Overexpression of the Peripheral Benzodiazepine Receptor Is a Relevant Prognostic Factor in Stage III Colorectal Cancer

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ABSTRACT

Purpose: The peripheral benzodiazepine receptor (PBR) has been implicated in the growth control of colorectal cancer, where PBR-specific ligand-binding is increased 3–4-fold. However, the prognostic relevance of PBR (over)expression has not yet been evaluated in colorectal cancer.

Experimental Design: A 5-year follow-up was performed in 116 consecutive patients undergoing surgery for colorectal cancer with regional or distant metastases [Union International Contre le Cancer (UICC) stage III, 59 patients; UICC stage IV, 57 patients]. The monoclonal anti-PBR antibody 8D7 was used for immunohistochemical examination of paraffin-embedded sections. PBR-specific staining was compared in cancer tissues and normal mucosa. Kaplan-Meier survival curves were calculated.

Results: Twenty-eight percent of the colorectal cancers strongly overexpressed PBR. The mean survival of patients with stage III cancer was 56.2 ± 9.2 months with and 86.8 ± 6.6 months without high overexpression of PBR (P = 0.006). Univariate and multivariate analyses revealed that high PBR overexpression is an independent unfavorable prognostic factor in stage III colorectal cancer. In stage IV, however, the PBR status did not correlate with different survival times.

Conclusions: Strong PBR overexpression is a new independent prognostic marker in stage III colorectal cancer. Evaluating PBR overexpression may be useful for stratifying risk and developing risk-adapted strategies of adjuvant therapy.

INTRODUCTION

Colorectal carcinoma is the second most common cause of cancer death in Western countries. The prognosis of colorectal carcinoma correlates closely with the pathological staging (1). However, even the Tumor-Node-Metastasis classification (UICC4 I-IV) predicts prognosis only within a wide range. Thus, additional prognostic markers are needed to predict survival more precisely.

The PBR has been implicated in tumor growth (2). Binding of PBR-specific ligands is increased in several tumor entities, including cancer of the colon (3), brain (4), breast (5), ovary (6, 7), and liver (8). PBR is an M sub 18,000 D protein located mainly in the outer mitochondrial membrane but also found in the plasma membrane and perinuclear region (2). Almost all tissues express PBR, although to widely varying degrees. Levels of PBR expression are particularly high for organs involved in steroidogenesis but low for normal gut mucosa (9). Although the functions of the PBR are not yet fully understood, there is good evidence for its involvement in steroid biosynthesis (10). Moreover, it does play an important role in the proliferation of cancer cells (5, 11–14). We showed recently that specific PBR ligands induced both apoptosis and cell cycle arrest in colorectal cancer (15).

Despite its overexpression and its emerging importance in the regulation of colorectal cancer growth, the PBR has not yet been investigated for its possible prognostic value. The aim of this study was to determine whether the extent of PBR overexpression correlated with survival in stage III-IV colorectal cancer.

PATIENTS AND METHODS

Patients. Between 1989 and 1991, 116 consecutive patients with colorectal carcinoma UICC III-IV underwent primary tumor resection at the University Hospital Benjamin Franklin (Free University of Berlin, Germany). A complete follow-up of all patients was documented for at least 5 years or until death. There were 59 patients (35 women and 24 men) in UICC stage III and 57 (26 women and 31 men) in UICC stage IV. The mean age was 63.4 years (range, 26–87 years) for UICC III patients and 66.1 years (range, 42–87 years) for UICC IV patients. Only 3 of the 59 patients with colorectal cancer stage UICC III received adjuvant chemotherapy. Clinicopathological parameters have already been described elsewhere (16)

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4 The abbreviations used are: PBR, peripheral benzodiazepine receptor; UICC, Union International Contre le Cancer.
Immunohistochemical Staining. Microsections (2–3 μm) of paraffin-embedded primary tumors were deparaffinized and rehydrated in a decreasing alcohol series (16, 17). Immunohistochemistry was performed using a robotic system (Chemo-mate; DAKO, Heidelberg, Germany). Sections were incubated with the anti-PBR antibody 8D7 (0.5 μg/ml) for 30 min at room temperature. The antibody was kindly provided by P. Carayon (Sanofi-Synthelabo, Montpellier, France) (18). After washing, samples were incubated with antimouse IgG (1:20 dilution) for 30 min at room temperature, and staining was detected by the “fast-red system” (DAKO). Samples were slightly counterstained in Mayer’s hematoxylin.

Semi-quantitative Evaluation of PBR Staining. Tissue staining was independently scored by two of the authors (K. M. and P. G.) with a variation of <10%. The staining intensity of tumor tissue was compared with that of the corresponding normal mucosa for each patient (0, no increase; 1, weak increase; 2, moderate increase; 3, strong increase). A score of 0–12 was calculated as the product of the increase in staining intensity and the frequency of stained cancer cells (0%; 1, 1–25%; 2, 26–50%; 3, 51–75%; 4, 76–100%). The overexpression was rated as low (score ≤6) or high (score >6).

Statistical Analysis. The nonparametric Mann-Whitney U test was used to compare data between groups. Overall survival was assessed by the Kaplan-Meier method, and the significance of differences was calculated by the log-rank test. Univariate and multivariate analyses were performed by the Cox regression model. Therefore, variables were used as dichotomized (categorical) variables: T-stage (T4 versus T1–3), N-stage (N2 versus N1), grading (G3 versus G1–2), tumor localization (rectum/rectosigmoid versus colon), age (>67 years versus ≤67 years), and PBR overexpression (PBRhigh versus PBRlow). Differences of P < 0.05 were considered to be significant. All statistical analyses were performed using SPSS software.

RESULTS

Survival of the Patients. All patients were followed-up for at least 5 years or until death. The median follow-up period was 17 months with a range of 1–112 months. The 5-year survival was 54.2% for UICC stage III and 2% for UICC stage IV patients. Because survival differed between UICC stage III and IV patients (Mantel-Cox log-rank test, P < 0.0001), the following analyses were performed separately for each stage.

PBR Overexpression in Colorectal Carcinomas. The immunohistochemical analysis showed that all colorectal tissues expressed PBR. PBR was detectable in the cytoplasm of both cancer cells and normal mucosa cells but not in the plasma membranes or nuclei (Fig. 1). PBR was homogeneously expressed in normal mucosal tissues (Fig. 1C, F, G, I), whereas the expression in tumor tissues was unevenly distributed (Fig. 1E). The expression levels of PBR in normal mucosa varied among different patients. Most normal epithelia displayed a low to intermediate PBR expression, and only four normal epithelia tissues showed high PBR expression. Therefore, the PBR staining score in tumor tissues was estimated in comparison with the corresponding normal mucosa of the same patient, and only tumor cells with a higher PBR expression than the correspond-
diolabeled PBR-specific ligands have been successfully used to image brain tumors (20).

Our findings in stage III colorectal cancer are the first to demonstrate a significant correlation between PBR expression and survival within a stage-corrected group of tumor patients. Previous studies correlated PBR expression with the degree of malignancy of brain tumors and thus indirectly with survival. Expression differed between low or intermediate but not between higher grades of malignancy (19). Our study likewise showed no difference in PBR overexpression between stage III and stage IV colorectal cancers.

Although its biological functions are not yet fully understood, PBR has been associated with apoptotic and mitotic processes. Antiapoptotic functions have been ascribed to the PBR protein itself. It was shown that transfection-induced PBR hyperexpression protected lymphocytes against UV-induced apoptosis (21). The ability of breast cancer cells to grow in severe combined immunodeficient mice correlated with PBR expres-

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Fig. 1 Immunohistochemical detection of PBR. Immunohistochemical detection of PBR (red) in colorectal cancers (A, B, E, G ca, and H) and corresponding normal mucosa (C, F, G mu, and I). D, negative control staining. A–D, stage IV cancer of the sigmoid colon, score (PBR) = 12. E and F, stage III cancer of the rectum, score (PBR) = 9; 70% of the tumor cells strongly overexpressed PBR (black arrow), and 30% showed no PBR overexpression (white arrow). G, stage IV cancer of the rectum, score (PBR) = 8. H and I, stage III cancer of the cecum, score (PBR) = 0. Bars: A and C–I, 100 μM; B, 20 μM.
Moreover, it was shown that PBR correlated positively with proliferation rate but inversely with spontaneous apoptosis in different glioma cell lines (data not shown). These PBR-mediated proliferative and/or apoptosis-protective effects might contribute to the poor prognosis of patients with PBR-overexpressing tumors. In a variety of tumor models, these functions of PBR can be reversed by PBR-specific exogenous ligands (5, 11–15).

The carcinogenesis of colorectal cancer is a well known multistep process requiring accumulating genetic alterations (23). It progresses from normal epithelial tissues to adenomas, and finally to invasive carcinomas or even metastases. Future studies should investigate the mechanisms regulating PBR overexpression and their possible interactions with already known abnormalities of transcriptional control in colorectal cancer.

In conclusion, this study identified high PBR overexpression as a strong independent predictor of poor survival of stage III colorectal cancer patients. PBR overexpression appears useful for developing risk-adapted strategies of adjuvant therapy. Future clinical studies should evaluate whether PBR overexpression similarly identifies stage II patients that are at high risk for recurrent or metastatic disease.

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