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Role of Micro-RNA in Colorectal Cancer Screening☆

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ABSTRACT

MicroRNAs are involved in carcinogenesis through postranscriptional gene regulatory activity. These molecules are involved in various physiological and pathological functions, such as apoptosis, cell proliferation and differentiation, which indicates their functionality in carcinogenesis as tumour suppressor genes or oncogenes. Several studies have determined the presence of microRNAs in different neoplastic diseases such as colon, prostate, breast, stomach, pancreas, and lung cancer. There are promising data on the usefulness of quantifying microRNAs in different organic fluids and tissues. We have conducted a review of the determinations of microRNAs in the diagnosis of colorectal cancer.

Papel de los micro-RNA en el cribado del cáncer colorrectal

Palabras clave:
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RESUMEN

Los micro-RNAs son responsables de la regulación de múltiples procesos biológicos de índole metabólica, de proliferación, de diferenciación, de apoptosis, del desarrollo y de la oncogénesis. En la carcinogénesis, los micro-RNA pueden ejercer su función a través de la alteración de los genes supresores de tumores o mediante la interacción con los oncogenes. Se ha determinado la presencia de diferentes micro-RNA en distintas enfermedades neoplásicas como cáncer de colon, próstata, mama, estómago, páncreas, pulmón, etc. Existen datos prometedores sobre la utilidad de cuantificar los micro-RNA en diferentes fluidos orgánicos y tejidos. Se ha realizado una revisión sobre las determinaciones de los micro-RNA en el diagnóstico del cáncer colorrectal.

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Introduction

Across the world, colorectal cancer is the third most frequent cancer in men, second in women and the second cause of death related to tumour disease.[1] In 2013, estimates in the United States showed over 140 000 new cases, with mortality in 50 000 colorectal cancer-related cases.[2]

Early detection of colorectal cancer may reduce the incidence and mortality figures; screening methods bear special importance, because the appearance of signs and symptoms are associated with advanced stages of the disease. Furthermore, follow-up of patients who have suffered colorectal cancer aims to detect those with a greater risk of disease relapse.

Unfortunately, the probability of cure after the onset of symptoms is 50%, whereas 80% can be reached with early diagnosis. Accepted screening methods for colorectal cancer are: occult blood detected in stools, double contrast opaque enema, colonoscopy, and rectal examination. Occult faecal blood testing is a low-cost and simple method, although only 50% of tumours and 10% of polyps bleed enough to be diagnosed by this method.

There is an ongoing debate on colon lesions not diagnosed by faecal blood testing or colonoscopy, because these are non-bleeding lesions, or located in the proximal colon, many with a rather aggressive progression (methylator phenotype, and instability of microsatellites).[3] In spite of the several available diagnostic methods, there is no consensus on new alternatives for detection, or guidelines on neoplastic disease precursors and early lesions. Promising advances in the field of molecular markers could play an important role at this level.

A tumour marker can be defined as an ''identifiable'' component in the tumour cell or secreted by the cellular clones.[4] A tumour marker in supraphysiological quantities indicates neoplasic disease, which contributes valuable information about the tumour's biological behaviour.[5,6] However, the absence of sensitivity and specificity in the initial phases of neoplastic disease strictly limits the systematic use of most tumour markers in screening asymptomatic patients. Disease staging at the time of diagnosis (determined by tumour size, degree of cytological differentiation and lymph node involvement) is the most-used prognostic indicator in colorectal cancer patients. However, certain prognostic markers are evaluated for this purpose, quantified and determined in tumour tissue and peripheral blood.[5]

In 1981, the *National Health Information Centre* (NHIC) proposed monitoring the carcinoembryonic antigen (CEA) as the best non-invasive technique to confirm relapses in patients with a previous diagnosis of colorectal cancer.[7] Later, in 1996, the *American Society of Clinical Oncology* (ASCO) published clinical guidelines based on evidence for using tumour markers in colorectal cancer.[8] In 2007, the *European Group on Tumour Markers* (EGTM) updated the guidelines for using serum, tissue and faecal markers in colorectal cancer.[9]

In spite of having studied a multitude of tumour markers in colorectal cancer, only a few of them are recommended for routine use. The various oncological guidelines accept and recommend: (1) faecal blood testing for early diagnosis in people over 50 years of age; (2) determination of CEA in post-operative follow-up of patients undergoing chemotherapy or surgical resections; (3) instability of microsatellites, through genetic study of MLH1, MSH2, MSH6, PMS2 to identify people susceptible for non-polyposis hereditary colorectal cancer and 4) APC mutation in the diagnosis of familial adenomatous polyposis.[7–9]

Physiologic epigenetic mechanisms that are able to change chromatin structure are, amongst others, modification of DNA methylation, histone and RNA; it has been demonstrated that epigenetic changes are just as important as genetic changes in the origin of neoplastic disease, and both contribute to the progression and development of neoplastic disease.[3] Micro-RNA are molecular structures with 20–22 nucleotides and post-transcriptional activity involved regulating genetic expression. Their participation in different physiological and pathological functions has been shown, such as apoptosis, cellular proliferation and differentiation, and their functionality as tumour suppressor genes or as proto-oncogenes in carcinogenesis has been demonstrated.[10] The medical literature includes various studies focusing on micro-RNA detection in tissue, in stools or peripheral blood, and their values have been linked to the diagnosis and prognosis of neoplastic disease.

Markers in Stools

Different studies have determined faecal DNA to quantify the various values of micro-RNA in stools. Link et al. verified the overexpression of miR-21 and miR-106a in colorectal neoplastic lesions and in adenomas, comparing them with healthy individuals.[11] In their study, Kalimutho et al. evaluated hypermethylation of miR-34b/c promoter in stools; they demonstrated that up to 75% of colorectal cancer patients had promoter hypermethylation correlated to the tumour stage, which led them to propose determining miR-34b/c aberrant methylation in stools as a diagnosis marker. In normal conditions, miR-34 functions as a tumour suppressor by participating in cellular ageing, inducing apoptosis and stopping the cellular cycle. Thus, miR-34b/c aberrant methylation, present in up to 97.5% of colorectal neoplasia, would allow cellular proliferation.[12]

Likewise, over-expression of miR-20a, miR-21, miR-92, miR-96, miR-106a, miR-203 and miR-326 in patients with colorectal tumour disease has been detected in stools, whereas advanced stages of the disease show low levels of

miR-16, miR-125b, miR-126, miR-143, miR-144, miR-145, miR-320 and miR-484-5p.[13] In the study of Koga et al., sensitivity values of 70 and 46% for miR-17-92 and miR-135 were determined with a specificity of 81% and 95%, respectively.[14]

Although different papers indicate that determining micro-RNA in stools could be used as a diagnostic marker in colorectal cancer, new studies validating these new markers are needed; furthermore, the collecting and processing of samples needs to be standardised.[15]

Tissue Markers

Since Michael et al. revealed the reduction in miR-143 and miR-145 reduction in colorectal cancer, many studies have looked for expressions of various micro-RNA in colorectal cancer. In general, almost 300 micro-RNA have been detected that are different in colon tumour tissue samples with respect to normal mucosa, with an important variability with respect to micro-RNA assessed, number of used samples and number of changed micro-RNA.[16]

Approximately 50 different micro-RNA have been described in colorectal cancer cells. Over-expressed micro-RNA relate to amplified chromosomic regions in this type of neoplasia, whereas micro-RNA expressed below physiological conditions relates to frequently deleted chromosomic regions.[15]

The medical literature lists several over-expressed micro-RNA in colorectal neoplasia, including, amongst others: miR-15b, miR-17-5p, miR-19a, miR-20, miR-21, miR-29a, miR-31, miR-92, miR-96, miR-135b, miR-148a, miR-181b, miR-182, miR-183, miR-191, miR-200b, miR-200c and miR-212. On the contrary, micro-RNA in values lower than existing ones in normal conditions would be: miR-1, miR-9-1, miR-30a-3p, miR-30a-5p, miR-30c, miR-34a, miR-34b, miR-34c, miR-126, miR-129, miR-133a, miR-133b, miR-137, miR-139, miR-143, miR-145, miR-195, miR-342, miR-422a, miR-422b and *let*-7a-1.[17–19]

Markers in Peripheral Blood

In peripheral blood, micro-RNA are arranged in structures known as exosomes or microvesicles. Aberrant expression of micro-RNA in blood detected in diverse tumours, including colorectal cancer, makes it possible to use circulating micro-RNA as tumour markers in colorectal cancer; they have been proposed as diagnosis and prognosis markers.[20]

In their study, Chen et al. detected several micro-RNA in the serum of colorectal cancer patients, and they are not present in healthy individuals; however, the common presence of these micro-RNA in lung cancer patients led to the search for specific micro-RNA for colorectal cancer.[21] Ng et al. identified 5 micro-RNA (miR-17-3p, -92, -95, -135b, -222) overexpressed in the serum and tumour tissue of colorectal cancer patients, and, additionally, verified that plasma values of miR-17-3p and miR-92a reduced their values during the post-operative period. They concluded that miR-92 has a reasonable sensitivity in colorectal cancer; therefore, it could be used for screening in this disease, as they detected 89% sensitivity and 70% specificity, with an area under the curve of 0.885.[22]

In a study that included 100 cases of colorectal cancer, 37 with adenoma, and 59 healthy patients, values with statistical significance for micro-RNA miR-92a and miR-29a were obtained ($P<.0001$), with 84% sensitivity and 71% specificity. Micro-RNA-92a showed an area under the curve of 0.838 (95% confidence interval: 0.775–0.900) in the discrimination between healthy individuals and colorectal cancer patients, additionally, linking the determination of miR-29a with TNM colorectal cancer stage.[23] Likewise, Pu et al. determined the expressions of miR-21, miR-221 and miR-222, by qRT-PCR, in 103 cases of colorectal cancer and 37 healthy volunteers, with significant ($P=.0021$) increase of miR-221 in colorectal cancer patients with respect to the control patients.[24]

The additional use of micro-RNA was also demonstrated to be able to detect advanced colorectal adenomas, which allows the diagnosis of the disease in early stages; the increase of miR-29a and miR-92a was significant with respect to normal checks ($P<.0001$); the ROC curve showed for both micro-RNA the possibility of differentiating advanced colorectal adenoma, with an area under the curve of 0.769 for miR-29a and 0.749 for miR-92a.[23] In the same type of research, Kanaan et al., while studying 380 micro-RNA, found that 8 micro-RNA (miR-532-3p, miR-331, miR-195, miR-17, miR-142-3p, miR-15b, miR-532 and miR-652) differentiated patients with colorectal polyps from healthy individuals, with an area under the curve of 0.868.[25]

Despite having determined a large amount of micro-RNA in plasma, those showing elevated concentrations in patients with colorectal tumours are miR-29a, miR-95, miR-135b, miR-221, miR-222 and miR-141, the latter being linked to stage IV tumours. It has been confirmed that determining miR-141 linked to CEA would increase detection of liver metastasis.[26] Recently, it has been suggested that miR-29a can also be used as a marker to differentiate colorectal cancer with liver metastasis, due to its increase in non-metastatic stages.[27]

These technological advances have helped perform a great variety of studies focused on determining various micro-RNA. Using micro-arrays has revealed a diversity of micro-RNA present in colorectal neoplastic disease, involved specifically with disease progression, survival, disease-free survival, and therapeutic response.[28] Vital pathogenic nexuses turn micro-RNA into potential markers for diagnosis, prognosis and therapeutic response. Early colorectal cancer diagnosis allows for treating the disease in the initial stages, rendering the neoplasia potentially curable; therefore, regardless of the method, any early diagnostic procedure is better than no screening method at all.

Conclusions

Currently, there are many important challenges in developing and implementing the determination of micro-RNA in the body as diagnostic markers for colorectal cancer symptoms. Nonetheless, there are encouraging results regarding the clinical potential of circulating micro-RNA as colorectal cancer diagnostic markers and for other types of neoplasia; results from the miR-92a study are encouraging in particular, as they are a ''significant'' way for determination in stools, plasma and tumour tissue from colorectal cancer patients.

Sensitivity for early detection of colorectal cancer and adenoma in advanced stages represent a relevant role in this context, since the combination of various micro-RNA and the conjugation of micro-RNA with other non-invasive markers, serum tumour markers or markers in stools can give way to a study aiming to establish the true diagnostic value of these new procedures. This implies including a reasonable number of individuals, comprising healthy subjects, patients with different tumour stages and adenomas, and several factors able to modify micro-RNA expression, such as comorbidities or different life styles.

Conflicts of Interest

The authors declare having no conflicts of interest.

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