Virchows Archiv A Pathol Anat (1993) 422:351–355

Virchows Archiv A
Pathological Anatomy and Histopathology

Distribution of S-100 protein-positive dendritic cells and expression of HLA-DR antigen in transitional cell carcinoma of the urinary bladder in relation to tumour progression and prognosis

Keiji Inoue[1], Mutsuo Furihata[2], Yuji Ohtsuki[2], Yukitoshi Fujita[1]

[1] Department of Urology, Kochi Medical School, Kochi 783, Japan
[2] Second Department of Pathology, Kochi Medical School, Kochi 783, Japan

Received December 3, 1992 / Received after revision March 1, 1993 / Accepted March 2, 1993

Abstract. The distribution of S-100 protein positive dendritic cells (S100-DCs) in cancer nests and the expression of HLA-DR antigen on cancer cells in 90 patients with transitional cell carcinoma of the urinary bladder were studied immunohistochemically. A dense infiltrate of S100-DCs (more than 10 S100-DCs/high power field) was detected in 47 out of 90 cases, while in the remaining tumours the infiltrate was sparse. HLA-DR positive cancer cells (DR-CCs) were detected in 24 cases, including 16 with dense DR-CCs (more than 100 DR-CCs/high power field); no expression was observed in the remaining tumours. In terms of the numbers of S100-DCs infiltrating the following statistically significant differences were observed: tumour grading G1 > G3, depth of penetration pT0 > pT3; ($p < 0.05$), G2 > G3, lymphatic invasion $- > +$ and venous invasion $- > +$; ($p < 0.01$). A multivariate analysis demonstrated that the most important factor affecting prognosis was distant organ and/or lymph node metastasis ($p < 0.01$) the number of S100-DCs, with a hazard ratio (HR) of 0.26 ($p < 0.01$), and the number of DR-CCs with HR of 0.18 ($p < 0.05$); these were statistically significant. S100-DCs and DR-CCs may be regarded as independent prognostic factors of tumour growth and progression.

Key words: Bladder cancer – S-100 protein – HLA-DR/dendritic cell – Multivariate analysis

Introduction

The prognosis of patients with transitional cell carcinoma (TCC) of the urinary bladder is predicted by the degree of malignancy, as determined by tumour grading (G), depth of penetration (pT), lymphatic invasion (ly), venous invasion (v), distant organ metastasis (m) and lymph node metastasis (n). However, there is not always agreement between the degree of malignancy suggested by these classifications and the prognosis. New prognostic factors which more accurately reflect the degree of malignancy and more accurately predict the prognosis are necessary.

We have studied S-100 protein positive dendritic cells (S100-DCs) in cancer nests, because their presence has been shown to be correlated significantly with the tumour growth and prognosis of several types of cancer such as lung cancer (Nakajima et al. 1985), and colorectal cancer (Ambe et al. 1989). In this study, we tested for the presence of S100-DCs in cancer nests of TCC of the urinary bladder, together with the expression of HLA-DR antigen, a subunit of major histocompatibility complex (MHC) class II, on dendritic cells using the immunohistochemical methods described previously (Furihata et al. 1992). We were particularly interested in certain cases in which the expression of HLA-DR was present on cancer cells. Thus in this study, we tested for the presence of S100-DCs in cancer nests immunohistochemically, and compared results obtained with those for presence of HLA-DR positive cancer cells (DR-CCs), using the Cox proportional hazards model for statistical analysis of the each population number relevant to prognostic factors in these cases, in relation to the prognosis.

Materials and methods

We studied 90 patients with TCC of the urinary bladder who had undergone radical or partial cystectomies at the Department of Urology of Kochi Medical School or the Division of Urology of Kochi-Takasu Hospital between October 1980 and June 1991. Table 1 shows the prognostic background of these 90 patients, ranging in age between 49 and 92 (mean: 68.42) years and including 70 men and 20 women.

Buffered-formalin fixed, paraffin-embedded tissues of each specimen were studied using polyclonal antibody to S-100 protein (S-100, dilution 1:800) and monoclonal antibody to HLA-DR alpha-chain (HLA-DRα, 1:30). Both antibodies were purchased from Dakopatts (Copenhagen, Denmark). Immunohistochemical testing for S-100 protein and HLA-DR α was performed using the strepta-

Correspondence to: K. Inoue

Table 1. Population numbers of S-100 positive dendritic cells (S100-DCs) and DR-positive cancer cells (DR-CCs) related to prognostic factors in transitional cell carcinoma of the urinary bladder

	Total	S100-DCs			DR-CCs			
		Few	Many	Mean ± SD	No	Few	Many	Mean ± SD
Age								
≦69	44	21	23	14.68 ± 13.41	30	5	9	73.91 ± 189.58
70≦	46	22	24	14.39 ± 13.07	36	3	7	68.02 ± 194.61
Sex								
Male	70	34	36	14.34 ± 12.60	53	8	9	62.79 ± 189.58
Female	20	9	11	15.20 ± 15.32	13	0	7	99.30 ± 198.67
Grade								
1	9	1	8	23.78 ± 13.09*	7	1	1	26.00 ± 69.12
2	43	17	26	15.47 ± 11.96*	33	4	6	75.30 ± 221.20
3	38	25	13	11.29 ± 13.60*	26	3	9	76.55 ± 175.32
Depth of penetration								
0 (a)	15	4	11	20.47 ± 13.93**	13	1	1	12.20 ± 40.85
1	35	13	22	15.83 ± 11.77	25	2	8	105.51 ± 247.64
2	5	3	2	11.60 ± 12.28	5	0	0	0.00 ± 0.00
3	23	15	8	10.39 ± 10.41**	17	2	4	81.78 ± 209.22
4	12	8	4	12.50 ± 18.91	6	3	3	52.00 ± 69.67
Lymphatic invasion								
(−) (0)	62	24	38	16.82 ± 13.65***	46	4	12	75.81 ± 200.37
(+) (1, 2)	28	19	9	9.46 ± 10.57***	20	4	4	60.04 ± 171.80
Venous invasion								
(−)	62	26	36	17.08 ± 14.12****	46	4	12	76.40 ± 200.10
(+)	28	17	11	8.89 ± 8.55****	20	4	4	58.71 ± 172.36
Nodal and distant metastases								
(−)	73	34	39	15.22 ± 13.82	55	5	13	66.53 ± 185.71
(+)	17	9	8	11.59 ± 9.62	11	3	3	89.65 ± 217.97
Radiation therapy								
(−)	62	29	33	13.79 ± 11.62	47	6	9	69.95 ± 200.38
pre-	13	6	7	16.69 ± 14.51	10	2	1	20.31 ± 45.86
post-	11	5	6	17.00 ± 20.01	5	0	6	161.82 ± 251.33
pre- & post-	4	3	1	12.25 ± 12.61	4	0	0	0.00 ± 0.00
Chemotherapy								
(−)	12	8	4	11.75 ± 10.11	10	0	2	88.25 ± 249.20
pre-	15	9	6	9.47 ± 8.30	11	2	2	25.40 ± 55.22
post-	36	14	22	15.30 ± 12.85	26	3	7	110.53 ± 254.84
pre- and post-	27	12	15	17.56 ± 16.18	9	3	5	35.63 ± 69.88
All cases	90	43	47	14.53 ± 13.16	66	8	16	70.90 ± 191.11

* G1 > G3 ($p < 0.05$), G2 > G3 ($p < 0.01$)
** pT0 > pT3 ($p < 0.05$)
*** ly(−) > ly(+) ($p < 0.01$)
**** v(−) > v(+) ($p < 0.01$)

vidin-biotin-peroxidase complex method as described previously (Furihata et al. 1992).

The number of S100-DCs was determined by counting, only in the tumour tissue, using a ×40 objective at voluntarily selected 10 high power fields (HPFs) in the most densely infiltrated areas, and averaged as 'population density'. All specimens examined were assigned to one of two groups; 'few', with 0 to 9 S100-DCs/HPF, or 'many', with more than 10 S100-DCs/HPF. For purposes of counting, an S100-DC was defined as an S-100 protein positive cell with an oval nucleus and cytoplasmic flame-like extensions present among adjacent tumour cells. Also for the purposes of counting, a DR-CC was defined as a TCC cell with cytoplasm and cell membrane stained for HLA-DR α. The number of DR-CCs was counted, using a ×40 objective in 10 HPFs voluntarily selected from within the most densely infiltrated regions, and the number per 1000 cancer cells determined. All specimens examined were assigned to one of three groups; 'no', with 0 DR-CC/HPF, 'few', with 1–99 DR-CCs/HPF, or 'many', with between 100 and 1000 DR-CCs/HPF.

Histological or clinical classifications including G, pT, ly, v, m and n were made using "The general rule for clinical and pathological studies on bladder cancer" (Japanese Urological Associa-

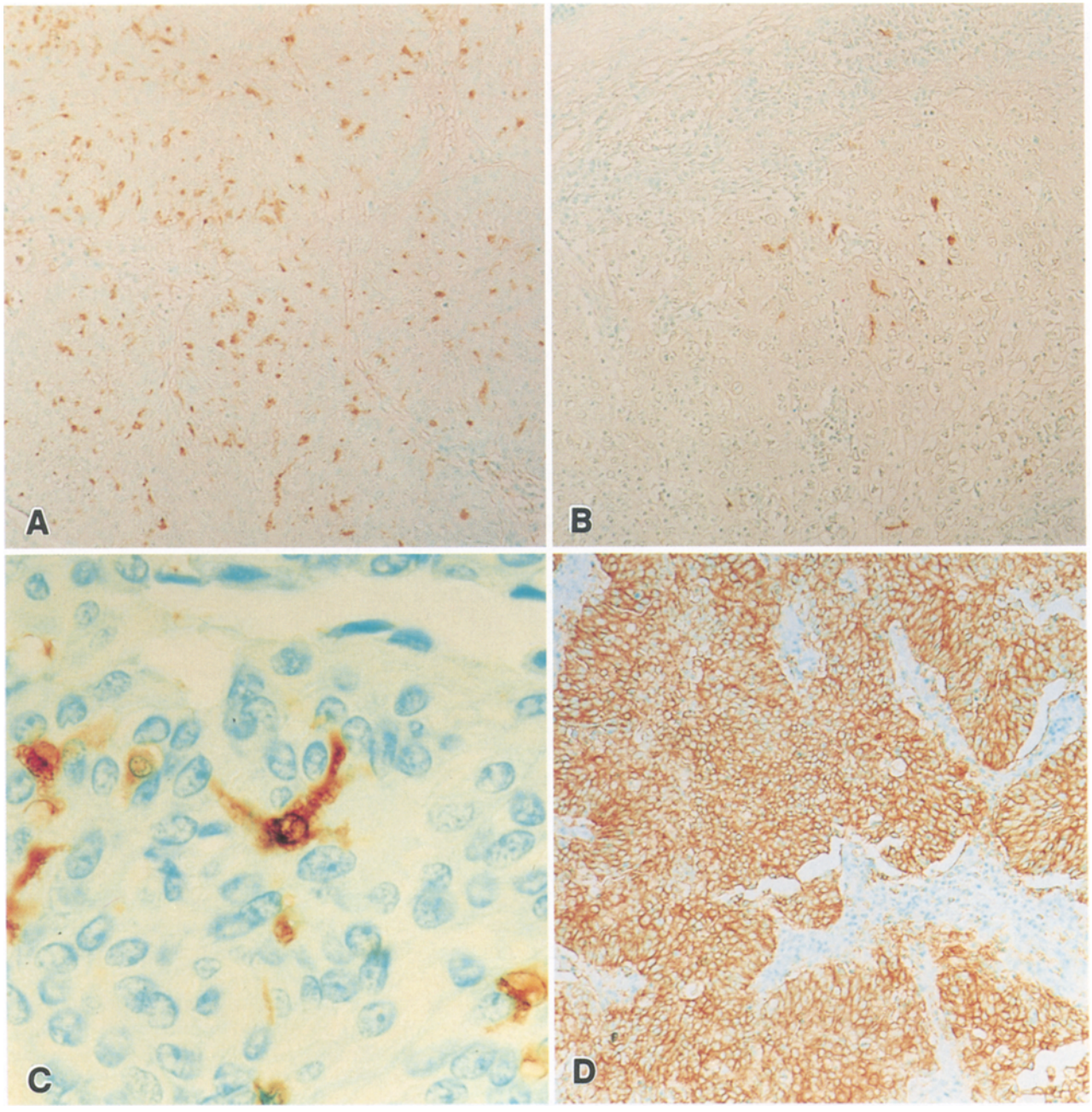

Fig. 1. **A** Dense infiltration of S-100 positive dendritic cells (S100-DCs) in a case of transitional cell carcinoma (TCC) G2, avidin-biotin complex (ABC) method (×20). **B** Sparse infiltration of S100-DCs in a case of TCC, G3. ABC method (×20). **C** Representative S100 DCs in a case of TCC, ABC method (×80). **D** HLA-DR α positive transitional carcinoma cells (DR-CCs) in a 'many' and 'homogenous' case of DR-CC. ABC method (×20)

tion and Japanese Pathological Society, 1980). In addition to these, patient age, sex and history of treatment with radiation and/or chemotherapy were evaluated as prognostic factors. For the purpose of comparing the numbers of S100-DCs and/or DR-CCs in each degree for each factor, the Wilcoxon rank sum test was also used. Kaplan-Meier methods were used for determination of prognosis. In addition, the Cox proportional hazard model was used to calculate and estimate the post-operative survival rate and to determine the statistical difference for each prognostic factor of histological or clinical classification. For multivariate analysis, variables were selected on condition that they were statistically significant and were only poorly correlated with each other (correlation coefficient $p < 0.4$).

Results

The number of S100-DCs infiltrating in cancer nests (Fig. 1A-C) and/or DR-CCs (Fig. 1D) by prognostic factor are listed in Table 2. Dense infiltrates of S100-DCs in cancer nests (more than 10 S100-DCs/HPF) were found in 47 of the 90 cases of TCC examined. Density ranged between 1 and 66, with an average of 14.53 ± 13.16 (mean $\pm$ SD) (Table 1). Expression of HLA-DR on cancer cells was detected in 24 cases, including the 16 with the dense existence of DR-CCs (more than 100

Table 2. Results of statistical analysis of prognostic factors relevant to S100-DCs and DR-CCs using proportional hazards model

Prognostic factors	Hazard ratio		95% Confidence limits		Statistical significance	
G1	1	1				
G2	0.84	2.10	0.10~7.40	0.26~17.20	0.875	0.493
G3	1.78	7.20	0.19~16.96	0.77~67.04	0.617	0.087
v(−)	1	1				
(+)	1.38	0.91	0.54~3.55	0.35~2.40	0.507	0.853
m+n(−)	1	1				
(+)	9.20	8.20	3.74~22.63	3.06~21.96	<0.001	<0.001
S100-DCs few	1					
many	0.26		0.11~0.64		0.004	
DR-CS no		1				
few		2.69		0.83~8.69		0.102
many		0.18		0.04~0.82		0.029

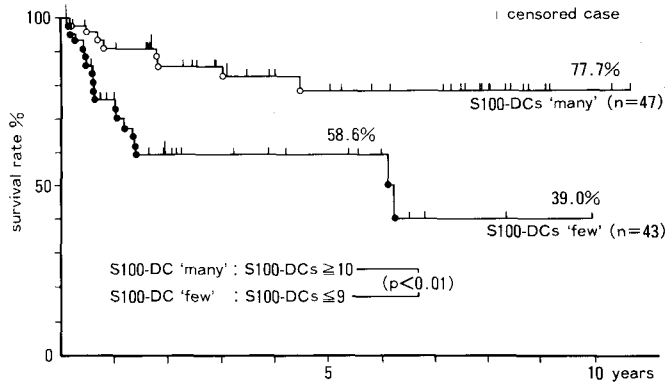

Fig. 2. Survival rate according to the number of S100-DCs

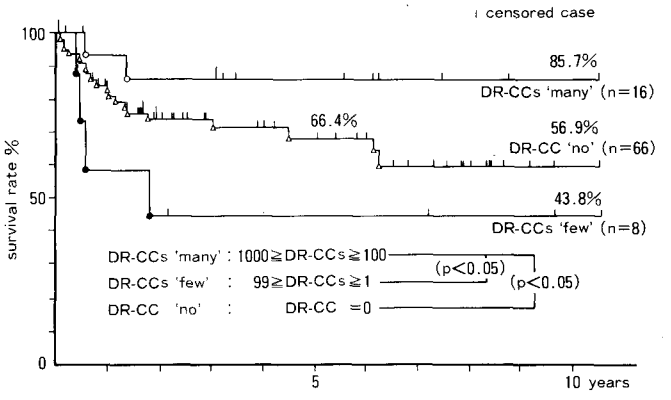

Fig. 3. Survival rate according to the number of DR-CCs

DR-CCs/HPF). It was not present in any of the remaining tumours; and ranged, as number per 1000 cancer cells, between 0 and 859, with an average of 70.90± 191.11 (mean±SD) (Table 1). Forty-seven cases had many S100-DCs, 16 cases had many DR-CCs, and 10 cases had many S100-DCs and many DR-CCs. Two cases positive for DR-CCs had a homogenous population, while other cases positive for DR-CCs had a heterogenous population. There was therefore no correlation in either population number of area between S100-DCs and DR-CCs.

In terms of the numbers of infiltrating S100-DCs, the following statistically significant differences were observed: G1>G3, pT0>pT3; ($p<0.05$), G2>G3, ly(−)>ly(+) and v(−)>v(+); ($p<0.01$). Statistical analysis detected no significant correlation between the number of DR-CCs and any of the prognostic factors including G, pT, ly, v, m and n (Table 1).

Regarding prognosis, the 1, 3, 5 and 10 year survival rates for the group of all 90 cases were 83.4%, 71.3%, 66.9% and 60.4%, respectively. The 10 year survival rate was 77.7% for patients having tumours with many S100-DCs and 39.0% for those with few S100-DCs; the difference between these rates was statistically significant ($p<0.01$) (Fig. 2). The 10 year survival rate was 85.7% for patients having tumours with many DR-CCs, 43.8% for those with few DR-CCs, and 56.9% for those with none (Fig. 3). The differences in survival rate between those with many DR-CCs and those with no DR-CC ($p<0.05$), and between those with many DR-CCs and few DR-CCs ($p<0.05$) were statistically significant. Those with many S100-DCs and many DR-CCs tended to have a better prognosis than those with many S100-DCs or many DR-CCs, but not both; the difference between these groups, however, was not significant.

Regarding survival rate, a multivariate analysis using the Cox proportional hazard model was used to calculate G, pT, ly, v, m and n, S100-DC and DR-CC, which were each statistically significant. A stepwise selection of these factors was made, based on the relative magnitude of their contribution to survival.

Analysis demonstrated that the most important factor affecting survival was m and n ($p<0.01$), the number of cases affected by S100-DCs with a hazard ratio (HR) of 0.26 ($p<0.01$), and the number of cases affected by DR-CCs with HR of 0.18 ($p<0.05$) in this order; these were statistically significant (Table 2). However, the differences between the few and no DR-CCs groups, and between the many and few DR-CCs groups, were not statistically significant (Table 2).

Discussion

In this study, S100-DC and DR-CC reflect the degree of malignancy by demonstration of a step in the immune response. The presence of S100-DCs in cancer nests has

been found to be significantly correlated with tumour growth and prognosis of several types of cancer (Nakajima et al. 1985; Nomori et al. 1986; Smolle et al. 1986; Tsujitani et al. 1987; Schroder et al. 1988; Ambe et al. 1989; Nakano et al. 1989); they may play roles as antigen presenting cells. HLA-DR is an MHC antigen class II subunit and is present in the immune-related cells such as macrophages and in in certain non-lymphatic tissues (Natali et al. 1981). It has been reported that HLA-DR is expressed in lung cancer (Wilson et al. 1984), breast cancer, malignant melanoma (Natali et al. 1983), colon cancer (Daar and Fabre 1983) and gastric cancer (Sakai et al. 1987) in a manner identical to that in TCC of urinary bladder in our study. The significance of HLA-DR expression is its demonstration of a step in cell differentiation, because HLA-DR expressed in active cells divided at gastric pit (Sakai et al. 1987) and differentiating stem cells in bone marrow (Robinson et al. 1981). It has been reported that HLA-DR antigen expression is induced and increased by interferon-γ in each cultured immune-related cell and in each cell of malignant melanoma (Basham and Merigan 1983), glioma (Takiguchi et al. 1985) and gastric adenocarcinoma (Sakai et al. 1987) and that HLA-DR antigen is expressed in tumour cells induced by lymphokines such as interferon-γ. Like macrophages, HLA-DR may have the ability to present antigens to helper-T lymphocytes. The significance and function of HLA-DR antigen expression are, however, still matters of conjecture.

This study revealed, regarding S100-DCs, the dense population cases (S100-DCs$\geqq$10/HPF) with a HR of 0.26 ($p<0.01$), had significantly better prognosis than the sparse, and S100-DCs significantly reflected any of the standard factors showing the degree of the malignancy such as G, pT, ly and v. It revealed, regarding DR-CCs, that the dense population cases (DR-CCs$\geqq$100/HPF) with HR of 0.18 ($p<0.05$) had significantly better prognosis than the non-expressed cases, and DR-CCs did not significantly reflect the standard factors showing the degree of malignancy. In this study, there are several cases in which the prognosis was poorly predicted using standard factors. For example, among the cases considered here was an 84-year-old man with TCC, G3, pT3b, ly(−), v(−), m and n(−) and a history of having undergone post-operative chemotherapy, who survived 2315 days after operation. On the other hand, a 71-year-old man with TCC, G2>G1, pT1b, ly(−), v(−), m and n(−) and a history of having undergone post-operative radiation and pre- and post-operative chemotherapy, who survived only 373 days after operation. The former patient had 40 S100-DCs/HPF and 552 DR-CCs/HPF, suggesting a good prognosis, while the latter had 5 S100-DCs/HPF and 0 DR-CC/HPF, suggesting a poor prognosis. For these 2 cases, therefore, standard prognostic factors were insufficient for prediction of prognosis, while numbers of S100-DCs and DR-CCs did accurately reflect prognosis. There was, however, no correlation in population number or area between S100-DCs and DR-CCs, and they therefore play roles independent of each other as prognostic factors. These cases with large numbers of both S100-DC and DR-CC did not fare significantly better than others, but according to increase the cases, synergism and additional effects for prognosis may have been expected.

Acknowledgements. We thank Dr. N. Terao and Dr. H. Hashimoto in the Division of Urology, Kochi-Takasu Hospital, Japan for providing the clinical data and materials of the cases studied.

References

Ambe K, Mori M, Enjoji M (1989) S-100 protein-positive dendritic cells in colorectal adenocarcinomas. Cancer 63:496–503

Basham TY, Merigan TC (1983) Recombinant interferon-γ increases HLA-DR synthesis and expression. J Immunol 130:1792–1794

Daar AK, Fabre JW (1983) The membrane antigens of human colorectal cancer cells: demonstration with monoclonal antibodies of heterogeneity within and between tumours and of anomalous expression of HLA-DR. Eur J Cancer Clin Oncol 19:209–220

Furihata M, Ohtsuki Y, Ido E, Iwata J, Sonobe H, Araki K, Ogoshi S, Ohmori K (1992) HLA-DR antigen- and S-100 protein-positive dendritic cells in esophageal squamous cell carcinoma – their distribution in relation to prognosis. Virchows Arch [B] 61:409–414

Japanese Urological Association and the Japanese Pathological Society (1980) The general rule for clinical and pathological studies on bladder cancer. 1

Nakajima T, Kodama T, Tsumuraya M, Shimosato Y, Kameya T (1985) S-100 protein-positive Langerhans cells in various human lung cancers, especially in peripheral adenocarcinomas. Virchows Arch [A] 407:177–189

Nakano T, Oka K, Arai T, Morita S, Tsunemoto H (1989) Prognostic significance of Langerhans' cell infiltration in radiation therapy for squamous cell carcinoma of uterine cervix. Arch Pathol Lab Med 113:507–511

Natali PG, Maetino CD, Quaranta V, Nicotra MR, Frezza F, Pellegrino MA, Ferrone S (1981) Expression of Ia-like antigens in normal human nonlymphoid tissues. Transplantation 31:75–78

Natali PG, Giacomini P, Bigotti A, Imai K, Nicotra MR, Ferrone S (1983) Heterogeneity in the expression of HLA and tumor-associated antigens by surgically removed and cultured breast carcinoma cells. Cancer Res 43:660–668

Nomori H, Watanabe S, Nakajima T, Shimosato Y, Kameya T (1986) Histiocytes in nasopharyngeal carcinoma in relation to prognosis. Cancer 57:100–105

Robinson J, Sieff C, Delia D, Edwards PAW, Greaves M (1981) Expression of cell-surface HLA-DR. HLA-ABC and glycophorin during erythroid differentiation. Nature 289:68–71

Sakai K, Takiguchi M, Karino K (1987) Malignant tumor and MHC. Oncologia 20(1):55–65

Schroder S, Schwarz W, Rehpenning W, Loning T, Bocker W (1988) Dendritic/Langerhans cells and prognosis in patients with papillary thyroid carcinomas. Am J Clin Pathol 89:295–300

Smolle J, Soyer HP, Ehall R, Bartenstein S, Kerl H (1986) Langerhans cell density in epithelial skin tumors correlates with epithelial differentiation but not with the peritumoral infiltrate. J Invest Dermatol 87:477–479

Takiguchi M, Ting JPY, Buessow SC, Boyer SC, Boyer C, Gillespie Y, Frelinger JA (1985) Response of glioma cells to interferon-gamma: increase in class II RNA, protein and mixed lymphocyte reaction-stimulating ability. Eur J Immunol 15:809–814

Tsujitani S, Furukawa T, Tamada R, Okamura T, Yasumoto K, Sugimachi K (1987) Langerhans cells and prognosis in patients with gastric carcinoma. Cancer 59:501–505

Wilson BS, Herzig MA, Lloyd RV (1984) Immunoperoxidase staining for Ia-like antigens in paraffin-embedded tissues from human melanoma and lung carcinoma. Am J Pathol 115:102–108