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Molecular biomarkers: multiple roles in radiotherapy

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Abstract: Preoperative chemoradiation therapy (CRT) is becoming the standard treatment for patients with locally advanced rectal cancer. However, individual differences in response to treatment range from a complete response to complete resistance. Predicting the tumor response to radiotherapy may improve the efficacy of radiotherapy. This review mainly summarizes recent studies about the molecular biomarkers that can predict the response to radiotherapy in rectal cancer. These studies have indicated that the molecular markers involved in the response to radiotherapy mainly include genes related to radiosensitivity, cancer stem cell-related markers, non-coding RNAs (ncRNAs), single-nucleotide polymorphisms (SNPs) and gene methylation, and other factors including carcinoembryonic antigen (CEA) level, anemia, lymphocytes, and signaling pathways. Many of these identified markers are mainly associated with DNA repair, apoptosis, and cell cycle, but some involve unknown cell mechanisms. We speculate that predictors of radiotherapy response may involve combinations of multiple molecular biomarkers that may be useful for the development of individualized therapy for rectal cancer patients.

Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide. In many developed countries, it is the third most frequently diagnosed cancer after lung and prostate cancer in males and lung and breast cancer in females (Provenzale *et al.*, 2015). In recent years, the incidence of CRC has been increasing in part because of an aging population (Center *et al.*, 2009). Among CRCs, nearly 50% are rectal cancers (Torre *et al.*, 2015). Because chemoradiation therapy (CRT) has been shown to be effective in local control of tumors and anal sphincter preservation (Sauer *et al.*, 2004), preoperative CRT has become popular in rectal patients with cT3-4 N0/+ disease, according to National Comprehensive Cancer Network (NCCN) guidelines (Engstrom *et al.*, 2009). However, a wide range of tumor responses, from pathological complete response (pCR) to disease progression, has been observed in response to CRT. According to previous investigations, 10–30% of patients who received preoperative CRT showed complete remission, and 60% showed a reduction in tumor size and N down-staging (Valentini *et al.*, 2002; Wheeler *et al.*, 2004). Unfortunately, some patients have shown poor response with little or no tumor reduction after preoperative CRT (Eich *et al.*, 2011; Rödel *et al.*, 2005). Therefore, predicting the tumor response to radiation has long been a goal of radiation oncologists and would be a major step toward personalized treatment of CRC.

Many previous studies have demonstrated that the response to CRT is highly individualized (Kuremsky *et al.*, 2009; Subbiah *et al.*, 2017), and this may partly be explained at the molecular level. Many molecular markers of radiosensitivity have been examined, including expression of radiation-associated genes, cancer stem cell-related markers, non-coding RNAs (ncRNAs), single-nucleotide polymorphisms (SNPs) and gene methylation, and other factors. They generally play important roles in regulating apoptosis, DNA repair, the cell cycle, and other important functions. This review will mainly summarize recent studies about the molecular markers of radiosensitivity in rectal cancer.

Summary of Radiosensitivity

As an important method of cancer treatment, radiation therapy can damage tumor cell membranes, plasma proteins, and DNA. As a result, it changes the properties of some biological macromolecules and interferes with the cell membrane and nuclear signals that regulate growth factors,

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signal transduction, the cell cycle, and cell adhesion. Thus, the expression of some genes is either activated, downregulated, or even turned off. As is well-known, the response to radiotherapy varies widely. Sensitivity to radiotherapy depends mainly on hypoxia (Vaupel *et al.*, 1991), anemia (Pasquier and Lartigau, 2003), infection (RICHAUD, 1950), tissue origin, differentiation, and inherent radiosensitivity. However, what is their internal mechanism? It is worth studying.

Molecular Biomarkers of Radiosensitivity

Radiotherapy primarily influences cell cycle arrest, DNA repair, and apoptosis through related genes and thus plays an important role in the treatment of tumors. With the application of preoperative radiotherapy in rectal cancer patients, the number of studies on radiosensitivity has increased. Relevant studies that have identified molecular biomarkers of radiotherapy will be discussed below.

Genes related to radiosensitivity

CRC patients respond differently to radiotherapy, which is reflected in differences in gene expression among these patients. The genes related to radiotherapy can be classified as either radiation-resistant genes (RRGs) or radiation-sensitive genes (RSGs). RRGs are defined as genes that are highly expressed in tumors with poor response to radiotherapy or weakly expressed in tumors with good response to radiotherapy. Conversely, RSGs are defined as genes that are highly expressed in tumors with good response to radiotherapy or weakly expressed in tumors with poor response to radiotherapy. Although a large number of genes are involved in the sensitivity of radiotherapy, most of them are ultimately functioning by influencing DNA repair, apoptosis, and cell cycle arrest, thereby affecting the radiosensitivity of rectal cancer.

RRGs

Many genes are associated with resistance to radiation. More than 40 different genes have been explored in the literature. Some of the more promising genes are discussed below.

Ku70 plays a critical role in DNA repair and cell death induction after damage (Fell and Schild-Poulter, 2015). Pucci *et al.* (2017) found nuclear localization of Ku70/80 in preoperative CRT tumor biopsies of responder patients and observed unconventional overexpression of Ku70 in the cytoplasm of the non-responder population. Komuro *et al.* (2003) performed preoperative irradiation biopsies using immunohistochemistry and found a significant correlation between the Ku70 expression and tumor radioresistance.

Phosphatidylethanolamine binding protein 4 (hPEBP4) is a novel anti-apoptotic gene associated with the resistance of tumors to apoptotic agents (Li *et al.*, 2007). Qiu *et al.* (2013) evaluated the expression of hPEBP4 in pretreatment biopsy specimens and the response of rectal cancer to radiotherapy using immunohistochemistry and tumor regression grading system. Their results indicated that the upregulation of hPEBP4 was a potential mechanism by which rectal cancer cells avoid the destructive effects of radiotherapy and suggested that hPEBP4 had independent predictive value for the response of rectal cancer to radiotherapy. They subsequently demonstrated the radioresistant effect of hPEBP4 *in vivo* and *in vitro* and thus confirmed that hPEBP4 was associated with radioresistance of rectal cancer (Qiu *et al.*, 2014a).

Survivin is expressed in a variety of human tumors and plays an important role in regulating apoptosis, cell division, and adaptation to stress (Dai *et al.*, 2012). Xiaoyuan *et al.* (2010) examined the expression of survivin in CRC specimens and concluded that the downregulation of survivin enhanced the radiosensitivity of CRC cells while elevated expression of survivin was an independent factor for predicting the prognosis of CRC patients. In another study, Rödel *et al.* (2008) inhibited the expression of survivin by the use of antisense oligonucleotides *in vitro* and *in vivo*. They found that inhibition of survivin improved the treatment response to radiotherapy in patients with rectal cancer. Furthermore, Capalbo's research showed that survivin accumulated in the nucleus following irradiation, where it subsequently interacted with members of the DNA double-strand break repair machinery in order to regulate the activity of DNA-dependent protein kinase (Capalbo *et al.*, 2010).

Gamma-H2A histone family, member X (γH2AX) plays an important role in repairing DNA damage after exposure to radiation (Sánchez-Flores *et al.*, 2015). Kao *et al.* (2006) found that radiosensitivity was increased when cells were treated with peptide inhibitors of γH2AX. This indicated that therapies that inhibit H2AX function may interfere with DNA damage repair processes and warrant further investigation as potential radiosensitizing agents. In another study, Kroeber *et al.* (2015) demonstrated that γH2AX assays were able to predict individual radiosensitivity.

Other radiation-resistant genes, based on their proven roles in the literature, which are related to DNA repair, apoptosis, cell cycle, or unknown functions, are listed in Tab. 1.

RSGs

Compared with RRGs, there are few studies on RSGs. Some of the main studies in the literature are listed in Tab. 1 and are summarized as follows.

Shin *et al.* (2013) concluded that rectal cancers with microsatellite instability may exhibit radiation sensitivity because of possible direct roles that mismatch repair proteins play in the DNA damage response to radiation. Sadahiro *et al.* (2017) examined the expression of gamma-glutamyl hydrolase (GGH) using RT-PCR assays in CRC patients. The expression levels of GGH were significant in pathological response patients, and the accuracy rate of the predictive model was 83.3%. Yokoi *et al.* (2017) identified cellular retinol-binding protein (CRBP1) as a radiation-sensitive predictor of rectal cancer by microarray analysis and verified that finding by transfection *in vitro*. Park *et al.* (2014) downregulated phosphoenolpyruvate carboxykinase (PEPCK) in tissues of patients with rectal cancer and noted a poor response to preoperative radiation therapy in these patients. They demonstrated that PEPCK was a useful predictor of the response to CRT in rectal cancer patients. Yang *et al.* (2014) examined the expression of granulocyte colony-stimulating factor receptor (G-CSFR) in biopsy specimens from 126 rectal cancer patients using

TABLE 1

Genes related to radiosensitivity classified by function

	RSG	RRG
DNA repair	PEDF (Yi *et al.*, 2016) Ku80 (Pucci *et al.*, 2017)	Ku70* (Komuro *et al.*, 2003; Pucci *et al.*, 2017) CCR6 (Chang *et al.*, 2018) PrxI (Chen *et al.*, 2010) γH2AX* (Kao *et al.*, 2006; Pouliliou and Koukourakis, 2014) XPO1 (Ferreiro-Neira *et al.*, 2016)
Apoptosis	PSMB8 (Ha *et al.*, 2017)	XIAP (Flanagan *et al.*, 2015) hPEBP4* (Qiu *et al.*, 2016, 2014a, 2013) RBBP6 (Xiao *et al.*, 2018) LIG4 (Song *et al.*, 2016) MRP3 (Yu *et al.*, 2014b) aBCC4 (Yu *et al.*, 2013) XIAP (Moussata *et al.*, 2012) PLK1 (Rödel *et al.*, 2010) Survivin* (Rödel *et al.*, 2008; Xiaoyuan *et al.*, 2010) XRCC2 (Qin *et al.*, 2015)
Cell cycle		RBBP6 (Xiao *et al.*, 2018) ABCC4 (Yu *et al.*, 2014a)
Unknown function	MSI (Shin *et al.*, 2013) GGH (Sadahiro *et al.*, 2017) CRBP1 (Yokoi *et al.*, 2017) PEPCK (Park *et al.*, 2014) SMAC (Pan *et al.*, 2008)	MMP-9 (Unsal Kilic *et al.*, 2007) Cyclooxygenase-2 (Shinto *et al.*, 2011) HMGB1 (Hongo *et al.*, 2015) SIRT7 (Tang *et al.*, 2017) SATB1 (Meng *et al.*, 2015) IGF-1R (Wu *et al.*, 2014) CIP2A (Birkman *et al.*, 2018) FXYD-3 (Loftas *et al.*, 2016) nuclear β-catenin (Wang *et al.*, 2013) REG4 (Kobunai *et al.*, 2011) BIRC5 (Kobunai *et al.*, 2011) NEIL2 (Kobunai *et al.*, 2011) MRP4 (Chai *et al.*, 2011) STAT3 (Spitzner *et al.*, 2010) ERBB2 (Spitzner *et al.*, 2010) VEGF (Zlobec *et al.*, 2008) G-CSFR (Yang *et al.*, 2014)

immunohistochemistry. They found that G-CSFR+ patients achieved better results from radiotherapy compared with G-CSFR-patients. Therefore, they concluded that the expression of G-CSFR before preoperative irradiation may predict the radiosensitivity of rectal cancer. Pan *et al.* (2008) detected the expression of Second mitochondria-derived activator of caspase (SMAC) in tumor tissues before irradiation and after operation by immunohistochemistry. They concluded that SMAC may be a useful marker of tumor response to preoperative radiotherapy because radiotherapy response rates were 83.3% in the patients with high SMAC immunoreactivity but only 25.0% in those with low SMAC immunoreactivity. Yi *et al.* (2016) overexpressed pigment epithelium-derived factor (PEDF) in cancer cell lines and observed enhanced radiosensitivity. Furthermore, by bioinformatics analysis, they found that PEDF performed functions via activation of p53 to regulate the double-strand break repair pathway and activate the G protein activation pathway. Pucci *et al.* (2017) examined the expression of Ku70/80 in preoperative CRT tumor biopsies. In the non-responder population, they observed that Ku80 expression in the tumor tissues was lost. Ha *et al.* (2017) described the expression profiles of tumors from 22 patients who were responders and nonresponders to preoperative CRT using RNA sequencing. They found proteasome subunit beta 8 (PSMB8) was upregulated in the responsive group and demonstrated that its overexpression enhanced radiosensitivity in cancer cell lines.

Controversial gene for chemoradiosensitivity

The genes listed below are the controversial gene for radiosensitivity at present. We speculate that the reason for the controversy may be the result of the complexity of regulation and interaction with each other.

p53

p53 is known to play an important role in the occurrence and treatment of tumors. Therefore, it has been studied as a potential biomarker more than any other gene. Komuro *et al.* (2003) examined the expression of p53 and Ku70 and

evaluated their association with tumor radiosensitivity. There was a significant correlation between the expression of p53/Ku70 and tumor radioresistance. They concluded that the positive expression of p53 and Ku70 may predict the radioresistance of rectal cancer before preoperative irradiation. In a further study, they evaluated the expression of Ku, p53, p21, and p16 in patients with rectal cancer by immunohistochemistry and found that Ku/p53-negative and p21/p16-positive patients were sensitive to radiotherapy (Komuro *et al*., 2005). Chai *et al.* (2011) examined multidrug resistance-associated protein 4 (MRP4) and p53 expression in rectal cancer specimens by immunohistochemistry and observed that patients with low expression of p53 and MRP4 had a higher response rate to radiation than high expression patients. They concluded that the expression of p53 and MRP4 was independently associated with the sensitivity of rectal cancer to radiation. Furthermore, Lin *et al.* (2006) demonstrated that tumor biopsies negative for p53 were predictive of complete tumor regression. Luna-Perez *et al.* (1998) found that tumors stained positively for p53 had a 65% residual tumor after radiation therapy compared with 25% residual for tumors negative for p53. Finally, Spitz *et al.* (1997) showed that samples lacking p53 staining had improved histopathological response to neoadjuvant chemoradiotherapy.

Contrary to the above studies, some investigators have shown little or no association between p53 and sensitivity to CRT. For example, Huerta *et al.* (2013) investigated the contribution of p53, p21, Bax, and the DNA-dependent protein kinase catalytic subunit, in response to radiation in CRC *in vitro* and *in vivo*. They found that p53-null tumors were relatively radioresistant *in vivo* compared with their wild-type counterparts. Esposito *et al.* (2001) also found that tumor biopsies with a higher degree of p53 expression were predictive of a better response to preoperative chemoradiotherapy. These results indicated that p53 may be a radiation-sensitive gene. However, because these studies have drawn conflicting conclusions, more in-depth studies will be needed to confirm the relationship between p53 and radiotherapy.

P21

In the study by Huerta *et al.* (2013) mentioned above, p21 deficiency was associated with a radiosensitive phenotype. Conversely, Komuro *et al.* (2005) studied the expression of Ku, p53, p21, and p16 in biopsy specimens of patients with rectal cancer by immunohistochemistry and concluded that p21-positive patients were sensitive to radiotherapy. Charara *et al.* (2004) found that 40% of p21-expressing tumors were complete responders, compared with none of the p21-negative tumors. Rau *et al.* (2003) investigated p21 in patients undergoing preoperative radiochemotherapy for rectal carcinoma and observed that lower p21 expression was associated with nonresponding tumors.

Bax

In the previously cited paper by Huerta *et al.* (2013), Bax deficiency was related to a radiosensitive phenotype. In contrast, Fucini *et al.* (2012) evaluated the expression of apoptotic Bax in biopsy samples and concluded that Bax was significantly associated with downstaging after radiation.

EGFR

Akimoto *et al.* (1999) investigated the relationship between epidermal growth factor receptor (EGFR) expression and radioresistance *in vivo*. They found that EGFR expression positively correlated with increased tumor radioresistance and that levels of EGFR were inversely correlated with radiation-induced apoptosis. Liang *et al.* (2003) transfected a full-length human EGFR expression vector into murine ovarian carcinoma cells and established stable clones expressing varying levels of EGFR. They demonstrated that overexpression of EGFR conferred cellular resistance to radiation. However, Zlobec *et al.* (2008) showed that the expression of EGFR and loss of vascular endothelial growth factor (VEGF) both demonstrated independent predictive value for a complete pathological response. Therefore, they suggested that EGFR-negative and VEGF-positive patients may be highly resistant to radiotherapy.

Gene expression profiling

Because of the limitations of single markers, many researchers have used DNA microarray-based gene expression profiling technology to analyze a large number of genes simultaneously and to search systematically for molecular markers to predict CRT responses and outcomes. Ghadimi *et al.* (2005) used microarrays to analyze 30 locally advanced rectal cancer patients stratified into two groups of responders and nonresponders. The results showed that 54 genes displayed significantly different expression between the two groups. The sensitivity and specificity in predicting response to therapy were 78% and 86%, respectively. They suggested that pre-therapeutic gene expression profiling might assist in predicting the response of rectal cancer to preoperative CRT. Watanabe *et al.* (2006) studied 52 rectal cancer patients who underwent preoperative radiotherapy. They found 33 novel differentially expressed genes between responder and nonresponder groups and established a model to predict response to radiotherapy with an accuracy of 82.4%. Among these discriminating genes, apoptosis inducers lumican, thrombospondin 2, and galectin-1 showed higher expression in responders, whereas apoptosis inhibitors cyclophilin 40 and glutathione peroxidase showed higher expression in nonresponders. The authors concluded that gene expression profiling might be useful in predicting the response to radiotherapy in order to establish individualized therapy for rectal cancer. Ha *et al.* (2017) analyzed gene expression profiles in tumors from 22 rectal cancer patients who were responders and nonresponders to preoperative CRT. They found eight differentially expressed genes, which were associated with the radiotherapy response. Among these genes, real-time RT-PCR showed that proteasome subunit beta type-8 (PSMB8) and putative sodium-coupled neutral amino acid transporter 7 (SLC39A7) were upregulated in the responsive group. In CRC cell lines, overexpressed PSMB8 was determined to reduce colony formation and increase apoptosis. The authors concluded that PSMB8 was a predictive marker of preoperative radiosensitivity in rectal cancer patients. Gantt *et al.* (2014) derived 812-gene and 183-gene signatures to separate nonresponders from responders. The classifiers were able to identify nonresponders with a sensitivity and

specificity of both 100% using the 812-gene signature, and sensitivity and specificity of 33% and 100%, respectively, using the 183-gene signature. Therefore, gene profiles could predict poor response to CRT in rectal cancer patients.

Many *in vitro* studies have also examined the association of gene expression profiles and radiotherapy sensitivity. Spitzner *et al.* (2010) found 4796 features in 12 CRC cell lines in which expression levels correlated significantly with the sensitivity to CRT by linear model analysis. They identified STAT3, RASSF1, DOK3, and ERBB2 as potential therapeutic targets by PCR. Therefore, this analysis revealed molecular biomarkers for the response of rectal cancers to CRT and demonstrated that it could serve as a potential method to improve the sensitivity of radiotherapy. Folkvord *et al.* (2010) analyzed tumor biopsy specimens using microarrays with kinase substrates in a subset of poor responders and good responders. Phospho-substrate signatures indicated high kinase activity and several discriminating phospho-substrate proteins derived from signaling pathways were implicated in radioresistance. The authors concluded that kinase activity profiling can identify functional biomarkers that could predict tumor response to preoperative CRT in rectal cancer. Eschrich *et al.* (2009) identified 10 genes, which were used to build a rank-based linear regression algorithm to predict the radiosensitivity index. They found that the predicted radiosensitivity index was significantly different in responders compared to nonresponders in rectal cancer. The sensitivity, specificity, and positive predictive value of this model were very high. Therefore, their work established a strong multigene expression model for tumor radiosensitivity in rectal cancer.

Cancer stem cell related markers

Previous studies had identified cancer stem cells as a contributor to radioresistance through the DNA damage checkpoint response and an increase in DNA repair capacity (Bao *et al.*, 2006). It was already known that CD133 was the most important marker of cancer stem cells. Therefore, many studies have investigated the effect of cancer stem cells on radiotherapy by examining the expression of CD133. Saigusa *et al.* (2010) investigated CD133 expression following CRT using immunohistochemistry *in vivo* and *in vitro*. The frequency of CD133 staining in CRT specimens was significantly higher than that of non-CRT specimens. Also, CD133 expression was significantly lower in histopathological responder patients than in patients lacking the histopathological response. *In vitro* studies showed that CD133 protein was increased in a radiation dose-dependent manner. The authors concluded that CD133 may be a marker of resistance to CRT in CRC that could be used in future cancer therapy. Qiu *et al.* (2014b) also demonstrated that CD133(+) cells have a radioresistant effect on SW 480 cells lines and in nude mice. Cai *et al.* (2017) detected mRNA and protein expression of hypoxia-inducible factor 1-alpha (HIF-1alpha) and CD133 before and after CRT using PCR and immunohistochemistry methods. They found HIF-1alpha mRNA expression was positively correlated with CD133 mRNA expression and HIF-1alpha mRNA and CD133 mRNA were significantly correlated with PCR. Therefore, they concluded that HIF-1alpha and CD133 could predict pCR and the survival of patients with rectal cancer.

Additional cancer stem cell markers have been related to radiotherapy. Maternal embryonic leucine zipper kinase (MELK) is known as an embryonic and neural stem cell marker (Liu *et al.*, 2017). Choi and Ku (2011) observed elevated MELK expression levels in a rectal cancer cell line treated with radiation and a reduction in cell proliferation after knockdown of MELK. They demonstrated that MELK was associated with increased resistance of CRC cells against radiation. G9a is a histone methyltransferase that plays a role in mediating phenotypes of cancer stem cells (Pan *et al.*, 2016). Luo *et al.* (2017) reported a significant positive correlation between G9a and CD133 in rectal cancer patients after CRT. The knockdown of G9a increased the sensitivity of cells to radiation treatment. Their study theorized that G9a might serve as a novel marker of preoperative CRT resistance in patients with CRC.

NcRNAs

MicroRNA (miRNAs) are one of the most common ncRNAs that regulate gene expression. It is known that miRNAs play an important role in tumorigenesis and development (Romero-Cordoba *et al.*, 2014). Many studies have shown that the expression of miRNA is associated with radiosensitivity. Kelley *et al.* (2017) revealed a decrease in the expression of miR-451a in nonresponders by using PCR. Therefore, they concluded that miRNAs may serve as biomarkers of radioresistance and offer treatment targets for radiotherapy in rectal cancer patients. Ma *et al.* (2015) found that miR-622, which inhibits Rb expression, was increased significantly in radiation-treated CRC cells and that overexpression of miR-622 induced radioresistance *in vitro*. They concluded that overexpression of miR-622 caused radioresistance and poor response to radiation therapy. MiR-622 is thus a potential biomarker for radiotherapy and a potential therapeutic target. Yu *et al.* (2016) analyzed global miRNA expression in groups of CRT-sensitive and CRT-resistant patients. They found that miR-345 was significantly elevated in the CRT-resistant group. Furthermore, they demonstrated that high miR-345 expression in the serum of CRT patients was significantly correlated with an unfavorable pathological response. Therefore, circulating serum miR-345 might be a promising biomarker for personalized treatment in CRT patients. Svoboda *et al.* (2012) selected 20 patients who underwent CRT for advanced rectal cancer and classified them as sensitive or resistant to the treatment. They compared the two groups using large-scale miRNA expression profiling. They found overexpression of miR-215, miR-190b, and miR-29b-2* in the radioresistant group and overexpression of let-7e, miR-196b, miR-450a, miR-450b-5p, and miR-99a* in the radiosensitive group. This study suggested that miRNAs are involved in the response of rectal cancer to CRT and that miRNAs may be promising predictive biomarkers for such patients. Wang *et al.* (2014) evaluated the role of lincRNA-p21 in radiotherapy for CRC and demonstrated that lincRNA enhances the sensitivity of radiotherapy for CRC by promoting cell apoptosis. Zhu *et al.* (2019) reviewed recent advances in the role and mechanism of long non-coding

RNAs (lncRNAs) in tumor radiosensitivity and found that lncRNAs influenced radiosensitivity by regulating various mechanisms, including DNA damage repair, cell cycle arrest, apoptosis, cancer stem cells regulation, epithelial-mesenchymal transition, and autophagy.

SNPs

Some studies have shown that gene polymorphisms are associated with radiotherapy response in patients with rectal cancer. Dzhugashvili *et al.* (2014) detected the polymorphisms rs28362491 (NFKB1), rs1213266/rs5789 (PTGS1), rs5275 (PTGS2) and rs16944/rs1143627 (IL1B) in 159 patients with locally advanced rectal cancer treated with radiation therapy. The NFKB1 del/del genotype was associated with pathological responses. Patients carrying the haplotype rs28362491-DEL/rs1143627-A/rs1213266-G/rs5789-C/rs5275-A/rs16944-G had a higher response rate to radiation therapy than patients with the rs28362491-INS/rs1143627-A/rs1213266-G/rs5789-C/rs5275-A/rs16944-G haplotype. They suggested that genetic variation in NFKB might influence sensitivity to primary chemoradiation for rectal cancer. Kim *et al.* (2013) identified nine SNPs associated with preoperative CRT responses. They identified candidate markers CORO2A rs1985859 and FAM101A rs7955740 as predictors of radiosensitivity to preoperative CRT using a cell-based functional assay for biological validation. Cecchin *et al.* (2011) analyzed 238 rectal cancer patients treated with CRT and found 25 genetic polymorphisms in 16 genes relevant in treatment-associated pathways. By multivariate analysis, the hOGG1-1245C>G polymorphism was associated with radiosensitivity.

Gene methylation

Gene methylation is known to play an important role in the regulation of gene expression. Aberrant DNA methylation of genomic regions, including CpG islands, is related to altered gene expression patterns in all human cancers. Some studies have shown that DNA methylation is associated with radiosensitivity. Ha *et al.* (2015) identified seven hypermethylated CpG sites (DZIP1 cg24107021, DZIP1 cg26886381, ZEB1 cg04430381, DKK3 cg041006961, STL cg00991794, KLHL34 cg01828474, and ARHGAP6 cg07828380) which were associated with preoperative CRT responses using genome-wide screening. Radiosensitivity in patients with hypermethylated KLHL34 cg14232291 was confirmed by pyrosequencing. The authors concluded that the methylation status of KLHL34 cg14232291 was a candidate for predicting sensitivity to preoperative CRT. Yokoi *et al.* (2017) identified that retinol-binding protein 1 (CRBP1) was a candidate radiation sensitivity marker gene, which was verified by using microarrays. They confirmed that CRBP1 was epigenetically silenced by hypermethylation of its promoter DNA and that the quantitative methylation value of CRBP1 was significantly correlated with a histological response in rectal cancer patients with CRT. Epigenetic modification is considered to be one of the important ways to regulate gene expression. Therefore, gene methylation, which is one of the epigenetic modifications, achieve their functions by regulating the expression of genes, thereby affecting the radiosensitivity of rectal cancer.

Network of molecular markers

Many studies showed that the combination of multiple molecular biomarkers formed regulatory networks in the radiosensitivity of rectal cancer.

Shao *et al.* (2018) demonstrated that EMT related transcription factor ZEB1 might take part in the radio-resistance induced by OCT4. Furthermore, the OCT4/ZEB1 axis could promote the resistance of human rectal cancer cells to radiation. As is well-known, the BRAF gene mutation was a poor prognostic factor for colorectal cancer patients. Spagnoletti *et al.* (2018) studied cyclin-dependent kinase 1 expression in colorectal tumor cells and represented it could improve radiosensitivity in BRAF mutation cells. Jiang *et al.* (2019) showed that BRAF and SMAD4 genetic mutations might be important molecular markers to predict resistance to radiotherapy. Therefore, we speculated that molecular markers for rectal radiotherapy formed a complex network to regulate each other. Therefore, the best predictors of radiotherapy might be some combination of multiple molecular biomarkers.

Biomarkers in other cancers

In addition to rectal cancer, biomarkers related to radiosensitivity have also been found in other tumors, such as GADD45G

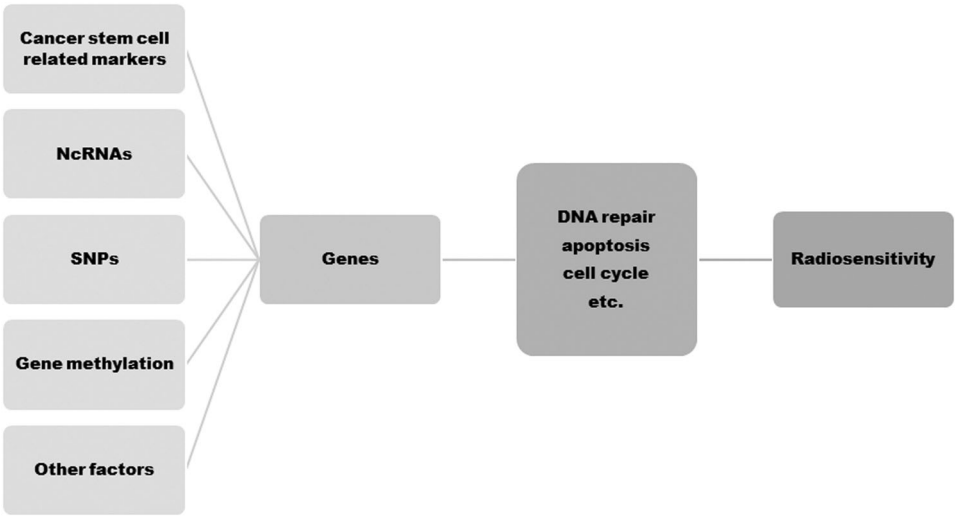

FIGURE 1. Relationship between molecular markers and radiosensitivity.

interacting protein 1 (CRIF1) (Ran *et al*., 2019), tripartite motif-containing 36 (TRIM36) (Man *et al*., 2019), miR-222 (Shi *et al*., 2019), miR-16-5p (Zhang *et al*., 2020), lncRNAs (Wang and Hu, 2019; Zhu *et al*., 2019) and other ncRNAs through regulating their target genes, as well as gene methylation (Wu *et al*., 2019) and many cell pathways (Huang *et al*., 2019; Li *et al*., 2019; Sun *et al*., 2019). So far, it is very clear that with the discovery of ncRNAs, more and more ncRNAs have been confirmed to involve in the radiosensitivity and will become a research hotspot in the future.

Other factors

Many studies have shown that other factors are associated with radiosensitivity, including CEA level (Colloca *et al*., 2017; Das *et al*., 2007; Lin *et al*., 2010), anemia (McGrane *et al*., 2017), lymphocytes (Ishihara *et al*., 2012; Kitayama *et al*., 2011; Yasuda *et al*., 2011), nuclear factor-kappa B (Sandur *et al*., 2009; Voboril and Weberova-Voborilova, 2007) and signaling pathway such as the PI3-K/AKT (Stegeman *et al*., 2014) and β-catenin signaling pathways (Wang *et al*., 2014). There are likely many other unknown factors that have yet to be identified.

Summary

This review has summarized most of the genes related to radiotherapy in rectal cancer from the perspective of RRGs, RSGs, gene expression profiling, cancer stem cell-related markers, ncRNAs, SNPs, gene methylation, and other factors. However, they ultimately regulate the radiosensitivity by affecting DNA repair, apoptosis, and cell cycle arrest, etc. (Fig. 1). In other cancers, molecular markers of radiosensitivity also exist, but they perform almost the same function.

Conclusion and Future Perspective

There are a large number of molecular markers involved in the regulation of radiosensitivity. Among them, molecular target genes, such as Ku70, hPEBP4, Survivin, and γH2AX, have the potential to be incorporated into treatments for rectal cancer, although this will require extensive validation and testing. However, there are a number of other genes including p53, P21, Bax, and EGFR, for which study results have been contradictory, and the exact mechanisms underlying their effect on radiosensitivity will require additional evaluation. With the study of ncRNAs, more and more ncRNAs have been confirmed to involve in the radiosensitivity, and have become a research hotspot in recent years. We concluded that molecular biomarkers for rectal radiosensitivity formed a complex regulatory network. Therefore, we speculated that the best predictors of radiotherapy response were likely to be some combination of multiple molecular biomarkers. More complex studies will thus be needed to identify these biomarkers in the future.

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