International Journal of Radiation Oncology biology • physics

www.redjournal.org

Clinical Investigation: Gastrointestinal Cancer

# Upregulation of Trefoil Factor 3 (TFF3) After Rectal Cancer Chemoradiotherapy Is an Adverse Prognostic Factor and a Potential Therapeutic Target

**Enrique Casado, MD,[*] Victor Moreno Garcia, MD,[†,‡] Jose Javier Sánchez, PhD,[¶] María Teresa Gómez del Pulgar, PhD,[**] Jaime Feliu, MD,[†,‡] Joan Maurel, MD,[††] Beatriz Castelo, MD,[†] Juan Moreno Rubio, PhD,[‡] Rocío A.B. López,[‡] Miguel Ángel García-Cabezas, MD,[§] Emilio Burgos, MD,[§] Javier de Castro, MD,[†,‡] Cristóbal Belda-Iniesta, MD,[†] Miriam López-Gómez, MD,[*] César Gómez-Raposo, MD,[*] Francisco Zambrana, MD,[*] María Sereno, MD,[*] Carlos Fernández-Martos, MD,[‡‡] Pilar Vázquez, MD,[‖] Juan Carlos Lacal, PhD,[**] Manuel González-Barón, MD,[†] and Paloma Cejas, PhD[‡]**

**Unidad de Oncología, Hospital Infanta Sofía, Madrid, Spain; [†]Servicio de Oncología Médica, [‡]Laboratorio de Oncología Traslacional, [§]Departamento de Anatomía Patológica, and [‖]Servicio de Gastroenterología, Hospital Universitario La Paz, Madrid, Spain; [¶]Departamento de Bioestadística, Universidad Autónoma de Madrid, Madrid, Spain; **Unidad de Oncología Traslacional, Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas, Madrid, Spain; [††]Departamento de Oncología, Hospital Clinic, Barcelona, Spain; and [‡‡]Fundación Instituto Valenciano de Oncología, Valencia, Spain*

Received Dec 23, 2011, and in revised form Jan 25, 2012. Accepted for publication Jan 28, 2012

**Summary**

Elucidating the dynamic of pathway activation after treatment could help to identify rectal cancer patients who will benefit from preoperative and

**Purpose:** Management of locally advanced rectal cancer (RC) consists of neoadjuvant chemoradiotherapy (CRT) with fluoropyrimidines, followed by total mesorectal excision. We sought to evaluate the expression of selected genes, some of which were derived from a previous undirected SAGE (serial analysis of gene expression)-based approach, before and after CRT, to identify mechanisms of resistance.

**Methods:** This retrospective cohort study included 129 consecutive patients. Quantitative polymerase chain reaction of 53 candidate genes was performed on the biopsy specimen before treatment and on the surgical specimen after CRT. A paired-samples *t* test was performed to

Reprint requests to: Enrique Casado, MD, Unidad de Oncología, Hospital Universitario Infanta Sofía, Avenida de Europa 34, San Sebastián de los Reyes, Madrid 28072, Spain. Tel: (34) 618-398-170; Fax: (34) 911-915-388; E-mail: enrique.casado@salud.madrid.org or Paloma Cejas, PhD, Laboratorio de Oncología Traslacional, Hospital Universitario La Paz (IdiPAZ), Paseo de la Castellana 261, Madrid 28046, Spain. Phone/fax: (34) 912071027; E-mail: paloma.cejas@idipaz.es

Funded by Fondo de Investigaciones Sanitarias PI021094 and Fundación Mutua Madrileña. P. Cejas is supported by Asociación Española Contra el Cáncer programa PAO 2010, and V. M. García is supported by Fundación Para la Investigación Biomédica del Hospital La Paz grant REX 09.

E. Casado and V. M. Garcia contributed equally to this work.

Conflict of interest: none.

Supplementary material for this article can be found at www.redjournal.org.

*Acknowledgment*—The authors thank Esther Díaz and María de Miguel for outstanding technical support.

Int J Radiation Oncol Biol Phys, Vol. 84, No. 5, pp. 1151–1158, 2012
0360-3016/$ - see front matter 
doi:10.1016/j.ijrobp.2012.01.083

postoperative treatment. We present the dynamic expression of 53 genes, before and after preoperative chemoradiotherapy, in a series of 129 consecutive patients. We show that upregulation of TFF3 is associated with a higher risk of relapse, pointing to TFF3 as a novel prognostic marker and a candidate therapeutic target.

determine genes that were significantly changed after CRT. The result was correlated with patients' disease-free survival.

**Results:** Twenty-two genes were significantly upregulated, and two were significantly downregulated. Several of the upregulated genes have roles in cell cycle control; these include CCNB1IP1, RCC1, EEF2, CDKN1, TFF3, and BCL2. The upregulation of TFF3 was associated with worse disease-free survival on multivariate analyses (hazard ratio, 2.64; $P = .027$). Patients whose surgical specimens immunohistochemically showed secretion of TFF3 into the lumen of the tumoral microglands had a higher risk of relapse (hazard ratio, 2.51; $P = .014$). *In vitro* experiments showed that DLD-1 cells stably transfected with TFF3 were significantly less sensitive to 5-fluorouracil and showed upregulation of genes involved in the transcriptional machinery and in resistance to apoptosis.

**Conclusion:** Upregulation of TFF3 after CRT for RC is associated with a higher risk of relapse. The physiological role of TFF3 in restoring the mucosa during CRT could be interfering with treatment efficacy. Our results could reveal not only a novel RC prognostic marker but also a therapeutic target. 

**Keywords:** TFF3, Rectal cancer, Neoadjuvant treatment, Response prediction, Prognosis

## Introduction

Colorectal carcinoma is one of the leading causes of cancer death worldwide, and rectal cancer (RC) accounts for 30%-35% of these cases (1). Approximately half of all RC cases are diagnosed in stage II or III, in which preoperative chemoradiotherapy (CRT), followed by total mesorectal excision, is currently the most accepted treatment. Preoperative CRT improves local control and reduces toxicity (2, 3) compared with postoperative CRT, but its impact on the patient's survival is unclear (4). Importantly, despite this treatment, distant metastases develop in one-third of patients (5). In addition, the local tumor response is highly variable, with 10%-15% of patients achieving a complete pathologic response and 15%-20% having no tumor response (2). Thus, there is an overwhelming need for new strategies that can overcome resistance to CRT and allow better customized therapy.

The reason for this variability in response is unknown. The variability could be partly explained by differences in the genetic background of the tumor and by the mechanisms of resistance acquired during treatment (6). A number of gene expression profiles obtained from pretreatment biopsy specimens have been developed with the aim of better identifying patients who are more likely to respond to the preoperative CRT (7-9). Although these models provide a valuable tool for selecting candidate patients for neoadjuvant treatment, they are not able to explain the molecular changes occurring within the tumor that might be responsible for treatment resistance. To this end, identifying RC markers that are altered in response to CRT could reveal new drivers of resistance and could provide tantalizing therapeutic targets to develop complementary treatments. We had previously conducted a small pilot study that dynamically evaluated the RC transcriptome by serial analysis of gene expression (SAGE) and found a small group of genes that are commonly regulated in patients who are refractory to CRT (10).

We sought to evaluate the differential expression of selected genes before and after CRT and to evaluate the impact of these changes in expression on patient prognosis, to identify potential mechanisms of resistance to CRT, as well as novel therapeutic targets.

## Methods

### Patients

One hundred twenty-nine patients with RC were included in this study. Both pre-treatment biopsies and surgical specimens were histologically confirmed to be colorectal adenocarcinomas. Values for various clinical and pathological variables were retrospectively obtained from a database of information on patients treated with neoadjuvant chemoradiotherapy (CRT) for rectal cancer at Hospital Universitario La Paz in Madrid and Hospital Clinic of Barcelona (108 and 21 patients, respectively). All patients underwent flexible endoscopy with rectal biopsy and magnetic resonance imaging (MRI) and/or endoscopic ultrasound for clinical staging. A chest radiograph was obtained and abdominal and pelvic computed tomography was performed to exclude TNM stage IV tumors. Patients with clinical stage II or III disease were considered eligible for this study. In 7 cases with low rectal stage I disease, neoadjuvant CRT was given to avoid abdominoperineal amputation. All patients received concurrent CRT before surgery. Radiotherapy was uniform across all of the patients and consisted of 45 Gy followed by a boost of 5 Gy to the gross tumor volume and the mesorectum. Concurrent chemotherapy consisted mainly of fluoropyrimidines (5-fluorouracil [5-FU], Tegafur-Uracil (UFT)-leucovorin, and capecitabine) or oxaliplatin-based chemotherapy (raltitrexed-oxaliplatin or oxaliplatin-capecitabine). Surgery was performed 6-8 weeks after the completion of radiotherapy, and total mesorectal excision was routinely performed. Tumor regression grade (TRG) was assessed according to Dworak et al (11). Follow-up examinations were performed according to the National Comprehensive Cancer Network guidelines. Disease-free survival (DFS) was defined as the time from diagnosis to the first evidence of disease recurrence, either locally or as metastases. Overall survival (OS) was defined as the time from diagnosis to death or last follow-up.

A flowchart of patients is shown in Fig. E1 (supplementary material).