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Original contribution

Altered *PTEN* function caused by deletion or gene disruption is associated with poor prognosis in rectal but not in colon cancer☆

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Summary Colorectal cancer is the third most common malignancy worldwide. Anti–epidermal growth factor receptor (EGFR)–targeted therapy shows clinical evidence in this malignancy and improves outcome. The tumor suppressor gene *phosphatase and tensin homologue* (*PTEN*) is considered a potential predictor of nonresponse to anti-EGFR agents. The purpose of this study was to assess whether associations between *PTEN* alterations (*PTEN* gene deletion or *PTEN* gene disruption) and clinical outcome could be caused by a prognostic (and not predictive) effect of *PTEN* inactivation. Therefore, we analyzed 404 colorectal cancers not previously treated with anti-EGFR drugs in a tissue microarray format. *PTEN* deletion and *PTEN* gene rearrangements were analyzed by fluorescence in situ hybridization. Heterogeneity analysis of all available large tissue sections was performed in 6 cases with genomic *PTEN* alteration. Twenty-seven (8.8%) of 307 analyzable colorectal cancer spots showed genomic *PTEN* alterations including 24 hemizygous and 1 homozygous deletion as well as 2 *PTEN* gene disruptions. Genomic *PTEN* alterations were associated with reduced patient survival in rectal cancer in univariate and multivariate analyses ($P = .012$; hazard ratio, 2.675; 95% confidence interval, 1.242-5.759) but not in colon cancer. Large-section evaluation revealed a homogeneous distribution pattern in all 4 analyzed cases with *PTEN* deletion and in both cases with a *PTEN* gene disruption. In conclusion, genomic *PTEN* gene alterations caused by deletion or gene disruption characterize a fraction of rectal cancers with particularly poor outcome.

1. Introduction

Colorectal cancer is the third most common malignancy worldwide [1]. Besides surgery and standard chemotherapy, anti–epidermal growth factor receptor (EGFR)–targeted therapy offers clinical benefit in advanced colorectal cancer [2].

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The tumor suppressor gene *phosphatase and tensin homologue* (*PTEN*), located on chromosome 10q23, is a negative regulator of the phosphatidylinositol 3-kinases (PI3K)/AKT pathway [3]. EGFR interacts with the cell nucleus through the PI3K/AKT pathway resulting in angiogenesis, proliferation, migration, and cell survival [4]. Loss of *PTEN* has been reported as a potential predictor of response to anti-EGFR therapy in colorectal cancer [5]. Several clinical trials suggested a potential linkage between loss of *PTEN* and chemotherapy resistance to cetuximab in advanced colorectal cancer [6-8].

However, associations between *PTEN* alterations and clinical outcome could also be caused by a trivial prognostic impact of *PTEN* inactivation rather than reflect an effect of nonresponse to therapy. To further evaluate this hypothesis, we analyzed a series of more than 400 colorectal carcinomas that were not treated by anti-EGFR drugs on a tissue microarray (TMA). The analysis not only included the search for *PTEN* deletions by fluorescence in situ hybridization (FISH) but was also extended to genomic *PTEN* disruption, an alternative way of *PTEN* inactivation, recently described in prostate cancer [9].

2. Patients and methods

2.1. Patients, samples, and follow-up

Tissue samples from a total of 404 patients with colorectal cancer who underwent surgical therapy at the Department of General, Visceral and Thoracic Surgery at the University Medical Center Hamburg-Eppendorf between 1993 and 2005 were taken for TMA construction. Written informed consent for the use of resected samples was obtained from all patients, and approval was obtained from the Ethics Committee of the Chamber of Physicians in Hamburg, Germany. Clinicopathologic features were evaluated by a review of medical records and pathologic records. Follow-up was done on a regular outpatient setting via contacting the patient or contacting the patient's primary care physician and included data on deaths of any cause. Patients were excluded from survival analysis if resection was not curatively intended (R2) or tumor-positive resection margins were present in final pathology (R1). Patients with 30-day mortality were also excluded to avoid blurring of survival estimation, as were patients with a follow-up shorter than 6 months.

2.2. TMA construction

Tissue samples were fixed in buffered 4% formalin, paraffin embedded, and used for TMA construction. Hematoxylin-eosin–stained histologic sections from resection specimens were used to mark representative tumor areas. A 0.6-mm tissue core was punched out from the tumor area of each specimen and was transferred in a TMA recipient block using a homemade semiautomated tissue arrayer. The TMA contained 615 tumor spots consisting of 380 primary colorectal cancers (201 colon cancers, 179 rectal cancers), 156 corresponding lymph node metastases, 55 corresponding liver metastases, and 24 samples of recurrent diseases. A standard control area consisting of 114 spots with 64 cancer spots of other organs, 20 normal colon mucosa spots, and 30 normal tissues spots from different sites completed the TMA.

2.3. Fluorescence in situ hybridization

For *PTEN* deletion analysis a Spectrum-Orange–labeled *PTEN* probe was manufactured using 2 bacterial artificial chromosome (BAC) clones (RP11-380G65 and RP11-813O3). BAC-DNA was extracted and labeled with Spectrum-Orange-dUTP (Vysis-Abbott, Chicago, IL) using a commercial nick translation kit (Vysis-Abbott). The *PTEN* probe was used together with a Spectrum-Green–labeled *centromere 10* (*CEP10*) reference probe (Vysis-Abbott). For *PTEN* gene rearrangement analysis, a 2-color *PTEN* break-apart FISH probe consisting of 2 BAC clones was done. One BAC clone set taps the sequence until the last base pair before the 5′-end of *PTEN* (5′-*PTEN*; Spectrum-Orange–labeled RP11-659F22 and RP11-79A15), and the other covers the last 3 kb at the 3′-end of *PTEN* (3′-*PTEN*; Spectrum-Green–labeled RP11-765C10 and RP11-813O3) with a genomic gap of approximately 105 kb between the 2 sets.

Before hybridization, sections were deparaffinized and then proteolytically pretreated with a commercial kit (paraffin pretreatment reagent kit; Vysis-Abbott). Sections were dehydrated in 70%, 85%, and 96% ethanol and air-dried, followed by denaturation for 10 minutes at 72°C in 70% formamide 2× SSC solution. After overnight hybridization at 37°C in a humidified chamber, slides were washed, counterstained with 0.2 μmol/L 4′,6-diamidino-2-phenylindole, and mounted in antifade solution (Vector Labs, Vectashield, CA). Each spot was manually interpreted with an epifluorescence microscope, and the predominant signal numbers were recorded for each FISH probe.

For *PTEN* deletion analysis, a homozygous deletion of *PTEN* was defined as the unequivocal complete absence of *PTEN* signal in all (100%) tumor cell nuclei of the tissue spot, but the presence of *CEP10* signals in tumor cells and presence of *PTEN* and *CEP10* signals in adjacent normal cells. A hemizygous deletion of *PTEN* was defined as the presence of fewer *PTEN* signals than *CEP10* signals (ratio *PTEN*/*CEP10* < 1) in 60% tumor cell nuclei or more. For *PTEN* gene rearrangement analysis, tumors were defined as "normal" when 2 pairs of overlapping orange and green signals were seen per cell nucleus. A (balanced) *PTEN* translocation was defined as the presence of at least 1 split signal consisting of separate orange and green signals observed per cell nucleus. A *PTEN* disruption with loss of either 5′-*PTEN* sequences or 3′-*PTEN* sequences equivalent of a heterozygous *PTEN* inactivation was defined if at least 1 green or orange signal per cell nucleus was lost. A tumor was