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The connection between *Fusobacterium nucleatum* levels and chemoresistance in colorectal cancer – a systematic review

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Introduction. The lack of response to chemotherapeutic drugs is one of the major challenges faced in the treatment of colorectal cancer. Several studies have indicated that the microbiome of the bowel affects the treatment response and specifically, certain bacterial species contribute to the development of chemoresistance. With *Fusobacterium nucleatum* being one of the bacterial species frequently found in the bowel of colorectal cancer patients, the present systematic review was undertaken to gather the existing literature on the relationship of *Fusobacterium nucleatum* with chemotherapy response.

Material and methods. Major online academic databases were searched using a combination of keywords and Boolean operators, in order to retrieve literature on the topic from inception until February 2023. Observational studies with relevant information were included in the present systematic review and their quality was assessed.

Results. A total of 7 studies with 2,280 colorectal cancer patients who underwent adjuvant or palliative chemotherapy were included in the qualitative synthesis. No study with a major risk of bias was found after a quality assessment. The majority of studies observed poorer prognosis in patients who had high levels of *Fusobacterium nucleatum* in their bowel, although, due to the small number of studies, a meta-analysis could not be performed.

Conclusions. High levels of *Fusobacterium nucleatum* result in a poorer response to chemotherapy in colorectal cancer. Nevertheless, to further verify this assertation, more observational and experimental studies must be undertaken in the clinical field.

Key words: colorectal cancer, colon cancer, *Fusobacterium nucleatum,* chemoresistance

Introduction

Colorectal cancer (CRC) remains one of the leading causes of cancer-related mortality globally, with its incidence rate predicted to be doubled in the upcoming decade [1]. One of the underlying reasons for its high mortality in some patients is the lack of response to chemotherapy, also known as chemoresistance, since adjuvant and palliative chemotherapy remain one of the main therapeutic strategies in the therapy of CRC [2–4]. There are many possible molecular mechanisms that can affect the response to chemotherapy in cancer cells, usually involving genetic mutations that occur during the tumor's progression [5]. Nevertheless, other factors may also result in the development of resistance, especially those which trigger genetic mutations. The bowel's microbial composition, typically known as the microbiome, has recently been found to be related to the formation of drug resistance

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in CRC and indeed increase the risk of occurrence of certain related mutations [6, 7].

One of the most commonly found bacteria in the microbiome of CRC patients is the anaerobic gram-negative species *Fusobacterium nucleatum* (*Fn*); it has been shown that the latter species affects the formation and progression of tumors [8]. For this reason, CRC patients are sometimes screened for *Fn* levels in the bowel and are classified as *Fn*-positive or *Fn*-negative based on the concentration of the species in biopsy or stool samples [9, 10]. More specifically, research has indicated that *Fn* is related to poor prognosis in CRC, suggesting that the bacterium may perhaps be an underlying cause of drug resistance [11, 12]. Hence, in this study, a systematic review was performed on all existing literature that relate levels of *Fn* with chemotherapy outcomes in colorectal malignancies, so as to assess whether there is a relation between chemoresistance and *Fn*-positivity. Such an association would certainly provide new insights for medical oncologists and researchers on how to combat drug resistance and improves the outcomes of chemotherapy in CRC.

Material and methods

The present systematic review has been registered in the OSF Registries platform on 16 January 2024, after the completion of the study.

Search strategy

A systematic literature search was performed in the electronic databases PubMed, SCOPUS and Embase from inception until January 2023, using a combination of keywords and Boolean operators. The keywords used were: "*F. nucleatum*", "*Fusobacterium nucleatum*", "colorectal", "colon", "bowel", "cancer", "carcinoma", "tumor", "chemoresistance" and "chemotherapy resistance". The search was limited to citations written in English.

After the retrieval of the literature, duplicate citations were removed by using the citation manger EndNote and subsequently, all remaining citations were assessed for eligibility by screening their titles and abstracts. The inclusion criteria for this systematic review were observational studies which compared outcomes between *Fn*-positive and *Fn*-negative bowel cancer patients who received chemotherapy. In turn, full-text versions of citations were assessed and studies which met the inclusion criteria were included in this review. The search and screening process was performed by two independent reviewers (DK and VT).

Data extraction and quality assessment

Data regarding the design of the studies, the number of participants, the stage and position of the tumors, the chemotherapy regimens used and the treatment outcomes were extracted from the eligible studies by two independent reviewers (DK and VT). In turn, the reviewers assessed the quality of the included studies using the Newcastle-Ottawa scale which evaluates the quality of the inclusion process of each study, the comparability between the cohorts and their respective outcomes [13]. Disagreements did not arise between the two reviewers during the whole selection and assessment process.

Results

Included studies

The electronic database search retrieved a total of 111 articles, out of which only a total of 63 articles remained after removal of duplicates. After screening the abstracts and titles of each citation, a total of 24 citations were deemed irrelevant and hence excluded from the study. From the remaining 39 citations, which were assessed based on the content of their full texts, a total of 12 citations did not contain relevant information on chemotherapy outcomes, 9 citations were review articles, 8 citations were animal studies and 3 citations were *in vitro* studies. Since the included studies were very heterogenous in their design and method of conduction, the presentation of the results varied and our review only contained a small number of studies, a meta-analysis was not conducted. Figure 1 presents a PRISMA diagram of the search strategy and inclusion process. The characteristics of the included studies are summarized in table I.

In general, the studies involved in this systematic review included a total of 2,280 patients with tumors in the colon or the rectum who underwent adjuvant or palliative chemotherapy. All studies, except one, found that *Fn*-positivity was associated with a higher risk of mortality and a lower survival expectancy in patients taking chemotherapeutic drugs, indicating that *Fn* colonies in the bowel are associated with a lower response to chemotherapy [14, 15, 17–20]. The study

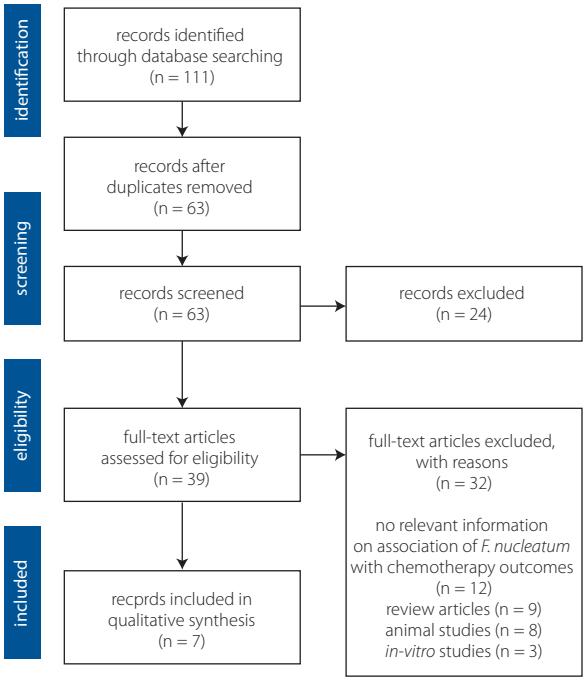

Figure 1. PRISMA diagram of the search strategy and inclusion process

Table I. Characteristics of the studies included in the qualitative synthesis

Study (author, year)	Type of study	Participants (n)	Tumor stage	Tumor location	Chemotherapy regimen	Association of *Fn*-levels and outcomes	p value
Borozan et al., 2022 [14]	retrospective cohort	total: 736 *Fn*-positive: 83 *Fn*-negative: 653	stage I, II, III and IV (distribution unknown)	colon and rectum	unknown	HR = 1.92 (CSM of *Fn*-positive *vs.* *Fn*-negative)	0.029
Chen et al., 2019 [15]	retrospective cohort	total: 91 *Fn*-positive: 25 *Fn*-negative: 66	stage II: 51 stage III: 40	colon: 77 rectum: 14	adjuvant FOLFOX or XELOX	HR = 2.09 (CSM of *Fn*-positive *vs.* *Fn*-negative)	0.032
Hanna et al., 2022 [16]	retrospective cohort	total: 38 *Fn*-positive: 12 *Fn*-negative: 26	stage II: 9 stage III: 29	rectum	unknown	no association found	–
Kim et al., 2018 [17]	retrospective cohort	total: 424 *Fn*-positive: 272 *Fn*-negative: 152	stage II and III (distribution unknown)	colon and rectum	adjuvant FOLFOX	association of high *Fn* levels with lower overall survival (only in right-sided colon cancers)	–
Lee et al., 2018 [18]	retrospective cohort	total: 118 (distribution unknown)	stage IV	colon and rectum	palliative FOLFOX, XELOX, SOX, FOLFIRI or capecitabine monotherapy	HR = 1.69 (CSM of *Fn*-positive *vs.* *Fn*-negative)	0.034
Oh et al., 2019 [19]	retrospective cohort	total: 593 *Fn*-positive: 204 *Fn*-negative: 389	stage II: 90 stage III: 503	colon and rectum	adjuvant FOLFOX or XELOX	HR = 0.4 (DFS of *Fn*-positive *vs.* *Fn*-negative)	0.043
Yan et al., 2017 [20]	retrospective cohort	total: 280 *Fn*-positive: 187 *Fn*-negative: 93	stage III: 218 stage IV: 62	colon: 150 rectum:130	adjuvant FOLFOX	HR = 2.13 (CSM of *Fn*-positive *vs.* *Fn*-negative)	<0.001

Fn – *Fusobacterium nucleatum*; HR – hazard ratio; CSM – cancer-specific mortality; DFS – disease-free survival; FOLFOX – folinic acid, fluorouracil, and oxaliplatin; XELOX – capecitabine and oxaliplatin; SOX – S-1 and oxaliplatin; FOLFIRI – folinic acid, fluorouracil, and irinotecan

which found no statistically significant difference, included patients with rectal cancer only [16]. One study by Kim et. al limited the results only to patients with right-sided carcinomas, in other words, carcinomas found within the cecum, the ascending or the transverse colon [17]. In all studies, a regimen of folinic acid, fluorouracil, and oxaliplatin (FOLFOX) or capecitabine and oxaliplatin (XELOX) was used for adjuvant post-surgery chemotherapy. However, in the case of palliative chemotherapy, the S-1 and oxaliplatin (SOX) or folinic acid, fluorouracil, and irinotecan (FOLFIRI) regimens were also used in some patients [18]. Overall, most studies found an approximately twofold hazard ratio of cancer-specific mortality (CSM) in patients who were *Fn*-positive [14, 15, 18, 20].

Quality assessment

The Newcastle-Ottawa scale was used by two reviewers (DK and VT) to evaluate the quality of each individual study included in this systematic review and the results have been recorded in table II. In general, the studies were classified as good quality in accordance with the Agency for Healthcare Research and Quality (AHRQ) standards, since for all studies, 3 or 4 stars were given in the selection domain, 1 or 2 stars in the comparability domain and 2 or 3 stars in the outcome domain [13]. This confirms that the conclusions of this systematic review are not highly affected by bias.

Discussion

The present systematic review evaluated all existing literature relating levels of *Fn* to the efficacy of chemotherapy in patients with CRC. As seen through the results, the existence of high levels of *Fn* lower the response to chemotherapy in CRC patients and are associated with poorer prognosis. Indeed, *in-vitro* studies have managed to discover that *Fn* can promote chemoresistance by triggering signaling pathways which result in the expression of drug efflux pumps, deactivation of apoptotic mechanisms and modulation of cellular autophagy [21, 22]. The results of this review verify the latter assertations in clinical studies since patients with *Fn* in their bowel have a poorer response to chemotherapy.

Nonetheless, as mentioned previously, one study did not find statistically significant results in the case of rectal cancer and another study only found significant results in right-sided carcinomas [16, 17]. This finding brings up the topic of tumor sidedness in CRC, which has been of great interest in recent years. In fact, a meta-analysis in 2017 concluded that tumors found in the right colon are associated with poorer prognosis results [23]. Therefore, it is rational for studies involving right-sided tumors to show poorer prognosis than left-sided tumors, which also include rectal tumors. On the other hand, researchers have discovered that *Fn*-positive cancers are much more frequent in right-sided carcinomas and quite rare in rectal tumors; therefore a lack of relationship between *Fn*-positivity and chemoresistance in rectal tumors does not significantly affect the conclusions of this review [24, 25].

It is also worth mentioning that some limitations exist in this systematic review, although it was performed in complete accordance with the Cochrane guidelines, and no potential bias was found in the quality assessment using the Newcastle-Ottawa scale [26]. Foremost, all included studies had a retrospective design, making them more prone to bias and therefore lowering the quality of the evidence [27]. Moreover, the whole review included only a few number of patients, lowering the statistical reliability of the results [28]. Simultaneously, the fact that the qualitative synthesis only included seven studies reporting their outcomes in different ways, made it difficult for a formal meta-analysis to be conducted.

Table II. Quality assessment of studies included in the review

Study (author, year)	Newcastle-Ottawa scale scores			
	Selection	Comparability	Outcome	Total
Borozan et al., 2022 [14]	4	2	3	9
Chen et al., 2019 [15]	3	2	1	6
Hanna et al., 2022[16]	3	1	2	6
Kim et al., 2018 [17]	3	2	2	7
Lee et al., 2018 [18]	3	2	2	7
Oh et al., 2019 [19]	3	2	2	7
Yan et al., 2017 [20]	3	2	2	7

Conclusions

The present study managed to collect evidence indicating that *Fn*-positivity is directly related to the development of chemoresistance. Hence, one of the novel strategies for better CRC chemotherapy outcomes would be to adjust the colorectal microbiome and eradicate the existence of the species *Fusobacterium nucleatum* within the bowel. There are several ways of achieving the latter, including the adjuvant administration of antibiotics such as metronidazole to eradicate anaerobes [29, 30]. Other methods of regulating the microbiome and eradicating such bacteria is through the use of probiotics and including specific foods to the patient's diet, such as yogurt, kefir and sourdough bread alongside anticancer treatments [31–33]. Indeed, a patient's diet has been found to be correlated with chemotherapy outcomes [34]. Nevertheless, there is an urgent need for more studies and clinical trials to be conducted in this field in order to evaluate the effectiveness of the forementioned methods and their results on chemotherapy response. More prospective studies should also be undertaken in order to collect stronger evidence that *Fn*-positivity contributes to

the development of chemoresistance in CRC, allowing researchers to conduct a meta-analysis confirming the assertation.

Article information and declarations

Author contributions

Datis Kalali – conceptualisation, methodology, investigation, project administration, supervision, formal analysis, manuscript draft.

Vasiliki Tzalili – methodology, investigation, formal analysis.

Doxakis Anestakis – manuscript revision and editing.

Conflict of interest

None declared

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