Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

NPTX2 is associated with neoadjuvant therapy response in rectal cancer

Georgios Karagkounis, MD,[a,b] *Leo Thai, MD,*[a,b] *Jennifer DeVecchio, BA,*[b] *Gerald A. Gantt, MD,*[a,b] *Leonardo Duraes, MD, PhD,*[a,b] *Rish K. Pai, MD, PhD,*[c] *and Matthew F. Kalady, MD*[a,b,*]

[a] *Department of Colorectal Surgery, Cleveland Clinic, Cleveland, Ohio*
[b] *Department of Stem Cell Biology and Regenerative Medicine, Cleveland Clinic, Cleveland, Ohio*
[c] *Department of Anatomic Pathology, Cleveland Clinic, Cleveland, Ohio*

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form
8 December 2015
Accepted 23 December 2015
Available online 30 December 2015

Keywords:
NPTX2
Rectal cancer
Chemoradiation
Resistance
Complete response

ABSTRACT

Background: Neoadjuvant chemoradiation (CRT) is recommended for locally advanced rectal cancer. Tumor response varies from pathologic complete response (pCR) to no tumor regression. The mechanisms behind CRT resistance remain undefined. In our previously generated complementary DNA microarrays of pretreatment biopsies from rectal cancer patients, *neuronal pentraxin 2* (*NPTX2*) expression discriminated patients with pCR from those with residual tumor. As tumor response is prognostic for survival, we sought to evaluate the clinical relevance of *NPTX2* in rectal cancer.

Materials and methods: Real-time quantitative polymerase chain reaction was used to evaluate *NPTX2* messenger RNA expression in individual rectal cancers before CRT. Tumors with *NPTX2* expression <50% of normal rectum were defined as NPTX2-low and those with >50% were defined as NPTX2-high. *NPTX2* levels were compared to response to therapy and oncologic outcomes using Mann–Whitney, Kruskal–Wallis, chi-square, and Mantel–Cox (log-rank) tests, as appropriate.

Results: Rectal cancers from 40 patients were included. The mean patient age was 56.8 years, and 30% were female. pCR was achieved in eight of 40 patients (20%). In these patients, messenger RNA *NPTX2* levels were significantly decreased compared to those with residual cancer (fold change 30.4, $P = 0.017$). Patients with NPTX2-low tumors ($n = 13$) achieved improved response to treatment ($P = 0.012$ *versus* NPXT2-high tumors), with 38.5% and 46.1% of patients achieving complete or moderate response, respectively. Of patients with NPTX2-high tumors ($n = 27$), 11.1% and 18.5% achieved complete or moderate response, respectively. No recurrence or death was recorded in patients with NPTX2-low tumors, reflecting more favorable disease-free survival ($P = 0.045$).

Conclusions: Decreased *NPTX2* expression in rectal adenocarcinomas is associated with improved response to CRT and improved prognosis. Further studies to validate these results and elucidate the biological role of *NPTX2* in rectal cancer are needed.

* *Corresponding author*. Department of Colorectal Surgery, Cleveland Clinic, 9500 Euclid Avenue, A30 Cleveland, OH 44195. Tel.: +1 216 445 2655; fax: +1 216 445 8627.
E-mail address: kaladym@ccf.org (M.F. Kalady).
0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2015.12.042

1. Introduction

Preoperative chemoradiation (CRT) reduces local recurrence for patients with locally advanced rectal cancer undergoing total mesorectal excision [1–3]. A wide range of responses is observed, with 10%–25% of patients achieving pathologic complete response (pCR), whereas others demonstrate partial or no treatment effect [4–6]. Considering this variability, predictive factors for CRT response could have profound implications in clinical practice, potentially sparing patients with resistant tumors from therapies that may offer them limited benefit, or expanding CRT indications to include highly sensitive early-stage tumors aiming to avoid proctectomy altogether. In addition, our group and others have showed that tumor regression closely correlates with local control and long-term survival, with pCR resulting in excellent long-term oncological outcomes [5–8]. However, there are no reliable predictors of pCR or CRT resistance.

Although patient and treatment factors contribute to CRT response, tumor biology is likely to be a major determinant of regression [5,9,10]. Nonetheless, our understanding of the biology of CRT resistant rectal tumors remains limited despite considerable efforts in the field. Uncovering the molecular characteristics of rectal adenocarcinomas that are sensitive to CRT could improve patient care by allowing for individualized treatment decisions, appropriate patient stratification in clinical trials, and pharmacologic manipulation of target pathways to increase sensitivity. We previously reported genetic profiles associated with resistance to CRT, which were determined by high-throughput nucleotide microarrays of pretreatment rectal adenocarcinoma samples [11]. *Neuronal Pentraxin 2 (NPTX2)* was selected from this data set, as it had the greatest difference (30-fold, $P = 0.02$) between patients with pCR after neoadjuvant CRT and those with residual tumor. This gene has been recently studied in the context of other malignancies, including glioblastoma, pancreatic cancer, and renal cell carcinoma [12–14]. Interestingly, in renal cell carcinoma, it was associated with aggressive disease, presumably via a mechanism involving the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunit GluR4 [14]. These studies, together with our *in silico* results, suggested a potential role for *NPTX2* in rectal cancer. The goal of this study was to further characterize *NPTX2* in patient tumor samples.

2. Materials and methods

2.1. Patient population

The Cleveland Clinic Department of Colorectal Surgery maintains an institutional review board–approved tissue bank of freshly frozen tumor samples. Patients with rectal cancer and available pretreatment biopsy samples who underwent long-course CRT followed by proctectomy between 2004 and 2012 were included in the study. All patients underwent 5040 cGy radiation in 30 fractions with 5-fluorouracil administered as radiation sensitizer. Curative proctectomy was performed approximately 8–10 weeks after completion of CRT.

2.2. Pathologic assessment

Pretreatment rectal cancer biopsies were reviewed by a gastrointestinal pathologist to confirm presence of greater than 60% tumor in the specimen. Histologic grade (degree of differentiation) was assessed in the pretreatment specimens. Regression scores after neoadjuvant CRT were assessed applying the American Joint Committee on Cancer (AJCC) criteria; 0: complete response, no viable cancer cells; 1: moderate response, single, or small groups of cancer cells; 2: minimal response, residual cancer outgrown by fibrosis; 3: poor response, minimal, or no tumor kill with extensive residual cancer.

2.3. RNA isolation and quantitative real-time polymerase chain reaction

RNA isolation for the microarray and real-time polymerase chain reaction (RT-PCR) experiments was performed as previously described [11,15]. Briefly, frozen tissue blocks were cut on a microtome to generate 20–30 × 5-μm slices. Using the RNAqueous Kit (Ambion, Austin, TX), RNA was isolated following the manufacturer's instructions. A spectrophotometer was used to quantify the isolated RNA and verify its purity and integrity. RNA was converted to complementary DNA using qScript cDNA SuperMix (Quanta Biosciences, MD). Quantitative RT-PCR was performed using SYBR Green PCR Master Mix (Life Technologies, NY) and ABI Prism 7900HT Sequence Detection System (Life Technologies, NY). *B-actin* was used as internal control. The oligonucleotide primers used for quantitative RT-PCR were *NPTX2* 5-CCT CCCACTCCGCACAAAC-3 (forward), 5-CACCGCATAGGAGAAG GGG-3 (reverse), and *B-actin* 5-AGAAAATCTGGCACCACACC-3 (forward), 5-AGAGGCGTACAGGGATAGCA-3 (reverse).

2.4. NPTX2 expression groups

To frame the discriminatory ability of *NPTX2* in a clinically relevant perspective, expression patterns were grouped based on their value relative to normal rectal mucosa. Normal rectal *NPTX2* expression was defined as the average expression in the proctectomy specimens obtained from five individuals with rectal adenocarcinoma (who did not undergo preoperative radiation or CRT), sampled at least 5 cm away from the tumor border. The samples of the 5 individuals demonstrated little variability in NPTX2 expression (Grubb's test $P > 0.05$). Tumors were classified as "NPTX2-low" if their *NPTX2* messenger RNA (mRNA) expression was less than 50% of normal rectum expression levels, whereas tumors with NPTX2 expression greater than 50% of normal rectum were classified as "NPTX2-high".

2.5. Statistical analyses

Gene expression comparisons were performed using nonparametric Mann–Whitney test (two comparison groups)