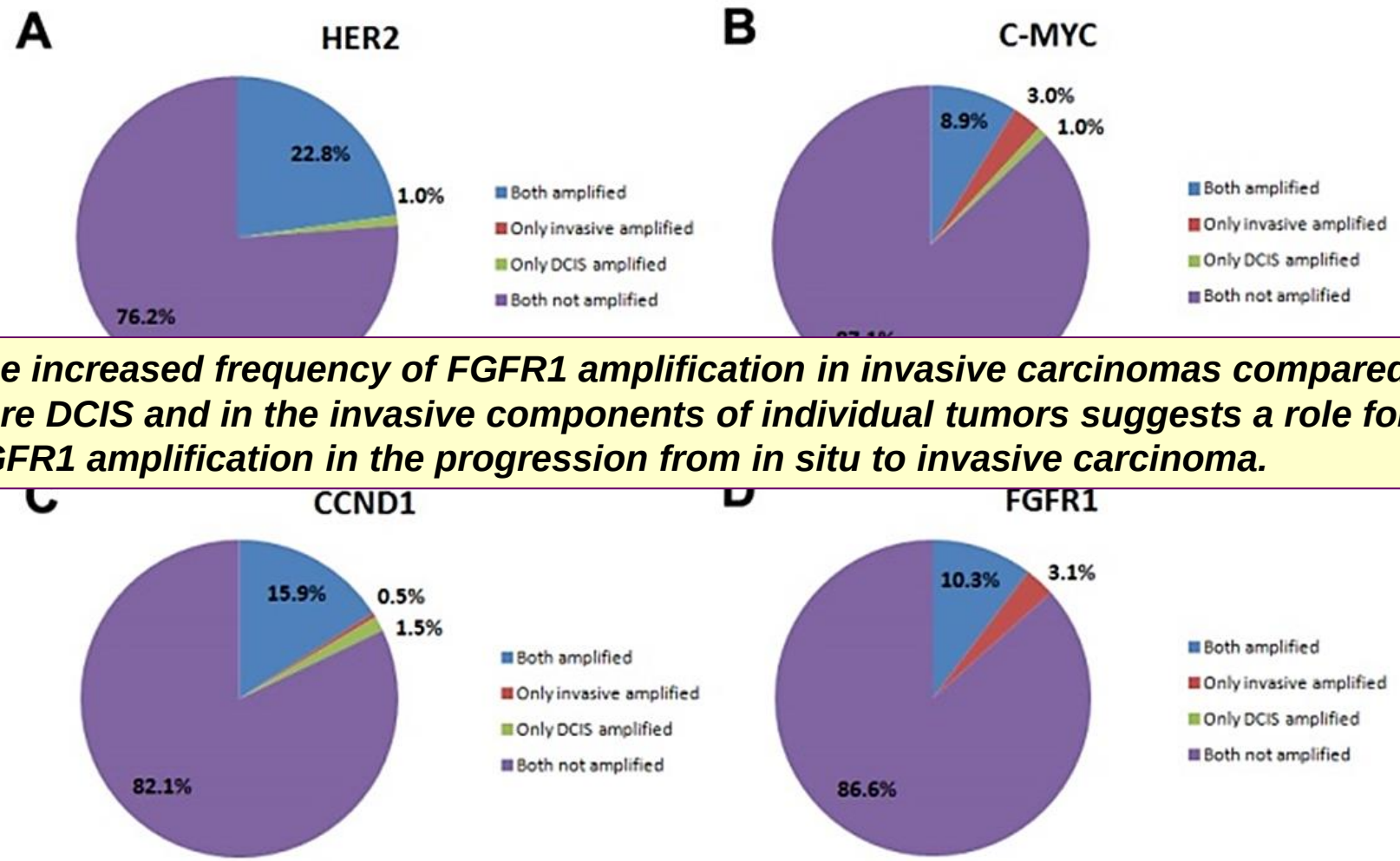
Histologic stage	Gene amplification	Molecular subtype					P value*
		Luminal A	Luminal B	HER2 enriched	Basal-like	TN-NB	
Invasive carcinoma (n=438)	**C-MYC**	13/208 (6.3)	15/103 (14.6)	7/42 (16.7)	14 /55 (25.5)	5/19 (26.3)	<0.001
	CCND1	19/205 (9.3)	**37/103 (35.9)**	4/42 (9.5)	**0/55 (0)**	1/18 (5.3)	<0.001
	FGFR1	21/202 (10.4)	**21/100 (21.0)**	3/42 (7.1)	7/55 (12.7)	0/18 (0)	0.025
DCIS associated with invasive carcinoma (n=216)	**C-MYC**	4/99 (4.0)	9/57 (15.8)	3/17 (17.6)	3/25 (12.0)	0/5 (0)	0.085
	CCND1	12/99 (12.1)	**20/56 (35.7)**	3/17 (17.6)	**0/25 (0)**	0/4 (0)	<0.001
	FGFR1	8/96 (8.3)	**9/55 (16.4)**	2/17 (11.8)	1/24 (4.2)	0/4 (0)	0.393
Pure DCIS (n=179)	**C-MYC**	6/98 (6.1)	4/24 (16.7)	6/34 (17.6)	1/9 (11.1)	0/8 (0)	0.198
	CCND1	11/99 (11.1)	**6/24 (25.0)**	4/35 (11.4)	**0/9 (0)**	1/8 (12.5)	0.298
	FGFR1	5/95 (5.3)	**4/23 (17.4)**	1/33 (3.0)	0/9 (0)	0/8 (0)	0.134

Jang M et al. Breast Cancer Res 2012;14:R155

Frequencies of gene amplification in pure DCIS, DCIS associated with invasive carcinoma, and invasive carcinoma

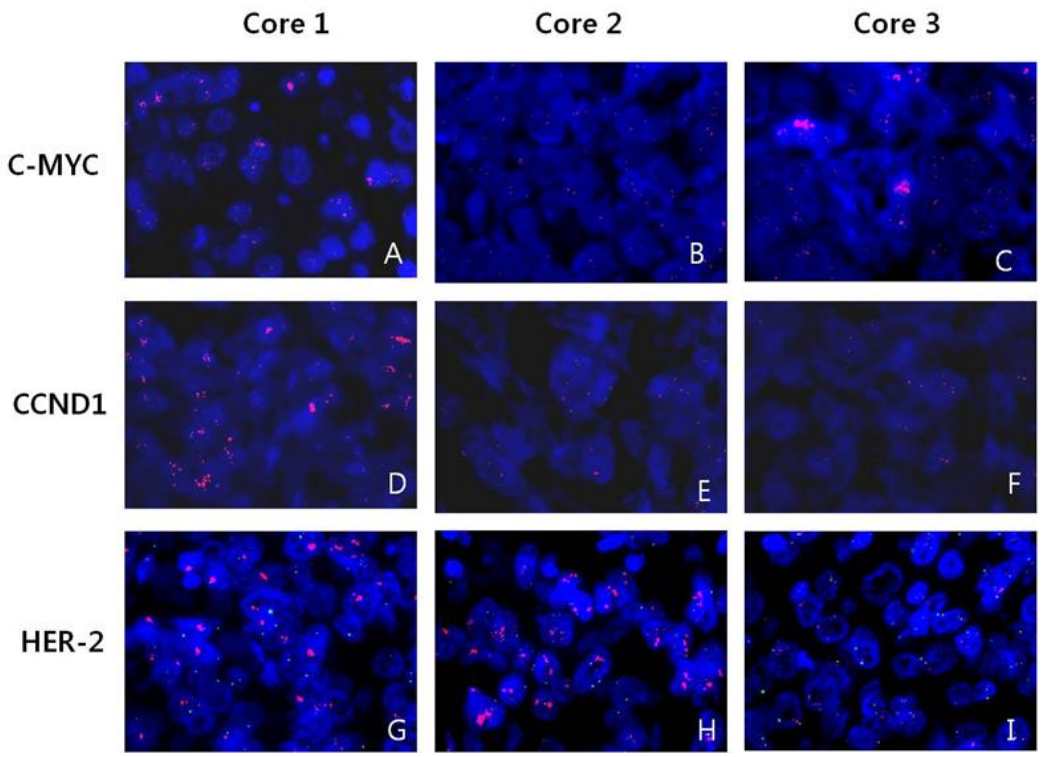


Comparison of gene amplification status in the invasive and DCIS components of the same tumors



The increased frequency of FGFR1 amplification in invasive carcinomas compared to pure DCIS and in the invasive components of individual tumors suggests a role for FGFR1 amplification in the progression from in situ to invasive carcinoma.

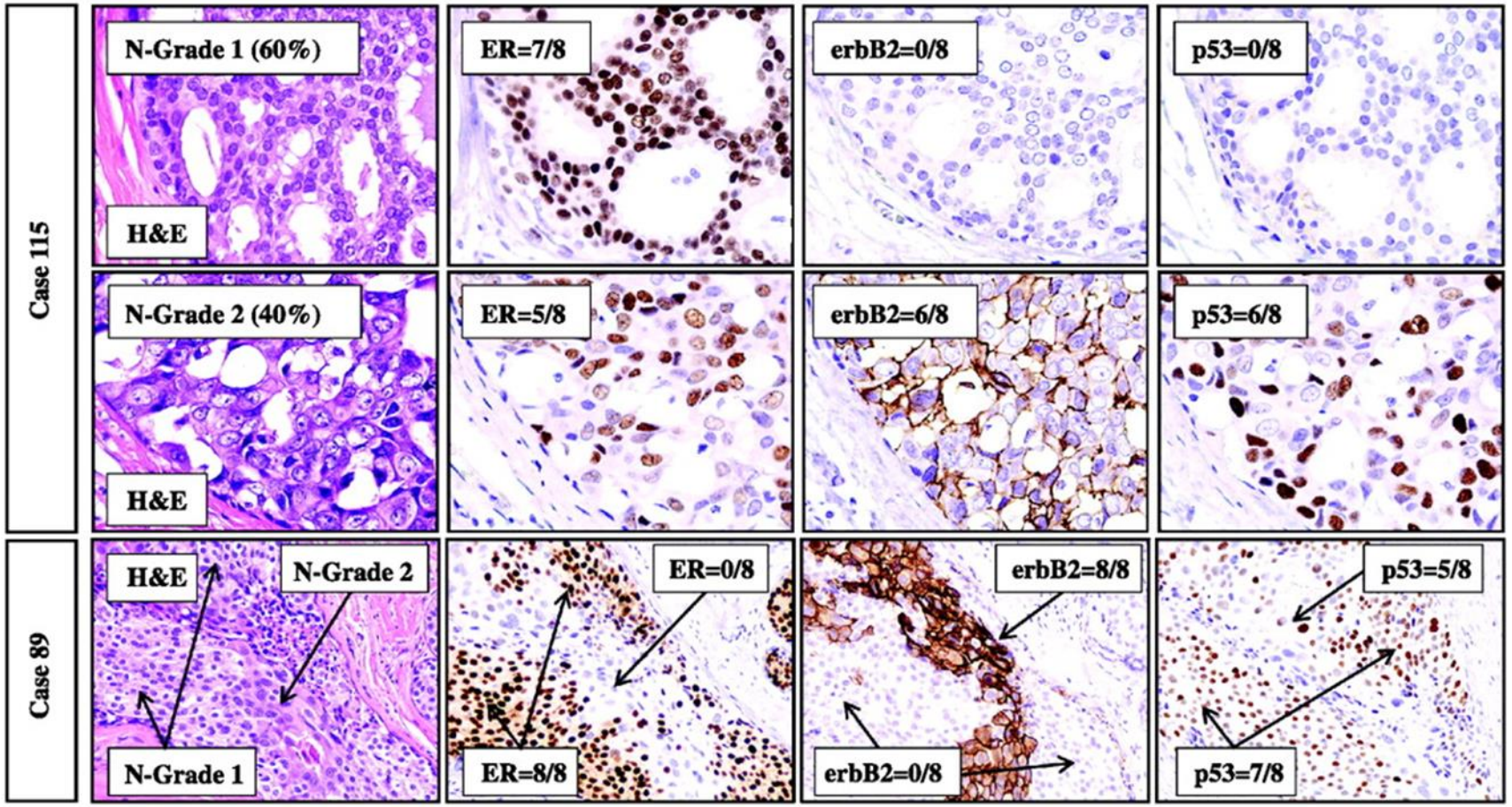
Heterogeneity of gene amplification in DICS



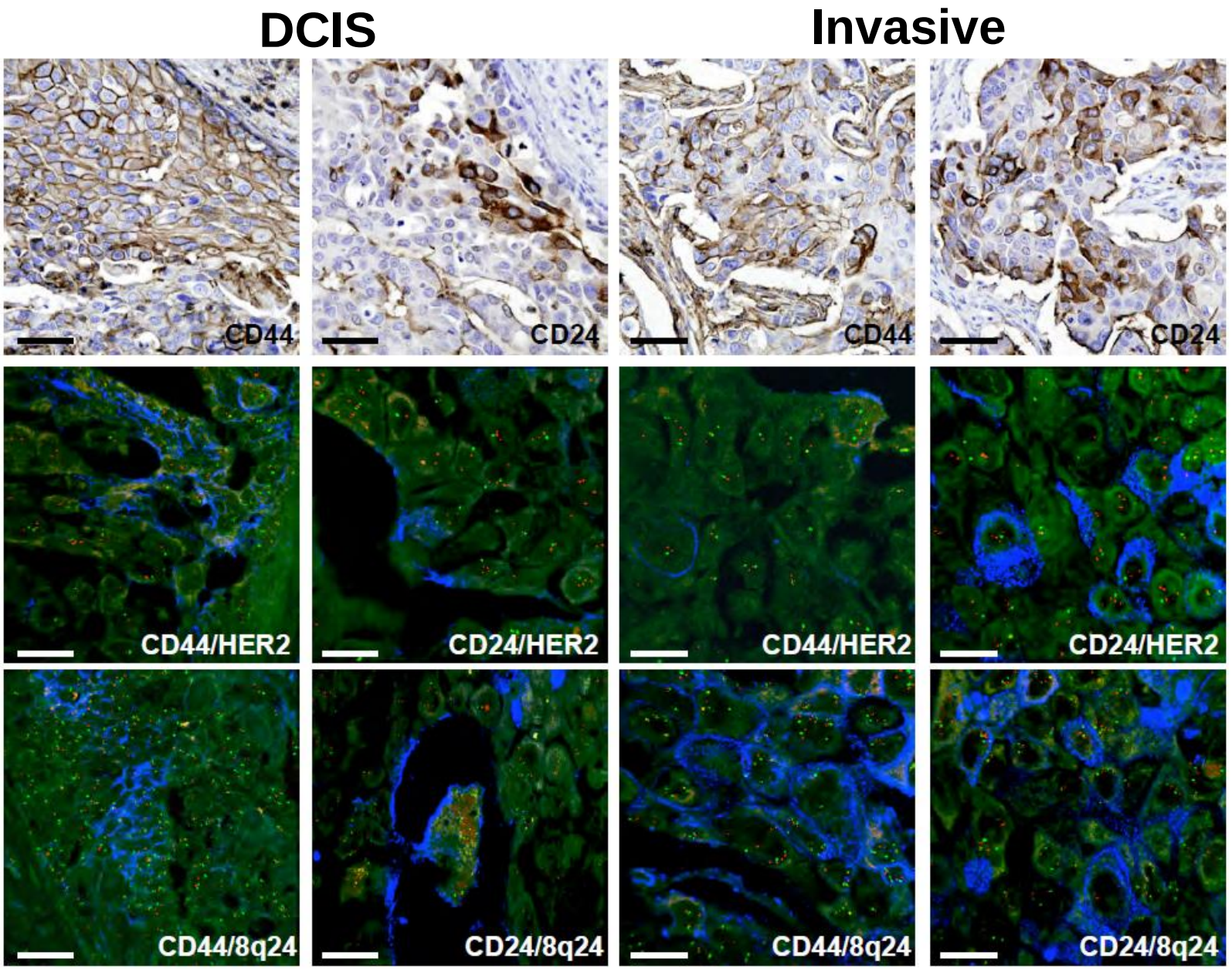
- In pure DCIS, heterogeneity of gene amplification was found in 3.7% (2/52) for *HER2*, 11.8 % (2/17) for *C-MYC*, 22.7% (5/22) for *CCND1*, and 10% (1/10) for *FGFR1* amplified cases.

- These findings suggest that intra-tumoral genetic heterogeneity is already present in the DCIS and that progression of DCIS to invasive carcinomas may result from selection of subpopulations of tumor cells.

Ductal Carcinoma *In situ* and the Emergence of Diversity during Breast Cancer Evolution

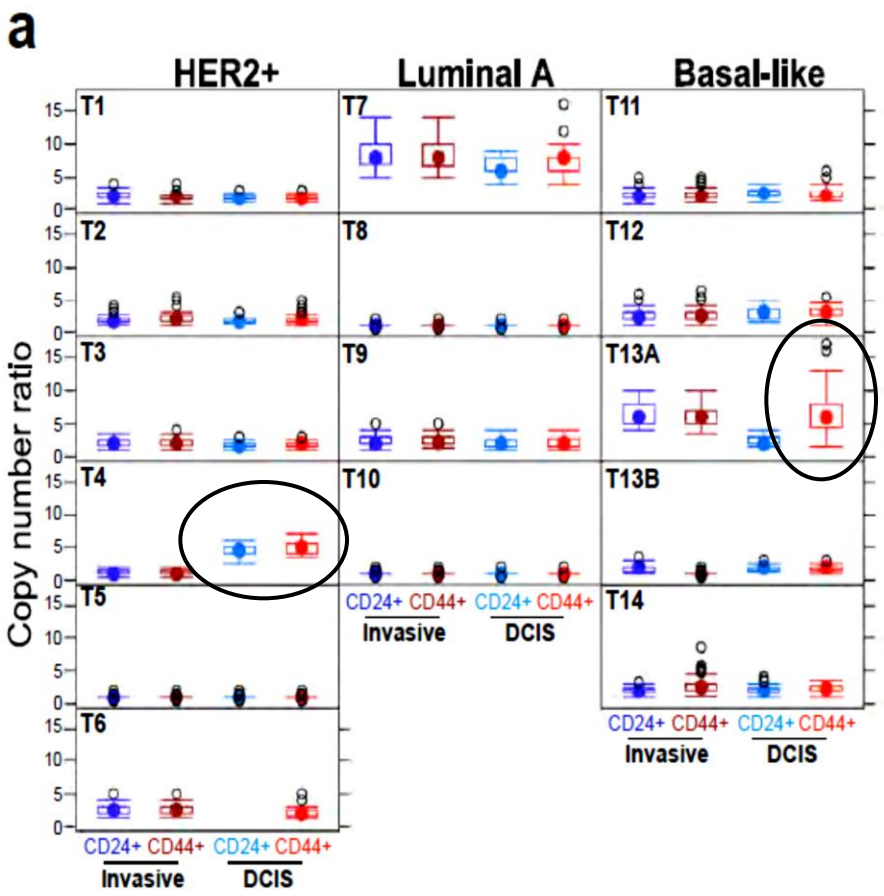


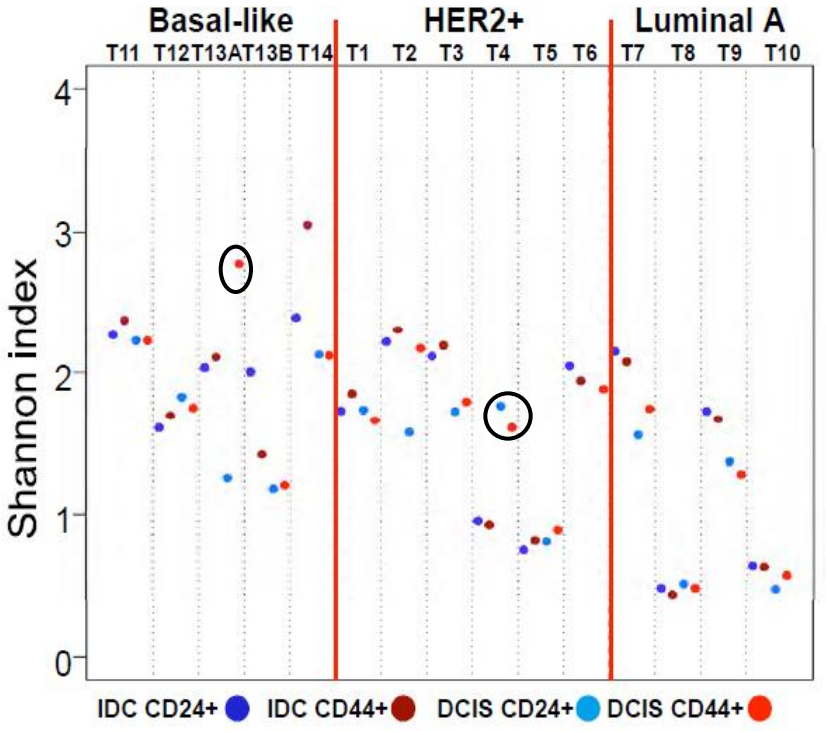
Allred DC et al. Clin Cancer Res 2008;14:370-378



Park SY et al., J Clin Invest 2010:120:636-44

Diversity for 8q24 copy number gain in breast cancers





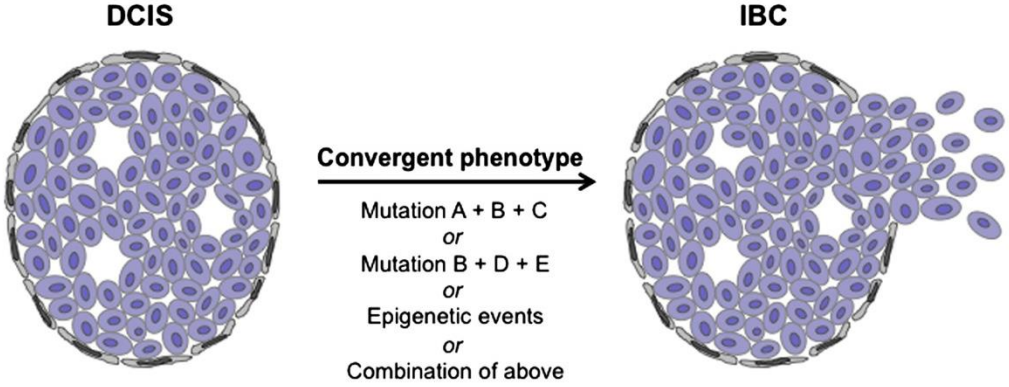
Shannon diversity index $H = -\sum_i p_i \ln(p_i)$

p_i = frequency of species i in the tumor sample

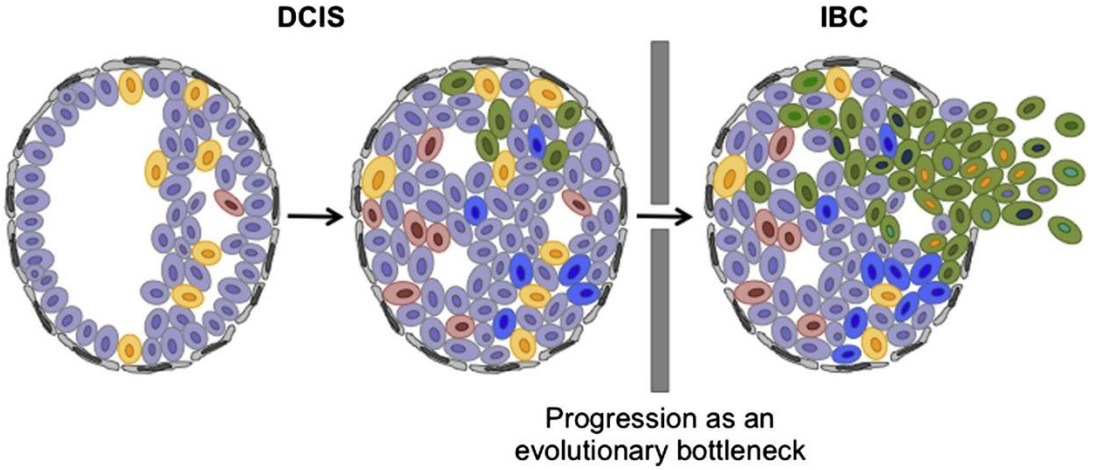
Park SY et al., J Clin Invest 2010:120:636-44

Hypothetical models of progression from in situ to invasive breast cancer

Progression from in situ to invasive breast cancer as a convergent phenotype

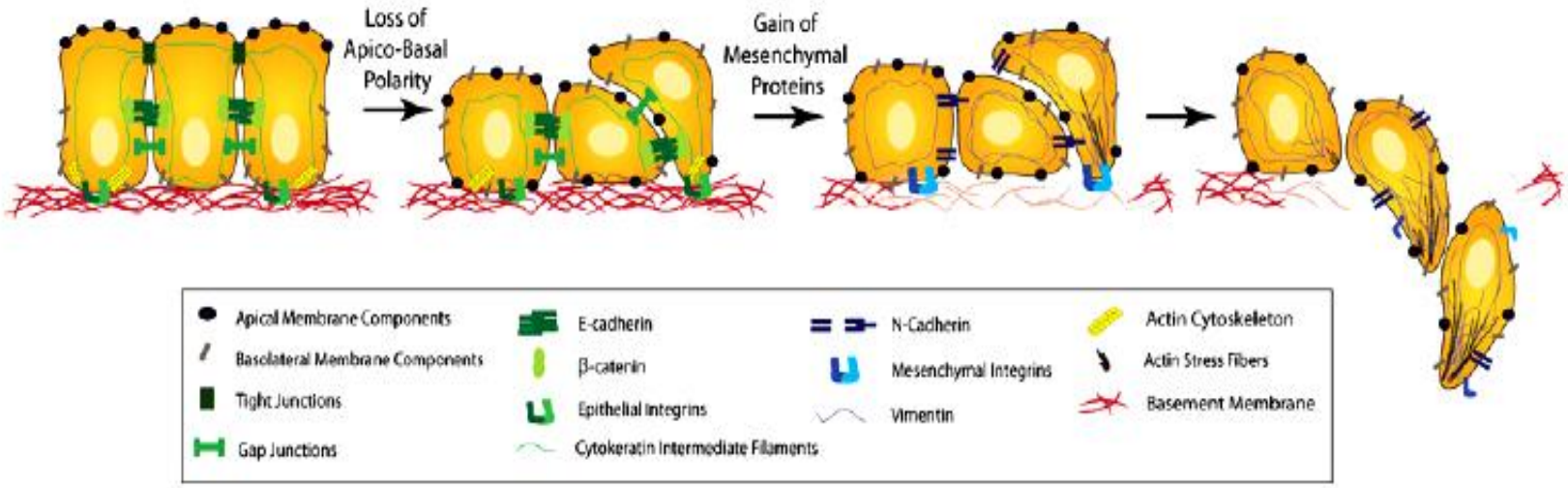


Progression from in situ to invasive breast cancer as an evolutionary bottleneck



Cowell CF et al., Med Oncol 2013:7:859-869

Epithelial-mesenchymal transition (EMT) and epithelial plasticity



Loss

Tight junctions
Gap junctions
E-cahderin/ β-catenin
Epithelial integrins
Cytokeratin

Gain

Mesenchymal integrins
N-cadherin
Vimentin
Actin

Micalizzi et al. J Mammary Gland Biol Neoplasia 2010;15:117-34

Relationships between the expression of EMT markers and molecular subtypes

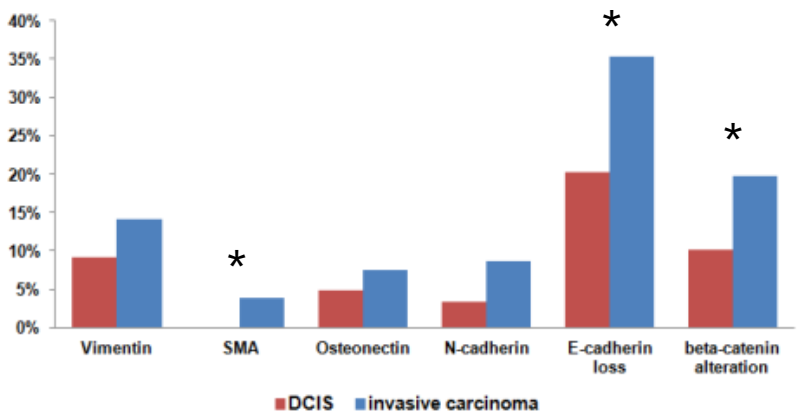
Group	Marker	Luminal A	Luminal B	HER2 positive	Basal-like	TNNB	P value*
Invasive	Vimentin	7/179 (3.9)	4/100 (4.0)	5/40 (12.5)	**35/51 (68.6)**	4/18 (22.2)	<0.001
carcinoma	SMA	0/179 (0)	0/98 (0)	1/49 (2.5)	**12/52 (23.1)**	2/18 (11.1)	<0.001
(n=438)	Osteonectin	4/180 (2.2)	6/99 (6.1)	2/40 (5.0)	**15/52 (28.8)**	2/18 (11.1)	<0.001
	N-cadherin	3/181 (1.7)	13/98 (13.3)	2/40 (5.0)	**13/52 (25.0)**	3/18 (16.7)	<0.001
	E-cadherin loss	38/177 (21.5)	29/96 (30.2)	16/40 (40.0)	**41/51 (80.4)[a]**	11/18 (61.1)	<0.001
	β-catenin alteration	8/172 (4.7)	18/97 (18.6)	12/40 (30.0)	**31/51 (60.8)[b]**	6/18 (33.3)	<0.001
Pure DCIS	Vimentin	7/93 (7.5)	1/20 (5.0)	3/35 (8.6)	**4/9 (44.4)**	0/6 (0)	0.005
(n=179)	SMA	0/84 (0)	0/20 (0)	0/32 (0)	0/9 (0)	0/4 (0)	NA
	Osteonectin	0/93 (0)	4/21 (19.0)	3/35 (8.6)	1/9 (11.1)	0/6 (0)	0.003
	N-cadherin	3/86 (3.5)	1/20 (5.0)	1/32 (3.1)	0 /9 (0)	0/4 (0)	0.959
	E-cadherin loss	14/84 (16.7)	8/20 (40.0)	6/32 (18.8)	**2/9 (22.2)[a]**	0/3 (0)	0.176
	β-catenin alteration	7/84 (8.3)	4/20 (20.0)	4/32 (12.5)	**0/9 (0)[b]**	0/4 (0)	0.390

[a]P=0.001 for E-cadherin, [b]P=0.001 for β-catenin in basal-like invasive carcinomas vs. basal-like DCIS

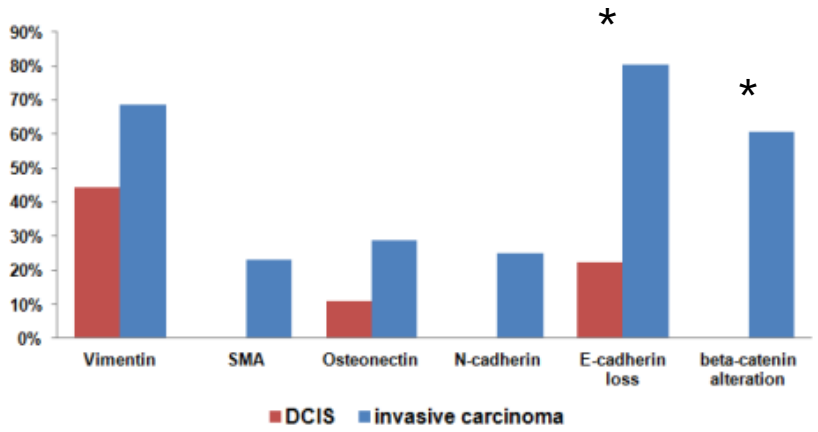
Choi Y et al., Human Pathol 2013;44:2581-9

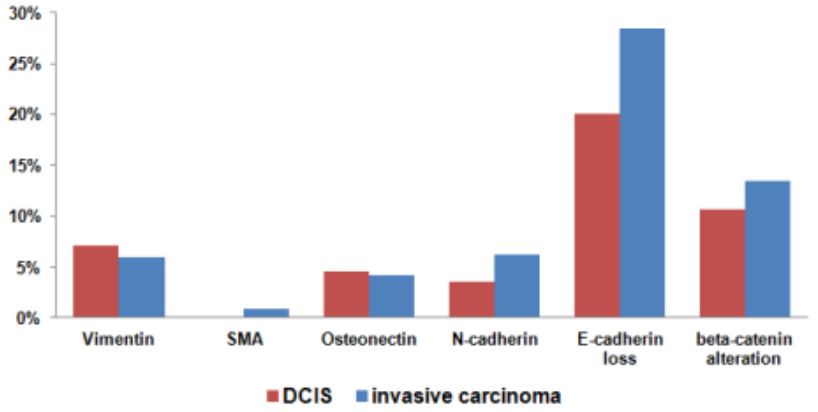
Comparison of the expression of EMT markers in pure DCIS and invasive carcinoma

Whole group



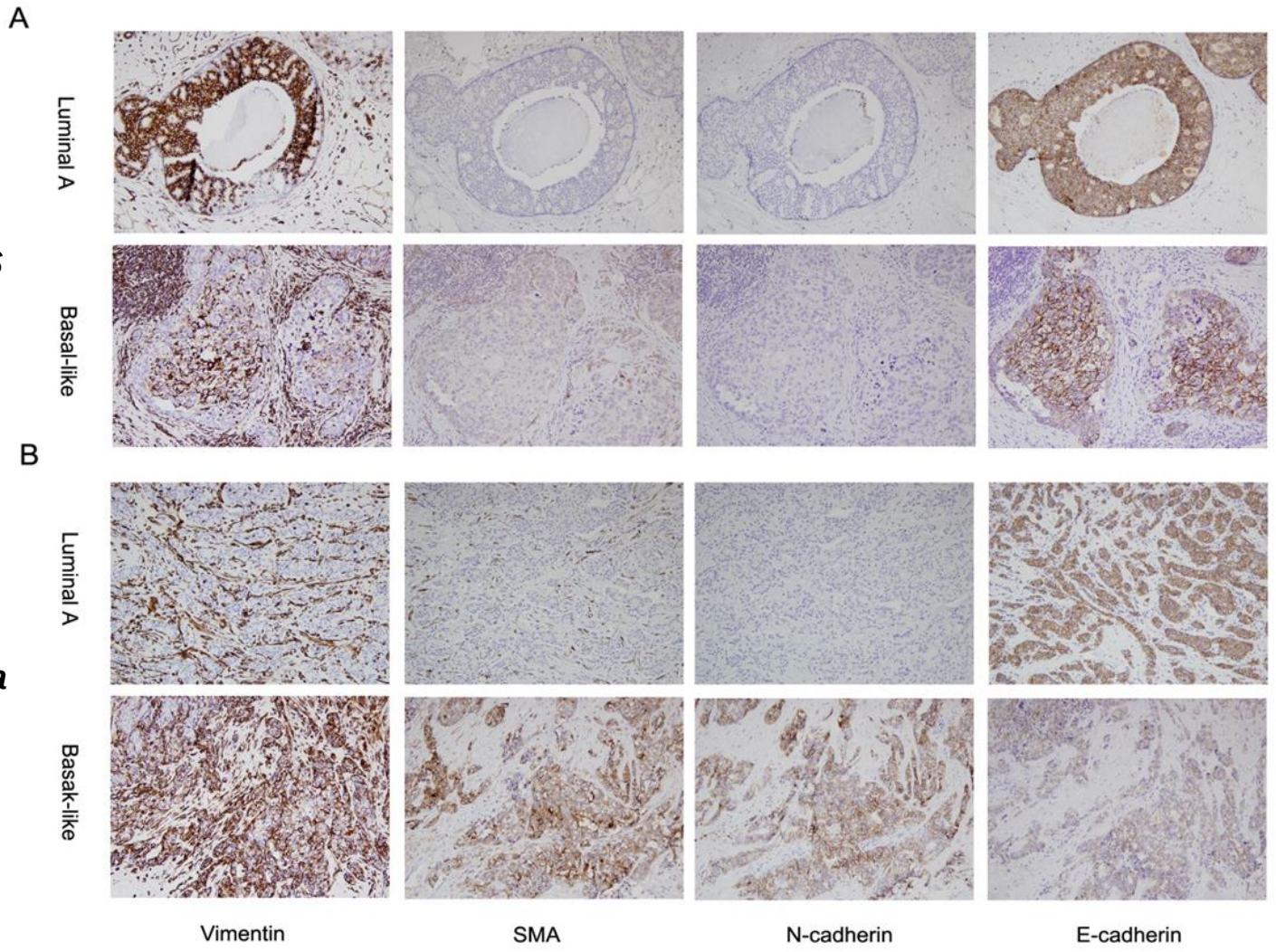
Basal-like subtype





Non-basal-like subtypes

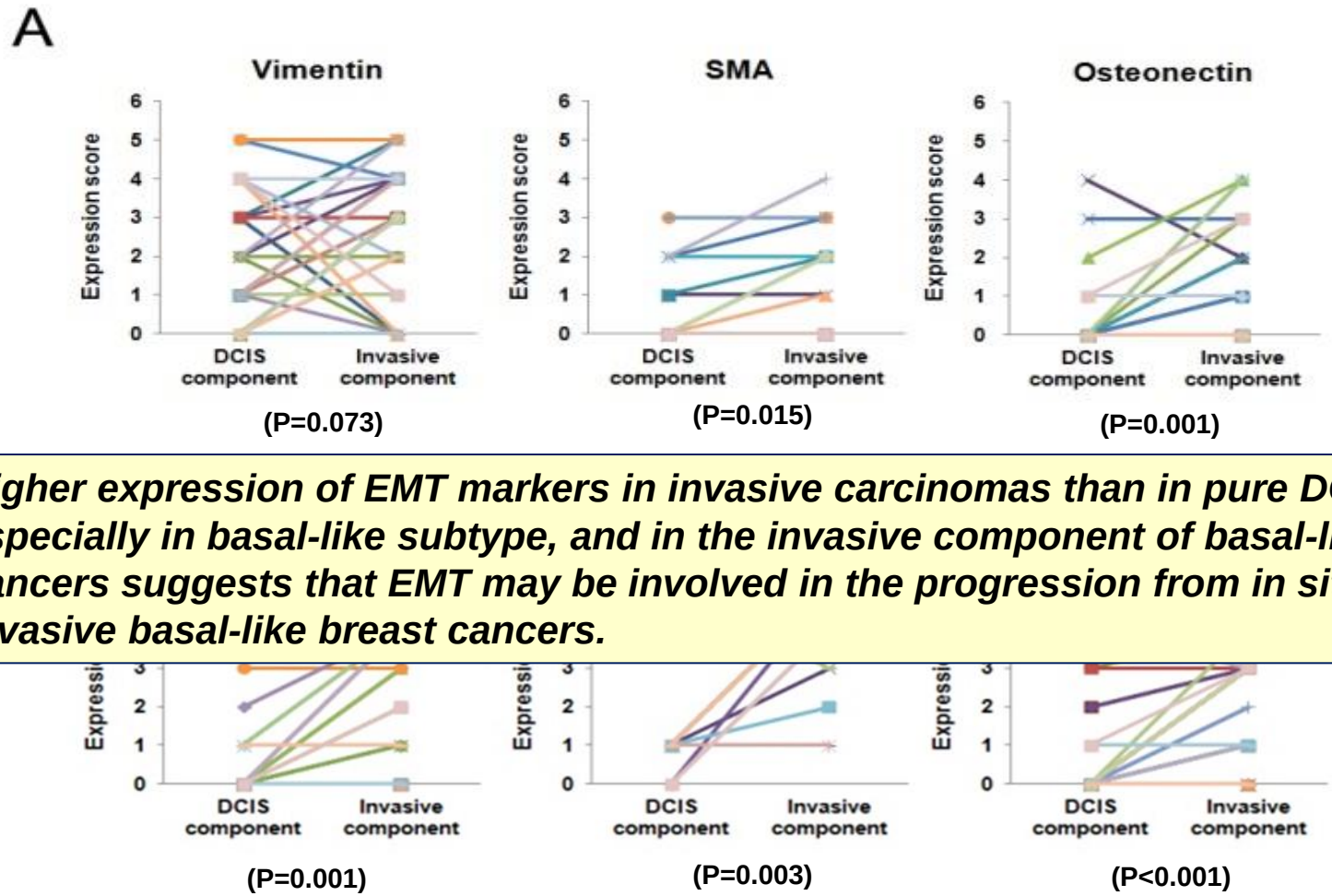
Expression of EMT markers according to breast cancer subtype in pure DCIS and invasive carcinoma



Pure DCIS

Invasive carcinoma

Comparison of the expression of EMT markers in the invasive and DCIS components of basal-like breast cancer



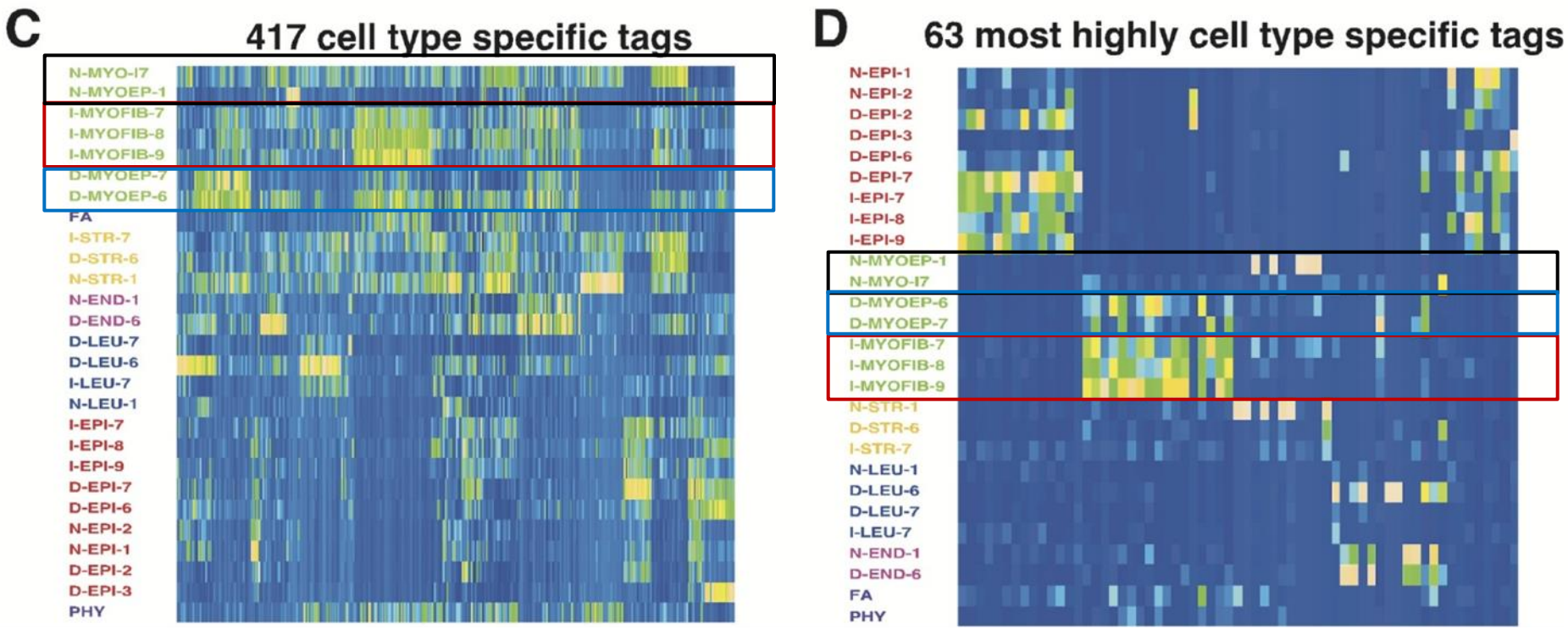
Higher expression of EMT markers in invasive carcinomas than in pure DCIS, especially in basal-like subtype, and in the invasive component of basal-like breast cancers suggests that EMT may be involved in the progression from in situ- to invasive basal-like breast cancers.

Mechanism of progression from in situ to invasive breast cancer

- **Tumor progression by genetic aberrations or altered expression in critical genes for invasion**

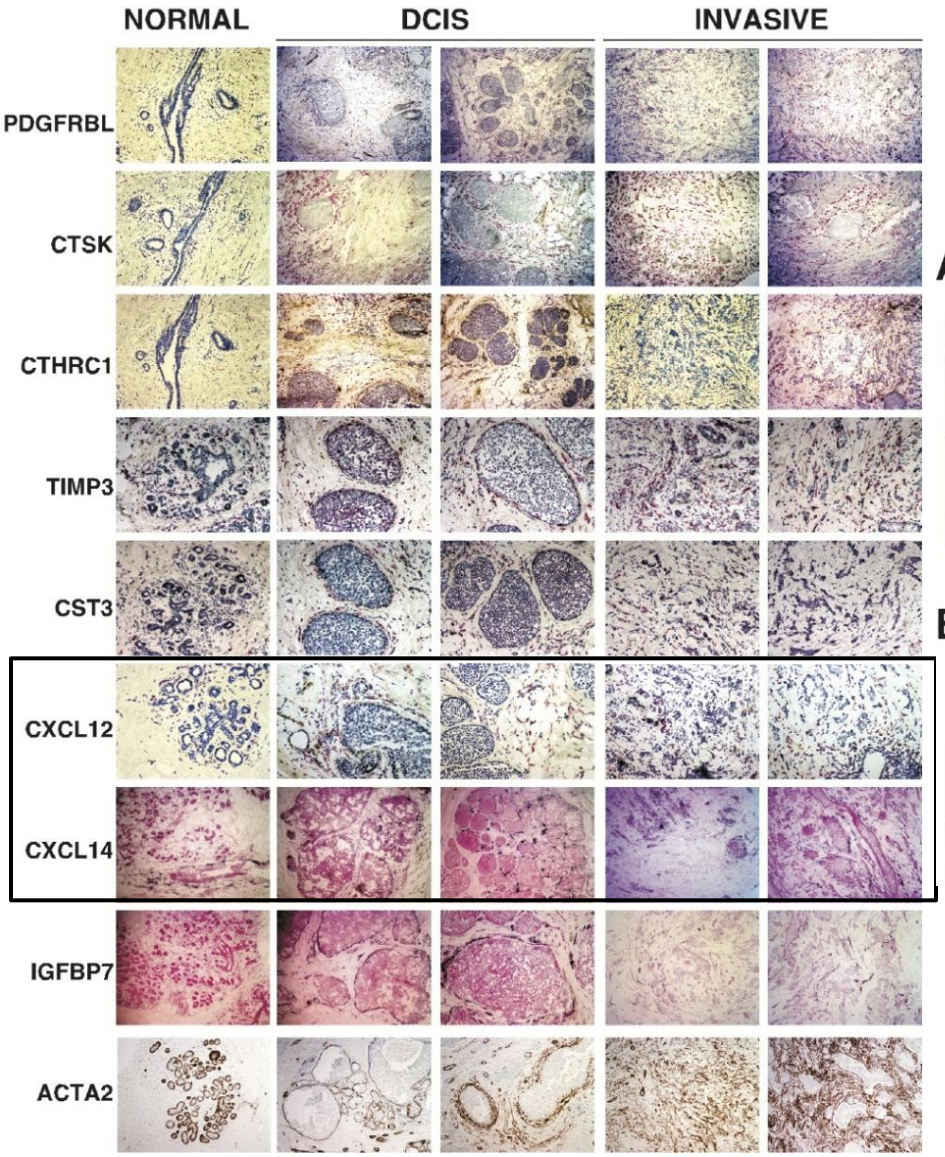
- **Tumor progression driven by tumor microenvironment**
  - **Myoepithelial cells**
  - **Stroma**

Molecular characterization of the tumor microenvironment in breast cancer

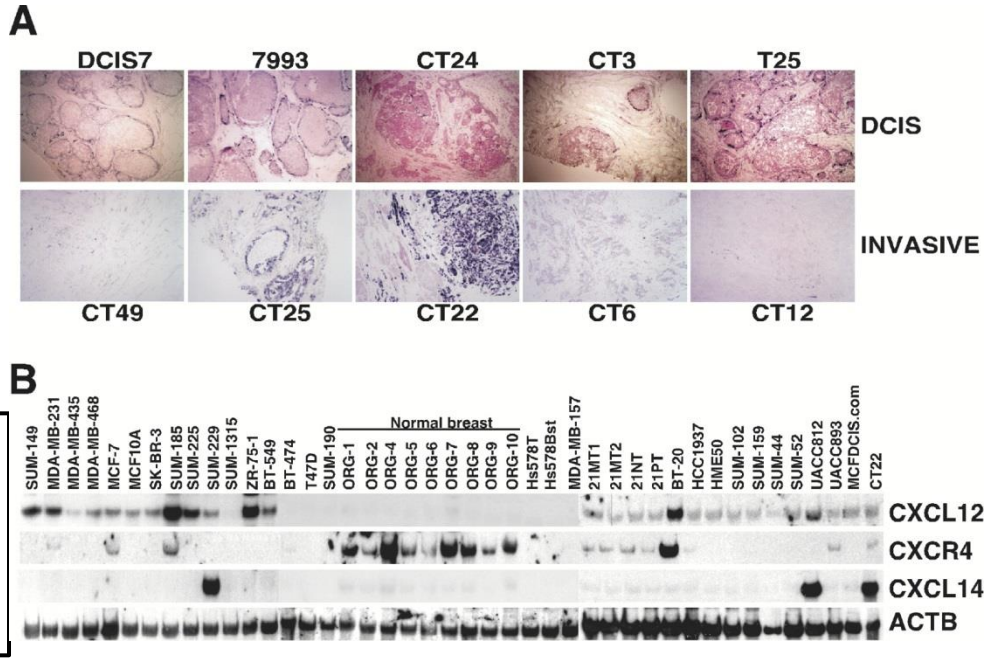


Color scheme: blue, downregulated (low tag counts); green, mean tag counts; yellow, upregulated (high tag counts).

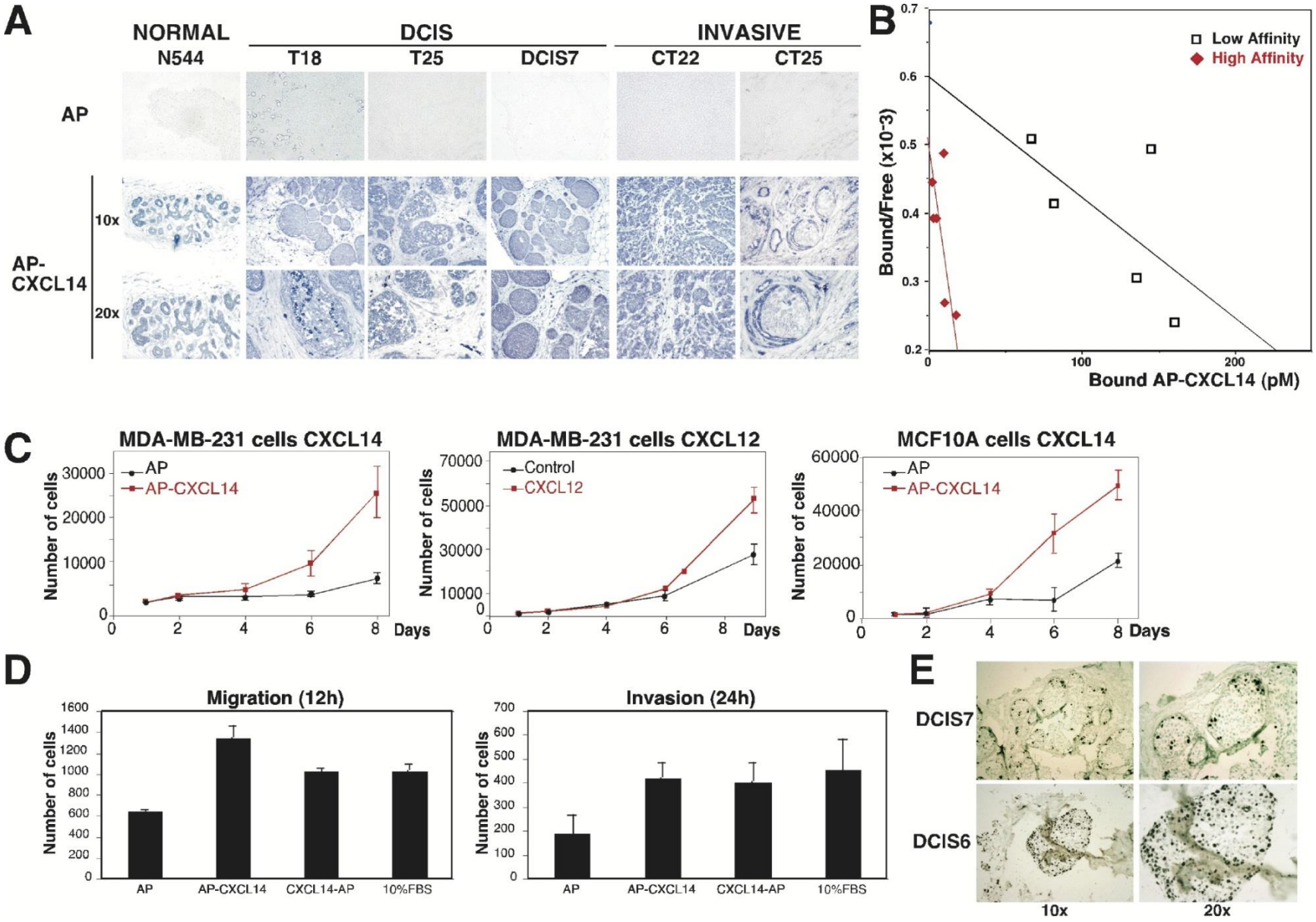
Allinen M et al., Cancer Cell 2004;6:17-32



mRNA in situ hybridization of CXCL14

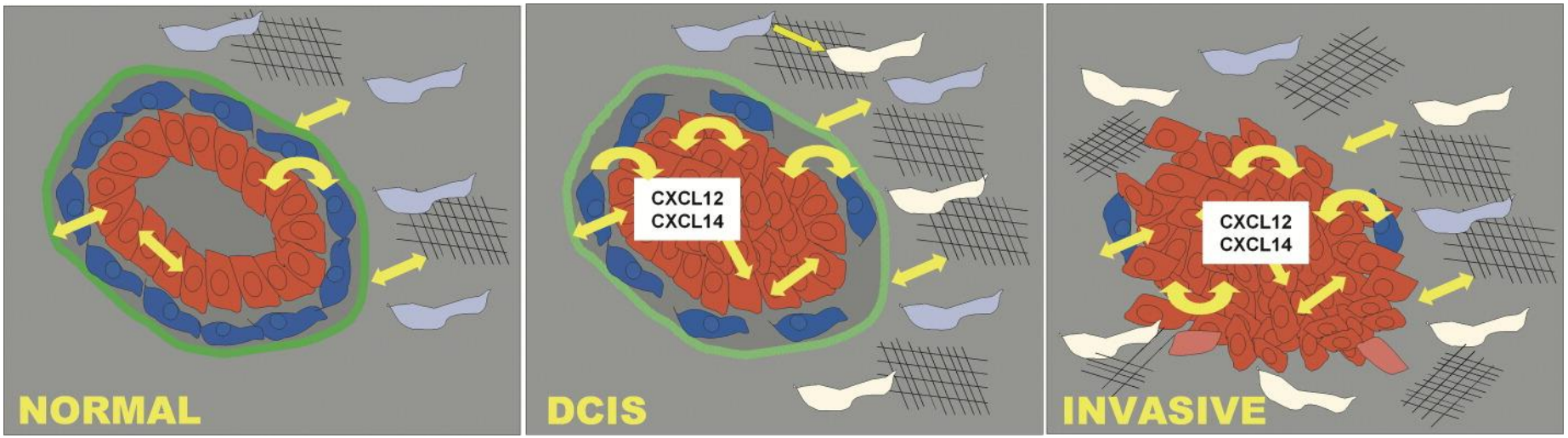


Allinen M et al., Cancer Cell 2004;6:17-32



Allinen M et al., Cancer Cell 2004;6:17-32

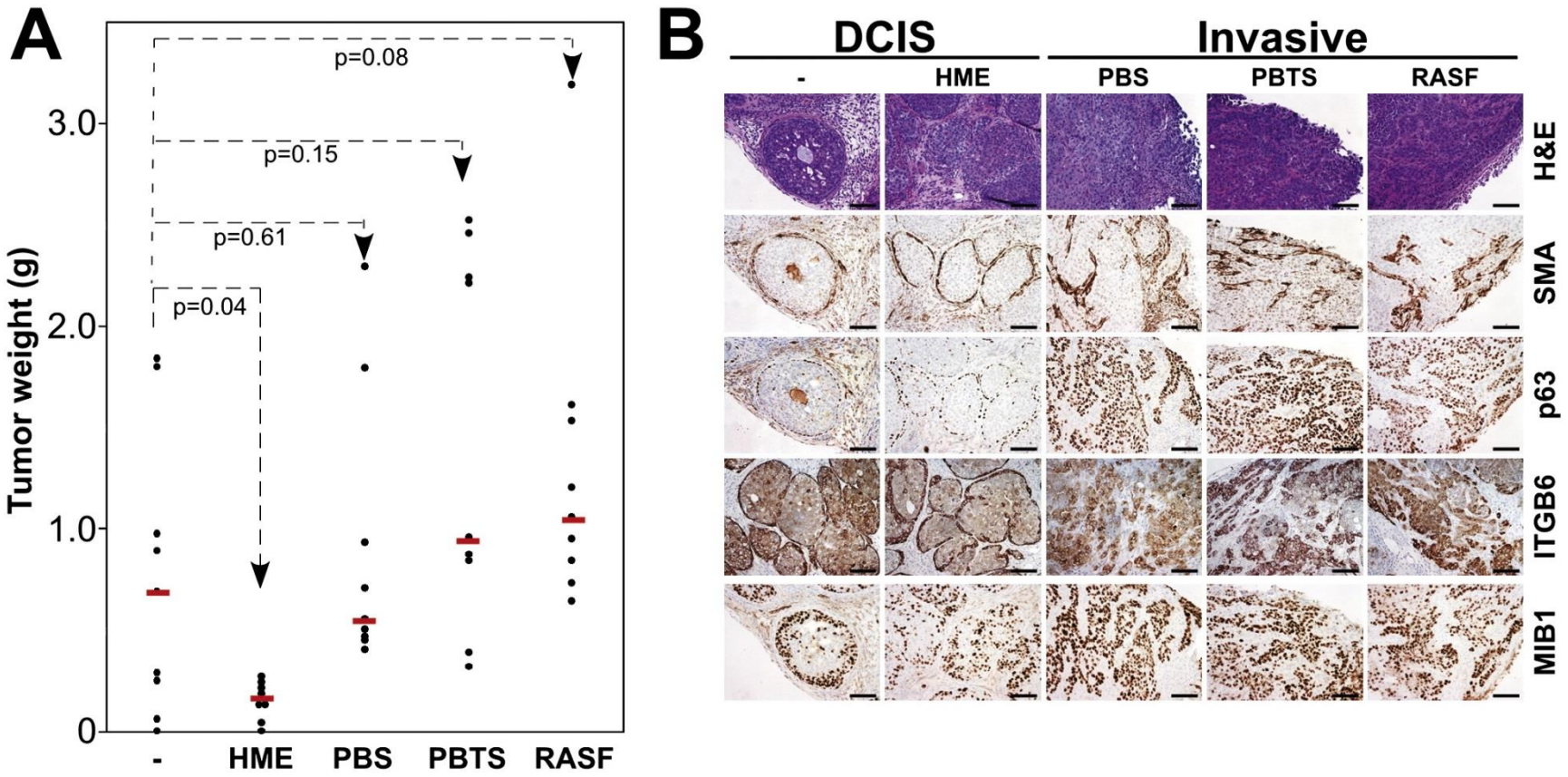
In situ to invasive breast carcinoma transition driven by chemokines released from myoepithelial cells and myofibroblasts



The CXCL14 and CXCL12 chemokines overexpressed in tumor myoepithelial cells and myofibroblasts, respectively, bind to receptors on epithelial cells and enhance their proliferation, migration, and invasion. Thus, chemokines may play a role in breast tumorigenesis by acting as paracrine factors.

Allinen M et al., Cancer Cell 2004;6:17-32
Rizki A & Bissell MJ, Cancer Cell 2004;6:1-2

Regulation of in situ to invasive breast carcinoma transition by myoepithelial cells



HME, Normal myoepithelial cells; PBS, fibroblast from normal breast; PBTS, fibroblast from breast tumor; RASF, fibroblasts form rheumatoid arthritis synovium

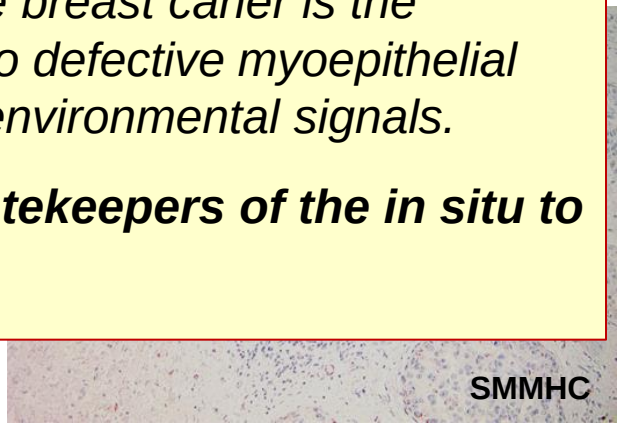
Hu M et al., Cancer cell 2008;13:394-406

Phenotypic alterations in ductal carcinoma in situ-associated myoepithelial cells: biologic and diagnostic implications

TABLE 3. Staining Intensity for 7 Myoepithelial Markers in DCIS-associated Myoepithelial Cells Compared With Staining Intensity of Myoepithelial Cells in Adjacent Normal Ducts and Lobules

Antibody (No. Evaluable)	Staining Intensity of DCIS-associated Myoepithelial Cells 3	2	1	0	No. (%) Cases With Decreased or no Expression in DCIS-associated Myoepithelial Cells (%)
SMA (100)	99	1	0	0	1/100 (1.0)
Calponin (98)	81	15	2	0	17/98 (17.4)
CD10 (88)	58	19	10	1	30/66 (34.0)
CK5/6 (96)	67	20	7	2	29/96 (30.2)
p63 (95)	83	8	3	1	12/95 (12.6)
SMMHC (98)	23	34	29	12	75/98 (76.5)
p75 (96)	92	4	0	0	4/96 (4.2)

- *A key event in the progression of in situ to invasive breast caner is the disappearance of the myoepithelial cell layer due to defective myoepithelial cell differentiation regulated by intrinsic and microenvironmental signals.*
- ***Thus, myoepithelial cells can be considered gatekeepers of the in situ to invasive carcinoma transition***



Hilson et al., Am J Surg Pathol 2009;33:227-232

Summary

- *Like invasive breast cancer, DCIS comprises a highly heterogeneous group of diseases with diverse histologic features, molecular alterations and risks of progression to invasive cancer.*
- *DCIS exhibit intra-tumoral phenotypic and genetic heterogeneity.*
- *DCIS are non-obligate precursors of invasive cancers of similar grade.*
- *Currently, it is not possible to stratify the aggressive forms of DCIS which will progress to invasive breast cancer from the indolent forms.*
- *Although the mechanisms by which DCIS progress to invasive carcinomas are not well understood, the progression from in situ to invasive carcinoma is thought to be a complex process, depending on changes in tumor cell properties and tumor microenvironment.*

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Collaborators

Seoul National University College of Medicine & Bundang Hospital

Gyeong Hoon Kang
Yu Jung Kim
Eunyoung Kang
Sun Mi Kim
Woo Kyung Moon
Jee Hyun Kim
Sung-Won Kim

Asan Medical Center

Hee Jin Lee
Gyeongyub Gong

Dana-Farber Cancer Institute

Kornelia Polyak

Lab members

Yoomi Choi
Min Hye Jang
Jae Moon Gwak
Yul Ri Chung
Mimi Kim
Eun Joo Kim
Hyun Jeong Kim