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Comprehensive evaluation of the effectiveness of gene expression signatures to predict complete response to neoadjuvant chemoradiotherapy and guide surgical intervention in rectal cancer

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Neoadjuvant chemoradiotherapy (nCRT) may lead to complete tumor regression in rectal cancer patients. Prediction of complete response to nCRT may allow a personalized management of rectal cancer and spare patients from unnecessary radical total mesorectal excision with or without sphincter preservation. To identify a gene expression signature capable of predicting complete pathological response (pCR) to nCRT, we performed a gene expression analysis in 25 pretreatment biopsies from patients who underwent 5FU-based nCRT using RNA-Seq. A supervised learning algorithm was used to identify expression signatures capable of predicting pCR, and the predictive value of these signatures was validated using independent samples. We also evaluated the utility of previously published signatures in predicting complete response in our cohort. We identified 27 differentially expressed genes between patients with pCR and patients with incomplete responses to nCRT. Predictive gene signatures using subsets of these 27 differentially expressed genes peaked at 81.8% accuracy. However, signatures with the highest sensitivity showed poor specificity, and vice-versa, when applied in an independent set of patients. Testing previously published signatures on our cohort also showed poor predictive value. Our results indicate that currently available predictive signatures are highly dependent on the sample set from which they are derived, and their accuracy is not superior to current imaging and clinical parameters used to assess response to nCRT and guide surgical intervention.

Keywords Rectal cancer, neoadjuvant chemoradiotherapy, complete response, gene signature, RNA-Seq

Neoadjuvant chemoradiotherapy (nCRT) is currently considered one of the preferred initial treatment strategies for locally advanced rectal cancer because of its benefits in long-term local disease control (1). In addition, nCRT may lead to primary tumor regression, ultimately resulting in complete pathological response (pCR) in a significant proportion of patients with rectal cancer (2). Reported pCR rates are variable, ranging from 0–42% in several phase II and III studies depending on the CRT regimen used (2–4). However, most series have reported pCR rates between 14–24% (5,6).

pCR has been associated with improved local disease control and survival, and there has been a progressive

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interest in alternative management of patients with rectal cancer who present with pCR to nCRT (5,6). The potential lack of oncological benefit of radical surgery, including total mesorectal excision (TME) with or without sphincter-preservation for patients with locally advanced rectal cancer and considerable risk for post-operative morbidity, have raised the possibility of offering organ-preserving strategies to these patients (7,8). Unfortunately, pCR is not accurately identified by any of the currently available clinical assessment or radiological imaging methods before or after nCRT (9). Precise up-front identification of these patients could potentially have a major impact in their actual clinical and surgical management by avoiding surgery and allowing a safe organ-sparing alternative (10,11).

Many studies have attempted to identify a clinically useful and reproducible gene expression signature capable of predicting response to nCRT using microarrays (12–17). Most studies have focused on the identification of predictive signatures to distinguish "good" responders from "bad" responders and were primarily interested in the identification of patients who would benefit the most from nCRT and spare others from the potential toxicity of CRT. However, definition of "good" response to nCRT may not be straightforward and is sometimes grouped together with pCR and residual ypT3 or ypN1 when tumor regression grade (TRG) alone was used for classification (12–18). Significant variations in definitions of responders and non-responders, in addition to the intrinsic subjectivity of these definitions, may be critical in this setting. Instead, the use of complete response to nCRT as an endpoint (defined for the purpose of the current study as either a pCR or a sustained complete clinical response (cCR) for at least 24 months to overcome any subjectivity in the clinical assessment of treatment response) provides an objective distinction of patients who benefit the most from neoadjuvant therapy with a potential clinically relevant impact of sparing patients from potentially unnecessary radical surgery.

In the present study, using RNA-Seq (19) gene expression profiles, we searched for gene expression signatures that were capable of predicting pCR in an attempt to identify candidates for this organ-preserving strategy. We have also evaluated the utility of previously published gene expression signatures in predicting complete response to nCRT in our cohort.

Materials and methods

Patients

A total of 25 consecutive patients with cT2-4N0-2M0 biopsy-proven rectal adenocarcinoma, which was located no more than 7 cm from the anal verge measured by a single experienced colorectal surgeon, were included in this study. Between 2006 and 2011, all pretreatment biopsies at Local Institute were routinely collected for a tumor-tissue bank after institutional review board approval and used for RNA-Seq analysis. Baseline staging included MRI and/or endorectal ultrasound (ERUS) for local assessment and abdominal/chest computed tomography (CT) for systemic staging. Briefly, radiation therapy consisted of 45 Gy of radiation delivered by a three-field approach, with daily doses of 1.8 Gy to the pelvis on weekdays, followed by a 5.4–9 Gy boost to the primary tumor and perirectal tissue (54 Gy total). Concurrently, patients received 5-fluoracil (425 mg/m^2/d) and folinic acid (20 mg/m^2/d) intravenously administered chemotherapy. Patients were assessed after 12 weeks from nCRT completion since the use of longer interval periods before clinical assessment has been shown to consistently increase pCR or cCR rates (20). Patients with clinical or radiological evidence of persistent cancer were referred to radical surgery (incomplete clinical response). Patients with no evidence of residual disease (cCR) were offered no immediate surgery and were closely followed (18).

Response to nCRT

Patients were grouped according to tumor response. Patients with incomplete clinical responses managed by radical surgery were grouped into three different categories, according to the final pathological staging: Group 1, patients with significant residual disease ($>$10% of cancer cells and $\geq$ypT2 or ypN1-2); Group 2, patients with intermediate or near-complete response ($\leq$10% of cancer cells or ypT1N0); and Group 3, patients with pCR who underwent radical surgery instead of observation alone due to the inability to rule out residual cancer by clinical/radiological assessment. Group 4 included patients with complete clinical response managed by observation alone and with sustained response without evidence of recurrence after at least 24 months follow-up. Patients with intermediate responses (Group 2) were excluded from the study. Exclusion of patients with intermediate responses eliminated the potential noise created by patients who could have responded completely if their clinical assessment had been performed later and those who transiently showed a significant response followed by significant tumor repopulation (21). Patients from groups 1, 3, and 4 were respectively defined as IR, pCR, and cCR, respectively, for the purpose of this study.

Tumor samples

All fragments were snap-frozen in liquid nitrogen and stored at -80^{o}C immediately after endoscopic biopsies. Before RNA extraction, all fragments were analyzed for the presence of at least 80% adenocarcinoma with H&E staining. Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA), and RNA quality was evaluated on a 2100 Bioanalyzer (Agilent, Santa Clara, CA). All samples had an RNA integrity number (RIN) $>$6.

RNA-Seq and differential expression analysis

Ribosomal RNA was depleted from total RNA using the RiboMinus Eukaryote Kit for RNA-Seq (Invitrogen). RNA-Seq libraries were prepared using SOLiD Total RNA-Seq Kit (Life Technologies, Carlsbad, CA), according to the manufacturer's recommendations, and were sequenced on the SOLiD sequencing platform (Life Technologies).