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ORIGINAL ARTICLE

Using Blood and Plasma MicroRNAs as a Non-Invasive Biomarker in Patients with Colorectal Cancer

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SUMMARY

Background: **A high percentage of oncological patients die yearly because of colorectal cancer (CRC). Worldwide, CRC represents the fourth leading cause of death among oncological patients. Numerous studies have been conducted in order to identify new biomarkers for the early diagnosis of patients with CRC. From this point of view, an ideal biomarker is represented by the expression of microRNAs. In this paper, we wish to summarize the expressions of microRNAs in CRC and to present the pathophysiological and genetic interactions that microRNAs have with protein systems in these patients.**
Methods: **For this paper, we looked into the studies available in scientific databases such as PubMed. For the search the following keywords have been used: "miRNAs expression", "colorectal cancer", "genetic polymorphisms in CRC", and "genetic biomarkers in CRC".**
Results: **Modifying the expression of microRNAs can be used successfully both in diagnosing patients with CRC and in following their response to chemotherapy. Numerous studies have shown high specificity for certain microRNA species in the case of CRC. An extraordinary advantage of these biomarkers is represented by their non-invasive sampling from urine and blood. Moreover, a series of connections of microRNAs in some mechanisms involved in the appearance and development of CRC have been shown. Therefore, microRNAs can be named as the biomarker of the future, as well as the epigenetic targeted treatment for patients with CRC.**
Conclusions: **The expression of microRNAs can be successfully used in the evaluation and non-invasive monitoring of patients with CRC. However, further studies are needed regarding the expression of microRNAs and the connections these species have in the pathological mechanisms specific for CRC.**
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INTRODUCTION

According to the latest reports published worldwide, colorectal cancer (CRC) is considered to be the second leading cause of death in cancer patients in the USA and fourth in cancer patients worldwide (Centers for Disease Control and Preventions, www.cdc.gov). Therefore,

CRC leads to over 600,000 deaths and over 1 million reported new cases every year [1].
From a morphological viewpoint CRC presents five stages of development. Moreover, most of the cases of CRC are sporadic, without any clear clinical causes behind them. Regarding the genetic involvement, studies have reported an incidence of over 20% for predisposing genetic modifications [2].
The evolution of CRC is slow, the time needed for the development of a tumor is sometimes as long as years. However, there are still numerous cases that are diagnosed in late stages, and the treatment in these cases does not present high accuracy and, therefore, the death rate remains high. Moreover, in this type of cancer, no new treatments have been developed that would present high specificity and selectivity.
In current medical practice, the classical treatment is surgical removal of the tumors followed by chemotherapy [3,4]. The main target of modern treatments is the suppression of tumor angiogenesis through the action on the vascular endothelial growth factor or the epidermal growth factor receptor. However, no high rate successes have been noted, especially in the case of tumors with involvement of KRAS oncogenic mutations. These types of tumors are resistant to treatment. Recent studies have reported on the importance of using microRNA species both for detection and for genetic treatments that would target the genes involved in the development of the tumor. Furthermore, research has shown a high specificity and selectivity for certain microRNA families that could be successfully used in the early detection of CRC, as well as for inhibiting some genetic proliferation mechanisms involved in the disease [5-7].
In this paper we wish to summarize the most important microRNAs that can be used as genetic biomarkers for the early detection of CRC. Moreover, we want to present as series of treatment opportunities proposed for blocking the development and proliferation mechanisms of CRC.

Morphopathological aspects for colorectal cancer (CRC)

From a morphopatological viewpoint, CRC is classified in four stages: invasion of the submucosa (stage I), penetration of the outer colonic wall (stage II), lymph node invasion (stage III), and metastasis (stage IV). The first genetic changes specific for the development of CRC begin in the intestinal epithelial stem cells or in the rectal mucosa and most frequently include modifications in the APC, BRAF or KRAS genes [8-10].

Biochemical and biogenesis aspects of microRNAs

From a biogenetic point of view, microRNA species are single-stranded non-coding RNAs, with 19 - 24 nucleotides [11,12]. The synthesis activity of the microRNA begins in the cell nucleus through the action of RNA polymerase II on the microRNA genes. Following this genetic reaction the first species are obtained, called pri-microRNAs [13]. Afterwards, the pri-microRNAs are attacked by the RNase III endonuclease (Drosha) leading to the formation of the next species called pre-microRNAs [14]. In order for this reaction to take place, Drosha needs a biocatalyzer or cofactor - DiGeorge Syndrome Critical Region 8 (DGCR8) [15]. One of the important steps in the formation process of these species is represented by the transportation of pre-microRNAs from the cell nucleus into the cytoplasm. This is made possible by the intervention of Exportin-5, a transporting protein. Once in the cytoplasm, the pre-microRNAs are attacked by the RNase III endonuclease (Dicer), together with another cofactor called trans-activator RNA binding protein (TRBP) [16-18]. This is followed by the introduction of the mature RNA in the RNA induced silencing complex (RISC). Following the aforementioned reactions, stable microRNA species are produced, that can be consequently released from the cell through two different mechanisms, passive release or active release (Figure 1).

CRC mutated genes and their relationship with microRNAs

A series of studies have reported a range of correlations between the action of microRNAs and the pathogenesis of CRC (Figure 2). Gregersen et al. have proven that microRNA-145 intervenes in the colorectal cancer cell line mechanisms - DLD-1. Moreover, they have also shown that microRNA-145 activated the YES system and signal transducer and activator of transcription 1 (STAT1) that is afterwards involved in CRC [19].
A similar study was carried out by Zhang et al. and has shown that microRNA-143 is also involved in the development of CRC through the activation of the metastasis-associated in colon cancer 1 (MACC1) [20].
Feng et al., have carried out a study regarding the mechanisms and the role microRNA-141 plays in the pathogenesis of CRC. Following this study, they observed that the expression of microRNA-141 was significantly decreased in the case of CRC. Moreover, they also observed that by decreasing the expression of microRNA-141, the levels of mitogen-activated protein kinase 4 (MAP4K4) increases aggressively. MAP4K4 is responsible for decreasing the activity of the anti-tumor response [21].
A similar study, carried out by Wan et al., shows the fact that the expression of microRNA-133a is significantly lower in the case of CRC. On the other hand, in the same study they have also shown that an increased expression in microRNA-133a can be strongly associated with poor prognosis [1]. Another important study carried out by Jinushi et al. has shown that there are a series of connections between microRNAs, the enhancer of zeste homolog 2 (EZH2) gene, and the milk fat globule-epidermal growth factor 8 (MFGE8) gene. Regarding the activity of MFGE8, it was proven that it is responsible for the tumor progression, while EZH2 is associated with metastasis and poor survival. If we were talking about the interaction with microRNAs, this study proved an increased expression in microRNA-124-5p as well as

Table 1. microRNA expressions in CRC.

Study	microRNAs	Expression	Comments	Reference
Liu et al.	microRNA-19a	Down	Associated with T-cell intracellular antigen 1 (TIA1 protein) activity Aberrant expression for microRNA-19a can accelerate the proliferation and migration in CRC cells	[30]
Miyoshi et al.	microRNA-139-5p	Up	Increased expression for microRNA-139-5p was associated with poor-recurrence-free survival Moreover, it was shown that through the upregulated expression of this microRNA species, strong correlation can be made with the epithelial-mesenchymal transition	[31]
Wang et al.	microRNA-622	Up	It was reported that microRNA-622 intervenes in the genetic proliferation and development mechanisms of CRC cells through the dual specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2)	[32]
Toiyama et al.	microRNA-21	Up	Associated with tumor size. Strongly correlated with poor survival. Sample from serum	[8]
Liu et al.	microRNA-92a	Up	Strongly associated with poor survival	[33]
Wang et al.	microRNA-21, microRNA-31, let-7g, microRNA-92a, microRNA-181b, microRNA-203	Up	Correlated with tumor stage Sample from serum	[34]
Kjersem et al.	microRNA-106a, microRNA-484, microRNA-130b, microRNA-148a, microRNA-326	Up	Strongly correlated with chemotherapy non-responders Associated with tumor stage	[35]
Colangelo et al.	microRNA-130b	Up	Associated with tumors in advanced stages	[36]
Zhang et al.	microRNA-155	Up	Correlated with advanced TNM stage and metastasis	[37]
Liao et al.	microRNA-244	Up	Associated with tumor growth and metastasis	[38]

a decreased expression for microRNA-26a [22].
Another important advantage of using microRNAs as diagnosis and prognosis biomarkers in CRC is given by the fact that these epigenetic species can bring important information regarding the response to chemotherapy. Zhang et al. carried out a study regarding the modifications that occur in microRNAs during chemotherapy treatment in patients with CRC. Following this study they have shown a strong correlation between microRNA-20a, microRNA-130, microRNA-145, microRNA-216, and microRNA-372 and the poor response to oxaliplatin based chemotherapy [23].

microRNAs as novel biomarkers in CRC

A series of studies in the field have shown significant changes from a statistical point of view regarding the expression of microRNAs in the case of CRC. Moreover, a high specificity has been proven both for this type of tissue as well as for the pathophysiological or mophopathological changes specific for CRC.
Xiao et al., in an original study carried out on a group of patients with CRC, reported aberrant changes for microRNA-15a and microRNA-16. Furthermore, they showed that microRNA-15a as well as microRNA-16 are strongly correlated with the lymph nodes positive for metastasis ($p < 0.05$) [24].
In a similar study, Ke et al. reported important changes for microRNA-224 in CRC. Moreover, they showed that microRNA-224 has a negative impact on cell migration in CRC [25]. Liu et al. observed that in the case of CRC the expression of microRNA-124 is significantly downregulated both in colorectal cancer tissue as well as in the cellular lines in comparison with healthy tissue. Moreover, in this study, they identified a strong correlation between the activity of microRNA-124 and the inhibitor of apoptosis-stimulating protein of p53 (iASPP). Regarding the genetic mechanism involved, it is related to the fact that microRNA-124 influences the protein ex-

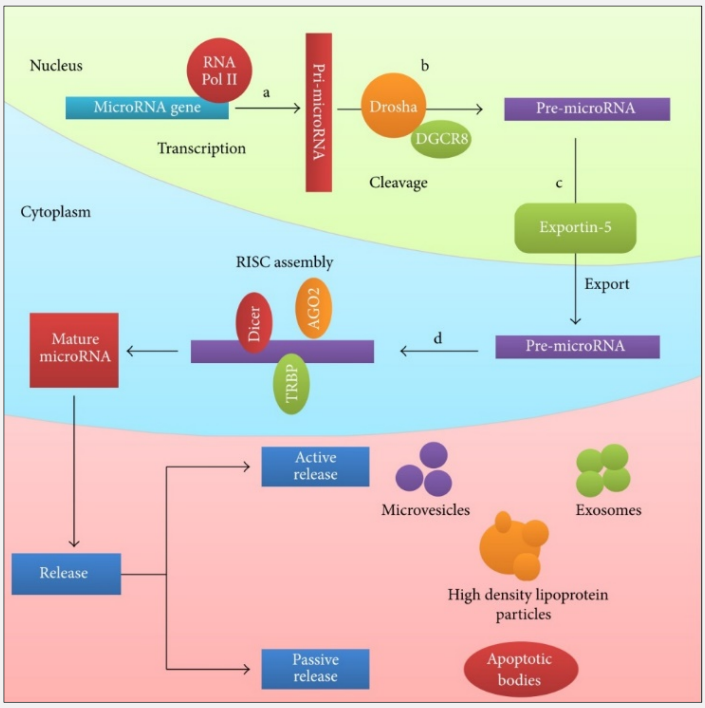

Figure 1. miRNA biogenesis mechanism. miRNA synthesis begins with the RNA polymerase II action on protein coding genes.

(a) Through the transformation phenomenon of the miRNA genes, pri-miRNA will be formed. (b) Through the action of RNase III endonuclease (Drosha) and of the DiGeorge Syndrome Critical Region 8 (DGCR8) cofactor, the pre-miRNA will be formed. (c) Transporting protein Exportin-5 will transfer the pre-miRNA from the nucleus into the cytoplasm. (d) In the cytoplasm, pre-miRNA is attacked by second RNase III endonuclease (Dicer) and transactivator RNA binding protein forming mature miRNA (double-stranded) and passenger strand. In what follows, mature miRNA induced silencing is taken in complex (RISC). The RISC complex contains mature miRNA and protein Argonaute 2 (AGO) that confers increased stability. After this, miRNAs are released from the cell through two mechanisms: active release (microvesicles, exosomes, and high density lipoprotein particles) and passive release (apoptotic bodies). Reproduced after Dumache et al. [13].

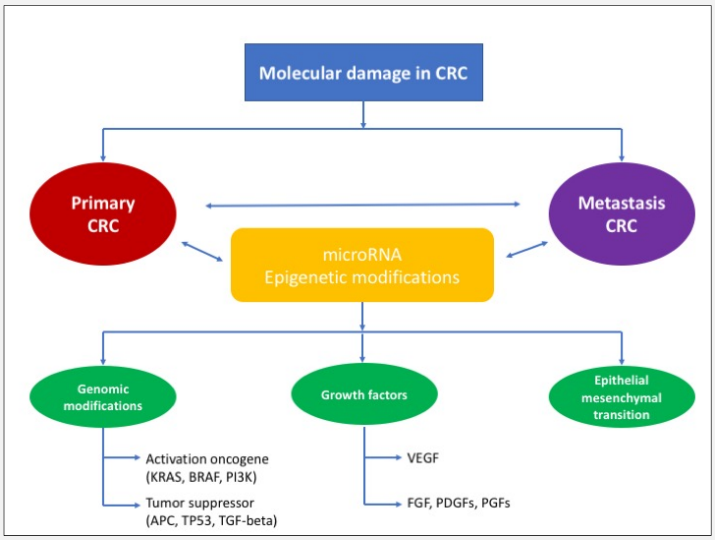

Figure 2. Molecular and epigenetic connections in the genesis of CRC.

pression of iASPP, leading to a decrease in signal activity for nuclear factor-kappa B (NF-kB) and, therefore, reducing the cell viability and the proliferation of CRC cells [26]. Wan et al. analyzed 125 pairs of tissue samples through genetic analysis using quantitative-real time polymerase chain reaction (qRT-PCR). Following this study, this group of authors showed that microRNA-133a had a decreased expression in CRC tissue. Moreover, by statistically correlating the result with the clinical data of patients from whom samples had been taken, they reported that microRNA-133a can be strongly associated with poor prognosis in CRC patients [1]. A similar study was carried out by Wang et al., who reported that the aberrantly increased expression for microRNA-31 is strongly correlated with the development and extension of CRC. An interesting fact is that, following this study, they were able to prove that microRNA-143 and microRNA-145 are involved in the development of CRC, but are not involved in the progression of the disease. Therefore, one can conclude that by using microRNAs as genetic biomarkers for cancer patients information regarding the stage and evolution of the disease can be obtained due to their high specificity and selectivity [27]. Zhang et al., by obtaining statistically significant results, correlated the aberrant expression for microRNA-195 with the development of CRC cells. Moreover, they proved the fact that the overexpression of microRNA-195 inhibits cell viability by its action on specific genetic mechanisms such as CyclinB1, CyclinD1, and CDK2. Following this study, this group of authors confirmed that by increasing the expression of microRNA-195, the proliferation of CRC cells can be stopped, especially through fibroblast growth factor 2 (FGF2) [28]. Li et al., in a similar study, showed an aberrant expression for microRNA-613 in the case of CRC [29]. A series of other studies reported different expressions in the case of CRC patients (Table 1).

Xu et al., in a similar study, also proved modified expressions for microRNA-130a, microRNA-26a-2, microRNA-148a, and microRNA-103-1. Moreover, following this study they validated microRNA-130a as having the anticancer effects because of the mechanisms it influences and because it blocks the proliferation of CRC cells [39].

CONCLUSION

In the case of CRC, a series of species of microRNAs present significant changes in the epigenetic expression. Moreover, there is an important panel of microRNAs that present high specificity and selectivity for the genetic mechanisms of CRC. Last but not least, following the studies, a series of connections of certain microRNAs in the development and progression of CRC have been proven.

In conclusion, we can state that microRNAs can be successfully used as diagnosis and prognosis biomarkers in the case of CRC patients. Furthermore, microRNAs can be used in the evaluation of the response to chemotherapy in different stages of the disease. Last but not least, according to the latest research, certain microRNA species can be used as a targeted epigenetic treatment by blocking genetic mechanisms and by suppressing the expression of some determining factors, all involved in the progression of CRC. However, further studies are needed both regarding the usage of microRNAs as biomarkers as well as regarding their use in diagnosis and prognosis, or as a targeted epigenetic treatment.

Declaration of Interest:
Nothing to declare.

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