Emmprin and Survivin Predict Response and Survival following Cisplatin-Containing Chemotherapy in Patients with Advanced Bladder Cancer


Abstract

Purpose: Cisplatin-containing chemotherapy is the standard of care for patients with locally advanced and metastatic transitional cell carcinoma of the urothelium. The response rate is ~50% and tumor-derived molecular prognostic markers are desirable for improved estimation of response and survival.

Experimental Design: Affymetrix GeneChip expression profiling was carried out using tumor material from 30 patients. A set of genes with an expression highly correlated to survival time after chemotherapy was identified. Two genes were selected for validation by immunohistochemistry in an independent material of 124 patients receiving cisplatin-containing therapy.

Results: Fifty-five differentially expressed genes correlated significantly to survival time. Two of the protein products (emmprin and survivin) were validated using immunohistochemistry. Multivariate analysis identified emmprin expression (hazard ratio, 2.23; P < 0.0001) and survivin expression (hazard ratio, 2.46; P < 0.0001) as independent prognostic markers for poor outcome, together with the presence of visceral metastases (hazard ratio, 2.62; P < 0.0001). In the clinical good prognostic group of patients without visceral metastases, both markers showed significant discriminating power as supplemental risk factors (P < 0.0001). Within this group of patients, the subgroups of patients with no positive, one positive, or two positive immunohistochemistry scores (emmprin and survivin) had estimated 5-year survival rates of 44.0%, 21.1%, and 0%, respectively.

Response to chemotherapy could also be predicted with an odds ratio of 4.41 (95% confidence interval, 1.91-10.1) and 2.48 (95% confidence interval, 1.1-5.5) for emmprin and survivin, respectively.

Conclusions: Emmprin and survivin proteins were identified as strong independent prognostic factors for response and survival after cisplatin-containing chemotherapy in patients with advanced bladder cancer.

Transitional cell carcinomas (TCC) are the most common urothelial tumors in the western countries and constitute ~95% of all cases of bladder cancer. This malignancy is among the five most common worldwide. Approximately one of four patients will, at the time of diagnosis or later, present with locally advanced (T4b and N2-3) or metastatic (M1) disease (1).

These patients cannot be cured by radiotherapy or surgery alone and systemic chemotherapy is the only option. For medically fit patients, cisplatin-containing combination chemotherapy is the treatment of choice.

Presently, there are two standard chemotherapeutic regimens: MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin) or GC (gemcitabine and cisplatin). Median survival is 14 to 15 months, and 5-year overall survival rate is between 13% and 15% (2). Although the gemcitabine and cisplatin combination has a significantly better toxicity profile, both regimens still carries risk for significant toxicity and toxic deaths (3) and a substantial fraction of patients will suffer from adverse reactions without achieving any benefit.

Traditional clinical prognostic factors, such as performance status, level of plasma alkaline phosphatase, and presence or absence of visceral metastases, are reported to correlate well with outcome of treatment (3, 4) and are presently included in the decision about treatment strategies for the individual patient. No prognostic biological marker has thus far been introduced into the clinic. However, recent reports have presented molecular signatures, based on microarrays, that objectively discriminate between bladder tumors having...
different clinical outcomes with respect to progression and recurrence of noninvasive tumors (5–8).

Molecular signatures of chemoresistance have been described for several different human carcinomas, such as lung (9), breast (10), colorectal (11), head and neck (12), and ovarian carcinoma (13). For bladder cancer, genome-wide expression profiling has been used to identify genes predictive of response in a neoadjuvant setting (14).

The purpose of the study was to identify molecular markers for survival in locally advanced and/or metastatic bladder cancer following cisplatin-based chemotherapy.

We used global gene expression profiling to identify possible prognostic molecular markers in a cohort of 30 patients. Two of the markers were validated using immunohistochemistry on tumor samples from an independent cohort of 124 patients.

Materials and Methods

Patient selection. The study consisted of an exploratory microarray study based on a prospectively collected material and a validation study based on immunohistochemistry on a retrospectively sampled material. All patients had histology verified locally advanced (T4b, and N2,3) or metastatic (M1) TCC and were treated in the period from March 1, 1995 to January 1, 2004. Patients had received cisplatin-containing combination chemotherapy (methotrexate, vinblastine, doxorubicin, and cisplatin combination or gemcitabine and cisplatin combination). Patients were required to have Eastern Cooperative Oncology Group performance status ≤2, adequate bone marrow reserve, and adequate renal function. Treatment was given according to standard protocols (3). Patients received a maximum of six cycles of treatment, unless they experienced disease progression or developed unacceptable toxicity. Response evaluation was done according to the WHO criteria (15). If bidimensional measurements were not possible, the patient was considered not evaluable and was only included in the survival analyses. Follow-up consisted of clinical examination and computed tomography scan every 3 months for 2 years and every 6 months for 3 additional years or until progression. All patients included in the project were followed clinically until progression. Time to progression and overall survival were recorded as time from first chemotherapy to the relevant event or censoring. Survival data were updated on February 1, 2006.

Informed consent was obtained from all 30 patients in the microarray study. Paraffin blocks were retrospectively collected from routine samples, and because the majority of the patients were dead at the time of the study, informed consent was not possible in this part of the study. The Scientific Ethics Committee of Aarhus County approved both protocols. All analyses are based on material from primary tumors; tissue from metastatic lesions was not included in this study. Tumor material used for microarray expression profiling was collected prospectively and consecutively for a tissue bank at Aarhus University Hospital, Skejby (Aarhus, Denmark).

All patients met the following inclusion criteria: histologic confirmed locally advanced or metastatic TCC of the urothelium; at least two cycles of cisplatin-containing chemotherapy; no neoadjuvant or adjuvant chemotherapy; at least 15 months of follow-up time; available specimen of freshly frozen tumor tissue suitable for RNA preparation (microarray study) or formalin-fixed paraffin-embedded (FFPE) tissue (immunohistochemistry validation study); and no antineoplastic therapy in the time interval between tissue sampling and chemotherapy. Tumor biopsies for microarray analyses were obtained directly from surgery at the Department of Urology, Aarhus University Hospital. After removal of tissue for histologic examination, the other half part of the biopsy was immediately frozen at -80°C in a guanidinium thiocyanate solution for preservation of the RNA. For immunohistochemistry analysis, we used FFPE tissue blocks from routine examination. All patients had chemotherapy at the departments of oncology at Aarhus University Hospital and Copenhagen University Hospital in Herlev. The primary surgical treatment and the FFPE procedure took place at 14 centers throughout the country of Denmark.

Array technology and analyses. Total RNA extraction, preparation of cDNA, labeling, hybridization to Affymetrix HU133A GeneChip, and scanning were done as described previously (5). Data were normalized and intensity measures were generated by robust multiarray analysis using ArrayAssist 3.0 (16).

For survival analysis of the microarray data, we used the Significance Analysis of Microarrays (SAM) program (Stanford University, Stanford, CA) with censored survival data (17). Significance Analysis of Microarrays program identifies genes most closely correlated with survival time in a Cox’s proportional hazards model. Permutation analysis was used to estimate the false discovery rate. We made 500 permutations and due to the exploratory character of this part of the study, we accepted a false discovery rate of 0.37.

Microarray data is available at Gene Expression Omnibus with series accession no. GSE5287.

Immunohistochemistry. FFPE bladder carcinoma samples were sectioned at 3 μm and mounted on glass slides. Primary antibodies were against emmprin (mouse monoclonal sc-21746; dilution 1:100; Santa Cruz Biotechnology) and survivin (rabbit polyclonal ab-469; dilution 1:400; Abcam Ltd.). Immunohistochemistry was done using a BenchMark XT automated stainer (Ventana Medical Systems, Inc.). Deparaffinization, epitope retrieval, and immunostaining were done according to the manufacturer’s instructions using Cell Conditioning solution (CC1) and the i-VIEW DAB detection system. Positive signals were enhanced using i-VIEW Copper, and sections were counterstained with hematoxylin. In each run was included negative controls with omission of primary antibody and positive control consisting of sections known previously to be positive for the antibody in question. Paraffin blocks were available in 25 of the 30 samples used in the microarray experiment. The following cut points were established as having optimal correlation with microarray expression values, as well as survival in these 25 patients: Emmprin membrane staining was scored as follows: negative if ≤10% of cells were stained and positive if ≥10% were stained. Survivin cytoplasmatic staining intensity was scored using a scale of 0 to 3 (0, no staining; 1, weak; 2, moderate; and 3, intense) and the number of positive cells was scored as <10% or ≥10%. Tumors were considered positive if ≥10% of cells stained with an intensity of 2 or 3. A total of 124 samples were used for independent validation. All slides were scored blinded and independently by two observers. In case of disagreement, slides were reevaluated and consensus was reached. There was a good interobserver agreement indicated by a value of 0.83 (P < 0.0001) and 0.72 (P < 0.0001) for emmprin and survivin immunohistochemistry scoring, respectively.

Western blotting. A Western blotting experiment on two cell lines documented the specificity of the antibodies used for immunohistochemistry. The blotting was carried out as described previously using HeLa and human bladder HU1609 cells (18). Membranes were incubated with mouse anti-emmprin (1:200) or rabbit anti-survivin (1 μg/mL).

Statistical analyses. Except for the microarray data analyses, all data calculations were done using the SPSS Statistical software (version 13.0). The distributions of baseline variables and immunohistochemistry results were analyzed by the χ2 test to test for associations between baseline variables and the obtained results. Clinical response was dichotomized as response (WHO complete response and partial response) versus no response (WHO no change or progressive disease). Patients not evaluable for response were excluded from the response analyses. Univariate and multivariate Cox’s regression analyses were done to evaluate the prognostic effect of pretreatment factors in relation to survival. Factors with a P value ≤0.1 in the univariate analyses were included in the multivariate analysis to identify factors with independent significance. The final model was developed by backward

patient characteristics. The clinical features of the patients are presented in Table 1A. All patients had locally advanced and/or metastatic bladder cancer and all had cisplatin-based chemotherapy (methotrexate, vinblastine, doxorubicin, and cisplatin combination or gemcitabine and cisplatin combination). The numbers of events were 25 (83.3%) for the microarray study and 101 (81.5%) for the immunohistochemistry study. For patients at risk at the time of analyses, the median follow-up-time was 81.8 months (range, 56.7-98.0) for the microarray study and 56.5 months (range, 19.5-129.8) for the immunohistochemistry validation study. No patients were lost to follow-up. The median number of cycles was 6 for both studies. The distribution of disease stages within the study population as well as the survival rates were as expected (2). There were no statistically significant associations between marker values and baseline characteristics (data not shown).

Survival analyses based on previously published clinical variables (4) showed expected and significant separation of patients (Table 1B). This indicates that the material was representative of patients with advanced bladder cancer receiving chemotherapy, as accepted prognostic factors behaved as reported elsewhere (2, 4). Among the 124 patients in the immunohistochemistry study, 106 (85%) patients were evaluable for response according to the WHO criteria.

Identification of prognosis-related genes. We used Affymetrix arrays to measure gene expression levels. Following scanning and normalization of the microarray data, we used Significance Analysis of Microarrays program to delineate the optimal markers for survival, and a set of 55 genes differentially expressed and with a high correlation to survival time was identified. A heat map of these genes is presented (Fig. 1). For visualization purposes, the gene expression values were median centered and normalized using Cluster and TreeView software (19). Interestingly, all genes in this set showed a low expression in the patients with long-term survival. The profile included several genes with biological relevance for resistance to chemotherapy like BIRC5 (survivin), BCL2L1, ERCC2, and BSG (emmprin).

In the microarray experiment, we included patients who received methotrexate, vinblastine, doxorubicin, and cisplatin combination or gemcitabine and cisplatin combination, both containing cisplatin, which is considered the key drug in combination chemotherapy for advanced bladder cancer (20). We did not find any differences in expression of the 55 selected genes within the two treatment groups, and the two treatment regimens resulted in similar survival rates in this study population.

Validation using immunohistochemistry. Immunohistochemistry analyses were used to evaluate if the differentially expressed genes showed prognostic value at the protein level. The two genes emmprin and survivin were selected for validation based on (a) high level of significance, (b) biological relevance, and (c) the availability of antibodies against their encoded proteins.

We found emmprin and survivin immunohistochemistry to be cancer cell specific (Fig. 2A). Western blotting revealed bands at relevant molecular weight position and confirmed the specificity of the antibodies (Fig. 2B).

We found a highly significant correlation with overall survival for both emmprin (P = 0.001) and survivin (P > 0.0001) expression and for the combination of the two proteins (P > 0.0001). The same was true for disease-specific survival as well as for progression-free survival (data not shown).

The median survival times were 18.7 months (95% CI, 14.8-22.9) versus 9.7 months (95% CI, 8.1-11.3; P = 0.001).
Eastern Cooperative Oncology Group performance status factors were included in the univariate analyses: sex, age, of pretreatment factors in relation to survival. The following hazards analyses were done to evaluate the prognostic effect categories, respectively (Fig. 3; Table 2).

The 5-year survival rates were 35.5% versus 13.3% versus 0% for the three (95% CI, 7.9-8.9), respectively (P < 0.0001). Five-year survival in months. Black-columns, dead patients; white-columns, survivors.

and the 5-year survival rates were 22.5% and 14.6% for emmprin-negative tumors and emmprin-positive tumors, respectively. For survivin, the median survival times were 18.4 months (95% CI, 16.5-25.8) versus 9.8 months (95% CI, 8.0-11.7; P < 0.0001), and the 5-year survival rates were 27.8% and 5.1% for negative and positive tumors, respectively. If the two immunohistochemistry scorings were added into three categories (both negative, one positive, and both positive), we found median centered and the color saturation indicates differences in gene expression across the tumor samples. Yellow, up-regulation compared with median expression (black); blue, down-regulation. Gene names are listed on the right. B, overall survival in months. Black-columns, dead patients; white-columns, survivors.

Predicting outcome in patients without visceral metastases. The multivariate analysis showed that presence or absence of visceral metastases was the strongest clinical prognostic factor. The protein expression added predictive power to this clinical variable as shown (Fig. 3E), where the group of patients without visceral metastases was further subdivided by the protein expressions. Patients without visceral metastases had a median survival time of 21.1 months (95% CI, 16.5-25.8), which was increased to 47.3 months (95% CI, 21.7-72.9), if the tumor was negative for both emmprin and survivin, and reduced to 17.5 (95% CI, 13.5-21.5) months, if the tumor was negative for only one of the two proteins. If the tumor was immunohistochemistry positive for both emmprin and survivin, the median survival time was 6.6 months (95% CI, 3.6-9.6; P < 0.0001). The latter is very similar to the median survival of 8.7 months (95% CI, 7.1-10.3) in the standard poor prognostic group of patients with presence of visceral metastases. The group of patients without visceral metastases was divided further by analyzing the protein expression, but because the prognosis is quite poor for patients with presence of visceral metastases, no distinct separations of survival curves were noted. Data are presented in Table 2.

Predicting response to chemotherapy. Protein expression assessed by immunohistochemistry was strongly correlated to response to chemotherapy. The response rate for all 124 patients was 51.6%. Among the 106 patients evaluable for response, the response rate was 60.4%.

The response rates in patients with emmprin-negative and emmprin-positive tumors were 74% and 39%, respectively, with an odds ratio of 4.41 (95% CI, 1.91-10.1). For survivin-negative and survivin-positive tumors, response rates were 70% and 47%, respectively, with an odds ratio of 2.48 (95% CI, 1.1-5.5). If the tumor was negative for both emmprin and survivin, the response rate was 82%, as opposed to 27%, if both immunohistochemistry results were positive [i.e., an odds ratio of 11.9 (95% CI, 3.2-42.3)].

Discussion

The clinical course of patients with locally advanced and/or metastatic bladder cancer varies and patients with the same disease stage have different outcomes from the same therapy. Combination chemotherapy is a strenuous treatment and factors that enable pretreatment evaluation of the probability of a survival benefit are of utmost importance. Presently, clinical variables, such as performance status, alkaline phosphatase level, and the presence or absence of visceral metastases, are the only useful prognostic factors for survival following chemotherapy (4). We present novel molecular factors of independent prognostic significance for the outcome of cisplatin-containing chemotherapy in advanced TCC. First, we identified a set of 55 genes, which expression correlated
with overall survival. Among these, several genes were biologically relevant for the effect of chemotherapy. Antiapoptosis is a core factor for chemoresistance (14, 21) and the 55 genes included collective down-regulation of several members of antiapoptotic pathways, such as survivin (22), Bcl-xL (23), Rho GDP dissociation inhibitor (24), tissue transglutaminase 2 (25), and GADD45 (26), among the long-term survivors. These findings implicate a critical role of antiapoptosis in chemoresistant TCC. Another interesting group of relevant genes is emmprin (27), ERCC2/XPD (28), and thrombospondin (29), which all have been correlated to drug resistance. Such evaluation of the biological relevance of the predictive genes is important for an understanding of the involved mechanisms, but it can never replace rigorous and independent validation using new samples and, if possible, an independent method.

Emmprin (BSG) is a 46.6-kDa membrane protein mapped to 19p13.3. It is a modulator of matrix metalloproteinases and is up-regulated in bladder carcinoma compared with benign urothelium (30). Recent work has shown that emmprin enhances growth and resistance to chemotherapy via the phosphatidylinositol 3-kinase/Akt pathway in a hyaluronan-dependent manner (31). Excess expression has also been correlated with tumor progression and the development of metastases in a range of different cancers (32–37). The results from our study support these findings.

Survivin (BIRC5) is localized to 17q25 and is a 16.6-kDa protein present in the cytoplasm and the nucleus. Several splice variants have been reported (22). The variant 3B is described to be located in the cytoplasm and is functionally relevant as it is described to inhibit apoptosis via cytoplasmic caspases (38, 39). High levels of survivin have been associated with poor prognosis in bladder cancer (38) and other human cancers (40–42). Survivin has been described to be a predictor of cisplatin resistance in gastric
Fig. 3. Overall survival rates and immunoreactivity for all patients. A, emmprin. B, survivin. C, combination of (A) and (B). D, presence and absence of visceral metastases. E, separation of survival curves based on emmprin and survivin expression for patients without visceral metastases (red curves) and with visceral metastases (black curves).
cancer and melanoma, as well as in different cell lines (43–45). Targeting survivin as an anticancer strategy by antisense oligonucleotides has been investigated in several cancer types (38). Validation was done by immunohistochemistry on an independent set of archival FFPE material. This is easily accessible and the methods do not require advanced laboratory facilities. Therefore, further validation in a prospective setting using immunohistochemistry is very feasible.

We found survivin and emmprin to be strong and independent prognostic markers for survival. Immunohistochemistry analyses of emmprin and survivin significantly added information to the prognostic assessment based on the presence or absence of visceral metastases.

In the favorable prognostic group of patients without visceral metastases, the expression of the two proteins had discriminatory power as supplemental risk factors. They discriminated subgroups with estimated median survival times of 47.3, 17.5, and 6.6 months, respectively.

The patient material selected for gene expression profiling as well as for validation is representative of patients with advanced bladder cancer, and all patients had complete clinical follow-up data. In the analyses, we considered survival a continuous variable, thereby avoiding arbitrary cutoff values for defining “short-term” and “long-term” survival.

In addition, we found it relevant to evaluate the markers with respect to tumor response as well. These analyses showed that the protein expression correlated not only to survival but also to response, which indicates that the prognostic value of the emmprin and survivin expression is associated with the treatment rather than the course of the disease. The presented protein expressions may therefore represent markers of the sensitivity of TCCs to chemotherapy.

It is strongly recognized that the prognostic value of the two markers presented here, as well as other markers from the profile, has to be tested in an independent prospective randomized study. If validated, these prognostic factors may help to identify patients with either a high or a low probability of benefit from cisplatin-containing chemotherapy. Patients with a high probability of achieving long-term survival should always be offered known effective combination chemotherapy. Patients with an expected poor outcome may be selected for novel and investigational treatment protocols or may achieve more benefit from palliative treatment. In addition, improved knowledge of prognostic factors may assist interpretation of

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<th>Table 2. Median and 5-y survival rates according to emmprin, survivin, and combination of markers</th>
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Table 3. Univariate and multivariate analyses of clinical and laboratory variables

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<td>P-alkaline phosphatase</td>
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*Lower than the lower limit of the normal reference range for male and female patients, respectively.
† Higher than the upper limit of the reference range at the specific period and hospital.
results from different clinical trials and improve information to patients and their relatives. In the future, availability of molecular profiles may permit a more rational allocation of targeted therapy. In clinical use of prognostic markers, immunohistochemistry represents a low cost and easily targeted therapy. In clinical use of prognostic markers, molecular profiles may permit a more rational choice of results from different clinical trials and improve information to patients with locally advanced and/or metastatic bladder cancer.

In conclusion, we have identified two genes whose transcript and encoded proteins may predict response and survival following cisplatin-containing chemotherapy in patients with locally advanced and/or metastatic bladder cancer.

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References

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