ORIGINAL ARTICLE

Clinical value of significance of Hypoxia Inducible Factor-1a, Glucose Transporter-1 and Carbonic Anhydrase IX in rectal cancer after preoperative chemoradiotherapy

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Summary

Purpose: The standard treatment of rectal cancer is surgery along with preoperative radiotherapy, administered alone or in combination with chemotherapy. Preoperative chemoradiotherapy (preCRT) is widely used as it allows better local control and the use of sphincter-saving surgery. Pathological response after preCRT has been shown to be a significant prognostic factor of rectal cancer recurrence and survival. In this review we will assess the value of Hypoxia Induced Factor 1a (HIF-1a), Carbonic Anhydrase IX (CA-9) and Glu*cose Transporter 1 (GLUT-1) genes as predictive markers of* the course of local advanced rectal cancer in patients who underwent pre-CRT.

Methods: We searched studies, from Pubmed and in English language, obtained the information by using "HIF-1 alpha",

"Carbonic Anhydrase IX (CA-9)", "Glucose Transporter 1 (GLUT-1)" and "rectal cancer" as key words.

Results: 27 relevant articles were retrieved in initial stage. After full-text review, 13 articles were selected for the final analysis.

Conclusions: HIF-1a, GLUT-1 and CAIX may be connected with tumor response to preCRT, however, there is still skepticism towards their clinical use as predictors of outcome. Therefore, there is a need to conduct larger and more extensive cohort studies in order to find whether these predictors can be used in practice.

Key words: HIF-1 alpha, carbonic anhydrase IX, glucose transporter 1, rectal cancer, systematic review

Introduction

agnosed malignancies and a major cause of cancer- in males rather than females [1]. related deaths in the Western world. Its incidence

Rectal cancer is one of the most commonly di- Europe, and North America and seems more often

The standard treatment of rectal cancer is is substantially higher in Australia, New Zealand, surgery along with preoperative radiotherapy, ad-

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ministered alone or in combination with chemotherapy. Preoperative chemoradiotherapy (preCRT) is widely used as it allows better local control and the use of sphincter-saving surgery [2]. Pathological response after preCRT has been shown to be a significant prognostic factor of recurrence and survival of rectal cancer [3]. Nonetheless, the response of individual tumors is not uniform and as a result there is a need to identify prognostic markers of response to preCRT. By establishing prognostic markers, cancer treatment can be improved by recognizing certain characteristics of malignancies, such as the aggressiveness and behavior of the tumors. Those markers will allow the identification of patients who will benefit the most from this treatment offering a patient tailored therapy [4]. However, despite the extensive search, no molecular marker has proved valid for clinical application so far [5,6].

It has been demonstrated that response to treatment is largely influenced by the tumor microenvironment, such as low oxygen levels (hypoxia) [7]. Hypoxia starts a cascade of events, including the stabilisation of the hypoxia induced factor 1-a (HIF-1a) which activates the transcription of numerous genes associated with angiogenesis, metabolism, cell proliferation, pH regulation, growth factor signal transduction and apoptosis [8-10]. Furthermore, the carbonic anhydrase IX (CAIX) and glucose transporter 1 (GLUT1) genes are downstream targets of HIF-1a. CAIX is involved in maintaining the acidic pH [9,11] and GLUT1 facilitates transport of glucose across the cellular membrane [12].

In this review we assessed the value of HIF-1a, GLUT1 and CAIX as predictive markers of the course of locally advanced rectal cancer (LARC) in patients who underwent pre-CRT.

Methods

Inclusion criteria

Patients 18 years old or older were included who had been diagnosed with local advanced rectal cancer (LARC) and treated surgically with preoperative chemoradiotherapy (preCRT), administered alone or in combination with chemotherapy. All of the patients were measured, in preoperative time, for the expression of HIF1-a or/and CIX (or CA-9) or/and GLUT-1 genes.

Exclusion criteria

Studies with limited information, inaccurate number of cases, literature reviews, case reports, etc were excluded.

Retrieval method

A literature search was conducted with the following key words such as "Inducible Factor-1 Alpha (HIF1-a)" and/or "Carbonic Anhydrase IX (CAIX or CA-9)" and/or "Glucose Transporter 1 (GLUT-1)" and "rectal cancer" from PubMed (until May 2016). PubMed and retrieval is limited to clinical studies. Searching language is limited to English.



Figure 1. Graphical presentation of general characteristics of included studies.

Study screening and data extraction

Two researchers separately screened the studies, collected and cross-checked data, and distinguished differences through discussion with another researcher. Missing materials were supplemented by contacting the authors through telephone or mails.

Statistics - Data synthesis and assessment of risk of bias

Data extracted from studies were summarized in tabular form to facilitate qualitative analyses of trends or patterns in the findings. Quality of included studies was based on the Cochrane Risk of Bias Tool, and the software RevMan 5.3 was used. The software Corel Draw was used to graphically display the data.

A p value<0.05 denoted statistical significance.

Results

According to the designated searching method, 27 relevant articles were retrieved in initial stage. After full-text review 13 articles were selected for the final analysis. (Table 1 and 2, Figure 1 and 2). The quality of the studies was medium-high (Figure 3 and 4).

HIF-la

HIF-1a is one of the subunits of the heterodimeric hypoxia inducible factor (HIF) and is regulated by the presence of oxygen. Under normoxic conditions, HIF-1a is propyl hydroxylated and then prepared for degradation through mitochondrial ubiquitination. Under hypoxic conditions this degradation cannot take place due to a reduction in propyl hydroxylation, which leads to HIF-1a accumulation [13]. This upregulation allows it to bind with HIF-1 β and become an active transcription inducer, causing its translocation to the nucleus and promoting the expression of numerous genes associated with angiogenesis, metabolism, cell proliferation, pH regulation, growth factor signal transduction and apoptosis [8-10].

HIF-1a mRNA has been demonstrated to be present in a significant number of colorectal adenoma and carcinoma specimens [14]. According to Novell et al. [15], cytoplasmic HIF-1a expression is higher in tumor than normal tissue of the diagnostic biopsy. Furthermore, cytoplasmic HIF-1a levels were decreased in the tumor after CRT whereas nuclear HIF-1a levels were increased. These findings do not correlate with the observations of many authors who demonstrated that the nuclear expression of HIF-1a decreased during CRT [16,17]. Additionally, Saigusa et al. [17] showed that HIF-1a increased with colony formation and that there was no significant association between the expression levels of HIF-1a and clinicopathological variables.

No significant correlation has been found between good response to CRT and high versus low expression of HIF-1a [17-19]. Additionally, no significant association has been found between HIF-1a expression and Tumor Regression Grade (TRG), or between TRG and HIF1a expression changes during CRT [16,19].

Novell et al. [15] showed that nuclear HIF-1a is an independent prognostic marker for progression-free survival. Interestingly, HIF-1a positivity retained a significant effect on cancer-specific survival independent of TNM stage and vascular in-



Figure 2. The geographical distribution and general characteristics of included studies. First author [reference], Year of publication, Biomarkers, Number of cases.

Table 1. Cha	racteristics c	of included si	tudies						
First author (reference)	Year of publication	Materials collection (period)	Country	Cases analyzed	Biomarker	CRT treatment	Age of patients	Statistical methods	Conclusion
Novell A et al (14)	2014	1998-2009	Spain	152 (107 M, 45 F)	HIF1-α	45-50.4 Gy + Fluorinated pyrimidines	71 (median)	Survival analysis (retrospective cohort study)	HIF1-a was identified as possible predictive/prognostic value on progression free survival of rectal cancer in stage II/III.
Havelund BM et al (18)	2012	2007-2011	Denmark	86 (62 M, 24 F)	HIF1-a GLUT-1	50.4-60 Gy + Uracil/Tegafur	62 (median)	Survival analysis (prospective cohort studv)	Although no significant predictive impact of the fluctuation of the markers was found, there appears to be an obvious biological dynamics in HIF1-0. which calls for further investigation.
Saigusa S et al (23)	2011	2001-2008	Japan	52 (42 M, 10 F)	HIF1-α	20-45 Gy + Uracil/Tegafur	64.5 (median)	Survival analysis (retrospective cohort study)	HIF1-a gene expression may be strongly associated with poor prognosis in rectal cancer after preoperative CRT.
Havelund BM et al (24)	2011	1998-2009	Denmark	86 (49 M, 37 F)	HIF1-α GLUT-1	50.4-60 Gy + Uracil/Tegafur	66.7 (median)	Survival analysis (retrospective cohort study)	The present study did not suggest any predictive or prognostic value of pretreatment HIF1-a or GLUT-1 expression in patients with rectal cancer treated with preoperative CRT.
Shioya M et al (25)	2011	2003-2006	Japan	50 (38 M, 12 F)	HIF1-α	40-50 Gy + 5-FU/LV	59 (median)	Survival analysis (retrospective cohort study)	There was no correlation between HIF1-a and pathological evaluation of rectal cancer.
Korkeila E et al (33)	2012	2000-2009	Finland	178	HIF1-α GLUT-1 CA-9	50.4 Gy		Survival analysis (retrospective cohort study)	The most remarkable panel consisting of moderate/strong expression of CA-9, positive HIF1-a expression and negative/ weak GLUT-1 expression was associated with risk of death from rectal cancer.
Korkeila E et al (12)	2011	2000-2009	Finland	178	GLUT-1	50.4 Gy		Survival analysis (retrospective cohort studv)	A trend towards longer disease-free survival among patients in favour of negative/weak GLUT-1 staining in the operative samples after long-course radiotherapy is demonstrated.
Guedj N et al (31)	2011	2006-2010	France	61 (35 M, 26 F)	GLUT-1 CA-9	45-50.4 Gy	60.5 (mean)	Survival analysis (prospective cohort study)	CAIX, but not GLUT-1, was shown as an effective molecular marker of post-CRT response.
Debucquoy et al (30)	2009	1996-2003	Belgium	99 (64 M, 35 F)	CA-9	45 Gy + FU (79) 30 Gy (20)	63 (median)	Survival analysis (retrospective cohort study)	For CA-9 no significant differences were seen before and after therapy.
Korkeila E et al (32)	2011	2000-2008	Finland	178	HIF1-α GLUT-1 CA-9	50.4 Gy		Principal component analysis	HIF1-a and CAIX were significant independent predictors of disease-specific survival. None of the biomarkers (HIF1-a, GLUT-1, CAIX) were significant predictors of disease-free survival.
Saigusa S et al (20)	2012	2001-2008	Japan	52 (42 M, 10 F)	GLUT-1	20-45 Gy + Uracil/Tegafur	64.5 (median)	Survival analysis (retrospective cohort study)	An elevated GLUT-1 expression may be a useful predictor of recurrence and poor prognosis in rectal cancer after preoperative CRT.
Brophy S et al (19)	2009	2000-2005	Ireland	69 (54 M, 15 F)	GLUT-1	46-54 Gy + FU	63 (median)	Retrospective correlative study	GLUT-1 may be a useful predictive marker of response to CRT in rectal cancer.
Shim BY et al (34)	2013	2005-2008	South Korea	104 (71 M, 33 F)	GLUT-1	50.4 Gy + FU + Leucovorin	62 (median)	Survival analysis (retrospective cohort study)	GLUT-1 expression is predictive and prognostic factor for pathologic complete response and recurrence in rectal cancer patients treated CRT.
CAIX: carbonic	anhydrase IX;	CRT: chemorad	liotherapy; H	IF-1A: hypoxia ii	nduced factor	l-α; GLUT-1: gluco	se transporte	rl	

vasion. In addition, Shioya et al. [19] demonstrated that the overall survival rate, the recurrence-free survival rate (RFS) and the metastasis-free survival rate at 3 years are significantly better in the HIF-1a negative group. Saigusa et al. [17] suggested an optimal cut off value of HIF-1a expression for recurrence-free survival and overall survival. Patients with HIF-1a levels above cut-off values showed a significantly worse overall survival than those with HIF-1a levels below cut-off values. However, the other authors have shown that HIF-1a was not significantly associated with disease-free survival



Figure 3. The risk of Bias summary (Cochrane Collaboration).

and that there is no prognostic impact of HIF-1a [18,22].

Carbonic anhydrase IX

Carbonic anhydrases (CAs) are zinc metalloenzymes, vital to many biological and physical functions. Carbonic anhydrase IX (CAIX) is a transmembrane isozyme involved in the control of cell proliferation and cellular transformation [21]. Increased expression of CAIX has been shown in a variety of tumor types compared with normal tissues [15]. CAIX expression has been proven to be strongly induced by hypoxia and plays an important role in the growth and survival of tumor cells under normoxic and hypoxic conditions [22].

A study by Debucquoy et al. [24] suggested that CAIX has no prognostic value. However, Guedj et al. [23] found that CAIX expression was significantly lower in biopsy specimens from responders than from non-responders. This finding is also reflected in the Dukes' stage with a higher rate of CAIX positivity in earlier cancers (Dukes' A and B) compared with later cases (Dukes' C). In addition, assessment of TNM stage reached statistical significance when comparing CAIX positivity between early (stage I and II) and late stage (stage III) rectal cancers. Korkeila et al. [25], showed that CAIX is significantly associated with disease-specific survival (DSS).

Glucose Transporter -1

GLUT-1 is a member of the protein-glucose transporters family. It allows the energy independent transport of glucose across the cell membrane and thereby provides the cell with substrate for glycolysis [28,29]. GLUT-1 is one of the downward targets of HIF-1a [16], as it facilitates the metabolic adaptation of cells to hypoxia [26,27]. GLUT-1 is essential for survival of the cells under hypoxic conditions [26,27]. It is expressed in a few normal tissues such as the blood-brain barrier and the perineum, but is extensively expressed in malignancies such as colon cancer, lung carcinomas and pancreatic cancer [28]. Since hypoxic conditions in several tumors have been shown to be indicators of poor prognostic outcome of CRT, GLUT-1 is of great interest as a biomarker related to tumor hypoxia and therefore resistance to therapeutic agents and finally poor prognosis [26].

GLUT-1 expression does not differ significantly between tumor stages nor is it associated with the degree of pathological malignancy [29]. Korkeila et al. [21] conducted a study investigating the GLUT-1 status in rectal tumors, while also comparing the GLUT-1 expression in preoperative tumors with their corresponding operative specimens. It was

Countries	HIF1-a	GLUT-1	CAIX
Spain	152 (107 M, 45 F)	-	-
Denmark	172 (111 M, 61 F)	172 (111 M, 61 F)	-
Japan	102 (80 M, 22 F)	52 (42 M, 10 F)	-
UK	90 (56 M, 34 F)	-	101 (61 M, 40 F)
Finland	178	178	178
France	-	61 (35 M, 26 F)	61 (35 M, 26 F)
South Korea	-	104 (71 M, 33 F)	-
Ireland	-	69 (54 M, 15 F)	-
Belgium	-	-	99 (64 M, 35 F)

Tabl	e 2.	Summary	of	cases	per	country	and	factor
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CAIX: carbonic anhydrase IX; HIF-1A: hypoxia induced factor 1-a; Glut-1: glucose transporter 1; M: male, F: female



Figure 4. The risk of bias summary (Cochrane Collaboration): review authors' judgements about each risk of bias item presented as percentages across all included studies.

observed that 62% of the GLUT-1 positive preoperative biopsies remained positive after radiotherapy (RT) or chemoradiotherapy (CRT), whereas the remaining 38% changed to negative in the operative specimen. Negative GLUT-1 expression in the biopsies remained negative after chemotherapy (CT) or CRT in 72% of the operative samples and 28% changed to positive.

As far as tumor regression and pathologic response after preCRT are concerned, there are conflicting findings. Others have not found significant association between GLUT-1 expression during therapy and pathological response to CRT [16]. However, other authors demonstrated that GLUT-1 is a biomarker related to chemoradiosensitivity, as a higher expression of GLUT-1 was associated with a significantly lower rate of pathological complete response (ypCR) compared to a low expression of GLUT1 protein [30]. According to Havelund et al. [19] no correlation was found between the TRG and GLUT-1 and no difference in GLUT-1 expression between responders and non-responders. These findings are in corroboration with the observations of Guedj et al. [23] who found similar results and in opposition to those made by Shim et al. [30] who observed that GLUT-1 was significantly higher in the poor response group.

In the study of Korkeila et al. [21], which was previously mentioned, no significant differences have been observed in disease-free survival (DFS) or disease-specific survival (DSS) in the shortcourse radiotherapy group in terms of GLUT-1 expression. In the long course radiotherapy group, the patients who had negative orweak staining intensity in the operative specimen, had a tendency towards better DFS as compared to the patients with moderate/strong staining intensity [21]. Other authors, nevertheless, have not found significant association between GLUT-1 and DFS [20]. Furthermore, the expression of GLUT-1 protein has demonstrated a significant correlation with the time to recurrence [30]. The prognostic impact of GLUT-1 is controversial, as some confirm that GLUT-1 is an independent unfavorable prognostic factor [30]

of GLUT-1 [19].

Discussion

Tumor response to chemoradiotherapy has been proven to be widely influenced by the tumor microenvironment, such as tumor hypoxia. Tumor hypoxia can lead to resistance to chemoradiation by depriving tumor cells of oxygen, which is essential for the cytotoxic effects of chemoradiotherapy agents. In this review, we have provided an overview of the predictive models of three hypoxia related factors, HIF-1a, CAIX and GLUT-1.

During this review, we have observed controversial findings concerning the effect of these factors on tumor regression, their predictive value and their connection with recurrence-free survival and overall survival. This may be a result of the usually limited number of patients in the studies, the high dimensionality in therapy regimens and the different histopathological evaluation of tumor response in those studies.

We concluded that HIF-1a, GLUT-1 and CAIX may be connected with tumor response to preCRT

whereas others have found no prognostic impact but there is still skepticism towards their clinical use as predictors of outcome. Therefore, there is a need to produce larger and more extensive cohort studies in order to find whether these predictors can be used in practice. Each parameter could also be combined with other models, thus, providing a more integrated way to predict a response to preCRT.

Authors' contributions

All colleagues listed as authors on the manuscript have contributed significantly to preparing the manuscript. DIV, MT and PL contributed to the conception and design of the study. NT and GR made the acquisition of data. CG and KP analyzed the data. AL revised the manuscript for important intellectual content. GK made the final approval of the version to be submitted. All authors have read and approved the final version of the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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