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Review

Potential predictive biomarkers in locally advanced rectal cancer treated with preoperative chemo-radiotherapy

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Summary. Fluorouracil-based preoperative chemoradiotherapy represents a standard option for the treatment of locally advanced rectal cancer. Randomized clinical trials have shown that fluorouracil concomitant to preoperative radiation enhances tumor shrinkage (with 10% to 15% of the patients showing a complete pathological tumor response) compared with preoperative radiation alone. A high response rate is of clinical importance in rectal cancer, since patients who achieve a complete pathological response may experience improved long-term survival. Adding oxaliplatin to fluorouracil-based preoperative chemoradiotherapy has no effect on response of the primary rectal tumor and single-agent fluoropyrimidine remains the standard chemotherapy in this setting. Despite novel biological insights and therapeutic advances, little is known about potential biological markers able to predict pathological tumor response before treatment and to subsequently impact patients' prognosis. This review focuses on the current available data on main molecular markers and molecular subtypes and the possible upcoming introduction of such analyses in the clinical setting. (www.actabiomedica.it)

Key words: rectal cancer, marker, chemo-radiotherapy, prognosis

Background

Preoperative radiation therapy alone (RT) or combined with chemotherapy (RCT) have improved the management of locally advanced rectal cancer patients (1, 2). With this approach, pathologic complete response (pCR), which is an important predictor for both local and disease-free survival, is achieved in up to 30% of patients (3). Furthermore, achieving a complete or near-complete pathologic response before surgery may increase the number of sphincter-sparing procedures (3). No benefit from adding oxaliplatin could be demonstrated on primary tumor response to preoperative chemoradiation (4-6) and chemotherapy with fluoropyrimidine remains the standard of care.

Only limited data are available regarding the role of biomarkers to predict complete pathological response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer patients (Table 1). Subgroup analyses are ongoing to investigate if there are patients gaining a greater benefit from investigational treatment. The ability to predict the pathological tumor response before treatment may significantly affect the selection of patients for preoperative combined therapy and may potentially adapt the choice of post-operative treatments. There is, therefore, an unmet need to improve individual treatment approaches in this setting.

Complex molecular and clinical phenotypes trigger the development and progression of rectal cancer, thus yielding different pathological responses to treat-

Table 1. Potential predictive biomarkers

Glossary of molecular markers			
Marker	Abnormality or abnormal gene	Functions of wild-type gene product	Reported prognostic or predictive value in CRC
TS	Overexpression	Pyrimidine metabolism	Adverse prognostic marker, adverse predictive marker
P53	Overexpression	Control of DNA topology	Adverse prognostic marker
ERCC1 (9, 25)	Overexpression	Repair of platinum agents-DNA adducts	Adverse prognostic marker, adverse predictive marker
HER-2 (26-28)	Overexpression	Cellular signal transduction	Predictive marker
MSI	Consequence of abnormal genes in mismatch repair family	Repair of nucleotide mismatches	Favorable prognostic marker, adverse predictive marker
PD-L1 (29, 30)	Overexpression	Immune checkpoint	Adverse predictive marker
PTEN (31, 32)	Loss of expression	Phosphatase activity	Adverse prognostic marker, adverse predictive marker
CD3	Overexpression	Cellular signal transduction	Favorable prognostic marker
CD4	Overexpression	Cellular signal transduction	Favorable prognostic marker
CD8	Overexpression	Cellular signal transduction	Favorable prognostic marker

ERCC1: excision repair cross-complementing 1; HER2: human epidermal growth factor receptor 2; MSI: microsatellite instability; PD-L1: programmed death-ligand 1; PTEN: phosphatase and tensin homolog

ment (7). Recent molecular analyses uncover that tumors arising in the rectum may carry distinctive genetic alterations from other colon cancers (8). Compared to left colon cancers, rectal cancers display a higher frequency of *TP53* (71% vs. 57%, p=0.03) and a higher expression of excision repair cross-complementing 1 (ERCC1) (29% vs. 15%, p=0.03) (8), which is a marker of resistance to platinum drugs (9). Additionally, approximately 50% of rectal cancers express high levels of thymidylate synthase (TS), which is involved in pyrimidine nucleotide synthesis and it is an important target for 5-fluorouracil (5-FU) (10, 11).

Finally, some studies focus on the importance of immune infiltration to predict the clinical outcome of untreated patients but also to predict the response to treatment (12, 13). The presence of immune cells may reveal a distinct biology of the tumor, as gene expression profiling and other assays have unveiled.

This review focuses on the current available data on some of the molecular markers and on comparative analyses that showed molecular variations among rectal tumors that might contribute to differences in clinical behavior of rectal cancer tumors.

TS (thymidylate synthase)

TS is an enzyme that catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) and is essential for 'de novo' DNA synthesis (14). The tissue expression of TS may affect tumor sensitivity to fluoropyrimidines, such as 5-FU (15). The role of TS in fluoropyrimidine cytotoxicity has been established in both preclinical and clinical studies (16, 17). Moreover, the association between TS levels and resistance to 5-FU could depend on the 5-FU schedules of treatment used and/or biochemical modulators and on the degree of incorporation into RNA (18). These may result in different mechanisms of cytotoxicity, potentially affecting the correlation between thymidylate synthase (TS) expression and the clinical response to the fluoropyrimidine.

Aschele and coll. (19) showed that TS levels predicted clinical response only for regimens involving continuous infusion with a higher response rate in patients with low and high TS levels compared with high TS levels (66% versus 24%, respectively). Conversely, TS expression failed to predict the clinical response within the group of patients treated bolus 5-FU.

To date, however, only a small number of retrospective heterogeneous studies have addressed the issue of TS expression levels and tumor response in rectal cancer patients, especially FU-based chemo-radiotherapy (20, 21). In rectal cancer, low TS gene expression has been found to correlate with pathological response to neoadjuvant FU-based CRT (20). In contrast, another study from our group showed a significant interaction between high TS level and the probability of achieving a pathological response (21). Several factors may account for these controversial results on the predictive role of TS expression. The first may be related to the different techniques used to assess TS levels. For example, a significant correlation between protein expression and tumor response in rectal cancer patients was seen only when both staining intensity and staining pattern were evaluated, with a significant association between high TS expression in tumor biopsies and resistance to therapy (20). Moreover, in contrast with previous data, in our study, FU was administered as continuous infusion and strong TS expression was found to be predictive of pathological tumor response to treatment. Therefore, the potential of TS expression levels to predict tumor response to preoperative combined-modality therapy remains to be proven.

p53

p53 mutations have been described in about 40% to 50 % of colorectal carcinomas and are associated with an aggressive behavior and resistance to chemoradiotherapy in several tumor models (22).

Microsatellite instability (MSI)

High microsatellite instability (MSI-H) status is a predictive marker for lack of response to 5-FU-based chemotherapy compared with microsatellite stable (MSS) disease (23). Moreover, MSI is a useful predictive criterion for irinotecan response in patients with colorectal cancer (24) (reviewed elsewhere).

Tumor infiltrating lymphocytes (TILs)

With the exclusion of MSI, which is limited to a small subgroup of rectal cancers, recent genetic and molecular studies did not identify any novel predictive biomarkers (33). One possible reason is that until recently research has been mainly focused on cell processes rather than on tumor microenvironment (34). Nowadays, a large body of data from retrospective cohorts of solid tumors has shown that the in situ immune infiltrate may have a strong impact on patients' outcome (35). The immune infiltrate has been shown to overcome the TNM scoring system in predicting survival and to influence the outcome also of colorectal cancer patients (36-38). To quantify the immune infiltrate, an "immunoscore" based on the enumeration of CD3 and CD8 lymphocytes within the core of the tumor and the invasive margin has been suggested (39). This applies also to rectal tumors, as an inverse relationship between tumor invasion and the extent of immune cell infiltration has been reported (40, 41). Moreover, the immunoscore seems to be a useful prognostic marker in rectal cancer patients treated by primary surgery (41). Studies on larger cohorts of patients are ongoing to validate the former results. In fact, a positive result could provide the rationale to assess the immune infiltrate in biopsies to predict potential responders to preoperative treatments and to select them for new strategies with minimal or even no surgery.

Conclusions

The overall landscape is multifaceted and our knowledge on this issue is still at the starting point.

Doubtlessly, analyzing and genotyping distinct tumor subtypes and setting apart patients with distinctive diseases represent the goal of future treatments to pave the way for precision medicine also in rectal can-

cer patients. Finally, accurate tools to predict response to therapies should probably consider both the genetic features and the immune components of the tumor.

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