Review

Network Mapping of Molecular Biomarkers Influencing Radiation Response in Rectal Cancer

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Abstract

Preoperative radiotherapy (RT) plays an important role in the management of locally advanced rectal cancer (RC). Tumor regression after RT shows marked variability, and robust molecular methods are needed to help predict likely response. The aim of this study was to review the current published literature and use Gene Ontology (GO) analysis to define key molecular biomarkers governing radiation response in RC. A systematic review of electronic bibliographic databases (Medline, Embase) was performed for original articles published between 2000 and 2015. Biomarkers were then classified according to biological function and incorporated into a hierarchical GO tree. Both significant and nonsignificant results were included in the analysis. Significance was binarized on the basis of univariate and multivariate statistics. Significance scores were calculated for each biological domain (or node), and a direct acyclic graph was generated for intuitive mapping of biological pathways and markers involved in RC radiation response. Seventy-two individual biomarkers across 74 studies were identified. On highest-order classification, molecular biomarkers falling within the domains of response to stress, cellular metabolism, and pathways inhibiting apoptosis were found to be the most influential in predicting radiosensitivity. Homogenizing biomarker data from original articles using controlled GO terminology demonstrated that cellular mechanisms of response to RT in RC—in particular the metabolic response to RT—may hold promise in developing radiotherapeutic biomarkers to help predict, and in the future modulate, radiation response.

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Introduction

Total mesorectal excision represents the surgical standard of care in rectal cancer (RC) and has led to significant improvements in local control over the past 3 decades.[1] The additive benefit of radiotherapy (RT) in reducing local recurrence in locally advanced RC (T3/T4 and/or node-positive tumors) has been confirmed by several landmark randomized controlled trials (RCTs).[2-5] In the majority of patients, RT leads to clinically meaningful tumor regression. However, there is considerable variability in terms of tumor response, such that up to 20% of tumors undergo minimal regression, while an estimated 15% exhibit a complete radiologic response. The remainder have an intermediate response, with varying degrees of residual viable tumor seen in the postoperative resection specimen. Correspondingly, patients will fall broadly into 3 groups: (1) those in whom RT has led to complete tumor destruction; in this group of patients, there is growing interest in the feasibility of organ preservation[6,7]; (2) those in whom RT will result in at least a moderate degree of tumor regression, which in turn may enhance the likelihood of R0 resection and/or sphincter preservation; and (3) those in whom preoperative RT will lead to negligible tumor shrinkage. Paradoxically, for patients in the last category, the delay in proceeding to tumor excision while completing RT may increase the likelihood of distant metastases. Thus, the development of reliable methods to predict response at the pretreatment phase represents a critical unmet need in order to personalize locally advanced RC treatment algorithms.

Current understanding of response to RT is limited by the lack of a unifying interpretation of the molecular pathways implicated in response mechanisms. Additionally, systematic reviews in this context have served little to no role in expanding current

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understanding of this topic, as they typically evaluate data as a narrative series of disconnected molecular entities, with varying experimental methodology and clinical outcome measures and no wider appreciation of biological interconnectivity. While biomarkers, commonly oncogenes, are important in cancer diagnosis, such genes do not occur in isolation, and therefore mechanisms such as those governing tissue response to RT are likely to involve multiple biological pathways and processes at genomic, transcriptomic, and metabolomic levels. Thus, it is desirable to map potential biomarkers more holistically, in such a way as to allow integration of heterogeneous molecular data and intuitive visualization of the resulting network in order to identify areas with greatest translational potential.

Here we present a systematic review and network analysis of biomarkers implicated in RC radiosensitivity, with the aim of mapping key molecular processes involved, statistically estimating their relative importance, and permitting visualization of how these are interconnected.

Methods

An electronic literature search of the Medline and Embase databases was performed for English-language articles published between January 2000 and November 2015, utilizing Boolean logic and controlled vocabularies (Medical Subject Headings [MeSH] and Emtree). No review protocol was published in advance of this review. The following keywords were used, with wildcards used to maximize article capture: rectal, cancer, neoplasm, response, pathological complete response, tumor response, radiotherapy, chemoradio*, neoadjuvant, predict*, biomarker, *omic, RNA, DNA. Both human in vivo and human colorectal cancer (CRC) cell-based in vitro studies were included. Inclusion criteria were studies examining potential biomarkers (specific genes, protein products, or metabolites) correlated with a documented tumor regression grade (TRG) after RT (either long-course chemoradiotherapy [LCCR] or short-course RT [SCRT]). Studies were excluded if they evaluated targeted monoclonal antibody therapy, gene expression panels obtained through microarrays, micro-RNAs (miRNA) where the gene targets were not defined, or pure animal model–derived data. miRNA studies were initially screened but were excluded from this review primarily because the corresponding gene targets of respective miRNAs were not defined in any of the studies. Irrespective of this, with the potential for a single miRNA to target multiple genes, the inability to assign each miRNA to a single gene classifier was deemed incompatible with the objectives of the present study. Similarly, there was poor congruence between studies that used tissue microarrays, and the resultant outputs from these were almost universally reported as panels of multiple genes providing an in silico means for predicting response, and as such, these data were noncompliant with our objectives.

The following information was extracted from each publication: (1) studied biomarker, (2) analytical methodology used, (3) patient numbers, and (4) statistical parameter used by the authors to categorize discovery as significant (or not), according to correlation with TRG (including *P* values and odds ratios [ORs] for univariate tests, and sensitivity and specificity for multivariate tests). Each biomarker was then classified according to the system proposed by the Gene Ontology (GO) Consortium into 1 of 5 domains of molecular function or biological purpose (cell cycle, cell death, response to stimuli, cell signaling, and cellular metabolism).[8] The GO approach represents a relatively recent development in biological computational science that offers a means of annotating and grouping molecular products of upstream gene expression according to functional/biological commonality. We chose to utilize a GO approach here in order to generate a biologically more interpretable panorama of processes governing radiation response.

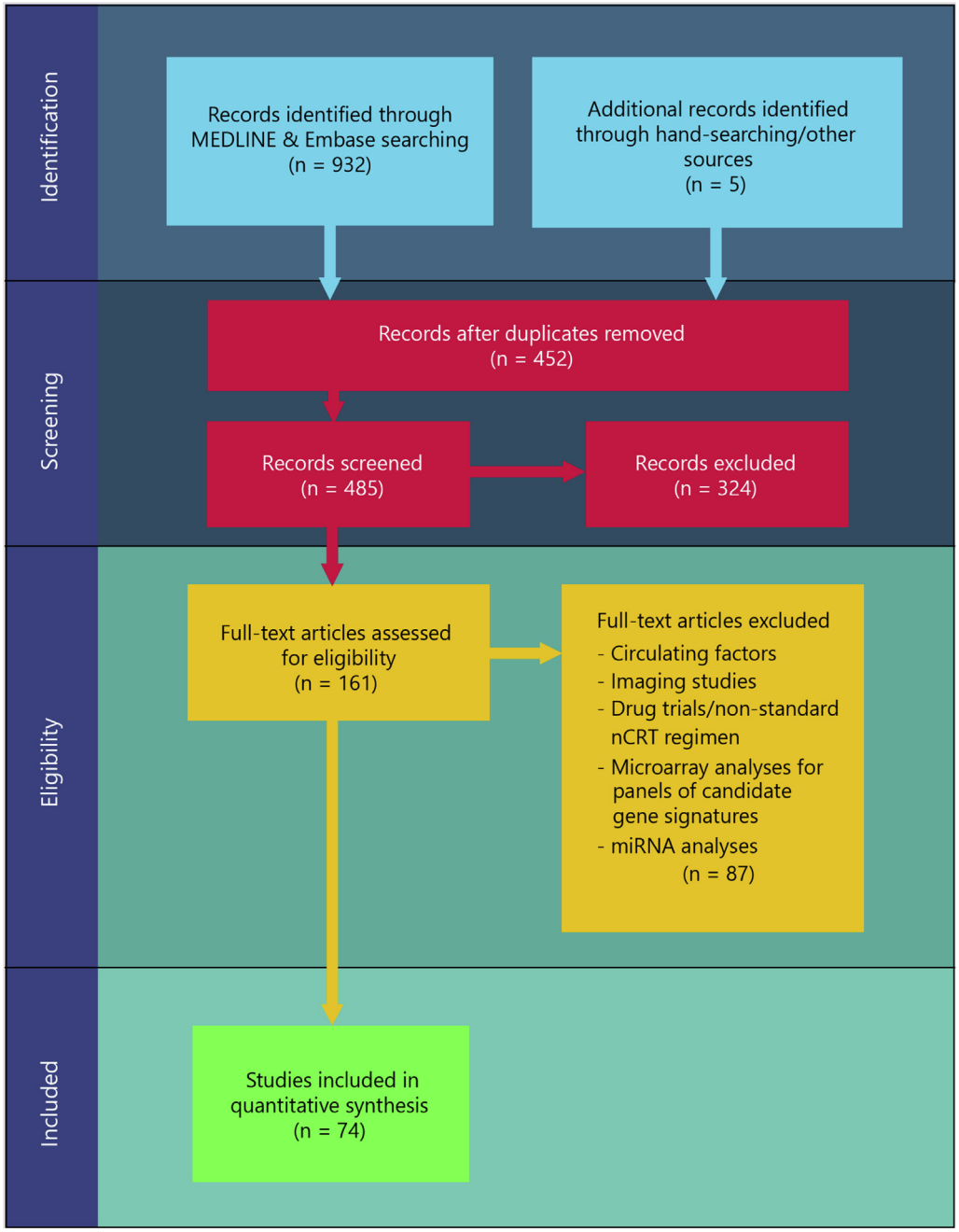
Depending on the statistical parameter used in the original study, significance was binarized (significant or not) based on *P* values ($P < .05$), OR (> 1), or sensitivity and specificity ($> 60\%$). A score summarizing the significance of a given biomarker was in turn calculated as a ratio of significant biomarkers relative to the total number of biomarkers under a given ontology heading. Nonunique biomarkers reported by different studies were included in this scoring, as the magnitude of supporting or conflicting evidence for a given biomarker by different studies needs to be represented. For each ontology node, a second score was computed to represent the total number of biomarkers and publications investigating this domain. This was calculated as the number of unique biomarkers for a given GO term (unlike in the first score, where nonunique biomarkers were considered). The 2 scores (each ranging between 0 and 1) represent 2 dimensions on the reported biomarkers we identified through systematic review: relative significance and popularity.

Results

A total of 452 studies were identified after removal of articles that did not include full text (mostly conference proceedings) and manual deduplication. The abstracts of these articles were further screened, and 373 were excluded because they did not address the question of tissue biomarkers predicting response to RT in RC, or because there was insufficient information on methodology or statistical analysis. A further 11 excluded articles comprised systematic reviews and meta-analyses, the bibliographies of which were hand searched, providing a further 5 articles (Figure 1). This left 74 articles that provided sufficient methodologic and statistical detail for both systematic review and to enable integration into a node-based biological network of tissue-based biomarkers. For ease of discussion, biomarkers evaluated in these studies were analyzed on the basis of biological and molecular function according to GO, and clustered under broad, highest-level biological function using controlled language, as previously described.[8,9] For many biomarkers with multiple GO annotations, clustered GO terms fell under different domains of biological function and so were recorded as separate entries in a data sheet of hierarchical ontology terms for each biomarker. It should be noted, however, that many of the biomarkers assessed have multiple functions and GO annotations; this is taken into account in the network-driven analytics.

The data obtained from the review process were extracted in such a way as to assign an identifier to each biomarker assessed by each study (Table 1). This generated a list of biomarkers with multiple entries for those that had been the subject of investigation for more than one study. Equally, to account for statistical power, separate entries were made for each statistical test (univariate or multivariate) applied to each biomarker. The full data extraction table is provided in the Supplemental Material.

Figure 1 Search Strategy (PRISMA) Outlining Search Methodology and Article Inclusion



By using GO terms, we were able to build a hierarchical classification for each biomarker that allowed for clustering under 1 of 5 subheadings (cell cycle, cell death, response to stimuli, cell signaling, and cellular metabolism). A network model was then developed (Figure 2). In this model, nodes are annotated according to their GO term and are clustered in spokes around the root term "biological process." Nodes are sized according to a significance score for that ontology term, calculated by taking into account the various statistical tests applied to each study (most commonly chi-square association or OR between biomarker and TRG in univariate and multivariate analysis, respectively). The size of the node represents the number of biomarkers investigated under that ontology term, and the color scale indicates the relative significance level. Therefore, a large red node would represent an ontology term with both a large number of biomarkers and high statistical significance.

The GO-based analytical approach used here shows that when the vast number of assessed biomarkers in the literature are

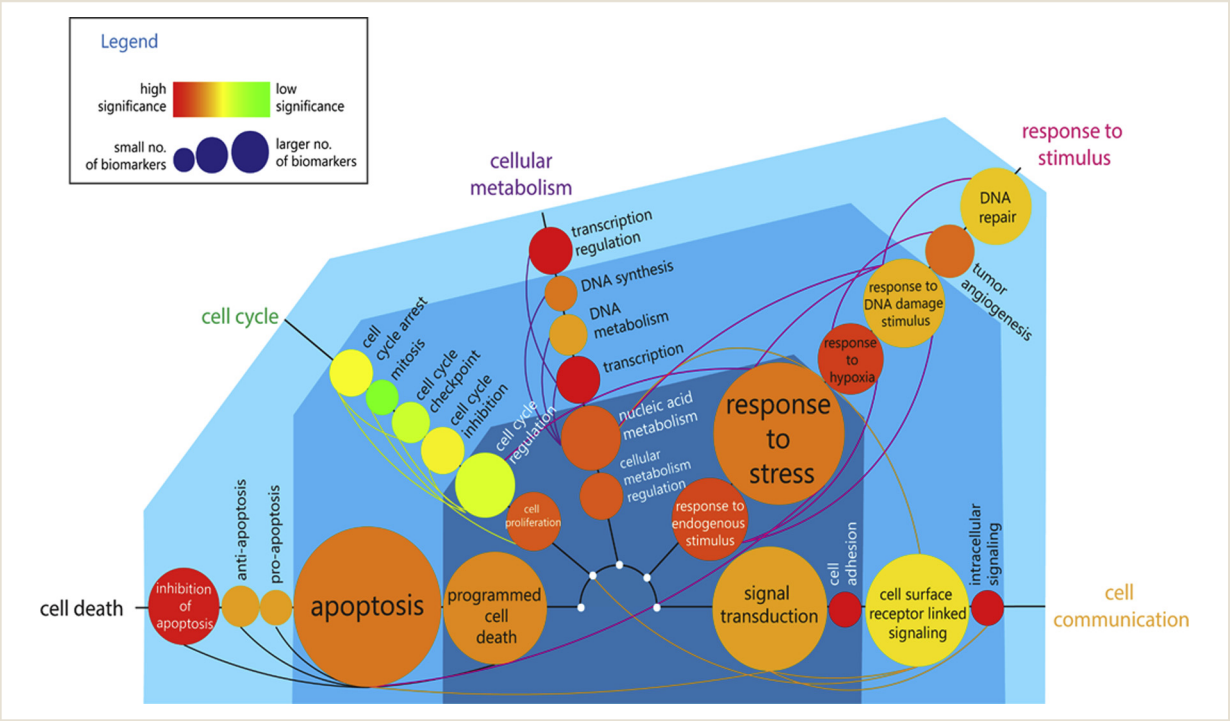
Table 1 Biomarkers Classified According to GO Hierarchical Tree (Biological Functions)

GO Cluster (Biological Function)	GO Node	Biomarker and References	Node Size	Node Significance (HSB Color Space)
Cell cycle and cell proliferation	Cell cycle arrest	***BIRC5***,[10-13] p21,[14-16] p53[14-19]	29.42857	57.77778
	Cell cycle checkpoint	p21,[14-16] p53[14-19]	25.71429	74.28571
	Cell cycle inhibition	p27,[16-20] BIRC5[10-13]	29.42857	29.42857
	Cell-cycle regulation	BIRC5,[10-13] p21,[14-16] p27,[16,20] p53,[14-19] phospho-Akt[21]	40.57143	68.8
	Cellular proliferation	Ki-67,[22-24] REG4,[11,25] Securin,[26] YKL-40[27]	36.85714	24
	Mitosis	β-Tubulin[28]	22	120
Cell death	Programmed cell death	Apoptotic index,[12,29] Bax,[14,16,30] BIRC5,[10-13] caspase-8,[31] hPEBP4,[32,33] M30,[34] MIB1,[15] p53,[14-19] PDCD4,[35] phospho-Akt,[21] XIAP,[36] YKL-40[27]	70.28571	35
	Apoptosis	Apoptotic index,[12,29] Bax,[14,16,30] BIRC5,[10-13] caspase-8,[31] hPEBP4,[32,33] M30,[34] MIB1,[15] p53,[14-19] PDCD4,[35] phospho-Akt,[21] XIAP,[36] YKL-40,[27] CD34,[37] calsenilin,[28] Smac[24,38]	100	31.11111
	Proapoptosis	Bax[14,16,30]	22	40
	Inhibition of apoptosis	BIRC5,[10-13] hPEBP4,[32,33] phospho-Akt,[21] XIAP,[36] YKL-40,[27] HIF-1,[39] HIF-1α[14]	48	5.714286
Response to stimuli, stress and DNA damage	Response to endogenous stimulus	CA9,[40] c-met,[27] CXCL10,[41] MSH2,[15] phospho-Akt,[21] thymidine phosphorylase,[37] VEGF,[15,16,24,37,39,42-45] YKL-40[27]	51.71429	18.94737
	Response to stress	ANXA1,[46] BIRC5,[10-13] DIABLO,[36] DNAJC12,[47] HIF-1,[39] HIF-1α,[14] hOGG1-1245C > G,[48] MTHFR 677T-1298A,[49] MTFHR-677C > T,[48] Hsp42,[28] Hsp90,[21] m-TIMP3,[50] NEIL2,[11] NF-κB,[10,51] p21,[14-16] p53,[14-19] sHsp16.2,[21] Smac,[24,38] XRCC2,[52] tropomodulin[28]	88.85714	31.11111
	Response to hypoxia	HIF-1,[39] HIF-1α,[14] CA9,[40] c-met,[27] CXCL10,[41] phospho-Akt,[21] thymidine phosphorylase,[37] VEGF,[15,16,24,37,39,42-45] YKL-40[27]	44.28571	16.47059
	Response to DNA damage stimulus	MSH2,[15] MTHFR 677T-1298A,[49] BIRC5,[10-13] hOGG1-1245C > G,[48] MTFHR-677C > T,[48] NEIL2,[11] NF-κB,[10,51] p53,[14-19] XRCC2[52]	55.42857	43.47826
	DNA repair	MSH2,[15] BIRC5,[10-13] hOGG1-1245C > G,[48] MTFHR-677C > T,[48] NEIL2,[11] p53,[14-19] XRCC2[52]	48	48
	Tumor angiogenesis	VEGF,[15,16,24,37,39,42-45] phospho-Akt,[21] thymidine phosphorylase,[37] YKL-40[27]	33.14286	28.57143
Cell communication	Signal transduction	ABCC4,[53] Bcl-2,[16,45] β-catenin,[45,54,55] CD44,[10,14] CD133,[14,56] CD166,[14] c-met,[27] CXCR4,[40] EGFR,[14,15,22,39] EpCAM,[14] GHRH-R,[21] HER-2,[45,57] IQGAP1,[58] MRP3,[59] TGF-β1,[60] THBS2[61]	77.71429	40
	Cell-surface receptor signaling	ABCC4,[53] CD34[37], CD44,[10,14] CD133,[14,56] CD166,[14] CXCR4,[40] EGFR,[14,15,22,39] EpCAM,[14] GHRH-R,[21] HER-2,[45,57] MRP3,[59] TGF-β1,[60] calsenilin[28]	70.28571	52.63158
	Intracellular signaling	IQGAP1[58]	22	0
	Cell adhesion	THBS2[61]	22	0
Cellular metabolism	Regulation of cellular metabolism	COX-2,[16,17,37,45,56,62] ALDH1,[14,63] HMGCS2,[64,65] HSD17B2,[65] MMP9,[43] phospho-Akt,[21] GLUT-1,[18,40] CPS1,[66] asparagine synthetase[67]	29.42857	24
	Nucleic acid metabolism	p-SMAD3,[60] SMAD3,[60] TCF4,[68] thymidine phosphorylase,[37] thymidylate synthase[10,14,15,22,23,69-72]	40.57143	24
	DNA synthesis	Thymidylate synthase[10,14,15,22,23,69-72]	22	31.11111
	DNA metabolism	Thymidine phosphorylase[37]	25.71429	40
	Transcription	p-SMAD3,[60] SMAD3,[60] TCF4[68]	29.42857	0
	Regulation of transcription	p-SMAD3,[60] SMAD3,[60] TCF4[68]	29.42857	0

Node size was calculated based on number of biomarkers within each GO term, accounting for sample size in each study. Number of references for each biomarker reflects number of studies retrieved. Significance was mapped to HSB color space to reflect overall significance of each node in resulting network, with lower relative value reflecting greater significance level based on multiple statistical tests used across studies.
Abbreviations: GO = Gene Ontology; HSB = hue, saturation, brightness.

clustered according to controlled language, it is possible to begin to identify patterns that may aid direction of future study by highlighting areas where there has been success to date. Figure 2 indicates that the biological function spoke "cell cycle" demonstrates a number of biomarkers that have shown negative results in experimental work. Thus, despite this being a fundamental component of cancer regulation, cell-cycle–based molecular dynamics may have little to do with radiation response. We

Figure 2 Static Knowledge Network Visualization of Biomarkers Clustered According to GO Terminology. As Per Data From Table 1. Each Node Represents GO Term, Clustered Under Group Headings (Spokes). Size of Nodes Was Calculated Based on Number of Biomarkers Under That GO Term, With Color/Hue/Saturation/Brightness Mapped to Aggregate Significance of Multiple Statistical Tests Used Across Studies



found that the greatest numbers of significant biomarkers lie in the domains of "response to stimulus" and the interplay of this domain with "cell death." In addition, while a relatively small number of biomarkers responsible for cellular metabolism have been evaluated to date, many of these demonstrate a high level of correlation with TRG.

Discussion

Cell Cycle

Numerous genes involved in cell-cycle regulation and cellular proliferation have been implicated in cancer development and progression. The p53 tumor suppressor network has been extensively studied and is ubiquitously implicated in almost all cancer subtypes. At its core, p53 can be broadly considered a tumor suppressor activated in response to stress, with subsequent effects on apoptosis, cell-cycle arrest, and senescence, with loss of p53 function being associated with decreased apoptosis.[16,73] Although certain radioresistant CRC cell lines demonstrate decreased expression of p53, multiple immunohistochemical (IHC) studies aimed at identifying it as a candidate biomarker for radioresistance have not shown a significant association between isolated p53 expression and TRG.[14,16-19,45] One 2014 study in which IHC was performed for a panel of biomarkers including p53, revealed a correlation between low p53 expression and pathologic complete response, but not with TRG,[42] whereas Kelley et al[31] found that intact p53 with deficiency in caspase-8 expression predicted inferior TRG.

These conflicting findings may in part relate to the complex interactions of the p53 network. In a study of 112 patients, both univariate and multivariate analysis found that p53 expression in pretreatment biopsy specimens did not correlate with RT response, whereas p21 expression—the product of which is a cyclin-dependent kinase (CDK) inhibitor acting through p53-dependent and independent pathways—was significantly associated with both TRG and disease-free survival.[14] By contrast, 2 further studies examining both p21 and p53 did not demonstrate any correlation between p21 expression and TRG.[15,16]

Downstream, a cross-regulatory network exists between p53 and protein kinase B (Akt), balancing the apoptotic regulation by p53 with the effects of the Akt signaling pathway. Phosphorylated Akt exerts subsequent effects on cell-cycle progression, metabolism, and inhibition of apoptotic pathways through Bad,[74] although its expression was not found to be predictive of response to RT when evaluated with IHC.[21] Other CDK inhibitors, notably p27, have also failed to demonstrate any correlation with response to RT.[16,20] Markers of cell-cycle progression are, however, useful surrogate markers of proliferation, and both Ki-67 and securin (pituitary tumor transforming gene 1, *PTTG1*) have been evaluated for correlation with response to RT. Whereas Ki-67 is detected in all active phases of the cell cycle, securin peaks in the G_2/M phases. Significant correlation has been demonstrated between Ki-67 expression and response to RT.[22-24] Although securin did not demonstrate a predictive capacity for TRG, expression levels in general were shown

to decrease after RT; furthermore, for those tumors with high securin expression after treatment, overall prognosis was poorer.[26]

The regenerating islet-derived gene 4 (*REG4*) is one of a family of genes (*Reg*) belonging to the calcium-dependent lectin superfamily, encoding a group of small secretory proteins. These have been found to be constitutively expressed in cell lines of the gastrointestinal tract with characteristic up-regulation in RC at both messenger RNA (mRNA) and peptide levels.[75,76] In cellular models, it has been shown to activate cell proliferation, in addition to in vitro migration and invasion of CRC cells.[77] Increased expression of *REG4* in radioresistant RC cell lines led to a subsequent up-regulation of *NEIL2* and survivin (*BIRC5*), suggesting a network correlation of expression of these genes and their products with radioresistance.[11] Additionally, IHC for *REG4* expression in 172 clinical specimens revealed increased *REG4* expression to be a surrogate marker for radioresistance.[25] Survivin acts as both a controller of mitotic progression and inhibition of apoptosis, and it has been demonstrated to play a role in the molecular pathogenesis of CRC.[78] Conflicting evidence exists, however, for survivin as a putative biomarker for radioresistance. Whereas McDowell et al[12] did not find survivin alone to be a predictor of response when assessed by both mRNA and protein expression, other studies have found a significant correlation in both in vivo and in vitro models with established radioresistant cell lines, including SW480 and HCT-15.[10,11,13]

Although the full role and molecular function of the glycoprotein YKL-40 (chitinase-3-like-1) is yet to be elucidated, it is understood to regulate a number of cellular processes, including cellular proliferation, by exerting effects on the tumor microenvironment to stimulate angiogenesis and tissue remodeling in CRC.[79,80] A multicenter study utilizing IHC to verify YKL-40 expression in biopsy specimens found a high positive predictive value for radioresistance. This predictive value was increased to 94% when evaluating for biomarker coexpression with the c-Met protooncogene.[27]

Cell Death

Cell death covers a range of biological processes by which the cell ceases to function, either as a result of an intrinsic, programmed pathway or in response to extrinsic stressful stimuli such as exposure to cytotoxic agents or RT. A number of mediators of the apoptotic pathway have shown potential as predictors of radiosensitivity.

A critical step in apoptotic signaling is the release of cytochrome *c* from mitochondria, which in turn activates apoptosis protease-activating factor 1 (APAF-1/apoptosome). After the activation of the initiator caspase-9, a series of reactions ensue that result in apoptosis and that are regulated by a balance of pro- and anti-apoptotic mediators. APAF-1 expression in pretreatment biopsy samples has been shown to correlate significantly with an improved TRG. Evidence for downstream mediators such as Bax (proapoptotic) and Bcl-2 (antiapoptotic) as predictive biomarkers has been inconclusive,[14,16,30] although one group found a significant association between Bcl-2 and TRG in both univariate and multivariate analysis.[45] Furthermore, programmed cell death 4 (PDCD4) was first shown to be up-regulated in apoptosis by Shibahara et al[81] and is now known to act as a nuclear mediator of apoptosis having interactions with factors downstream of Akt. In a recent study PDCD4 expression was shown to correlate positively with TRG on multivariate analysis.[35]

Although some correlation has been observed between apoptotic rate in pretreatment CRC biopsy samples and response to RT,[12,29] expression levels of M30 (caspase-cleaved keratin 18 cytoskeletal protein) have not been shown to be predictive of response to RT.[34] Similarly, annexin V—an established marker for apoptosis, as it binds to phosphatidylserine molecules on the cell surface (an event observed in apoptotic cells)—was not found to be predictive of response.[28] In spite of this, there is increasing evidence that the presence of higher levels of complex lipids is not only characteristic of the tumor microenvironment but may also contribute to radioresistance. Levels of phosphatidylethanolamine binding protein 4 (hPEBP4) have been shown to correlate strongly with increased resistance to RT in a cohort of prospectively recruited patients who received SCRT.[33] Functionally, hPEBP4 is a secreted glycoprotein that has been shown to be involved in the activation of Akt.[82] Overexpression of hPEBP4 in SW480 cell lines has been shown to enhance clonogenic survival, a finding mirrored in mouse models subjected to ionizing radiation. Inhibition of Akt activation additionally appeared to reverse the radioresistance effect of hPEBP4.[32]

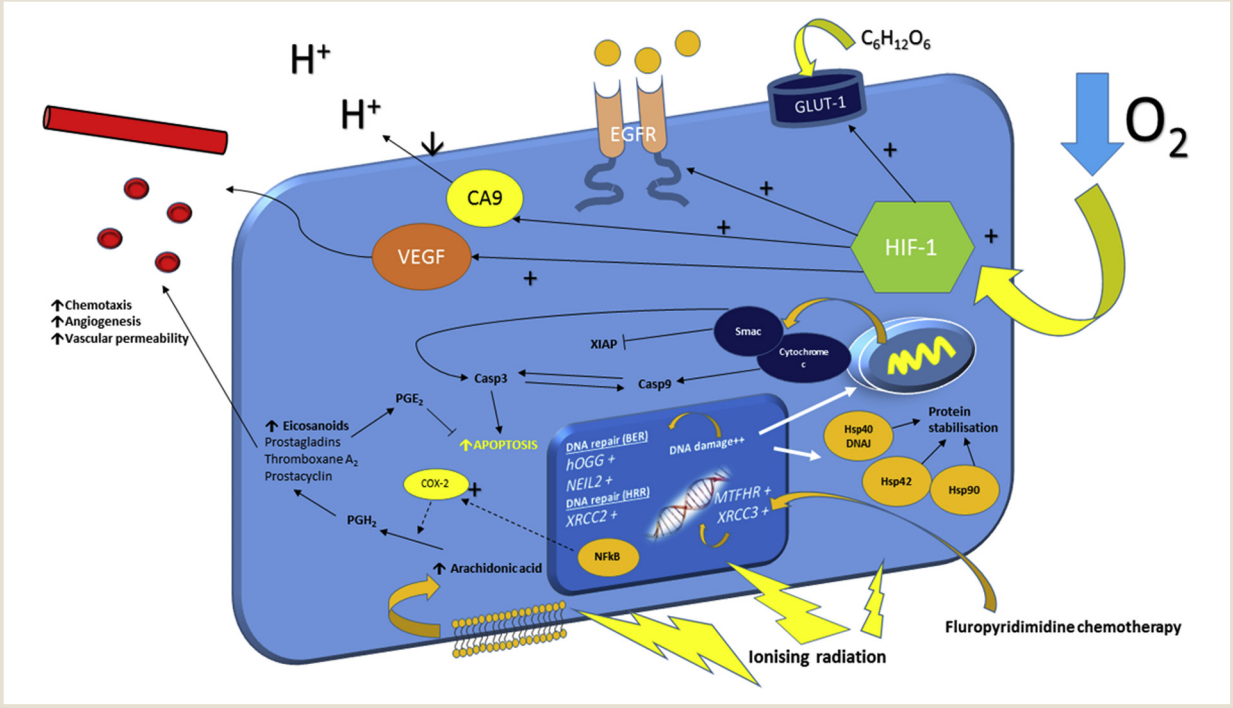
Response to Stimuli

Ionizing radiation leads to DNA strand breaks and ultimately cell death. Mediators involved in maintaining cellular homeostasis and responding to changes in the tumor microenvironment have shown variation in expression levels in relation to radiosensitivity. The presence of relative tumor hypoxia has been implicated in radioresistance in a number of malignancies. Central to the regulation of over 100 genes involved in the adaptive response to hypoxia is hypoxia-inducible factor 1 (HIF-1).[83,84] The effects of this transcription factor, stabilized at low oxygen tensions, include regulation of apoptosis, angiogenesis, glycolysis, and the cell cycle. A number of these downstream genes and products have subsequently been investigated as putative biomarkers for radiation response. Using quantitative PCR to evaluate HIF-1 expression in pretreatment tumor biopsy samples, Toiyama et al[39] demonstrated not only significantly lower levels of HIF-1 in those with significant tumor regression but also downstream induced factors epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) on both univariate and multivariate analyses.

The effects of hypoxia include further biochemical changes in the tumor microenvironment. CA9 (carbonic anhydrase 9), coding for carbonic anhydrase isoenzyme 9, plays an important role in regulating extracellular pH under both hypoxic and normoxic conditions, and has been found to be underexpressed in tumors with a pathologic complete response.[40] This study broadly examined a number of mechanisms of the adaptive response of RC to hypoxia, including glucose transporter 1 (GLUT-1) expression, which had been shown in an earlier study to be significantly associated with radioresistance. Conversely, GLUT-1—negative tumors were found to have a 70% probability of a good response to RT. These findings[18] were not corroborated by Guedj et al,[40] however, who found no significant association between GLUT-1 expression and TRG. It is worth noting that these 2 studies used differing intervals between RT and surgery, and also used different TRG grading systems.

Figure 3 summarizes the factors identified through this review that have been found to correlate with response to RT, based on

Figure 3 Factors Implicated in Response to Cellular Damage Due to Unfavorable Tumor Microenvironmental Conditions (Acidosis, Hypoxia) and RT. Individual Variation in These Pathways Are Those Shown to Be Implicated in Differing Responses to RT. (A) At Low Oxygen, Tension HIF-1 Is Up-regulated, With Corresponding Increased Expression of Downstream Cofactors; EGFR Is Up-regulated on Cell Surface; Carbonic Anhydrases Are Released in Order to Stabilize Extracellular pH, With CA9 Particularly Shown to Be Independent Predictor for RR; VEGF Stimulates Angiogenesis in Tumor Microenvironment. GLUT-1 Is Not Expressed in Normal Colonic Mucosa But Has Been Shown to Be Present in up to 80% of Colorectal Tumors. (B) Direct DNA Damage Leads to Activation of Number of DNA Repair Factors (hOGG, MTFHR, NEIL2) in Addition to Increased Levels of Heat Shock Proteins (Particularly Hsp40 [DNAJ], Hsp42 and Hsp90), Increased Levels of All of Which Have Shown Direct Relationship With RR. DNA Damage Stimulates Release of Cytochrome *c* and Activated Smac Proteins From Mitochondria, and Subsequent Activation of Apoptosome and Direct Inhibition of XIAP Leads to Apoptosis Under Normal Circumstances. XIAP Expression in Turn Has Been Demonstrated as Independent Predictor of Radioresistance.[36] Decreased Relative Levels of Smac Were Shown to Be Associated With RR. There May Be Additional Interplay With Smac Contributing to Direct Activation of Caspase-3. (C) Ionizing Radiation Leads to Increased Degradation of Complex Lipids in Cell Membrane and Subsequent Release of Higher Levels of Arachidonic Acid. Higher Levels of COX-2 Have Been Shown to Correlate With RR, With Subsequent Increased Levels of Eicosanoids and Their Downstream Effects in Both Intra- and Extracellular Environment. Within Cytosol, Both COX-2 and Eicosanoids Have Been Shown to Inhibit NF-κB, While in Tumor Microenvironment PGs and Thromboxane A_2 Have Well-established Effects on Chemotaxis, Vascular Permeability, and Angiogenesis as Part of Local and Systemic Inflammatory Process. Arrows Denote Signaling Pathway or Up-regulation; Hammerheads Denote Inhibition



Abbreviations: BER = base excision repair; CA9 = carbonic anhydrase 9; Casp3/Casp9 = caspase 3/9; COX-2 = cyclo-oxygenase 2; EGFR = endothelial growth factor receptor; GLUT-1 = glucose transporter 1; HIF-1 = hypoxia-inducible factor 1; HRR = homologous recombinant repair; Hsp = heat shock protein; PGE2/PGH2 = prostaglandin E_2/H_2; RT = radiotherapy; VEGF = vascular endothelial growth factor; XIAP = X-linked inhibitor of apoptosis protein.

individual variations. Pathway A outlines how HIF-1 relates to other markers identified though these studies in the presence of hypoxia. The pathways directed at mounting a local response to ionizing radiation, repairing DNA double-strand breaks and stabilizing protein structures, or activating apoptosis are summarized in pathways B and C.

The primary aim of RT is to cause lethal double-stranded DNA (DS-DNA) breaks resulting in cell death. A number of genes involved in base-excision repair pathways have already been well studied. *NEIL2* belongs to a family of DNA glycosylases that initiates the first step in base exicision repair. *NEIL2* has been shown to be up-regulated in radioresistant RC cell lines as well as in pretreatment clinical biopsy samples of patients whose disease had a poor response to RT.[11] Equally implicated in the base excision repair pathway, polymorphisms within the *hOGG* gene—specifically the *hOGG1 1245C > G* polymorphism—have been shown to be associated with inferior TRG when extracted from peripheral blood and RC tissue in 238 RC patients.[48] It is thought that the 1245C > G polymorphism codes for a low-functioning isoform of the protein, resulting in less DS breaks as part of excision repair, leading to a smaller proportion of cells undergoing cell death as part of lethal DS-DNA breaks.

The X-ray repair cross-complementing proteins 2 and 3 (XRCC2 and XRCC3) are involved in a separate DNA repair pathway

(homologous recombinant repair) and have been separately evaluated as markers of radiosensitivity. Knockdown of *XRCC2* in vitro results in impaired repair of DS-DNA breaks, and Qin et al[52] concluded that intact XRCC2 proteins resulted in radioresistance by facilitating DNA repair after RT. In addition to nuclear repair mechanisms, DNA damage signals the release of cytochrome *c* and activated Smac proteins from the mitochondria, with subsequent activation of the apoptosome, as previously described. As well as preventing the inhibition of apoptotic promoters, it is postulated that Smac could also play a role in both activation and enhancement of caspase-3. Correspondingly, increased expression of Smac has been found to correlate with improved TRG.[24,38] Within the cytosol, a number of small stabilizing proteins (heat-shock proteins, Hsps) have been found to to have roles in stabilizing protein structures and maintaining cellular homeostasis. The gene *DNAJC12* codes for a 40 kDa Hsp, increased expression of which was shown to be significantly associated with inferior TRG as well as with increased vascular and perineural invasion and posttreatment nodal involvement,[47] with selected Hsps being correlated with radioresistance. Increased Hsp42 expression, verified by protein separation with 2-D gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, was associated with radioresistance in 17 clinical specimens sampled before RT.[28] Similarly, increased expression of Hsp90 in pretreatment biopsy samples has been shown to correlate with a poor response to RT.[21]

Cell Signaling

Many of the factors already discussed play a role in both cell−cell signaling and intracellular signal cascade mechanisms. Cell-surface signaling molecules have long been established as molecular fingerprints for cancer immunotyping, such as the family of clusters of differentiation (CD). This large class of molecules act as receptors or ligands, which either initiate a signaling cascade once activated or play a role in processes such as cell−cell adhesion,[10,14,37,40,56,57] although the evidence for these correlating with radiosensitivity has been inconclusive (Table 2). CXCL10 (CXC motif chemokine 10) is the ligand for the CXCR3 receptor, although this is found predominantly in T cells.[85] Presence of increased expression of CXCL10 mRNA in pretreatment RC biopsy samples was shown to be predictive of sensitivity to RT, although IHC did not corroborate this.[41] CXCL10 has been shown to act as an angiostatic, inhibiting VEGF,[41] but the correlation between mRNA expression and protein expression in this case, and hence the implication for CXCL10 to act as a surrogate biomarker is not clear.

Although numerous cell-surface adhesion molecules play a major role in the interaction between tumor and neighboring microenvironment, only the glycoprotein thrombospondin 2 (THBS2) has been shown to be significantly associated with response to RT.[14] THBS2 demonstrates antiangiogenic effects upon interaction with stromal endothelial cells, and low or absent levels of THBS2 expression in CRC biopsy samples has been shown to be linked to inferior TRG. In this study, however, 118 of the 172 specimens examined fell into an intermediate TRG (Dworak TRG 2-3), with a near 50-50 split between high and low expression of THBS2 on IHC.[61]

As with the cellular response mechanisms to stress, expression levels of a number of receptor tyrosine kinases (RTKs) have been shown to increase in RC and may be implicated in radioresistance. The downstream effects of activation of RTKs and the mitogen-activated protein kinase (MAPK) pathway on gene regulation, cellular proliferation and apoptosis as described, have highlighted these pathways as potential players in radioresistance. IQGAP1 (Ras GTPase-activating-like protein) has been evaluated as a potential biomarker on the basis of its being an essential scaffold protein in extracellular signal-regulated kinase signaling, which is a key step in the MAPK cascade.[86] It is known that MAPKs are activated—and MAPK signaling up-regulated—in response to a variety of stimuli.[87] Although this study did not evaluate levels of IQGAP1 in pretreatment biopsy samples as a stand-alone predictor of response to RT, the authors found that apical cell IQGAP1 expression increased most dramatically before and after treatment in those patients with a poor TRG (< 50% regression).[58]

Components of other signaling pathways activated by membrane receptor binding (notably the Wnt and transforming growth factor [TGF]-β signaling cascades) have failed to demonstrate conclusive results. SMAD3 transcriptionally regulates TGF-β target genes upon accumulation in the nucleus, and SMAD3 mutations in murine models have been linked to the development of CRC.[88] The phosphorylated isoform of SMAD3 is considered as a hallmark for SMAD3 activation, increased expression of which has been shown to be associated with a poor TRG.[60] β-Catenin can be detected at the membranous, cytoplasmic, and nuclear levels, representing its function within the Wnt cascade, and counts activation of genes linked to the *APC* gene among its downstream effects. A shift in treatment response has been demonstrated that is dependent on nuclear versus cytoplasmic expression of β-catenin, with increased nuclear expression being association with radioresistance.[55] However, conflicting evidence was provided in 2 other studies; no significant association was found between membranous β-catenin expression and Dworak TRG, but when correlated with the Cologne grading system, statistical significance was reached on univariate analysis.[54] A further multivariate analysis of 130 samples also demonstrated no significant correlation of β-catenin with Mandard TRG.[45] By contrast, in a single study, expression of T-cell factor 4 (TCF4)—another downstream mediator of the Wnt cascade and implicated in the adenoma−adenocarcinoma sequence—demonstrated inverse correlation with TRG.[68]

EGFR is one of a group of the RTKs that are activated and up-regulated by the stabilization of HIF-1. As with a number of other studies, conflicting results abound, with only one IHC study out of 3 identified demonstrating a link between low EGFR expression and complete pathologic response.[14,15,22] Evaluation of gene expression levels with quantitative PCR corroborated this finding, demonstrating a significant association between low expression levels of the *EGFR* gene and increased tumor regression.[39]

Growth-hormone-releasing hormone (GHRH), when bound to its receptor, stimulates local tissue proliferation via release of insulin-like growth factor 1 (IGF-1) and has been demonstrated as a feature of tumor aggressiveness, with effects on both malignant transformation and metastasis.[89] Work on RC cell lines has demonstrated that when administered in combination with a cytotoxic agent, GHRH antagonists can induce S-phase arrest and thus apoptosis in part by inhibition of these mechanisms.[90] Correspondingly, increased expression of the GHRH receptor in pretreatment RC biopsy samples has been found to correlate with radioresistance.[21]

Table 2 Association of Cell-Surface Clusters of Differentiation Molecules With Response to NCRT

Biomarker	Function	Cell Type	Study	Association With NCRT Response
CD34	Cell-surface glycoprotein, cell—cell adhesion	Hematopoietic stem cells	Min et al 2008; IHC of pretreatment biopsy samples[37]	No significant correlation
CD44	Cell-surface glycoprotein	Some cancer stem cells	Huh et al 2014; RT-PCR and Western blot analysis for 13 markers including those for angiogenesis, apoptosis, proliferation, cell adhesion, and collagenases[10]	Low CD44 correlates with improved TRG (OR 4.694, $P = .027$)
	Cell—cell adhesion and migration	Most mammalian cell lines	Sim et al 2014; IHC for markers of cell cycle, proliferative index, apoptosis, cell adhesion and response to hypoxia[14]	No significant correlation
CD133	Cell-surface glycoprotein	Hematopoietic stem cells	Shinto et al 2011; IHC for CD133, COX-2, p53, p27, p21, EGFR in pretreatment biopsy samples of patients undergoing short-course NCRT (20 Gy/5 fractions)[56]	CD133 expression correlates with RR (OR 3.32, $P = .03$)
	Uncertain function	Cancer stem cells (brain, breast, liver, medulloblastoma, melanoma); glandular tissue cells (including GI tract)	Sim et al 2014; IHC for markers of cell cycle, proliferative index, apoptosis, cell adhesion and response to hypoxia[14]	No significant correlation with pCR
CD166	Cell-surface glycoprotein; putative roles in cell—cell adhesion and activation of matrix metalloproteinase cascades	Neurons, fibroblasts, endothelial cells, keratinocytes	Sim et al 2014; IHC for markers of cell cycle, proliferative index, apoptosis, cell adhesion and response to hypoxia[14]	High CD166 expression associated with poor prognosis (DFS) but not specifically with TRG
CD184 (CXCR4)	Chemokine receptor; lymphocyte chemotaxis	Hematopoietic stem cells; over 20 cancer subtypes (including colorectal)	Guedj at al 2011; IHC for panel of biomarkers by function; CXCR4 believed to have role in tumor cell migration and metastatic potential[40]	No significant correlation with TRG
CD340 (HER-2, ERBB2)	EGFR family cell-surface protein	Important biomarker for breast cancer	Drebber et al 2011; IHC for both HER-2/neu and β-catenin and correlated with TRG[54]	No association of HER-2 expression with TRG, although HER-2 positivity associated with survival benefit
	Activation of signal transduction pathways, including MAPK and PI3K/Akt	Up-regulation of ERBB2 gene associated with certain breast, lung, gastric, uterine and ovarian cancer cells	Meng et al 2014; IHC and FISH for HER-2 in pretreatment biopsy samples[57]	No association between HER-2 expression in tumors and TRG; positive HER-2 status associated with poorer 5-year survival

In study by Guedj et al, CD184 labeled as CXCR4 (C-X-C chemokine receptor 4). CD340 is more commonly referred to as HER-2.
Abbreviations: DFS = disease-free survival; EGFR = epidermal growth factor receptor; FISH = fluorescence in-situ hybridization; GI = gastrointestinal; HER-2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; NCRT = neoadjuvant chemotherapy; OR = odds ratio; pCR = pathologic complete response; RT-PCR = reverse transcriptase PCR; TRG = tumor regression grade.

Cellular Metabolism

The cytotoxic backbone of the majority of chemo-radiotherapeutic regimens is 5-fluorouracil (5-FU), which functions as an antimetabolite and pyrimidine analog, irreversibly inhibiting the action of thymidylate synthase. Thymidylate synthase in turn plays a key role in nucleic acid metabolism, catalyzing the conversion of deoxyuridine monophosphate (dUMP) to deoxyuridine TMP, a key nucleoside required in DNA replication. Most groups have found a correlation between low-level thymidylate synthase expression and enhanced TRG,[10,23,69,70,72] although this has not been universally reported.[14,15,71] Microarray analysis of published RC transcriptomic data sets has identified candidate genes involved in both nucleotide and amino acid metabolism that may be implicated in radioresistance, including CPS1 (carbonyl phosphate synthetase 1, an enzyme involved in glutamine metabolism and in catalyzing the initial steps in ammonia detoxification). CPS1 was identified as the most significantly up-regulated gene in the glutamine pathway in radioresistant RC; additionally, IHC staining of clinical specimens demonstrated significant correlation between CPS1 expression and radioresistance.[66]

Another enzymatic marker being explored is asparagine synthetase (ASNS), which has recently been found to serve functionality beyond amino acid synthesis; accumulation of both aspartate and glutamine in ASNS-deficient tissues leads to an increase in nucleotide synthesis and thus cellular proliferation.[91] Consequently, a significant correlation between low ASNS expression and radioresistance was observed in RC specimens, after identification of both the *ASNS* and *PAH* genes as being differentially expressed between response and nonresponse in a transcriptomic data set.[67]

As described, the distinguishing features of invasive cancer involve a multitude of genetic and proteomic anomalies, which in turn have profound downstream effects on normal cellular metabolism and bioenergetics. Metabolic phenotyping approaches (metabonomics/metabolomics) are showing increasing promise in

elucidating the metabolic factors that govern radiosensitivity. Modifications to lipid biosynthetic pathways and lipid metabolism are now established as hallmark features of cancer,[92] and intracellular lipid mediators have now been implicated in mechanisms of response to ionizing radiation and cytotoxicity. Through analysis of the gene expression database of RC tissue, *HSD17B2* and *HMGCS2* were identified as key lipid biosynthesis-associated genes with a predictive capacity for response to RT.[65] The enzyme product HSD17B2 (hydroxysteroid 17-beta dehydrogenase 2) is known to catalyze the reduction of E_2 estrogens, testosterone to androstenedione, and 5α-androstenediol to dehydroepiandrosterone.[93] HMGCS2 (3-hydroxy-3-methylglutaryl-CoA synthase 2) catalyses the formation of HMG-CoA from both acetyl-CoA and aceto-acetyl coenzyme A (CoA), a key early step in the malonate and ketogenesis pathways. This is in keeping with the Warburg phenomenon consistently displayed by solid cancers, where a propensity for anaerobic fermentation of ketone bodies is displayed, even in the presence of abundant oxygen.[94] Overexpression of both genes and their products was found to be associated with poor response to RT.

Cyclooxygenase-2 (COX-2; prostaglandin endoperoxidase synthase) converts arachidonic acid to prostaglandin H_2, which is the precursor to all other prostanoids that act principally as inflammatory mediators.[92,95] For this reason, and because arachidonic acid is released from the cell membrane after direct damage by ionizing radiation, a number of groups have evaluated the role of COX-2 in radiosensitivity.[16,17,37,45,56,62,96] In addition to the functional effects of these bioactive lipids (angiogenesis, increased vascular permeability, and chemotaxis; Figure 2), COX-2 has also been shown to directly stimulate VEGF synthesis.[37] This supports a theory of COX-2 playing an important role in the response to the cellular injury sustained after RT, although whether this is purely due to an increased amount of substrate (ie, arachidonic acid) as a result of cell membrane damage or an additional up-regulated pathway remains to be fully elucidated. Moreover, a cell-wide inflammatory response mechanism, portended by up-regulation of nuclear factor kappa B (NF-κB) in the nucleus, which in turn acts as a transcription factor for (among others) COX-2, TGF- β, VEGF, and EGFR, has been proposed.[97] However, evaluation of NF-κB as a predictive biomarker reveals conflicting results. Nuclear IHC staining for NF-κB in 74 pretreatment samples demonstrated up-regulation of NF-κB correlated with poorer overall survival, but not with TRG.[51] This may have been partly due to a type I error, however, as a subsequent larger study found that lower NF-κB expression correlated with improved TRG.[10] Furthermore, despite previous evidence that annexin 1 (ANXA1) is an endogenous inhibitor of NFκB, Sheu et al[46] found that high levels of expression of ANXA1 correlated with a poor TRG. ANXA1 exerts its effects as both a potent anti-inflammatory mediator and a calcium ion/phospholipid binder that has effects on tumourigenesis and progression.

Conclusion

Locally advanced RC represents an excellent case model for personalized multimodality therapy, although to date molecular phenotyping has had little direct impact on the RC treatment algorithm. Currently an estimated 15% to 20% of patients with locally advanced RC experience complete tumor regression after up-front RT. The value of radical surgery in these patients is increasingly challenged, and selected patients in this group could instead be offered organ-preserving treatment, such as local excision or a watch-and-wait approach. A model predictive of the RT response, applied at the pretreatment stage, will be critical to personalizing RC treatment and would facilitate organ preservation, perhaps even in patients for whom up-front RT would not otherwise be routinely considered. Molecular biomarker discovery offers the strongest opportunity for development of a radiation response predictive model. However, the inherent complexities of radiation oncobiology, coupled with the vast heterogeneity of existing works in the literature, have made it difficult to precisely define the molecular drivers of radiosensitivity. In the present study, we sought to present the RC radiation response molecular landscape as holistically as possible; we used a GO approach to group biomarkers together according to biological commonality for ease of interpretation. A more targeted search for relevant biomarkers in this context is essential, and as this work appears to demonstrate, there are several key areas that appear to be showing genuine promise and others—perhaps surprisingly—that do not.

Insofar as analyzing a heterogenous landscape of biomarkers, the generation of a knowledge-based GO network is a concise way of displaying data acquired from a comprehensive systematic review while providing both an appreciation of the statistical analyses of the original studies and insight into potential overall biological impact. We believe the work presented here is the first such analysis of its type and offers an intuitive method of visually appraising statistical data gathered from published experimental work by mapping identified biomarkers using GO terms. One benefit of such analysis is that it can take into account all published data, including nonsignificant findings, and can aid in identifying gaps in knowledge and potential targets for future work. We have shown that by clustering biomarkers according to GO terms and integrating them into a network analysis based on biological function, the ontologies under "stress response" and "cellular metabolism" appear to be of greatest significance. Conversely, biomarkers of cell-cycle regulation and progression have been found to be of low relative significance despite large numbers of studies. The work presented here should allow for more targeted direction of enquiry for future RT biomarker discovery studies, and the biostatistical methodology we used will undoubtedly be applicable to other clinical questions in which a biomarker-driven end point is the ultimate aim.

The data presented herein are subject to a number of inherent limitations. Although the methodology used has sought to mitigate the impact of these, it is important to highlight them. First, it is possible that subtle differences exist in terms of the molecular mechanisms that govern response to SCRT (25 Gy, 5 Gy in 5 fractions) and LCCR (50.4 Gy, 1.8 Gy in 28 fractions), and studies evaluating both approaches have been included in the present review. Unfortunately, the current literature on biomarkers in RC radiation response does not permit these distinctions to be defined. However, despite differences in timing and fractionation, the recent Stockholm III RCT demonstrated equivalent oncologic outcomes after SCRT and immediate surgery, SCRT and delayed surgery, and LCCR.[4] This suggests that in spite of methodologic variability, different approaches lead to relatively homogeneous oncologic end points, which most likely is because the central constituent of each

Table 3 Definitions of Tumor Regression Grading Systems as Used by Studies in This Review

Dworak et al[98]	Mandard et.al[99]	Rödel et al[100]	Ryan et al[101]	Wheeler et al[102]
0. No regression.	1. Complete regression (fibrosis without detectable tissue of tumor—pCR).	0. No regression.	1. No viable cancer cells.	1. Sterilisation or only microscopic foci of adenocarcinoma remaining, with marked fibrosis.
1. Predominantly tumor with significant fibrosis and/or vasculopathy.	2. Fibrosis with scattered tumor cells.	1. Regression of <25% of tumor mass.	2. Residual cancer outgrown by fibrosis.	2. Marked fibrosis but macroscopic disease present.
2. Predominantly fibrosis with scattered tumor cells (slightly recognizable histologically).	3. Fibrosis and tumor cells with preponderance of fibrosis.	2. Regression of 25-50% of tumor mass.	3. Significant fibrosis outgrown by cancer.	3. Little or no fibrosis, with abundant macroscopic disease.
3. Only scattered tumor cells in the space of fibrosis with/ without acellular mucin.	4. Fibrosis and tumor cells with preponderance of tumor cells.	3. Regression of >50% of tumor mass.		
4. No vital tumor cells detectable.	5. Tissue of tumor without changes of regression.	4. Complete regression (pCR).		

Many of these were not originally determined for rectal cancer but have been validated for such use.
Abbreviation: pCR = pathologic complete response.

approach is pelvic RT. We suggest that this fundamental radiobiological commonality will compensate for differences in approach, and therefore all eligible studies evaluating LCCR (n = 67), SCRT (n = 5), and a combination of these (n = 2) were included in the present study. Finally, multiple tumour regression grading systems were used across studies, and this lack of congruence may lead to difficulties in interpreting data (Table 3)

In the context of LCCR, 5-FU–based chemotherapy was almost universally used for radiosensitization, combined with fractionated long-course RT. However, we acknowledge that a minority of included studies also included use of oxaliplatin and/or leucovorin as induction agents.[10,22,24,30,35,37,57,64] Although we appreciate that this represents a further source of data heterogeneity, it is interesting to note that a number of the biomarkers assessed in these papers (eg, EGFR, Smac, and Ki-67) demonstrated congruent results with other studies where 5-FU alone was used as the radiation sensitizer.[22,24,35,57] In this context, we believe that the data were sufficiently comparable for collective inclusion in the present review.

Although previous studies have suggested that radiation response per se is not a reliable surrogate end point in recent clinical trials, we would argue that the development of reliable methods with which to predict radiation response will allow more precise treatment planning in the future for locally advanced RC.[103,104] It is feasible to surmise that were a favorable phenotype to be revealed through biomolecular profiling, an individual would be offered pelvic RT followed by radical surgery (conventional practice) or organ-preserving treatment (in the case of a complete response). In the patient in whom an unfavorable phenotype (ie, disease likely to be poorly responsive or nonresponsive to RT) is found, a different treatment approach would be required, potentially involving either beyond total mesorectal excision surgery (without preoperative therapy) or neoadjuvant chemotherapy, which is the subject of the ongoing Prospect multicenter RCT (ClinicalTrials.gov NCT01515787). Furthermore, it is important to acknowledge that the clinical landscape is evolving in RC; the introduction of watch-and-wait strategies, given our currently limited understanding of the biology underpinning radiation response, is somewhat controversial. The ability to robustly predict response to RT based on tumor biology will be an essential stratifier to guide patient selection for organ preserving treatment. The final point to consider in terms of the value of radiation response characterization is the potential to modulate radiation response once the key molecular drivers are defined.

In conclusion, what has been demonstrated by this work is that the goal of a single biomarker remains both out of reach and unrealistic based on current evidence. However, as for the implications of radiation response biomarkers going forward, one can posit a number of clinical end points. Foremost, as debates on watch and wait and selection of optimal up-front therapy continues (chemoradiation vs. chemotherapy), the ability to accurately predict radiation response (and more importantly to be able to adequately survey the patient in a longitudinal fashion) would solidify the oncobiological credibility of these approaches. For practical purposes, this would obviously require a clinically translatable, bedside, or office-based test (rather than tissue biopsy), and work would need to be guided to establish how tissue-based biomarkers may be reflected in a more readily accessible liquid biopsy source, be that from stool, blood, or urine.

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Supplemental Data

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References

1. Heald RJ, Husband EM, Ryall RD. The mesorectum in rectal cancer surgery—the clue to pelvic recurrence? *Br J Surg* 1982; 69:613-6.
2. Kreis ME, Ruppert R, Ptok H, et al. Use of preoperative magnetic resonance imaging to select patients with rectal cancer for neoadjuvant chemoradiation—interim analysis of the German OCUM trial (NCT01325649). *J Gastrointest Surg* 2016; 20:25-32.
3. Sebag-Montefiore D, Stephens RJ, Steele R, et al. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. *Lancet* 2009; 373:811-20.
4. Erlandsson J, Holm T, Pettersson D, et al. Optimal fractionation of preoperative radiotherapy and timing to surgery for rectal cancer (Stockholm III): a multicentre, randomised, non-blinded, phase 3, non-inferiority trial. *Lancet Oncol* 2017; 18:336-46.
5. van Gijn W, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. *Lancet Oncol* 2011; 12:575-82.
6. Creavin B, Ryan E, Martin ST, et al. Organ preservation with local excision or active surveillance following chemoradiotherapy for rectal cancer. *Br J Cancer* 2016; 20:417.
7. 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; 56:1109-17.
8. AmiGO 2. The Gene Ontology database, Available at: http://amigo.geneontology.org/amigo. Accessed April 10, 2016.
9. Hu P, Bader G, Wigle DA, et al. Computational prediction of cancer-gene function. *Nat Rev Cancer* 2007; 7:23-34.
10. 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; 259:508-15.
11. Kobunai T, Watanabe T, Fukusato T. *REG4, NEIL2*, and *BIRC5* gene expression correlates with gamma-radiation sensitivity in patients with rectal cancer receiving radiotherapy. *Anticancer Res* 2011; 31:4147-53.
12. McDowell DT, Smith FM, Reynolds JV, et al. Increased spontaneous apoptosis, but not survivin expression, is associated with histomorphologic response to neoadjuvant chemoradiation in rectal cancer. *Int J Colorectal Dis* 2009; 24:1261-9.
13. Rodel F, Hoffmann J, Distel L, et al. Survivin as a radioresistance factor, and prognostic and therapeutic target for radiotherapy in rectal cancer. *Cancer Res* 2005; 65:4881-7.
14. Sim SH, Kang MH, Kim YJ, et al. P21 and CD166 as predictive markers of poor response and outcome after fluorouracil-based chemoradiotherapy for the patients with rectal cancer. *BMC Cancer* 2014; 14:241.
15. 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; 68:1455-61.
16. Edden Y, Wexner SD, Berho M. The use of molecular markers as a method to predict the response to neoadjuvant therapy for advanced stage rectal adenocarcinoma. *Colorectal Dis* 2012; 14:555-61.
17. Kobayashi H, Hashiguchi Y, Ueno H, et al. Absence of cyclooxygenase-2 protein expression is a predictor of tumor regression in rectal cancer treated with preoperative short-term chemoradiotherapy. *Dis Colon Rectum* 2007; 50:1354-62.
18. Brophy S, Sheehan KM, McNamara DA, et al. GLUT-1 expression and response to chemoradiotherapy in rectal cancer. *Int J Cancer* 2009; 125:2778-82.
19. Lopez-Crapez E, Bibeau F, Thezenas S, et al. p53 status and response to radiotherapy in rectal cancer: a prospective multilevel analysis. *Br J Cancer* 2005; 92:2114-21.
20. Gunther K, Dimmler A, Rodel F, et al. P27 does not predict histopathological response to radiochemotherapy in rectal cancer. *J Surg Res* 2003; 113:179-88.
21. Farkas R, Pozsgai E, Schally AV, et al. Possible predictors of histopathological response to neoadjuvant chemoradiotherapy for rectal cancer. *J Cancer Res Clin Oncol* 2012; 138:387-95.
22. Carlomagno C, Pepe S, D'Armiento FP, et al. Predictive factors of complete response to neoadjuvant chemoradiotherapy in patients with rectal cancer. *Oncology* 2010; 78:369-75.
23. Jakob C, Liersch T, Meyer W, et al. 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; 14:1060-6.
24. Yan H, Wang R, Yu J, et al. Predictive value of Smac, VEGF and Ki-67 in rectal cancer treated with neoadjuvant therapy. *Oncol Lett* 2010; 1:641-7.
25. He HL, Lee YE, Shiue YL, et al. Overexpression of REG4 confers an independent negative prognosticator in rectal cancers receiving concurrent chemoradiotherapy. *J Surg Oncol* 2014; 110:1002-10.
26. Avoranta ST, Korkeila EA, Minn HRI, et al. Securin identifies a subgroup of patients with poor outcome in rectal cancer treated with long-course (chemo) radiotherapy. *Acta Oncol* 2011; 50:1158-66.
27. Senetta R, Duregon E, Sonetto C, et al. YKL-40/c-met expression in rectal cancer biopsies predicts tumor regression following neoadjuvant chemoradiotherapy: a multi-institutional study. *PLoS One* 2015; 10:e0123759.
28. Allal AS, Kahne T, Reverdin AK, et al. Radioresistance-related proteins in rectal cancer. *Proteomics* 2004; 4:2261-9.
29. Rodel C, Grabenbauer GG, Papadopoulos T, et al. Apoptosis as a cellular predictor for histopathologic response to neoadjuvant radiochemotherapy in patients with rectal cancer. *Int J Radiat Oncol Biol Phys* 2002; 52:294-303.
30. Chang HJ, Jung KH, Kim DY, et al. Bax, a predictive marker for therapeutic response to preoperative chemoradiotherapy in patients with rectal carcinoma. *Hum Pathol* 2005; 36:364-71.
31. Kelley ST, Coppola D, Yeatman T, et al. Tumor response to neoadjuvant chemoradiation therapy for rectal adenocarcinoma is mediated by p53-dependent and caspase 8-dependent apoptotic pathways. *Clin Colorectal Cancer* 2005; 5:114-8.
32. Qiu J, Yang G, Lin A, et al. Human phosphatidylethanolamine-binding protein 4 promoted the radioresistance of human rectal cancer by activating Akt in an ROS-dependent way. *PLoS One* 2014; 9:e90062.
33. Cao WT, Zhou ZY, Deng YH, et al. Clinical value of MR diffusion weighted imaging in prediction of pathological complete response of rectal cancer after neoadjuvant therapy. *Zhonghua Wei Chang Wai Ke Za Zhi* 2013; 16:1164-8.
34. Saigusa S, Inoue Y, Tanaka K, et al. Lack of M30 expression correlates with factors reflecting tumor progression in rectal cancer with preoperative chemoradiotherapy. *Mol Clin Oncol* 2014; 2:99-104.
35. Dou X, Wang RB, Meng XJ, et al. PDCD4 as a predictor of sensitivity to neoadjuvant chemoradiotherapy in locally advanced rectal cancer patients. *Asian Pac J Cancer Prev* 2014; 15:825-30.
36. Moussata D, Amara S, Siddeek B, et al. XIAP as a radioresistance factor and prognostic marker for radiotherapy in human rectal adenocarcinoma. *Am J Pathol* 2012; 181:1271-8.
37. Min BS, Choi YJ, Pyo HR, et al. Cyclooxygenase-2 expression in pretreatment biopsy as a predictor of tumor responses after preoperative chemoradiation in rectal cancer. *Arch Surg* 2008; 143:1091-7.
38. Yan H, Yu J, Wang R, et al. Prognostic value of Smac expression in rectal cancer patients treated with neoadjuvant therapy. *Med Oncol* 2012; 29:168-73.
39. Toiyama Y, Inoue Y, Saigusa S, 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* 2010; 22:272-80.
40. Guedj N, Bretagnol F, Rautou PE, et al. Predictors of tumor response after preoperative chemoradiotherapy for rectal adenocarcinomas. *Hum Pathol* 2011; 42:1702-9.
41. Li C, Wang Z, Liu F, et al. CXCL10 mRNA expression predicts response to neoadjuvant chemoradiotherapy in rectal cancer patients. *Tumour Biol* 2014; 35:9683-91.
42. Hur H, Kim NK, Min BS, et al. Can a biomarker-based scoring system predict pathologic complete response after preoperative chemoradiotherapy for rectal cancer? *Dis Colon Rectum* 2014; 57:592-601.
43. Kurt A, Yanar F, Asoglu O, et al. Low Mmp 9 and VEGF levels predict good oncologic outcome in mid and low rectal cancer patients with neoadjuvant chemoradiation. *BMC Clin Pathol* 2012; 12:27.
44. Zlobec I, Steele R, Compton CC. VEGF as a predictive marker of rectal tumor response to preoperative radiotherapy. *Cancer* 2005; 104:2517-21.
45. Garcia-Florez LJ, Gomez-Alvarez G, Frunza AM, et al. Predictive markers of response to neoadjuvant therapy in rectal cancer. *J Surg Res* 2015; 194:120-6.
46. Sheu MJ, Li CF, Lin CY, et al. Overexpression of ANXA1 confers independent negative prognostic impact in rectal cancers receiving concurrent chemoradiotherapy. *Tumour Biol* 2014; 35:7755-63.
47. He HL, Lee YE, Chen HP, et al. Overexpression of DNAJC12 predicts poor response to neoadjuvant concurrent chemoradiotherapy in patients with rectal cancer. *Exp Mol Pathol* 2015; 98:338-45.
48. Cecchin E, Agostini M, Pucciarelli S, et al. Tumor response is predicted by patient genetic profile in rectal cancer patients treated with neo-adjuvant chemoradiotherapy. *Pharmacogenomics J* 2011; 11:214-26.
49. Terrazzino S, Agostini M, Pucciarelli S, et al. A haplotype of the methylenetetrahydrofolate reductase gene predicts poor tumor response in rectal cancer patients receiving preoperative chemoradiation. *Pharmacogenet Genomics* 2006; 16:817-24.
50. Molinari C, Casadio V, Foca F, et al. Gene methylation in rectal cancer: predictive marker of response to chemoradiotherapy? *J Cell Physiol* 2013; 228:2343-9.
51. Berardi R, Maccaroni E, Mandolesi A, et al. Nuclear factor-kappaB predicts outcome in locally advanced rectal cancer patients receiving neoadjuvant radiochemotherapy. *Dig Liver Dis* 2012; 44:617-22.
52. Qin CJ, Song XM, Chen ZH, et al. XRCC2 as a predictive biomarker for radioresistance in locally advanced rectal cancer patients undergoing preoperative radiotherapy. *Oncotarget* 2015; 6:32193-204.
53. Yu Z, Zhang C, Chai R, et al. Prognostic significance and molecular mechanism of ATP-binding cassette subfamily C member 4 in resistance to neoadjuvant radiotherapy of locally advanced rectal carcinoma. *PLoS One* 2014; 9:e85446.
54. Drebber U, Madeja M, Odenthal M, et al. beta-Catenin and Her2/neu expression in rectal cancer: association with histomorphological response to neoadjuvant therapy and prognosis. *Int J Colorectal Dis* 2011; 26:1127-34.
55. Wang L, Zhang XM, Li Z, et al. Overexpression of nuclear beta-catenin in rectal adenocarcinoma is associated with radioresistance. *World J Gastroenterol* 2013; 19:6876-82.
56. Shinto E, Hashiguchi Y, Ueno H, et al. Pretreatment CD133 and cyclooxygenase-2 expression as the predictive markers of the pathological effect of

chemoradiotherapy in rectal cancer patients. *Dis Colon Rectum* 2011; 54:1098-106.
57. Meng X, Wang R, Huang Z, et al. Human epidermal growth factor receptor-2 expression in locally advanced rectal cancer: association with response to neoadjuvant therapy and prognosis. *Cancer Sci* 2014; 105:818-24.
58. Holck S, Nielsen HJ, Hammer E, et al. IQGAP1 in rectal adenocarcinomas: localization and protein expression before and after radiochemotherapy. *Cancer Lett* 2015; 356(2 pt B):556-60.
59. Yu Z, Zhang C, Wang H, et al. Multidrug resistance-associated protein 3 confers resistance to chemoradiotherapy for rectal cancer by regulating reactive oxygen species and caspase-3-dependent apoptotic pathway. *Cancer Lett* 2014; 353:182-93.
60. Huang MY, Lin CH, Huang CM, et al. Relationships between SMAD3 expression and preoperative fluoropyrimidine-based chemoradiotherapy response in locally advanced rectal cancer patients. *World J Surg* 2015; 39:1257-67.
61. Lin CY, Chang IW, Sheu MJ, et al. Low thrombospondin 2 expression is predictive of low tumor regression after neoadjuvant chemoradiotherapy in rectal cancer. *Am J Transl Res* 2015; 7:2423-32.
62. Smith FM, Reynolds JV, Kay EW, et al. COX-2 overexpression in pretreatment biopsies predicts response of rectal cancers to neoadjuvant radiochemotherapy. *Int J Radiat Oncol Biol Phys* 2006; 64:466-72.
63. Avoranta ST, Korkeila EA, Ristamaki RH, et al. ALDH1 expression indicates chemotherapy resistance and poor outcome in node-negative rectal cancer. *Hum Pathol* 2013; 44:966-74.
64. Yeo SG, Kim DY, Kim KH, et al. Hydroxymethylglutaryl-coenzyme a synthase 2 expression is associated with chemoradiotherapy responses in colorectal cancer. *Dis Colon Rectum* 2012; 55:686-94.
65. Lee YE, He HL, Shiue YL, et al. The prognostic impact of lipid biosynthesis-associated markers, HSD17B2 and HMGCS2, in rectal cancer treated with neoadjuvant concurrent chemoradiotherapy. *Tumour Biol* 2015; 36:7675-83.
66. Lee YY, Li CF, Lin CY, et al. Overexpression of CPS1 is an independent negative prognosticator in rectal cancers receiving concurrent chemoradiotherapy. *Tumour Biol* 2014; 35:11097-105.
67. Lin CY, Sheu MJ, Li CF, et al. Deficiency in asparagine synthetase expression in rectal cancers receiving concurrent chemoradiotherapy: negative prognostic impact and therapeutic relevance. *Tumour Biol* 2014; 35:6823-30.
68. Dou X, Wang R, Meng X, et al. The prognostic role of TCF4 expression in locally advanced rectal cancer patients treated with neoadjuvant chemoradiotherapy. *Cancer Biomark* 2015; 15:187-94.
69. Jakob C, Liersch T, Meyer W, et al. Immunohistochemical analysis of thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase in rectal cancer (cUICC II/III): correlation with histopathologic tumor regression after 5-fluorouracil–based long-term neoadjuvant chemorodiotheropy. *Am J Surg Pathol* 2005; 29:1304-9.
70. Saw RPM, 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; 46:192-202.
71. 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; 18:2442-52.
72. 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; 204:562-8.
73. Perez RE, Shen H, Duan L, et al. Modeling the etiology of p53-mutated cancer cells. *J Biol Chem* 2016; 291:10131-47.
74. Carnero A, Paramio JM. The PTEN/PI3K/AKT pathway in vivo, cancer mouse models. *Front Oncol* 2014; 4:252.
75. Bishnupuri KS, Luo Q, Sainathan SK, et al. Reg IV regulates normal intestinal and colorectal cancer cell susceptibility to radiation-induced apoptosis. *Gastroenterology* 2010; 138:616-26.
76. Matsumoto S, Konishi H, Maeda R, et al. Expression analysis of the regenerating gene (Reg) family members Reg-IIIbeta and Reg-IIIgamma in the mouse during development. *J Comp Neurol* 2012; 520:479-94.
77. Rafa L, Dessein AF, Devisme L, et al. REG4 acts as a mitogenic, motility and pro-invasive factor for colon cancer cells. *Int J Oncol* 2010; 36:689-98.
78. Kim PJ, Plescia J, Clevers H, et al. Survivin and molecular pathogenesis of colorectal cancer. *Lancet* 2003; 362:205-9.
79. Shao R. YKL-40 acts as an angiogenic factor to promote tumor angiogenesis. *Front Physiol* 2013; 4:122.
80. Kawada M, Seno H, Kanda K, et al. Chitinase 3-like 1 promotes macrophage recruitment and angiogenesis in colorectal cancer. *Oncogene* 2012; 31:3111-23.
81. Shibahara K, Asano M, Ishida Y, et al. Isolation of a novel mouse gene *MA-3* that is induced upon programmed cell death. *Gene* 1995; 166:297-301.
82. He H, Liu D, Lin H, et al. Phosphatidylethanolamine binding protein 4 (PEBP4) is a secreted protein and has multiple functions. *Biochim Biophys Acta* 2016; 1863(7 pt A):1682-9.
83. Moeller BJ, Dewhirst MW. HIF-1 and tumour radiosensitivity. *Br J Cancer* 2006; 95:1-5.
84. Moeller BJ, Richardson RA, Dewhirst MW. Hypoxia and radiotherapy: opportunities for improved outcomes in cancer treatment. *Cancer Metastasis Rev* 2007; 26:241-8.
85. Karin N, Wildbaum G, Thelen M. Biased signaling pathways via CXCR3 control the development and function of $CD4^+$ T cell subsets. *J Leukoc Biol* 2016; 99:857-62.
86. Jameson KL, Mazur PK, Zehnder AM, et al. IQGAP1 scaffold-kinase interaction blockade selectively targets RAS-MAP kinase-driven tumors. *Nat Med* 2013; 19:626-30.
87. Munshi A, Ramesh R. Mitogen-activated protein kinases and their role in radiation response. *Genes Cancer* 2013; 4:401-8.
88. Taketo MM, Takaku K. Gastrointestinal tumorigenesis in Smad4 (Dpc4) mutant mice. *Hum Cell* 2000; 13:85-95.
89. Schally AV, Varga JL, Engel JB. Antagonists of growth-hormone-releasing hormone: an emerging new therapy for cancer. *Nat Clin Pract Endocrinol Metab* 2008; 4:33-43.
90. Rick FG, Seitz S, Schally AV, et al. GHRH antagonist when combined with cytotoxic agents induces S-phase arrest and additive growth inhibition of human colon cancer. *Cell Cycle* 2012; 11:4203-10.
91. Balasubramanian MN, Butterworth EA, Kilberg MS. Asparagine synthetase: regulation by cell stress and involvement in tumor biology. *Am J Physiol Endocrinol Metab* 2013; 304:E789-99.
92. Tuncer S, Banerjee S. Eicosanoid pathway in colorectal cancer: recent updates. *World J Gastroenterol* 2015; 21:11748-66.
93. Labrie F, Luu-The V, Lin S, et al. Role of 17beta-hydroxysteroid dehydrogenases in sex steroid formation in peripheral intracrine tissues. *Trends Endocrinol Metab* 2000; 11:421-7.
94. Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci* 2016; 41:211-8.
95. Yarla NS, Bishayee A, Sethi G, et al. Targeting arachidonic acid pathway by natural products for cancer prevention and therapy. *Semin Cancer Biol* 2016; 40-41:48-81.
96. Bouzourene H, Yan P, Sandmeier D, et al. The role of COX-2 in rectal cancer treated with preoperative radiotherapy. *Virchows Arch* 2008; 452:499-505.
97. Di Maggio FM, Minafra L, Forte GI, et al. Portrait of inflammatory response to ionizing radiation treatment. *J Inflamm (Lond)* 2015; 12:14.
98. Dworak O, Keilholz L, Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. *Int J Colorectal Dis* 1997; 12:19-23.
99. Mandard AM, Dalibard F, Mandard JC, et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. *Cancer* 1994; 73:2680-6.
100. Rodel C, Grabenbauer GG, Papadopoulos T, et al. Phase I/II trial of capecitabine, oxaliplatin, and radiation for rectal cancer. *J Clin Oncol* 2003; 21:3098-104.
101. Ryan R, Gibbons D, Hyland JM, et al. Pathological response following long-course neoadjuvant chemoradiotherapy for locally advanced rectal cancer. *Histopathology* 2005; 47:141-6.
102. Wheeler JM, Warren BF, Mortensen NJ, et al. Quantification of histologic regression of rectal cancer after irradiation: a proposal for a modified staging system. *Dis Colon Rectum* 2002; 45:1051-6.
103. Fokas E, Liersch T, Fietkau R, et al. Downstage migration after neoadjuvant chemoradiotherapy for rectal cancer: the reverse of the Will Rogers phenomenon? *Cancer* 2015; 121:1724-7.
104. Appelt AL, Vogelius IR, Ploen J, et al. Long-term results of a randomized trial in locally advanced rectal cancer: no benefit from adding a brachytherapy boost. *Int J Radiat Oncol Biol Phys* 2014; 90:110-8.