Tissue micro-RNAs associated with colorectal cancer prognosis: a systematic review

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Abstract

Colorectal cancer (CRC) is a multifactorial disease commonly diagnosed worldwide, with high mortality rates. Several studies demonstrate important associations between differential expression of micro-RNAs (miRs) and the prognostic of CRC. However, only a few systematic reviews emphasize the major miRs able to contribute to the establishment of new prognostic biomarkers in CRC patients. The present study aimed to identify differentially expressed tissue miRs associated with prognostic factors in CRC patients, through a systematic review of the literature. Using the PubMed database, Cochrane Library and Web of Science, studies published in English evaluating miRs differentially expressed in tumor tissue and significantly associated with the prognostic aspects of CRC were selected. All the included studies used RT-PCR (Taqman or SYBR Green) for miR expression analysis and the period of publication was from 2009 to 2018. A total of 115 articles accomplished the inclusion criteria and were included in the review. The studies investigated the expression of 102 different miRs associated with prognostic aspects in colorectal cancer patients. The most frequent oncogenic miRs investigated were miR-21, miR-181a, miR-182, miR-183, miR-210 and miR-224 and the hyperexpression of these miRs was associated with distant metastasis, lymph node metastasis and worse survival in patients with CRC. The most frequent tumor suppressor miRs were miR-126, miR-199b and miR-22 and the hypoexpression of these miRs was associated with distant metastasis, worse prognosis and a higher risk of disease relapse (worse disease-free survival). In general, lymph node metastases and lower overall survival in patients with CRC were

the most frequently investigated prognostic variables associated with the expression of tumor miRs. Specific tissue miRs are shown to be promising prognostic biomarkers in patients with CRC, given their strong association with the prognostic aspects of these tumors, however, new studies are necessary to establish the sensibility and specificity of the miRs in order to use them in clinical practice.

Introduction

Colorectal cancer (CRC) is one of the most prevalent neoplasms in the world, being the second type of cancer more frequent in women and the third type more frequent in men. In Brazil, for the 2018-2019 biennium, it's estimated the occurrence of 36.030 new colorectal cancer cases with 17.380 cases in men and 18.980 cases in women[1].

The survival of patients with colorectal is directly associated with the pathological stage (pTNM) of the disease which is determined after microscope analysis of the sample obtained by biopsy or surgical resection of the tumor [2,3]. The pTNM stage is a system of stage classification proposed by the American Joint Committee on Cancer which determines the degree of tumor development according with the T, N and M categories. The T category informs about tumor growth, depth and stage of adjacent tissues invasion. The N category informs about lymph nodes metastasis and the M category describes the quantity of affected anatomical sites by distant metastasis [3].

It´s estimated that the survival of patients diagnosed at the first stages of the disease is up to 90%, in the other hand, 30 to 45% of the patients with late diagnosis

will present disease recurrence [4,5]. Factors such as low degree of differentiation and high levels of serum carcinoembryonic antigen (CEA) are also described as important for the increase of CRC recurrence after tumor resection [2,6], however, results from recent studies have demonstrated that the analysis of micro-RNA expression in CRC can be used as a more sensible prognostic biomarker due to it's potential of predicting disease recurrence, survival and therapeutic response [7–12].

MicroRNAs (miRs) are small non-coding RNA molecules of approximately 21 to 24 nucleotides in length that modulate gene expression by connecting with the non translational region 3 (3'UTR) of messenger RNAs resulting in it's translational repression or cleavage [13]. In the literature, it's been already described more than 2000 miRs which demonstrate an essential role in the regulation of eukaryotic cells and in the pathogenesis of several diseases, including cancer [14].

Biogenesis of miRs begins with the activity of RNA polymerase enzyme II which transcribes the primary miRs (pri-miRs) recognized afterwards by DGCR8 protein associated with DROSHA enzyme creating a complex which allows the cleavage of the pri-miRs originating the precursor miRs (pre-miRs). The pre-miRs are carried to the cell cytoplasm by Exportin-5 and cleaved by DICER, originating double strand miRs which will be recognized by Argonaut proteins promoting the release of one strand of the mature miR. Argonaut proteins in association with the mature miR and other proteins will originate the RNA-Induced Silencing Complex (RISC) whose targets will be specific mRNAs. [13,15,16]. Fig 1 outlines the miRs biogenesis.

Fig 1. **miRNAs biogenesis and proteins involved in the process.**

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93 Recent studies report that the miRs regulate cellular growth and apoptosis,
94 therefore by silencing or upregulating of specific miRs can trigger the carcinogenic
95 process. [17,18]. MiRs are classified as oncogenic or tumoral suppressor when they inhibit
96 the expression of tumoral suppressor genes or oncogenes respectively. Oncogenic
97 miRs are usually upregulated in the neoplastic process while tumor supressor miRs
98 are usually downregulated in cancer [13,18,19]. Consequently, studies have demonstrated
99 by means of analysis of miRs expression, that they can be valuable diagnostic and
100 prognostic biomarkers in cancers, including the CRC. [7,20–22].

101 Despite the recent improvements in the diagnostic methods for CRC detection,
102 the overall survival for CRC patients is only 65% while the disease recurrence after
103 tumor resection is approximately 45%, emphasizing the importance of describing new
104 methods in order to guarantee a better prognosis for this patients [4,23].

105 There are innumerable studies investigating the significance of miRs expression
106 in CRC prognosis [7–10,12,21,22]. However, there is a lack of systematic reviews in the
107 literature integrating and identifying the main miRs significantly associated with the
108 prognostic aspects of CRC.

109 Given the relationship between the expression of miRs and the prognosis of
110 CRC, the present study aims to highlight such association by means of a systematic
111 review of the literature, in order to identify the main miRs associated with each
112 prognostic aspect of CRC, in order to contribute for the innovation of new prognostic
113 biomarkers and to help the development of new therapies based on the understanding
114 of the molecular biology of this disease.

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Methods

Study design and search strategy

This study comprehends a systematic review of the literature. For the elaboration of this project a reference review in the PubMed database, Cochrane Library and Web of Science database was performed in order to identify the relevant studies that evaluated miR expression associated with CRC prognosis. The time period of the search was from February 2009, date from the last found and selected study, to May 2018, date from the first found and selected study. The following search strategy was used for the identification and selection of the articles: miRNA, colorectal cancer, prognosis. The search was performed in November 2018 and it was limited to English published articles. At the end of the search, all the results were compared and reviewed for the inclusion of relevant publications about the subject.

Inclusion criteria

As a strategy of including relevant articles for the review, criteria related to study classification and design were considered. The relevant criteria related to the classification of the studies were: primary and descriptive studies, studies related to CRC patients and studies completely available online. The relevant criteria related to study design were: articles that evaluate CRC prognosis by overall survival and disease free survival; articles that evaluate miR expression in tumoral tissues

compared to normal tissues; articles that evaluate miR expression by Real Time Polymerase Chain Reaction (RT-PCR) using Taqman and/or SYBR Green; articles that evaluate miR expression related to tumor growth/size, lymph nodes metastasis, distant metastasis and TNM stage. As exclusion criteria, publications belonging to case report, systematic reviews and meta-analysis categories were not included.

Data Extration

The data extraction was performed by means of a collection form pre-developed by the authors in which the following data of each publication were collected: name of the first author, year of publication, micro-RNA studied, sample group, origin of cases, type of specimens analyzed, type of methodology used to evaluate the expression of micro-RNAs, target genes and association with pathological and prognostic clinical aspects.

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations.

Quality assessment and publication bias

The present study comprehends a systematic review without meta-analysis, therefore the results extracted from the selected articles were analyzed qualitatively, thus the assessment of publication bias might not be practically applicable.

Problems with the design and execution of individual studies of healthcare interventions raise questions about the validity of their findings, and therefore, the quality of the primary studies were accessed by evaluating eight attributes extracted

162 from a checklist provided by the STROBE platform: description of the studying setting,
163 description of study participants, definitions of all variables, description of the data
164 sources and measurement tools, justification of sample size, description of statistical
165 methods, description of results and discussion and interpretation of results. After
166 evaluating the studies, each attribute were pointed in a range from 0 to 2 points (0 =
167 not described, 1 = poor described, 2 = well described), with maximum quality score of
168 16 points in total.

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170 Data analysis

171 The data were reviewed and synthesized in semi-structured tables of
172 Microsoft® Office Excel version 2013. The results were analyzed by descriptive
173 statistics and qualitatively tabulated.

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175 Results

176 Articles selection

177 After searching for the terms: microRNA, colorectal cancer and prognosis in all
178 three data bases, 924 articles were found. By reading the titles, 29 duplicates were
179 removed and 215 abstracts were selected for complete reading. After analyzing the
180 abstracts, 134 articles which evaluate miR expression associated with CRC prognosis
181 were selected for complete reading. After reading, 19 articles that did not achieve the
182 proposed inclusion criteria were excluded. At the end, 115 articles were included in the
183 systematic review. Fig. 2 outlines the study selection process.

184 **Fig 2. Flow diagram outlining the study selection process.**

185 The quality scores of the studies ranged from 14 to 16 with a mean quality rating of
186 15.7 (S2 File).
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188 ## Characteristics of the selected studies

189 In total, 14.894 CRC patients were studied in the 115 included articles. The
190 number of patients varied from 10 to 765 patients per study. The studies were
191 performed in institutes and universities in Greece, South Korea, United States, Czech
192 Republic, Iran, Sweden, Denmark, Japan and China with most of the research
193 performed in the last two countries. The studies evaluated the expression of 102 miRs
194 associated with CRC prognostics aspects. MiR-21 was the most frequent one, being
195 analyzed in six studies [24–29]. MiR-181a [30–32] , miR-16 [33–35] and miR-133b [36–38] were
196 individually investigated in three studies. The miRs: miR-126 [9,39], miR-133a [40,41], miR-
197 182 [42,43], miR-183 [44,45], miR-199b [46,47], miR-22 [48,49], miR-224 [50,51], miR-24-3p [52,53], miR-
198 7 [54,55], were separately analyzed in two studies. Seven researches studied two miRs
199 concomitantly [29,33,37,38,56–58], while the others analyzed a single miR. All studies
200 analyzed miR expression performing RT-PCR by TaqMan and/or SYBR Green
201 methods in which 64 studies used SYBR Green while 51 studies used Taqman. The
202 characteristics of the selected studies in this review were summarized in the Table 1.
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208 **Tabela 1.** Características dos estudos incluídos na revisão sistemática.

Ref.	Author / Year	Studied micro-RNA	Target Gene	Cases (n)	Cases Origin	Quantification Method	Results
(105)	Cui, 2018	miR-6852	TCF7	CRC (n=44) and normal adjacent tissue (n=44)	Harbin, China	RT-qPCR SYBR Green	Downregulation was associated with lymph node metastasis and worse overall survival.
(83)	Tao, 2018	miR-1296	SFPQ	CRC (n= 80) and normal adjacent tissue (n= 80)	China and Japan	RT-qPCR TaqMan	Upregulation was associated with advanced TNM stage, tumor growth, lymph node metastasis and worse overall survival
(63)	Gao, 2017	miR-888	Smad4	CRC (n= 126) and normal adjacent tissue (n= 126)	Jiangsu, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, distant metastasis, worse overall survival and worse disease free-survival.
(66)	Ma, 2018	miR-302c	AP-4	CRC (n= 90) and adjacent normal tissue (n=90)	Shaanxi, China	RT-qPCR TaqMan e SYBY Green	Downregulation was associated with advanced TNM stage, tumor depth, lymph node metastasis and worse overall survival.
(131)	Tsiakanikas, 2018	miR-28-5p	/	CRC (n= 182) and adjacent normal tissue (n= 86)	Athens, Greece	RT-qPCR SYBR Green	Upregulation was associated with worse overall survival and worse disease-free survival.
(96)	Wang, 2018	miR-552	/	CRC (n= 183) and adjacent normal tissue (n= 83)	Shijiazhuan, China	RT-qPCR TaqMan	Upregulation was associated with advanced TNM stage, lymph node metastasis and worse overall survival.
(44)	Chen, 2018	miR-183	/	CRC (n=42) and adjacent normal tissue (n=42)	Tianjin, China	RT-qPCR SYBR Green	Upregulation was associated with worse overall survival and worse disease-free survival.
(113)	Roh, 2018	miR-200c	/	CRC (n=109) and adjacent normal tissue (n=109)	Changwon, South Korea	RT-qPCR TaqMan	Upregulation was associated with lymph node metastasis, worse overall survival and worse disease-free survival.
(67)	Tong, 2018	miR-466	Cyclin D1, BAX e MMP-2	CRC (n=100) and adjacent normal tissue (n=100)	Zhejiang, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, tumor growth, lymph node metastasis, distant metastasis and worse overall survival.

(28)	Wu, 2017	miR-21	PTEN	CRC (n=105) and adjacent normal tissue (n=105)	Sichuan, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage and lymph node metastasis.
(84)	Chen, 2017	miR-130a	Forkhead box F2	CRC (n=53 and adjacent normal tissue (n=53)	Zhejiang, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage and lymph node metastasis.
(105)	Lin, 2017	miR-193a-3p	E-cadherin	CRC (n=90) and adjacent normal tissue (n=90)	Taizhou, China	RT-qPCR SYBR Green	Upregulation was associated with tumor groth and downregulation associated with worse overall survival.
(68)	Zhang, 2017	miR-340	RLIP76	CRC (n=91) and adjacent normal tissue (n=91)	Beijing, China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage and lymph node metastasis.
(132)	Rapti, 2017	miR-34a	/	CRC (n=133) and adjacent normal tissue (n=61)	Athens, Greece	RT-qPCR SYBR Green	Upregulation was associated with worse overall survival and worse disease-free survival.
(122)	Zhou, 2017	miR-650	AKT2/GSK3β/ E-cadherin pathway	CRC patients with better prognosis (n=48) and CRC patients with worse prognosis (n=40)	Shanghai, China	RT-qPCR Taqman	Downregulation was associated with worse overall survival.
(69)	Stiegelbauer, 2017	miR-196b-5p	HOXB7 e GALNT5	Two cohorts: CRC (n=110) and CRC (n=182)	Graz, Austria e Czech Republic	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage, worse overall survival and worse disease-free survival.
(70)	Wen, 2017	miR-944	MACC1	CRC (n=86) and adjacent normal tissue (n=86)	Guangzhou, China	RT-qPCR TaqMan	Downregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis, worse overall survival and worse disease-free survival.
(71)	Yan, 2017	miR-30d	LRH-1	CRC (n=80) and adjacent normal tissue (n=80)	Xi'an, China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis, worse overall survival and worse disease-free survival.
(49)	Xia, 2017	miR-22	Sp1 (e PTEN/AKT)	CRC (n=118) and adjacent normal tissue (n=118)	Sichuan, China	RT-qPCR TaqMan	Downregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis and worse overall survival.

(133)	Kontos, 2017	miR-15a-5p	RECK	CRC (n=182) and adjacent normal tissue (n=86)	Athens, Greece	RT-qPCR SYBR Green	Upregulation was associated with worse overall survival and worse disease free-survival.
(72)	He, 2017	miR-296	S100A4	CRC (n=90) and adjacent normal tissue (n=90)	Beijing, China	RT-qPCR TaqMan	Downregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis, worse overall survival and worse disease-free survival.
(134)	Yamazaki, 2017	miR-181c	/	Two cohorts of CRC patients stage II: CRC (n= 80) and adjacent normal tissue (n=80) and CRC (n=60) and adjacent normal tissue (n=60)	Tokyo, Japan	RT-qPCR TaqMan	Upregulation was associated with worse overall survival and worse disease-free survival.
(52)	kerimis, 2017	miR-24-3p	/	CRC (n=182) and adjacent normal tissue (n=86)	Athens, Greece	RT-qPCR SYBR Green	Upregulation was associated with worse overall survival and worse diasease-free survival.
(34)	Diamantopoulos, 2017	miR-16	/	CRC (n=182) and adjacent normal tissue (n=86)	Athens, Greece	RT-qPCR SYBR Green	Upregulation was associated with worse overall survival and worse diasease-free survival.
(54)	Nagano, 2016	miR-7	/	CRC (n=196) and adjacent normal tissue ((n=14)	Mie, Japan	RT-qPCR TaqMan	Upregulation was associated with advanced TNM stage, lymph node metastasis and worse overall survival.
(118)	Yu, 2016	miR-140-5p	ADAMTS5, IGFBP5	CRC (n=60) and adjacent normal tissue (n=60)	Dalian, China	RT-qPCR TaqMan	Downregulation was associated iwth tumoral stage and distant metastasis.
(85)	Rapti, 2016	miR-96	/	CRC (n=108) and adjacent normal tissue (n=54)	Athens, Greece	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, lymph node metastasis, worse overall survival and worse disease-free survival.
(73)	Wang, 2016	miR-384	KRAS, CDC42	CRC (n=100) and adjacent normal tissue (n=100)	Guangdong, China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage.
(46)	Cristóbal, 2017	miR-199b	PP2A	CRC (n=97)	Madrid, Spain	RT-qPCR TaqMan	Downregulation was associated with lymph node metastasis, worse overall survival and worse disease-free survival.

(114)	Liu , 2016	miR-1260b	/	CRC (n=120) and adjacent normal tissue (n=120)	Gansu, China	RT-qPCR SYBR Green	Upregulation was associated with lymph node metastasis, worse overall survival and worse disease-free survival.
(123)	Wang, 2016	miR-187	CD276	CRC (n=32) and adjacent normal tissue (n=32) CRC (n=80) and adjacent normal tissue (n=80)	Shanghai, China	RT-qPCR SYBR Green	Downregulation was associated with worse overall survival and worse disease free-survival.
(62)	Cheng, 2016	miR-20a-5p	Smad4	CRC (n=544) and adjacent normal tissue (n=544)	Shanghai, China	RT-qPCR TaqMan	Upregulation was associated with advanced TNM stage, distant metastasis, worse overall survival and worse disease-free survival.
(97)	Liu, 2016	let-7a-5p	HMGA2	CRC (n=192) and adjacent normal tissue (n=192)	Taipei, Taiwan	RT-qPCR TaqMan	Downregulation was associated with tumor growth, lymph node metastasis, worse overall survival and worse diasease-free survival.
(47)	Shen, 2016	miR-199b	SIRT1; CREB/KISS1	CRC (n=60) and adjacent normal tissue (n=60)	Beijing, China	RT-qPCR SYBR Green	Downregulation associated with advanced TNM stage, distant metastasis and worse overall survival.
(86)	Ma, 2016	miR-517a	FOXJ3	CRC (n=90) and adjacent normal tissue (n=90)	Xi'an, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis, worse overall survival and worse disease-free survival.
(135)	Noguchi, 2016	miR-503	CaSR	CRC (n=20), adenomas (n=20) and normal tissue (n=20)	Tsu , Japan	RT-qPCR TaqMan	Upregulation was associated with worse overall survival and worse disease-free survival.
(24)	Mima, 2016	miR-21	/	CRC (n=765)	Boston, MA, USA	RT-qPCR SYBR Green	Upregulation was associated with worse overall survival and worse disease-free survival.
(98)	Mokutani, 2016	miR-132	ANO1	CRC (n=21 and adjacent normal tissue (n=21)	Osaka, Japan	RT-qPCR TaqMan	Downregulation was associated with tumor growth, lymph node metastasis, worse overall survival and worse disease-free survival.

(32)	Li, 2016	miR-181A	PTEN/AKT	CRC (n=72) and adjacent normal tissue (n=69)	Shandong, China	RT-qPCR SYBR Green	Upregulation in hepatic metastasis patients was associated with worse overall survival.
(102)	Bin, 2016	miR-30c	/	CCR (n=192) and adjacent normal tissue (n=192)	Baoding, China	RT-qPCR SYBR Green	Downregulation was associated with tumor growth, lymph node metastasis and worse overall survival
(106)	Wang, 2016	miR-497	KSR1	CRC (n=62) and adjacent normal tissue (n=62)	Nanjing, China	RT-qPCR TaqMan	Downregulation was associated with lymph node metastasis and advanced CRC stages.
(99)	Sun, 2015	miR-206	/	CRC (n=80) and adjacent normal tissue (n=80)	Jilin, China	RT-qPCR SYBR Green	Downregulation was associated with tumor growth, lymph node metastasis and worse overall survival.
(124)	Hibino, 2015	miR-148a	MMP7	CRC (n=159) and adjacent normal tissue (n=159)	Hiroshima , Japan	RT-qPCR TaqMan	Downregulation was associated with advanced CRC stages and worse overall survival in CRC patients stage III.
(74)	Wu, 2015	miR-128	IRS1	CRC (n=45) and adjacent normal tissue (n=45)	Changchun, China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage and lymph node metastasis.
(75)	Liao , 2015	miR-33b	/	CRC (n=60) and adjacent normal tissue (n=60)	Shanghai, China	RT-qPCR TaqMan	Downregulation was associated with advanced TNM stage, tumor growth and worse overall survival.
(76)	Qu, 2015	miR-155-5p	/	CRC (n=372) and adjacent normal tissue (n=372)	Xinjiang, China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage and distant metastasis.
(119)	Li, 2015	miR-99b-5p	mTOR	CRC (n=56) and adjacent normal tissue (n=56)	Shanghai, China	RT-qPCR TaqMan	Downregulation was associated with distant metastasis and worse overall survival.
(87)	Zhang, 2015	miR-106b	DLC1	CRC (n=93) and adjacent normal tissue (n=93)	Sichuan, People's Republic of China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, lymph node metastasis, worse overall survival and worse disease-free survival.
(136)	Sümbül, 2015	miR-211	/	CRC (n=65), and adjacent normal tissue (n=65) and normal tissue (n=65)	Hatay, Turkey	RT-qPCR SYBR Green	Upregulation was associated with worse overall survival.

(77)	Kai, 2015	miR-154	/	CRC (n=169) and adjacent normal tissue (n=169)	Wenzhou, China	RT-qPCR TaqMan	Downregulation was associated with advanced TNM stage, tumor growth, lymph node metastasis, distant metastasis and worse overall survival.
(88)	Perez-Carbonell, 2015	miR-320e	/	CRC (n=100)	Barcelona, Spain	RT-qPCR TaqMan	Up regulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis, worse overall survival and worse disease-free survival.
(125)	Wang, 2015	miR-217	AEG-1	CRC (n=50)	Beijing, P.R. China	RT-qPCR SYBR Green	Downregulation was associated with worse overall survival.
(89)	Liu, 2015	miR-592	/	CRC (n=89) and adjacent normal tissue (n=89)	Shanghai, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, tumor growth, distant metastasis and worse overall survival.
(78)	Xu, 2015	miR-149	FOXM1	CRC (n=78) and adjacent normal tissue (n=20)	Jiangsu, PR China	RT-qPCR TaqMan	Down regulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis and worse overall survival.
(79)	Wang, 2015	miR-194	MAP4K4/c-Jun/MDM2	CRC (n=50)	Beijing, PR China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage, tumor growth and worse overall survival.
(56)	Ge, 2014	miR-196a, miR-196b	/	CRC (n=126) and adjacent normal tissue (n=126)	Hunan, China	RT-qPCR TaqMan	Upregulation was associated with advanced TNM stage, lymph node metastasis, worse overall survival and worse disease-free survival.
(55)	Suto, 2014	miR-7	EGFR	CRC (n=105) and adjacent normal tissue (n=105)	Maebashi, Japan	RT-qPCR TaqMan	Down regulation was associated with worse overall survival.
(53)	Gao, 2015	miR-24-3p	/	CRC (n=95) and adjacent normal tissue (n=95)	Shandong, China	RT-qPCR SYBR Green	Downregulation was associated with lymph node metastasis and worse overall survival.
(36)	Guo, 2015	miR-133b	CTGF	CRC (n=71)	Hunan, China	RT-qPCR SYBR Green	Downregulation was associated with lymph node metastasis and advanced TNM stage.

(126)	Inoue, 2015	miR-29b	MCL1 e CDK6	CRC (n=42) and adjacent normal tissue (n=42)	Osaka, Japan	RT-qPCR TaqMan	Downregulation was associated with worse overall survival and worse disease-free survival.
(33)	Xiao, 2014	miR-15a, miR-16	/	CRC (n=126) and adjacent normal tissue (n=126)	Hunan, China	RT-qPCR TaqMan	Downregulation was associated with advanced TNM stage, lymph node metastasis, worse overall survival and worse disease-free survival.
(115)	Mo, 2014	miR-376a	/	CRC (n=53) and adjacent normal tissue (n=53)	Changchun, China	RT-qPCR SYBR Green	Upregulation was associated with lymph node metastasis and worse overall survival.
(25)	Fukushima, 2014	miR-21	/	CRC (n=306) and adjacent normal tissue (n=306)	Tokyo, Japan	RT-qPCR TaqMan	Upregulation was associated with tumor growth, lymph node metastasis, distant metastasis, worse overall survival and worse disease-free survival.
(50)	Adamopoulos, 2015	miR-224	/	CRC (n=115) and adjacent normal tissue (n=66)	Athens, Greece	RT-qPCR SYBR Green	Upregulation was associated with worse overall survival and worse disease-free survival.
(64)	Gao, 2015	miR-34a-5p	p53	CRC (n=10) and adjacent normal tissue (n=10)	Beijing, China	RT-qPCR TaqMan	Downregulation was associated with worse overall survival.
(127)	Yin, 2014	miR-204-5p	RAB22A	CRC (n=272) and adjacent normal tissue (n=272)	Shanghai, China	RT-qPCR SYBR Green	Downregulation was associated with worse overall survival.
(90)	Wang, 2014	miR-720	/	CRC (n=96) and adjacent normal tissue (n=96)	Suzhou, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, tumor growth, lymph node metastasis, distant metastasis and worse overall survival.
(60)	Sun, 2014	miR-494	PTEN	CRC (n=247) and adjacent normal tissue (n=247)	Shaanxi, china	RT-qPCR TaqMan	Upregulation was associated with lymph node metastasis, distant metastasis, worse overall survival and worse disease-free survival.
(137)	Li, 2014	miR-223	/	CRC (n=62) and adjacent normal tissue (n=62)	Jinan, China	RT-qPCR	Upregulation was associated with worse disease-free survival.
(120)	Ress, 2015	MiR-96-5p	KRAS	CRC (n=80)	Graz, Austria	RT-qPCR SYBR Green	Downregulation was associated with distant metastasis and worse overall survival.

(107)	Zhang, 2014	miR-193a-5p	/	CRC (n=69) and adjacent normal tissue (n=69)	Beijing, China	RT-qPCR TaqMan	Downregulation was associated with lymph node metastasis, worse overall survival and worse disease-free survival.
(80)	Chen, 2014	miR-100	/	CRC (n=138) and adjacent normal tissue (n=138)	Zaozhuang, China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage, tumor growth, lymph node metastasis and worse overall survival.
(40)	Wang, 2014	miR-133a	/	CRC (n=169) and adjacent normal tissue (n=169)	Shandong, China	RT-qPCR	Downregulation was associated with advanced TNM stage, lymph node metastasis and worse overall survival.
(128)	Song, 2014	miR-139-5p	AMFR and NOTCH1	CRC (n=158) and adjacent normal tissue (n=158)	Shanghai, China	RT-qPCR SYBR Green	Downregulation was associated with worse overall survival.
(41)	Wan, 2014	miR-133a	/	CRC (n=125) and adjacent normal tissue (n=125)	Hong Kong, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, distant metastasis and worse overall survival.
(129)	Jinushi, 2014	miR-124-5p	SMC4	CRC (n=71)	Sapporo, Japan	RT-qPCR SYBR Green e TaqMan	Downregulation was associated with worse overall survival.
(37)	Ellermeier, 2014	miR-133b e miR-210	/	CRC (n=19 and adjacent normal tissue (n=19)	Providence, RI, U.S.A	RT-qPCR TaqMan	Upregulation of both was associated with worse overall survival. Upregulation of miR-210 and downregulation of miR-133b were associated with distant metastasis.
(91)	Chu, 2014	miR-630	/	CRC (n=206) and adjacent normal tissue (n=206)	Xi'na, China	RT-qPCR TaqMan	Upregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis and worse overall survival.
(108)	Orang, 2014	miR-205	/	CRC (n=40) and adjacent normal tissue (n=40)	Tabriz, Iran	RT-qPCR SYBR Green	Downregulation was associated with lymph node metastasis.
(59)	Fang, 2014	miR-17-5p	PTEN	CRC (n=295)	Guangzhou, China	RT-qPCR SYBR Green	Upregulation was associated with distant metastasis and worse overall survival.
(81)	Sun, 2014	miR-335	ZEB2	CRC (n=80) and adjacent normal tissue (n=80)	Xi'na, China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage, lymph node metastasis and worse overall survival.

(30)	Ji, 2014	miR-181a	WIF-1	CRC (n=137) and adjacent normal tissue (n=137)	Beijing, China	RT-qPCR TaqMan	Upregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis and worse overall survival.
(61)	Zhang, 2014	miR-20a	SMAD4	CRC (n=86) and adjacent normal tissue (n=86)	Nanchong, China	RT-qPCR SYBR Green	Upregulation was associated with lymph node metastasis and worse overall survival.
(92)	Qu, 2014	miR-210	VMP1	CRC (n=193) and adjacent normal tissue (n=193)	Shandong, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, tumor growth, lymph node metastasis and worse overall survival.
(42)	Rapti, 2014	miR-182	/	CRC (n=116) and adjacent normal tissue (n=60)	Athens, Greece	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, lymph node metastasis and worse overall survival.
(100)	Zhang, 2014	miR-378	Vimentin	CRC (n=84) and adjacent normal tissue (n=84)	Sichuan, China	RT-qPCR SYBR Green	Downregulation was associated with tumor growth, lymph node metastasis and worse overall survival.
(39)	Liu, 2014	miR-126	/	CRC (n=92) and adjacent normal tissue (n=92)	Zhanjiang, China	RT-qPCR SYBR Green	Downregulation was associated with distant metastasis and worse overall survival.
(57)	Li, 2014	miR-378a-3p, miR-378a-5p	/	Two cohorts: CRC (n=96) and adjacent normal tissue (n=96); CRC (n=12) and adjacent normal tissue (n=12)	Guangzhou, China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage, tumor growth and worse overall survival.
(82)	Yu, 2013	miR-218	/	CRC (n=26) and adjacent normal tissue (n=26)	Jiangsu, China	RT-qPCR SYBR Green	Downregulation was associated with advanced TNM stage, lymph node metastasis and worse overall survival.
(93)	Li, 2014	miR-25	/	CRC (n=186) and adjacent normal tissue (n=186)	Shaanxi, China	RT-qPCR TaqMan	Upregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis and worse overall survival.
(94)	Qin, 2014	miR-191	TIMP3	CRC (n=136)	Xinxiang, China	RT-qPCR TaqMan	Upregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis and worse overall survival.

(109)	Long, 2013	miR-138	TWIST2	CRC (n=187) and adjacent normal tissue (n=187)	Hunan,China	RT-qPCR TaqMan	Downregulation was associated with lymph node metastasis, distant metastasis, worse overall survival and worse disease-free survival.
(45)	Zhou, 2014	miR-183	/	CRC (n=94) and adjacent normal tissue (n=94)	Sichuan, China	RT-qPCR SYBR Green	Upregulation was associated with lymph node metastasis, distant metastasis and worse overall survival.
(35)	Qian, 2013	miR-16	/	CRC (n=143) and adjacent normal tissue (n=18)	Jiangsu, China	RT-qPCR TaqMan	Downregulation was associated with advanced TNM stage, lymph node metastasis and worse overall survival.
(9)	Hansen, 2013	miR-126	/	CRC (n=126)	Odense, Denmark/ Lund, Sweden	RT-qPCR SYBR Green	Downregulation was associated with worse overall survival.
(110)	Lou, 2013	miR-625	/	CRC (n=96) and adjacent normal tissue (n=96)	Shanghai, China	RT-qPCR SYBR Green	Downregulation was associated with lymph node metastasis, distant metastasis and worse overall survival.
(51)	Liao, 2013	miR-224	PHLPP1, PHLPP2	CRC (n=43) and adjacent normal tissue (n=43)	Guangzhou, China	RT-qPCR TaqMan	Upregulation was associated with distant metastasis and worse overall survival.
(26)	Chen, 2013	miR-21	/	CRC (n=195)	Changhua, Taiwan	RT-qPCR TaqMan	Upregulation was associated with worse overall survival.
(27)	Toiyama, 2013	miR-21	/	CRC (n=166) and adjacent normal tissue (n=166)	Japan	RT-qPCR TaqMan	Upregulation was associated with tumor growth, distant metastasis and worse overall survival.
(109)	Meng, 2013	miR-212	MnSOD	CRC (n=30) and adjacent normal tissue (n=30)	Guangzhou, China	RT-qPCR TaqMan	Downregulation was associated with lymph node metastasis, distant metastasis, worse overall survival and worse disease-free survival.
(43)	Liu, 2013	miR-182	/	CRC (n=152) and adjacent normal tissue (n=152)	Jinan, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, tumor growth, lymph node metastasis and worse overall survival.
(116)	Wan, 2013	miR-199a-3p	/	CRC (n=92) and adjacent normal tissue (n=92)	Shanghai, China	RT-qPCR SYBR Green	Upregulation was associated with lymph node metastasis, distant metastasis and worse overall survival.

(104)	Li, 2012	miR-429	SOX2	CRC (n=107) and adjacent normal tissue (n=107)	Shandong, China	RT-qPCR	Upregulation was associated with tumor growth, lymph node metastasis and worse overall survival.
(31)	Nishimura, 2012	miR-181a	PTEN	CRC (n=162)	Beppu, Japan	RT-qPCR TaqMan	Upregulation was associated with worse overall survival and worse disease-free survival.
(130)	Wang, 2013	miR-124	/	CRC (n=96) and adjacent normal tissue (n=96)	Chengdu City, China	RT-qPCR TaqMan	Downregulation was associated with worse overall survival and worse disease-free survival.
(95)	Zhou, 2012	miR-92a	/	CRC (n=82) and adjacent normal tissue (n=82)	Nanchong, China	RT-qPCR SYBR Green	Upregulation was associated with advanced TNM stage, lymph node metastasis, distant metastasis and worse overall survival.
(48)	Zhang, 2012	miR-22	/	CRC (n=86) and adjacent normal tissue (n=86)	Nanchong, China	RT-qPCR SYBR Green	Downregulation was associated with distant metastasis and worse overall survival.
(121)	Yamashita, 2012	miR-372	/	CRC (n=144)	Beppu, Japan	RT-qPCR TaqMan	Upregulation was associated with distant metastasis and worse overall survival.
(117)	Nishida, 2012	miR-10b	BIM	CRC (n=88)	Osaka, Japan	RT-qPCR TaqMan	Upregulation was associated with lymph node metastasis and worse overall survival.
(101)	Song, 2011	miR-148b	CCK2R	CRC (n=96) and adjacent normal tissue (n=96)	Shenyang City, China	RT-qPCR SYBR Green	Downregulation was associated with tumor growth.
(38)	Akçakaya, 2011	miR-185, miR-133b	/	CRC (n=50)	Stockholm, Sweden	RT-qPCR SYBR Green e TaqMan	Upregulation of miR-185 and downregulation of miR-133b were associated with metastasis and worse overall survival.
(29)	Shibuya, 2011	miR-21 e miR-155	PDCD4 and TP53INP1	CRC (n=156) and adjacent normal tissue (n=156)	Tokyo, Japan	RT-qPCR TaqMan	Upregulation of miR-21 was associated with distant metastasis, worse overall survival and worse disease-free survival. Upregulation of miR-155 as associated with lymph node metastasis, worse overall survival and worse disease-free survival.

(65)	Nishida, 2011	miR-125b	p53 e p21	CRC (n=89)	Osaka, Japan	RT-qPCR TaqMan	Upregulation was associated with tumor growth and worse overall survival.
(112)	Wang, 2012	miR-195	/	CRC (n=85) and adjacent normal tissue (n=85)	Nanjing, China	RT-qPCR SYBR Green	Downregulation was associated with lymph node metastasis and worse overall survival.
(58)	Wang, 2009	miR-31, miR-143, miR-145	/	CRC (n=98) and adjacent normal tissue (n=98)	Chengdu, China	RT-qPCR TaqMan	Upregulation of miR-31 was associated with advanced TNM stage.

miR: micro-RNA. CRC: colorectal cancer. RT-qPCR: real-time polymerase chain reaction

221 Studies that investigated the prognosis of CRC patients by means of overall
222 survival and disease-free survival demonstrated that upregulated miRs associated with
223 worse prognosis were more frequent than downregulated miRs. In terms of pathologic
224 clinical aspects, downregulated miRs associated with advanced TNM stage, tumor
225 growth and lymph node metastasis were more frequent than upregulated miRs.
226 Nonetheless, upregulated miRs were more associated with distant metastasis. The
227 association of the miRs differentially expressed in CRC and their respective prognostic
228 aspects are summarized in Table 2.

229 **Tabela 2**. Micro-RNAs diferentially expressed in CRC and associated prognostic aspects.

Prognostic Aspects	Downregulated micro-RNAs	Upregulated micro-RNAs
Advanced TNM stage	miR-302c [66], miR-466 [67], miR-340 [68], miR-196b-5p [69], miR-944 [70], miR-30d [71], miR-22 [49], miR-296 [72], miR-384 [73], miR-199b [47], miR-128 [74], miR-33b [75], miR-155-5p [76], miR-154 [77], miR-149 [78], miR-194 [79], miR-133b [36], miR-15a [33], miR-100 [80], miR-133a [40], miR-335 [81], miR-378a-3p [57], miR-378a-5p [57], miR-218 [82], miR-16 [33]	miR-182 [42,43], miR-1296 [83], miR-888 [63], miR-21 [28], miR-130a [84], miR-7 [54], miR-96 [85], miR-20a-5p [62], miR-517a [86], miR-106b [87], miR-320e [88], miR-592 [89], miR-196a [56], miR-196b [56], miR-720 [90], miR-133a [41], miR-630 [91], miR-181a [30], miR-210 [92], miR-25 [93], miR-191 [94], miR-92a [95], miR-31[58], miR-552 [96]
Tumor Growth	miR-302c [66], miR-466 [67], let-7a-5p [97], miR-132 [98], miR-206 [99], miR-33b [75], miR-154 [77], miR-194 [79], miR-100 [80], miR-378 [100], miR-378a-3p [57], miR-378a-5p [57], miR-148b [101], miR-30c [102]	miR-1296 [83], miR-193a-3p [103], miR-592 [89], miR-720 [90], miR-210 [92], miR-182 [43], miR-429 [104],miR-125b [65], miR-21 [25,27]
Lymph Node Metastasis	miR-6852 [105], miR-302c [66], miR-466 [67], miR-340 [68], miR-944 [70], miR-30d [71], miR-22 [49], miR-296 [72], miR-199b [46], let-7a-5p [97], miR-132 [98], miR-497 [106], miR-206 [99], miR-128 [74], miR-154 [77], miR-149 [78], miR-24-3p [53], miR-15a [33], miR-16 [33,35], miR-193a-5p [107], miR-100 [80], miR-133a [40], miR-205 [108], miR-335 [81], miR-378 [100], miR-218 [82], miR-138 [109], miR-625 [110], miR-212 [111], miR-195 [112], miR-133b [36], miR-30c [102]	miR-1296 [83], miR-200c [113], miR-21 [25,28], miR-130a [84], miR-7 [54], miR-96 [85], miR-1260b [114], miR-517a [86], miR-106b [87], miR-320e [88], miR-196a [56], miR-196b [56], miR-376a [115], miR-720 [90], miR-494 [60], miR-630 [91], miR-181a [30], miR-20a [61], miR-210 [92], miR-182 [42,43], miR-25 [93], miR-191 [94], miR-183 [45], miR-199a-3p [116], miR-429 [104], miR-92a [95], miR-10b [117], miR-155 [29], miR-552 [96]
Distant Metastasis	miR-466 [67], miR-944 [70], miR-30d [71], miR-22 [48,49], miR-296 [72], miR-140-5p [118], miR-199b [47], miR-155-5p [76], miR-99b-5p [119], miR-154 [77], miR-149 [78], miR-96-5p [120], miR-133b [37,38], miR-126 [39], miR-138 [109], miR-625 [110], miR-212 [111]	miR-888 [63], miR-20a-5p [62], miR-517a [86], miR-320e [88], miR-592 [89], miR-720 [90], miR-494 [60], miR-133a [41], miR-210 [37], miR-630 [91], miR-17-5p [59], miR-181a[30], miR-25 [93], miR-191 [94], miR-183 [45], miR-224 [51], miR-199a-3p [116], miR-92a [95], miR-372 [121], miR-185 [38], miR-21 [25,27,29]
Worse Overall Survival	miR-199b [46,47], miR-6852 [105], miR-302c [66], miR-466 [67], miR-650 [122], miR-196b-5p [69], miR-944 [70], miR-30d [71], miR-296 [72], miR-187 [123], let-7a-5p [97], miR-132 [98], miR-206 [99], miR-148a [124], miR-33b [75], miR-99b-5p [119], miR-154 [77], miR-217 [125], miR-149 [78], miR-194 [79], miR-7 [55], miR-24-3p [53], miR-29b [126], miR-15a [33], miR-16 [33,35], miR-204-5p [127], miR-96-5p [120], miR-193a-5p [107], miR-100 [80], miR-133a [40], miR-139-5p [128], miR-124-5p [129], miR-335 [81], miR-378 [100], miR-126 [39], miR-378a-3p [57], miR-378a-5p [57], miR-218 [82], miR-138 [109], miR-625 [110], miR-212 [111], miR-124 [130], miR-22 [48], miR-133b [38], miR-193a-3p [103], miR-195 [112], miR-30c [102]	miR-181a [30–32], miR-182 [42,43], miR-1296 [83], miR-888 [63], miR-28-5p [131], miR-183 [44,45], miR-200c [113], miR-34a [132], miR-15a-5p [133], miR-181c [134], miR-24-3p [52], miR-16 [34], miR-7 [54], miR-96 [85], miR-1260b [114], miR-20a-5p [62], miR-517a [86], miR-503 [135], miR-106b [87], miR-211 [136], miR-320e [88], miR-592 [89], miR-196a [56], miR-196b [56], miR-376a [115], miR-224 [50,51], miR-720 [90], miR-494 [60], miR-223 [137], miR-133a [41], miR-133b [37], miR-210 [37,92], miR-630 [91], miR-17-5p [59], miR-20a [61], miR-25 [93], miR-191 [94], miR-183 [44,45], miR-199a-3p [116], miR-429 [104], miR-92a [95], miR-372 [121], miR-10b [117], miR-185 [38], miR-21 [24–27,29], miR-155 [29], miR-125b [65], miR-552 [96]
Worse Disease-Free Survival	miR-196b-5p [69], miR-944 [70], miR-30d [71], miR-22 [49], miR-296 [72], miR-199b [46], miR-187 [123], let-7a-5p [97], miR-132 [98], miR-29b [126], miR-15a [33], miR-16 [33], miR-34a-5p [64], miR-193a-5p [107], miR-138 [109], miR-126 [9], miR-212 [111], miR-124 [130]	miR-888 [63], miR-28-5p [131], miR-183 [44], miR-200c [113], miR-34a [132], miR-15a-5p [133], miR-181c [134], miR-24-3p [52], miR-16 [34], miR-96 [85], miR-1260b [114], miR-20a-5p [62], miR-517a [86], miR-503 [135], miR-106b [87], miR-320e [88], miR-196a [56], miR-196b [56], miR-224 [50], miR-494 [60], miR-181a [31], miR-21 [24,25,29], miR-155 [29]

Some of the selected studies investigated the target genes of the miRs associated with the prognostic aspects of CRC. In total, 65 target genes were analyzed in which the most frequent ones were: PTEN, investigated in six studies [28,31,32,49,59,60], SMAD4, investigated in three studies [61–63], and TP53 [64,65] investigated in two studies.

Micro-RNAs and the prognosis of colorectal cancer

Micro-RNAs associated with advanced TNM stage

Twenty-four studies demonstrated the downregulation of twenty-five miRs associated with advanced TNM stage. Such marks were: miR-302c [66], miR-466 [67], miR-340 [68], miR-196b-5p [69], miR-944 [70], miR-30d [71], miR-22 [49], miR-296 [72], miR-384 [73], miR-199b [47], miR-128 [74], miR-33b [75], miR-155-5p [76], miR-154 [77], miR-149 [78], miR-194 [79], miR-133b [36], miR-15a [33], miR-100 [80], miR-133a [40], miR-335 [81], miR-378a-3p [57], miR-378a-5p [57], miR-218 [82] and miR-16 [33]. In the other hand, two studies demonstrated the upregulation of miR-182 [42,43] associated with advanced TNM stage. Futhermore, others 22 studies pointed 23 different upregulated miRs associated with advanced TNM stage. Those markers were: miR-1296 [83], miR-888 [63], miR-21 [28], miR-130a [84], miR-7 [54], miR-96 [85], miR-20a-5p [62], miR-517a [86], miR-106b [87], miR-320e [88], miR-592 [89], miR-196a [56], miR-196b [56], miR-720 [90], miR-133a [41], miR-630 [91], miR-181a [30], miR-210 [92], miR-25 [93], miR-191 [94], miR-92a [95], miR-31[58] and miR-552 [96].

Micro-RNAs associated with tumor growth

253 Tumor growth was associated with the downregulation of 14 miRs demonstrated in
254 13 studies. Those markers were: miR-302c [66], miR-466 [67], let-7a-5p [97], miR-132 [98],
255 miR-206 [99], miR-33b [75], miR-154 [77], miR-194 [79], miR-100 [80], miR-378 [100], miR-378a-
256 3p [57], miR-378a-5p [57], miR-148b [101] and miR-30c [102]. However, ten studies verified the
257 upregulation of miR-1296 [83], miR-193a-3p [103], miR-592 [89], miR-720 [90], miR-210 [92],
258 miR-182 [43], miR-429 [104],miR-125b [65] and miR-21 [25,27] associated with tumor growth as
259 well.

260

261 **Micro-RNAs associated with the presence of lymph node metastasis**

262 A total of 32 studies demonstrated the association between downregulation of 32
263 miRs and the presence of lymph node metastasis such as miR-6852 [105], miR-302c [66],
264 miR-466 [67], miR-340 [68], miR-944 [70], miR-30d [71], miR-22 [49], miR-296 [72], miR-199b [46],
265 let-7a-5p [97], miR-132 [98], miR-497 [106], miR-206 [99], miR-128 [74], miR-154 [77], miR-149 [78],
266 miR-24-3p [53], miR-15a [33], miR-16 [33,35], miR-193a-5p [107], miR-100 [80], miR-133a [40], miR-
267 205 [108], miR-335 [81], miR-378 [100], miR-218 [82], miR-138 [109], miR-625 [110], miR-212 [111],
268 miR-195 [112], miR-133b [36] and miR-30c [102]. In contrast, 30 studies verified the
269 upregulation of miR-1296 [83], miR-200c [113], miR-21 [25,28], miR-130a [84], miR-7 [54], miR-96
270 [85], miR-1260b [114], miR-517a [86], miR-106b [87], miR-320e [88], miR-196a [56], miR-196b [56],
271 miR-376a [115], miR-720 [90], miR-494 [60], miR-630 [91], miR-181a [30], miR-20a [61], miR-210
272 [92], miR-182 [42,43], miR-25 [93], miR-191 [94], miR-183 [45], miR-199a-3p [116], miR-429 [104],
273 miR-92a [95], miR-10b [117], miR-155 [29] and miR-552 [96] associated with the presence of
274 lymph node metastasis.

275

276 **Micro-RNAs associated with the presence of distant metastasis**

277 Downregulation of miR-466 [67], miR-944 [70], miR-30d [71], miR-22 [48,49], miR-296 [72],
278 miR-140-5p [118], miR-199b [47], miR-155-5p [76], miR-99b-5p [119], miR-154 [77], miR-149 [78],
279 miR-96-5p [120], miR-133b [37,38], miR-126 [39], miR-138 [109], miR-625 [110] and miR-212 [111]
280 was associated with the presence of distant metastasis in 19 studies. In contrast,
281 downregulation of miR-888 [63], miR-20a-5p [62], miR-517a [86], miR-320e [88], miR-592 [89],
282 miR-720 [90], miR-494 [60], miR-133a [41], miR-210 [37], miR-630 [91], miR-17-5p [59], miR-
283 181a[30], miR-25 [93], miR-191 [94], miR-183 [45], miR-224 [51], miR-199a-3p [116], miR-92a [95],
284 miR-372 [121], miR-185 [38] and miR-21 [25,27,29] was associated with the presence of distant
285 metastasis in 23 studies.

286

287 **Micro-RNAs associated with worse overall survival**

288 Among de evaluated studies, 49 demonstrated the downregulation of miR-199b
289 [46,47], miR-6852 [105], miR-302c [66], miR-466 [67], miR-650 [122], miR-196b-5p [69], miR-944 [70],
290 miR-30d [71], miR-296 [72], miR-187 [123], let-7a-5p [97], miR-132 [98], miR-206 [99], miR-148a
291 [124], miR-33b [75], miR-99b-5p [119], miR-154 [77], miR-217 [125], miR-149 [78], miR-194 [79], miR-7
292 [55], miR-24-3p [53], miR-29b [126], miR-15a [33], miR-16 [33,35], miR-204-5p [127], miR-96-5p [120],
293 miR-193a-5p [107], miR-100 [80], miR-133a [40], miR-139-5p [128], miR-124-5p [129], miR-335
294 [81], miR-378 [100], miR-126 [39], miR-378a-3p [57], miR-378a-5p [57], miR-218 [82], miR-138 [109],
295 miR-625 [110], miR-212 [111], miR-124 [130], miR-22 [48], miR-133b [38], miR-193a-3p [103], miR-
296 195 [112] and miR-30c [102] associated with worse prognosis (lower overall survival) of
297 CRC patients. Conversely, 56 studies identified the upregulation of miR-181a [30–32],
298 miR-182 [42,43], miR-1296 [83], miR-888 [63], miR-28-5p [131], miR-183 [44,45], miR-200c [113],
299 miR-34a [132], miR-15a-5p [133], miR-181c [134], miR-24-3p [52], miR-16 [34], miR-7 [54], miR-96
300 [85], miR-1260b [114], miR-20a-5p [62], miR-517a [86], miR-503 [135], miR-106b [87], miR-211 [136],

301 miR-320e [88], miR-592 [89], miR-196a [56], miR-196b [56], miR-376a [115], miR-224 [50,51], miR-
302 720 [90], miR-494 [60], miR-223 [137], miR-133a [41], miR-133b [37], miR-210 [37,92], miR-630 [91],
303 miR-17-5p [59], miR-20a [61], miR-25 [93], miR-191 [94], miR-183 [44,45], miR-199a-3p [116], miR-
304 429 [104], miR-92a [95], miR-372 [121], miR-10b [117], miR-185 [38], miR-21 [24–27,29], miR-155 [29],
305 miR-125b [65] and miR-552 [96] was also associated with worse overall survival of CRC
306 patients.

307

308 **Micro-RNAs associated with worse disease free-survival**

309 In total, 17 studies demonstrated the downregulation of eighteen miRs associated
310 with worse disease free-survival such as miR-196b-5p [69], miR-944 [70], miR-30d [71], miR-
311 22 [49], miR-296 [72], miR-199b [46], miR-187 [123], let-7a-5p [97], miR-132 [98], miR-29b [126], miR-
312 15a [33], miR-16 [33], miR-34a-5p [64], miR-193a-5p [107], miR-138 [109], miR-126 [9], miR-212
313 [111] and miR-124 [130]. However, 21 studies associated the upregulation of miR-888 [63],
314 miR-28-5p [131], miR-183 [44], miR-200c [113], miR-34a [132], miR-15a-5p [133], miR-181c [134],
315 miR-24-3p [52], miR-16 [34], miR-96 [85], miR-1260b [114], miR-20a-5p [62], miR-517a [86], miR-
316 503 [135], miR-106b [87], miR-320e [88], miR-196a [56], miR-196b [56], miR-224 [50], miR-494 [60],
317 miR-181a [31], miR-21 [24,25,29] and miR-155 [29] with worse disease free-survival.

320 ## Target genes

321 In addition to demonstrating the association of miRs and the clinical and prognostic
322 aspects of colorectal cancer, 60 studies also investigated the possible target genes for
323 these miRs. 65 target gens were identified, however PTEN, SMAD4, P53 and KRAS were
324 the most frequent ones. The downregulation of PTEN was associated with de
325 upregulation of miR-181a [31,32], miR-17-5p [59], miR-494 [60], miR-22 [49] and miR-21 [28]. The
326 downregulation of SMAD4 was associated with de upregulation of miR-20a [61], miR-20a-
327 5p [62], miR-888 [63]. The downregulation of P53 was related to the upregulation of miR-
328 125b [65] and to the downregulation of de miR-34a-5p [64]. The upregulation of KRAS was
329 associated with the downregulation of miR-96-5p [120] and miR-384 [73].

330

331 # Discussion

332 Colorectal cancer is one of the most prevalent neoplasms in the world and it's
333 estimated that the five-year overall survival for CRC patients is only 65%[23]. Several
334 factors associated with CRC prognosis have been already studied, however sensible and
335 specific prognostic biomarkers capable of predicting survival and recurrence of the
336 disease are still unknown [138].

337 Recent reviews have demonstrated strong correlations between the prognostic
338 aspects of breast cancer [139] and leukemia [140] with miR expression, yet there are few
339 systematic reviews integrating the main miRs associated with the prognostic aspects of
340 CRC. The present study evaluated 113 articles that investigated the differential
341 expression of 100 tissue miRs associated with the main clinical-pathological and

342 prognostic aspects of CRC, demonstrating that such molecules may be useful as valuable
343 prognostic biomarkers in CRC.

344 Expression of the miRNAs can be analyzed in different biological samples such as
345 tumor tissue [45], blood (serum or plasma) [141], feces [142], or urine [143]. In the present study,
346 articles that evaluated the expression of miRs in the tumor tissues were prioritized and
347 selected. Tumor tissues can be stored in liquid nitrogen or paraffin, allowing greater
348 stability to the miRs molecules for later analysis in retrospective studies that aim to
349 evaluate patients' survival. The analysis in the tumor tissues confers greater specificity to
350 the results since circulating miRs (present in serum or plasma) may come from other
351 anatomical sites. In addition, tumor samples allow the analysis of miRs expression in
352 comparison to the adjacent normal tissue of the same patient providing greater reliability
353 to the results. In addition, studies indicate that factors associated to the tumor
354 microenvironment influence the expression of miRs making the analysis in tumors more
355 adequate for this type of study [144,145].

356 The expression of miRs can be evaluated by different methods such as northern
357 blotting, immunohistochemistry, real-time PCR (RT-PCR), microarray, and others [146].
358 Each method has its own particularities and limitations. In the present study, only articles
359 that evaluated the expression of miRs in the CRC by means of RT-PCR using TaqMan
360 or SYBR Green were selected, because these techniques often present hight sensitivity
361 and specificity, besides allowing the comparison of a specific miR in relation to an
362 endogenous control.

363 The miRs are classified as oncogenic or tumor suppressors according to their
364 respective target genes. MiRs that inhibit the expression of tumor suppressor genes are

365 considered oncogenic and are generally upregulated in neoplasms. The most frequent
366 oncogenic miRs in this study were miR-21, miR-181a, miR-182, miR-183, miR-210 and
367 miR-224 and the upregulation of most of these miRs was associated with distant
368 metastasis, lymph node metastasis and poorer prognosis in patients with CRC.

369 On the other hand, miRs that inhibit the expression of oncogenes are considered
370 tumor suppressors and are generally downregulated in the carcinogenic processes. The
371 most frequent tumor suppressor miRs in this study were miR-126, miR-199b and miR-22
372 and downregulation was associated with the presence of distant metastasis, worse
373 prognosis and a greater risk of disease relapse (worse disease-free survival). Particularly,
374 downregulation of miR-199b and miR-22 was also associated with more advanced TNM
375 stage and the presence of lymph node metastasis. Other miRs such as miR-133a, miR-
376 133b, miR-16, miR-24-3p and miR-7 were also relatively frequent in the present study,
377 however, investigations analyzing the expression of these miRs have shown controversial
378 results. The downregulation of these five miRs was associated with a worse prognosis
379 and with the exception of miR-7, all the others also had downregulation associated with
380 the presence of lymph node metastasis.

381 In addition, another 84 miRs were also identified in the present review and their
382 differential expression was associated with distinct clinicopathological and prognostic
383 aspects of CRC, however, these miRs were evaluated in a single study. In general, it was
384 observed that down and upregulated miRs associated with lymph node metastasis and
385 worse prognosis (overall survival) in CRC were the most frequently studied.

386 Among the miRs identified, miR-21 was the most frequent one being analyzed in
387 six studies. In these studies, an upregulation of miR-21 in the tumor compared to the

388 adjacent normal tissue was found in addition to a strong correlation with more aggressive
389 CRC characteristics such as tumor size, lymph node metastasis, distant metastasis and
390 worse prognosis. A systematic review with meta-analysis conducted by Peng et al.
391 selected studies that analyzed the role of miR-21 as diagnostic biomarker and prognostic
392 of CRC, and statistically demonstrated that miR-21 upregulation in the tumor was
393 associated with worse overall survival and worse disease-free survival, as in the present
394 review [147]. The findings in both studies suggest the potential of miR-21 to predict survival
395 and relapse of the disease making it promising biomarker for CRC prognosis.

396 Another systematic review with meta-analysis conducted by Gao et al. evaluated
397 the prognostic value of miRs in colorectal cancer as in the present review. However, in
398 that review, studies that analyzed the expression of miRs in different types of samples
399 such as serum, plasma and tissue and performing other methods of detection, as
400 immunohistochemistry, were considered, so they presented miRs not identified in the
401 present study. Nevertheless, the findings of the review by Gao et al. and the present
402 review demonstrate similar results regarding the analysis of miRs expression in tumor
403 tissues associated with the prognostic aspects of CRC, that is, the upregulation of miR-
404 181a, miR-224, miR-21 and miR-126 downregulation were also present associated with
405 worse overall survival in CRC patients [21].

406 The molecular mechanisms by which polyps and adenomas develop to CRC are
407 diverse. However, the major altered signal pathways responsible for this progression in
408 CRC are: MAPK / EGFR pathway, TGFβ pathway and via P13K / AKT pathway [148].
409 Coincidentally, the most frequent target genes for the miRNAs identified in the present
410 study were also directly associated with these pathways.

411 The phosphatase and tensin homolog (PTEN) protein is encoded by a tumor
412 suppressor gene responsible for the inactivation of pathways associated with cell
413 proliferation. PTEN negatively regulates the PI3K / AKT pathway by hydrolyzing the
414 Phosphatidylinositol-3,4,5–trisphosphate (PIP3) into Phosphatidylinositol 4,5-
415 bisphosphate (PIP2) inactivating AKT, a protein that has antiapoptotic action. Similarly,
416 PTEN inactivates the MAPK pathway by ERK (Extracellular Regulated Kinase)
417 dephosphorylation, inactivating the transmission of signals through this pathway and
418 consequently inhibiting cell proliferation. In addition, PTEN activates RAD51, a DNA-
419 repairing protein, and CENPC (Centromere Protein C), a protein responsible for the
420 stability of centromere and chromosomal integrity [149]. Situations in which PTEN is
421 downregulated result in dysregulation in the cell cycle and destabilization of the cellular
422 DNA and can trigger carcinogenic processes like CRC. In the present study, the
423 upregulation of miR-181a, miR-17-5p, miR494, miR-22 and miR-21 was correlated with
424 the downregulation of PTEN gene and worse prognosis of CRC patients.

425 The transforming growth factor β (TGFβ) is a tumor suppressor cytokinin protein
426 known to exert important functions in the regulation of apoptosis, differentiation, migration
427 and cell proliferation. Through the binding of TGFß to its TGFR2 receptor, numerous
428 proteins of this pathway are phosphorylated and activated, including the SMAD4 protein.
429 However, studies indicate that mutations or downreglation of SMAD4, can hyperactivate
430 TGFβ pathway conferring it the potential to promote tumor growth, invasion and
431 metastasis [148,150]. In the present study, upregulation of miR-20a, miR-20a-5p and miR-
432 888 was associated with the downregulation of SMAD4 and the presence of distant

433 metastasis, advanced TNM stage, worse overall survival and worse disease-free survival
434 in patients with CRC.

435 The TP53 gene encodes the tumor suppressor protein P53 that plays important roles
436 in cell cycle control and apoptosis. MiRs belonging to the miR-34 family can modulate the
437 antiproliferative and pro-apoptotic activity of P53 by inhibiting oncogenes and cell cycle
438 associated genes. Thus, downregulation of miR-34 or overexpression of oncogenic miRs
439 that negatively regulate p53 results in a dysregulation of the cell cycle leading to a
440 carninogenic process [151]. In the present study, the downregulation of P53 was correlated
441 with the upregulation of miR-125b and downregulation of miR-34a-5p, resulting in a worse
442 prognosis for patients with CRC.

443 KRAS is a protein belonging to the RAS family of proteins that control the activation
444 of several pathways related to cell proliferation, such as the MAPK pathway. MiRs
445 considered to be tumor suppressors may act as regulators of KRAS expression. Thus,
446 the downregulation of these miRs may increase the activation of KRAS resulting in a
447 dysregulated cellular proliferation [152]. The present review demonstrated that upregulation
448 of KRAS was associated with downregulation of miR-96-5p and miR-384, which in turn
449 was associated with an advanced TNM stage, presence of distant metastasis and worse
450 overall survival of patients with CRC. Fig 3 outlines the differential expression of the miRs
451 and their respective target genes.

452

453

454 **Fig 3. Upregulation and downregulation of miRs associated with their respective target genes**
455 **and cell functions.**

By analyzing the results of the present review fully, it was possible to observe the large number of miRs associated with the different prognostic aspects of CRC, making them eligible as possible prognostic markers. However, few articles have analyzed the expression of two or more miRs concomitantly. Thus, the development of studies that analyze these miRs synergistically, would allow the identification of network interactions, making them more sensitive and specific in predicting the prognosis of patients with CRC and assisting in their therapeutic behavior.

Some limitations were observed during the elaboration of the present study. The large number of studies and miRs investigated made it difficult to conduct a quantitative and qualitative analysis capable of demonstrating the most sensitive and specific differentially expressed miRs associated with each clinical-pathological and prognostic aspect of the CRC. In addition, the study was limited to articles published in English, making it harder to obtain data from studies published in other languages.

Conclusion

After analyzing the results of the present systematic review, specific tissue miRs demonstrate to be promising CRC prognostic biomarkers due to their strong correlation with prognostic aspects of this disease, including advanced TNM stage, presence of distant metastasis and lymph node metastasis, tumor growth, prediction of worse prognosis (worse overall survival) and recurrence of the disease (worse disease-free survival).

The miR-21 was the most frequent studied miR in the present review among the miRs identified and its upregulation was associated with tumor size, lymph node metastasis, distant metastasis and worse prognosis making it a promising biomarker of CRC prognosis. However, new studies are needed to systematize and identify the most sensitive and specific miRs associated with each prognostic aspect of CRC in order to choose the most relevant ones and apply them into clinical practice.

In addition, most studies have shown strong correlations between the expression of miRs and their respective target genes, so the development of functional studies aiming to elucidate these associations becomes important as a way to help in the description of new therapeutic targets of this disease.

The vast number of miRs identified in the present study also translates the complexity and heterogeneity of CRC, emphasizing the importance of new studies that clarify the regulatory mechanisms of the expression of these miRs in this tumor, allowing a better understanding of the molecular biology of this disease.

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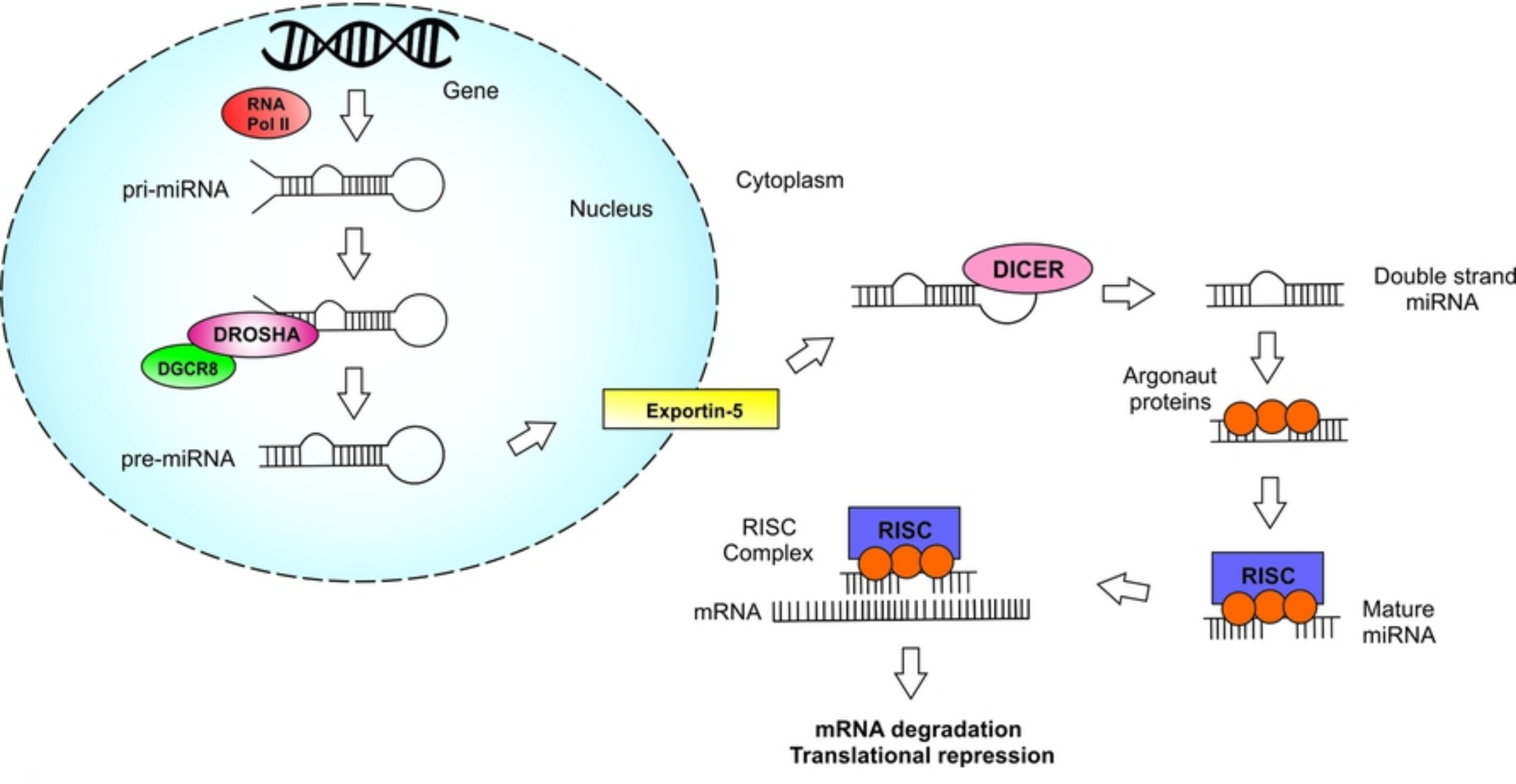
986

987 # Supporting Information

988 **S1 File. PRISMA Checklist**

989 **S2 File. Quality assessment of the selected studies for the systematic review**

990


Gene
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pri-miRNA
Nucleus
DROSHA
DGCR8
pre-miRNA
Exportin-5
Cytoplasm
DICER
Double strand miRNA
Argonaut proteins
RISC
Mature miRNA
RISC Complex
RISC
mRNA
mRNA degradation
Translational repression

Fig1

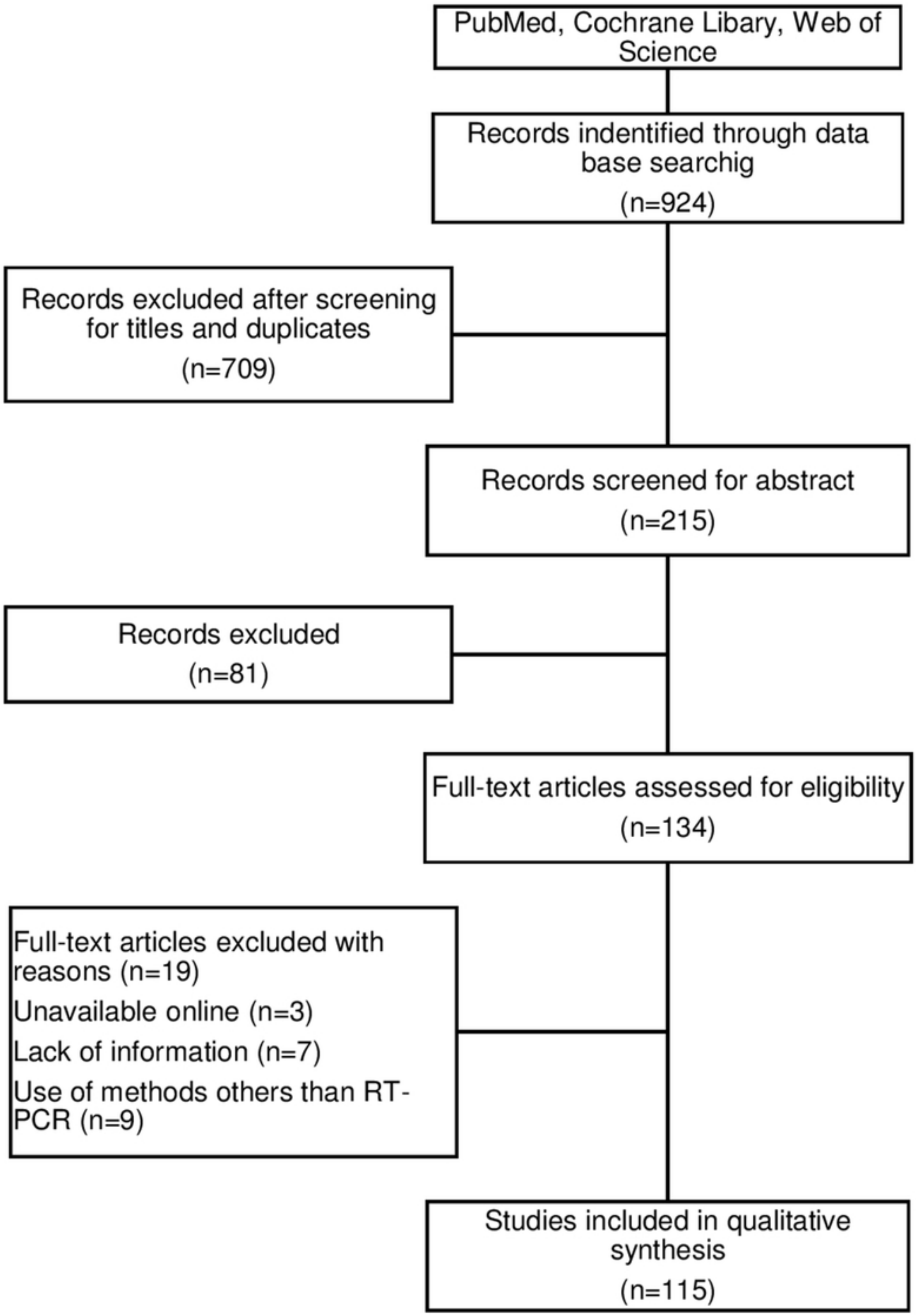
PubMed, Cochrane Libary, Web of Science
Records indentified through data base searchig
(n=924)
Records excluded after screening for titles and duplicates
(n=709)
Records screened for abstract
(n=215)
Records excluded
(n=81)
Full-text articles assessed for eligibility
(n=134)
Full-text articles excluded with reasons (n=19)
Unavailable online (n=3)
Lack of information (n=7)
Use of methods others than RT-PCR (n=9)
Studies included in qualitative synthesis
(n=115)

Fig2

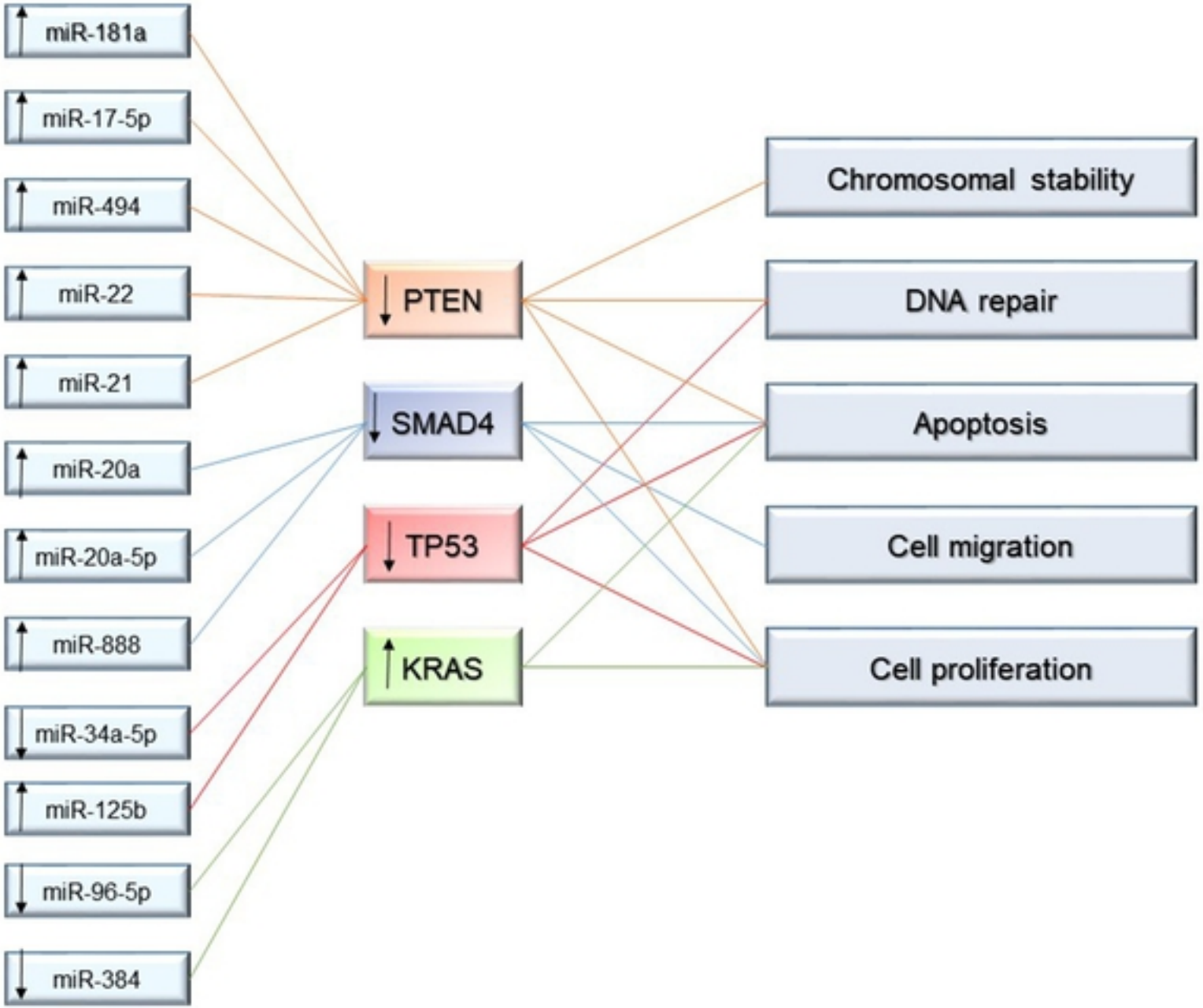
miR-181a
miR-17-5p
miR-494
miR-22
miR-21
miR-20a
miR-20a-5p
miR-888
miR-34a-5p
miR-125b
miR-96-5p
miR-384
PTEN
SMAD4
TP53
KRAS
Chromosomal stability
DNA repair
Apoptosis
Cell migration
Cell proliferation

Fig3