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Original Article

Comparison of serum and tissue values of miRNAs related to autophagy in glial brain tumors and metastases other than lmphoma

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Abstract

Aim: Central nervous system tumors are seen in both children and adults, and most of these tumors cause disability and death. Current studies are focused on the molecular pathogenesis to identify new targets for the diagnosis and follow-up of patients. In this study, we investigated whether serum micro ribonucleic acid (miRNA) values could be used as biomarkers of these tumors by examining miRNA levels associated with autophagy in serum and tissue.

Material and Methods: We included 27 patients who underwent surgery at our clinic and were diagnosed with glial tumor or metastasis other than lymphoma after pathological examination. The serum and tissue levels of miRNAs associated with autophagy were compared, and correlations between the obtained values were determined using Student's t-test.

Results: Based on histopathological examination, there were 14 glioblastomas, 3 oligodendrogliomas, 2 anaplastic astrocytomas, 2 carcinoma metastases, 2 pilocytic astrocytomas, 1 anaplastic ependymoma, 1 anaplastic oligodendroglioma, 1 diffuse astrocytoma, and 1 ependymoma. Serum Ct values were significantly higher than tissue Ct values. Correlations were found between serum and tissue levels of 5 miRNA subtypes. In addition, we could not determine the Ct value in some serum samples for 5 of the 12 miRNA subtypes.

Conclusion: Although correlations were found between serum and tissue miRNA levels in the literature, we did not find any reasonable correlation, which might be explained by the less number of patients in our study. Therefore, considering the results of this study, we believe it is too early to determine that miRNAs cannot be biomarkers of brain tumors.

Keywords: Glial Tumor; Mirna; Biomarker; Autophagy.

INTRODUCTION

Tumors of the central nervous system are seen in both children and adults and most of these tumors cause disability and death. Current studies are focused on the molecular field to identify new targets for the diagnosis and follow-up of patients with these tumors. One of the most popular molecules in the genetic field over the last decade is micro ribonucleic acid (miRNA) which represents gene expression and function in multiple pathways in human physiology. Therefore the question "Do they play a role in tumor development?" comes to mind. The effectiveness of various miRNAs on cancer pathways in many systems including the nervous system has been determined in different studies.

MicroRNAs are conserved small (containing 17–24 nucleotides) and genetically non-coding and they bind to protein-encoding mRNAs and affect gene expression (1-3). miRNAs can be present in many body fluids such as urine tissue blood and saliva (13).

Each miRNA influences multiple functions in the body and these miRNAs regulate one-third of the human genome (2). These biological functions include cell division cell differentiation internal stimulation cell growth cell death I (apoptosis) cell death II (autophagy) and biological functions (4). The Ct value was defined as the number of cycles in which the threshold value was exceeded in fluoroscopic measurements (5).

This study aimed to investigate whether serum miRNA

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levels particularly those associated with autophagy (programmed cell death) could be used as a biomarker of the central nervous system tumors by evaluating miRNA levels in tissue and serum of patients with glial brain tumor or with cranial metastases other than lymphoma.

MATERIAL and METHODS

Patients who underwent surgery in the Neurosurgery Department of Gaziantep University Medical Faculty were included in sampling. Serum samples were obtained by collecting preoperative blood samples from patients. A part of the pathological tissue obtained by mass resection during surgery was obtained as a sample and stored immediately at −80°C. We included patients diagnosed with glial tumor or cranial metastasis other than lymphoma by pathological examination of the intracranial mass.

miRNeasy Mini Kit (Qiagen GmbH Hilden Germany) was used to isolate total RNA (containing miRNA) from fresh tissue.

The serum samples stored at −80°C were thawed and numbered. QIAzol Lysis Reagent was added to the samples in a 5:1 (v/v) ratio (i.e. 1 ml QIAzol Lysis Reagent was added for 200 µL sample).

cDNAs (template) were synthesized using Qiagen miScript Reverse Transcription (RT) Kit II (Hilden Germany). Nine RT mixtures were prepared on ice. The amount of RNA was adjusted to 200 ng/µL in a volume of 20 µL. RNA was added to each tube containing the RT mixture and the tubes were briefly centrifuged and placed on ice again.

Reaction components for quantitative real-time polymerase chain reaction (PCR) were prepared using Qiagen miScript SYBR Green PCR kit. The threshold value fit to kit used in fluoroscopic measurements during cycling in PCR was determined. Figure 1 shows our Ct values.

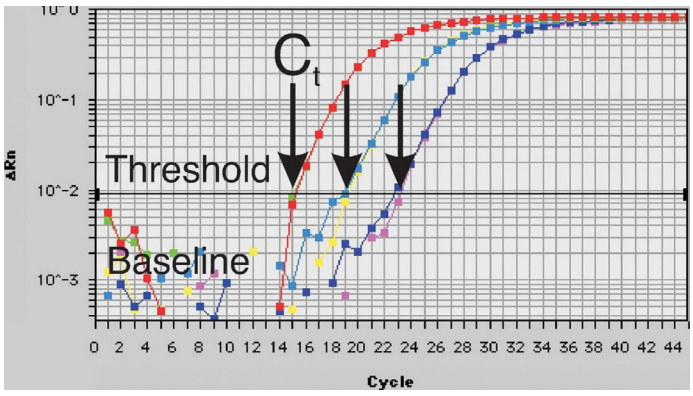

Figure 1. Determination of Ct value

Data Analysis

The normality of data was tested. Independent sample t-test was used to analyze normally distributed continuous variables. The results were expressed as mean ± standard deviation or median (min–max). P-values <0.05 were considered statistically significant. The data were analyzed using the SPSS 22.0 program.

RESULTS

We included 27 patients with suitable serum and tissue samples for research from those who underwent surgery at our clinic and were found to have glial tumor or non-lymphoma metastasis. The distribution of patient pathologies was as follows: glioblastoma (14 patients) oligodendroglioma (3 patients) anaplastic astrocytoma (2 patients) carcinoma metastasis (2 patients) pilocytic astrocytoma (2 patients) anaplastic ependymoma (1 patient) anaplastic oligodendroglioma (1 patient) diffuse astrocytoma (1 patient) and ependymoma (1 patient) (Figure 2).

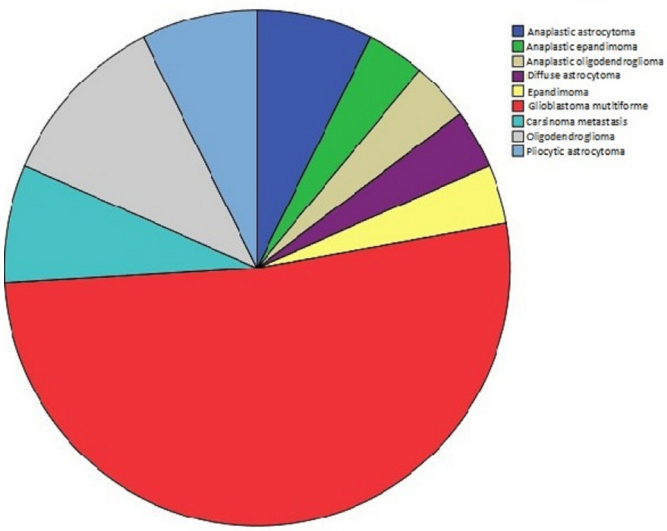

Figure 2. Distribution of tumor pathologies in patients

cDNA was obtained by RT from miRNAs (Table 1). The mean cDNA concentrations were found to be 1469.13 ± 582.07 and 510.00 ± 43.54 µg/L for tissue and serum samples respectively.

A statistically significant difference (Student's t-test) was found between tissue Ct (21.07 ± 2.82) and serum Ct (32.04 ± 2.80) values ($p < 0.005$).

Considering the correlation between the serum and tissue Ct values a positive or negative relationship was found among 5 miRNA subtypes and no relationship was found in 7 miRNA subtypes. The correlations between serum and tissue levels were as follows: negative correlation among hsa_miR_370 hsa_miR_429 and hsa_miR_203 ($r = -0.553$ $p = 0.003$; $r = -0.52$ $p = 0.005$; $r = -0.589$ $p = 0.010$ respectively) and positive correlation between hsa_let_7b and hsa_let_7c ($r = 0.404$ $p = 0.037$; $r = 0.390$ $p = 0.044$ respectively) (Table 1).

Table 1. Correlation analysis between serum and tissue values

		hsa_let_7a_5p	hsa_let_7b_5p	hsa_let_7c_5p	hsa_miR_26a_5p	hsa_miR_34a_5p	hsa_miR_96_5p	hsa_miR_101_3p	hsa_miR_128_3p	hsa_miR_150_5p	hsa_miR_203a_3p	hsa_iR_370 3p	hsa_miR_429
hsa_let_7a_5p	r	179	502**	420*	327	096	066	349	263	225	135	-171	256
	p	371	008	029	096	633	744	074	186	260	503	394	197
hsa_let_7b_5p	r	129	404*	372	301	059	092	166	135	045	097	-252	089
	p	523	037	056	127	769	650	408	503	823	632	205	660
hsa_let_7c_5p	r	187	382*	390*	357	103	088	290	239	220	179	-220	217
	p	349	049	044	067	610	664	143	229	270	371	271	277
hsa_miR_26a_5p	r	-101	255	235	046	020	-172	030	010	136	-020	-099	162
	p	616	199	239	818	920	390	881	962	500	921	623	419
hsa_miR_34a_5p	r	-242	339	201	-004	-156	-063	-085	-106	-007	-114	-019	-059
	p	224	084	315	984	438	756	675	597	972	570	925	772
hsa_miR_96_5p	r	055	-102	-111	182	143	-299	274	164	015	-279	-700**	-146
	p	840	708	683	501	596	261	305	544	957	295	003	591
hsa_miR_101_3p	r	-015	039	-072	-022	-142	-032	-055	-109	-221	118	-053	039
	p	941	849	721	913	481	875	787	587	267	557	793	847
hsa_miR_128_3p	r	-129	111	-034	354	-086	-095	255	063	009	-246	-260	-041
	p	621	671	896	163	743	718	323	812	974	341	313	876
hsa_miR_150_5p	r	-036	328	252	199	052	-034	151	133	235	154	-131	196
	p	859	095	204	320	796	868	451	508	238	444	516	328
hsa_miR_203a_3p	r	-331	-076	-268	-111	-341	-387	-069	-161	-024	-589*	-294	-427
	p	179	766	282	660	166	113	785	524	926	010	237	077
hsa_miR_370_3p	r	-100	038	-041	-039	103	-076	-193	-243	-299	-151	-553**	143
	p	620	850	838	846	608	705	336	222	130	451	003	477
hsa_miR_429	r	-366	-234	-343	-276	-251	-238	-102	-199	-284	-568**	-236	-52**
	p	060	240	080	164	207	232	611	320	151	002	235	005

**. Correlation is significant at the 0.01 level (2-tailed)
*. Correlation is significant at the 0.05 level (2-tailed)

DISCUSSION

Blood samples have been used since many years for the diagnosis of many diseases and treatment follow-up. Many biomarkers are used for this purpose. However biomarkers used for brain tumors are limited. The aim of this study was to discover new biomarkers. Therefore we aimed to investigate the feasibility of using miRNAs which have been discovered and studied over the last 20 years in this study.

Many studies about miRNA levels in all cancer types have been conducted and many positive results have been achieved (16).

Free circulating (extracellular) miRNAs in serum were first described in 2008. Blood cells and serum miRNA profiles were observed to be similar in studies on healthy controls. Based on this the change in serum miRNA profile was speculated to reflect pathological change (6). Lawrie et al. first detected tumor-specific regulation disorder of circulating miRNAs and identified high miRNA21 levels in diffuse large B-cell lymphoma (7). Another study on miRNA21 reported that miRNA21 inhibits autophagy in gliomas and miRNA21 is positively correlated with expression level thus increasing radiotherapy resistance in glioma cells (8). Mitchell et al. reported that miRNAs from epithelial tumors were also elevated in serum and that the number of circulating miRNAs was significantly higher in metastatic prostate cancer than in a healthy control group (9). In a study on colon cancer cells 5 miRNA subtypes were significantly overexpressed in tumor cells and were found to be significantly higher in serum from patients than that from healthy volunteers. The same study also showed that that miRNA92 and miRNA-17-3p were confirmed as diagnostic markers for colon cancer (10). Wang et al. demonstrated that miRNA182 concentration in glioma patients was 3.1 times higher than that in healthy controls (11). In a study conducted on serum miRNA29 in glioma patients with various grades patients with grades I and II were compared with those with grades III and IV glioma. Researchers found differences between their miRNA29 expression and indicated that miRNA29 is predictive for high-grade gliomas but the same finding cannot be established for low-grade gliomas (12). Wei et al. showed that miRNA125b could be a potential biomarker for gliomas (13). They observed that the serum miRNA125b level in glioma patients was lower than that in the healthy population and this level tends to significantly decrease throughout the tumor grade. The area under the curve was greater in World Health Organization (WHO) grade II (0.868) and WHO grades III–IV (0.959) than in WHO grade I (0.691) (13). Comparing the miRNA210 levels in blood between patients with glioma and the healthy control group the serum miRNA210 level in patients with glioblastoma was found to be 7 times higher and a relationship was found between serum miRNA210 expression level with the tumor grade and poor patient outcomes (20). When serum samples of normal controls and patients with meningioma were compared with preoperative serum samples of patients with glioma low miRNA128 levels were found in patients with glioma. An important finding of the study was the increase in miRNA128 expression postoperatively. However low miRNA128 levels in tissue and serum were associated with pathologic grade (15). Lavon et al. found that increased miRNA21 and decreased miRNA124 and miRNA137 were effective on gliomagenesis (16). Silber et al. similarly confirmed that miRNA124 and miRNA137 levels were significantly lower in patients with glioma compared with normal brain tissue (17). The miRNA expression pattern was found to change completely from low-grade gliomas to anaplastic gliomas or to secondary GBM (18). In a review conducted in 2016 256 miRNAs were reported to be overexpressed and 95 miRNAs were underexpressed in patients with GBM (19). Considering that miRNAs were associated with autophagy miRNA17 which targets ATG7 the main enzyme of the autophagic pathway was observed to be frequently overexpressed in gliomas. In contrast anti-miRNA17 treatment was speculated to increase the efficacy of radiotherapy and temozolomide treatment and to be significantly advanced in terms of GBM progression (20). Despite all serum and tissue miRNA findings it the mechanism of how miRNAs get out of the cancer cell and overcome the blood–brain barrier has not been fully clarified (3). Turchinovich et al. suggested that sources of most circulating miRNAs other than those actively released are dead cells (21).

Some studies have reported that pre- and postoperative serum miRNA levels were detected to be high in the postoperative period due to tumor damage during surgery (13).

The increased importance of miRNAs and the autophagic pathway in malignancy development detected in studies on glial tumors has led us to this study. The method used to detect miRNA-targeting enzymes in the autophagy pathway in our study was similar to the methods used in previous studies (18). In our study RNA levels isolated from serum and tissue were measured before transforming into cDNA and the quality of the obtained RNA was verified. Some miRNA subtypes (hsa_mir_96_5p hsa_mir_101_3p hsa_mir_128_3p hsa_mir_203a_3p hsa_mir_429) could not be detected in some serum samples by qPCR after cDNA measurement suggesting that these miRNA subtypes could not be a reliable biomarker for serum measurements. Moreover significantly higher Ct values in serum compared with values in tissue suggested that the miRNAs studied here could not be used as suitable biomarkers. However considering the number of patients in our study sample the number of samples should be increased to identify the biomarkers. Increasing the number of samples can provide both generalization and results based on tumor subtypes and grades in the future. The detection of miRNA in serum is a great source of hope for brain tumor diagnosis follow-up and grade prediction. This great potential is being tested with the developments in this area and recent extensive studies. However which of these miRNAs should be selected for the study is unclear yet and which miRNAs are specific

to which tissues and which are better obtained in serum are still controversial. The fact that miRNAs are affected not only directly but also indirectly is another cause of error in measurement (6). For this reason the necessary processes should be to establish a standard and simple method to detect circulating miRNAs and to investigate the underlying mechanism of extracellular miRNA.

CONCLUSION

The Ct values inversely proportional to expressed miRNA level were significantly higher in the serum for our subtypes in this study of 27 patients. A correlation was found between tissue and serum levels in 5 of the 12 miRNA subtypes studied suggesting that the miRNA subtypes included in this study are not suitable for use as biomarkers in serum. However the number of patients in this study is small to make a generalization.

A large study sample more carefulness in sample handling and storage and additional or different miRNA subtypes may lead to more positive results in terms of biomarker use of miRNAs in the future.

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