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Good cop-bad cop: different roles of hsa-miR-93-5p in colorectal cancer

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Background: Colorectal cancer (CRC) is a heterogeneous disease that ranks third and second globally in terms of incidence and mortality rates, respectively. Five-year survival of patients with CRC is approximately 90% if diagnosed in the early stages, and only 13% if the advanced disease is present. About 25% of patients already have CRC metastases (mCRC) with the primary CRC diagnosis, while half of the patients will develop metastases with further disease progression. The most common organ in which CRC metastasizes is the liver (colorectal cancer liver metastasis, CRLM). Almost half of CRC patients will die due to complications caused by the presence of metastases, so it is extremely important to discover new therapeutic approaches, as well as prognostic and predictive biomarkers, in order to reduce such a high mortality rate. In the era of personalized medicine, various treatment modalities are available to CRC and mCRC patients, including resective surgery, systemic chemotherapy, and novel targeted biologics, which significantly improve the outcome of CRC patients. The main goal of neoadjuvant systemic chemotherapy is to render currently unresectable disease amenable to resection. The standard cytotoxic drugs used in systemic chemotherapy for the treatment of CRC are: 5-fluorouracil (5-FU), oxaliplatin, and irinotecan applied as single agents or combined. It has been shown that the combination of systemic chemotherapy with targeted biological agents (e.g., bevacizumab which targets vascular endothelial growth factor) leads to a better therapy response compared to the use of systemic chemotherapy alone. MicroRNA (miRNA) molecules belong to a large class of small regulatory non-coding single-stranded RNA molecules that exert negative post-transcriptional regulation of gene expression. MiRNAs are involved in the regulation of fundamental cellular processes such as cell proliferation, differentiation and death, thus these molecules have been proposed as one of the regulators of oncogenesis, considering that they can have an oncogenic or tumor-suppressive role, which can be tumor-specific. The miRNA expression pattern is consistently and reproducibly altered in CRC compared with normal intestinal mucosa, and this expression pattern changes during the progression from normal colon, through adenoma to colorectal cancer. Not surprisingly, microRNAs have been implicated in the CRC growth, progression, metastasis, and response to therapy. MiRNAs have also been studied as potential diagnostic, prognostic and predictive biomarkers, and therapeutic agents or targets. To date, a small number of molecular biomarkers have been identified that can predict a patient's response to therapy and thus help doctors in decision making to select the right therapy for a given patient. Identification of new validated predictive and prognostic biomarkers will be necessary to improve the quality of life and outcome of CRC patients. Hsa-miR-93-5p, together with hsa-miR-106b and hsa-miR-25, belongs to the miR-106b-25 cluster located on the 515 bp long region of chromosome 7q22, within intron 13 of the MCM7 gene. Interestingly, hsa-miR-93-5p has been reported to have oncogenic and tumor-suppressive roles in different tumor types. This systematic review aims to present the current knowledge on the

role of hsa-miR-93-5p in the processes related to colorectal carcinogenesis, metastasis, and response to chemotherapy in patients with primary and metastatic colorectal cancer. Also, the role of hsa-miR-93-5p as a potential prognostic and predictive biomarker is described. **Material and Methods:** PubMed database was searched using keywords hsa-miR-93-5p, colorectal cancer, metastatic colorectal cancer, response to therapy, 5-fluorouracil, oxaliplatin, irinotecan, bevacizumab, predictive biomarker, and prognostic biomarker for available relevant literature data. **Results:** Literature data shows no consensus regarding the direction of hsa-miR-93-5p expression in CRC compared to normal mucosa, with most studies indicating elevated hsa-miR-93-5p expression, while others emphasize decreased hsa-miR-93-5p expression. In various tumors, the oncogenic or tumor-suppressive role of hsa-miR-93-5p has been demonstrated. As for CRC, several studies have shown that hsa-miR-93-5p inhibits proliferation, invasion, migration, autophagy and tumor formation in vivo, reduces viability, induces apoptosis, and may suppress the immune evasion of CRC cells, indicating a tumor-suppressive role. Some of the molecular mechanisms by which hsa-miR-93-5p regulates these processes include Wnt/β-catenin and PI3K/AKT signaling pathways, also downregulation of several genes including ERBB2, p21 and VEGF, and reduction of MMP-1, MMP-2, and MMP-9 proteins. Results of our still unpublished work are in contrast to the aforementioned studies where the anti-tumor effect of hsa-miR-93-5p in CRC was proven. We have shown that hsa-miR-93-5p stimulates in vitro tube formation thereby participate in promoting CRC angiogenesis, but does not affect viability, cell cycle, anoikis and migration in vitro, as well as tumor growth in the in vivo chick embryo model. MiRNA expression is often altered by anti-tumor drugs. A number of studies associate hsa-miR-93-5p with response to standard and targeted chemotherapeutic drugs used for the treatment of CRC and mCRC. One study showed downregulation of hsa-miR-93-5p after treatment with 5-FU and oxaliplatin for 24 h, while other showed increase of hsa-miR-93-5p after treatment with 5-FU for 24 h in a different CRC cell line. Results on the metastatic CRC cell line SW620 showed that the hsa-miR-93-5p expression 72 h after treatment with individual chemotherapeutic drugs (5-FU, oxaliplatin, irinotecan) and their combinations (5-FU + oxaliplatin and 5-FU + irinotecan) was reduced compared to the control treatment. When comparing these studies, one should definitely pay attention to the used CRC cell line, as well as the used concentration and the treatment duration. Besides standard chemotherapeutic drugs, the effect of targeted molecular agent bevacizumab on hsa-miR-93-5p expression was also analyzed in vitro, applied alone or in combination with 5-FU + oxaliplatin. However, neither bevacizumab nor bevacizumab in combination with 5-FU + oxaliplatin for 72 h did not change the hsa-miR-93-5p expression in SW620 metastatic CRC cell line. miRNAs present in the tissue or as freely circulating molecules in the blood have been proposed as promising biomarkers for predicting response to systemic and targeted therapy in CRC patients. One study investigated whether the hsa-miR-93-5p expression was altered in patients who received neoadjuvant chemotherapy based on the combination of 5-FU + oxaliplatin, compared to those who did not receive neoadjuvant chemotherapy using samples of metastatic tissue and serum from patients with CRLM. Also, in order to examine the translational potential of hsa-miR-93-5p as a predictive biomarker for response to neoadjuvant chemotherapy in patients with CRLM, the association of high and low expression of hsa-miR-93-5p in metastatic tumor tissue and serum was analyzed in patients who showed a chemotherapy response compared to those who did not. These results showed that neoadjuvant chemotherapy based on the combination of 5-FU + oxaliplatin did not affect the expression of hsa-miR-93-5p in metastatic tumor tissue or serum in CRLM patients, and that hsa-miR-93-5p has no predictive potential in patients with CRLM. Numerous studies indicate that miRNAs expressed in the tumor or in various body fluids could be potential prognostic biomarkers for CRC and mCRC and thus predict the disease outcome. It was suggested that miRNAs in combination with carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) tumor markers could improve the discrimination of patients in relation to the disease outcome. One study investigated whether hsa-miR-93-5p expressed in CRLM or serum of these patients correlates with the levels of CEA and CA 19-9 tumor markers. The results showed that hsa-miR-93-5p expressed in CRLM shows a moderately negative correlation with CEA level. So far, four studies investigated the potential prognostic utility of hsa-miR-93-5p in CRC patients. All studies found that decreased hsa-miR-93-5p expression was associated with early disease recurrence and worse overall and disease-free survival. Regarding the role of hsa-miR-93-5p in mCRC, hsa-miR-93-5p expression is known to be higher in CRLM compared to normal colon tissue and compared to primary CRC. One study investigated the prognostic significance of hsa-miR-93-5p for one-year disease-free survival and early disease recurrence in CRLM patients. The results showed that there was no difference in one-year disease-free survival in patients with increased or decreased hsa-miR-93-5p expression in CRLM or serum. However, it was shown that the elevated hsa-miR-93-5p expression in the serum was significantly associated with early disease recurrence during one-year follow-up. **Conclusion:** This systematic review summarizes the role of hsa-miR-93-5p in colorectal carcinogenesis, response to therapy and disease prognosis. Tumor-suppressive role of hsa-miR-93-5p has been demonstrated in CRC. Standard chemotherapeutics for the treatment of CRC affect the expression of hsa-miR-93-5p, but different in vitro studies have shown conflicting results. Numerous studies confirm the prognostic utility of hsa-miR-93-5p in CRC patients, as it has been shown that reduced hsa-miR-93-5p expression is associated with early disease recurrence and worse overall and disease-free survival. In mCRC, hsa-miR-93-5p has been shown to participate in promotion of angiogenesis in CRC and mCRC by stimulating in vitro tube formation. It has also been shown that standard

chemotherapeutical drugs 5-FU, oxaliplatin, irinotecan and their combined regimens, but not the targeted agent bevacizumab, affect the hsa-miR-93-5p expression in SW620 cells in vitro, but the effect of neoadjuvant chemotherapy on hsa-miR-93-5p expression was not observed in CRLM patients. Hsa-miR-93-5p has no predictive potential in CRLM patients treated with 5-FU + oxaliplatin. Interestingly, hsa-miR-93-5p in CRLM showed a moderately negative correlation with the level of the tumor marker CEA, but further research should confirm the usefulness of their combination for the disease prognosis. Finally, elevated hsa-miR-93-5p expression in the serum of CRLM patients was significantly associated with the early disease recurrence, which is why it was proposed as a potential prognostic biomarker for the early disease recurrence.

Keywords: colorectal cancer, hsa-miR-93-5p, metastasis, predictive biomarker, prognostic biomarker, response to therapy

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