Journal of Cellular Biochemistry, Supplement 16I:69–71 (1992)

Altered RB Expression Is a Prognostic Clinical Marker Involved in Human Bladder Tumorigenesis

William F. Benedict, MD

Center for Biotechnology, Baylor College of Medicine, The Woodlands, Texas 77381

Abstract We are now beginning to understand the development of bladder cancer at the molecular level. Tumor evolution involves the interaction of both oncogenes and tumor suppressor genes. One of the key tumor suppressor genes in this process is the retinoblastoma (RB) gene. Much has been learned recently about the role of this gene in the tumor progression and prognosis of bladder cancer, although several questions are still unanswered. The progress made on this subject in our laboratory as well as others will be the focus of this report.

Key words: bladder cancer, retinoblastoma gene, tumor progression

Altered retinoblastoma (RB) function plays a significant role in bladder cancer. Initially, approximately one-third of bladder carcinoma cell lines were shown to have an absence of RB protein expression [1]. These results implicated the loss of RB function in bladder carcinogenesis, but where the alteration in RB protein expression occurred in the tumorigenic process still needs to be determined. The present status of this issue as well as the prognostic implications of altered RB function will be discussed.

ALTERED RB FUNCTION IS INVOLVED IN THE PROGRESSION OF BLADDER TUMORS

Evidence for the importance of the RB gene in bladder cancer progression has come from several studies and approaches. In two initial papers, immunohistochemical and Western blot protein analyses were done on a limited number of frozen tumor samples at various stages of progression [2,3]. In both of these studies an increased frequency of altered RB protein expression was found in high stage disease. However, these studies were only preliminary in nature.

The next approach examining the status of the RB gene in bladder cancer was the study of the loss of heterozygosity (LOH) at the RB locus in primary tumors. It was reported that LOH at the RB locus occurred considerably more frequently in advanced stage bladder carcinoma than in earlier stages; 26 of the 28 tumors having LOH were muscle invasive, and of the two others, one rapidly progressed to invasive disease [4]. Although this study was quite convincing, it did not definitively prove that there was actual loss of RB protein expression in the tumors where LOH at the RB locus occurred. In order to show that LOH in these tumors correlated with actual altered RB protein function, the same cohort, together with some additional cases, were examined for both LOH at the RB locus and their RB protein status. Protein studies were done on paraffin-embedded tumor sections using the RB polyclonal antibody, WL-1 [5]. Absence of RB protein expression was found in 15 of 17 tumors in which LOH could be identified [6]. In contrast, 31 of 36 tumors from informative patients with no LOH showed a normal RB protein pattern [6]. These findings were of high statistical significance ($p < 0.001$), and for the first time, showed a correlation between actual structural changes at the RB locus and the absence of RB protein expression in primary bladder tumors. In addition, in this same study [6] altered RB protein expression was seen more frequently in higher stage tumors ($p < 0.001$). Moreover, three of the pT_1 tumors which had altered RB contained a large area of RB-positive tumor cells, along with another RB-negative tumor component. Such

findings further supported the premise that loss of RB function is a factor in the progression of bladder cancer.

Similarly, using their RB staining criteria, Cordon-Cardo and his colleagues [7] examined frozen tumor sections as opposed to paraffin-embedded bladder tumor material and found that the tumors from 13 of 38 patients diagnosed as having muscle invasive disease were RB negative, whereas only 1 of 10 superficial carcinomas was scored as RB negative. These results again suggested that altered RB protein expression occurred in all stages of bladder cancer, but was more common in advanced disease. Consistent with these conclusions was a report published concurrently in which 43 consecutive patients with well-characterized, locally advanced bladder carcinomas were studied. Altered RB protein expression was found in 37% of the primary tumors analyzed [8]. In addition, some tumors were documented to have large areas of RB-positive tumor cells, whereas in other areas all the tumor cells were negatively stained. These results indicated that rather than being an initiation factor in tumor formation, in these specific high stage tumors RB functional loss was a secondary event associated with progression [8].

ALTERED RB PROTEIN EXPRESSION MAY ALSO BE AN EARLY EVENT IN CERTAIN BLADDER CARCINOMAS

Although the studies presented above strongly suggest that the loss of normal RB protein expression occurs more frequently in advanced tumors and thus plays an important role in progression of bladder malignancies, 10 of 45 low stage bladder carcinomas (pT_a or pT_1) have also been found with altered RB protein expression [6]. This documented that actual altered RB function can occur in a portion of low stage tumors as initially suggested by LOH studies at the RB locus [4]. Consequently, the loss of RB function may not always be a late event. However, as mentioned previously, in some low stage cancers, large areas which were both RB positive and RB negative have been documented [6], again indicating that even in early stage disease functional RB protein loss can be associated with tumor progression rather than initiation. Nevertheless, the fact that bladder tumors can develop as secondary nonocular malignancies in patients with hereditary retinoblastoma [8,9] suggests that in certain cases, RB functional loss may be an initiating event.

ALTERED RB PROTEIN EXPRESSION IS AN IMPORTANT INDEPENDENT INDICATOR OF POOR PROGNOSIS

Since the previous studies had strongly suggested that the loss of normal RB protein expression was a key factor in the progression of bladder cancer, the next logical step was to determine whether altered RB protein expression could be used as a prognostic marker signaling an unfavorable outcome. Two independent studies done concurrently indicate that indeed loss of RB protein expression is an important prognostic change in bladder cancer. The first study examined frozen sections of 48 primary bladder tumors obtained from cystectomy or transurethral resection. In this study, computerized image analysis was used to quantify the level of RB protein in each tumor cell. The 5-year survival for this group was 66% with a median follow-up of 42 months. Thirteen of 38 patients with muscle invasive tumors were categorized as having altered RB protein expression [7]. When these patients were examined for overall survival rate, it was found that there was significantly worse prognosis ($p < 0.001$) in those individuals with altered RB protein [7].

In the second study, 43 consecutive patients with locally advanced bladder carcinoma were examined. Each patient received the same chemotherapeutic agents as well as a cystectomy. Paraffin-embedded tumor tissue was examined and a different RB antibody was used. However, similar results were reported. Altered RB protein expression was found in 37% of these advanced stage patients [8]. When disease-free survival greater than 3 years and RB status were compared, absence of RB protein expression was found to be an independent negative prognostic marker in this cohort, and was even more significant when combined with evidence of vascular invasion [8].

Both of these studies largely examined the long-term survival of patients with muscle invasive disease. It is also important to determine if altered RB protein expression is an indicator of a poor prognosis in patients with low stage (pT_a

and pT_1) disease. In one study, 22% of low stage tumors were found to have altered RB protein expression [6], and since this is approximately the percentage of patients who do poorly with low stage bladder cancer, it is tempting to speculate that loss of RB function has a direct influence on the overall survival of patients with early stage tumors as well. Studies are now underway to determine if such speculation is well-founded. If so, this will likely result in new approaches to the treatment of early stage bladder tumor. For example, patients with less advanced disease whose tumors have normal RB expression may require less aggressive therapeutic approaches, whereas those with altered RB expression may need more aggressive therapy including cystectomy and/or chemotherapy. The possibility of RB gene replacement in RB-negative bladder tumors is also now being contemplated, since it has already been shown that reintroduction of normal RB protein expression into RB-negative bladder tumors markedly suppresses their tumorigenic potential [10,11]. Moreover, a nude mouse human bladder tumor model exists to test the efficiency of such an approach [12] before human gene therapy is begun.

Although several other genes, both oncogenes and tumor suppressor genes, have been implicated in human bladder carcinogenesis, it should be apparent that the RB gene has a critical role in this common malignancy. Future directions will include determining if loss of RB function can also occur in *in situ* tumors and whether altered RB function can have prognostic significance in even the earliest lesions identified as having a malignant component. The possibility of considering RB gene therapy and other modalities of therapy based in part on the RB status in a given tumor ensures that the RB gene will continue to be of long-term interest to both basic researchers and clinicians alike who are involved in understanding bladder carcinogenesis and its treatment.

ACKNOWLEDGEMENT

The work done in our laboratory was funded by NIH grant CA-54672 and the Texas Advanced Technology Program.

REFERENCES

1. Horowitz JM, Park S-H, Bogenmann E, Cheng J-C, Yandell DW, Kaye FJ, Minna JD, Dryja TP, Weinberg RA: Frequent inactivation of the retinoblastoma anti-oncogene is restricted to a subset of human tumor cells. Proc Natl Acad Sci USA 87:2775–2779, 1990.
2. Ishikawa J, Xu H-J, Hu S-X, Yandell DW, Maeda S, Kamidono S, Benedict WF, Takahashi R: Inactivation of the retinoblastoma gene in human bladder and renal cell carcinomas. Cancer Res 51:5736–5743, 1991.
3. Presti JC Jr, Reuter VE, Galan T, Fair WR, Cordon-Cardo C: Molecular genetic alterations in superficial and locally advanced human bladder cancer. Cancer Res 51:5405–5409, 1991.
4. Cairns P, Proctor AJ, Knowles MA: Loss of heterozygosity at the RB locus is frequent and correlates with muscle invasion in bladder carcinoma. Oncogene 6:2305–2309, 1991.
5. Xu H-J, Hu S-X, Cagle PT, Moore GE, Benedict WF: Absence of retinoblastoma protein expression in primary non-small cell lung carcinomas. Cancer Res 51:2735–2739, 1991.
6. Xu H-J, Cairns P, Hu S-X, Knowles MA, Benedict WF: Loss of RB protein expression in primary bladder cancer correlates with loss of heterozygosity at the RB locus and tumor progression. Int J Cancer (in press).
7. Cordon-Cardo C, Wartinger D, Petrylak D, Dalbagni G, Fair WR, Fuks Z, Reuter VE: Altered expression of the retinoblastoma gene product is a prognostic indicator in bladder cancer. J Natl Cancer Inst 84: 1251–1256, 1991.
8. DerKinderen DJ, Koten JW, Nagelkerke NJD, Tan KEWP, Beemer FA, Den Otter W: Non-ocular cancer in patients with hereditary retinoblastoma and their relatives. Int J Cancer 41:499–504, 1988.
9. Sanders BM, Jay M, Draper GJ, Roberts EM: Non-ocular cancer in relatives of retinoblastoma patients. Br J Cancer 60:358–365, 1989.
10. Takahashi R, Hashimoto T, Xu H-J, Hu S-X, Matsui T, Miki T, Bigo-Marshall H, Aaronson SA, Benedict WF: The retinoblastoma gene functions as a growth and tumor suppressor in human bladder carcinoma cells. Proc Natl Acad Sci USA 88:5257–5261, 1991.
11. Banerjee A, Xu H-J, Hu S-X, Araujo D, Takahashi R, Stanbridge EJ, Benedict WF: Changes in growth and tumorigenicity following reconstitution of retinoblastoma gene function in various human cancer cell types by microcell transfer of chromosome 13. Cancer Res 52:6297–6304, 1992.
12. Ahlering TE, Dubeau L, Jones PA: A new *in vivo* model to study invasion and metastasis of human bladder carcinoma. Cancer Res 47:6660–6665, 1987.