Review

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Prediction of response to preoperative chemoradiotherapy in patients with locally advanced rectal cancer

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Summary **Preoperative chemoradiotherapy (CRT) combined with surgery has become a standard treatment strategy for patients with locally advanced rectal cancer (LARC). The pathological response is an important prognostic factor for LARC. The variety of tumor responses has increased the need to find a useful predictive model for the response to CRT to identify patients who will really benefit from this multimodal treatment. Magnetic resonance imaging (MRI), positron emission tomography-computed tomography (PET-CT), serum carcinoembryogenic antigen (CEA), molecular biomarkers analyzed by immunohistochemistry and gene expression profiling are the most used predictive models in LARC. The majority of predictors have yielded encouraging results, but there is still controversy. Diffusion-weighted MRI may be the best model to detect the dynamic changes of rectal cancer and predict the response at an early stage. Gene expression profiling and single nucleotide polymorphisms hold considerable promise to unveil the underlying complex genetics of response to CRT. Because each parameter has its own inherent shortcomings, combined models may be the future trend to predict the response.**

Keywords: Rectal cancer, preoperative chemoradiotherapy, prediction, response

1. Introduction

Rectal cancer is one of the leading causes of cancer related deaths in the world (*1*). Over the last two decades, advances in new treatment strategies have contributed significantly to the improvement of the outcome of patients with locally advanced rectal cancer (LARC). Compared with postopertive chemoradiotherapy (CRT), preoperative CRT (pre-CRT) reduced toxicity, improved local recurrence control and disease free survival (*2,3*). For the true benefits, pre-CRT combined with surgery has been implemented as a standard treatment strategy for patients with LARC (*3,4*). The pathological complete response (pCR) is associated with a high 5-year overall survival rate and disease-free survival (DFS) rate (*5-7*), but there is a wide spectrum of responses to preoperative CRT, ranging from none to complete. The variety of tumor responses increased the need to find a useful predictive model for the response to preoperative CRT, which may be helpful in the design of individualized treatment for rectal cancer and allow an early surgery in nonresponders. In this review, we will discuss the current predictive models of the response to pre-CRT in patients with LARC.

2. Functional or molecular imaging techniques

2.1. *Magnetic resonance imaging (MRI)*

With the advancement of MRI techniques, recent studies are no longer only reliant on staging rectal cancer patients, but also on prognostic and predictive functions (*8,9*). Diffusion-weighted MRI (DW-MRI) provides information about microscopic structures through the detection of water proton mobility in biologic tissues (*9,11*). In DW-MRI, the apparent diffusion coefficient (ADC) provides a tool for absolute

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quantitative image analysis. The ADC is related to tissue cellularity, tissue organization, extracellular space tortuosity, tumor proliferation, tumor grade, and tumor necrosis (*9-11*). With respect to ADC as a quantitative biomarker in rectal cancer, several investigations have reported promising results for the prediction and monitoring of therapeutic responses (*10-18*) (Table 1).

Concerning the pre-CRT ADC value (ADC-pre) as a predictor for response, Sun *et al*. observed that the mean ADC-pre value in the T-downstaged group was lower than that in the T-non-downstaged group (p = 0.013) (*10*). Based on the tumor regression grade (TRG), ADC-pre showed a positive predictive value of 42% for pCR and 67% for a good response (GR, pCR, and near-pCR) (*11*). Lambrecht *et al*. confirmed a low ADC-pre value was significantly correlated with pCR yielding a sensitivity of 100% and specificity of 86% (*12*). In a recent study, ADC for predicting response was confirmed using the area under the receiver-operating characteristic (ROC) curve analysis with a sensitivity of 75% and specificity of 48% (*13*). Despite the promising results, there is no consensus whether low ADC-pre value should be used as a predictor for response to CRT because in some studies the ADC-pre value could not reliably discriminate CR from non-CR (*14,15*). Therefore, relying on ADC-pre seems to be insufficient to select in advance poor responders who might need to undergo early surgery.

Regarding the post-CRT ADC value (ADC-post) as a predictor for response, several studies showed that it could differentiate patients with pCR from those without (*14,15,17*). Moreover ADC-post value measurements are reliable and reproducible (*17*). It might be used as a non-invasive tool to evaluate response to CRT as an ADC-post value presenting good performance to select good responders.

The percentage change in the ADC (ΔADC) was also a useful predictor for pCR. Sun *et al*. observed that the ΔADC was significantly higher in the T-downstaged group than that in the nondownstaged group ($p < 0.001$) (*10*). An increase of ADC had a high positive predictive value for pCR (*11,15*). During treatment, the mean percentage of ADC increase was significantly greater in the responders than nonresponders ($p < 0.0001$) and a > 23% ADC increase had a negative predictive value of 96.3% for TRG4 (*18*). For dynamic observation ΔADC during (ΔADC-during) and after CRT (ΔADC-post) showed a significantly higher value in patients

Table 1. Recent studies of ADC as a predictor for response to CRT of patients with LARC

Ref.	No.	Parameters	Cut-off value ($\times 10^{-3}$ mm^2/s)	*p* value	Conclusion
Sun *et al*. (*10*)	37	ADC-pre ADCchange	1.07 23%	0.013 < 0.0001	Early increase of mean ADC and low mean ADC-pre correlate with good response to CRT.
Intven *et al*. (*11*)	59	ADC-pre ΔADC	0.97 41%		Low ADC-pre and high ΔADC correspond to pCR.
Lambrecht *et al*. (*12*)	20	ADC-pre ΔADC-during ΔADC-post ΔV-during ΔV-post	0.94 ± 0.12 72 ± 14% 88 ± 35% -62 ± 16% -86 ± 12%	0.003 0.0006 0.0011 0.015 0.012	ADC-pre, ΔADC-during, and ΔADC-post may be useful for prediction and early assessment of pathologic response to pre-RCT, with higher accuracy than volumetric measurements.
Barbaro *et al*. (*13*)	49	ADC-pre	0.833		Low ADC-pre may be an early biomarker for predicting treatment response.
Kim *et al*. (*14*)	76	ADC-pre ADC-post ADCchange	0.85 ± 0.10 1.43 ± 0.10 70.0 ± 23.5%	0.409 < 0.0001 < 0.0001	ADC-post alone can reliably differentiate pCR from non-pCR.
Genovesi *et al*. (*15*)	28	ADC-pre ADC-post % ADC	1.01 ± 0.061 1.79 ± 0.51 29.5%	0.33 0.003 0.001	The mean % ADC increase appears to be a reliable tool to differentiate CR from non-CR.
Kim *et al*. (*16*)	40	Mean ADC-post	1.62 +/- 0.36	< 0.0001	The mean ADC-post value of the CR group was significantly higher than that of the non-CR group.
Monguzzi *et al*. (*17*)	31	ADC-post	1.294	AUC of 0.833	Post-CRT ADC measurements are reliable and reproducible to evaluate response to therapy.
Elmi *et al*. (*18*)	62	ADC-pre ΔADC	< 1.0 > 23%	0.0011 < 0.001	Low ADC-pre was correlated with TRG 4, the increase in ADC was greater in the responders.

ADC: apparent diffusion coefficient; CRT: chemoradiotherapy; LARC: locally advanced rectal cancer; pCR: pathological complete response; ADC-pre: pre-CRT ADC values; ADC-post: post-CRT ADC;ΔADC :change in the ADC;ΔV-during: volume reduction during CRT; ΔV-post: volume reduction after CRT. ADC% = (ADC-post−ADC-pre)/ADC-pre×100%.

with pCR than those without, yielding a sensitivity and specificity of 100% for the ΔADC-during and, respectively, 100% and 93% for the ΔADC-post (*12*).

With its great development, MRI has potential to assess and predict the response to pre-CRT. First, most studies provided promising results to confirm the predictive value with high sensitivity and specificity. Second, tumors appear hypointense on ADC maps for the diffusion restriction of proton motion which can help differentiate tumorous lesions from non-tumorous lesions such as radiation-induced fibrosis and inflammation. Furthermore, MRI is known to enable the most accurate and useful assessment of tumor (T) staging before CRT (*19*). So it is convenient to acquire an ADC value. Despite some inconsistent results, ADC values hold great potentiality to be a useful predictor for response. However, all previous studies suffer the same issue that the sample size was too small and lack of standardization in ADC acquisition. Therefore, before ADC can be used as a predictor clinically, large cohort studies are needed.

2.2. *Positron emission tomography-computed tomography (PET-CT)*

PET-CT has become increasingly used for staging and evaluating therapeutic response in oncology (*20*). Over the past decades, lots of studies have implemented PET-CT to assess the response to CRT in LARC. Various PET-CT parameters have been investigated: mean standardized uptake value (SUVmean), maximum SUV (SUVmax), ΔSUVmax (SUVmax-pre – SUVmax-post), response index [RI, $(SUV_{pre} - SUV_{post})/SUV_{pre}$), metabolic tumor volume (MTV), ΔMTV% ($MTV_{pre} - MTV_{post})/MTV_{pre}$, visual response assessment (VRA), and total lesion glycolysis (TLG, SUVmean × MTV) (Table 2) (*19-32*). These studies were able to establish a correlation between PET-CT results and CRT response (*22-30*). Most studies used several parameters but found just one or two parameters correlated with CRT response (*21-27*). Kim *et al*. used SUVmax-pre, SUVmax-post, ΔSUVmax and RI to assess tumor response. Univariate and multivariate analysis revealed

Table 2. Recent studies of SUV as a predictor for response to CRT of patients with LARC

Ref.	No.	Parameters	Sensitivity (%)	Specificity (%)	Cut-off value	Conclusion
Amthauer *et al*. (*21*)	22	ΔSUVmax	93%	100%	36%	ΔSUVmax was significantly greater in responders than in non-responders.
Hur *et al*. (22)	37	SUVmean-post	84.6	79.2	3.35	SUV-post and RR were significantly associated with pathological treatment response, especially in pCR.
Shanmugan *et al*. (*23*)	70	SUVmax-post SUV decrease	58 60	78 84	4.0 63%	SUVpost and %SUV decrease correlate with pCR.
Melton *et al*. (*24*)	21	ΔSUV	86	85	75%	Tumor downstaging and CR are associated with greater RI.
Capirci *et al*. (*25*)	87	RI	84.5	80	65%	RI seems the best predictor to identify CRT response.
Martoni *et al*. (*26*)	80	SUVmax-post RI	88 94	34 31	5.0 66%	SUVmax-post supplies limited predictive information.
Chennupati *et al*. (*27*)	35	ΔSUVmax	93	19	64%	SUVmax, MTV and ΔMTV are not correlated with TRG.
Kim *et al*. (*28*)	151	SUVmax-post	73.7	63.7	3.55	SUVmax-post independently predicts pCR.
Maffione *et al*. (*29*)	69	SUVmax-post MTVpost TLGpost RI ΔMTV% ΔTLG VRA	85.7 65.3 85.7 83.7 69.4 69.4 86	80 80 75 70 80 80 55	5.1 2.1 cm^3 23.4 cm^3 61.8% 81.4% 94.2%	SUVmax ,MTV and TLG after CRT, RI, ΔMTV% and ΔTLG% parameters were significantly correlated with pathological treatment response. SUVmax-post demonstrated the highest AUC, sensitivity and specificity.
Everaert *et al*. (*32*)	45	%ΔSUVmax %ΔSUVmean	90 80	60 72	39% 24.5%	%ΔSUVmax and %ΔSUVmean correlate with histopathologic response.

SUV: standardized uptake value; CRT: chemoradiotherapy; LARC: locally advanced rectal cancer; pCR: pathological complete response; SUVmean: mean SUV; SUVmax: maximum SUV; SUVmean-post: SUVmean of post CRT; SUVmax-post: SUVmean of post CRT; ΔSUVmax: SUVmax-pre-SUVmax-post; RI: response index $(SUV_{pre} - SUV_{post})/SUV_{pre}$); MTV: metabolic tumor volume; ΔMTV%($MTV_{pre} - MTV_{post})/MTV_{pre}$; TLG: total lesion glycolysis (SUVmean × MTV); VRA: visual response assessment;%ΔSUVmax: the percentage differences (%Δ) between SUVmax-pre and SUVmax-post; %ΔSUVmean: the percentage differences (%Δ) between SUVmax-pre and SUVmax-post.

SUVmax-post was a significant factor for prediction of downstaging and pCR (*28*). Maffione *et al*. used 8 parameters to predict TRG and found SUVmax, MTV, TLG-post, RI, ΔMTV%, and ΔTLG% were significantly correlated with pathological treatment response ($p < 0.01$) while SUVmax-post had the highest sensitivity in predicting TRG (*29*). SUVmax is the most commonly studied metabolic parameter for semiquantitative analysis of glucose metabolism with PET-CT. However, SUV can be influenced by the nuclear medicine physicians and acquisition protocols, the reproducibility was poorer than those of RI and the percentage differences. Two studies revealed the mean RI was significantly higher in responders than in nonresponders and concluded that RI may be best for assessing the CRT response (*25,27*). A meta-analysis derived a threshold for RI of 36-52% for predicting response to CRT with a sensitivity and specificity of 86% and 80%, respectively. In the subgroup analysis, the accuracy of the group that underwent PET during therapy (sensitivity 86% and specificity 80%) was statistically higher than that acquired after completion of the therapy (sensitivity 78% and specificity 62%) (*31*). Everaert *et al*. investigated the potential value of sequential PET in assessing the response to radiation therapy (RT). The percentage differences between pre- and post-RT scans in SUVmax (%ΔSUVmax), SUVmean (%ΔSUVmax), %ΔMV, and total glycolytic volume (%ΔtGV) were calculated. Significant differences in %ΔSUVmax and %ΔSUVmean were observed between responders and nonresponders (*32*). However, it remains to be investigated whether these results obtained from patients treated with preoperative RT can be extrapolated to CRT.

Over the past decades lots of PET-CT parameters have been implemented to assess the CRT response in LARC. RI might be the best parameter for response assessment, especially acquired during therapy (*25-27*). The percentage differences in SUV between pre- and post-CRT, especially %ΔSUVmean, can be considered as valuable markers and worth further study (*32*). However, it cannot meet clinical use because of its own limitations. First, up to now the results of the predicting value of PET-CT are still not uniform. Second, PET-CT scans were not successful in determining nodal status (*24*). Third, PET-CT has difficulty in distinguishing between residual cancer and intraluminal or physiologic mucosal activity uptake (*30*). Fourth, the specificity of predictive value is too low to justify modification of the standard treatment protocol for an individual patient.

3. Serum carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA) is the most widely used tumor marker in patients with rectal cancer. Compared with other potential predictive markers, measurement of serum CEA levels are inexpensive, standardized, widely used and easily performed (*33*). In recent years, many studies have focused on the predictive value of CEA levels in patients with rectal cancer receiving pre-CRT (Table 3) (*34-40*). Most studies showed low pre-CRT CEA (CEA-pre) levels with different cut-off values associated with good tumor response or pCR (*34-36*), but concerning the CEA-pre

Table 3. Recent studies of CEA as a predictor for response to CRT of patients with LARC

Ref.	No.	Parameters	Cut-off value	*p* value	Conclusion
Park *et al*. (*34*)	352	CEA-pre	3 ng/mL	< 0.001	CEA-pre levels could be of clinical value as a predictor of response to pre- CRT.
Wallin *et al*. (*35*)	469	CEA-pre	3.4 ng/mL	0.008	Low CEA-pre was significantly associated with pCR.
Lee *et al*. (*36*)	345	CEA-pre	5 ng/mL	0.002	CEA-pre was found to be significant for prediction of pCR.
Perez *et al*. (*37*)	170	CEA-post CEA-pre	5 ng/mL	0.009 (clinical CR) 0.05 (pCR)	Low CEA-pre level was associated with an increased rate of complete clinical response but not with pCR.
		CEA-reduction	5 ng/mL	0.015 (clinical CR) 0.06 (pCR)	There was no correlation between reduction in CEA and CR.
Jang *et al*. (*38*)	109	CEA-post	2.7 ng/mL	0.001	CEA-post was an independent predictor of good tumor regression.
Yang *et al*. (*39*)	138	CEA-post CEA-ratio	2.61 ng/mLl 0.22		CEA-post < 2.61ng/mL predicted pCR (sensitivity 76.0%; specificity 58.4%), CEA ratio predicted pCR (sensitivity 87.5%, specificity 76.7%) for those with CEA-pre ≥ 6 ng/mL.

CEA: Carcinoembryonic antigen; CRT: chemoradiotherapy ; LARC: locally advanced rectal cancer; pCR: pathological complete response; CEA-pre: pretreatment CEA (CEA-pre) level; CEA-post: post-CRT CEA level; CEA ratio: CEA-post divided by CEA-pre; CEA –reduction: CEA-pre-CEA-post.

predictive values, the results were controversial. Perez *et al*. didn't find a correlation between initial CEA-pre level and pCR (*37*).

Recent studies confirmed the predictive value of post-CRT CEA (CEA-post) levels for response to CRT (*37-39*). Perez *et al*. reported that a CEA-post level < 5 ng/mL was associated with increased rates of clinical CR and pCR (*37*). CEA-post with a different cut-off value of 2.7 ng/mL was also proved to be an independent predictor of good tumor regression (p = 0.001) (*38*). In a recent study, CEA-post < 2.61 ng/mL also showed a strong predictive value for pCR with a sensitivity of 76.0% and specificity of 58.4% in patients with a low CEA-pre level or in patients with a high CEA-pre level but normalized CEA-post levels (*39*).

CEA-change as a predictor was first evaluated in a retrospective study, they found patients with a lower CEA-pre level or higher CEA-pre level but CEA reduction ratio ≥ 70% would have a better 5-year DFS. However, it was unknown whether this ratio was related to pCR or not (*40*). To make sure that the CEA ratio (defined as CEA-post divided by CEA-pre) could be used as a predictor for pCR, Yang *et al*. found that when CEA-pre levels ≥ 6 ng/mL, the CEA ratio was a significant predictor for pCR, and the optimal cutoff value of CEA ratio was 0.22 with a sensitivity of 87.5% and specificity of 76.7% (*39*).

Compared with other potential prognostic and predictive markers, measurement of serum CEA levels is inexpensive, widely used and easily performed. There is controversy if CEA-pre could be a predictive marker for pCR or not (*34-39*). CEA change groups were relevant to pCR, but may not be significant enough (*37,38*). CEA-post was an independent predictor for response to CRT (*37-39*), but different studies used different cut-off values and most studies did not mention the sensitivity and specificity of CEA-post as a predictor for CRT response.

4. Molecular markers

Many molecular markers were assessed for response prediction to CRT by immunohistochemistry (IHC) or direct gene sequencing analysis. Recent studies are listed in Table 4.

4.1. *p53*

Several studies assessed the ability of p53 status to predict response to CRT (*41-45*). Of these, some studies found that p53 could significantly predict response (*43-45*). In contrast, with similar sample size and pathological endpoints, some studies found no association between over-expression of the p53 protein and treatment response (*41,45*). Interestingly, one study found p53 genotype but not a p53 IHC result could predict response to preoperative short-term radiotherapy in rectal cancer (*42*). A meta-analysis found the wild-type *p53* gene was significantly associated with complete response (p = 0.003), and low expression of p53 protein was not significantly associated with complete response (p = 0.124) (*46*). Due to inconsistencies in different studies, p53 still cannot be considered a reliable predictor for treatment modalities.

4.2. *p21*

P21 protein has been studied as a response predictor because of the disruption of regulatory networks, in particular those involved in cell death signaling, which may be a causative factor of resistance to radiotherapy (*44*). Rau *et al*. reported that lower p21 expression in pre-treatment biopsies was correlated with poor response (*47*). Another study found p21 and apoptosis together with histologic changes on biopsy specimens obtained 7 days after starting CRT were strong predictors for response to CRT (*48*).

4.3. *K-ras*

K-ras plays an important role in colorectal carcinogenesis. Luna-Perez *et al*. analyzed codons 12, 13, and 61 of K-ras and found that tumors with wild-type K-ras were more likely to be responsive than tumors with mutant K-ras (*49*). In contrast, in two retrospective studies the K-ras mutation status was not found to be correlated with response or TRG (*50,51*).

4.4. *Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF)*

EGFR expression is an indicator of poor response to CRT in LARC (*52*). Kim *et al*. found a low level of EGFR expression may be a significant predictive molecular marker for increased tumor downstaging after CRT (*53*). Opposite to the former studies, Zlobec *et al*. found EGFR-positive tumors were six times more likely to undergo pCR compared with EGFR-negative cases (*54*). The conflicting results among different studies might be due to IHC methodological differences and heterogeneity of EGFR expression. Those shortcomings may be overcome by gene polymorphism. The most common single nucleotide polymorphism is Sp1 -216 G/T polymorphism in the EGFR promoter region. Evaluating for EGFR Sp1 -216 G/T polymorphism from blood samples and EGFR expression on primary tumor biopsies simultaneously, the major response rate in patients with Sp1 -216 T containing variants is significantly higher in Sp1 -216 GG homozygote patients, but in regard to EGFR expression by IHC no correlation was observed with response rates (*55*). Low VEGF expression levels also indicated a good pathological response (*56*). Zlobec *et*

Table 4. Recent studies of biomarkers as a predictor for response to CRT of patients with LARC

Ref.	No.	Biomarker	Analysis methods	*p* value	Conclusion
Rebischung *et al*. (*41*)	86	p53	GE	< 0.01	P53 status is an independent prognostic factor of response to radiotherapy.
Kandioler *et al*. (*42*)	64	p53	GE IHC		P53 genotype but not p53 immunohistochemistry is predictive for response to preoperative short-term radiotherapy.
Komuro *et al*. (*43*)	111	p53	IHC	0.045	There was a significant correlation between the expression pattern of p53 and tumor radiosensitivity.
Fu *et al*. (*44*)	49	p53 p21	IHC IHC	0.01	The majority of p53(-) or p21(+) tumors were radiosensitive.
Huh *et al*. (*45*)	123	13 markers	PCR	0.03	Only CD44 expression was found to be significant independent predictive factors for tumor regression grade response.
Chen *et al*. (*46*)	1830	p53	meta-analysis	0.003 0.124	Wild-type p53 gene was significantly associated with CR. Low expression of p53 protein was not significantly associated with CR.
Rau *et al*. (*47*)	66	p53, p21, Ki67	PCR		Lower p21 expression in pre-treatment biopsies correlated to poor response.
Suzuki *et al*. (*48*)	101	p21, apopstosis	IHC	0.04 < 0.01	P21 with tumor regression P21 and apoptosis together obtained 7 days after starting CRT are strong predictors of the response to CRT.
Luna-Perez *et al*. (*49*)	37	K-ras	GE		K-ras mutations is an indicator of tumor response
Bengala *et al*. (*50*)	146	K-ras EGFR	GE		Neither EGFR nor K-ras status was statistically correlated to TRG
Gaedcke *et al*. (*51*)	94	K-ras	GE	> 0.05	The presence of K-ras mutations was not correlated neither with tumor response.
Kim *et al*. (*53*)	183	EGFR	IHC	0.012	The significant predictive factor for increased tumor downstaging was a low level of EGFR expression
Zlobec *et al*. (*54*)	104	EGFR VEGF	IHC	0.01 0.009	Loss of VEGF and positive EGFR are an independent predictor for pCR.
Spindler *et al*. (*55*)	77	EGFR	IHC SNP	> 0.05 0.023	EGFR Sp1-216G/T polymorphism are potential markers for response to CRT.
Kurt *et al*. (*56*)	29	Several markers including VEGF	IHC	0.05	VEGF level was higher in the non-pCR group than the pCR group.
Saigusa *et al*. (*57*)	50	CD133	IHC	< 0.05	The ratio of histopathological responder in cases with CD133 expression was significantly lower than that without it.
Hiroishi *et al*. (*58*)	50	12 biomarkers including CD133	IHC	0.003	CD133 was significantly associated respectively with sensitivity to pre-operative CRT.
Shinto *et al*. (*59*)	96	CD133	IHC	0.002 (uni) 0.003 (multi)	Positivity for CD133 expression was associated with chemoradioresistance on univariate and multivariate analyses.
Sprenger *et al*. (*60*)	126	CD133	IHC	< 0.01	Increased fraction of CD133-expressing cells after preoperative CRT was associated with lower histopathologic tumor regression.
Vaupel *et al*. (*61*)	86	HIF-1α, GLUT-1	IHC		HIF-1α and GLUT-1 expression had no predictive impact regarding response measured by TRG.
Havelund *et al*. (*62*)	50	HIF-1α	IHC		There were no significant differences between the HIF-1α-positive group and HIF-1α-negative group for pathological grading and pCR.

CRT: chemoradiotherapy; LARC: locally advanced rectal cancer; pCR: pathological complete response; GE:gene expression;IHC: immunohistochemistry; PCR: polymerase chain reaction .TRG: tumor regession grade; EGFR: Epidermal growth factor receptor; VEGF: vascular endothelial growth factor; SNP: single nucleotide polymorphisms; HIF-1α:hypoxia-inducible factor 1α ; GLUT-1:glucose transporter-1

al. applied ROC curve derived cut-off scores to VEGF and confirmed VEGF negative tumors were four times more likely to undergo complete tumor regression (*54*). Despite different results, EGFR or VEGF, especially EGFR Sp1-216G/T polymorphism are potential new markers for assessing response to CRT in LARC.

4.5. *Cancer stem cell markers*

CD133, CD44, and CD24 have been described as cancer stem cell markers. Several studies confirmed elevated CD133 expression was associated with resistance to CRT in LARC (*57-60*). The status of CD24 was also found to be significantly associated with response to CRT ($p = 0.029$) (*58*). Huh *et al*. revealed that among 13 molecular markers, only elevated CD44 mRNA levels in pretreatment biopsies might be predictive of poor tumor regression and CD133 level had no significant correlation with the response to CRT (*45*).

4.6. *Markers of tumor hypoxia*

Tumor hypoxia can lead to resistance to radiation and chemotherapy by depriving cells of oxygen essential for the cytotoxic activities of these agents (*61*). Hypoxia-inducible factor 1α (HIF-1α) and glucose transporter-1 (GLUT-1) are intrinsic markers of tumor hypoxia. It has been considered that HIF-1α and GLUT-1 expression may be predictors for poor response. Different from the hypothesis, the HIF-1α and GLUT-1 expressions had no predictive value regarding response to CRT based on TRG in a study carried by Havelund *et al*. (*62*). Shioya *et al*. detected HIF-1α expression in 42.0% of samples but found no significant correlation between the HIF-1α-expression and pathological response (*63*).

Among molecular markers based on tumor tissues, the vast majority of studies have assessed single or multiple markers. A limited number of promising markers have been identified, including p53, p21, EGFR, VEGF, CD133, HIF-1α and so forth. The majority of markers assessed, however, have yielded disappointing results. No specific molecular marker has yet been proven to be a definitive predictor of the response to CRT. The failure of IHC methods as a means of biomarker discovery is that this assesses small numbers of pre-defined protein markers per tissue section.

5. Gene expression profiling

Instead of focusing on specific factors, recent advances in deoxyribonucleic acid (DNA) microarray-based gene expression profiling technology make it possible to analyze a large number of genes simultaneously, and search systematically for molecular markers to predict responses and outcomes (*64*). Consequently, several investigators have used gene expression profiling to analyze the genetics of rectal cancer and their predictive potential in terms of response to CRT (Table 5). Ghadimi *et al*. used two different microarray platforms to analyze pretreatment biopsies and identified 54 genes that were significantly differentially expressed between responders and non-responders based on T-down-staging. The genes were able to predict tumor behavior correctly in 83% of patients (*65*). Using an Affymetrix U95Av2 Gene Chip, 33 novel discriminating genes related to transcription, cell growth, signal transduction and apoptosis were identified based on TRG in another study. Among the 33 genes, 20 genes expression increased and 13 genes expression decreased in responders as compared to nonresponders (*66*). In the following studies, differentially expressed genes related to cell cycle and/or cell signaling were also successfully identified between responders and non-responders (*67-69*). The model based on the identified genes predicted

Table 5. Recent studies using gene expression profiling to analyze the genetics for response to CRT of patients with LARC

Ref.	No.	No. of genes	Accuracy (%)	Conclusion
Ghadimi *et al*. (*65*)	30	54	82.4	Pretherapeutic gene expression profiling may assist in response prediction to preoperative CRT.
Watanabe *et al*. (*66*)	52	33	82.4	Gene expression profiling may be useful in predicting response to radiotherapy.
Kim *et al*. (*67*)	46	95	84	Microarray gene expression analysis was successfully used to predict CR to preoperative CRT.
Rimkus *et al*. (*68*)	43	42	86	Pretherapeutic prediction of response to CRT by gene expression analysis may represent a new valuable and practical tool of therapeutic stratification.
Nishioka *et al*. (*69*)	17	17		Gene expression patterns of diagnostic biopsies can predict pathological response to preoperative CRT.
Supiot *et al*. (*81*)	6	31 (up) 6 (down)		Micro-arrays can efficiently assess early transcriptomic changes during preoperative radiotherapy for rectal cancer.

CRT: chemoradiotherapy; LARC: locally advanced rectal cancer; CR: complete response.

the response to CRT at an accuracy of over 80% (*65-68*).

It seems reasonable to apply microarray gene profiling to identify novel molecular markers to predict response to CRT. Each study generated gene expression classifiers capable of high predictive accuracy (*65-68*), but the use of this microarray data in clinical practice is still limited for several reasons. First, microarray profiling relies on the prompt collection of fresh tissue samples and tumor biopsies consist of varying amounts of stroma, blood vessels and lymphocytes which contribute to the gene expression profiles and thus introduce a potential source of error. Second, the previously reported gene signatures differed considerably in terms of gene composition among different studies which make it difficult to compare the effectiveness of different genes. Third, the numbers of patients in each study were relatively limited. The current reported genes analyzed by microarray technology are not robust enough for clinical utility at this point. However, considering the promising data and usefulness of gene profiling in breast and lung cancer, gene expression profiling holds considerable promise to unveil the underlying complex genetics of response to CRT of rectal cancer if candidate genes are carefully validated in the future.

6. Thymidylate synthase (TYMS)

TYMS is an essential enzyme for cell proliferation and DNA synthesis (*70*). TYMS is considered the indirect target of 5-fluorouracil (5-Fu) and it was evaluated in several studies as a predictor of response to 5-Fu based pre-CRT in LARC (*71-76*), but there is much debate about the results. Saw *et al*. (*71*) observed that pretreatment biopsy specimens negative for TYMS were predictive of tumor down-staging in the CRT group but not in the radiotherapy group. In a contrasting study, patients with high TYMS IHC staining were more likely to achieve complete and partial response in the CRT group only (*72*). Even no correlation between TYMS expression and treatment response was observed in a study carried by Bertolini *et al*. (*73*). All the above studies used the IHC method to determine the TYMS levels, and limitations of IHC (heterogeneity in the TS assay and classification criteria) may contribute partly to the conflicting results. A genetic approach was used to quantify different TYMS genotype activities (2R/2R,2R/3R, and 3R/3R). By evaluating the number of tandem repeats of the *TYMS* gene, patients with either 2R/2R, 2R/3R are more likely to achieve downstaging and pCR than patients with 3R/3R (*74*). According to 2R/3R and 3R/3R tandem repeat polymorphisms in the *TYMS* gene, TYMS polymorphisms were classified into a low expression group (2R/2R, 2R/3RC, or 3RC/3RC) and high expression group (2R/3RG,3RC/3RG, or 3RG/3RG) but no correlation was found between TYMS SNPs and tumor response (*75,76*). However, in a further study, patients in the low-expression group with a G>C SNP exhibited a significantly greater tumor downstaging rate ($p = 0.001$) (*76*). The TYMS gene is complicated and regulation is still not fully understood. The underlying reason for previous contradictory results according to TYMS polymorphisms on the clinical response need to be investigated, and additional novel polymorphisms might be identified to understand the complete role of TYMS genotyping for predicting a 5-Fu based pre-CRT response in rectal cancer.

7. Single-nucleotide polymorphism (SNP) markers

SNP analysis has been used to tailor special gene sites to predict response to CRT (*55,75,76*). Instead of focusing on specific genes, genome-wide association studies were capable of genotyping thousands of SNPs. It is theoretically possible to identify SNP markers predicting a response to pre-CRT in LARC. A quite recent study has implemented a human 3-step genome-wide SNP strategy for the determination of CRT sensitivity in LARC. In the first step, the screening group was performed using the Genome-Wide Human SNP Array with 906,600 probes in 43 patients with LARC. Then the results of the genotyping analysis were associated with the responses to pre-CRT. USP20rs227450, FAM101Ars795574, ZNF281rs424414, OR2T4rs153870, SLC10A7rs41398848, CORO2Ars198585, ASZ1 rs7808424 MED4 rs157125, and CDC42BPA rs192986 were identified as CRT-responsive SNPs. In the second step, the above nine candidate SNPs were genotyped by pyrosequencing for clinical validation in a total of 113 patients. The patients carrying the reference allele(C) of the SNP CORO2A rs1985859 were more likely to obtain a positive response (TRG1-3) than the substitution allele (T) ($p = 0.01$). This was confirmed by the *in vitro* assay of ionizing radiation cytotoxicity and the clonogenic assay in the third step. However, there are controversial findings with respect to FAM101A, no specific genotype or allelotype showing significant CRT sensitivity was identified in the clinical association study, but downregulation of FAM101A reduced early apoptosis, enhanced colony formation and increased cell survival or viability in RKO cells (*77*). Despite the controversial findings in the current study and poor concordance between GWA studies, the finding is novel and SNPs are worth further study.

8. Dynamic analyzing

Increasing evidence shows that the way in which tumors respond to radiation is dynamic by analyzing sequential core biopsies (*78,79*). Recently, the same studies were carried out on rectal cancer. Based on biopsies taken before CRT, after 2, 4, and 6 weeks of CRT and in specimens from the operation, decreasing expressions

of HIF-1α, Bcl-2 and Ki-67 were observed during CRT, but unfortunately no association was seen between the fluctuations of any of the markers and response to CRT (*80*). In another study, based on biopsy specimens obtained 7 days after starting CRT, the expressions of p21 and apoptosis have been proven to be strong predictors of the response to CRT in rectal cancer (*48*). Gene expression changes detected on biopsies after a dose of 7.2 Gy at a median time of 1 hour following irradiation found 31 genes significantly up-regulated and 6 genes down-regulated (*81*). This potentially means that the response may be more accurately predicted after initial treatment cycles rather than only before or after treatment of tissues, but performing serial biopsies is an invasive and unpleasant procedure and has the risk of increasing toxicity from repeated biopsies. As such, a non-invasive means of monitoring early-stage responses would be beneficial. DW-MRI, PET-CT and CEA can detect dynamic changes of rectal cancer during CRT easily and non-invasively. However, the price of PET-CT testing is so high that many patients are unable to afford repeating PET-CT tests and the serum CEA levels of parts of patients are always at a normal level so that CEA detection has no use for them at all. A study including twenty patients underwent MRI before CRT, after 10-15 fractions and 1 to 2 weeks before surgery showed ΔADC-during had a significantly higher value in patients with pCR compared with patients without (*12*). Due to the above evidences, DW-MRI may be the best model to detect the dynamic change of rectal cancer and predict the response at an early stage.

9. Dual or more models combined

As each parameter has its own inherent shortcomings, a combination of dual or more models may improve the accuracy of a response prediction. A prospective study investigated the combination of PET-CT and DW-MRI for the prediction of a pathological response. Twenty two patients had PET-CT before CRT, after 10 to 12 fractions of CRT, 5 weeks after CRT and had DW-MRI before CRT. Both during and after CRT, the number of false positive results were decreased and consequently specificity was increased by the combination of ΔSUV max and ADC. During CRT the combination of ΔSUVmax > 40% and ADC-pre < 1.06×10^3 mm²/s can predict pCR with a sensitivity of 100% and specificity of 94%. Also, the combination of the provided threshold for ΔSUV max after CRT and ADC-pre was able to predict the response in all 22 patients (*82*). In a recently published study, the predictive value of PET-CT and CEA was simultaneously evaluated in terms of downstaging and pCR. SUV-post < 3.7 and CEA-pre < 5.3ng/mL are independent predictors for response to CRT in univariate and multivariate analysis. The combination of SUV-post < 3.7 and CEA-pre < 5.3 ng/mL increased the specificity from 65% and 51.4% to 84.4% for downstaging and the specificity from 63.6% and 81.8% to 91.6% for pCR (*29*).

Despite the small number of patients in the studies, the combination of two modalities provide us with complementary information of the tumor and yielded higher accuracy and specificity than the individual investigations, which holds great potential for response prediction. The integration of functional imaging, CEA, together with the numerous potential molecular markers and identified genes will provide us with a bulk of information on each individual patient and make individualized treatment therapy possible. Combined models may be the future trend to predict a response.

10. Conclusion and prospects

The high level of variation in response to CRT and the potentially toxic side effects increased the need for better patient selection in LARC. A major focus of rectal cancer research has been the identification of predictive factors allowing clinicians to predict responses. However, to be able to base treatment decisions, prediction should be effective and applicable in current clinical practice. From the update of current status, prediction is still in its infancy: First, there is still controversy between different results. Second, until now virtually all biomarkers have been identified in retrospective studies and predictions were not effective enough. Third, the number of patients is naturally limited. Fourth, there is the problem of high dimensionality of the therapy regimens: long or short term radiation and different chemotherapy regimens. Fifth, the histopathological evaluation of tumor response is different, such as complete response, partial response, TRG and downstaging. Differences among studies make a direct comparison of the results impossible. So before any predictor is used clinically, tumor response evaluation should be standardized and large consistent cohort studies are needed.

In this review, we have provided an overview of the different predictive models. The ADC of MRI, SUV of PET-CT, CEA, gene expression profiling and SNPs analysis are the most potential predictors for response to CRT from the evidence today. Due to the evidence that the way in which tumors respond to radiation is dynamic, DW-MRI may be the best model to detect the dynamic change of rectal cancer and predict the response at early stage. Considering the promising data and usefulness of gene profiling in other cancers, gene expression profiling and SNPs analysis hold considerable promise to unveil the underlying complex genetics of response to CRT in rectal cancer. As each parameter has its own inherent shortcomings and a combination of dual or more models may provide us with complementary information, combined models may be the future trend to predict a response. Before any predictor is used clinically, large consistent cohort studies are needed.

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