Tailoring treatment of rectal adenocarcinoma: immunohistochemistry for predictive biomarkers

Payal Kapur

Over the past couple of decades, multimodality treatment for the management of resectable rectal cancer has substantially improved the outcome of affected patients. However, the broad and unpredictable response to tumor of patients with rectal cancer treated with preoperative chemoradiotherapeutic interventions shows that our understanding of the molecular events leading to radioresistance in patients affected with this malignancy remains sparse. Multiple attempts by individual molecular markers in gene array and tissue microarray studies have emerged with the goal of identifying predictors of a response to chemoradiation in patients with rectal cancer. In this report, we discuss the status of the markers currently available in an attempt to tailor specific targeted therapies for rectal cancer in the neoadjuvant setting. *Anti-Cancer Drugs* **22:362–370**

Anti-Cancer Drugs 2011, **22**:362–370

Keywords: biomarkers, chemoradioresistance, rectal adenocarcinoma

Department of Pathology, UT Southwestern Medical Center, Dallas, Texas, USA

Correspondence to Payal Kapur, MD, Department of Pathology, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9110, USA
Tel: +1 214 590 8683; fax: +1 214 590 1411;
e-mail: payal.kapur@utsouthwestern.edu

Received 12 October 2010 **Revised form accepted** 30 November 2010

Introduction

Over the past two decades, neoadjuvant combined radiation therapy with or without sensitizing chemotherapy has been increasingly used together with surgery in the primary management of patients with rectal adenocarcinoma. The CAO/ARO/AIO-94 trial has established neoadjuvant chemoradiotherapy (CRT) followed by total mesorectal excision as the treatment of choice for stage II–III rectal cancer [1]. In this study, although no survival benefit was reported with preoperative compared with postoperative CRT, the preferred modality was preoperative CRT for patients with locally advanced rectal cancer, because of the superior overall compliance rate of preoperative CRT, superior rate of local control, reduced toxicity, and higher rate of sphincter preservation in patients with low-lying tumors. Approximately 40–60% of these patients with neoadjuvant CRT achieve some degree of pathological downstaging, but only 10% of these patients had pathologically complete response (pCR) [2]. Despite these recent advances in multimodality treatment, the overall rates of tumor recurrence, morbidity, and mortality remain high. Furthermore, individual rectal tumors show a wide range of radiosensitivity, and there is no currently established robust predictor of response to neoadjuvant CRT [3]. Prediction of response to CRT is particularly important because CRT is accompanied by significant side effects and cost. There is evidence to suggest that differences in intrinsic radiosensitivity exist and understanding their biological bases could help develop practical predictive assays and novel treatment strategies. Prospective identification of patients with a high likelihood of response to CRT is of interest for multiple reasons. First, it would significantly reduce morbidities associated with CRT as unnecessary toxic therapy need not be given to patients with intrinsically radioresistant tumors. Second, alternative adjuvant treatment modalities could be pursued early in the course of disease in the patients with intrinsically radioresistant tumors. Third, molecular characteristics of tumor could be used as potential targets of novel drug interventions. Fourth, patients with pCR who may not need surgical intervention after CRT can be identified and, finally, it would reduce health costs by limiting unnecessary treatment. Moreover, CRT is a common denominator in numerous cancer therapies, which may benefit from similar bioassays. Personalized medicine holds the promise that diagnosis, prevention, and treatment will be based on individual assessment of risk [4]. A successful radiosensitivity biomarker panel is essential to the development of biologically guided personalized radiation oncology treatment.

The rationale for radiotherapy is based on the finding that radiation inhibits cell proliferation, induces apoptotic cell death, and inhibits tumor growth [5]. In solid tumors, radiation induces two different modes of cell death termed mitotic or clonogenic cell death and apoptosis [6]. Among various mechanisms of action exerted by ionizing radiation, it creates a class of DNA lesions called DNA double-stranded breaks, either directly or by production of free radicals. Although some of these lesions might be repaired by intact DNA repair mechanisms, misrepaired lesions can lead to mutations deleterious to the cell. Subsequent cell death may occur after a number of cell cycles, probably from failure to repair DNA damage. Apoptosis after radiation appears to be significant in embryonal and hematopoietic cells. The most common

chemotherapeutic drug used in neoadjuvant therapy in rectal cancer is 5-fluorouracil (5-FU). 5-FU, a pyrimidine antagonist, is similar in structure to uracil and thymine. It functions to inhibit DNA synthesis both by blocking the formation of normal pyrimidine nucleotides through enzyme inhibition and by interfering with DNA synthesis after incorporation into a growing DNA molecule. 5-FU also interferes with the production of RNA. With this understanding of the mechanism of radiation and chemotherapy-induced cell death, it is not surprising that most researchers have focused their investigations on molecules that regulate the cell cycle and apoptosis as putative markers to explore chemoradioresistance. The main goal of this study is to review predictors of the histological response to neoadjuvant CRT for rectal cancer.

Genomic and proteomic studies

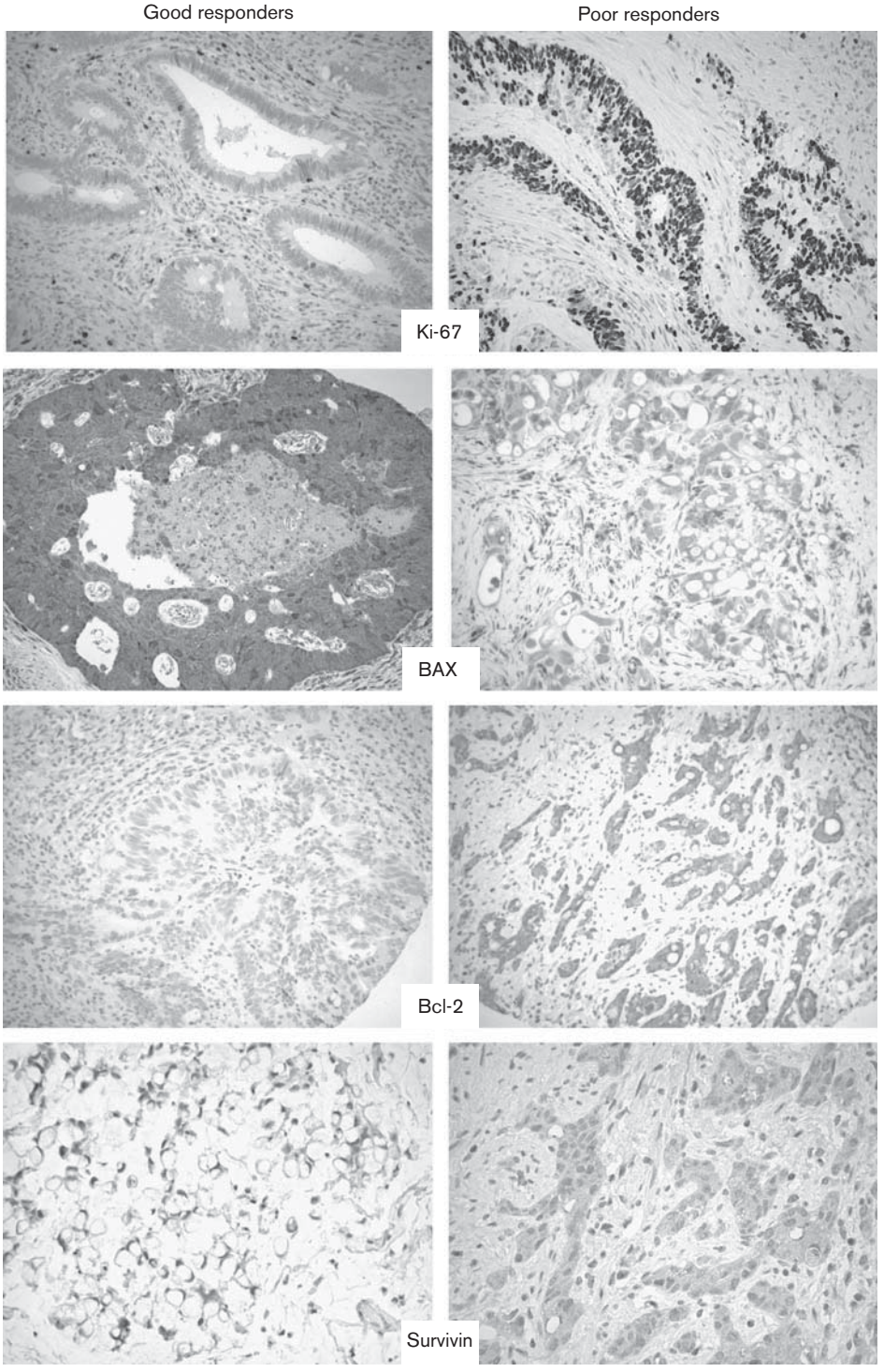
The value of gene expression profiling based on microarray technologies for the prediction of drug response has been tested in several model systems. The results from these studies show some promise that, for some tumors and drugs, pretherapeutic gene expression profiles might predict treatment response. Ghadimi *et al.* [7] compared gene expression differences between CRT responders and nonresponders from pretreatment rectal tumor biopsies from 30 patients. On the basis of T-level downsizing, 54 genes that were most significantly differentially expressed were identified. Some of these genes are associated with DNA damage repair pathways (SMC1) and microtubule organization (calmin, Cdc42BPA, filamin B, villin, kinectin 1). They generated an algorithm that allowed tumor behavior to be predicted with 78% sensitivity and 86% specificity. Watanabe *et al.* [8] identified 33 novel discriminating genes from 52 patients using the human U95Av2 gene chip. Using this gene set they established a model to predict CRT response with an accuracy of 82.4%. The discriminating genes included apoptosis regulators (lumican, thrombospondin 2, galecton 1, cyclophilin 40, and glutathione peroxidase). Kim *et al.* [9] similarly used top-ranked 95 differentially expressed genes to predict CRT response with 88.6% accuracy. The predictor genes in this study included thymidylate synthetase, RAD23B, RAP1A, LMNB2, MLF2, DDX1, EIF4A1, FOX03A, and ILF3. Eschrich *et al.* [10] built a rank-based linear regression algorithm to predict radiosensitivity. They validated this model in multiple cohorts of patients totaling 118 patients with a positive predictive value of 86%. These reports have indicated that the majority of the genes related to chemoradiosensitivity is associated with apoptosis, DNA repair, growth factor, signal transduction, cell cycle, adhesion, invasion, metastasis, angiogenesis, and hypoxia. It is interesting that many of the predictor genes are different in each study with a limited overlap. These apparent discrepancies may reflect differences in therapeutic regimens, cancer types, and/or methods of analyses. Extending this genomic-based approach to proteomics, Allal *et al.* [11] used two-dimensional polyacrylamide gel electrophoresis to correlate protein patterns from 17 patients with and without radioresistance. Of the 56 landmark proteins, those of particular interest included tropomodulin, heat shock protein, β-tubulin, annexin, calsenilin, keratin type 1, Notch 2 protein homolog, and DNA repair protein RAD51L3. Smith *et al.* [12] identified a cohort of 14 protein peaks that differentiated good and poor responders to neoadjuvant therapy with 87.5% sensitivity and 80% specificity by using low molecular weight proteome analysis. It should be noted that all genome-wide and proteome-wide studies performed on fresh/frozen tumor samples are limited by the use of whole tissue samples that include, in addition to tumor cells, non-neoplastic elements such as stroma, blood vessels, inflammatory cells, and potential foci of necrotic tissue. These could introduce errors in the final results and could be one explanation of the lack of agreement noted among various such studies.

Tissue microarray studies

The availability of simple, financially feasible, and high-throughput technology such as tissue microarray (TMA)-based immunohistochemistry (IHC) provides an opportunity to develop such a panel of biomarkers. To this end we evaluated the differential expression of molecular markers in TMA constructs from archived stage II/III rectal tumors and matching adjacent mucosa ($n = 38$) from patients treated with preoperative CRT [13]. IHC was carried out for Ki-67, cyclin E, p21, p27, p53, survivin, Bcl-2, and BAX (Figs 1 and 2). Immunoreactivity for Ki-67, p53, Bcl-2, and BAX was associated with a pathological response (all P values < 0.001). Forward stepwise logistic regression analysis showed that MIB-1 was an independent predictor of a response to CRT ($P = 0.001$). A similar study was conducted by Debucquoy *et al.* [14] on TMA constructed from 99 patients and evaluated for epidermal growth factor receptor, carbonic anhydrase IX, Ki-67, vascular endothelial growth factor, cyclooxygenase 2, and cleaved cytokeratin 18. In patients with good tumor regression, they found a lower expression of Ki-67 and cleaved cytokeratin 18 ($P = 0.0007$) in the resection specimens. They did a hierarchical cluster analysis on nonresponders and good responders, but found no significant difference in the patterns of expression associated with response to CRT. Intrinsic apoptosis (i.e. apoptosis in preirradiated tissue) was shown to be a marker of reduced local recurrence in 1198 patients for the Dutch Total Mesorectal Excision Trial in which the tissue was subjected to terminal deoxynucleotidyl transferase dUTP nick end labeling in TMA samples [15].

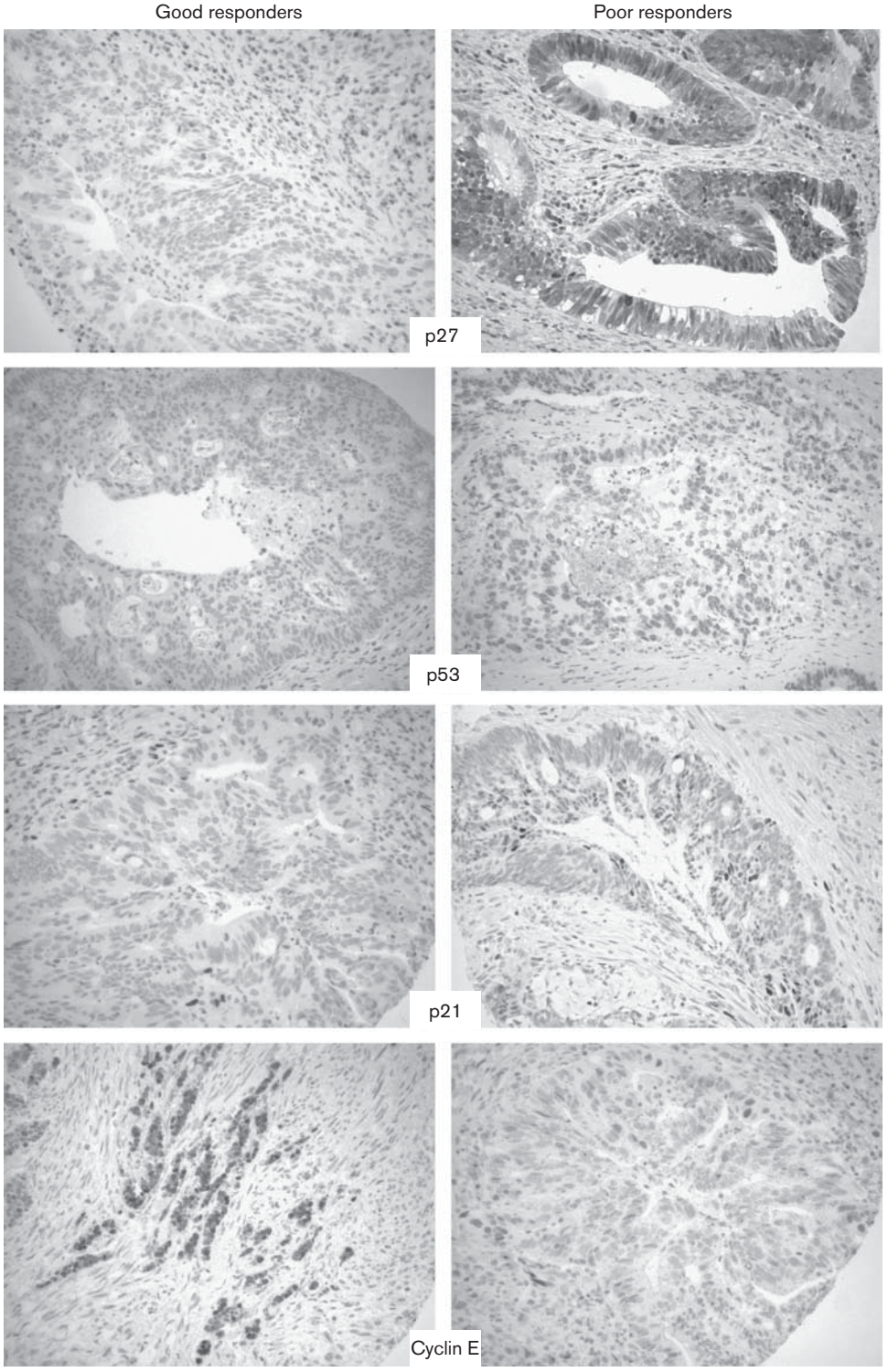
In the last decade, numerous studies have shown a plethora of differentially expressed genes and proteins; however, determination of just a handful of differentially expressed proteins might be sufficient for the purpose

Fig. 1



Representative photomicrographs of rectal adenocarcinomas on tissue microarray. The photomicrographs on the left panel represent immunoreactivity for apoptotic markers from cases with good response to chemoradiotherapy and the right panel includes representative immunoreactivity from cases with poor response. Magnification, $\times$ 200.

Fig. 2



Representative photomicrographs of rectal adenocarcinomas on tissue microarray. The photomicrographs on the left panel represent immunoreactivity for cell-cycle markers from cases with good response to chemoradiotherapy and the right panel includes representative immunoreactivity from cases with poor response. Magnification, $\times$ 200.

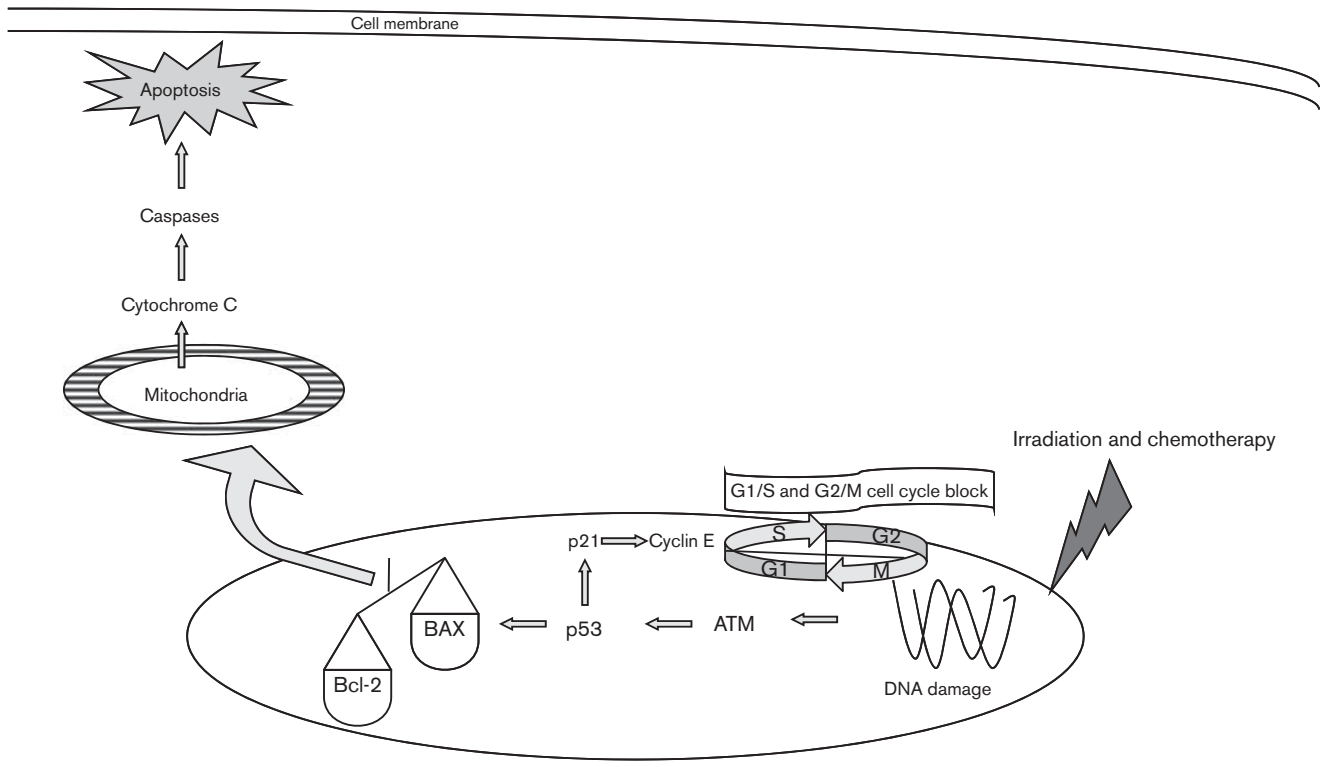
of routinely predicting chemoradioresistance in rectal cancer. More than 40 different biomarkers have been explored in the literature with conflicting results in predicting the outcome of CRT [2]. Some of the more promising markers are discussed below.

Specific markers for a response to chemoradiotherapy

Cell cycle regulators

One of the most extensively studied markers is the tumor suppressor, p53. It plays a vital role in the regulation of the cell cycle and is important for genetic stability, cell proliferation, apoptosis, and inhibition of angiogenesis [16]. A defect in p53 leads to loss of p53-dependent apoptosis and gives a proliferation advantage. It has been shown that conformational changes of the p53 protein resulting from mutations lead to protein stability and a longer half-life [17,18]. This allows for increased detection by the antibodies and thus p53 immunoreactivity by IHC signifies a mutated status of the protein [19]. Bertolini *et al.* [20] ($n = 91$) and Chang *et al.* [21] ($n = 130$) studied a large number of rectal cancers and showed no correlation between p53 expression and treatment outcome. However, similar to our findings, Luna-Perez *et al.* [22] ($n = 26$), Spitz *et al.* [23] ($n = 42$) and Esposito *et al.* [24] ($n = 38$) showed a positive correlation between the expression of p53 and poor response. However, stepwise logistic regression analysis in our study failed to identify p53 as an independent predictor of response to CRT [13]. In contrast, Lin *et al.* [25] ($n = 70$) showed that p53 staining predicted a good response to CRT. Wild-type p53 protein induces the expression of p21, a product of the WAF1/CIP1 gene. P21 and p27 are cyclin-dependent kinase inhibitors that inhibit cells from entering the G1 to S phase (Fig. 3). Wild-type p21 suppresses apoptosis in the presence of DNA damage caused by CRT. In-vitro studies suggest that mutated p21 serves to sensitize human colorectal cancer cells to CRT [26,27]. Charara *et al.* [28] ($n = 57$) and Rau *et al.* [29] ($n = 66$) found p21 expression to be associated with good/complete response. In contrast, Reerink *et al.* [30] ($n = 34$) showed higher p21 expression to be associated with worse survival, whereas Bertolini *et al.* [20] ($n = 91$) found no correlation with pathological response but showed decreased disease-free survival with high p21 expression. In our cohort, although low expression of p21 was seen more often in good responders, it did not reach statistical

Fig. 3



Diagrammatic representation showing radiotherapy and chemotherapy causing DNA damage, which activates p53 through ataxia telangiectasia mutated. Activated p53 causes activation of BAX, which then permeates from the nucleus to the mitochondria and allows the release of a number of proapoptotic proteins such as cytochrome c, which in turn activates the caspase cascade resulting in apoptosis. Activated p53 also results in activation of p21 leading to cell-cycle arrest at G1–S phase.

significance [13]. Similarly, Lin *et al.* [25] ($n = 70$) reported that p27-positive and p53-negative tumors showed a fair response to CRT.

Proliferative markers and mitotic index

Ki-67 and proliferating cell nuclear antigen have been used to assess proliferation. Kim *et al.* [31] ($n = 23$) found that good responders have a higher Ki-67 index, whereas Jakob *et al.* [32] ($n = 22$) showed the opposite result. Many studies have not found any association. In our cohort, the Ki-67 index was significantly higher in post-therapy specimens of nonresponders. Furthermore, Ki-67 was an independent predictor of a response to CRT [13]. Willett *et al.* [33] ($n = 90$) reported high proliferating cell nuclear antigen levels to be associated with greater chance of downstaging after CRT. Other reports have found no apparent correlation between these markers and response to CRT [34,35].

Apoptotic markers

Apoptosis is an important mechanism by which CRT mediates its response. It is hypothesized that tumors with intact apoptotic mechanisms may be more prone to undergo cell death with CRT. Defects in apoptosis are known mechanisms that render rectal cancers radioresistant [15]. The BAX and Bcl-2 proteins have been investigated extensively. BAX is a proapoptotic counterpart of Bcl-2, which inhibits cellular apoptosis. Loss of the BAX function has been linked with resistance to chemotherapeutic agents in colorectal cancer. Chang *et al.* [21] ($n = 130$) found that tumors with pCR showed significantly higher BAX positivity when compared with partial responders. Bcl-2 overexpression has been linked with resistance to many chemotherapeutic agents and has been shown to protect cells from radiation-induced apoptosis [36,37]. Kudrimoti *et al.* [38] ($n = 17$) showed 60% of tumors with cPR to express Bcl-2 compared with 16% of partial responders. However, other investigators have not been able to confirm these findings. Survivin is another antiapoptotic molecule that inhibits members of the caspase family of enzymes. It is a member of the inhibitors of apoptosis (IAP) family that also includes X-linked inhibitor of apoptosis (XIAP). Sarela *et al.* [39] ($n = 49$) showed a correlation between survivin expression and decreased survival. Expression of survivin is regulated in part by SMAC (second mitochondria-derived activator of caspase)/DIABMO (direct inhibitor of apoptosis-binding protein with low isoelectric point), a mitochondrial protein. Its mimics (SMAC-mimetics) are potential therapeutic agents. Our group has shown that JP-1201 (SMAC-mimetic) radiosensitized HT-29 colorectal cancer cells by inducing apoptosis and reducing the levels of XIAP [40]. In addition, HT-29 xenografts, when treated with JP-1201 and radiotherapy, shows decreased tumor growth compared with those treated with radiotherapy or JP-1201 alone. Similar in-vitro and in-vivo preliminary results from our group have shown very encouraging results. One of these studies involved colorectal cancer SW480 cells derived from primary resection for a stage II tumor and colorectal cancer SW620 cells derived from the same patient from a lymph node metastasis. Compared with SW480 cells, SW620 cell have low levels of SMAC/DIABMO and high levels of natural killer κB, survivin, and XIAP [41]. Consistent with this data, we observed that SW620 cells are more resistant to radiotherapy-induced apoptosis and cell death [42].

Angiogenesis and hypoxia

Angiogenesis is crucial for tumor growth and survival. Therefore, vascular endothelial growth factor (VEGF) is a subject of investigation in cancer biology. Zlobec *et al.* [43] ($n = 59$) found low levels of VEGF to correlate with radioresponsiveness in rectal cancer. Targeted therapy using VEGF inhibitors (bevacizumab) have shown an encouraging response [44]. It has also been established that oxygen is vital in the response of cells to the DNA-damaging effects of radiotherapy. Tumors with poor oxygenation are generally found to be more radioresistent. Dietz *et al.* [45] showed that rectal tumors that did not downstage with the CRT had higher levels of hypoxia. It is believed that hypoxia prevents the degradation of hypoxia-inducible factor-1α (HIF-1α). HIF-1α in turn regulates a number of processes including angiogenesis (through VEGF) and cell growth [46]. Toiyama *et al.* [47] ($n = 40$) showed that rectal tumors with elevated HIF-1α expression had decreased chances of tumor regression after CRT.

Other biomarkers

Cyclooxygenase 2 is an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins. Tumor cells can use these compounds as survival factors and they are important mediators of tumor invasiveness and metastatic potential. Smith *et al.* [48] found patients with cyclooxygenase 2 overexpression to more likely show moderate-to-poor response to CRT ($P = 0.026$).

Mismatch repair proteins have also been investigated to predict response to treatment. Microsatellite instability reflects a defect in DNA mismatch repair pathway. Tumors with microsatellite instability have been found to have a better prognosis. However, the two studies that assessed IHC overexpression of mismatch repair proteins did not find these to predict response to CRT [28,29]. Recently, single-nucleotide polymorphism in the *XRCC1* gene was shown to be predictive of the CRT therapy response ($P = 0.039$; $n = 81$) [49].

Epidermal growth factor receptor (EGFR) is involved in numerous cellular events including cell proliferation, apoptosis, and differentiation. As EGFR antibodies have shown efficacy in the treatment of metastatic colorectal cancer, many investigators have examined the association of EGFR expression and response to therapy. In-vitro

studies have found EGFR overexpression to be associated with CRT resistance. Giralt *et al.* [50] ($n = 87$) found a significantly higher number of EGFR-negative patients to achieve pCR compared with EGFR-positive patients. Using multivariate analysis, Kim *et al.* [51] ($n = 183$) found that low EGFR expression significantly predicted increased tumor downstaging with therapy. Spindler *et al.* [52] ($n = 60$) found that tumors with replacement of G by a T nucleotide at position 216 of the EGFR promoter (GT or TT genotypes) resulted in improved response compared with tumors with GG genotype.

Independent of these results, cetuximab therapy has not been found to be a recommended strategy in the neoadjuvant setting [53].

Another target, thymidylate synthetase (TS) has been studied widely. TS is critical in DNA synthesis and is a target of 5-FU. Okonkwo *et al.* [54] ($n = 25$) and Saw *et al.* [55] ($n = 58$) found the lack of TS expression to correlate with tumor downstaging after CRT. However, Negri *et al.* [56] ($n = 57$) reported more complete response with high TS expression, whereas Bertolini *et al.* [20] ($n = 125$) did not find any correlation.

Recently, intensified preoperative chemotherapy regimens have been evaluated for increasing the rate of downstaging or downsizing. The chemotherapeutic armamentarium used in the treatment of colorectal adenocarcinoma has increased from one option, that is, 5-FU to five with the recent availability of irinotecan, oxaliplatin, cetuximab, and bevacizimab. Recent studies are focused on identifying profiles and biomarkers to predict treatment efficacy of these drugs. Irinotecan is a synthetic derivative of camptothecin and is a topoisomerase I inhibitor. Horisberger *et al.* [57] ($n = 38$) recently showed a significantly higher expression of topoisomerase I using real-time PCR in pretreated tumors of patients receiving neoadjuvant CRT with irinotecan and capecitabine among responders compared with nonresponding patients ($P = 0.015$). Oxaliplatin is a platinum compound thought to inhibit DNA synthesis. Negri *et al.* [56] ($n = 57$) found IHC expression of TS in pretreatment biopsy to be the only marker among a variety of biomarkers evaluated to have a potential to predict response to preoperative oxaliptatin-based RCT. Cetuximab is a monoclonal antibody directed against EGFR. Debucquoy *et al.* [58] ($n = 41$) showed that cetuximab has a significant impact on the expression of genes involved in tumor proliferation and inflammation. Tumor downstaging was associated with upregulated TGF-α and downregulated Ki-67 expression after a cetuximab-loading dose. However, proteomic and microarray analysis did not predict a pathological response. Duda *et al.* [59] have found pretreatment plasma soluble VEGF receptor-1 (sVEGFR-1), an endogenous inhibitor of VEGF, to be associated with tumor regression after neoadjuvant bevacizumab (VEGF inhibitor) and CRT.

Conclusion

In summary, a large number of biomarkers have been studied to date. The data, although promising but often contradictory, have not resulted in the establishment of individual biomarkers or a panel of biomarkers that could help identify the radiosensitivity of rectal cancers. There may be multiple reasons for this. First, the evaluation of response of primary tumor to CRT is problematic and not standardized. This is reflected in the numerous different evaluation parameters that have been used in the literature. The most commonly used is downsizing defined as reduction of tumor stage by at least one T level assessed either by rectal ultrasound, magnetic resonance imaging, or histopathology [60]. The accuracy of this technique may be limited by the imaging modalities used and the interpretive inaccuracies. The second commonly used method is histopathological tumor regression and various semiquantitative approaches have been used [61]. In addition , rectal cancer, unlike colonic carcinoma, is treated with both chemotherapy and radiotherapy, which brings in more variables that may regulate response to therapy. There are variations in the assessment of these markers that are intrinsic to IHC evaluation parameters, methodology, evaluation of pretherapy versus posttherapy tumor, and different treatment protocols. Finally, it is possible that the missing link in radioresistance remains unidentified. An ideal molecule that would predict with certainty which group of patients will experience a particular response to CRT. Alternatively, gene array analysis could identify a particular pathway that moves in the expected direction in terms of the response to CRTs, our understanding of tumor biology grows and with the advent of more sophisticated high-throughput techniques, basic science studies will get translated to clinical tests. Ideally, pretherapy predictive marker evaluation of an individual tumor combined with individualized therapy would help significantly in reducing morbidity and mortality in the era of personalized medicine.

Acknowledgements

The author thanks Dr Dinesh Rakheja for his critical review, insightful comments, and sagacious suggestions.

The authors report no conflict of interest.

References

1 Sauer R, Becker H, Hohenberger W, Rodel C, Wittekind C, Fietkau R, *et al.* Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med* 2004; **351**:1731–1740.
2 Kuremsky JG, Tepper JE, McLeod HL. Biomarkers for response to neoadjuvant chemoradiation for rectal cancer. *Int J Radiat Oncol Biol Phys* 2009; **74**:673–688.
3 Wong RK, Tandan V, De Silva S, Figueredo A. Pre-operative radiotherapy and curative surgery for the management of localized rectal carcinoma. *Cochrane Database Syst Rev* 2007; **2**:CD002102.
4 Dalton WS, Friend SH. Cancer biomarkers – an invitation to the table. *Science* 2006; **312**:1165–1168.
5 Hendry JH, West CM. Apoptosis and mitotic cell death: their relative contributions to normal-tissue and tumour radiation response. *Int J Radiat Biol* 1997; **71**:709–719.

6 Ross GM. Induction of cell death by radiotherapy. *Endocr Relat Cancer* 1999; **6**:41–44.
7 Ghadimi BM, Grade M, Difilippantonio MJ, Varma S, Simon R, Montagna C, *et al.* Effectiveness of gene expression profiling for response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy. *J Clin Oncol* 2005; **23**:1826–1838.
8 Watanabe T, Komuro Y, Kiyomatsu T, Kanazawa T, Kazama Y, Tanaka J, *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**:3370–3374.
9 Kim IJ, Lim SB, Kang HC, Chang HJ, Ahn SA, Park HW, *et al.* Microarray gene expression profiling for predicting complete response to preoperative chemoradiotherapy in patients with advanced rectal cancer. *Dis Colon Rectum* 2007; **50**:1342–1353.
10 Eschrich SA, Pramana J, Zhang H, Zhao H, Boulware D, Lee JH, *et al.* A gene expression model of intrinsic tumor radiosensitivity: prediction of response and prognosis after chemoradiation. *Int J Radiat Oncol Biol Phys* 2009; **75**:489–496.
11 Allal AS, Kahne T, Reverdin AK, Lippert H, Schlegel W, Reymond MA. Radioresistance-related proteins in rectal cancer. *Proteomics* 2004; **4**:2261–2269.
12 Smith FM, Gallagher WM, Fox E, Stephens RB, Rexhepaj E, Petricoin EF III, *et al.* Combination of SELDI-TOF-MS and data mining provides early-stage response prediction for rectal tumors undergoing multimodal neoadjuvant therapy. *Ann Surg* 2007; **245**:259–266.
13 Huerta S, Hrom J, Gao X, Saha D, Anthony T, Reinhart H, *et al.* Tissue microarray constructs to predict a response to chemoradiation in rectal cancer. *Dig Liver Dis* 2010; **42**:679–684.
14 Debucquoy A, Goethals L, Libbrecht L, Perneel C, Geboes K, Ectors N, *et al.* Molecular and clinico-pathological markers in rectal cancer: a tissue micro-array study. *Int J Colorectal Dis* 2009; **24**:129–138.
15 De Bruin EC, Van de Velde CJ, Van de Pas S, Nagtegaal ID, Van Krieken JH, Gosens MJ, *et al.* Prognostic value of apoptosis in rectal cancer patients of the dutch total mesorectal excision trial: radiotherapy is redundant in intrinsically high-apoptotic tumors. *Clin Cancer Res* 2006; **12**:6432–6436.
16 Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature* 2000; **408**:307–310.
17 Baas IO, Van den Berg FM, Mulder JW, Clement MJ, Slebos RJ, Hamilton SR, *et al.* Potential false-positive results with antigen enhancement for immunohistochemistry of the p53 gene product in colorectal neoplasms. *J Pathol* 1996; **178**:264–267.
18 Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ. Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. *Mol Cell Biol* 1988; **8**:531–539.
19 Huerta S, Gao X, Saha D. Mechanisms of resistance to ionizing radiation in rectal cancer. *Expert Rev Mol Diagn* 2009; **9**:469–480.
20 Bertolini F, Bengala C, Losi L, Pagano M, Iachetta F, Dealis C, *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**:1455–1461.
21 Chang HJ, Jung KH, Kim DY, Jeong SY, Choi HS, Kim YH, *et al.* Bax, a predictive marker for therapeutic response to preoperative chemoradiotherapy in patients with rectal carcinoma. *Hum Pathol* 2005; **36**:364–371.
22 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**:203–208.
23 Spitz FR, Giacco GG, Hess K, Larry L, Rich TA, Janjan N, *et al.* P53 immunohistochemical staining predicts residual disease after chemoradiation in patients with high-risk rectal cancer. *Clin Cancer Res* 1997; **3**:1685–1690.
24 Esposito G, Pucciarelli S, Alaggio R, Giacomelli L, Marchiori E, Iaderosa GA, *et al.* P27kip1 expression is associated with tumor response to preoperative chemoradiotherapy in rectal cancer. *Ann Surg Oncol* 2001; **8**:311–318.
25 Lin LC, Lee HH, Hwang WS, Li CF, Huang CT, Que J, *et al.* P53 and p27 as predictors of clinical outcome for rectal-cancer patients receiving neoadjuvant therapy. *Surg Oncol* 2006; **15**:211–216.
26 Waldman T, Lengauer C, Kinzler KW, Vogelstein B. Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. *Nature* 1996; **381**:713–716.
27 Tian H, Wittmack EK, Jorgensen TJ. P21WAF1/CIP1 antisense therapy radiosensitizes human colon cancer by converting growth arrest to apoptosis. *Cancer Res* 2000; **60**:679–684.
28 Charara M, Edmonston TB, Burkholder S, Walters R, Anne P, Mitchell E, *et al.* Microsatellite status and cell cycle associated markers in rectal cancer patients undergoing a combined regimen of 5-FU and CPT-11 chemotherapy and radiotherapy. *Anticancer Res* 2004; **24**:3161–3167.
29 Rau B, Sturm I, Lage H, Berger S, Schneider U, Hauptmann S, *et al.* Dynamic expression profile of p21WAF1/CIP1 and Ki-67 predicts survival in rectal carcinoma treated with preoperative radiochemotherapy. *J Clin Oncol* 2003; **21**:3391–3401.
30 Reerink O, Karrenbeld A, Plukker JT, Verschueren RC, Szabo BG, Sluiter WJ, *et al.* Molecular prognostic factors in locally irresectable rectal cancer treated preoperatively by chemo-radiotherapy. *Anticancer Res* 2004; **24**:1217–1221.
31 Kim NK, Park JK, Lee KY, Yang WI, Yun SH, Sung J, *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**:418–424.
32 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**:1060–1066.
33 Willett CG, Warland G, Cheek R, Coen J, Efird J, Shellito PC, *et al.* Proliferating cell nuclear antigen and mitotic activity in rectal cancer: predictor of response to preoperative irradiation. *J Clin Oncol* 1994; **12**:679–682.
34 Desai GR, Myerson RJ, Higashikubo R, Birnbaum E, Fleshman J, Fry R, *et al.* Carcinoma of the rectum. Possible cellular predictors of metastatic potential and response to radiation therapy. *Dis Colon Rectum* 1996; **39**:1090–1096.
35 Tannapfel A, Nusslein S, Fietkau R, Katalinic A, Kockerling 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**:585–591.
36 Miyashita T, Reed JC. Bcl-2 gene transfer increases relative resistance of S49.1 and WEHI7.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. *Cancer Res* 1992; **52**:5407–5411.
37 Fukunaga-Johnson N, Ryan JJ, Wicha M, Nunez G, Clarke MF. Bcl-2 protects murine erythroleukemia cells from p53-dependent and -independent radiation-induced cell death. *Carcinogenesis* 1995; **16**:1761–1767.
38 Kudrimoti M, Lee EY, Kang Y, Ahmed M, Mohiuddin M. Genetic markers predictive of response to induction chemoradiotherapy for locally advanced rectal cancers. *J Ky Med Assoc* 2007; **105**:18–22.
39 Sarela AI, Scott N, Ramsdale J, Markham AF, Guillou PJ. Immunohistochemical detection of the anti-apoptosis protein, survivin, predicts survival after curative resection of stage II colorectal carcinomas. *Ann Surg Oncol* 2001; **8**:305–310.
40 Huerta S, Gao X, Livingston EH, Kapur P, Sun H, Anthony T. In vitro and in vivo radiosensitization of colorectal cancer HT-29 cells by the smac mimetic JP-1201. *Surgery* 2010; **148**:346–353.
41 Bonavida B, Khineche S, Huerta-Yepez S, Garban H. Therapeutic potential of nitric oxide in cancer. *Drug Resist Updat* 2006; **9**:157–173.
42 Huerta S, Baay-Guzman G, Gonzalez-Bonilla CR, Livingston EH, Huerta-Yepez S, Bonavida B. In vitro and in vivo sensitization of SW620 metastatic colon cancer cells to CDDP-induced apoptosis by the nitric oxide donor DETANONOate: involvement of AIF. *Nitric Oxide* 2009; **20**:182–194.
43 Zlobec I, Steele R, Compton CC. VEGF as a predictive marker of rectal tumor response to preoperative radiotherapy. *Cancer* 2005; **104**:2517–2521.
44 Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, *et al.* Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 2004; **10**:145–147.
45 Dietz DW, Dehdashti F, Grigsby PW, Malyapa RS, Myerson RJ, Picus J, *et al.* Tumor hypoxia detected by positron emission tomography with 60Cu-ATSM as a predictor of response and survival in patients undergoing Neoadjuvant chemoradiotherapy for rectal carcinoma: a pilot study. *Dis Colon Rectum* 2008; **51**:1641–1648.
46 Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. *Nat Rev Cancer* 2008; **8**:425–437.
47 Toiyama Y, Inoue Y, Saigusa S, Okugawa Y, Yokoe T, Tanaka K, *et al.* Gene expression profiles of epidermal growth factor receptor, vascular endothelial growth factor and hypoxia-inducible factor-1 with special reference to local responsiveness to neoadjuvant chemoradiotherapy and disease recurrence after rectal cancer surgery. *Clin Oncol (R Coll Radiol)* 2010; **22**:272–280.

48 Smith FM, Reynolds JV, Kay EW, Crotty P, Murphy JO, Hollywood D, *et al.* COX-2 overexpression in pretreatment biopsies predicts response of rectal cancers to neoadjuvant radiochemotherapy. *Int J Radiat Oncol Biol Phys* 2006; **64**:466–472.
49 Grimminger PP, Brabender J, Warnecke-Eberz U, Narumiya K, Wandhofer C, Drebber U, *et al.* XRCC1 gene polymorphism for prediction of response and prognosis in the multimodality therapy of patients with locally advanced rectal cancer. *J Surg Res* 2010; **164**:e61–e66.
50 Giralt J, De las Heras M, Cerezo L, Eraso A, Hermosilla E, Velez D, *et al.* The expression of epidermal growth factor receptor results in a worse prognosis for patients with rectal cancer treated with preoperative radiotherapy: a multicenter, retrospective analysis. *Radiother Oncol* 2005; **74**:101–108.
51 Kim JS, Kim JM, Li S, Yoon WH, Song KS, Kim KH, *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**:195–200.
52 Spindler KL, Nielsen JN, Lindebjerg J, Brandslund I, Jakobsen A. Prediction of response to chemoradiation in rectal cancer by a gene polymorphism in the epidermal growth factor receptor promoter region. *Int J Radiat Oncol Biol Phys* 2006; **66**:500–504.
53 Glynne-Jones R, Mawdsley S, Harrison M. Cetuximab and chemoradiation for rectal cancer–is the water getting muddy? *Acta Oncol* 2010; **49**:278–286.
54 Okonkwo A, Musunuri S, Talamonti M, Benson A III, Small W Jr, Stryker SJ, *et al.* Molecular markers and prediction of response to chemoradiation in rectal cancer. *Oncol Rep* 2001; **8**:497–500.
55 Saw RP, Morgan M, Koorey D, Painter D, Findlay M, Stevens G, *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**:192–202.
56 Negri FV, Campanini N, Camisa R, Pucci F, Bui S, Ceccon G, *et al.* Biological predictive factors in rectal cancer treated with preoperative radiotherapy or radiochemotherapy. *Br J Cancer* 2008; **98**:143–147.
57 Horisberger K, Erben P, Muessle B, Woernle C, Stroebel P, Kaehler G, *et al.* Topoisomerase I expression correlates to response to neoadjuvant irinotecan-based chemoradiation in rectal cancer. *Anticancer Drugs* 2009; **20**:519–524.
58 Debucquoy A, Haustermans K, Daemen A, Aydin S, Libbrecht L, Gevaert O, *et al.* Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer. *J Clin Oncol* 2009; **27**:2751–2757.
59 Duda DG, Willett CG, Ancukiewicz M, di Tomaso E, Shah M, Czito BG, *et al.* Plasma soluble VEGFR-1 is a potential dual biomarker of response and toxicity for bevacizumab with chemoradiation in locally advanced rectal cancer. *Oncologist* 2010; **15**:577–583.
60 Gavioli M, Bagni A, Piccagli I, Fundaro S, Natalini G. Usefulness of endorectal ultrasound after preoperative radiotherapy in rectal cancer: comparison between sonographic and histopathologic changes. *Dis Colon Rectum* 2000; **43**:1075–1083.
61 Dworak O, Keilholz L, Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. *Int J Colorectal Dis* 1997; **12**:19–23.