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Predictive biomarkers in radioresistant rectal cancer: A systematic review

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ABSTRACT

Background and aims: The treatment of locally advanced rectal cancer often consists of neoadjuvant chemoradiotherapy followed by surgery. However, approximately 15% of patients show no response to this neoadjuvant chemoradiotherapy. This systematic review aimed to identify biomarkers of innate radioresistant rectal cancer.

Method: Through a systematic literature search, 125 papers were included and analyzed using ROBINS-I, a Cochrane risk of bias tool for non-randomized studies of interventions. Both statistically significant and nonsignificant biomarkers were identified. Biomarkers mentioned more than once in the results or biomarkers with a low or moderate risk of bias were included as the final results.

Results: Thirteen unique biomarkers, three genetic signatures, one specific pathway, and two combinations of two or four biomarkers were identified. In particular, the connection between HMGCS2, COASY, and PI3K-pathway seems promising. Future scientific research should focus on further validating these genetic resistance markers.

1. Introduction

The incidence of rectal cancer in Europe is 125,000 per year (Glynne-Jones, 2017), with a 5-year survival rate of approximately 60% (Allemani, 2015). Most patients with locally advanced rectal cancer receive standardized treatment consisting of neoadjuvant chemoradiotherapy (nCRT), followed by surgery. nCRT is either short-course radiotherapy (25 Gy in 5 fractions per week) or fluorouracil as a radiosensitizer combined with long-course radiotherapy (45–50.4 Gy in 25–28 fractions over 5–6 weeks) (Glynne-Jones, 2017).

Clinical outcomes after nCRT vary from 20% of patients with a complete pathological response to 15% of patients with no response or even disease progression (Poynter, 2019; Park, 2012). A patient with a complete response will have a significantly longer 5-year disease-free survival than a patient without a complete response (Maas, 2010). Patients with radioresistant tumors are at risk of unnecessary toxicity (Birgisson et al., 2007; Thong, 2011; Bruheim, 2010; Peeters, 2005; Bruheim, 2010). These patients experience delays in curative surgery, which may lead to tumor progression or metastatic growth. If biomarkers of radioresistance are identified, patients with such tumors could avoid nCRT.

Several reviews have provided an overview of radioresistance predictions. The CEA biomarker has been thoroughly investigated previously with controversial results and has not been included in this review (Meng et al., 2014; Fischer et al., 2021; Dayde et al., 2017; Alkan et al). Clinical markers, including clinicopathological and radiological variables, have also been previously reported and are not included here (Meng et al., 2014; Fischer et al., 2021).

Studies on biomarkers for predicting radioresistant rectal cancer have shown conflicting results. These conflicting results could be explained by the different uses of biological materials, such as pre- or post-therapeutic tissue samples or blood (Machackova et al., 2019; Huerta et al., 2009). In addition, methods can vary from single-protein identification with immunohistochemistry to investigation of large gene panels. Differences in the radiotherapy dose, chemotherapy regimen, and time interval from the end of nCRT to surgery could also affect the results (Meng et al., 2014; Dayde et al., 2017).

This systematic review aims to identify biomarkers indicating innate

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radioresistant rectal cancer.

2. Materials and methods

A systematic literature search was performed on the 7th of August 2020 and updated on the 21st of July 2021, using the following research databases: PubMed, Embase, Cochrane, and Web of Science. Prospero ID CRD42020210023 (PROSPERO. ⟨https://www.crd.york.ac.uk/prospero/⟩ (accessed Feb. 22, 2023).).

Literature was included based on the following criteria: English written articles that were available in full-text and peer-reviewed; patients had to be diagnosed with rectal cancer and treated with neoadjuvant radiotherapy, radiotherapy had to be with curative preoperative dosing equivalent to the radiobiological effect of 25 Gy in 5 fractions per week or above 42 Gy given in fraction doses of 1.8–2 Gy (authors were contacted if the radiotherapy dose was unspecified); concurrent chemotherapy was allowed, if the prescribed dose was as a radiosensitizer; if the results were based on the investigation of pre-therapeutic tumor biopsies and/or blood samples combined with the tumor regression grade of the surgical specimen; and if it was noted that any form of validation was performed, such as an external dataset, mouse xenograft, or cell lines.

No timeline restrictions were imposed in the literature search. A minimum of five patients had to be included in the final analysis. The exclusion criteria were as follows: meta-analysis, systematic review, inclusion of patients with metastatic disease, immediate surgery after radiotherapy, and results exclusively based on a public dataset.

A full description of the search protocol is provided in Supplementary Material 1. The PRISMA guidelines were followed (Page, 2021). A chief librarian from the Aalborg University Hospital conducted the literature search. Titles and abstracts were uploaded to Covidence (Covidence - Better systematic review management, 2023) and duplicates were removed. The abstracts were independently screened by two authors (A.S and S.N.H). Any disagreements were resolved through discussion with the review group. Afterwards, a full-text screening, including an assessment of risk of bias (RoB), was performed by two of the authors, A.S and S.N.H.

The ROBINS-I tool is a RoB tool for assessing nonrandomized studies of interventions (Sterne, 2016). It was used to evaluate all included studies' RoB due to confounding factors, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of reported results (Supplementary Material). Each RoB domain can be rated as low, moderate, serious, or critical. A study's overall RoB was judged based on the highest RoB from each domain. A study with a low RoB includes a description of the study design with a sufficient dose of radiotherapy and a duration of 4–12 weeks between radiotherapy and surgery to shrink and prevent regrowth of a rectal cancer tumor (Gambacorta, 2021; Ryan, 2019; Du et al., 2018; Veenhof et al., 2009), chemotherapy as a radiosensitizer, a pathological assessment of a systematic defined tumor regression grade, a minimum 50% of tumor parenchyma in the pre-therapeutic biopsy, and a clear overview of the tumor regression grade.

Both statistically significant and nonsignificant markers were included in the review results. When assessing tumor regression grade after radiotherapy, a statistically significant result was defined as $p < 0.05$. Biomarkers with nonsignificant results were defined as those with no association with radioresistance. The results of the included studies were divided into two groups according to A) biomarkers mentioned more than once in the results or biomarkers from studies with a low or moderate RoB, and B) biomarkers mentioned once in the results and with a serious or critical RoB (Fig. 1). Group A was further divided into three subgroups; Table 1: Biomarkers indicating radioresistance or radiosensitivity, Supplementary Material 1, Table A: Biomarkers with conflicting results, Supplementary Material 1, Table B: Biomarkers with no association to radioresistance. Furthermore, a Table C with all included studies' key characteristics is also available in Supplementary Material 1.

3. Results

We generated 1552 articles in our literature search (Fig. 2). After the removal of duplicates and papers that did not meet our inclusion criteria, 159 articles remained. An additional 30 articles were identified from the reference lists of the chosen articles and included in our review. In the second literature search, 24 of the 189 full-text screened articles were included. Finally, a total of 125 articles were included and evaluated for RoB using ROBINS-I (Supplementary Material 2).

Table 1 shows the identified radioresistant/radiosensitive biomarkers, thirteen unique biomarkers, three genetic signatures, one specific pathway, and two combinations of two or four biomarkers. All markers were mentioned in one article, except HMGCS2, RAD23B, and REG4, which were mentioned in two, two, and three papers, respectively. The RoB varied from low to critical (Table 1). Studies of COASY, NPTX2, and two gene signatures of 812 and 183 genes had a low RoB. Biomarkers with conflicting or no association with radioresistance are

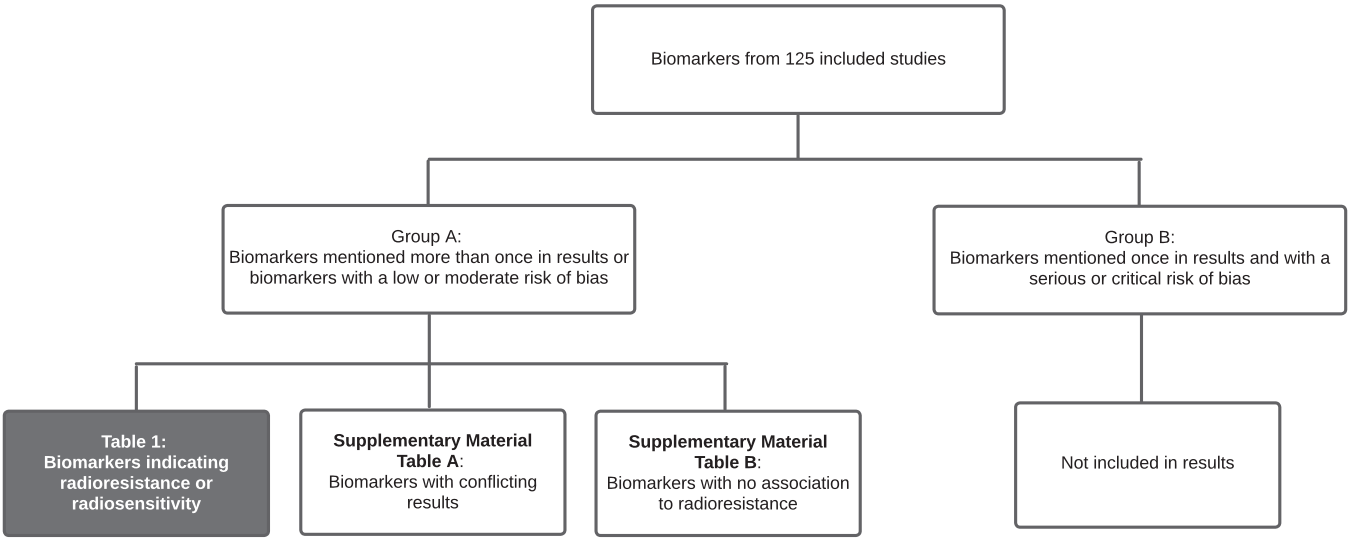

Fig. 1. Biomarkers from the 125 included studies

Table 1
Biomarkers indicating radioresistance or radiosensitivity

Biomarker	Ref.	Type of biomarker	Material	Cohort	Result	Validation	Risk of bias	Conclusion
C-MET	(Senetta, 2015)	Protein	Tissue	75	Expression = radioresistance	No	Moderate	Radioresistant
COASY / Coenzyme A synthase	(Ferrandon, 2020)	RNA + protein	Tissue	33	High expression = radioresistance	Yes	Low	Radioresistant
Gene signature	(Rimkus, 2008)	RNA	Tissue	43	Gene expression signature of 42 genes = radioresistance	Yes	Moderate	Radioresistant
	(Millino, 2017)	RNA	Tissue	59	*TMEM188, ITGA2, NRG1, TRAM1, BCL2L13, MYO1B, KLF7, GTSEI* = predictors of response	Yes	Moderate	Predictors of response
	(Gantt et al., 2014)	RNA	Tissue	33	Gene signature of 812 and 183 genes identifying non-responders	Yes	Low	Radioresistant
HMGCS2	(Lee, 2015)	Protein	Tissue	172	High expression = radioresistance	Yes	Serious	Radioresistant
	(Yeo, 2012)	Protein	Tissue	45	No expression = radiosensitivity	Yes	Serious	
Ki67 + p53 + VEGF + p21	(Hur, 2014)	Protein	Tissue	81	4-point scoring system predicts pathological complete response	No	Moderate	Radiosensitive
KLF5	(Kim et al., 2019)	Protein	Tissue	60	High expression = radioresistance	Yes	Moderate	Radioresistant
LC3β	(Shim, 2016)	Protein	Tissue	101	High expression = radioresistance	No	Moderate	Radioresistant
MIR17HG	(Molinari, 2016)	DNA	Tissue	108	MIR17HG amplifikation = radioresistance	No	Moderate	Radioresistant
MRP3	(Yu, 2014)	Protein	Tissue	144	High expression = radioresistance	Yes	Moderate	Radioresistant
NPTX2	(Karagkounis, 2016)	RNA	Tissue	40	Low expression = radiosensitivity	No	Low	Radiosensitive
PI3K pathway related genes	(Abdul-Jalil, 2014)	DNA	Tissue	201	PI3K pathway-related genes mutations = absence of pathological complete response	No	Moderate	Radioresistant
RAD23B	(Troncarelli Flores, 2019)	Protein	Blood	30	RAD23B on CTCs: expression = radioresistance	No	Serious	Radioresistant
	(Silva, 2021)	Protein	Blood	56	RAD23B on CTC: no expression = pathological complete response	No	Moderate	
REG4/REG4	(He, 2014)	Protein	Tissue	172	High expression = radioresistance	Yes	Serious	Radioresistant
	(Kobunai et al., 2011)	RNA	Tissue	22	High expression = radioresistance	Yes	Critical	
	(Gao, 2021)	Protein	Tissue	146	Low expression = radiosensitivity	Yes	Critical	
RSF-1	(Lin, 2012)	Protein	Tissue	172	High expression = radioresistance	No	Moderate	Radioresistant
SPINK4	(Chen, 2021)	Protein	Tissue	172	High expression = radioresistance	Yes	Moderate	Radioresistant
VEGF + COX-2	(Edden et al., 2012)	Protein	Tissue	152	Overexpression = radioresistance	No	Moderate	Radioresistant
YKL-40	(Senetta, 2015)	Protein	Tissue	75	Expression = radioresistance	No	Moderate	Radioresistant

provided in Supplementary Material 1.

4. Discussion

This systematic review summarizes and evaluates statistically significant innate radioresistant biomarkers reported at least once. Four unique radioresistant biomarkers, COASY, HMGCS2, RAD23B, and REG4, and one radiosensitive biomarker, NPTX2, were identified (Table 1). These unique biomarkers have either a low RoB or results supported by more than one study. The PI3K pathway from Table 1 is further discussed in the following section because of its connections to COASY and HMGCS2.

4.1. COASY, HMGCS2, and PI3K-pathway related genes

Ferradon et al. (Ferrandon, 2020) discovered that high COASY expression indicates radioresistance. This study had a low RoB according to ROBINS-I criteria. Patients received 50 Gy in 25 fractions with a radiosensitizer and underwent surgery 8–12 weeks after the completion of nCRT. The tumor biopsy samples contained at least 60% tumor cell parenchyma. The College of American Pathologists guidelines were used for systematic scoring of tumor regression grade, which was performed by a specialized gastrointestinal pathologist. The number of patients in each tumor regression grade group is stated. Microarrays, RT-qPCR, gene set enrichment, and immunohistochemistry were used for RNA and protein analyses of tissue samples from the patient cohort. Immunohistochemistry was performed. The validation was performed using an external database, cell lines, and mouse xenografts. We evaluated this study to provide high evidence. Furthermore, coenzyme A synthase overexpression was shown to increase the activation of the PI3K pathway through p-AKT and p-mTOR in colorectal cell lines. This study concluded that this is a potential mechanism for radioresistance.

Two studies have shown, HMGCS2 expression as a protein that indicates radioresistance (Table 1). Lee et al. (Lee, 2015) associated high HMGCS2 expression levels with radioresistance after nCRT. Yeo et al. (Yeo, 2012) found that no HMGCS2 expression was associated with a better nCRT response. These results validated the discovery using external databases or cell lines. Both studies had a serious RoB in ROBINS-I due to either missing information regarding the duration from radiotherapy to surgery or the amount of tumor tissue in biopsies and pathological assessment of tumor regression grade. However, their findings were in concordance with those of the two independent patient cohorts strengthening the potential of high HMGCS2 expression as biomarker of radioresistance.

Abdul-Jalil et al. showed (Abdul-Jalil, 2014) that the mutational activation of the PI3K pathway related genes is associated with the absence of a pathological complete response. The study had a moderate RoB due to missing information regarding the amount of tumor cell parenchyma in the tumor biopsy samples.

4.1.1. Interpretation

HMGCS2 catalyzes the first reaction to generate β-OHB during ketogenesis. This catalysis creates coenzyme A synthase as a biproduct (HEGARDT, 1999), meaning that a high amount of HMGCS2 produces a high amount of coenzyme A synthase. High expression of coenzyme A synthase is associated with the activation of the PI3K pathway (Ferrandon, 2020), and mutational activation of PI3K pathway-related genes is associated with the absence of a pathological complete response (Abdul-Jalil, 2014). This connects HMGCS2, coenzyme A synthase, and the PI3K pathway, all supporting the findings that activation and high

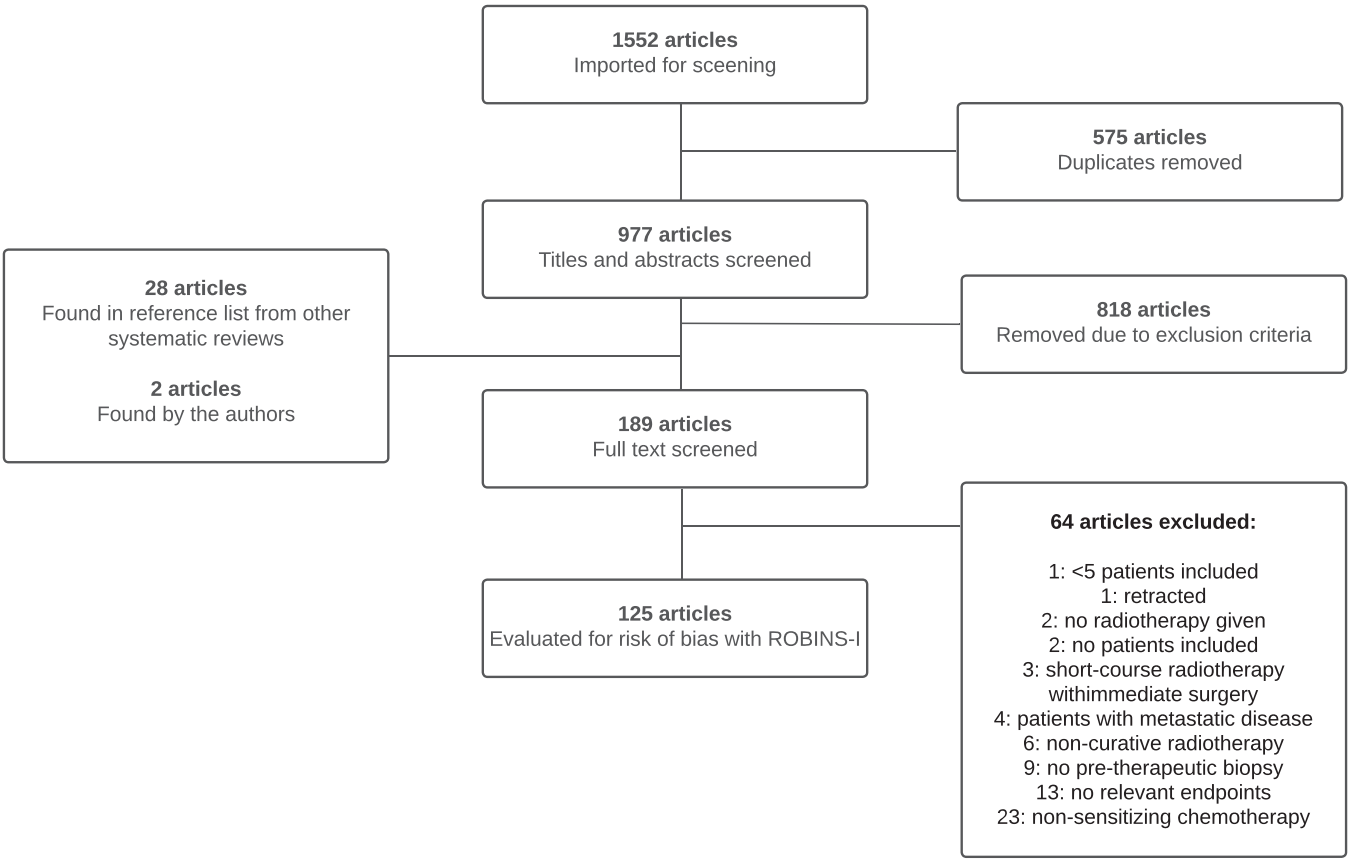

Fig. 2. Flowchart

expression indicate radioresistance (Table 1).

Both HMGCS2 and coenzyme A synthase are involved in fasting-induced ketogenesis. Fasting protects mice from lethal DNA damage by promoting the survival of small intestinal epithelial stem cells (Tinkum, 2015). Under fasting conditions, the analysis of small intestine crypts conditionally deleted for HMGCS2 revealed a marked decrease in H3K9bhb-associated loci and an altered gene expression profile. H3K9bhb enrichment in the crypt of the small intestine might be dependent on the local production of β-OHB (Terranova, 2021). Thus, it could be speculated HMGCS2 expression could potentially protect the tumor stem cell pool from radiotherapy induced DNA damage through this epigenetic modification.

4.2. NPTX2

Karagkounis et al. (Karagkounis, 2016) showed that low NPTX2 levels are associated with a better response to nCRT in rectal adenocarcinomas. Among the included studies, only NPTX2 was investigated in this study. However, it is one of the few studies evaluated as having a low RoB. However, the results were not validated.

NPTX2 plays an important oncogenic role in various malignancies (Wang, 2020). In colorectal cancer cells, NPTX2 promotes proliferation and metastasis by activating the Wnt/β-catenin pathway (Xu, 2019). It is also associated with the p53/PTEN/Akt/NF-κB signaling pathway, which is involved in oncogenesis, tumor progression, and chemoresistance (Shukla, 2013). Furthermore, NPTX2 has been reported to play a role in diseases of the nervous system. In central nervous system development, it is assumed that the interaction of NPTXs with AMPA receptor is associated with tumorigenesis (Wang, 2020). Thus, the biological function of NPTX2 does not contradict the fact that it may play a role in the radiotherapy response using a mechanism similar to that observed in malignant cells and identified pathways in other malignancies.

4.3. RAD23B

Two studies from our systematic literature search linked RAD23B to radioresistance, both as a protein expressed on circulating tumor cells (CTC) in the blood. Flores et al. (Troncarelli Flores, 2019) reported that RAD23B-positive CTC were associated with radioresistance. The RoB was serious, because the duration between radiotherapy and surgery exceeded 12 weeks. Silva et al. (Silva, 2021) showed that RAD23B expression reduces the chance of a pathological complete response. A moderate RoB was observed owing to missing data. Neither of the studies validated their findings, but again, it is a strength that two independent studies found the same high protein expression association to correlate with radioresistance.

RAD23B is a DNA-repair gene. Recently, Priya et al. demonstrated that 2 Gy of radiotherapy induces methylation of the RAD23B promoter, indicating transcriptional repression of a DNA-repair protein (Priya and Das, 2022). In a study of miRNAs that sensitize cancer cells to radiation, miR-744–3p was found to be a potent radiation-sensitizing miRNA (Hatano, 2015). MiR-744–3p significantly delays radiation-induced DNA damage repair by directly targeting RAD23B. Thus, it can be speculated that RAD23B plays a significant role in radioresistance through its direct involvement in DNA-repair.

4.4. REG4

Three studies from our systematic literature search showed that REG4 expression, as either a protein or a gene, indicates radioresistance. All three studies were discovered in the external dataset or cell lines and validated in their own cohort. He et al. (He, 2014) showed that high expression of REG4 is associated with a lower degree of tumor

regression, which indicates radioresistance. A serious RoB was evaluated because of missing information regarding the time duration between radiotherapy and surgery. Kobunai et al. (Kobunai et al., 2011) observed significantly higher REG4 expression in radioresistant patients than in radiosensitive patients. Critical RoB due to lack of proper definition or pathological assessment of tumor regression grade, and missing information regarding the amount of tumor tissue in biopsies. Gao et al. (Gao, 2021) showed that low expression of REG4 is related to a better effect on nCRT. It was rated as a critical RoB due to missing information regarding the time duration between radiotherapy and surgery, dose of radiotherapy, and use of chemotherapy.

REG4 is a protein involved in the cell cycle and proliferation and is enriched in the intestinal mucosa, mainly in mucus-secreting cells. A report on proliferation and stemness in cancer cells found that the pro-proliferative and pro-stemness effects of REG4 are mediated through γ-secretase-mediated CD44/CD44ICD signaling (Bishnupuri et al., 2022). These findings have increased the focus on strategies to disrupt the Reg4-CD44-γ-secretase-CD44ICD signaling axis, which may be involved in cancer cell susceptibility to radiotherapy.

4.5. Strengths and limitations

The reason why the abundance of original research as well as review articles reporting on the response to radiotherapy in rectal cancer patients have resulted in no clinical impact, to this day remains unknown. However, in this systematic review, several differences were observed in the original studies. In terms of clinical features (dose of radiotherapy, allowance of combination chemotherapy regimens, timing of surgery, number of samples, and selected cohorts for discovery and validation), histopathological features (grading of response, grouping of different responders, fresh frozen vs. FFPE material), molecular biological features (different methods and cut-offs for RNA and protein expression) as well as different bioinformatics applied, this lack of consensus might partially be explained.

The methods applied in this systematic review attempted to solve most of these issues by applying a combination of clinical and methodological features. Most of the genetic radioresistant markers from the included studies were placed in Supplementary Material 1 or excluded from tables due to lack of repetition of markers or results, as well as the RoB (see Fig. 1). These demands might have also excluded potential candidate markers that have either been reported with ambiguous results, only once, or poorly reported regarding the RoB. On the other hand, Table 1 includes a manageable list of main results, that are highly relevant for verification. Therefore, the subjective element in the assessment of molecular radioresistant biomarkers is a strength that allows future scientific research to be conducted with a low RoB while focusing on these selected specific markers.

5. Conclusion

This systematic review identified radioresistant biomarkers in patients with rectal cancer, including thirteen unique biomarkers, three genetic signatures, one specific pathway, and two combinations of two or four biomarkers. In particular, the connection between HMGCS2, COASY, and PI3K-pathway seems promising. Future scientific research should focus on further validating these genetic resistance markers with a prospective study design with well-defined clinical parameters defined upfront, ensuring a low RoB.

Ethical approval

Not required.

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CRediT authorship contribution statement

Anna Slipsager: Data curation, Formal analysis, Methodology, Investigation, Visualization, Project administration, Writing – original draft, Writing – review & editing, **Sofie N. Henrichsen:** Conceptualization, Data curation, Formal analysis, Methodology, Investigation, Writing – original draft, Writing – review & editing, **Ursula G. Falkmer:** Conceptualization, Methodology, Investigation, Supervision, Writing – original draft, Writing – review & editing, **Karen Dybkær:** Supervision, Writing – review & editing, **Mattias Belting:** Supervision, Writing – review & editing, **Laurids Ø. Poulsen:** Conceptualization, Methodology, Investigation, Supervision, Writing – original draft, Writing – review & editing.

Conflict of Interest

We declare that there is no financial or personal relationships that could inappropriately influence our work.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.critrevonc.2023.103991.

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