Acta Oncologica Vol. 30 No. 8 1991

FROM THE DEPARTMENT OF UROLOGY, INSTITUTE OF PATHOLOGY, UNIT OF EPIDEMIOLOGY, AND UNIT OF IMMUNOLOGY AND MICROBIOLOGY, THE SOROKA MEDICAL CENTER AND FACULTY OF HEALTH SCIENCES, BEN GURION UNIVERSITY OF THE NEGEV, BEER-SHEVA, AND THE DEPARTMENT OF ONCOLOGY, BEILINSON MEDICAL CENTER, PETAH TIKVA AND THE SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY, TEL AVIV, ISRAEL.

CELLULAR B-2 MICROGLOBULIN EXPRESSION AS A PROGNOSTIC INDICATOR IN RENAL CELL CARCINOMA

I. LEVIN, O. KUPERMAN, J. GOLDSTEIN, L. NEUMANN, S. SEGAL, J. KANETI, L. LISMER, A. MACHPUD and B. KLEIN

Abstract

The expression of B-2 microglobulin (B-2M) on tumor cells and their normal cell counterparts in 39 patients with renal cell carcinoma was studied and correlated to tumor stage and survival. The median survival time of patients with localized disease (stage I) whose tumors expressed B-2M was 10.2 years while the median survival time for patients whose tumors did not express B-2M was only 3.6 years ($p < 0.001$). For patients with more advanced disease (stages II, III, IV) whose tumors expressed B-2M, median survival time was 3.6 years compared to 2.0 years in patients whose tumors did not express B-2M, a non-significant difference. It is suggested that the tumor cell membrane expression of B-2M may serve as an indicator of good prognosis in early renal cell carcinoma.

Key words: Renal cell carcinoma, B-2 microglobulin, survival.

The major histocompatibility complex (MHC) in humans, as well as in other species, in addition to non-immune functions, plays a pivotal role in controlling the various arms of the immune response against a given antigen, at the level of inductive as well as effective processes (1). MHC class I (HLA-A-B-C in humans) molecules serve as restriction elements for T-cell mediated cytotoxicity and other T-cell mediated functions (2).

Qualitative and quantitative alterations of MHC expression have been observed in murine tumor cells in numerous studies. These alterations include imbalances of expression between the different loci at the MHC, and either a decreased or increased expression of class I MHC encoded antigens (3–7). In a variety of human tumors, reduced expression of HLA class I has been reported (8–10) and in some tumors this attenuation was correlated with the degree of tumor differentiation (11), aggressiveness (12) and invasiveness (13).

Normal kidney cells express HLA class I antigens (14). In renal cell carcinoma Heinemann et al. (15) reported absent expressions of class I antigens in 8 out of 10 patients. On the other hand, Natali et al. (16) reported positive expression of HLA class I in 9 out of 10 patients with renal cell carcinoma.

Due to the importance of HLA class I antigens in immune regulation we report on the expression of HLA class I antigens in renal cell carcinoma in relation to patients' survival.

Material and Methods

Patients. Thirty-nine patients (22 males and 17 females) with renal cell carcinoma were treated by radical nephrectomy at the Soroka Medical Center between 1968 and 1984. Their ages ranged from 16–77 years with a median of 59 years. Pathology reports were reviewed by one of the pathologists. Sixteen patients had stage I, 14 patients stage II, 7 patients stage III and 2 patients stage IV disease. The patients were classified into group A, 'localized disease' (stage I), and group B, 'extended disease' (stages II–IV). Since routine work-up of these patients changed considerably during the years, the patients were allocated to the stages on the basis of the surgical findings according to the following system; stage I: tumor confined to the kidney; stage II: tumor extended to Gerota's fascia; stage III: tumor involving the renal vein, inferior vena cava or hilar lymph nodes; stage IV: tumor spread to local adjacent organs or distant metastasis.

Pathology and histochemistry. Paraffin-embedded blocks from the 39 cases were reviewed. These included routine

Submitted 12 November 1990.
Accepted for publication 19 July 1991.

hematoxylin and eosin stained sections from the tumor and adjacent non-neoplastic renal tissues. From each tumor 1–5 slides were examined by the same pathologist. Histologic sections were chosen based on the degree of tissue preservation and quality and the presence of tumor and normal kidney in each section.

The expression of B-2M in neoplastic and non-neoplastic renal tissue was evaluated by an avidin-biotin complex immunoperoxidase technique as described elsewhere (17). Briefly, histologic sections were cut at 4–6 μm from available paraffin-embedded tissue, dewaxed and rinsed in absolute alcohol. Endogenous peroxidase activity was blocked by 0.5% hydrogen peroxide in methanol. The slides were then rinsed in distilled water and TRIS-buffered saline of pH 7.6, before incubation in 20% (V/V) normal swine serum in TRIS-buffered saline for 30 min at room temperature. After the blocking steps, the tissue sections were incubated with the primary antibody, which was Dakopatts polyclonal rabbit antihuman B-2M (code No.: A-072) 1 : 1 000 for 1 h at room temperature in humid chamber. Unbound material was washed from the slide, and the tissue sections were then incubated with biotin-conjugated secondary antibody. Unbound material was again washed from the slides, and the tissue sections were incubated with streptauidin-peroxidase conjugate. After another wash the enzyme substrate and chromogen were added to the slide, and after incubation of 10–15 min in a humid chamber, an insoluble, highly chromogenic product was deposited at the site of the antigen after exposure to 3-3,diaminobenzidine (DAB). Negative controls were obtained by excluding the primary antibody from the staining procedure. A negative control was used for each case. B-cells from peripheral blood lymphocytes of the patients were used as positive controls.

Two tissue samples from each block were stained in each slide. Two slides from each case were evaluated for immunohistochemical reactivity. Positive staining was determined by comparing each case with its negative control. A case was considered to show positive staining if specific cellular elements, stained more strongly than their comparable negative control, were found. Staining was considered positive if more than 20% of cells were stained positively. This number was chosen arbitrarily to eliminate non-specific staining errors. The specificity of class I antisera was ascertained by (Dako) affinity chromatography on a column coupled with human B-2M antibodies of high affinity. The specificity to B-2M was ascertained by crossed immunoelectrophoresis.

Statistical analysis. Survival was calculated using the Kaplan-Meier method and the differences in survival time was analyzed by the log-rank test.

Results

The expression of B-2M on tumor cells and normal tissue adjacent to the tumor is presented in Table 1.

Table 1

Expression of B-2 microglobulin on renal cell carcinoma and normal renal tissue

B-2M expression	Histologically normal tissue from affected kidney	Renal cell carcinoma
Positive	5 (14.3%)	19 (48.5%)
Negative	30 (85.7%)	20 (51.5%)
Total	35*(100%)	39 (100%)

* In 4 cases non-neoplastic tissue was not available for evaluation.

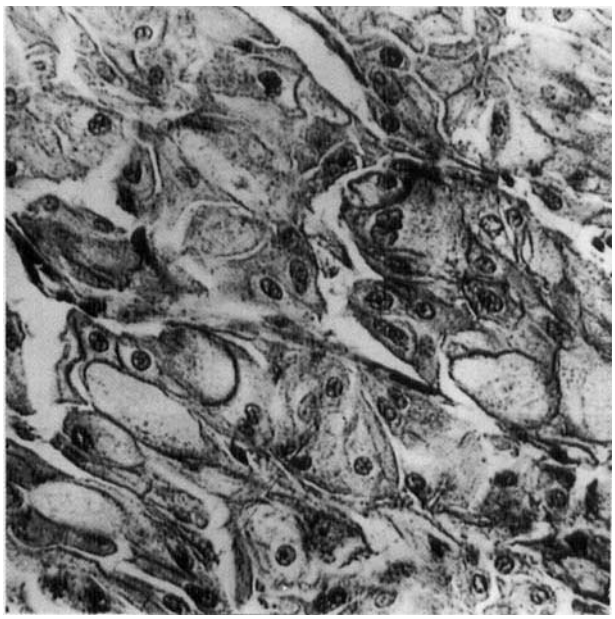

Fig. 1. Renal cell carcinoma, granular type, demonstrating cytoplasmic and membrane staining with anti B-2M (IP × 250).

Nineteen out of 39 tumors were positively stained, whereas the normal adjacent tissue was positively stained only in 5 cases. The staining in the tumor cells was noted mainly as membrane staining (Figs 1, 2), while in the normal tissue renal tubular cells were stained, usually from the proximal convoluted tubules.

The expression of B-2M in relation to stage of the disease is shown in Table 2. Nine cases in group A and 10 in group B were positive for B-2M staining on the tumors, a non-significant difference. Similarly, there was no significant difference in B-2M negative staining between groups A and B. In 15% of all the cases, the normal adjacent tissue was positively stained for B-2M.

The relationship between B-2M expression and survival is shown in Table 3. In group A, the median survival time for B-2M positive patients was 10.2 years compared to 3.6 years for patients whose tumors were B-2M negative ($p < 0.001$). On the other hand, in group B there was no significant difference in the median survival time of patients between B-2M positive and negative tumors. Median

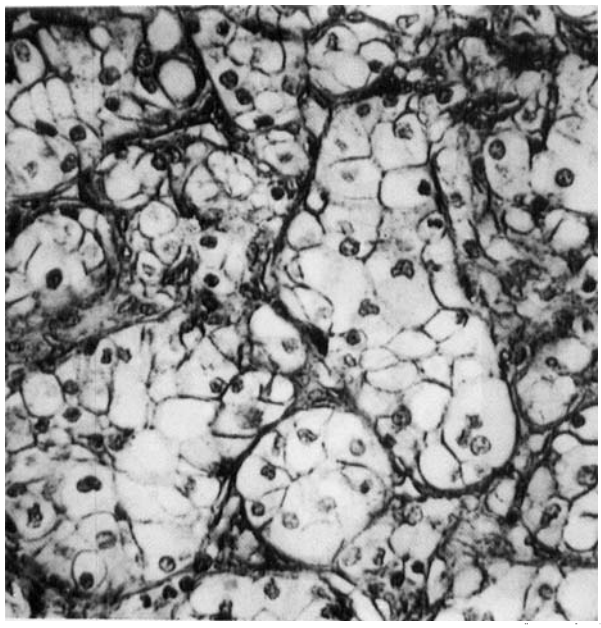

Fig. 2. Renal cell carcinoma, clear cell type, demonstrating positive membrane and focal cytoplastic staining with anti B-2M (IP × 250).

Table 2

Expression of B-2 microglobulin on renal cell carcinoma and normal tissue by stage of disease

B-2M expression	Renal cell carcinoma		Normal renal tissue	
	Group A	Group B	Group A	Group B
Positive	9 (53.3%)	10 (43.5%)	3 (14.3%)	3 (15%)
Negative	7 (46.7%)	13 (56.5%)	12 (85.7%)	17 (85%)
Total	16 (100%)	23 (100%)	15 (100%)	20 (100%)
	(19/39)		(5/35)	

Group A = Stage I.
Group B = Stages II, III and IV.

Table 3

Median survival time (in years) and its relation to the expression of B-2M on cell surface of renal cell carcinoma

B-2M expression	Group A	Group B	p-value
Positive	10.2	3.6	<0.001
Negative	3.6	1.9	N.S.
p-value	<0.001	N.S.	

survival of group A patients with B-2M positive tumors was significantly longer ($p < 0.001$) than of group B patients with B-2M positive tumors. However, there was no significant difference in the median survival time between groups A and B in B-2M negative tumors.

Discussion

B-2M is the 12 kd light chain non-covalently associated with the 45 kd heavy chain of MHC class I antigens. It is generally accepted that class I and B-2M chains must be simultaneously synthesized in order to be expressed on the cell surface (18, 19), and that expression of B-2M reflects the degree of class I expression on the cell surface.

In the present study 48.5% of renal cell carcinoma and 15% of normal tissue adjacent to the tumor expressed B-2M. This is in agreement with the results reported by Heinemann et al. (15). The observation that in 14% of the cases also normal tissue adjacent to the tumor expressed B-2M may be explained by the theory that MHC antigens are lost during malignant transformation. This process starts in the normal tissue and then proceeds to the tumor. In another study from our laboratory (12) the normal tissue adjacent to the tumor expressed less HLA class I antigen.

Since class I antigens serve as restriction elements for cytotoxic T cells it is conceivable to assume that alteration in class I antigen expression on tumor cells might lead to abrogation of the immune response and thus to dissemination of the tumor. Indeed several investigators have reported that the reduction in HLA class I expression in human tumors is correlated to tumor aggressiveness (13) and differentiation (20). However, little data exists regarding the relationship between reduced HLA class I expression and survival. A striking feature in our study was that the median survival of patients with early localized disease whose tumors expressed B-2M, was significantly longer than that of patients with early disease whose tumors did not express B-2M. In patients with no expression of tumor B-2M there was no significant difference in survival between early and advanced disease which suggests that the negative expression of HLA rather than the stage of the disease may be the dominant factor in predicting survival.

In the past several years, numerous studies have reported alteration of MHC class I antigens in both murine and human tumor cells. For example, the virally induced AKR T cell leukemia line K36.16 expresses negligible levels of H-2K antigens (21). The loss of H-2K was also found in the methylcholantherene-induced sarcoma T-10. H-2 gene transfection restored the expression of H-2K antigens in the variant subclones of the T-10 sarcoma and reduced the tumorigenicity and rate of metastases of such subclones (22). Relationships between MHC class I expression, oncogenes and viral transformed tumors have been demonstrated in several studies. Thus, a differential expression of Ki-ras oncogenes was found in $H\text{-}2D^K$ negative non-metastatic and $H\text{-}2K^K$ positive metastatic subclones of the T-10 sarcoma (23). Schrier et al. (24) found that the expression of class I antigens was switched off by the highly oncogenic adenovirus 12 in transformed rat cells while other strains of adenovirus (Ad2 and Ad5) did not

induce such down-regulation and were able to progress to tumors. Amplification of N-myc also causes down-modulation of MHC class I antigens in neuroblastoma (25). On the other hand, various factors, such as interferons and tumor necrosis factor are known to increase the level of class I antigens (26, 27).

On the basis of the discussion above and the results obtained in our present study, one is tempted to speculate about the possible mechanism behind the findings. The possibility exists that there are two major etiologically different types of renal carcinomas. The first, which is rapidly progressive and highly metastatic, might be caused by an agent which leads to the suppression of MHC expression, which in turn results in a rapid progression and metastatic dissemination. The other type may represent a less malignant disease which does not abolish MHC expression. This second type might induce a strong local and possibly systemic immunity which eradicates potential metastatic cells.

A similar correlation between HLA expression, metastatic spread, and clinical course of the disease was reported in patients with melanoma by Van Duinen et al. (28). These findings and our present study may indicate the possible existence of variable constitutive immunity in some patients with melanoma or renal carcinoma. The immunoreactivity of renal cell carcinoma is corroborated by the studies of Rosenberg et al. (29), who employed immunotherapy with LAK cells in advanced renal cell carcinoma with relative success.

In conclusion, B-2M expression on tumor cells seems to be a prognostic factor in early stage renal cell carcinoma and may be an indicator for the use of immunotherapeutic approaches in the treatment of early disease. Further studies on larger material are called for, however.

ACKNOWLEDGEMENT

This work was supported by The Rosen Foundation.

Corresponding author: Dr. Israel Levin, Department of Urology, Soroka Medical Center, PO Box 151, Beer-Sheva, Israel

REFERENCES

1. Benacerraf B. Role of MHC gene products in immune regulation. Science 1981; 212: 1229–38.
2. Zinkernagel RM, Doherty PC. MHC cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T cell restriction: specificity, function and responsiveness. Adv Immunol 1979; 27: 51–177.
3. Blackmore M, Thompson S, Turner G. Forum: The MHC and cancer. Med Sci Res 1987; 15: 807–10.
4. Festenstein H, Garido F. MHC antigens and malignancy. Nature 1986; 322: 502–3.
5. Festenstein H. The biological consequence of altered MHC expression on tumors. Br Med Bull 1987; 43: 217–27.
6. Garcia-Espejo R, Alonso MC, Solana R, Pera C, Pena J. Expression of HLA molecules on cells from fresh explants of human digestive tract cancer. J Immunogenet 1986; 13: 211–7.
7. Goodenow RS, Vogal JM, Linsk RL. Histocompatibility antigens on murine tumors. Science 1985; 230: 777–83.
8. Doyle A, Martin JW, Funa K. Markedly decreased expression of class I histocompatibility antigen proteins on mRNA in human small cell lung cancer. J Exp Med 1985; 161: 1135–51.
9. Kabawat SE, Bast RC Jr, Welch WR, Knapp RC, Bahn AK. Expression of major histocompatibility antigens and nature of inflammatory cellular infiltrate in ovarian neoplasms. Int J Cancer 1983; 32: 547–52.
10. Lopez Nevot MA, Garcia E, Pareja E, et al. Differential expression of HLA antigens in primary and metastatic melanomas. J Immunogenet 1986; 13: 219.
11. Eyal A, Levin I, Segal S, Levi I, Klein B, Kuperman O. Variation of HLA-ABC cells surface antigens expression on adenolarcarcinoma on the colon in correlation to the degree of differentiation. Nat Immun Cell Growth Regul 1990; 193: 1–7.
12. Klein B, Klein T, Konichezky M, et al. The expression of HLA-class I antigens in germ cell testicular cancer. Am J Clin Pathol 1990; 93: 202–7.
13. Klein B, Klein T, Niska A, et al. The expression of HLA class I and class II in gastrocarcinoma in relation to pathological stage. Tumour Biol 1991; 12: 68–74.
14. Williams KA, Hart DN, Fabre JW, Morris PJ. Distribution and quantitation of HLA-ABC and DR (Ia) antigens on human kidney and other tissues. Transplantation 1980; 29: 274–9.
15. Heinemann D, Smith PYB, Symes MO. Expression of histocompatibility antigens and characterization of mononuclear cell infiltrate in human renal cell carcinoma. Br J Cancer 1987; 56: 433–7.
16. Natali G, Bigotti A, Nicotra MR, Viora M, Manfredi D, Ferrone S. Distribution of human class I (HLA-A, B, C) histocompatibility antigens in normal and malignant tissues of nonlymphoid origin. Cancer Res 1984; 44: 4679–87.
17. Hsu SM, Raine L, Fanger H. A comparative study of the peroxidase–antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 1981; 75: 734–8.
18. Eliott BE, Carlow DA, Rodricks AM, Wades A. Prospectives on the role of MHC antigens in normal and malignant cell development. Adv Cancer Res 1989; 53: 182–236.
19. Ploegh HT, Orr HT, Strominger JL. Major histocompatibility antigens: the human (HLA-A, B, C) and murine (H-2K, H-2D) class I molecules. Cell 1981; 24: 287–99.
20. Momburg F, Muller P, Moldenhauer G, Hammerling G. Loss of HLA-A, B, C in colorectal carcinoma is related to the degree of differentiation. J Immunogenet 1986; 13: 195–9.
21. Schmidt W, Festenstein H. Resistance to cell mediated cytotoxicity is correlated with reduction of H-2K gene products in AKR leukemia. Immunogenetics 1982; 16: 257–64.
22. Wallach R, Bulbul N, Hammerling G, Katzav S, Segal S, Feldman M. Abrogation of metastatic properties of tumor cells by de novo expression of H-2K antigens following H-2 gene transfection. Nature 1985; 315: 301–5.
23. Alon J, Hammerling GJ, Segal S, Bar-Eli M. Association in the expression of kirsten-ras oncogene and the major histocompatibility complex class I antigens in fibrosarcoma tumor cell variants exhibiting different metastatic capabilities. Cancer Res 1987; 47: 2553–7.
24. Schrier I, Bernards R, Vaessen R, Houweling A, Eb A. Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. Nature 1983; 305: 771–3.

25. Bernards R, Desoain S, Weinberg R. N-myc amplification causes down-modulation of MHC class I antigens expression in neuroblastoma. Cell 1986; 47: 667–74.
26. Giacomini P, Aguzzi A, Pestka S, Fischer P, Ferrone S. Modulation by recombinant DNA leucocyte (A) and fibroblast (B) interferons of the expression and shedding of HLA and tumor associated antigens by human melanoma cells. J Immunol 1984; 133: 1649–55.
27. Strominger JL. Human major histocompatibility complex genes: class I antigens and tumor necrosis factor. Cold Spring Harbour Symposia on Quantitative Biology, 1986; LI: 63–6.
28. Van Duinen S, Ruiter D, Broecker E, et al. Level of HLA antigens in locoregional metastases and clinical course of the disease in patients with melanoma. Cancer Res 1988; 48: 1019–25.
29. Rosenberg S, Lotze M, Muul L. A progress report on the treatment of 157 patients with advanced cancer by using lymphokine activated killer cells and interleukin 2 or high-dose interleukin alone. N Engl J Med 1987; 316: 889–94.