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ORIGINAL ARTICLE

Plasma miR-21 and miR-31 as Predictive Biomarkers for Evaluation of Therapeutic Efficacy in Metastatic Colorectal Cancer

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SUMMARY

Introduction. Colorectal cancer (CRC) is the third most common cancer worldwide. Evaluation of patient response to the applied therapy regime is still challenging. Routine laboratory tests during follow-up do not provide necessary information on therapy efficacy. To improve life expectancy and quality of life for patient with CRC ongoing researches is concentrated to discovering new, reliable biomarkers which could contribute to define patients prognosis and choice of therapy. One of topical research field for searching new biomarkers is mi-RNS.

Aim of the study. The aim of this study was to analyze relative expression changes of miR-21 and miR-31 as potential biomarkers for evaluation of therapy efficacy in mCRC.

Material and methods. In the present study seven patients with mCRC diagnosis were included. After surgery all patients received first line therapy with oxaliplatin. Blood samples for present study were collected every 2-3 weeks. Relative expression of miR-31 and miR-21 assessed with real time PCR. Data analysis carried out by delta delta Ct ($\Delta\Delta$Ct) algorithm. Correlation analysis was performed by R programme ver. 3.1.2 using Pearson correlation coefficient.

Results. Our results in the correlation analysis for CEA serum level and relative expression level of miR-21 and miR-31 did not confirm significant correlation. MiR-31 showed increased expression soon after administration of therapy with a drop of relative expression closer to the CDP I. Acquired results revealed trend of increase in relative expression of miR-21 in PFS II compared to PFS I. Correlation analysis for miR-31 and miR-21 did not reach statistical reliability.

Conclusions. The relative expression alteration pattern of miR-31 and miR-21 in plasma during the therapy is promising biomarker for evaluation of therapy efficacy. These findings need to be validated in large cohort sample set.

Key words: miR-21; miR-31; mCRC; therapy efficacy

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide (2; 4). Approximately 25% of patients have already developed metastases at the moment of diagnosis. 50% of all CRC patients will develop metastases while the disease progresses. To the best knowledge the mechanisms of formation of metastasis in CRC are not fully understood (2; 7). The 5-year survival rates for early CRC patients are approximately 90% but in patients with distant metastases less than 10% (3).

Treatment for patients with metastatic CRC (mCRC) in majority of cases is palliative rather than curative. The main goals of treatment in these patients are to prolong survival and to support quality of life for as long as possible. Treatment of CRC is based on radical tumour resection and radio- or chemotherapy (11). Treatment choice is made by the tumor, node and metastasis (TNM) classification of the Union for International Cancer Control (UICC) (6). Despite of new developments in therapeutical agents and combination of different therapy regimes (such as FOLFOX and FOLFIRI) it has not given massive improvement in patient's survival (2; 4; 7). Over the recent years the new therapies of monoclonal antibodies targeting the vascular endothelial growth factor (VEGF) and the epidermal growth factor receptor (EGFR) has been introduced in mCRC patients care and have significantly improved median overall survival (OS) of patients (7).

The developments in personalised therapy based on distinct genetic biomarkers such as *KRAS, PIC3CA, p53, EGFR* and *BRAF* mutations, microsatellite instability (MSI), 18q chromosome deletion in tumour tissues contribute to the choice of therapy modality for improvement of progression-free survival (PFS) and OS (11;12).

Evaluation of patient response to the applied therapy regime is still challenging. Routine laboratory tests (complete blood cell counts, liver function tests, coagulation profiles, and chemistry panels) during follow-up do not provide necessary information on therapy efficacy. One of the most commonly used blood biomarkers of chemotherapeutical response in CRC is carcinoembryonic antigen (CEA). Pre-treatment CEA levels are used for prediction of prognosis, and post-operative serial assays of CEA level provide an opportunity for early detection of recurrent disease. Reduction of the CEA level after radical resection has been associated with improved survival in rectal cancer (10; 5).

To improve life expectancy and quality of life for patient with CRC ongoing topical research is concentrated to discovering new, reliable biomarkers for cost-effective and non-invasive diagnosis of disease as well as biomarkers which could contribute to define patient`s prognosis and choice of therapy. CRC along with other neoplastic diseases is phenotypic expression of multiple molecular pathways which include micro-satellite instability (MSI) or micro-satellite stability (MSS), epigenetic changes and chromosomal instability (3). Mi-RNAs are one of known mechanisms for epigenetic regulation of genes and consist of small, non-coding RNA fragments (18-24 nucleotides). It is suggested that up to 30% of protein-genes are regulated by mi-RNAs (9). Most of research concentrates on expression alterations of mi-RNAs in tumour tissue compared to plasma as diagnostic, prognostic and predictive marker (15). Along with many other mi-RNAs, miR-31 and miR-21 are described as regulatory molecules involved in pathogenesis of CRC. Sun and colleagues reported miR-31 role in activating the RAS signalling pathway through the inhibition of RASA1 translation, thereby stimulating tumorigenesis in case of CRC (14). Upregulated expression of mir-31 is confirmed in CRC tumour tissue and positively related to advanced TNM stage (9; 1; 8; 13). MiR-21 is well described as onco-miR. High miR-21 expression in cancer tissues is reported in association with advanced TNM staging, poor survival, and poor therapeutic outcome (9; 16). Upregulation of miR-21 is also reported in plasma and feces (9).

AIM OF THE STUDY

The aim of this study was to analyze relative expression changes of miR-21 and miR-31 as potential biomarkers for evaluation of therapy efficacy in mCRC.

MATERIAL AND METHODS

Patients. In the present study seven patients with mCRC diagnosis were included. All patients were diagnosed and underwent radical surgery at Pauls Stradins Clinical University Hospital, Riga, Latvia. Informed consent was obtained from all individual participants included in the study. After surgery all patients received first line therapy with oxaliplatin, with follow-up serum CEA level every 2-3 weeks, CT and MRI based on RECIST 1.1 criteria every 8-10 weeks during the therapy and thereafter. For the correlation analysis for CEA serum concentration and mi-RNA relative expression patients were divided in two groups based on CEA level during the therapy – CEA informative group (CEA >5 ng/ml) and non-informative group (CEA <5 ng/ml) (Table 1.). Blood samples for present study were collected every 2-3 weeks and matched with CEA level analysis. Data points during the course of the disease varied among patients, the shortest one consisted of nine data points, the longest – 33 respectively.

Mi-RNA analysis. Circulating nucleic acids were extracted from frozen plasma samples with mirVana miRNA isolation kit (Ambion, Life technologies, USA). RT-PCR was carried out using TaqMan MicroRNA Reverse Transcription Kit (Life Technologies, USA) according to the manufacturer protocol. Mi-RNA relative expression was assessed by TaqMan MicroRNA Assay (Life Technologies, USA) using TaqMan Universal PCR Master Mix (Life Technologies, USA) on Applied Biosystems ViiA 7 real time PCR platform. Two endogenous controls (miR-486 and let-7a) were used for normalisation of acquired mi-RNA data. Data analysis carried out by delta delta Ct (ΔΔCt) algorithm. The relative expression of mi-RNA was calculated against the first sample obtained before therapy started.

Statistical analysis. Correlation analysis was performed by R programme ver. 3.1.2 using Pearson correlation coefficient.

RESULTS

The correlation of miR-21 and miR-31relative expression with CEA concentration in plasma was analyzed on the start of therapy, during the first progression free survival (PFS I), on the first clinically detectable progression (CDP I), during the second progression free survival (PFS II), on the second clinically detectable progression (CDP II) and during palliative care (PC).

In the group of CEA informative patients one out of three patients (Fig. 1.) demonstrated statistically significant correlation between CEA concentration in serum and relative expression of miR-21 ($r = 0.64$; $p = 0.01$). In the group of CEA non-informative patients one out of four showed similar trend of correlation between CEA and miR-21, but did not reach the statistical significance ($r = 0.53$; $p = 0.08$). Between CEA and miR-31 in both groups correlation was not confirmed. Analysis of miR-21 and miR-31 relative expression in both groups did not reveal correlation between those markers, although two patient sample sets from CEA non-informative group demonstrated correlation trend, but there was no statistical reliability achieved.

The relative expression of miR-31 was analysed in both patient groups according to the course of the disease. Out of seven patients four of them demonstrated increased miR-31 expression after the start of therapy – PFS I, compared to relative expression level at the start of therapy, CDP I, PFS II and CDP II (Fig. 2.). Despite the result did not succeed to meet statistic significance, the relative expression level trend during the PFS I showed differences against CDP I, PFS II and CDP II.

Analysis of miR-21 relative expression during the course of the disease revealed five patients out of seven with increased miR-21 expression trend on PFS II compared to PFS I (Fig. 3.).

DISCUSSION

CEA is considered to be a sensitive biomarker for detecting cancer progression, however high level of serum CEA is not specific to CRC and can be caused by another disease (Gonzales-Pons et al., 2015). Also our patient group with non-informative CEA level showed the marker is not enough informative for evaluation of therapy efficacy. Mi-RNAs are considered as sensitive biomarker for diagnostics as well as

prognostic and predictive marker. Most of published research concentrates on analysis of mi-RNA expression alteration in cancer tissue and/or plasma, in some cases also in feces. To the best of our knowledge there are very few if any publications concentrated on expression level changes of miR-31 and miR-21 during the therapy course. Based on knowledge about upregulation of miR-21 and miR-31 in cancer tissue we hypothesize alteration in expression pattern in plasma of these mi-RNAs during the applied therapy.

Our results in the correlation analysis for CEA serum level and relative expression level of miR-21 and miR-31 did not confirm significant correlation. This can be explained by already discussed factors contributing to the CEA concentration fluctuation. We suggest that miR-31 and miR-21 expression alterations during the therapy course in plasma in combination with CEA concentration are not informative.

More promising results were acquired by observation of mi-RNA expression alterations during the therapy. MiR-31 showed increased expression soon after administration of therapy with a drop of relative expression closer to the CDP I. This observation can be explained with already described upregulation of miR-31 in CRC cancer tissues and involvement in tumorogenesis. Applied therapy trigger autophagy of cancer cells and by this process the high concentration of miR-31 from tumour cells are released. We assume that high relative expression of miR-31 in plasma during the therapy is promising marker for evaluation of therapy efficacy. Observed increase in relative expression of miR-21 in PFS II could be indicative for disease progression and also to the development of therapy resistance. Correlation analysis for miR-31 and miR-21 did not reach statistical reliability.

Based on results of present study we suggest that analysis of miR-31 and miR-21 relative expression during the therapy course could be informative for evaluation of therapy efficacy and disease progression. To achieve statistical and clinical reliability of our results it is necessary to replicate this study in larger study group. Choice of CRC patient group without metastasis would contribute complementary data to validation of mentioned biomarkers in clinical practice.

CONCLUSIONS

The relative expression alteration pattern of miR-31 and miR-21 in plasma during the therapy is promising biomarker for evaluation of therapy efficacy. These findings need to be validated in large cohort sample set.

Table 1. Serum CEA values (ng/ml) during the therapy

Sample	Start of therapy	PFS I mean	n	CDP I	PFS II mean	n	CPD II	PC mean	n
CEA informative patient group									
Z01	46.7	18.6±9.8	8	49.8	56.4±27.4	4	91.1	-	-
Z11	958.8	1034.0±239.2	9	846.4	>1500.0±0	2	>1500.0	-	-
Z28	25.9	22.2±5.9	6	12.8	16.4±5.5	3	-	-	-
CEA non-informative patient group									
Z02	2.6	2.2±0.3	18	2.0	2.2±0.3	13	-	-	-
Z04	1.6	1.3±0.2	2	1.6	1.2±0.3	3	1.1	1.0±0.3	3
Z12	2.6	3.3±0.7	7	1.4	-	-	-	-	-
Z22	3.3	2.0±1.1	14	3.5	1.5±0.3	16	-	-	-

Start of therapy – first plasma sample acquired on start of the therapy; CEA – carcinoembryonic antigen; PFS – progression free survival; n – number of sample collection points; CDP – clinically detectable progression; PC – palliative care.

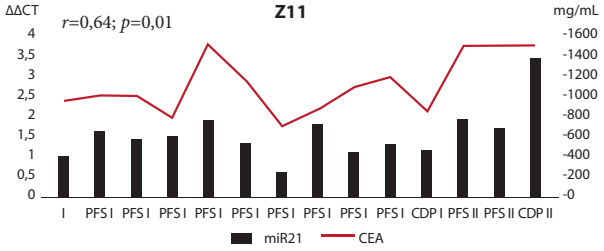

Fig. 1. Correlation of CEA concentration and relative expression of miR-21 in patient Z11 during the course of the disease. I – the first plasma sample acquired on start of the therapy; CEA – carcinoembryonic antigen; PFS I – the first progression free survival; CDP I – the first clinically detectable progression; PFS II – the second progression free survival; CDP II – the second clinically detectable progression

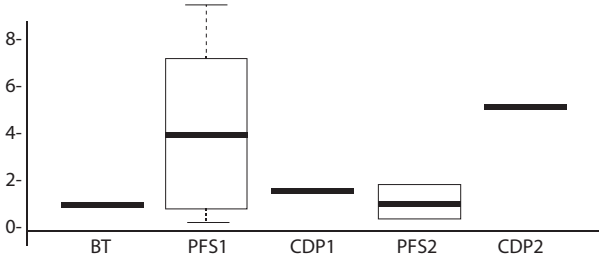

Fig. 2. Relative expression of miR-31 in patient Z11 during the course of the disease. BT – expression level before the therapy start; PFS I – the first progression free survival; CDP I – the first clinically detectable progression; PFS II – the second progression free survival; CDP II – the second clinically detectable progression. Box-plot diagram with the median, first quartile, third quartile, and non-outlier range

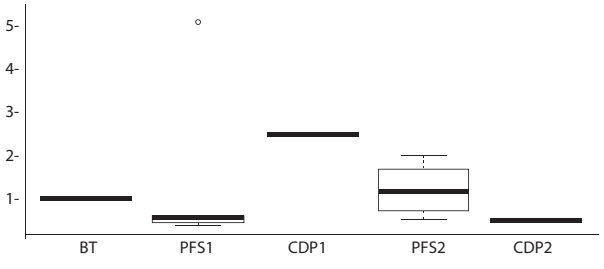

Fig. 3. Relative expression of miR-21 in patient Z01 during the course of the disease. BT – expression level before the therapy start; PFS I – the first progression free survival; CDP I – the first clinically detectable progression; PFS II – the second progression free survival; CDP II – the second clinically detectable progression. Box-plot diagram with the median, first quartile, third quartile, and non-outlier range

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