



Tumour Review

Identification of prognostic DNA methylation biomarkers in patients with gastrointestinal adenocarcinomas: A systematic review of epigenome-wide studies

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ABSTRACT

This systematic review aims to summarize epigenome-wide studies on aberrant DNA methylation and its association with survival in patients with gastrointestinal adenocarcinoma. The 15 studies identified showed a large variety of methodological approaches for the identification of prognostic epigenetic markers from genome-wide methylation analyses. None of the findings were reported by more than one study in this systematic review. Further validation studies, a better reporting of methods and results are needed, as well as a clearer definition of investigated outcomes. At present, no conclusions can be drawn on the clinical relevance of the reported epigenetic markers.

Introduction

Cancers of the gastrointestinal (GI) tract are among the cancers with the highest incidence and the leading cause of cancer death worldwide [1]. They include malignant pathology of the oesophagus, stomach, small bowel, colon, rectum, anus, and accessory organs of the digestive system, such as pancreas, liver and gallbladder [1]. Despite the progress in screening and therapies, this group of cancers remains an important problem for public health, in both developed and developing countries [2]. The TNM Classification of Malignant Tumours (TNM) is still the most important classifier for treatment and prognosis of these cancers [3]. However, the consideration of molecular tumour characteristics becomes increasingly important in the perspective of personalized oncology.

The influence of epigenetic changes on tumour progression and clinical outcomes has been widely described for different cancers [2]. For example, the CpG island methylator phenotype (CIMP) is an epigenetic pattern with a high frequency of aberrant methylation whose role has been investigated in cancer development and in the classification of cancer patients [4]. DNA methylation holds great potential as a diagnostic biomarker, as it can be easily translated from a laboratory

setting into hospital routine, due to the stable nature of DNA and its amplification [5]. Particularly, disease-associated CpG methylation has been associated with nearly every type of GI cancer [6,7]. However, the strategies employed to investigate epigenetic biomarkers are very heterogeneous across the literature, ranging from candidate gene approaches to genome-wide methods [5].

The development of CpG microarray platforms constituted a milestone in the field of epigenetic research, allowing high-throughput analysis of epigenetic changes in tumour tissue. Another important contribution comes from public databases, such as The Cancer Genome Atlas (TCGA) project, a widely-used platform to assess the effect of differentially methylated CpGs (dmCpGs) on prognosis in different tumours, including those of the GI tract [8–10]. Among the GI cancer subtypes, adenocarcinomas constitute the most common malignancy for stomach, small bowel, pancreas, colon, rectum and gallbladder [1,11–14] and the most common type of oesophageal cancer in developed countries [15], while it accounts for only 20% of anal cancers [16] and it is absent in liver cancer [11]. Adenocarcinomas are cancers of the epithelial tissues that arise from specialized secreting cells and can spread to other areas of the human body [17]. Considering the prevalence of adenocarcinomas in GI cancers and the heterogeneity of

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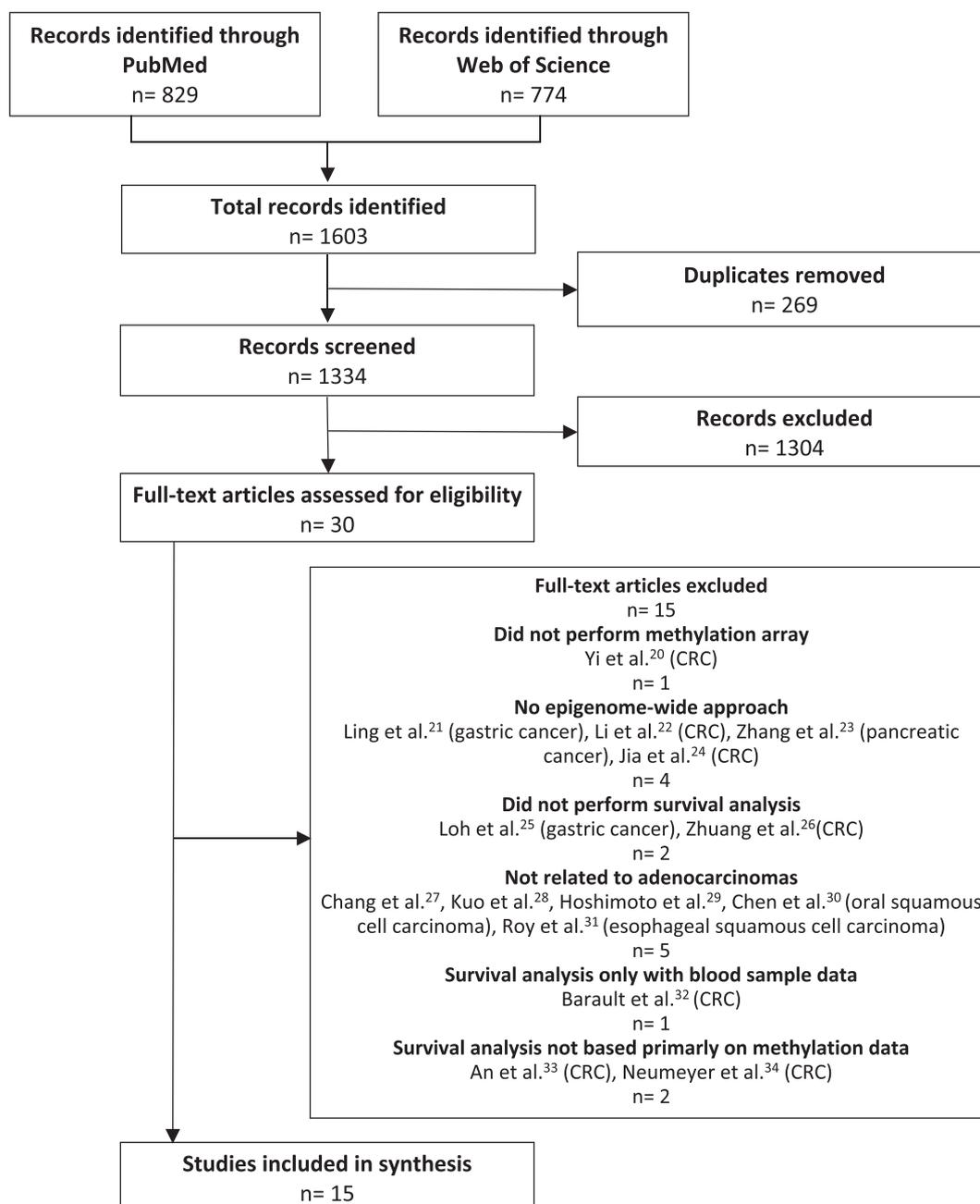


Fig. 1. Systematic literature search, adapted from PRISMA 2009 statement.

methodological strategies, we aimed to systematically summarize studies that investigated the role of DNA methylation patterns in the prognosis of gastrointestinal adenocarcinomas using an epigenome-wide approach.

Methods

Systematic review protocol and information sources

This systematic review was conducted and reported according to a protocol developed in line with the PRISMA statement [18]. The PubMed and Web of Science (WoS) databases were searched from inception until August 2019 by one author (MD) and the search strategy was generated using MeSH terms and free-text words related to cancer, epigenetic signatures and survival (search term list in [Suppl. File 1](#)). Filters were applied to exclude books and editorials and limit the search to articles written in English language. Finally, the reference list of

relevant papers was scanned to find potential additional eligible articles. Duplicate records obtained from PubMed and WoS were removed.

Eligibility criteria and selection of studies

The title and abstract of the identified records were screened by one author (MD) and selected according to the following eligibility criteria: (1) studies that included patients of intestinal adenocarcinomas at any stage, (2) studies that adopted an epigenome-wide approach and performed a methylation array, (3) studies that investigated the association between aberrant methylation (of single CpGs, genes, genetic regions) and cancer survival, prognosis and/or therapy response, (4) studies performed in humans and (5) studies that identified dmCpGs by analysing tumour tissue samples. Reviews and studies with a candidate-gene approach were excluded, as were studies on diagnostic methylation markers and studies that analysed blood samples. Also, descriptive studies on tumour subtypes and clustering were not considered for

inclusion in the review.

Data extraction

Assessment of study characteristics was defined prior to the data extraction and included: year of publication, cancer location, disease stages, data source, methylation array type, population sample-size, type of validation, sample-size of the validation data set, follow-up time, correction for multiple testing, type of outcome and outcome measurements (Hazard Ratio and 95% Confidence Interval), type of tissues compared for obtaining the dmCpGs, list of CpGs (when provided), cut-point used to define a “differentially methylated” site, risk/prognostic score (when provided). Data was extracted by two authors independently (MD, EA) and compared, and differences were resolved in consensus.

Data quality assessment

The quality evaluation was performed independently by two authors (MD, EA). The thirteen criteria for the quality assessment were based on the *Newcastle-Ottawa Quality Assessment Scale* (NOS) and on the *REporting recommendations for tumour MARKer prognostic studies* (REMARK)[19] guideline. The records were analysed and one point was assigned for each of the following items: clear explanation of the study objective, clear statement of the data source, clear description of the patient selection and enrolment, report of the sample size of the validation data set, adjustment for major confounders (sex, age and tumour stage) in the survival analysis, clear description of the methodological approach, correction for multiple testing, reporting of the identified dmCpGs or of a prognostic score, follow-up ≥ 3 years and discussion of study limitations. Notably, one point was awarded for internal validation and two points in case of external validation of results. The records were classified into three “quality classes”. Studies that obtained a total score of up to five points were considered “low quality”, studies with a score between six and eight points as “medium quality”, and studies with a score from nine to thirteen were defined as “high quality”.

Summary of findings

The main characteristics and findings of each study were summarized, and the different methodological approaches used by the studies were illustrated in a figure. No meta-analysis was performed because of the heterogeneous methodologies and the assorted definitions of differentially methylated sites across the included studies.

Results

Study selection

The search identified 774 records in WoS and 829 records in PubMed. After duplicate removal, the titles and abstracts of 1334 articles were screened (Fig. 1). After exclusion of records not in accordance with the eligibility criteria, 30 potentially relevant articles were retrieved for full-text assessment. In total, 15 studies were excluded because: did not use a methylation array [20], did not use an epigenome-wide approach [21–24], did not perform survival analysis [25,26], were not related to adenocarcinomas [27–31], performed survival analysis only based on differentially methylated sites detected in blood samples [32], did not base the survival analysis primarily on methylation data [33,34]. Finally, 15 studies were eligible for inclusion and summarized in this review.

Study characteristics

The characteristics of the included studies are summarized in Table 1. Five studies were based on tumour tissue samples from patient

cohort studies, nine conducted the analysis based on data from the TCGA database and one did not clearly state the source of the tissue samples. Notably, two studies used data from the GEO database in addition to the TCGA database. Among the studies that used the TCGA database, Hao et al. [35], Li et al. [36] and Yang et al. [10] investigated the potential of prognostic epigenetic biomarkers in multiple cancer locations. However, only the data (e.g. sample size and outcome measurements) relevant to GI adenocarcinomas were considered for the review. Only five studies (30% of the total) reported a sample size ≥ 300 patients. Most studies used the Illumina 450 K BeadChip array, except for Gaedke et al. [37], who employed a CpG island array, and Chen et al., that did not report the array type used in the study [38]. The most frequent outcome was overall survival (OS), considered as main outcome by 65% of the studies. Gaedke et al. [37] investigated disease-free survival (DFS) and Martinez-Cardus et al. [39] analysed relapse-free survival (RFS) together with OS. Notably, Zhang et al. reported DFS in the article body, but indicated OS in the figures. Considering the overall limited size of the patient populations, the validation of the findings constituted a key point of our data quality assessment. Gaedke et al. [37], Lin et al. [41], Chen et al. [38], and Zhang et al. [40] validated the genes or dmCpGs in an external data set, whereas Gündert et al. [42], Nones et al. [43], Martinez-Cardus [39], and Hu et al. [44] performed internal validation. Hao et al. [35] performed both internal and external validation while Doecke et al. [9], Kim et al. [45], Li et al. [36], Hou et al. [46], and Liang et al. [47] did not perform any validation of the survival analysis results. Disease stage of the patient populations differed widely across the studies and was not reported in four studies [9,10,36,47].

dmCpGs, genes and risk scores

Despite the common study objective, the 15 studies used different definitions of what the methylation of interest is: single sites (CpGs), gene promoters or genetic regions. Five studies investigated the role of significantly dmCpGs and prognosis [10,35,36,42,46], while eight studies highlighted the prognostic potential of gene aberrant methylation, defined as the differential methylation of one CpG on one gene or as the mean methylation value of multiple CpGs on one gene [9,37,40,41,43–45,47]. Notably, Chen et al. [38] focused mainly on gene expression, exploring DNA methylation as a complementary factor to support the findings. The study by Martinez-Cardus et al. [39] was the only study that focused on intra-tumour heterogeneity rather than investigating dmCpGs between tumour tissue and normal tissue. Two studies developed a classifier based on their findings, that was employed to predict survival in CRC patients: Gündert et al. proposed the ProMCol classifier, based on 20 dmCpGs [42], and Li et al. the Iterative Deletion Feature Optimal (IDFO) pipeline [36]. A prognostic index was calculated by Yang et al. [10] and Hou et al. [46], a risk score was developed by Hu et al. [44] and Chen et al. [38], and a coefficient of epigenetic homogeneity constituted the core of the survival analysis performed by Martinez-Cardus et al. [39]

Methodological strategies

In general, the included studies proposed nine different methodological strategies, that we tried to illustrate from their description in Fig. 2. Four studies started by performing the epigenome-wide methylation array in tumour tissue and normal tissue in a small group of patients, that ranged from eleven to twenty-six individuals [37,40,41,45]. After selecting the CpGs that were significantly differentially methylated between tumour tissue and normal tissue, the hits were validated in a larger set and their prognostic potential was assessed in survival analysis. Notably, Kim et al. [45] did not perform validation of the findings. A similar approach was used by Yang et al. [10] and Hao et al. [35], who used whole-genome methylation data from the TCGA database from multiple cancer locations (colon,

Table 1
Characteristics and summary of findings.

Authors, year	Country	Data Source	Cancer Location	Type of Array	N	Tumor Stage	Outcomes	Type of Validation	HR reported for	HR, 95% CI	Quality of Evidence
Kim et al. [45] 2014	Multi	TCGA database	Colon-rectum	Illumina HM 27 K/450 K	524	II-IV	Survival	Not performed	High vs low SRFP1 methylation hyper vs hypo	1.93 (1.12–3.33)	Low
Gaedke et al. [37] 2014	Netherlands	Patient cohort study	Rectum	CpG Island Array	61	II-IV	DFS	External	gene methylation High vs low	4.09 (1.12–14.87)	High
Nones et al. [43] 2014	Australia	Not reported	Pancreas	Illumina HM 450 K	196	I-IV	Survival	Internal	gene methylation greater than 2 years vs < 2 years survival	Not reported	Medium
Li et al. [22] 2015	Multi	TCGA database	Colon	Illumina HM 27 K/450 K	255	Not reported	Survival	Internal	Aberrant vs normal gene expression	1.77; CI not reported	Low
Doecke et al. [9] 2016	Multi	TCGA database	Colon	Illumina HM 450 K	173	Not reported	Survival	Not performed	High vs. low EH coefficient	0.48 (0.17–0.87)	Low
Martinez-Cardus et al. [39] 2016	Spain	Patient cohort study	Colon-rectum	Illumina HM 450 K	79	I-III	RFS; OS	Not performed	High vs low BEND5 methylation	2.91 (1.03–8.16) not reported	Medium
Lin et al. [41] 2017	Taiwan	Patient cohort study	Colon-rectum	Illumina HM 450 K	135	I-IV	OS	External	High vs low Prognostic Index (PI)	1.28 (1.04–1.58)	High
Yang et al. [10] 2017	Multi	TCGA database	Colon, pancreas, rectum	Illumina HM 450 K	339, 156, 106	Not reported	OS	Not performed	High vs low risk, based on	HR for each dmCpG (Suppl. Material) no association	Medium
Hao et al. [35] 2017	China	TCGA, 2 patient cohort studies	Colon	Illumina HM 450 K	275	I-IV	OS	Internal and external	High vs low risk		High
Hou et al. [46] 2018	Multi	TCGA database	Colon-rectum	Illumina HM 450 K	379	II-IV	OS	Internal	High vs low risk	1.32; (1.29–1.36)	Medium
Chen et al. [38] 2019	Multi	APGI, TCGA and GEO	Pancreas	not reported	102	I-IV	OS	External	High vs low risk, based on a Risk Score	1.82; (1.09–3.05)	Medium
Gundert et al. [42] 2019	Germany	Patient cohort study	Colon-rectum	Illumina HM 450 K	572	I-III	OS; DSS	Internal	ProMCo Classifier	0.51; (0.41–0.63) 0.50; (0.38–0.67)	High
Hu et al. [44] 2019	Multi	TCGA database	Stomach	Illumina HM 450 K	194	I-III	OS	Internal	High vs low risk	1.49; (1.17–1.91)	Medium
Liang et al. [47] 2019	Multi	TCGA database	Colon	Illumina HM 450 K	314	Not reported	OS	Not performed	High vs low GDNF,RELN methylation	not reported	Low
Zhang et al. [40] 2019	Multi	GEO Database, TCGA	Colon-rectum	Illumina HM 27 K/450 K	Not reported	I-IV	OS	External	High vs low TGFBI expression	not reported	Low

Abbreviations: HR = Hazard Ratio; 95% CI = 95% Confidence Interval; TCGA = The Cancer Genome Atlas; DACHS = Darmkrebs: Chancen der Verhütung durch Screening; OS = Overall Survival; DFS = Disease Free Survival; RFS = Relapse Free Survival; EH coefficient = Epigenetic Homogeneity Coefficient, PI = Prognostic Index; dmCpG = differentially methylated CpG.

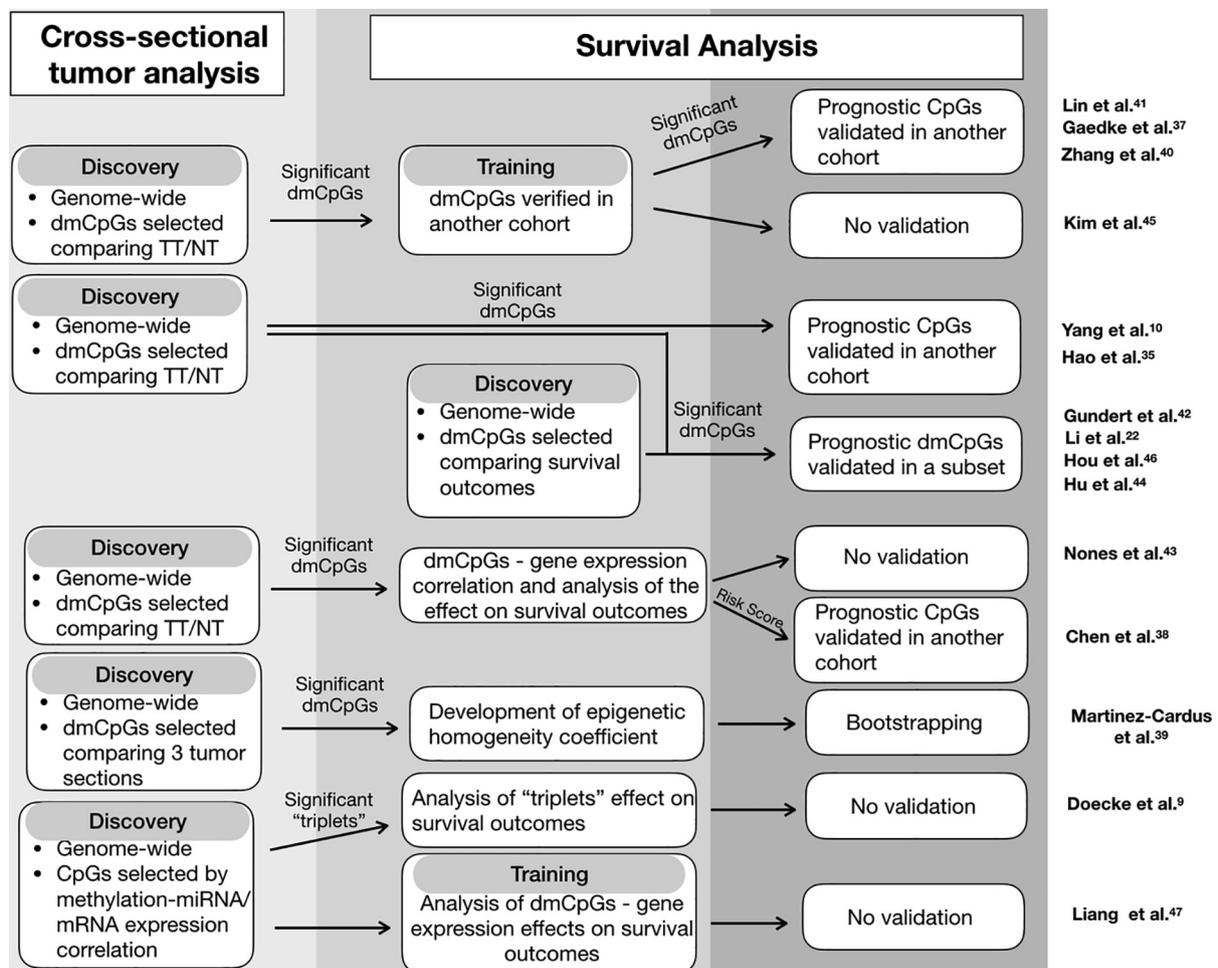


Fig. 2. Summary of methodological strategies adopted by the studies included in the review.

pancreas and rectum). After comparing tumour/normal tissue and selecting the dmCpGs, they performed survival analysis to see if the identified dmCpGs were associated with survival, and subsequently validated associated dmCpGs in another cohort. Moreover, Hao et al. [35] replicated the findings in a separate TCGA data set and in an independent patient cohort. Nones et al. [43] had a similar approach to the one of Yang et al. [10] and Hao et al. [35], that included the comparison of tumour/normal tissue samples to identify the dmCpGs, that were subsequently used in the survival analysis. In addition, they used the GenomeStudio Methylation module to calculate the correlation between dmCpGs and gene expression. After observing a significant correlation between methylation and gene expression, they observed how this correlation was associated with survival by performing survival analysis. However, they did not perform validation of the findings. Chen et al. [38] adopted an analogous methodology to the one of Nones et al. [43], except for validating their risk score in an external dataset. Gündert et al. [42] developed a prognostic classifier, analysing whole-genome methylation and clinical data, selecting the top-ranked dmCpGs combining survival analysis and tumour tissue – normal tissue comparison, and validating the findings in subsets of the patient cohort. Li et al. [36], Hou et al. [46], and Hu et al. [44] explored dmCpGs directly with respect to survival after cancer diagnosis with validation of findings in a subset. Martinez-Cardus et al. [39] analysed three different tumour tissue sections from each patient (tract surface, central bulk and invasive front) and developed a coefficient of epigenetic homogeneity. The methodology used by Doecke et al. [9] analysed correlations between DNA methylation, miRNA expression and mRNA expression in TCGA data of multiple tumour types, including

colon adenocarcinomas. The CpG-miRNA-mRNA “triplets” with the highest co-localized regulation were selected and their impact on CRC survival was evaluated performing survival analysis. Likewise, Liang et al. [47] explored the correlation between dmCpGs and gene expression, combining RNA and DNA methylation data.

Summary of findings

The main findings and relative outcome measurements are reported in Table 1. Notably, most of the identified studies focused on cancer of the colon and rectum, while two articles focused on pancreatic cancer, one investigated both colorectal and pancreatic cancer, and one focused on gastric cancer. Nine articles [9,37,38,40,41,43–45,47] investigated the role of aberrant gene methylation on different survival outcomes. The list of the relevant genes and corresponding methylation status is reported in Supplementary Table 3. In summary, the hypermethylation of *SFRP1*, *BEND5*, *AP000357.4*, *GZMA*, *GDNF* and *RELN*, and the hypomethylation of *MET*, *ITGA1*, *SULT1E1*, *IGF2BP3*, *MAP4K4*, *SERPINA*, *AC004702.2*, *GREB1L*, *TGFBI* and *TCN1* was associated with poorer survival in GI adenocarcinoma patients [38,40,41,43–45,47]. Also, a ten-gene panel, including *ADAP1*, *BARHL2*, *CABLES2*, *DOT1L*, *ERAS*, *ESRRG*, *RNF220*, *ST6GALNAC5*, *TAF4* and *SLC20A2*, was significantly associated with DFS ($p < 0.05$). Patients were grouped into a low methylation and high methylation group, with the latter associated with poorer prognosis (HR = 4.09, 95% CI = [1.12–14.87])[37]. The study by Doecke et al. [9] described the association between *C9orf3* and *GABRE* methylation and survival in colorectal adenocarcinomas, but not when correcting for multiple testing. No further details were

provided on the aberrant methylation gene status.

Eight articles reported either prognostic classifiers or an epigenetic homogeneity coefficient [9,35,40,42,43,45,46] consisting of multiple dmCpGs, or investigated the association between each dmCpG and survival specifically [10] (relevant CpGs reported in Supplementary Table 2). Gündert and collaborators developed the ProMCol classifier derived from independent hypothesis weighting (IHW), which integrated tumour/normal tissue comparison of CpGs and CpG associations with survival: the 20 dmCpGs with the highest score were used to build the classifier that was significantly associated with OS in the screening and validation cohort [42]. Interestingly, Gündert et al. [42] (CRC) and Nones et al. [43] (pancreatic cancer) both reported the dmCpG cg11056055 among their hits, representing the only case of shared findings of the review. Li et al. [36] proposed the iterative deletion feature optimal (IDFO) pipeline as a signature evaluation methodology, which was developed in three steps: pre-biomarker ranking strategy, building of the model that ranked the potential prognostic molecules in relation to different cancer types and the selection of the prognostic features. This signature was associated with poorer survival in CRC patients (HR 1.77, 95% CI not reported), but the lack of confidence interval reporting limits the interpretation of this result. Martinez-Cardus et al. [39] focused on the prognostic potential of intra-tumour epigenetic status and calculated a coefficient of epigenetic homogeneity, based on epigenetic changes across three tumour regions for each patient. A higher coefficient was associated with poorer RFS in CRC patients with an HR of 2.91 (95% CI 1.03–8.16). Yang et al. [10] developed a prognostic index, by calculating the sum of the products of the Cox regression coefficients and the methylation levels for 62 dmCpGs, that could distinguish colorectal adenocarcinoma patients into high and low prognostic risk groups. Finally, Chen et al. [38] developed a risk score based exclusively on gene expression, including differentially expressed genes that were previously selected by comparing gene expression and DNA methylation data.

Discussion

In this systematic review, we summarized epigenetic markers that have been associated with prognosis in GI adenocarcinomas in studies using a genome-wide methylation array. The development of genome-wide methylation arrays enabled the capturing of dmCpGs that could have never been identified in candidate-gene studies offering the most complete information for investigations of disease mechanisms and treatment response related to DNA methylation. We identified 15 published studies, 14 of which reported and described CpG sites differentially associated with survival. The majority of the studies focused on cancers of the colon and rectum. Despite the promising results, the variations in methodology, study design and investigated outcomes made it difficult to compare the existing studies.

Methodological flaws were observed in more than one study, such as the imprecise dichotomization of patients, e.g. into patients with good prognosis and bad prognosis. Also, some of the studies did not accurately report effect estimates (e.g. HR, CI) and/or did not clearly define their outcome. For example, four studies [9,36,43,45] reported patient survival as outcome, without providing further details or a more specific definition (e.g. DFS, OS, RFS). Also, one study referred to DFS in the abstract and body of the article, but indicated OS in the figures that described the survival analysis [40]. Moreover, only one dmCpG, among the methylation sites that were significantly associated with cancer survival, was reported in two studies. The scarcity of common findings among the included studies could be due to the differences in study designs and in the selection of patients, as well as the different definitions of exposures in the survival analysis (e.g. normal vs aberrant gene methylation).

Another reason for the inconsistent study results could be the variation in the disease stage distribution of the patient populations, as GI tumour tissues at different disease stages could display different genetic

and epigenetic patterns [45,48]. Tumour stage is a very strong predictor of prognosis and only methylation markers that predict survival beyond stage would be meaningful. Likewise, if studies did not adjust for tumour stage as a confounder when investigating the association of aberrant DNA methylation and cancer survival, it is not clear whether such associations would be independent from tumour stage. Indeed, seven [9,10,36,40,43,45,47] among the 15 studies identified in this systematic review did not adjust or did not state clearly whether they adjusted for tumour stage which may lessen the relevance of their findings.

Different reasons may apply for the different approaches selected by the studies. A comparison of tumour and normal tissue methylation enables reduction of the number of CpGs to be tested in a larger study population, which goes along with better statistical power due to the lower number of tests to account for and lower cost, as the genome-wide methylation array is performed at first in a smaller population. Hence, a comparison of tumour and normal tissue methylation seems to be a plausible approach to detect potentially meaningful aberrant methylation with impact on prognosis. On the other hand, the exploration of dmCpGs in tumour tissue, based on the outcome in a model adjusted for potentially related other factors, could directly point to potentially prognostic CpG sites with or without consideration of normal tissue methylation. It might be necessary to apply more than one approach, in order to identify the most meaningful predictive and prognostic dmCpGs.

Based on the studies included in this review, it was not possible to clarify which one is the best approach. Also, different concepts were followed to define potentially relevant aberrant methylation: from single CpG sites, to CpG scores and full gene methylation. Further validation studies will be required to confirm the findings of single studies and to elaborate the potential clinical utility of such findings.

The Illumina HumanMethylation450K BeadChip array was the most widely used technique in 14 cases out of 15 of the included studies. Generally, the sample sizes of the publications reported in this review were relatively small: only five studies (33%) performed the epigenome-wide array in a population larger than 300 patients. However, it is important to note that three studies, namely Hao et al. [35], Li et al. [36] and Yang et al. [10], aimed to evaluate epigenetic changes across multiple cancer types, therefore having a bigger total sample size than the one reported in this review. Studies with a smaller sample size are not able to detect associations with moderate effect strengths and thus will detect fewer associations. However, regardless of the sample size, correction for multiple testing and validation in an independent dataset is required, in order to provide results potentially reproducible in an independent patient cohort. Consequently, one of the key points of our data quality assessment was represented by the validation type performed in the studies, as the exploratory research approaches require validation in other studies.

Methodological heterogeneity was also found regarding the validation strategies, with five studies validating the prognostic methylation markers in an external cohort [33,36,38–40], four studies performing internal validation [35,41,42,44], and seven studies not performing validation of the results obtained in the survival analysis [9,10,37,39,45–47]. Moreover, the attempt of comparing and summarizing the study findings was challenged by the absence of essential steps in the statistical analysis, almost two thirds of the studies did not perform correction for multiple testing, thus not accounting for false positive findings in the analysis.

Despite the marked heterogeneity in the experimental settings, nine studies focused on the effect of epigenetic changes on gene expression, (Supplementary Table 3), one of which did not report the gene aberrant methylation status associated to poor clinical outcome. The hypermethylation of the secreted frizzled related protein 1 (SFRP1) gene, resulting in the inactivation of its expression [49], showed a significant association with poorer survival of CRC patients [45]. Interestingly, the role of SFRP1 hypermethylation in the development of GI

adenocarcinomas is widely described in the literature [50–52]. In addition to previous publications, Kim et al. [45] demonstrated the link between SFRP1 promoter hypermethylation and poorer survival in CRC.

On the other hand, the role of the BEN domain containing 5 (BEND5) gene methylation reported by Lin et al. [41] was not discussed in prior literature. Nones et al. [43] described the association of the hypo-methylation of the MET proto-oncogene and the integrin subunit alpha 2 (ITGA2) with poorer survival in pancreatic ductal adenocarcinoma patients. This result is in line with previous studies that reported MET hypomethylation is inversely correlated with MET expression in CRC metastases [53] and associated with poor prognosis in hepatocellular carcinomas [54]. Gaedke et al. [37] found a significant association between the overall hypomethylation of a 10-gene panel and DFS in rectal cancer patients. Among them, BARHL2, ERAS and DOT1L were previously described to have a potential role in the development, detection and treatment of GI cancers [55–57]. However, previous studies did not investigate the role of these genes in GI cancer prognosis.

Chen et al. [38] developed a three-gene prognostic model for pancreatic cancer patients with metastatic lymph nodes. The association of overexpression of MAP4K4 and SULT1E1 and increased number of metastatic lymph nodes is documented in previous literature [58,59]. On the contrary, no evidence on the role of IGF2BP3 is available, except for a study by Ishii et al. [60], that described the H19-PEG10/IGF2BP3 axis as an important player in metastasis progression in gastric cancer.

Little is known about the five genes that Hu et al. [44] reported in their results, thus providing an interesting starting point for further investigation into the biological function of these prognostic genes in gastric adenocarcinoma. Similarly, the hypermethylation of glial cell line-derived neurotrophic factor (GDNF) indicated by Liang et al. [47] does not appear in previous studies, whereas the prognostic role of Reelin (RELN) hypermethylation is supported by a study that observed RELN epigenetic silencing in gastric carcinogenesis [61]. Finally, the upregulation of transforming growth factor-beta induced (TGFB1) highlighted in the study by Zhang et al. [40] has been previously found in GI cancers of the esophagus, stomach, pancreas and CRC [62,63].

While the abovementioned studies identified the significant dmCpGs by comparing tumour and normal tissue samples, Martinez-Cardus et al. [39] used another approach in investigating the role of intra-tumour epigenetic homogeneity in cancer prognosis with the development of a coefficient of epigenetic homogeneity that was inversely associated with DFS in CRC patients. Notably, a number of studies put a particular emphasis on the methylation of single CpGs, rather than focusing on gene specific methylation. After identifying dmCpGs, Gündert et al. [42] and Li et al. [36] developed prognostic classifiers based on a restricted number of these epigenetic biomarkers, that predicted patient prognosis in CRC patients. Yang et al. [10] calculated a prognostic index and reported a CpG-panel that separated patients with colorectal adenocarcinomas in high and low prognostic index patients.

In summary, we found a large variety of methodological approaches for the identification of prognostic epigenetic markers from genome-wide methylation analyses. Also, none of the findings were reported by more than one study in this systematic review. Studies were often lacking a comprehensible description of the study methodology and a complete reporting of the identified methylation sites or methylation patterns, as well as an accurate definition of the investigated outcomes. Although the reported epigenetic markers and classifiers may have potential as they could improve patient stratification in the light of personalized medicine, further validation studies are required. At present, no conclusions can be drawn on the clinical relevance of the reported epigenetic markers. This systematic review highlights the strong potential of genome-wide methylation analyses in the detection of promising biomarkers, albeit calling for larger studies and better reporting of methodologies and results, to support clinical decision-making in patients with gastrointestinal cancer.

Disclosures

The authors have no conflict of interest to declare.

Author contributions

Study concept and design: MD, MH; acquisition of data: MD, MH, EA; analysis and interpretation of data: MD, MH, EA, HB; drafting of manuscript: MD, MH; critical addition of important intellectual content to manuscript: MD, MH, EA, YZ, HB, DE; all authors approved the final version of the manuscript.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ctrv.2019.101933>.

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