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Cytokeratin profiles of male breast cancers

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Aims: The prognostic factors and expression of molecular markers in male breast carcinomas are similar to those in female breast cancers. The identification of distinct cytokeratin (CK) profiles (basal as opposed to luminal cells) helps to identify subsets of tumours with different clinical behaviour. The aim of this study was to investigate CK expression in male breast cancer.

Methods and results: Thirty-two cases of male breast cancer were studied. The panel of CKs studied by immunohistochemistry included: 5/6, 14, 17, 18 and 19. Pathological findings and CK expression were analysed in all cases. Histological patterns included ductal carcinoma *in situ*, invasive ductal carcinoma and mixed patterns. Four cases were positive for CK5/6 and CK14, identifying a basal-like phenotype. CK17 was negative in all but two cases. All cases expressing either CK5/6 or CK14 were invasive carcinomas of high nuclear and histological grade and were also larger compared with the tumours not expressing CK5/6 and CK14. All tumours except three (also negative for CK5/6) expressed CK18 and CK19. The four basal-like tumours were negative for Her-2 expression.

Conclusions: Male breast carcinomas have a basal-like phenotype that is similar in frequency to that of female breast carcinomas. The expression of CK5/6 and CK14 identifies a subset of pathologically aggressive male breast cancers.

Keywords: cytokeratin expression, immunohistochemistry, male breast cancer

Abbreviations: CK, cytokeratin; ER, oestrogen receptor

Introduction

Male breast cancer represents < 1% of all breast cancer diagnoses. However, a mortality of 31% is considerably higher than for breast cancer in women.[1,2] The incidence of male breast cancer has remained stable and such tumours are usually detected at an advanced stage compared with female breast cancers that are detected more frequently as subclinical tumours. Male breast cancers are treated following the same therapeutic guidelines applied to female cancers with a combination of chemotherapy and adjuvant hormonal therapy.[1,2]

Pathologically, the vast majority of male breast cancers are invasive ductal carcinomas.[3] The *in situ* carcinomas are of ductal subtype with a predominance of papillary subtypes. Only a small number of lobular carcinomas have been reported.[3,4] Also, a higher percentage of male breast carcinomas are oestrogen-receptor (ER) positive compared with their female counterparts.[3,5] The same prognostic factors for female breast carcinomas such as tumour size, histological grade and lymph node status are important in male

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breast carcinomas.[1–3,5,6] Recent studies have shown that male breast cancers have a similar prognosis to their female counterparts when matched for age and stage.[7]

Compared with female breast carcinoma, there is relatively little information about the molecular mechanisms involved in male breast carcinoma. For example, specific gene expression profiles and cytokeratin (CK) phenotypes have allowed characterization of female breast carcinomas into separate groups showing different behaviour and response to therapy.[8–16] The expression of CK5/6 and CK17, markers that identify a basal-like phenotype, have been linked to more aggressive carcinomas in women.[8] Those carcinomas characterized by a luminal-like CK expression profile (CKs 5/6 and 17 negative, and 8, 18 and 19 positive) are regarded as less aggressive breast carcinomas, especially if they are also ER+.[8]

In this study, we used immunohistochemistry to investigate CK expression profiles of 32 male breast carcinomas to elucidate the CK composition of these tumours and its possible significance.

Materials and methods

All the male breast cancers were obtained from the files of the Departments of Pathology of Thomas Jefferson University Hospital; Girard Medical Center, Philadelphia, PA; University of Texas Medical Branch at Galveston; Ospedale S. Eugenio, Rome, Italy and Ospedale di Melegnano, Milan, Italy. Approval of the study protocol was obtained from the Jefferson Internal Review Board and from each participating institution. Fresh frozen samples were obtained from four male breast cancers that underwent surgery at Thomas Jefferson Hospital.

A total of 32 cases were reviewed to confirm the diagnosis and to characterize each tumour. The following information was obtained in each case: age of the patient, tumour subtype, size, nuclear and histological grades and ER status. The histopathological evaluation of the tumours was carried out following the guidelines of the Breast Cancer Consensus Conference.[17] Briefly, low-grade tumours (histological and nuclear grading) are considered 1, high-grade cancers are assigned a 3 and intermediate grade tumours a 2.

The following CK immunohistochemistry was performed: 5/6, 14, 17, 18 and 19 (Dako, Carpinteria, CA, USA) in a Dako autostainer. For the CK immunohistochemistry, only cytoplasmic immunoreactivity was considered positive. The stains were interpreted as follows: 1+, up to 25% of cells staining; 2+, between 25 and 50% of cells staining; and 3+, > 50% of cells staining. Cytoplasmic staining with CK5/6 and CK14 of any intensity was considered positive. Whether the stains were positive in the *in situ*, invasive or both components was also analysed.

ER was interpreted as positive if > 20% of the cells were staining. Normal skin and tonsils were used as positive controls for the CK and a known breast cancer for the ER immunohistochemistry. The four cases that expressed the basal-like phenotype were stained for Her-2 using the HercepTest™ (Dako Corp., Hamburg, Germany). For the negative controls the same steps were followed except that the primary antibodies were omitted.

Results

All of the male tumours, *in situ* and invasive, were of the ductal type. Four of the 32 cases were purely *in situ* ductal carcinomas while the other 28 cases showed only invasive or a mixture of invasive and *in situ* ductal carcinomas. The size of the tumours ranged from 2 mm (*in situ* ductal carcinoma) to 40 mm (invasive carcinoma).

Luminal- and basal-like cytokeratins were positive in the invasive and *in situ* components. However, in one of the basal-like tumours the *in situ* component showed no expression of CK5/6. All the cancers except three expressed CK18 and CK19 and only two cases were positive for CK17 (including one with a basal-like phenotype). CK18 and CK19 showed diffuse positive expression with > 80% of the cells staining. Four cases showed staining of the invasive component with CK5/6 and CK14, thus identifying these tumours as having a basal-like phenotype.[9] In three of the four basal-like tumours, 25–50% of tumour cells stained with CK5/6. In the fourth case, < 25% of tumour cells stained with CK5/6. In two of the four basal-like tumours, CK14 was expressed in 50–75% of tumour cells. In the remaining two cases, 25% of tumour cells stained with CK14. These four tumours measured ≥ 20 mm, had *in situ* and invasive components and were of intermediate or high nuclear and histological grades (2 or 3).

Of the four cases that expressed a basal-like phenotype, three cases expressed ER. The four cases were negative for Her-2. Two of the four patients with basal-like phenotype tumours showed clinical evidence of disseminated metastatic disease beyond the axillary lymph nodes. The other two cases had metastases in the axillary lymph nodes. The pathological and immunohistochemical features of all tumours are summarized in Table 1. Examples of CK immunoreactivity representative of basal-like tumours are illustrated in Figures 1 and 2.

Table 1. Pathological and immunohistochemical features of all male breast cancers

Age, years	Diagnosis	Size, mm	Nuclear grade	Histological grade	CK5/6	CK14	CK18	TNM stage
69	IDC + DCIS	20	III	II	++	+++	+++	pT1N2Mx
61	IDC + DCIS	23	III	III	++	+++	+++	pT2N1Mx
76	IDC + DCIS	25	II	II	++	+	+++	pT2NxM2
80	IDC + DCIS	25	II	III	+	+	++	pT2NxM2
64	DCIS	2	II	II	–	–	+++	Tis
69	DCIS	30	II	II	–	–	+++	pTisN1Mx
76	IDC + DCIS	7	I	II	–	–	–	pT1N0Mx
91	IDC	30	II	II	–	–	+++	pT2N0Mx
72	IDC + DCIS	10	II	II	–	–	+++	pT1NxMx
88	IDC + DCIS	60	II	II	–	–	+ F	pT3NxMx
78	IDC + DCIS	12	III	III	–	–	++	pT1NxMx
63	DCIS	7	II	II	–	–	+++	Tis
63	IDC + DCIS	11	II	II	–	–	+++	pT1N0Mx
68	IDC	14	II	II	–	–	+++	pT1NxMx
61	IDC	17	III	III	–	–	+++	pT1N1M0
67	IDC	7	II	II	–	–	+++	pT1N1M0
41	DCIS	7	II	II	–	–	+	Tis
82	IDC	10	III	II	–	–	+++	pT1N0M0
65	IDC	17	I	I	–	–	+++	pT1N0M0
80	IDC	25	I	I	–	–	+++	pT2N1M0
79	IDC + DCIS	37	III	III	–	–	+++	pT2N1M0
59	IDC	35	I	I	–	–	+++	pT2N0Mo
76	IDC	40	III	III	–	–	+++	pT2N1M0
55	IDC + DCIS	22	II	II	–	–	+++	pT2N0Mo
73	IDC	20	II	III	–	–	+++	pT1N1Mx
73	DCIS	10	II	II	–	–	–	Tis
59	IDC	25	III	III	–	–	++	pT2N0Mx
65	IDC	20	II	II	–	–	+++	pT1N1Mx
71	DCIS	30	II	II	–	–	+++	Tis
78	IDC + DCIS	8	II	II	–	–	+++	pT1N0Mx
74	IDC	27	II	II	–	–	+++	pT2N1Mx
70	IDC + DCIS	17	III	III	–	–	+++	pT1N0Mx

IDC, Invasive ductal carcinoma; DCIS, ductal carcinoma *in situ*; –, negative; +, ++ and +++, <25%, 25–50% and >50%, respectively.

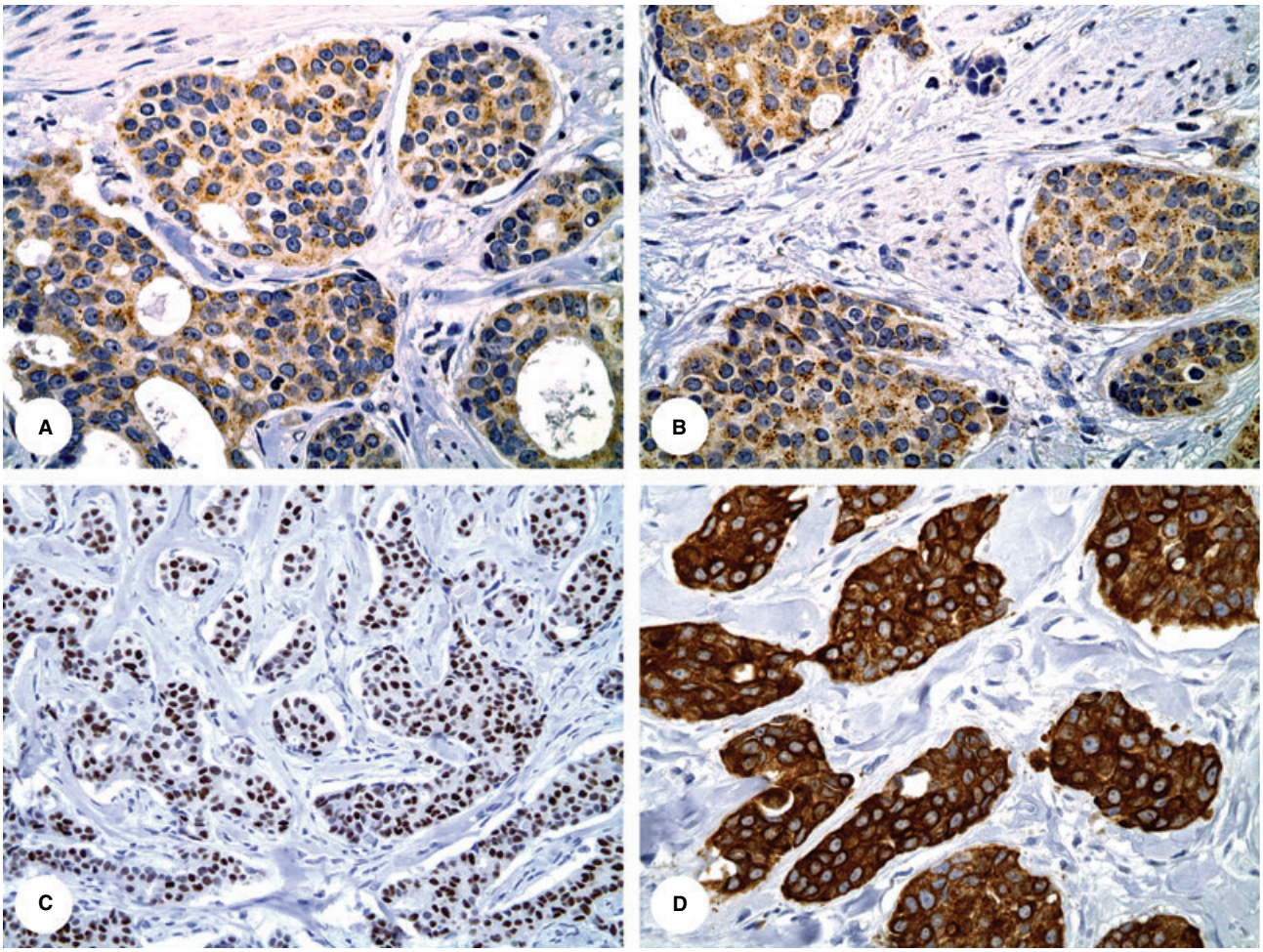

Figure 1. Male breast cancer with a basal phenotype. **A, B, C and D,** Cytokeratins (CK) 5/6, 14, oestrogen receptor and CK18, respectively. The invasive carcinoma cells show diffuse cytoplasmic immunoreactivity.

Discussion

Male breast cancers comprise a small percentage of all breast tumours. Markers of increased risk for male breast cancer include a positive family history of cancer, gynaecomastia and Klinefelter's syndrome.[1] Male breast cancers are usually invasive ductal carcinomas with fewer *in situ* carcinomas. The parameters associated with a worse prognosis in male breast cancer patients are lymph node status, the number of positive lymph nodes and nipple involvement.[1,2]

Recent studies of breast cancers have identified non-morphological parameters as predictors of survival of breast cancer patients.[12] Several studies have analysed markers to determine their prognostic significance in male breast cancer.[5,6,18]

The immunohistochemical expression of CK subtypes and the analysis of gene expression by profiling studies have revealed important aspects in female breast cancers.[8–16,19] CKs have been shown to play a role in female breast carcinogenesis and the invasive properties of breast tumours.[8,14,20] CK expression is tightly regulated and correlates with the origin of the cells in the ducts. Basal cells express CKs 5, 6, 14 and/or 17 (basal/myoepithelial cell phenotype) and luminal-like cells express CKs 8, 18 and 19. A shift occurs in the expression of CKs during breast tumorigenesis with increased CK18 expression and a marked decreased of CK5 expression.[13,14]

Using Her-2 and gene expression profiles, four groups of breast carcinoma have been identified: Her-2+, Her-2–, hormone receptor negative with a basal phenotype, and Her-2– with hormone receptor expression, favourable prognosis; and Her-2– with hormone receptor expression, unfavourable prognosis.[9,10]

To our knowledge, this is the first study to analyse this group of cytokeratins in male breast cancers. Our findings illustrate the complex interactions involving

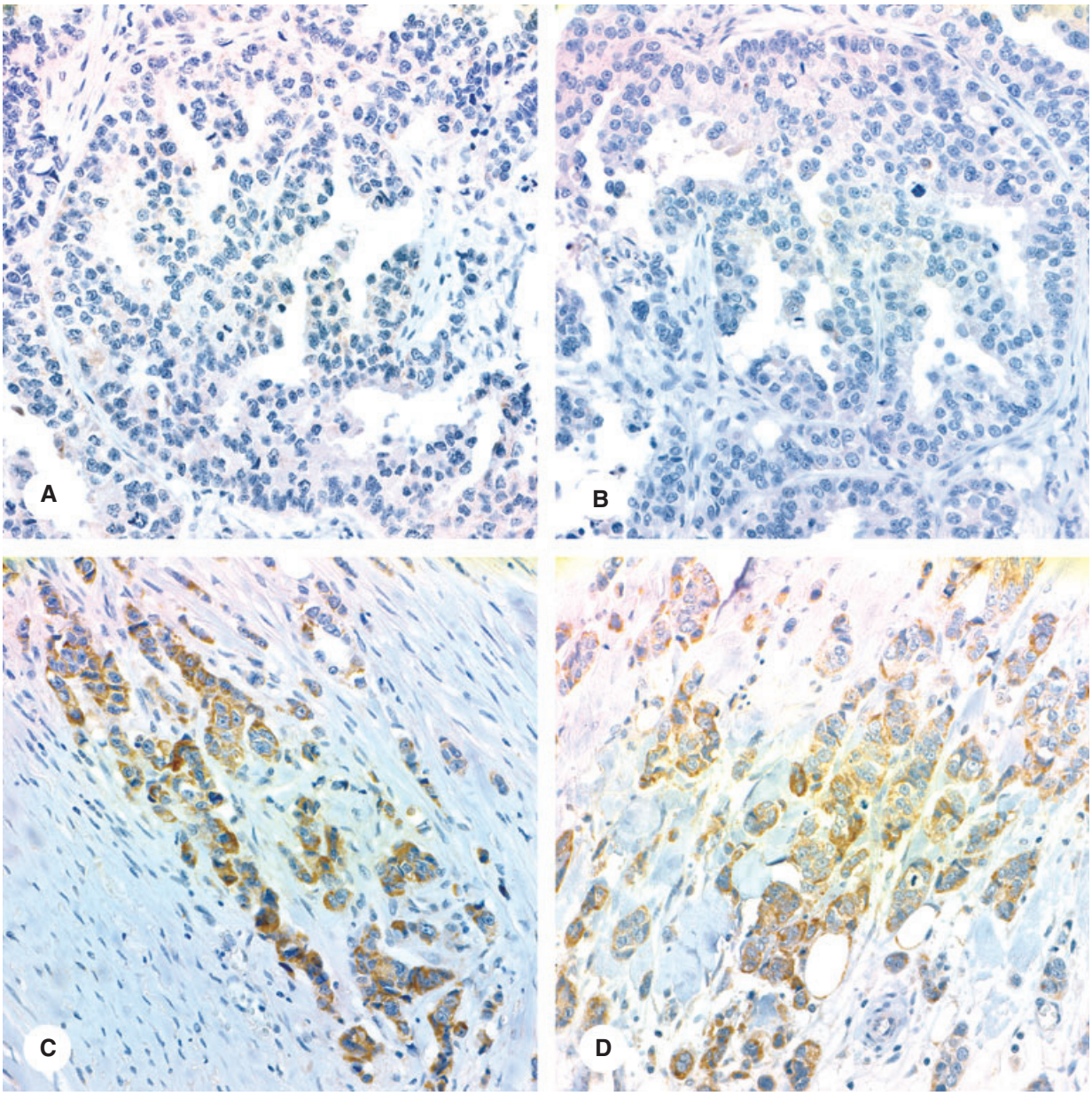

Figure 2. *In situ* and invasive male breast cancer: *in situ* component showing lack of expression of cytokeratin (CK) 5/6 (**A**) and CK14 (**B**). Invasive carcinoma which shows cytoplasmic staining of CK5/6 (**C**) and CK14 (**D**).

cytokeratins in male breast cancer initiation and progression. We have found that the vast majority of these tumours express the more common phenotype of luminal-like CKs. The expression profiling of four of our cases, showing a luminal-like phenotype, confirmed the up-regulation of the genes controlling these cytokeratins (data not shown).

A small subset of tumours expresses a distinct pattern of CKs (5/6 and 14) characteristic of basal-like tumours. From the number of male cases expressing CK5/6 and CK14, the incidence of basal-like tumours is similar to that of female breast cancers (approximately 10–15% with a higher incidence in African-Americans).[8] These tumours were of larger size, higher nuclear grade and presented initially at an advanced stage compared with those tumours that expressed a luminal-like phenotype. This finding is similar to the association between basal-like tumours and higher-grade cancers in female patients.[8]

In our study, CK5/6 and CK14 were identified in the *in situ* and invasive components of the basal-like tumours except for one tumour which did not express

CK5/6 in the *in situ* component. This is an interesting finding and suggests that CK5/6 and CK14 play an important role in the progression of breast cancers, with consistent expression of this phenotype in invasive tumours. Another interesting aspect of this group of tumours is that three of the four cases expressed ER. In female breast cancers, there is an inverse correlation between the basal-like phenotype and the expression of ER, with only 30% of basal-like tumours expressing ER.[8] In our series of luminal-like tumours we were able to stain 10 cases for ER and they were all positive. Abd El Rehim *et al*. found that 33% of tumours that expressed CK5/6 also expressed ER in female breast cancers.[11] We do not know if ER expression in male breast cancers is unique to these tumours, even though they seem to share other CK profiles with female cancers. Given the small number of tumours in our series expressing this phenotype, it is possible that there are two independent mechanisms of cell growth and differentiation. This would explain the difference in male breast cancers from the usual expression of basal-like CK and lack of ER expression described in female tumours. Regarding the Her-2 findings in the four cases expressing a basal-like phenotype, our results are similar to those in a series that reported Her-2 expression in only 15% of all male cancers.[21] The only study that has analysed Her-2 expression in female cancers with a basal-like phenotype also found a consistent lack of Her-2 expression.[16]

We conclude that a subset of male breast cancers shows a basal-like phenotype as detected by the expression of CK5/6 and CK14. Compared with the more common luminal-like tumours, a basal-like phenotype is associated with more aggressive tumours. Two of the four patients with basal-like tumours presented initially with clinical evidence of metastatic disease. The lack of expression of Her-2 parallels the finding in female breast cancers and this should be analysed for its prognostic significance. Based on morphology alone, distinguishing male cancers that are luminal or basal-like cannot be determined; however, the analysis of a panel of CKs can help identify a subset of patients with more aggressive tumours.

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References

1. Giordano SH, Buzdar AU, Horobagyi GN. Review. Breast cancer in men. *Ann. Intern. Med.* 2002; **137;** 678–687.
2. Donegan WL, Redlich PN, Lang PJ, Gall MT. Carcinoma of the breast in males. A multiinstitutional survey. *Cancer* 1998; **83;** 498–509.
3. Tavassoli FA. *Pathology of the breast*, 2nd edn, Chapter 16. New York: McGraw-Hill 1999; 829.
4. Hittmair AP, Lininger RA, Tavassoli FA. Ductal carcinoma *in situ* (DCIS) in the male breast. A morphologic study of 84 cases of pure DCIS and 30 cases of DCIS associated with invasive carcinoma—a preliminary report. *Cancer* 1998; **83;** 2139–2149.
5. Joshi MG, Lee AKC, Loda M *et al.* Male breast carcinoma: an evaluation of prognostic factors contributing to a poorer outcome. *Cancer* 1996; **77;** 490–498.
6. Wang-Rodriguez J, Cross K, Gallagher S *et al.* Male breast carcinoma: correlation of ER, PR, Ki-67, Her-2 Neu, and p53 with treatment and survival, a study of 65 cases. *Mod. Pathol.* 2002; **15;** 853–861.
7. Anderson WF, Althuis MD, Brinton LA *et al.* Is male breast cancer similar or different than female breast cancer? *Breast Cancer Res. Treat.* 2004; **83;** 7–10.
8. Van de Rijn M, Perou CM, Tibshirani R. *et al.* Expression of cytokeratins 17 and 5/6 identifies a group of breast carcinomas with poor clinical outcome. *Am. J. Pathol.* 2002; **161;** 1991–1996.
9. Perou CM, Sorlie T, Eisen MB *et al.* Molecular portraits of human breast tumors. *Nature* 2000; **406;** 747–752.
10. Burstein H. The distinctive nature of Her2-positive breast cancers. *N. Engl. J. Med.* 2005; **353;** 1652–1654.
11. El-Rehim DMA, Pinder SE, Paish CE *et al.* Expression of luminal cytokeratins in human breast carcinoma. *J. Pathol.* 2004; **203;** 661–671.
12. van de Vijver MJ, He YD, van't Veer L *et al.* A gene-expression signature as a predictor of survival in breast cancer. *N. Engl. J. Med.* 2002; **347;** 1999–2009.
13. Trask DK, Band V, Zajchowski DA *et al.* Keratins as markers that distinguish normal and tumor-derived mammary epithelial cells. *Proc. Natl Acad. Sci. USA* 1990; **87;** 2319–2323.
14. Malzahn K, Mitze M, Thoenes M *et al.* Biological and prognostic significance of stratified epithelial cytokeratins in infiltrating ductal breast carcinomas. *Virchows Arch.* 1998; **433;** 119–129.
15. Jones CJ, Grigoriadis A, Cossu A *et al.* Expression profiling of purified normal human luminal and myoepithelial breast cells: identification of novel prognostic markers for breast cancer. *Cancer Res.* 2004; **64;** 3037–3045.
16. Nielsen TO, Hsu FD, Jensen K *et al.* Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin. Cancer Res.* 2004; **10;** 5367–5374.
17. Schwartz GF, Lagies MD, Carter D *et al.* Consensus conference on the classification of ductal carcinoma *in situ*. *Cancer* 1997; **80;** 1796–1802.
18. Pich A, Margaria E, Chiusa L. Oncogenes and male breast carcinoma: c-erb-2 and p53 coexpression predicts a poor survival. *J. Clin. Oncol.* 2000; **18;** 2948–2956.
19. van't Veer LJ, Dai H, van de Vjijver MJ *et al.* Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002; **415;** 530–535.
20. Korsching E, Packeisen J, Agelopoulos K *et al.* Cytogenetic alterations and cytokeratin expression patterns in breast cancer: integrating a new model of breast differentiation into cytogenetic pathways of breast carcinogenesis. *Lab. Invest.* 2002; **82;** 1525–1533.
21. Rudlowski C, Friedrichs N, Faridi A *et al.* Her-2/neu gene amplification and protein expression in primary male breast cancer. *Breast Cancer Res. Treat.* 2004; **84;** 215–223.

