Total loss of MHC class I is an independent indicator of good prognosis in breast cancer

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Tumours can be recognised by CTL and NK cells. CTL recognition depends on expression of MHC Class I loaded with peptides from tumour antigens. In contrast, loss of MHC Class I results in NK activation. In our study a large set of samples from patients with primary operable invasive breast cancer was evaluated for the expression of MHC Class I heavy and light by immunohistochemical staining of 439 breast carcinomas in a tissue microarray. Forty-seven percent (206 of 439) of breast carcinomas were considered negative for HLA Class I heavy chain (HC10), whereas lack of anti-β2m-antibody staining was observed in 39% (167 of 424) of tumours, with only 3% of the β2m-negative tumours expressing detectable HLA Class I heavy chain. Correlation with patient outcome showed direct relationship between patient survival and HLA Class I expression. In those studies, loss of MHC Class I molecules has been associated with poor differentiation, aggressiveness and metastatic potential.6,9,11,13,14 To our knowledge, however, there has been no previous study examining whether there is any prognostic significance of HLA Class I loss on a large number of paraffin embedded invasive breast carcinomas. To analyse haplotype or allelic loss on paraffin sections of tumours it would be necessary to know the HLA type of all of the patients. This is not possible, however, in a retrospective study as many of these patients are no longer alive. In contrast, antibodies to polymorphic heavy chains and β2m are available allowing us to study complete loss of MHC Class I.

To obtain more insight into the prognostic value and mechanisms that tumour cells use to escape from immune system, we analysed HLA Class I expression in a large series of breast cancer tissue arrays using a monoclonal antibody to the polymorphic heavy chain (HC10) and a polyclonal anti-β2 microglobulin antibody. Expression of HLA heavy and light chains was then associated with various clinicopathological parameters and patient outcome. Our study group consisted of 439 primary operable breast cancer with a mean follow up of 7 years.

Material and methods

Patients and tumour characteristics
The original study group consisted of 439 primary operable invasive breast carcinoma from patients 27–70 years of age (median = 54 years) diagnosed between 1987–1992 and obtained from the Nottingham Tenvous Primary Breast Carcinoma Series. Patient characteristics, including age and menopausal status, were also collected and information on local, regional and distant recurrence and survival was also retrieved from a prospective database (Table I). Patients were followed up at 3-month intervals initially, then 6-month intervals, then annually for a median period of 83 months with a mean survival of 77 months (1–151 months).

Patient management was based on tumour characteristics by calculating the Nottingham Prognostic Index (NPI). Women with an NPI score ≤3.4 received no adjuvant therapy, those with a NPI score >3.4 received tamoxifen if ER positive (±Zoledex if premenopausal) or classical cyclophosphamide, methotrexate and 5-fluorouracil (CMF) if ER negative and fit enough for chemotherapy to be appropriate.

Tissue preparation
Breast cancer tissue microarrays were prepared as described previously.15 Each case was sampled in 3 copies, each containing one sample from a different region of the tumour.16 Specimens

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from 160 tumours could not be included in the study because of lack of tumour in the arrayed sample (due to sampling error or previous sectioning).

This is a well characterized series of primary operable breast cancer treated in a uniform manner and has been used to study a wide range of potential prognostic factors and markers including CD59, CD55 and CD46 expression. Tumour characteristics, including histological grade, tumour type, vascular invasion, menopausal status, tumour size, lymph node stage and Nottingham Prognostic Index are assessed routinely and recorded in a database. The Nottingham Prognostic Index (NPI) score was calculated for each patient based on the following equation: 

\[ \text{NPI} = 0.2 \times \text{tumour size (cm)} + \text{grade (1–3)} + \text{lymph node stage (1–3)}. \]

This index predicts survival of patients with invasive breast cancer and is utilised clinically in 3 groups: good (NPI ≤ 3.4), moderate (3.41 < NPI ≤ 5.4) or poor (NPI > 5.4) prognosis according to the score obtained.

### Antibodies

The mouse mAb HC10 (provided by Professor H. Ploegh, Harvard Medical School, USA) recognizes free (non ligand associated/non-B2m associated) human HLA Class I heavy chains with preferential binding to HLA-B and HLA-C alleles. The anti-heavy chain mAb HC10 (IgG2a) reacts with denatured Class I antigens and showed strong reactivity in Western blots and conventional light microscopical analysis of formalin-fixed, paraffin-embedded sections. This mAb precipitated 4 bands of molecular mass 78–105 kDa and additional higher molecular mass material, seen by nonreducing SDS-PAGE. HC10 recognizes an epitope in the domain of unfolded HLA-B and -C heavy chains that are not assembled with β2m. In contrast β2m is essential for the structural stability and optimal function of Class I molecule, and is invariant and therefore independent of allelic variants. The polyclonal rabbit-anti-β2-microglobulin (Dako, Cambridge, Ely, UK) recognizes the light chain of MHC Class I molecule.

### Table I – Correlation of HLA expression in invasive breast carcinoma with clinicopathological parameters (Pearson \( r^2 \))

| Tumour and patient characteristics | Proportion % (n) | Intensity of staining (p-value) | Percentage of cells stained (p-value) |
|-----------------------------------|-----------------|-------------------------------|-------------------------------------|
|                                   |                 | HC10                          | Anti-β2m                            |
| Age (years)                       |                 |                               |                                     |
| <40                               | 8 (39)          | 0.452                         | 0.19                                |
| 41–50                             | 28 (150)        |                               | 0.072                               |
| 51–60                             | 36 (193)        |                               | 0.341                               |
| >61                               | 28 (151)        |                               |                                     |
| Menopausal status (% of recorded cases) | 36 (184/507)    | 0.567                         | 0.765                               |
| Pre-menopausal                    | 64 (322/507)    |                               | 0.625                               |
| Post-menopausal                   |                 |                               |                                     |
| Histological Grade                |                 |                               |                                     |
| Grade 1                           | 21 (111)        | <0.001                        | <0.001                              |
| Grade 2                           | 35 (186)        |                               | <0.001                              |
| Grade 3                           | 44 (236)        |                               | <0.001                              |
| Lymph node status                 |                 | 0.223                         | 0.145                               |
| LN negative                       | 64 (340)        |                               | 0.682                               |
| LN positive                       | 36 (193)        |                               | 0.216                               |
| Tumour size (mm)                  |                 |                               |                                     |
| <10, 11–20, 21–30, 31–40, 41–50   | NA              | 0.285                         | 0.197                               |
| NPI2                              |                 |                               | 0.464                               |
| Good                              | 35 (181)        |                               | 0.093                               |
| Moderate                          | 55 (285)        |                               |                                     |
| Poor                              | 10 (55)         |                               |                                     |
| Tumour type3                      |                 |                               |                                     |
| Excellent                         | 6 (31)          |                               |                                     |
| Good                              | 21 (113)        |                               |                                     |
| Moderate                          | 12 (64)         |                               |                                     |
| Poor                              | 61 (325)        |                               |                                     |
| Vascular invasion                 |                 |                               |                                     |
| None or probable                  | 71 (370)        | 0.615                         | 0.149                               |
| Definite                          | 29 (154)        |                               | 0.515                               |
| Distant metastases                |                 | 0.615                         | 0.149                               |
| Absent                            | 88 (470)        |                               | 0.045                               |
| Present                           | 12 (63)         |                               | 0.032                               |
| Any recurrence                    |                 |                               |                                     |
| Absent                            | 77 (409)        | 0.242                         | 0.141                               |
| Present                           | 23 (124)        |                               | 0.214                               |
| Regional recurrence               |                 | 0.242                         | 0.141                               |
| Absent                            | 91 (484)        |                               | 0.017                               |
| Present                           | 9 (49)          |                               | 0.018                               |
| Local recurrence                  |                 | 0.007                         | 0.028                               |
| Absent                            | 90 (480)        | 0.885                         | 0.545                               |
| Present                           | 10 (53)         |                               | 0.268                               |
| Overall survival                  |                 | 0.004                         | 0.021                               |

1NA, none applicable.

2NPI (The Nottingham Prognostic Index) is an integrated prognostic index used to predict patient survival for women with invasive breast cancer based on tumour size, lymph node stage and tumour grade. It is often clinically utilised in three groups: good (≤ 3.4), moderate (3.41–5.4), and poor prognosis (> 5.4). Tumour sections were classified in four prognostic type groups as described previously. Excellent prognosis type (>80% 10-year survival) includes tubulo-lobular, tubular, mucinous and invasive cribriform carcinoma. Good prognosis types (60-80% 10-year survival) includes tubular mixed, mixed ductal with special type and alveolar lobular carcinoma. Moderate prognosis types (50-60% 10-year survival) includes classical lobular, medullary, atypical medullary and lobular mixed carcinoma. Poor prognosis types (<50% 10-year survival) includes ductal NST, solid lobular, mixed ductal and lobular carcinoma.
Immunohistochemistry

Sections 4-μm thick were cut and stained using a standard avidin–biotin complex method as described previously. Microwave pre-treatment was carried out in citrate buffer (pH = 6.0) 10 min at high power followed by 10 min at low power to retrieve antigenicity for staining of sections with HC10, whereas no pre-treatment was needed for staining of β2m, based on manufacturer’s recommendations. The primary antibodies were incubated on the slides for 1 hr with the optimal dilution found to be 1:200 (HC10) and 1:3,000 (anti-β2m).

**FIGURE 1** – HLA heavy chain (HC10) expression. Immunohistochemical detection of HLA Class I heavy chain (HC10) in normal mammary epithelium and invasive carcinoma. (a) Normal epithelium of ducts and inflammatory cells consistently express HLA. (b) Lack of staining of tumour cells with strong staining of lymphocytes. (c) weak and (d) moderate staining of HC10. (e) Strong staining of invasive breast tumours.
Negative controls, consisting of normal swine serum (NSS) (Harlan Sera Lab, UK) instead of primary antibody, confirmed the specificity of the staining. Breast tumours that were associated strongly MHC Class I immunopositive inflammatory cells were used as internal positive control. Many studies have shown that, contrary to expectations, tissue heterogeneity does not negatively influence the predictive power of the TMA results. To assess heterogeneity of the immunoreactivity with HC10, a pilot study of 20 cases were examined on full size tissue sections. A comparison of tissue array and original full size tissues showed similarity in intensity and the area of positive of staining.

**Evaluation of immunostaining**

All of immunostained tissue arrays were evaluated using a semi-quantitative system by one author (Z.M.) in a coded manner without knowledge of the clinical and pathological parameters of the patients. The obtained results were confirmed by 2 observers (Z.M. and S.E.P.) using a multi-headed microscope and, in difficult cases, a consensus was achieved. The intensity of the staining was scored on a scale as 0 (absent), 1 (weak), 2 (moderate) or 3 (strong). The percentage of tumour cells showing positive staining was also assessed semi-quantitatively as 1 (<25%), 2 (25–50%), 3 (51–75%) or 4 (>75%). In addition the histochemical score (H score) of immunoreactivity was obtained by multiplying the intensity and percentage scores. The histochemical score were sub-grouped into 3 groups of equal range for analysis and a score of <100 was considered weak, 100–200 was moderate and 201–300 was strong.

**Statistical analysis**

Statistical analysis of data was carried out using the SPSS package (version 11 for Windows, Chicago, IL). The significance of associations was determined by means of the Pearson R-test or Pearson $\chi^2$ test. In survival analysis, Kaplan-Meier curves were derived and the statistical significance of differences between the survival of groups with different HLA Class I expression was determined using the log-rank test. Survival was censored if the patients were still alive or died from other causes. Cox regres-

**TABLE II – INTENSITY OF EXPRESSION OF HLA CLASS I HEAVY AND LIGHT CHAINS**

| Immunohistochemical score | % Tumours (n) |
|---------------------------|---------------|
|                           | HC10          | Anti-β2m Ab |
| None                      | 47 (206)      | 39 (167)    |
| Weak (+)                  | 34 (150)      | 36 (153)    |
| Moderate (+ +)            | 11 (48)       | 16 (66)     |
| Strong (+ + +)            | 8 (35)        | 9 (38)      |
| Total (n)                 | 439           | 424         |

Significant association was found between expression of HC10 and anti-β2m (p-value = 0.001).

**FIGURE 2 – β2-Microglobulin expression in invasive breast carcinoma.** (a) No staining of tumour cells. (b) Weak and (c) moderate staining of breast carcinomas with β2-m. (d) Strong staining of invasive breast tumours.
Results

Co-expression of MHC Class I light and heavy chains in breast carcinomas

Only 439 of 539 arrayed samples contained sufficient tumour cells for assessment by HC10, whereas for β2m staining fewer samples (424/539) contained adequate tumour cells. Difference in number of cases in 2 series is due to tissue damage, either tissue loss or inadequate tumour tissue, a problem associated with tissue microarrays. Samples (113 of 539) contained stroma and inflammatory infiltrating cells. Inflammatory cells including lymphocytes, exhibited strong positive staining indicating the success of the immunohistochemical technique (Fig. 1a). Non-malignant breast tissues adjacent to carcinoma often showed strong expression of HLA heavy chain (Fig. 1a) as well as β2m, however, considerable variations occurred in breast carcinomas. Forty-seven percent (206 of 439) breast carcinomas were considered negative for HC10, whereas for β2m staining fewer samples (424/539) contained adequate tumour cells. 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Complete loss of MHC Class I light and heavy chains (no staining) and MHC Class I light and heavy chains positive tumours (weak, moderate or strong staining, Log-rank test for HC10 and β2 microglobulin: 0.0047 and 0.021, Fig. 3a, b). As can be seen in Figure 3a, b, patients whose breast tumours lost MHC Class I showed a more favourable outcome than those tumours with immunohistochemically detectable expression of MHC Class I. Similar results was found for the percentage of cells expressing HC10 and β2 microglobulin: 0.0047 and 0.021, Fig. 3a, b). Similarly, patients with absent or a low percentage of HC10 positive cells (<25%) showed significantly longer survival times than patients with >25% positive tumour cells (p = 0.0043, c), whereas it was not significant for percentage of β2m positive cells (d)

Multivariate analysis
To investigate whether HLA expression had independent prognostic significance, Cox multivariate regression analysis, including the parameters of tumour size, lymph node stage, grade and intensity of HC10 and β2m expression, was carried out. In this analysis, lymph node stage (p < 0.001), tumour grade (p = 0.005) and intensity of MHC Class I light and heavy chains expression were shown to be independent prognostic factors predictive of overall survival (p-values for HC10 and β2m are:...
Discussion

We found weak expression or absence of MHC Class I molecules in the majority of breast carcinomas studied. In 47% of primary breast carcinomas no staining for MHC Class I was observed, similarly lack of staining for β2m was observed in 39% of tumours. Importantly analysis of individual tumours indicated that concordant results were obtained using antibodies to either Class I heavy chain (HC10) or β2m, with only 3% of β2m negative tumours showing any expression of MHC Class I heavy chain. Conversely only 8% of tumours stained positively for MHC Class I but were absent for β2m expression. This supports earlier studies on paraffin embedded breast carcinomas that showed a good correlation between β2m and heavy chain expression with only 4 of 56 (7%) β2m negative tumours staining for cytoplasmic expression of free MHC Class I heavy chain. A similar lack of expression of HLA Class I has been reported in a range of tumours including breast carcinomas.

Although both haplotype loss and allelic loss of HLA can have profound consequences for T cell recognition, our study only addressed the issue of total HLA loss. There are several steps involved in HLA-Class I expression and any of these could potentially be defective leading to complete loss in tumour cells. Two types of defects, (i) mutations in the HLA-Class I genes or abnormal regulation of their expression and (ii) abnormal antigen processing such as TAP (transporter associated with antigen processing) or tapasin loss have received the most attention. In a recent study on breast tumours it was shown by RT-PCR and biochemical analysis that loss of HLA Class I expression is associated with expression of the anti-apoptotic bcl-2 gene. This loss of MHC Class I may therefore be associated with an increased resistance to apoptosis. Alternatively immune surveillance by T cells may result in tumour escape by downregulation of MHC molecules a process known as “immune editing.”

Our study shows that on a large series of breast tumours with long-term clinical follow-up there is a selective loss of expression of MHC Class I heavy and light chains that is related to improved prognosis. This loss may be a consequence of dedifferentiation as expression correlated with tumour stage and grade although loss of MHC heavy and light chains was shown to be an independent prognostic factor. Alternatively it may be due to immunosurveillance that in some patients selects against the more aggressive tumours leaving the more indolent HLA negative tumours to grow through. The growth of these tumours may then be further controlled by NK cells. In contrast tumour cells may downregulate specific HLA alleles protecting them from specific T cell attack while not making them susceptible to NK recognition. This issue was not addressed in our study.

In conclusion, loss of MHC heavy and light chains has been shown to be an independent indicator of good prognosis in breast tumours. It could be added to the other independent prognostic factors to help further stratify patients for adjuvant therapy. Patients expressing HLA could be candidates for aggressive chemotherapy as they have a poor predicted outcome. Alternatively they may be candidates for cancer vaccines that stimulate new cytotoxic T cell responses. In contrast patients lacking Class I HLA may be appropriate for monoclonal antibody therapy such as Herceptin as lack of HLA would enhance NK killing by antibody dependent cellular cytotoxicity (ADCC).

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References

1. Ochsnebin AF, Klenerman P, Karrer U, Ludewig B, Pericin M, Hengartner H, Zinkernagel RM. Immune surveillance against a solid tumor fails because of immunological ignorance. Proc Natl Acad Sci USA 1999;96:2233–8.

2. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immuno editing. Annu Rev Immunol 2004;22:529–60.

3. Garrido F, Ruiz-Cabello F, Cabrera T, Perez-Villar JJ, Lopez-Botet M, Duggan-Keen M, Stern PL. Implications for immunosurveillance...
of altered HLA Class I phenotypes in human tumours. Immunol Today 1997;18:89–95.
4. Fleming KA, McMichael A, Morton JA, Woods J, McGee JO. Distribution of HLA class I antigens in normal human tissue and in mammary cancer. J Clin Pathol 1981;34:779–84.
5. Natali PG, Giacomini P, Bigotti A, Imai K, Nicotra MR, Ng AK, Ferrone S. Heterogeneity in the expression of HLA and tumor-associated antigens by surgically removed and cultured breast carcinoma cells. Cancer Res 1983;43:660–8.
6. Perez M, Cabrera T, Lopez Nevot MA, Gomez M, Peran F, Ruiz-Cabello F, Garrido F. Heterogeneity of the expression of Class I and II HLA antigens in human breast carcinoma. J Immunogenet 1986;13:247–53.
7. Wintzer HO, Benzing M, von Kleist S. Lacking prognostic significance of beta-2-microglobulin. Histopathology 1995;27:219–26.
8. Concha A, Cabrera T, Ruiz-Cabello F, Garrido F. Can the HLA phenotype be used as a prognostic factor in breast carcinomas? Int J Cancer Suppl 1991;6:146–54.
9. Concha A, Esteban F, Cabrera T, Ruiz-Cabello F, Garrido F. Tumor aggressiveness and MHC Class I and II antigens in laryngeal and breast cancer. Semin Cancer Biol 1991;2:47–54.
10. Klein G, Klein E. Evaluation of tumours and the impact of molecular oncology. Nature 1985;315:190–95.
11. Garrido F, Cabrera T, Concha A, Glew S, Ruiz-Cabello F, Stern PL. Natural history of HLA expression during tumour development. Immunol Today 1993;14:491–9.
12. Hicklin DJ, Marincola FM, Ferrone S. HLA Class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. Mol Med Today 1999;5:178–86.
13. Eyal A, Levin I, Segal S, Levi I, Klein B, Kuperman O. Variation of HLA-ABC surface antigen expression on adenocarcinoma of the colon in correlation with the degree of differentiation. Nat Immun Cell Growth Regul 1990;9:222–7.
14. Levin I, Klein T, Goldstein J, Kuperman O, Kanetti J, Klein B. Expression of Class I histocompatibility antigens in transitional cell carcinoma of the urinary bladder in relation to survival. Cancer Res 1991;68:2591–4.
15. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Sauter G, Kallioniemi OP, Sauter G. Tissue microarrays for rapid linking of molecular changes to clinical endpoints. Am J Pathol 2001;159:72–9.
16. Perosa F, Luccarelli G, Prete M, Favino E, Ferrone S, Dammacco F. Beta-2-microglobulin-free HLA Class I heavy chain epitope mimicry by monoclonal antibody HC-10-specific peptide. J Immunol 2003;170:1918–26.
17. Pedersen LO, Hansen AS, Olsen AC, Gerwien J, Nissen MH, Buaas F. The interaction between beta-2-microglobulin (beta 2m) and purified class-I major histocompatibility (MHC) antigen. Scand J Immunol 1994;39:64–72.
18. Petersen BL, Petersen CL, Braendstrup O, Mouritsen S, Engel AM, Svane IM, Werdelin O. Expression of beta-2-microglobulin by premalignant epithelium. APMIS 1993;101:529–36.
19. Bubendorf L, Nocto A, Moch H, Sauter G. Tissue microarray (TMA) technology: miniaturized pathology archives for high-throughput in situ studies. J Pathol 2001;195:72–9.
20. Torhorst J, Bucher C, Kononen J, Haas P, Zubler M, Kochli OR, Mross F, Dieterich H, Moch H, Mihaitsch M, Kallioniemi OP, Sauter G. Tissue microarrays for rapid linking of molecular changes to clinical endpoints. Am J Pathol 2001;159:2249–56.
21. McCarty KS, Jr., Miller LS, Cox EB, Kunrath J, McCarty KS, Sr. Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch Pathol Lab Med 1985;109:716–21.
22. Redondo M, Garcia J, Villar E, Rodrigo I, Serrano A, Morell M. Major histocompatibility complex status in breast carcinoma: association with prognosis. Int J Cancer 1990;26:133–9.
23. Cudmannsdottir I, Gunnlaugur Jonasson J, Sigurdsson H, Olafsdottir K, Tryggvadottir L, Ogmundsdottir HM. Altered expression of HLA Class I antigens in breast cancer: association with prognosis. Int J Cancer 2000;89:500–5.
24. Palmisano GL, Pistillo MP, Capanni P, Pera C, Nicolgi G, Salvi S, Perelli L, Pascucci G, Ferrara GB. Investigation of HLA Class I downregulation in breast cancer by RT-PCR. Hum Immunol 2001;62:139–9.
25. Rodriguez F, Peran F, Garrido F, Ruiz-Cabello F. Upmodulation by estrogen of HLA Class I expression in breast tumor cell lines. Immunogenetics 1994;39:161–7.
26. Pereira H, Pinder SE, Sibbering DM, Galea MH, Elston CW, Blamey RW, Robertson JF, Ellis IO. Pathological prognostic factors in breast cancer. IV. Should you be a typer or a grader? A comparative study of two histological prognostic features in operable breast carcinoma. Histopathology 1995;27:219–26.