Molecular Biology: Are We Getting Any Closer to Providing Clinically Useful Information?

Georgios Karagkounis, MD¹ Matthew F. Kalady, MD¹

¹Department of Colorectal Surgery, Digestive Disease and Surgery Institute, Cleveland Clinic, Cleveland, Ohio

Clin Colon Rectal Surg 2017;30:415–422.

Address for correspondence Matthew F. Kalady, MD, Department of Colorectal Surgery, Digestive Disease and Surgery Institute, Cleveland Clinic, 9500 Euclid Avenue, A30, Cleveland, OH 44195 (e-mail: kaladym@ccf.org).

Abstract

Keywords

- rectal cancer
- biomarkers
- neoadjuvant chemoradiation
- prognosis
- molecular markers
- gene signatures

Advances in molecular biology and biomarker research have significantly impacted our understanding and treatment of multiple solid malignancies. In rectal cancer, where neoadjuvant chemoradiation is widely used for locally advanced disease, most efforts have focused on the identification of predictors of response in an attempt to appropriately select patients for multimodality therapy. A variety of biomarkers have been studied, including genetic mutations, chromosomal copy number alterations, and single as well as multigene expression patterns. Also, as transanal resection of rectal tumors requires accurate preoperative detection of lymph node metastasis, the identification of biomarkers of regional nodal involvement has been another important field of active research. While preliminary results have been promising, lack of external validation means has a limited translation to clinical use. This review summarizes recent developments in rectal cancer biomarker research, highlighting the challenges associated with their adoption, and evaluating their potential for clinical use.

Advances in molecular biology and our understanding of neoplasia and its underlying mechanisms have revolutionized oncology over the last few decades. From breast cancer to leukemia and from glioblastoma to colon cancer, the clinician currently has more tools available than ever before, allowing the identification of high-risk patients, the prediction of treatment response, and the use of highly effective targeted therapies. Considering the incidence, mortality, and impact on quality of life associated with rectal adenocarcinoma, it is not surprising that extensive basic science and translational research to provide more individualized treatments have grown exponentially. Also, the widespread use of combined modality highlights the need for a deeper understanding of disease biology to improve patient selection and tumor response.

The current treatment paradigm for locally advanced rectal adenocarcinoma includes neoadjuvant chemoradiation (CRT). Response to treatment is associated with prognosis,^{1,2} and complete response may even allow nonoperative management

using aggressive, active observation protocols.^{3,4} Given that both CRT and surgery are independently associated with significant morbidity, being able to avoid either treatment may be beneficial to patients. For example, if a biomarker were able to predict that the tumor will not respond to CRT, then the patient would be better served by avoiding the morbidity associated with it and directly undergo surgery. Similarly, if molecular markers could be utilized to predict or help confirm a complete clinical and pathological response, then surgery could be safely avoided in those patients. This is particularly relevant when determining sphincter preservation or the ability to achieve an adequate oncologic outcome using transanal local excision techniques. Molecular and genetic techniques have allowed for an explosion of research in this field, and large-scale gene signatures associated with response to radiation were reported approximately 10 years ago.⁵ Studies have evaluated specific genes and mutations, single nucleotide polymorphisms, chromosomal copy number alterations, and single or multigene expression patterns. Despite the vast

amount of knowledge gained about rectal cancer biology, there have been challenges applying this information as clinically useful tools. This article reviews various biomarkers studied in the treatment of rectal cancer and provides an opinion about the current and future state of the field.

Chemoradiation Response Biomarkers

The ability to predict and/or achieve enhanced response to CRT is one of the most pressing challenges in rectal cancer treatment. Several genetic polymorphisms and mutations, single gene expression profiles, and multigene signatures have been used as CRT response biomarkers during the last two decades. The goal of predicting which patients will benefit from CRT has led multiple groups to test pretreatment samples for genes that are of known importance in molecular oncology and attempt to correlate response to a particular mutation or expression. However, despite initially encouraging results in single-institution retrospective studies, external prospective validation has remained elusive, limiting their use in patient care. This section will discuss some of the more prominent biomarkers studied, along with their limitations.

Thymidylate Synthase

Thymidylate synthase (TS) is an enzyme that converts deoxyuridine monophosphate to deoxythymidine monophosphate, a nucleotide necessary for DNA synthesis.⁶ Considering that the antineoplastic function of 5-fluorouracil (5-FU) is mediated via TS inhibition, it is natural that TS has been investigated as a predictive biomarker for chemoresistance and CRT resistance in colorectal cancer (CRC). The results, however, have been inconsistent. Both TS expression levels and genetic polymorphisms have been studied in rectal cancer; with the latter showing more promising results. In recent studies by Negri et al and Conradi et al, low TS expression was correlated with worse tumor response and survival, respectively, in patients with rectal cancer who underwent preoperative radiotherapy or CRT.^{7,8} However, a smaller prior report from Germany suggested an inverse correlation between TS levels and response, questioning the generalizability of these findings.⁹ Perhaps, more importantly, differences in methodology in both the processing of tissue and the measurement of TS may at least partly account for the variation in the outcomes between these studies.

While TS expression levels have performed inconsistently as biomarkers of CRT response, polymorphisms of the TS gene have been more successful in predicting rectal cancer regression after neoadjuvant CRT. The most well-characterized of those was first reported by Kaneda et al in 1987.¹⁰ This group studied a polymorphism involving either two or three repeats of a 28 bp tandem sequence, the latter of which is translated into protein more efficiently, increasing TS protein levels without influencing transcription.¹¹ In turn, a higher number of repeats has been linked to a worse response to 5-FU-based chemotherapy.¹² Spindler et al demonstrated that patients with rectal cancer and three repeats in both alleles had significantly lower rates of pathological complete response

(pCR) after neoadjuvant CRT compared with those bearing at least one allele with two repeats.¹³ In a pioneering study of biomarker-guided CRT therapy, Tan et al used these TS polymorphisms to guide neoadjuvant therapy for locally advanced (T3–T4) rectal cancer in the setting of a prospective phase II trial.¹⁴ Patients classified as “good risk” (at least one allele with two repeats) received standard CRT with infusional 5-FU. Those classified as “poor risk” (both alleles with at least three repeats) received the same regimen with the addition of irinotecan. The addition of irinotecan appeared to result in better than expected outcomes for the “poor risk” group, with pCR rates similar to the good risk group, highlighting a possible role for genotype-directed neoadjuvant CRT. However, in the absence of further studies validating these results, the use of this promising marker currently has place only in the context of clinical trials.

p53

p53 is a well-characterized tumor suppressor gene that has a pivotal role in CRC, functioning as a regulator of apoptosis. Early in vitro studies suggested that CRC cell radiation and chemotherapy sensitivity is dependent on the presence of a wild-type p53.^{15,16} However, similar to TS discussed above, clinical studies have reported mixed results. In one of the first such studies, using 42 pretreatment biopsies, Spitz et al reported that mutant p53 expression was inversely related to pCR.¹⁷ Later, Luna-Perez et al evaluated the p53 mutational status in 26 pretreatment samples from rectal cancer patients who underwent preoperative CRT and found that tumors with mutant p53 had a higher percentage of residual tumor in the resection specimen (65 vs. 25%; $p = 0.018$), though an actual regression score/grade was not reported.¹⁸ Similar findings were also reported by other groups.^{19,20} However, several reports during the same period did not identify a correlation between p53 status and CRT response.^{21–24} In a recent meta-analysis seeking to address this discrepancy, Chen et al evaluated the predictive ability of p53 status compiling data from 18 studies.²⁵ Wild-type p53 was associated with both pCR and good response to CRT, with a relative response rate of 1.92 (95% confidence interval [CI]: 1.26–2.91) and 1.20 (95% CI: 1.01–1.43), respectively. Based on these findings, the authors concluded that p53 status could be a predictor of tumor response to neoadjuvant radiation-based therapy. Despite these encouraging results, variations in the methodology used in each study and the lack of validation in prospective external cohorts have so far precluded the use of p53 as a CRT response biomarker in the clinical setting.

KRAS

The role of KRAS in CRC tumorigenesis and growth, via constitutive activation of the mitogen-activated protein kinases pathway, has been acknowledged for almost three decades.²⁶ Also, a KRAS gene mutation (most commonly in codon 12 or 13) is detected in approximately 40% of CRC cancers and has been associated with resistance to certain antiepidermal growth factor receptor (EGFR) agents.²⁷ Its role as a predictive biomarker of CRT response, however,

remains uncertain. In one of the largest studies in the field, Garcia-Aguilar et al studied 132 patients with locally advanced rectal cancer who received neoadjuvant CRT and found that *KRAS* mutations were associated with worse response and lower pCR rates. In fact, none of the 27 patients with a combination of *p53* and *KRAS* mutation had a pCR.²⁸ Similar results were reported by Duldulao et al, who noted that, in addition to the association between pCR and the presence of a wild-type *KRAS*, specific *KRAS* mutations in codon 13 were particularly CRT resistant.²⁹ Even though these studies provide evidence that *KRAS* may be a predictor of residual disease after CRT in rectal cancer, others did not identify a correlation between *KRAS* status and response to CRT. In the context of a phase II study of 57 patients treated with cetuximab, irinotecan, and capecitabine plus radiation, Erben et al found no correlation between *KRAS* mutation and tumor response or patient survival despite what would be otherwise expected considering the use of an anti-EGFR agent (cetuximab) in the regimen.³⁰ A larger study by Gaedcke et al, using CRT (5-FU + oxaliplatin), attempted to identify specific mutations in the *KRAS* gene that may be predictive of response.³¹ Even though the presence of any *KRAS* mutation did not correlate with response, the authors noted that specific mutations conferred more favorable tumor biology (e.g., G12V were associated with higher rates of tumor regression than G13D mutations). Taken together, these data suggest that while mutations in the *KRAS* gene are a promising biomarker of response, further work evaluating specific codon and amino acid changes is necessary to identify the exact mutations that have an impact on tumor regression and that may eventually guide clinical decision-making.

Epidermal Growth Factor Receptor

EGFR is expressed in the majority of CRC, with up to 80% of tumors being positive on immunohistochemistry.³² Studies have indicated a role for EGFR in CRC radioresistance, both in vitro,³³ and in the clinical setting. In a study of 45 patients, EGFR positivity was found to be associated with decreased pCR rates (4 vs. 23%; $p = 0.006$) and worse survival.³⁴ Similarly, in another study by Kim et al, greater levels of EGFR expression in pretreatment tissues independently correlated with decreased downstaging after preoperative CRT.³⁵ In direct contradiction with these findings, however, Zlobec et al studied a cohort of 104 patients with rectal cancer who received preoperative CRT and found that the presence of EGFR expression was actually predictive of pCR, while EGFR negative tumors were radioresistant (odds ratio: 3.82, 95% CI: 1.37–10.6; $p = 0.01$).³⁶ This variability in outcomes indicates the EGFR alone is not reliable enough to predict CRT response; however, its incorporation in an array of multiple biomarkers could be of value, especially in a context of standardized measurements.

K_i-67

K_i-67 is a cell-cycle protein that is absent from resting (G0) cells and, as such, is an excellent marker of cell proliferation.³⁷ Its appeal as a potential biomarker is even greater

considering that most surgical pathology laboratories are already using K_i-67 staining for other malignancies and is routinely available. As radiotherapy is known to primarily target actively dividing cells, the results reported by Kim et al in 2002, namely, that K_i-67 is higher for complete and partial responders compared with nonresponders ($p = 0.029$), seems logical.³⁸ Unfortunately, subsequent studies failed to replicate these findings, limiting the clinical value of K_i-67 expression as predictor of CRT response in rectal cancer.³⁹

Neuronal Pentraxin 2

Neuronal pentraxin 2 (*NPTX2*), a gene previously associated with neuronal development, has been recently linked to rectal cancer response to CRT.⁴⁰ Identified as a predictor of pCR in high-throughput nucleotide microarrays of pretreatment samples,⁴¹ a study of 40 patients indicated that pretreatment messenger RNA (mRNA) *NPTX2* levels were significantly decreased in patients that had a pCR compared with those with residual cancer (fold change, 30.4; $p = 0.017$). Patients with low *NPTX2* expression in their tumors (<50% of normal rectal mucosa) had 38.5 and 46.1% rates of complete or moderate response, respectively; high *NPTX2* expression resulted in only 11.1 and 18.5% rates of complete or moderate response. Before clinical use, however, these encouraging results would need to be validated in larger, multi-institutional studies.

Cancer Stem Cell Markers

One of the most promising developments in our understanding of CRC biology in recent years has been mediated by the adoption of the stem cell model. Colorectal tumors are composed of a heterogeneous hierarchical collection of cells, with a rare subset of cells (known as cancer-initiating cells or cancer stem cells, CSCs) that are responsible for tumor initiation, progression, and response to therapy, including resistance to chemotherapy and radiation.^{42,43} As these cells are thought to represent a quiescent cell population that is resistant to CRT and remains viable after therapy; many groups have sought to evaluate the use of CSC markers as predictors of response. Analyzing pretreatment samples in a 123 patient cohort, Huh et al identified putative CSC marker CD44 as one such biomarker, with elevated CD44 mRNA levels in biopsies predictive of poor tumor regression.⁴⁴ Similarly, CD133, another CSC marker, has been reported to correlate with decreased sensitivity to CRT.⁴⁵ However, in a study by Sprenger et al evaluating pre- and posttreatment samples from 126 rectal cancer patients, the absolute pretreatment CD133 expression was not associated with response, though the authors noted that patients who had an increase in CD133 after therapy had less tumor regression and worse survival.⁴⁶ While the use of CSC-based biomarkers in CRC is still in its infancy, there is exciting potential in terms of predicting treatment efficacy, as well as providing novel therapeutic targets.

Chromosomal Copy Number Alteration

Chromosomal instability is involved in the pathophysiology of the majority of CRC.⁴⁷ Therefore, copy number alterations

of genomic segments (gains and losses of chromosomal segments) occur frequently in colorectal tumors and have been the focus of several studies investigating rectal cancer response to CRT.^{48,49} Grade et al analyzed 42 rectal tumors and found that gains in chromosomal locations 7q32–q36 and 7q11–q31, as well as amplification of 20q11–q13, were predictors of improved response to CRT.⁴⁸ In a later study of 95 patients, Chen et al identified a different pattern of copy number alterations that were associated with response: loss of 15q11.1–q26.3, 11q24.3–q25 and 8p12 were more common in tumors with incomplete response, while loss of 12p13.31 was more common in patients that had a pCR.⁴⁹ This information allowed the authors to develop a model that predicted pCR with 76% sensitivity, 97% specificity, and 92% accuracy. However, to our knowledge, external validation has not been done. Despite these promising results, chromosomal copy number alterations have not been as extensively studied as genomic mutations and altered gene/protein expression profiles, and therefore remain multiple steps away from clinical application.

Multigene Signatures

Considering the complexity of tumor biology, where the interplay of thousands of genes determines the phenotype and behavior of each cancer, the pursuit of a single gene whose expression is the ideal biomarker of therapy response or outcome may be overly simplistic. Over the last decade, the development of high-throughput arrays has allowed determining the expression level of thousands of genes in parallel, enabling researchers to classify tumors based on the expression patterns of multiple genes rather than a single one. Several studies have leveraged this technology to identify expression patterns, often termed “signatures,” that predict rectal cancer response to neoadjuvant CRT.^{41,50–53} Unfortunately, the overlap of identified target genes between these studies has overall been poor, while differences in patient population and experimental assays further constrain the comparison of their findings.

In 2005, Ghadimi et al reported the first major study that attempted to identify a signature predictive of CRT response in rectal cancer.⁵⁰ Using 23 pretreatment biopsy samples from the CAO/ARO/AIO-94 German Rectal Cancer Trial, they developed a gene expression profile including 54 genes that were able to predict responders (defined as T classification downstaging and few or no tumor cells on pathology) with 78% sensitivity and 86% specificity. The authors then tested this profile on an internal validation group of seven patients using a different assay to measure expression (oligonucleotide arrays instead of cDNA arrays) and determined that the profile was still able to predict responders with 86% accuracy. Using a similar methodology, a group from Japan led by Watanabe et al studied 35 pretreatment samples of rectal tumors that subsequently underwent CRT and developed a 33-gene signature that predicted response.⁵¹ When tested on a validation cohort of 17 patients, this signature had a sensitivity and specificity of 50 and 100%, respectively, in predicting response.

Kim et al approached the same issue but rather than focusing on response versus nonresponse, they instead com-

pared tumors that achieve a pCR to those that do not.⁵² Using 31 samples to develop their signature and 15 more to validate it subsequently, the group identified 95 differentially expressed genes between those who have a pCR (defined here as no tumor cells in the pathologic specimen) and those who do not. When applied to the validation set, the signature showed 100% sensitivity and 82% specificity in predicting pCR.

In a methodologically distinct study, Casado et al used high-throughput techniques to identify 24 genes that are differentially expressed before and after neoadjuvant CRT.⁵⁴ They subsequently used quantitative polymerase chain reaction (PCR) to study the expression of these genes, combined with an additional 29 that were selected from the literature, on 94 pretreatment tumor samples (57 in the training and 37 in the validation cohort). This allowed the generation of a 13-gene signature that predicted rectal cancer response to neoadjuvant CRT with 73% sensitivity and 86% specificity, while also predicting recurrence and survival after multimodality therapy.

A more recent study by Gantt et al aimed to develop a signature that predicts poor, rather than partial or complete response.⁴¹ Based on the assumption that poor response is easier to define and more consistent to score, the authors attempted to eliminate some of the difficulties and inconsistencies of response assessment, especially when using different scoring systems. Using this approach with a 23-sample training set, an 812-gene signature was identified, and this was capable of predicting poor responders in a 10-sample validation set with 100% sensitivity and specificity. Even though these results are encouraging, further validation in a larger and/or external cohort is necessary before clinical use.

Finally, one of the most interesting studies in the field of using multigene signatures to predict rectal cancer CRT response was published by Brettingham-Moore et al.⁵³ This group studied 51 patients who received neoadjuvant CRT followed by proctectomy and used high-throughput arrays to quantify gene expression before therapy in both the tumor and the surrounding normal mucosa. They then attempted to generate signatures that predicted several relevant endpoints, including pathological response versus nonresponse, TNM downstaging, and metabolic response measured by positron emission tomography scanning. The accuracy of prediction for any of the response parameters was deemed inadequate by the authors, with sensitivity and specificity ranging between 0 to 82% and 30 to 89%, respectively. Arguably an even more important contribution of their study, however, was to provide an external validation cohort for the signatures published by other groups (including those described earlier by Ghadimi et al and Kim et al) using their own 51 patient samples.^{50,52,55} Unfortunately, they determined a sensitivity of 21 to 50% and a specificity of 30 to 70% for these signatures, suggesting that their potential for clinical use may be limited. Overall, based on the limited accuracy of their own signature as well as those published by other groups, the authors concluded that gene expression profiling in its current state could not offer a reliable prediction of CRT response in rectal cancer.

Based on the previously described studies, it is clear that the field of gene signatures in rectal cancer remains in early development. The heterogeneity and inconsistencies between the studies were discussed in a recently published review by Akiyoshi et al, which found that there was minimal overlap of genes between the five signatures they assessed.⁵⁶ Before this technology and approach are ready for patient care, it is necessary to develop a common methodology for tissue processing and response assessment that is reproducible across institutions and health care systems, along with larger scale training and validation cohorts to identify the optimal signatures.

Biomarkers of Lymph Node Positivity

The increasing use of local excision in the management of rectal cancer, whether in the form of transanal endoscopic microsurgery or transanal minimally invasive surgery, relies on the accurate preoperative identification of patients with high likelihood of lymph node metastasis. Magnetic resonance imaging is the primary modality that serves this purpose. However, its sensitivity is estimated at about 70%.⁵⁷ This has led multiple groups to investigate biomarkers that predict lymph node involvement, with the ultimate goal to properly channel patients to proctectomy with total mesorectal excision when necessary. In 2012, Kalady et al reported a study of 55 patients with stage II and 22 patients with stage III rectal adenocarcinomas that did not receive CRT.⁵⁸ High-throughput gene expression analysis of these samples demonstrated the presence of two major clusters representing stage II and stage III cancers, respectively. In one cluster, 11 of 12 patients (92%) had stage III cancer; in the other, 54 of 65 patients (83%) had stage II ($p < 0.001$). Another study with similar goals was conducted by Fan et al, who hypothesized that epithelial-mesenchymal-transition (EMT) is a prerequisite for lymph node metastasis, and sought to correlate the expression of multiple EMT markers, using immunohistochemistry, with lymph node involvement.⁵⁹ The result was a set of eight variables (six EMT biomarkers and two clinicopathological characteristics), which in their internal validation cohort had a sensitivity, specificity, and overall accuracy in predicting regional lymph node metastasis of 68.3, 81.1, and 72.3%, respectively. Finally, Chen et al assessed the use of chromosomal copy number alterations, which were described above as biomarkers of CRT response, to predict persistent lymph node metastasis after preoperative CRT.⁶⁰ The authors noted that losses of 28 chromosomal regions, most notably in chromosome 4, were significantly associated with lymph node metastasis and achieved 68% sensitivity and 93% specificity in this setting. Pending external validation, the results of these three studies could have significant implications for the clinical algorithms of patients who are candidates for local excision of rectal tumors.

Challenges and Future Directions

Despite considerable efforts from multiple scientific groups, there are currently no genetic or molecular biomarkers in

rectal cancer that is widely used to guide CRT treatment in clinical practice. Multiple factors are limiting current attempts and would have to be overcome to allow the development of robust predictive biomarkers. Demographic and ethnic variation in patient populations managed at each center suggests that a multi-institutional approach is necessary to generate generalizable results. Similarly, different treatment regimens (e.g., radiotherapy alone vs. CRT, duration of neoadjuvant treatment, the time interval between treatment completion and surgery) should not be grouped together in analyses, as tumor response to each one is likely to be distinct. Technical differences in sample processing and experimental assays introduce further inconsistencies and limit reproducibility. Perhaps more importantly, the heterogeneity within tumor specimens needs to be acknowledged. Cancer cells from distinct areas of the tumor have been reported to have different genetic and epigenetic abnormalities, and sampling can greatly influence results.⁶¹ Finally, variability in the definition of endpoints, for instance in the quantification of CRT response using different scales, further limits comparisons between studies. Standardization across all these variables is necessary to drive the field forward; as otherwise, it will remain difficult to incorporate prior progress to new research.

Another major impediment to the advancement of our molecular understanding of rectal cancer and the development of clinically relevant knowledge is the tendency of basic science and translational studies to investigate “colorectal” cancer rather than colon and rectal cancer individually. Over the last decade, it has become well established that colon and rectal tumors are separated by genetic and epigenetic differences, which in addition to distinct anatomic and embryologic characteristics, confer them a unique phenotype.⁶² Despite this, the majority of studies investigating prognostic biomarkers and therapeutic targets do not distinguish between the colon and rectal primaries, and as colonic tumors outnumber rectal cancers, most of the information that is available is derived from colon cancer, and its applicability in rectal cancer should be inferred with caution. It is perhaps for this reason that our understanding of rectal cancer biology is primarily related to CRT, which is not part of the colon cancer therapeutic paradigm and has thus allowed a focused investigation of rectal tumors. As we attempt to bring more molecular biology advances to clinical care, future experimental studies will need to appropriately differentiate between colon and rectal cancer and seek biomarkers and therapeutic targets specific to rectal tumors. Collaboration across institutions and from international groups will be necessary to characterize the utility of these models moving forward truly.

Conclusion

Our understanding of rectal cancer biology and its molecular basis has evolved substantially over the last decade. So yes, we are getting closer to clinically useful information, but there are still miles to travel. Even though they have yet to shift the current clinical paradigm, specific gene mutations

and protein expression patterns that predict CRT response and lymph node metastasis have shed light on the biology of rectal cancer. These findings provide a basis for the next round of research investigators attempt to leverage this knowledge to both establish clinical-grade assays and develop new targeted therapies. With more standardized tissue processing techniques and experimental methodology, we can be optimistic that rectal cancer will join other malignancies in the era of personalized cancer care.

References

- Maas M, Nelemans PJ, Valentini V, et al. Long-term outcome in patients with a pathological complete response after chemoradiation for rectal cancer: a pooled analysis of individual patient data. *Lancet Oncol* 2010;11(09):835–844
- Mace AG, Pai RK, Stocchi L, Kalady MF. American Joint Committee on Cancer and College of American Pathologists regression grade: a new prognostic factor in rectal cancer. *Dis Colon Rectum* 2015;58(01):32–44
- Habr-Gama A, Perez RO, Nadalin W, et al. Operative versus nonoperative treatment for stage 0 distal rectal cancer following chemoradiation therapy: long-term results. *Ann Surg* 2004;240(04):711–717, discussion 717–718
- Habr-Gama A, Sabbaga J, Gama-Rodrigues J, et al. Watch and wait approach following extended neoadjuvant chemoradiation for distal rectal cancer: are we getting closer to anal cancer management? *Dis Colon Rectum* 2013;56(10):1109–1117
- Ghadimi BM, Grade M, Difilippantonio MJ, et al. Effectiveness of gene expression profiling for response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy. *J Clin Oncol* 2005;23(09):1826–1838
- Heidelberger C, Chaudhuri NK, Danneberg P, et al. Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature* 1957;179(4561):663–666
- Conradi LC, Bleckmann A, Schirmer M, et al. Thymidylate synthase as a prognostic biomarker for locally advanced rectal cancer after multimodal treatment. *Ann Surg Oncol* 2011;18(09):2442–2452
- Negri FV, Campanini N, Camisa R, et al. Biological predictive factors in rectal cancer treated with preoperative radiotherapy or radiochemotherapy. *Br J Cancer* 2008;98(01):143–147
- Jakob C, Aust DE, Meyer W, et al. Thymidylate synthase, thymidine phosphorylase, dihydropyrimidine dehydrogenase expression, and histological tumour regression after 5-FU-based neo-adjuvant chemoradiotherapy in rectal cancer. *J Pathol* 2004;204(05):562–568
- Kaneda S, Takeishi K, Ayusawa D, Shimizu K, Seno T, Altman S. Role in translation of a triple tandemly repeated sequence in the 5'-untranslated region of human thymidylate synthase mRNA. *Nucleic Acids Res* 1987;15(03):1259–1270
- Kawakami K, Salonga D, Park JM, et al. Different lengths of a polymorphic repeat sequence in the thymidylate synthase gene affect translational efficiency but not its gene expression. *Clin Cancer Res* 2001;7(12):4096–4101
- Villafraña E, Okruzhnov Y, Dominguez MA, et al. Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. *J Clin Oncol* 2001;19(06):1779–1786
- Spindler KL, Nielsen JN, Lindebjerg J, Jakobsen A. Germline polymorphisms may act as predictors of response to preoperative chemoradiation in locally advanced T3 rectal tumors. *Dis Colon Rectum* 2007;50(09):1363–1369
- Tan BR, Thomas F, Myerson RJ, et al. Thymidylate synthase genotype-directed neoadjuvant chemoradiation for patients with rectal adenocarcinoma. *J Clin Oncol* 2011;29(07):875–883
- Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 1993;74(06):957–967
- McIlwrath AJ, Vasey PA, Ross GM, Brown R. Cell cycle arrests and radiosensitivity of human tumor cell lines: dependence on wild-type p53 for radiosensitivity. *Cancer Res* 1994;54(14):3718–3722
- Spitz FR, Giacco GG, Hess K, et al. p53 immunohistochemical staining predicts residual disease after chemoradiation in patients with high-risk rectal cancer. *Clin Cancer Res* 1997;3(10):1685–1690
- Luna-Perez P, Arriola EL, Cuadra Y, Alvarado I, Quintero A. p53 protein overexpression and response to induction chemoradiation therapy in patients with locally advanced rectal adenocarcinoma. *Ann Surg Oncol* 1998;5(03):203–208
- Lin LC, Lee HH, Hwang WS, et al. p53 and p27 as predictors of clinical outcome for rectal-cancer patients receiving neoadjuvant therapy. *Surg Oncol* 2006;15(04):211–216
- Esposito G, Pucciarelli S, Alaggio R, et al. P27kip1 expression is associated with tumor response to preoperative chemoradiotherapy in rectal cancer. *Ann Surg Oncol* 2001;8(04):311–318
- Tannapfel A, Nüsslein S, Fietkau R, Katalinic A, Köckerling F, Wittekind C. Apoptosis, proliferation, bax, bcl-2 and p53 status prior to and after preoperative radiochemotherapy for locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 1998;41(03):585–591
- Elsaleh H, Robbins P, Joseph D, et al. Can p53 alterations be used to predict tumour response to pre-operative chemo-radiotherapy in locally advanced rectal cancer? *Radiother Oncol* 2000;56(02):239–244
- Saw RP, Morgan M, Koorey D, et al. p53, deleted in colorectal cancer gene, and thymidylate synthase as predictors of histopathologic response and survival in low, locally advanced rectal cancer treated with preoperative adjuvant therapy. *Dis Colon Rectum* 2003;46(02):192–202
- Bertolini F, Bengala C, Losi L, et al. Prognostic and predictive value of baseline and posttreatment molecular marker expression in locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2007;68(05):1455–1461
- Chen MB, Wu XY, Yu R, et al. P53 status as a predictive biomarker for patients receiving neoadjuvant radiation-based treatment: a meta-analysis in rectal cancer. *PLoS One* 2012;7(09):e45388
- Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988;319(09):525–532
- Normanno N, Tejpar S, Morgillo F, De Luca A, Van Cutsem E, Ciardiello F. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. *Nat Rev Clin Oncol* 2009;6(09):519–527
- Garcia-Aguilar J, Chen Z, Smith DD, et al. Identification of a biomarker profile associated with resistance to neoadjuvant chemoradiation therapy in rectal cancer. *Ann Surg* 2011;254(03):486–492, discussion 492–493
- Duldulao MP, Lee W, Nelson RA, et al. Mutations in specific codons of the KRAS oncogene are associated with variable resistance to neoadjuvant chemoradiation therapy in patients with rectal adenocarcinoma. *Ann Surg Oncol* 2013;20(07):2166–2171
- Erben P, Ströbel P, Horisberger K, et al. KRAS and BRAF mutations and PTEN expression do not predict efficacy of cetuximab-based chemoradiotherapy in locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 2011;81(04):1032–1038
- Gaedcke J, Grade M, Jung K, et al. KRAS and BRAF mutations in patients with rectal cancer treated with preoperative chemoradiotherapy. *Radiother Oncol* 2010;94(01):76–81
- Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 1995;19(03):183–232
- Liang K, Ang KK, Milas L, Hunter N, Fan Z. The epidermal growth factor receptor mediates radioresistance. *Int J Radiat Oncol Biol Phys* 2003;57(01):246–254

- 34 Giral J, Eraso A, Armengol M, et al. Epidermal growth factor receptor is a predictor of tumor response in locally advanced rectal cancer patients treated with preoperative radiotherapy. *Int J Radiat Oncol Biol Phys* 2002;54(05):1460–1465
- 35 Kim JS, Kim JM, Li S, et al. Epidermal growth factor receptor as a predictor of tumor downstaging in locally advanced rectal cancer patients treated with preoperative chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2006;66(01):195–200
- 36 Zlobec I, Vuong T, Compton CC, et al. Combined analysis of VEGF and EGFR predicts complete tumour response in rectal cancer treated with preoperative radiotherapy. *Br J Cancer* 2008;98(02):450–456
- 37 Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol* 2000;182(03):311–322
- 38 Kim NK, Park JK, Lee KY, et al. p53, BCL-2, and Ki-67 expression according to tumor response after concurrent chemoradiotherapy for advanced rectal cancer. *Ann Surg Oncol* 2001;8(05):418–424
- 39 Jakob C, Liersch T, Meyer W, Becker H, Baretton GB, Aust DE. Predictive value of Ki67 and p53 in locally advanced rectal cancer: correlation with thymidylate synthase and histopathological tumor regression after neoadjuvant 5-FU-based chemoradiotherapy. *World J Gastroenterol* 2008;14(07):1060–1066
- 40 Karagkounis G, Thai L, DeVecchio J, et al. NPTX2 is associated with neoadjuvant therapy response in rectal cancer. *J Surg Res* 2016;202(01):112–117
- 41 Gantt GA, Chen Y, Dejulus K, Mace AG, Barnholtz-Sloan J, Kalady MF. Gene expression profile is associated with chemoradiation resistance in rectal cancer. *Colorectal Dis* 2014;16(01):57–66
- 42 O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;445(7123):106–110
- 43 Lotti F, Jarrar AM, Pai RK, et al. Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17A. *J Exp Med* 2013;210(13):2851–2872
- 44 Huh JW, Lee JH, Kim HR. Pretreatment expression of 13 molecular markers as a predictor of tumor responses after neoadjuvant chemoradiation in rectal cancer. *Ann Surg* 2014;259(03):508–515
- 45 Hiroishi K, Inomata M, Kashima K, et al. Cancer stem cell-related factors are associated with the efficacy of pre-operative chemoradiotherapy for locally advanced rectal cancer. *Exp Ther Med* 2011;2(03):465–470
- 46 Sprenger T, Conradi LC, Beissbarth T, et al. Enrichment of CD133-expressing cells in rectal cancers treated with preoperative radiochemotherapy is an independent marker for metastasis and survival. *Cancer* 2013;119(01):26–35
- 47 Teixeira MR, Heim S. Multiple numerical chromosome aberrations in cancer: what are their causes and what are their consequences? *Semin Cancer Biol* 2005;15(01):3–12
- 48 Grade M, Gaedcke J, Wangsa D, et al. Chromosomal copy number changes of locally advanced rectal cancers treated with preoperative chemoradiotherapy. *Cancer Genet Cytogenet* 2009;193(01):19–28
- 49 Chen Z, Liu Z, Li W, et al. Chromosomal copy number alterations are associated with tumor response to chemoradiation in locally advanced rectal cancer. *Genes Chromosomes Cancer* 2011;50(09):689–699
- 50 Ghadimi BM, Grade M, Difilippantonio MJ, et al. Effectiveness of gene expression profiling for response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy. *J Clin Oncol* 2005;23(09):1826–1838
- 51 Watanabe T, Komuro Y, Kiyomatsu T, et al. Prediction of sensitivity of rectal cancer cells in response to preoperative radiotherapy by DNA microarray analysis of gene expression profiles. *Cancer Res* 2006;66(07):3370–3374
- 52 Kim IJ, Lim SB, Kang HC, et al. Microarray gene expression profiling for predicting complete response to preoperative chemoradiotherapy in patients with advanced rectal cancer. *Dis Colon Rectum* 2007;50(09):1342–1353
- 53 Brettingham-Moore KH, Duong CP, Greenawalt DM, et al. Pre-treatment transcriptional profiling for predicting response to neoadjuvant chemoradiotherapy in rectal adenocarcinoma. *Clin Cancer Res* 2011;17(09):3039–3047
- 54 Casado E, García VM, Sánchez JJ, et al. A combined strategy of SAGE and quantitative PCR Provides a 13-gene signature that predicts preoperative chemoradiotherapy response and outcome in rectal cancer. *Clin Cancer Res* 2011;17(12):4145–4154
- 55 Rimkus C, Friederichs J, Boulesteix AL, et al. Microarray-based prediction of tumor response to neoadjuvant radiochemotherapy of patients with locally advanced rectal cancer. *Clin Gastroenterol Hepatol* 2008;6(01):53–61
- 56 Akiyoshi T, Kobunai T, Watanabe T. Predicting the response to preoperative radiation or chemoradiation by a microarray analysis of the gene expression profiles in rectal cancer. *Surg Today* 2012;42(08):713–719
- 57 Al-Sukhni E, Milot L, Fruitman M, et al. Diagnostic accuracy of MRI for assessment of T category, lymph node metastases, and circumferential resection margin involvement in patients with rectal cancer: a systematic review and meta-analysis. *Ann Surg Oncol* 2012;19(07):2212–2223
- 58 Kalady MF, Coffey JC, Dejulus K, Jarrar A, Church JM. High-throughput arrays identify distinct genetic profiles associated with lymph node involvement in rectal cancer. *Dis Colon Rectum* 2012;55(06):628–639
- 59 Fan XJ, Wan XB, Huang Y, et al. Epithelial-mesenchymal transition biomarkers and support vector machine guided model in pre-operatively predicting regional lymph node metastasis for rectal cancer. *Br J Cancer* 2012;106(11):1735–1741
- 60 Chen Z, Liu Z, Deng X, Warden C, Li W, Garcia-Aguilar J. Chromosomal copy number alterations are associated with persistent lymph node metastasis after chemoradiation in locally advanced rectal cancer. *Dis Colon Rectum* 2012;55(06):677–685
- 61 Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* 2012;366(10):883–892
- 62 Kalady MF, Sanchez JA, Manilich E, Hammel J, Casey G, Church JM. Divergent oncogenic changes influence survival differences between colon and rectal adenocarcinomas. *Dis Colon Rectum* 2009;52(06):1039–1045