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Original contribution

Preoperative radiotherapy modulates ezrin expression and its value as a predictive marker in patients with rectal cancer☆

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Summary The purpose of this study was to assess the value of ezrin expression as a predictor of disease outcome in rectal cancer treated by preoperative radio- or chemoradiotherapy. Operative samples from 176 rectal cancer patients and 76 diagnostic preoperative biopsies from the same cohort were analyzed for ezrin expression using immunohistochemistry. The patients had received short- (n = 76) or long-course radiotherapy with (n = 36) or without chemotherapy (n = 10) or no treatment preoperatively (n = 54). The direct effect of radiation on ezrin expression was studied in cultured cells by Western blot analysis. The biopsies and respective operative samples were significantly different ($\kappa = -0.010$ for 4-tier scoring and $\kappa = 0.028$ for dichotomized scoring) in their ezrin expression. Most preoperative biopsies (61/76, 80%) had negative/weak ezrin expression compared with 56% (43/76) of the corresponding operative samples. After preoperative treatment, negative expression in the biopsies of 18 (82%) of 22 patients turned positive, whereas positive expression in 6 (11%) of 54 biopsies turned negative in the operative samples. In univariate analysis, disease-free survival and disease-specific survival were significantly longer ($P = .027$ and $P = .002$) when ezrin expression in the preoperative biopsy was negative/weak compared with moderate/strong expression. Such prognostic association was lost in the radiated operative specimens. In multivariate regression model, ezrin was not a predictor of disease-free survival. No direct effect of radiation on ezrin expression was seen in vitro. In conclusion, radiotherapy increases ezrin expression in rectal cancer. In pretreatment biopsies, negative/weak ezrin expression correlates with favorable disease outcome, suggesting that ezrin expression modulates tumor aggressiveness and/or response to treatment.

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1. Introduction

The ability to invade and metastasize is characteristic of malignant growth [1]. The prerequisite for both phenomena is impaired cell-cell and cell-matrix adhesion [2]. Ezrin belongs to the ezrin-radixin-moesin family of proteins [3,4], which function as cross-linkers between the plasma membrane and the cytoskeleton [5-8].

Ezrin, also known as *cytovillin*, is widely expressed in several normal and malignant tissues of both epithelial and nonepithelial origin [8,9]. It is located in cell surface structures, such as membrane ruffles, in various cell types [3,10]. Stimulation leads to ezrin phosphorylation and translocation to the ruffles, which in turn leads to increased motility and proliferation [9]. In cultured cells, ezrin is distributed both underneath the plasma membrane and in the cytoplasm, where it links transmembrane proteins, especially adhesion molecules, to actin cytoskeleton [7,11]. Apart from linking the plasma membrane and the cytoskeleton, ezrin is involved in the control of cell morphology, motility, proliferation, and survival, as well as signal transduction (reviewed in [12-14]. The cell signaling pathways associated with ezrin include many oncogenic routes, such as protein kinase C, Rho-kinase, EGFR, Src, mammalian target of rapamycin (mTOR), and PI3 kinase/Akt [13].

In malignant melanoma, ezrin expression is related to higher mortality [15]; and it is essential for the metastatic spread of osteosarcoma cell lines [16]. High ezrin expression has also been associated with poor prognosis in colorectal cancer [17] and in several other malignancies [18,19]. However, there are no data on ezrin specifically in rectal cancer.

In rectal cancer, preoperative radiotherapy is generally given to patients with tumors extending through the bowel wall or to adjacent structures, very large tumors, or tumors with lymph node metastases to improve operability and/or local disease control. Radiotherapy causes cancer cell damage or death via a variety of mechanisms [20,21] causing tumor shrinkage [22]. Radiotherapy can also modify the tumor microenvironment, leading to more extensive membrane ruffling [23], which is a known function of ezrin [24]. In our previous research, we demonstrated that preoperative treatment modulated the expression of hypoxia-inducible factor–1α [25] and affected disease outcome. The current study was designed to assess the effect of preoperative radiotherapy on ezrin expression and clinical outcome of rectal cancer.

2. Patients and methods

2.1. Study population

This study consists of 176 archival operative tumor specimens from rectal cancer patients treated at Turku University Hospital according to the standard treatment protocols. Patients in the control group were operated on during the years 2000-2008; and those in the preoperative radiotherapy groups, in 2003-2008. Only adenocarcinomas of the lower and middle rectum were included to build up a biologically and therapeutically homogenous patient population. Superficial tumors treated by excision only were also excluded. Preoperatively, the patients were staged by computerized tomography (CT) or magnetic resonance imaging and digital examination of the rectum, CT of the abdomen, and x-ray or CT of the chest. Since 2005, rectal cancer treatments in this hospital were planned by a multidisciplinary team. This study is a direct continuation of our previous work testing other biomarkers in this cohort [25,26].

The patients were treated by short- or long-course radiotherapy or received no preoperative treatment (n = 54), depending on the stage of the tumor and following the standard treatment protocols, based upon the judgment of the multidisciplinary team. Short-course radiotherapy consisted of 5 fractions of 5 Gy in a week, with surgery on the following week (n = 76). Long-course radiotherapy was given in 6 weeks for a total dose of 50.4 Gy, with (n = 36) or without (n = 10) concomitant chemotherapy. The chemotherapy regimens were either bolus 5-fluorouracil (n = 5) or capecitabine (n = 31). A total of 122 patients had received preoperative radiotherapy. Of these patients, 76 preoperative biopsies were available for study. Postoperative adjuvant chemotherapy was given to patients with lymph node–positive or high-risk lymph node–negative tumors (72/174, 41%), according to the standard practice [27]. As a control group (n = 54), we studied another series of patients who had not received any treatment before surgery. Postoperative radiotherapy, chemoradiotherapy, or adjuvant chemotherapy was given to eligible control group patients when indicated (19/53, 36%). After completion of the treatment protocols, all patients were followed up at the Department of Surgery. The median follow-up time of the patients was 35 months, and the mean follow-up time was 40 months (range, 2-113 months).

The clinical characteristics of the patients are shown in Table 1. Tumors in this study were adenocarcinomas; about 5% of these were mucinous. Of the operations, 95 (54%) were performed by anterior resection. Surgery was macroscopically radical in 174 (99%) of 176 and microscopically radical (R0 resection) in 107 (72%) of 148 of the patients. Vessel invasion was seen in 36 (30%) of 119 of the tumors. The number of examined lymph nodes was less than 12 in 87 (50%) and at least 12 in the rest (87; 50%) of the operative specimens.

The study protocol was approved by the local Ethics Committee. The collection and use of archival tissue material were approved by the National Authority for Medico-Legal Affairs. The study was conducted in accordance with the Declaration of Helsinki.

2.2. Immunohistochemistry of ezrin

Ezrin protein expression was analyzed in all preoperative diagnostic biopsies (n = 76) available for study and in all