Discordant Marker Expression Between Invasive Breast Carcinoma and Corresponding Synchronous and Preceding DCIS

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Background

- **Ductal carcinoma in situ, DCIS 8500/2**
- 乳腺导管原位癌（DCIS），又称导管原位癌。是一种肿瘤性导管内病变。特征是上皮增生显著，细胞具有轻度到重度的异型性。
- DCIS平均好发年龄50-59，X线检查多数患者有显著微小钙化，近年的随访显示死亡病例的死因主要为最初诊断DCIS时未被发现的浸润性癌、残留的DCIS发展为浸润性癌或者在乳腺其他部位又发生了浸润性癌。
- **Invasive breast carcinoma, IBC 8500/3**
- 乳腺浸润性癌占乳腺癌的85%以上
- 组织学形态不一，肿瘤细胞一般体积较大，异型性明显，可呈条索状、巢状或大片实性分布。
- 部分病例可见显著的淋巴细胞、浆细胞浸润甚至出现肉芽肿反应，钙化也是常见的现象。
- 癌组织常侵犯血管、淋巴管，也常见神经周围浸润。

Background

- **Ductal carcinoma in situ (DCIS) is generally accepted as a nonobligate precursor of invasive breast carcinoma (IBC).This is because they are frequently found next to each other sharing the genetic alterations and risk factors (eg, age, family history of breast carcinoma, etc.).**
- **It has been shown that the histological grade of the DCIS component adjacent to invasive disease (synchronous DCIS) and the grade of the IBC lesion are significantly correlated, that is, well-differentiated DCIS relates to grade I IBC and poorly differentiated DCIS to grade III IBC.**
- **Several studies aimed to find markers involved in DCIS progression to IBC, mostly by comparing IBC lesions and an adjacent DCIS component, referred to as synchronous DCIS.**

- **However, it has never been investigated whether the synchronous DCIS and IBC comparisons are a good surrogate for primary DCIS and subsequent IBC.**
- **Therefore, we performed a comparative analysis between primary DCIS and subsequent ipsilateral IBC, and between this IBC and the adjacent synchronous DCIS component on the basis of immunohistochemical marker expression.**
- **With this, we aimed to (1) assess the concordance in marker expression between primary DCIS and subsequent ipsilateral IBC, and IBC and synchronous DCIS and (2) to identify factors that may explain the potential discordance in marker expression.**

Background

- **COX-2（Cyclooxygenase-2）环氧化酶-2**
- **环氧化酶2型(COX-2)在许多人类恶性肿瘤中过表达，并已被发现与肿瘤的发生发展(*Liu et al, 2001*)，肿瘤存活(*Tsujii et al, 1998*)，侵袭(*Tsujii et al, 1997*)和转移(*Tsujii and DuBois, 1995;Costa*等人，*2002*)密切相关**
- **COX-2的上调多发生在癌变过程的早期，在许多癌前病变和非乳腺腺上皮原位癌中均有过表达(*Eberhart*等，*1994;Kirschenbaum*等人，*2000;Shirahama, 2000;Morris*等，*2001*)**
- **研究发现Cox-2表达升高与乳腺癌不良预后相关。**

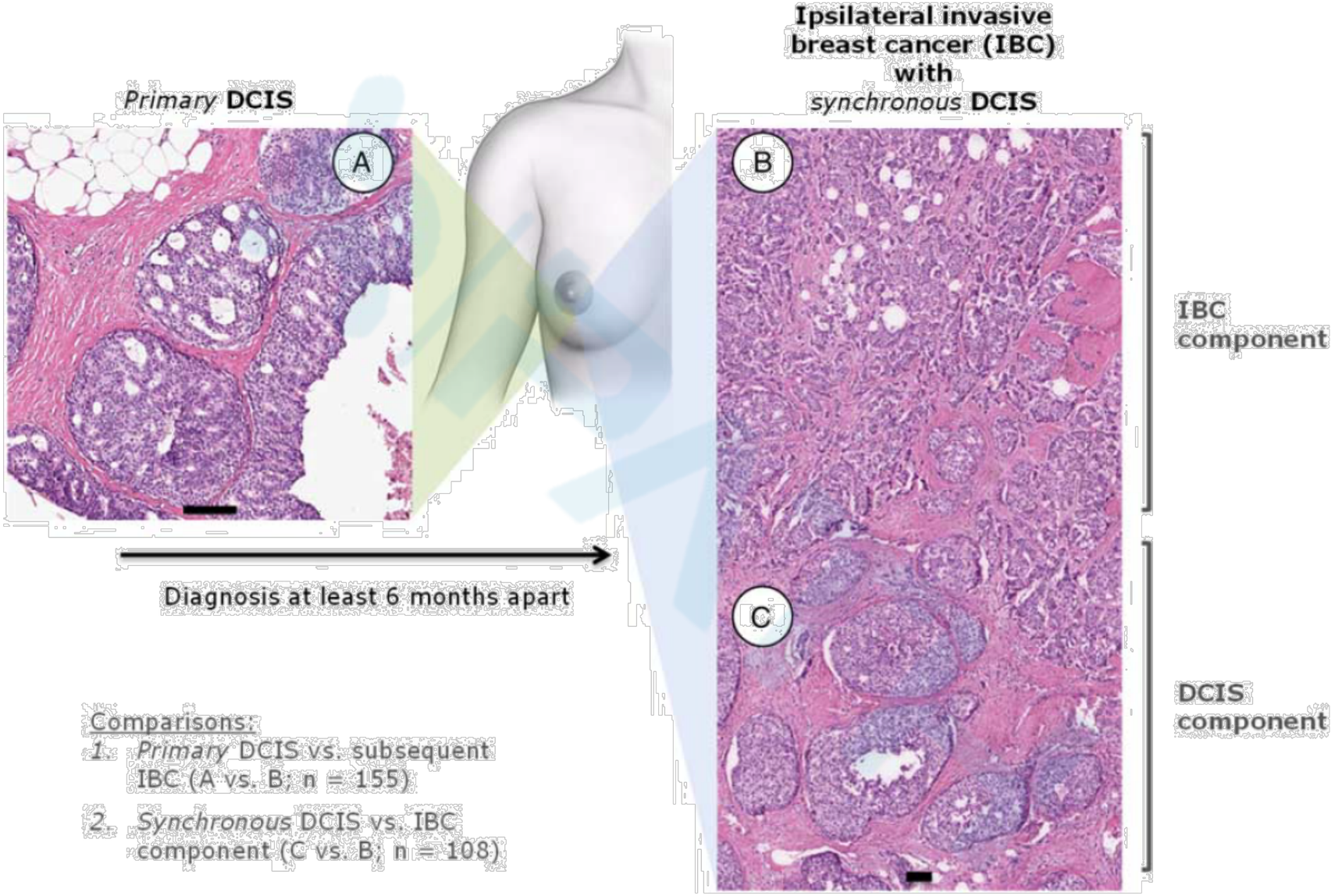


FIGURE 1. Set-up of the study and the number of included lesions. Of 155, there were 108 IBC lesions that harbored a synchronous DCIS component. A, Primary DCIS. B, IBC. C, Synchonous DCIS component.

Patients and Methods

- **Study Population and Design**

✓ **Source: Netherlands Cancer Registry (NCR);**

✓ **All women diagnosed with primary DCIS within the Netherlands and treated with breast-conserving surgery alone between January 1, 1989 and December 31, 2004 (n =2,658);**

✓ **The median follow-up was 12.0 years (interquartile range, 9.0 to 15.3);**

✓ **This resulted in 155 patient-matched primary DCIS and subsequent IBC pairs.**

Patients and Methods

- **DCIS and IBC lesions were categorized into the following IHC subtypes:**
- **hormone receptor (HR)+ HER2−, HR+ HER2+, HR− HER2+, and HR− HER2−.**
- **Lesions were classified as HR+ when ER and/or PR were/was scored as positive.**
- **Lesions were classified as HR− when both ER and PR were negative.**

Results-Baseline Characteristics

- A total of 108 of these 155 lesions (69.7%) had a DCIS component adjacent to the invasive disease (synchronous DCIS). The mean time to invasive recurrence was 6.3 years (range 0.5 to 17.0 y). An overall, 79.4% of the invasive recurrences occurred in the same quadrant as the initial DCIS.
- Immunohistochemical staining was performed for 142 of 155 primary DCIS and subsequent IBC pairs and 81 of 108 IBC and synchronous DCIS pairs. The frequency of ER, PR, and COX-2 positivity was similar in primary DCIS, IBC, and synchronous DCIS (Table 1).

Results

TABLE 1. Overview of Immunohistochemical Marker Expression of Primary DCIS, Invasive Breast Cancer, and Synchronous DCIS

	n (%)		
Characteristics	**Primary DCIS (n = 142)**	**Invasive BC (n = 142)**	**Synchronous DCIS (n = 81)**
IHC subtype			
HR+ HER2−	90 (63.4)	96 (67.6)	56 (69.1)
HR+ HER2+	28 (19.7)	23 (16.2)	8 (9.9)
HR− HER2+	21 (14.8)	14 (9.9)	12 (14.8)
HR− HER2−	3 (2.1)	9 (6.3)	5 (6.2)
ER			
Negative	24 (16.9)	23 (16.2)	17 (21.0)
Positive	118 (83.1)	119 (83.8)	63 (77.8)
NA	0 (0.0)	0 (0.0)	1 (1.2)
PR			
Negative	51 (35.9)	58 (40.8)	31 (38.3)
Positive	90 (63.4)	84 (59.2)	50 (61.7)
NA	1 (0.7)	0 (0.0)	0 (0.0)
HER2			
Negative	93 (65.5)	105 (73.9)	61 (75.3)
Positive	49 (34.5)	37 (26.1)	20 (24.7)
p53			
0% positive cells (mutant)	12 (8.5)	18 (12.7)	6 (7.4)
1%-70% positive cells (WT)	108 (76.1)	101 (71.2)	65 (80.2)
> 70% positive cells (mutant)	21 (14.8)	23 (16.2)	10 (12.4)
NA	1 (0.7)	0 (0.0)	0 (0.0)
COX-2			
Low	16 (11.3)	17 (12.0)	13 (16.0)
High	125 (88.0)	124 (87.3)	67 (82.7)
NA	1 (0.7)	1 (0.7)	1 (1.2)

- **Four IBC and synchronous DCIS pairs consisted of ER-positive IBC and an ER-negative synchronous DCIS component (P=0.046; Table 2), although this number is too small to draw any conclusions from.**

TABLE 2. Marker Expression of Synchronous DCIS Related to Invasive Component

	Invasive Component (n)			
	Negative/ Low/WT	Positive/ High/ Mutant	Agreement (%)	Symmetry (*P*)
Synchronous DCIS (n)				
ER				
Negative	13	4		
Positive	0	63	95.0	0.046
PR				
Negative	25	6		
Positive	10	40	80.3	0.32
HER2				
Negative	58	3		
Positive	1	19	95.1	0.32
P53				
WT	57	8		
Mutant	2	14	87.7	0.06
COX-2				
Low	7	6		
High	4	63	87.5	0.53

Agreement was calculated by nonweighted κ; *P*-values were calculated by asymptotic symmetry test.

Total number of pairs included: ER n = 80; PR n = 81; HER2 n = 142; p53 n = 140; COX-2 n = 140.

ER/PR positive: > 10% positive cells; HER2 positive: membrane score 3 or membrane score 2 confirmed by CISH; p53 wild-type (WT): 1% to 70% positive cells; p53 mutant: > 70% positive cells or complete lack of p53 expression; COX-2 high: score 2 to 3.

- **Notably, 18 of 49 patients (36%) had an HER2-positive primary DCIS, which was followed by an HER2-negative IBC (P =0.014; Table 3).**

TABLE 3. Immunohistochemical Marker Expression of Primary DCIS Related to Subsequent Invasive Breast Cancer

	Invasive Breast Cancer (n)			
	Negative/ Low/WT	Positive/ High/ Mutant	Agreement (%)	Symmetry (*P*)
Primary DCIS (n)				
ER				
Negative	15	9		
Positive	8	110	88.0	0.81
PR				
Negative	35	16		
Positive	23	67	72.3	0.26
HER2				
Negative	87	6		
Positive	18	31	83.1	0.014
P53				
WT	88	29		
Mutant	12	21	77.3	0.16
COX-2				
Low	3	13		
High	14	110	80.7	0.85

Discordant marker expression is more frequently observed between primary DCIS and subsequent IBC, as compared with synchronous DCIS and IBC

- **IBC and synchronous DCIS were discordant for ER, PR, HER2, p53, and COX-2 marker expression in 5.0%, 19.7%, 4.9%, 12.3%, and 12.5% of the pairs, respectively.**
- **Marker expression of primary DCIS and the subsequent ipsilateral IBC was discordant for ER, PR, HER2, p53, and COX-2 expression in 12.0%, 27.7%,16.9%, 22.7%, and 19.3% of the pairs, respectively.**

TABLE 4. Percentage Discordance in Grade and Marker Expression Between Pairs of DCIS and IBC

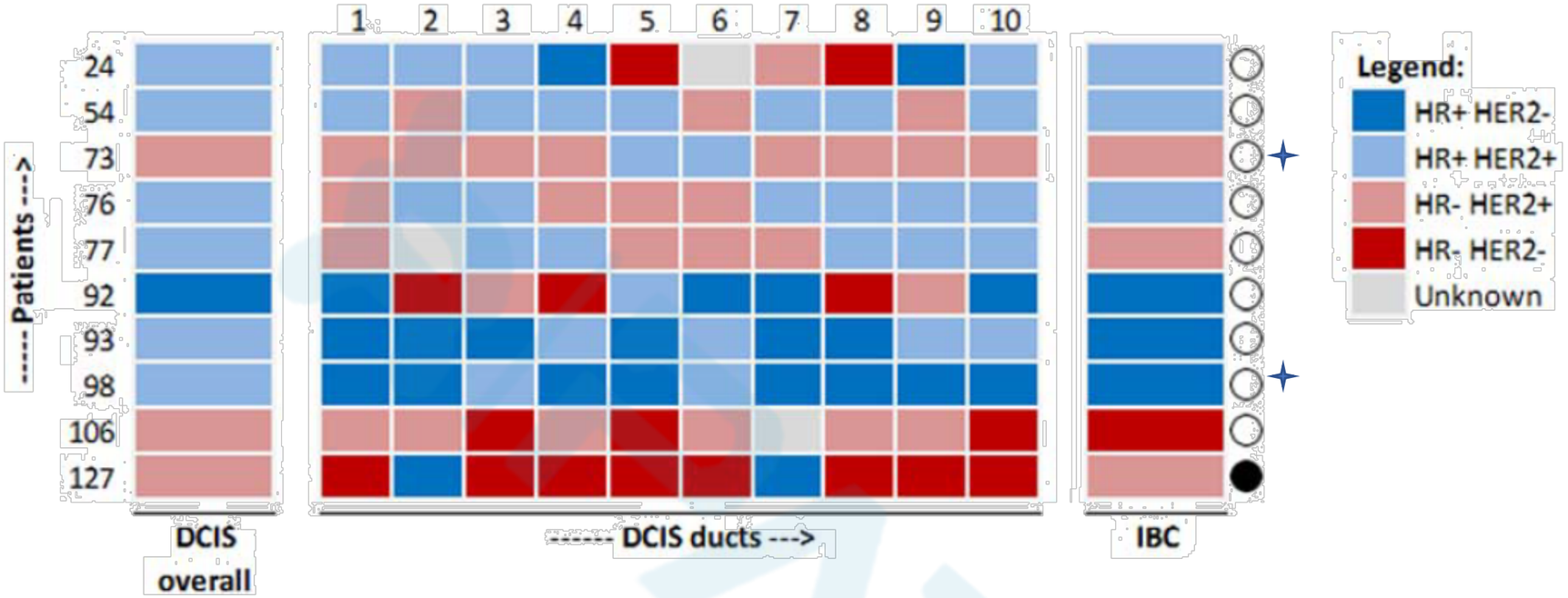
	sDCIS vs. IBC		✡ pDCIS vs. IBC ✡		pDCIS vs. sDCIS	
	Discordance (%)	Symmetry (*P*)	Discordance (%)	Symmetry (*P*)	Discordance (%)	Symmetry (*P*)
IHC						
ER	5.0	0.046	12.0	0.81	13.7	0.76
PR	19.7	0.32	27.7	0.26	29.6	0.68
HER2	4.9	0.32	16.9	0.014	13.6	0.007
p53	12.3	0.06	22.7	0.16	22.5	0.64
COX-2	12.5	0.53	19.3	0.85	25.0	0.37
Subtype	9.9	0.09	23.2	0.040	16.0	0.17

Discordance of marker expression is not associated with time to event

- **The study group was divided by the median time to IBC. While 56.9% of the women who developed IBC within 6.1 years after their DCIS diagnosis showed discordant marker expression between primary DCIS and subsequent IBC involving at least 1 IHC marker, this was 64.3% in the group of women who developed IBC after >6.1 years after their DCIS diagnosis (P = 0.37).**
- **These data suggest that the probability of discordant marker expression between the primary DCIS and subsequent IBC does not increase with longer time to IBC.**

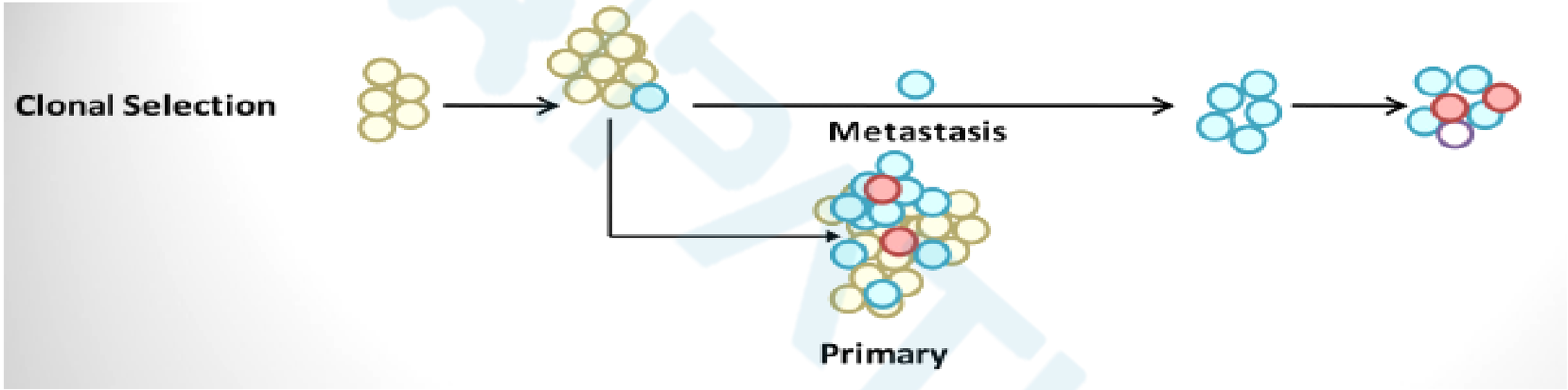
Specific subclones might be responsible for the invasive outgrowth

- **Discordant marker expression could be caused by heterogeneity within the DCIS lesion.**
- **Therefore, we assessed IHC staining in 10 individual ducts per DCIS lesion.**
- **In 10 of 94 DCIS lesions (10.6%), we observed heterogeneity, defined by the presence of multiple IHC subtypes, or subclones, within one DCIS lesion.**



- **In 9 of the 10 pairs, the subtype of the IBC lesion was shared with a subclone of the DCIS lesion. In 2 of these DCIS lesions, 4 different IHC subtypes were present, and 7 DCIS lesions consisted of 2 different IHC subtypes. In one pair, the subtype of the IBC lesion was not shared with any of the DCIS subclones.**
- **These results show that intralesional heterogeneity exists within DCIS lesions. This may be causative for the discordant marker expression between DCIS and IBC.**

The clonal Selection of tumor



Talmadge, Cancer Research. 2010

DISCUSSION

- **In this study, we demonstrated that comparative analysis between primary DCIS and subsequent ipsilateral IBC versus IBC and adjacent synchronous DCIS yields different results. This was most prominently illustrated for HER2, as we found that 36% of HER2-positive primary DCIS lesions were followed by HER2-negative IBC.**
- **If the overexpression of HER2 plays a major role in DCIS progression, the overexpression of HER2 in IBC might be expected to be equal or exceeding the level of the preceding DCIS.**

DISCUSSION

- **It could be hypothesized that HER2 overexpression promotes a higher proliferative rate, but does not lead to a higher invasive potential of DCIS.**
- **In the current study, we showed that the level of COX-2 expression is almost similar when comparing primary DCIS and subsequent IBC. This may suggest that COX-2 could play a role in the invasive outgrowth of DCIS.**
- **Yet, the frequency of discordant marker expression between primary DCIS and subsequent IBC did not increase with longer time to IBC.**

Limitations

- **First, our study group consisted of women who were all treated for DCIS by BCS alone. DCIS treated by BCS carries a risk of recurrent disease, but the origin of the subsequent IBC after primary DCIS could be (1) from residual DCIS that was left behind after BCS, or (2) unrelated to the preceding DCIS, and thus be a second primary tumor.**
- **Second, we cannot exclude the possibility of receptor measurement error as the source of discordance in marker expression.**
- **Third, for the intralesional heterogeneity analysis, inclusion of more heterogeneously expressed IHC markers would be more informative when assessing heterogeneity within DCIS lesions, as now we only found 10 cases of heterogenous DCIS based on IHC subtype.**

Summary

- **Marker expression between primary DCIS and subsequent IBC is less concordant than synchronous DCIS and IBC.**
- **HER2 marker expression showed the largest discrepancy: 36% of HER2-positive primary DCIS lesions were followed by HER2-negative IBC.**
- **The frequency of discordant marker expression between primary DCIS and subsequent IBC did not increase with longer time to IBC.**

Summary

- **Intralesional heterogeneity was identified as a possible cause of the observed discordant marker expression.**
- **We suggest that future studies investigating the progression of DCIS to IBC, should study primary DCIS and subsequent IBC, instead of synchronous DCIS and IBC lesions.**

Thanks For Your Attention