Loss of antigen-presenting molecules (MHC class I and TAP-1) in lung cancer

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Summary: Presentation of endogenous antigenic peptides to cytotoxic T lymphocytes is mediated by the major histocompatibility complex (MHC) class I molecules. For the stable assembly of MHC class I complex it is necessary that the antigenic peptide is transported by the MHC-encoded transporters TAP-1 and TAP-2 into a pre-Golgi region. T-cell-mediated host-vs-tumour response might therefore depend on the presence of these molecules on tumour cells. The presence of MHC class I antigens and TAP-1 was studied in 93 resection specimens of non-small-cell lung carcinomas (NSCLCs) by immunohistochemical methods using antibodies against the assembled class I molecule, beta₂-microglobulin (β₂-m), heavy-chain A locus, A2 allele and TAP-1 protein. Eighty-six patients were included in the survival analysis. Total loss of class I molecule was observed in 38% of the cases and was usually accompanied by loss of β₂-m and of heavy chain A locus. Selective loss of A locus was seen in 8.3% and of A2 allele in 27% of the cases. TAP-1 loss was always combined with β₂-m and/or heavy chain A locus loss. No correlation was found between the expression status of any of the above molecules, including the selective A2 allelic loss and histological type, degree of differentiation, tumoral stage, nodal stage and survival. Our findings suggest that loss of antigen-presenting molecules (including both MHC class I alleles and TAP-1) is a frequent event in lung cancer. However, the immunophenotypic profile of MHC class I and TAP-1 seems to be unrelated in vivo to the phenotype, growth or survival of NSCLC.

Keywords: MHC class I; TAP-1; lung carcinomas

The major histocompatibility complex (MHC) comprises an array of genes located on chromosome 6 in humans and encodes several sets of immunoregulatory molecules— the classical transplantation antigens (class I), the immune response-associated antigens (class II) and complement genes (class III) (Dausset, 1981). MHC class I molecules are polymorphic transmembrane glycoproteins composed of two polypeptide chains. The heavy chain (mol. wt. 4.5 kDa) is highly polymorphic and encoded by a group of closely linked loci, HLA-A, -B and -C. Its extracellular portion forms three domains α₁, α₂, α₃ (each approximately 90 amino acids long), which are coded by separate exons, while β₂-m is non-polymorphic and encoded by a different gene on chromosome 15. The interaction of β₂-m with the α₂ extracellular domain of the heavy chain plays a crucial role in the functional expression of the final product. Equally important in the formation of functional MHC class I molecules is the interaction of heavy-chain β₂-m with the antigenic peptides (Arce-Gomez et al., 1978; Ploegh et al., 1981; Bodmer, 1987; Townsend et al., 1990).

MHC class I molecules are widely distributed on most nucleated cells, with the exception of sperm, trophoblast, neurons and hepatocytes (Daar et al., 1984). They regulate the ability of cytotoxic T lymphocytes (CTLs) to recognise antigens (Zinkernagel et al., 1979) whereas natural killer cell cytotoxicity has been shown to be inversely correlated with the degree of class I expression (Kärre et al., 1986). MHC class I molecules present predominantly endogenous antigens, which are derived from the cytoplasmic pool and assembled within the endoplasmic reticulum with newly synthesised class I and β₂-m (Townsend et al., 1990). These antigenic peptides are transported by a protein complex carrier into the pre-Golgi regions. These transporters of antigenic peptides are heterodimers composed of the products of two genes (TAP-1 and TAP-2) located in the class II region of the MHC. Recently it was also shown that a chaperone molecule, calnexin, mediates heavy-chain β₂-m dimerisation and binding of the dimers to TAP molecules facilitates their assembly with TAP-transported peptides. (Trowsdale et al., 1990; Kleijmeer et al., 1992; Spies et al., 1992; Ortman et al., 1994).

There is an ever increasing body of evidence that suggests that surface MHC class I antigen expression is altered on human tumours, in the sense of a loss or down-regulation of these molecules (Orgad et al., 1985; Festenstein and Garrido, 1986; Pestka et al., 1992; Lopet Negro et al., 1989; Wiinzer et al., 1990; Goepel et al., 1991). Recently similar findings were also described regarding the immunophenotype of TAP-1 in cervical and colorectal tumours (Cromme et al., 1994; Kaklamani et al., 1994). There have been only a few studies on the expression of these antigens in lung cancer (Doyle, 1985; Funá et al., 1986; Dämmlrich et al., 1990; Redondo et al., 1991a, b) and these deal mainly with alterations of β₂-m and heavy chains.

The present study was undertaken to investigate the expression of MHC class I antigens along with that of TAP-1 protein in a large series of non-small-cell lung carcinomas (NSCLCs) and to examine its relationship with clinicopathological data.

Materials and methods

Patients

Ninety three specimens from patients undergoing resection for lung carcinomas at the John Radcliffe Hospital between 1984 and 1988 were studied. The characteristics of all patients studied are shown in Table I. Patients had undergone surgery if their tumour was apparently limited to one lobe with no evidence of metastasis and their residual lung function was good. The pathological stages of the tumours were T1 and T2 and the nodal status N0 and N1, according to the TNM classification. The patients had not received radiotherapy or chemotherapy before surgery. Survival data were available in all cases but patients dying within the first post-operative month or those dying of other causes were...
eliminated from survival analysis. There were 73 men and 20 women with a mean age of 60.3 years (s.d. 7.8, range 35–74). Survival analysis was based on 86 patients. By the time this study was undertaken 35 patients had died after a mean (± s.d.) post-operative survival of 459 (± 512) days. Table I shows the characteristics of the 86 patients included in the survival analysis according to the expression status of MHC class I molecules.

Table I Characteristics of 86 patients with non-small-cell lung carcinoma in which survival was studied in relation to HLA class I and TAP-1 protein expression

| Characteristic | Non-small-cell lung carcinoma | Squamous cell carcinoma | Adenocarcinoma |
|----------------|-------------------------------|-------------------------|---------------|
|                | W6/32 | TAP-1 | + | +/− | + | +/− | + | +/− |
| No. of patients| 49/27 | 64/22 | 30/27 | 40/17 | 19/10 | 24/5 |
| Male           | 36/32 | 50/18 | 22/25 | 33/14 | 14/7 | 17/4 |
| Female         | 13/5  | 14/4  | 8/2  | 7/3  | 5/3  | 7/1  |
| Mean age at surgery | 60.1/ | 59.7/ | 60.0/ | 60.7/ | 61.8 | 64.4 |
| s.d.           | 7.9/8.0 | 8.2/7.0 | 8.4/7.0 | 7.6/6.9 | 7.7/10.1 | 8.0/4.2 |
| F-value        | 1.025* | 1.372* | 1.21* | 1.44* | 1.721* | 0.86 |
| T stage 1      | 20/18 | 27/11 | 13/16 | 20/9 | 7/2 | 7/2 |
| T stage 2      | 29/19 | 37/11 | 17/11 | 20/8 | 12/8 | 17/3 |
| N stage 0      | 35/27 | 43/19 | 24/19 | 29/14 | 11/8 | 14/5 |
| N stage 1      | 14/10 | 21/3  | 6/8  | 11/3 | 8/2  | 10/0 |
| Differentiation| Good  | 3/1   | 3/1 | 1/1 | 1/1 | 2/0 | 2/0 |
|               | Moderate | 19/16 | 25/10 | 10/11 | 14/7 | 9/5 | 11/3 |
|               | Poor | 27/20 | 36/11 | 19/15 | 25/9 | 8/5 | 11/2 |

*Statistically non-significant (P > 0.05).

Immunohistochemistry

Cryostat sections (7 mm thick) were fixed in acetone for 10 min at room temperature, left to dry overnight and stored at −20°C until required for staining. Immunohistochemical staining was performed using the alkaline phosphatase–anti-alkaline phosphatase (APAAP) method as described previously (Cordell et al., 1984). For the polyclonal antibody AKI-7 a single modification of this technique was made, using an incubation step of mouse-anti-rabbit immunoglobulin.

Assessment of staining

Microscopic examination of immunohistochemically stained sections was carried out by two observers. The whole section was screened for the distribution of HLA antigens but areas of obvious tumour necrosis were avoided for counting. Normal respiratory epithelium and inflammatory lymphoid cells were used in each case as a control. Thus, a particular antigen was only considered to be lost by the tumour if it was still expressed by the adjacent normal respiratory epithelium and the lymphocytes. The evaluation was semiquantitative. A tumour was scored as negative (−) if less than 10% of the cells were labelled and as positive (+) if more than 75% of the cells were strongly stained. When the percentage of positive neoplastic cells was between 10% and 75% irrespective of the staining intensity the tumour was recorded as showing reduced expression.

Statistical analysis

The association between HLA expression and tumour type, degree of differentiation, T stage and N stage was investigated by the use of frequency tables (Altman, 1991). Survival was measured in days from the date of surgery. Actuarial survival curves were plotted using the Kaplan–Meier method (Kaplan and Meier, 1958). The statistical significance was calculated using the log-rank test (Peto et al., 1977) and the hazard ratio with a 95% confidence interval was calculated as described by Machin and Gardner (1989). The homogeneity of age in the various subgroups was assessed by calculating the F-value with one-way analysis of variances (Armitage and Berry, 1987).

Results

Tables I and II summarise the results of the immunohistochemical expression of HLA class I and TAP-1 in the groups of SQC and AC.
Table II  Survival of 86 patients with non-small-cell lung carcinoma according to MHC class I and TAP-1 protein expression

| Results of staining | No. of patients | 5 year survival (%) | \( \chi^2 \)-square | P-value | Hazard ratio (95% CI) |
|---------------------|-----------------|---------------------|---------------------|---------|----------------------|
| W6/32 positive      | 49              | 56.6                | 0.0001              | >0.95   | 0.99 (0.5–1.96)      |
| W6/32 negative      | 37              | 55                  |                     |         |                      |
| W6/32 positive MA2.1 positive | 30 | 57.6 | 0.2839 | >0.5 | 0.75 (0.26–2.16) |
| W6/32 positive MA2.1 negative | 13 | 53.9 |       |       |                      |
| AK1-7 positive      | 64              | 54.7                | 0.1773              | >0.5    | 1.18 (0.55–2.5)      |
| AK1-7 negative      | 22              | 56.3                |                     |         |                      |

Figure 1  (a) W6/32 expression in a squamous cell carcinoma (bar 100 mm). (b) MA2.1 selective loss from the same case (bar 50 mm). (c) Loss of W6/32 in an adenocarcinoma (bar 100 mm). (d) Expression of TAP-1 in the above case (bar 100 mm). Lymphocytes and stromal cells are positive in all the cases shown above.

**HLA class I and TAP-1 expression**

In the normal lung class I antigens, TAP-1, HCA2, BBM.1 and MA2.1 were expressed by the endothelial cells, lymphocytes, bronchiolar and alveolar epithelium and alveolar macrophages. As far as the tumours are concerned, 37 out of 93 cases showed loss of the framework antigenic determinant, either partial or complete, evidenced by reduced (five cases) or negative (32 cases) staining with W6/32 antibody. The loss was commoner in SQC (27 out of 61 cases) than in AC (10 out of 32 cases), although no statistically significant difference could be reached. Loss of the framework antibody W6/32 was usually accompanied by loss of \( \beta_2\)-m (28 out of 37 cases) and loss of A locus (19 out of 37 cases). Selective loss of A locus was detected in 2 out of 24 cases positive with W6/32. Selective loss of A2 allele was seen in 13 out of 43 cases in which A2 was present in the adjacent lung. All 13 cases were positive for W6/32.

TAP-1 protein was lost in 22 cases (17 SQC and 5AC). These cases also showed synchronous loss of \( \beta_2\)-m and/or heavy chain. A locus-isolated TAP-1 defect was not identified in our series. All cases showing loss of TAP-1 molecule were negative for W6/32 as well. No relationship could be found between the mode of MHC class I antigen and TAP-1 expression on the one hand and histological type, degree of differentiation, tumoral or nodal stage on the other, even when the last three parameters were examined with each histological group separately (Figure 1).

**Survival analysis**

The results of the survival analysis on the series of 86 non-small-cell lung carcinoma according to the staining with the antibodies W6/32, TAP-1 and MA 2.1 are summarised in Table II and the survival curves are shown in Figure 2a, b and c. Furthermore we examined whether the selective loss of the A2 allele was of any prognostic value: for this purpose we compared the survival of patients with W6/32- and MA2.1- positive tumours with that of patients with W6/2-
Discussion

In the present study we detected two types of alterations in the surface expression of MHC class I antigens by the neoplastic cells: total loss of MHC class I molecule and selective losses of HLA-A locus and A2 allele. Total loss of MHC class I molecule as evidenced by negative reaction to W6/32 was detected in 38% of our cases, a figure higher than those quoted in previous studies (Redondo et al., 1991a, b). Selective losses of A locus and A2 allele were identified in 8.3% and 27% respectively. Our findings show that loss of the assembled molecule is not only due to loss of β2-m but also to loss of TAP1 molecules and/or heavy chains.

Previous studies of lung cancer have shown no difference between β2-m and heavy chain expression (Doyle, 1985; Redondo et al., 1991b). However, in our series we have seen such a difference in a small proportion of cases (9.1%). Such uncoordinated expression of β2-m and heavy chains has also been observed in colon carcinomas (Momburg et al., 1989). It is also worthy of note that the failure to detect A locus or A2 allele is not necessarily associated with loss of the assembled class I molecule. This is similar to the situation in colon carcinomas (Rees et al., 1988; Kaklamanis et al., 1992).

Interestingly, loss of the transporter protein was always combined with β2-m and/or A locus loss and was invariably associated with lack of expression of the assembled class I molecule. This implies that in the absence of the transporter protein the antigenic peptide is not able to join the MHC class I molecule rendering the assembly of the heavy chains and β2-m impossible.

The mechanisms by which total or partial losses of HLA antigens occur are not yet well known. Theoretically, they might reflect underlying chromosomal abnormalities (e.g. translocations or deletions) in the short arm of chromosome 6 for the heavy chains and TAP-1 or chromosome 15 for β2-m. However, there is no current evidence to support this. In fact, molecular studies in lung carcinomas have failed to demonstrate rearrangements of class I genes in cases with abnormal surface expression of these antigens (Doyle et al., 1985; Redondo et al., 1991a). A more plausible mechanism is that of transcriptional down-regulation of MHC class I genes, which could be related to the action of cellular...
oncogene products, such as c-myc oncoprotein. This has been shown to operate in SLCL tumours and cell lines (Doyle, 1985). However, in NSCLC expression of class I antigens appears to be independent of c-myc expression. Alternatively, it has been hypothesised that a post-transcriptional mechanism may be involved in the differential expression of HLA-A, B, C products in NSCLC, since there is not always a close relation between the surface expression of these antigens and their mRNAs (Redondo et al., 1991a). Moreover, γ-interferon-mediated regulation of HLA-gene subsets has been documented (Hakan et al., 1989; Schmidt et al., 1990).

Based on these findings it has been suggested that MHC class I loss is an indication of a more aggressive phenotypic and of a more rapid tumour growth. However, no relationship with the tumoral or nodal stage was found in the studies of Dammrich et al. (1990) and Redondo et al. (1991b), in concordance with the results of the present study.

Experiments in murine models have shown that the loss of MHC class I antigen expression allows tumour growth and metastasis formation by escape from T-cell-mediated surveillance (Hui et al., 1984; Tanaka et al., 1985; Wallich et al., 1985). Following this line of argument it was tempting to speculate that tumours lacking the above antigens would be prone to pursue a more unfavourable clinical course as compared with those with normal expression. This idea was further strengthened by the association of MHC class I loss with a poorer degree of differentiation as reported for breast (Wintzer et al., 1990), colon (Momburg et al., 1986) and laryngeal (Lepez-Nevot et al., 1989) carcinomas. Clinical studies however have failed to confirm this idea, at least as far as colorectal carcinomas are concerned (Stein et al., 1988; Müller et al., 1991). In the case of breast cancer, however, the question of whether the relevancy of MHC class I expression is still open (Wintzer et al., 1990; Concha et al., 1991).

This study shows that down-regulation of antigen-presenting and antigen-transporting molecules is a common phenomenon in NSCLC. Specific allelic loss (A2) was also frequently detected and it might be of interest to study the expression of the entire allelic repertoire present on tumour cells. Although no correlation was found with clinicopathological parameters, the understanding of the underlying mechanisms that are responsible for this defective expression, would be of paramount importance.

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