Original Article
Plasma levels of miRNA-155 as a powerful diagnostic marker for dedifferentiated liposarcoma

Aleksandar Boro, David Bauer, Walter Born, Bruno Fuchs

Laboratory for Orthopedic Research, Department of Orthopedics, Balgrist University Hospital, University of Zurich, Forchstrasse 340, 8008 Zurich, Switzerland

Abstract: Atypic lipomatous tumors (ALT) and dedifferentiated liposarcomas (DDLS) are closely related liposarcoma subtypes, often difficult to distinguish but they exhibit an entirely different clinical outcome. Recently discovered regulatory functions of miRNAs in liposarcoma progression prompted us to investigate miRNAs as potential diagnostic biomarkers in liposarcoma with a main focus on circulating miRNAs for fast and reliable differential diagnosis. Tumor and blood samples of 35 patients with lipomatous lesions collected between June 2011 and September 2014 were analyzed by qRT-PCR. They included 10 lipomas, 7 ALT, 5 DDLS and 13 myxoid liposarcomas (MLS). Ten samples of normal fat tissue and blood from 20 healthy volunteers were used as controls. A meta-analysis of public data on miRNA expression in liposarcoma revealed 9 miRNAs with potential diagnostic power. Out of these, miRNA-155 was found significantly elevated in the circulation of DDLS patients as compared to the plasma levels detected in all other liposarcoma subtypes and in healthy subjects. miRNA-155 levels in the plasma samples correlated significantly (r=0.41, p=0.02) with those in corresponding tumor extracts. This correlation was even more pronounced in an analysis of plasma and tumor extracts of malignant liposarcoma subtypes alone (r=0.51, p=0.02). Receiver operating characteristic analysis indicated that plasma miRNA-155 levels have a high diagnostic accuracy for distinguishing DDLS from healthy subjects (AUC=0.91, p=0.005) and from lipomas (AUC=0.86, p=0.02), MLS (AUC=0.92, p=0.006) and most importantly ALT (AUC=0.91, p=0.01) patients. In conclusion, this study identified miRNA-155 as a first blood biomarker for the differential diagnosis of DDLS.

Keywords: Liposarcoma, miRNA, plasma biomarker, diagnostic biomarker

Introduction

Liposarcomas (LS) are a heterogeneous group of adipocytic tumors that represent approximately 13% of all sarcomas [1-3]. The WHO classification distinguishes four histologically distinct LS subtypes: atypic lipomatous tumors (ALT) (synonymously well differentiated liposarcoma, WDLS), dedifferentiated liposarcomas (DDLS), myxoid liposarcomas (MLS) and pleomorphic liposarcomas (PLS) [4].

Atypic lipomatous tumors represent more than 40% of all LS and the most frequent LS subtype. They are low grade, locally aggressive and clinically often difficult to distinguish from benign lipomas. Karyotypically, ALT are characterized by supernumerary rings and giant chromosomes, which involve amplification of the 12q13-15 chromosomal region, containing the oncogenes MDM2 and CDK4 [5, 6]. However, ALT can undergo dedifferentiation, particularly those located in the retro-peritoneum, and turn into high grade tumors with high metastatic potential. These tumors, defined as DDLS, are much more aggressive and, consequently, the patients have a much worse prognosis and an almost 3-fold higher mortality than those with ALT [7, 8]. Karyotypic rearrangements in DDLS and ALT result in the expression of MDM2 and CDK4 that are used as important immunohistochemical markers to distinguish these two LS subtypes from lipomas or pleomorphic LS.

Myxoid liposarcoma is the second most frequent LS subtype and, if its histology does not show round cell components, it is a low grade tumor that rarely metastasizes. However, along the progression of the disease, accumulation of round cell components indicates a more

aggressive high grade tumor phenotype and is therefore a crucial prognostic marker [9]. In more than 95% of all MLS cases a reciprocal chromosomal translocation t(12;16)(q13;p11) is observed, which results in the expression of a FUS-CHOP aberrant transcription factor that influences adipocyte differentiation and stimulates adipocytic proliferation and tumorigenesis [10-12].

Pleomorphic LS is a high grade sarcoma and the least frequent of all LS subtypes. It is often difficult to distinguish from other pleomorphic sarcomas [13]. It is characterized by complex chromosomal rearrangements and a very aggressive phenotype [14].

Considering the differences in clinical outcome of the different LS subtypes, the exact classification is of the utmost importance for the clinician when decisions on the treatment of individual patients need to be made. Even with multimodal treatment including surgery, radiation and chemotherapy, high rates of recurrence and of metastasis in LS patients result in an overall survival of only 50% [15]. However, the differences in treatment success between LS subtypes are quite considerable. MLS are more sensitive to chemo- and radiotherapy than other LS subtypes, and the sensitivity of different tumor types drops with dedifferentiation [15]. Up to now, careful histology and tumor grading based on the degree of dedifferentiation remain the best prognostic indicators. Accordingly, DDLS is a high grade tumor with high rates of recurrence and metastasis compared to its low grade well differentiated counterpart ALT. DDLS exhibits a short median progression free survival of only 2 months when compared to 11 months observed for ALT [15]. With such clear differences in prognosis for different LS subtypes, an accurate diagnosis is of great importance. Unfortunately, common genetic rearrangements observed in ALT and DDLS and the lack of suitable specific diagnostic markers for the two LS subtypes require extensive and laborious histopathological analyses including IHC, FISH, CGH and qRT-PCR for a meaningful diagnosis. Consequently, there is a need for novel, preferably non-invasive diagnostic markers that will facilitate the diagnostic procedures. Interestingly, in LS, unlike in many other cancer types, neither diagnostic nor prognostic noninvasive biomarkers have so far been reported.

A recently rapidly growing volume of data indicates that small non coding RNAs (miRNAs) are useful biomarkers for both, a biological as well as a clinical characterization of tumors. miRNAs mainly target 3'-UTRs of mRNAs and thereby repress target gene expression [16]. In sarcomas, miRNA profiling in tumor tissue so far proved to be useful for tumor subtype classification, as well as for the assessment of aggressiveness, response to therapy and differentiation [17, 18]. Importantly, miRNAs have been shown to be remarkably stable in blood, making them suitable non-invasive biomarkers. The biology of miRNAs in LS is not well explored and, so far, only a few studies reporting miRNA expression in LS have been published [18-22]. In these studies, a comparison between miRNA expression profiles in tumor tissue of different LS subtypes and in normal fat tissue established distinct subtype specific miRNA signatures and, in some studies, the biological role of certain miRNAs was further investigated [20-22]. However, there are considerable differences between the results presented in these studies. A minimal overlap of miRNA expression profiles reported in these studies for distinct LS subtypes asks for additional validation studies. Finally, to our knowledge, a study investigating circulating miRNAs as diagnostic or prognostic biomarkers in LS has so far not been reported. Consequently, we decided to address this issue in the present study and performed a meta-analysis of the published miRNA expression profiles in LS and screened selected miRNAs as diagnostic markers in plasma of LS patients. The study led to the discovery of miRNA-155 as a powerful non-invasive diagnostic marker for DDLS.

Patients and methods

Patients

The present study, approved by the local ethics committee (Ref. Nr. EK10/2007), included 13 patients with MLS, 5 patients with DDLS, 7 patients with ALT and 10 patients with lipomas (**Table 1**). The 35 patients were admitted for treatment to the University Hospital Balgrist, University of Zurich, Switzerland, between July 2011 and October 2014. Upon surgical resection of the primary tumor, tumor tissue specimens were snap frozen in liquid nitrogen and stored at -80°C. The remaining tumor tissue was examined by soft tissue pathologists for LS

Table 1. Characteristics of liposarcoma patients and healthy control group

Variable	Healthy subjects	Myxoid liposarcoma	Dedifferentiated liposarcoma	Atypical lipomatous tumor	Lipoma	P value
Number	20	13	5	7	10	
Age (years)						
Mean	47.2	45.6	62.6	57.9	51.5	0.067
SD	14.1	13.6	14.2	9.9	10.1	
Gender						
Male	13	9	4	3	4	0.402
Female	7	4	1	4	6	
Tumor site						
Lower extremity		12	2	7	4	
Upper extremity					4	
Thorax		1	1		1	
Retroperitoneal			1			
Groin			1			
Back					1	
Metastasis		3/13	2/5	0/7	0/10	

subtype classification according to current standard histopathological criteria together with immunohistochemical and molecular analyses defined by the WHO guidelines for the classification of tumors [23]. Blood was collected from individual patients 24h before surgery in EDTA-coated tubes and plasma was prepared and frozen at -80°C within 2 hours. Control blood samples were collected from 20 healthy age and sex matched volunteers. Normal control white fat tissue from upper and lower extremities was collected upon written consent from 7 healthy subjects.

RNA isolation

RNA was isolated from 200 µL of plasma with an miRNeasy Serum/Plasma Kit (Qiagen) according to the manufacturer's instructions. Briefly, 1 ml of denaturing lysis reagent was added to 200 µL of plasma and kept at room temperature for 5 min. 4 µL miRNeasy spike-in control miRNA-39 from C. elegans at $1.6×10^8$ copies/µL was then added as an internal control for RNA extraction efficiency. RNA extracts were stored at -80°C. RNA from freshly frozen tumor samples was extracted with an miRNeasy Micro Kit (Qiagen) according to the manufacturer's instructions. Between 20 and 30 mg of frozen tumor tissues were homogenized with TissueLyser LT (Qiagen) and further processed according to the manufacturer's proto-

col. RNA extracts were stored at -80°C. RNA concentrations and purity were analyzed by OD 260/280 measurements in a NanoDrop device (Thermo Scientific, USA).

Reverse transcription and qRT-PCR

miRNA was reverse transcribed from 3 µL of individual RNA extracts in miScript HiSpec Buffer with an miScript II RT Kit (Qiagen) according to the manufacturer's instructions. After cDNA synthesis, the samples were diluted with 100 µL of water and 1 µL was then used as substrate for qPCR. Quantitative PCR was done using an miScript SYBR Green PCR Kit (Qiagen) according to the manufacturer's instructions. The universal reverse primer provided in the kit was used in all reactions in combination with the following miScript Primer Assays forward primers: Hs_RNU6-2_11, Hs_miR-128_1, Hs_miR-93_1, Hs_miR-214_2, Hs_miR-210_1, Hs_miR-16_2, Hs_miR-221*_1, Hs_miR-21*_1, Hs_miR-593*_1, Hs_miR-155_2, Hs_miR-181a_2. The PCR reactions were run on an Applied Biosystems Cycler (AB) under the following cycling conditions: initial denaturation at 95°C for 10 min, followed by 40 PCR cycles at 95°C for 15 s and at 60°C for 1 min. The levels of the investigated miRNAs in plasma samples were corrected for miRNA extraction efficiency, determined with the spiked-in C. elegans miRNA-39, and normalized to the levels of miRNA-128 that was determined with Norm Finder algorithm to be the least variable and therefore a suitable endogenous control [24]. In tissue samples, RNU6-2 was considered as a housekeeping reference and used for normalization. Relative expression of miRNAs was calculated using 2-ddCt [25].

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc, USA). Continuous variables are shown as mean ± SD. Differences between miRNA levels observed in samples of healthy

Table 2. Expression levels of selected miRNAs in plasma of patients shown as fold change in comparison to expression in plasma of healthy control group

miRNA	Myxoid liposarcoma		Dedifferentiated liposarcoma		Atypical lipomatous tumor		Lipoma	
	Up/Down	Fold change	Up/Down	Fold change	Up/Down	Fold change	Up/Down	Fold change
miRNA-16	↑	1.0	↑	1.1	↑	1.1	↑	1.2
miRNA-21	↓	1.8*	↓	1.1	↓	1.2	↓	1.6
miRNA-93	↑	1.1	↓	1.2	↑	1.2	↑	1.2
miRNA-155	↓	1.1	↑	3.9*	↓	1.1	↑	1.0
miRNA-181a	↓	1.6	↓	3.7*	↓	1.2	↓	1.3
miRNA-210	↓	1.3	↓	1.1	↑	1.8	↓	1.1
miRNA-214	↓	1.5	↑	1.5	↓	1.4	↓	1.0
miRNA-593	↓	2.3*	↑	1.1	↑	1.1	↓	2.2

*-fold change is statistically significant, p < 0.05.

subjects and of LS patients were analyzed for statistical significance with the student's t-test. One-way analysis of variance (ANOVA) was used when more than two groups were compared and differences between groups were subsequently determined by the Bonferroni test. The correlation of miRNA levels detected in LS tumor tissue and in corresponding plasma samples was determined by Spearman Correlation analysis. The standard formulas were used for the calculation of sensitivity and specificity. The receiver operating characteristic (ROC) curves were plotted to calculate the area under the curve (AUC) in order to assess the predictive power of plasma levels of miRNA-155 for LS. All *p* values are two sided and results were considered significant when p < 0.05.

Results

Patient characteristics

The characteristics of the patients and of healthy individuals included in this study are summarized in **Table 1**. The 35 patients investigated included 10 patients with lipoma, 7 patients with ALT, 5 patients with DDLS and 13 with MLS. In addition we analyzed plasma samples from 20 healthy individuals, who were age and sex matched to the patient cohort, and normal fat tissue from 7 healthy individuals. As expected from the higher incidence of DDLS in older patients, this group had the highest mean age. The group of patients suffering from MLS, on the other hand, known to predominantly affect younger subjects, exhibited the lowest mean age (**Table 1**). However, the distribution of age and sex did not significantly differ

between the LS subgroups investigated here. In the majority of patients, primary tumors were located in the extremities, predominantly in the legs. Only DDLS showed a wider distribution, including patients with tumors located in extremities. Patients with lipoma and ALT had no metastases, but in the groups of patients with malignant tumors, e.g. MLS and DDLS 23% (3/13) and 40% (2/5), respectively, suffered from metastatic disease.

Plasma levels of selected miRNAs in liposarcoma patients and healthy subjects

We performed a meta-analysis of four publically available microRNA expression profiles for LS in order to assemble a list of candidate miRNAs to be tested in plasma of LS patients and healthy subjects [18, 20-22]. All four of these studies investigated miRNAs extracted from fresh frozen LS tumor samples, including different LS subtypes and normal fat tissue as a control. Considering that we were interested in identifying and analyzing miRNA that was released by the tumor cells into the circulation, we focused on those miRNAs that were found upregulated in any LS subtype when compared to benign lipomas and/or normal fat tissue. The criterion for selection was more than 2-fold higher expressed of miRNA in liposarcomas in comparison to normal fat or lipoma. Further selection was based upon the presence of miRNA in at least 2 of the 4 published studies analyzed or that it was confirmed in a validation cohort of the same study. Despite the high variation between miRNA expression profiles in the four studies, our meta-analysis provided us with the following 9 miRNAs for our study: miRNA-16, miRNA-21, miRNA-93, miRNA-155,
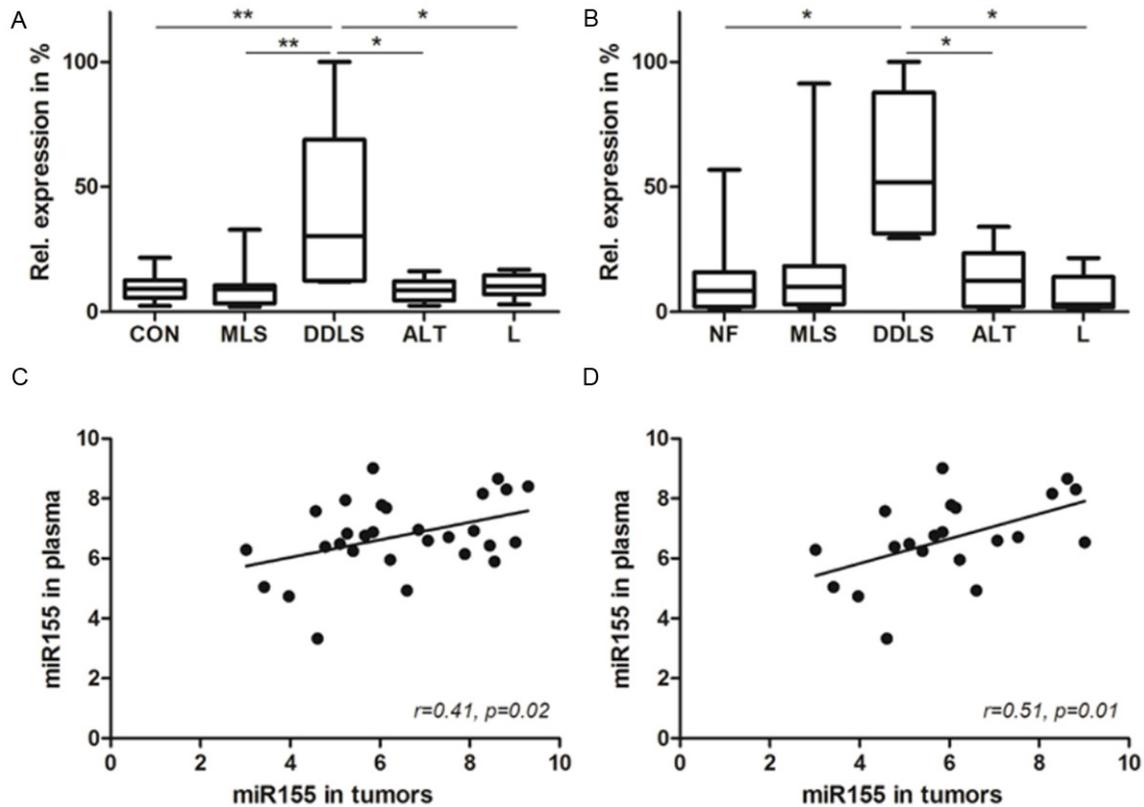
Figure 1. The levels of miRNA-155 in the circulation of liposarcoma patients correlate with the levels in corresponding tumor tissue extracts. A. Median (± SD) levels of miRNA-155 in plasma of indicated liposarcoma patients and healthy control subjects measured by qRT-PCR and normalized to miRNA-126 levels, plotted as % of maximum value. B. Median (± SD) levels of miRNA-155 measured by qRT-PCR in extracts of indicated liposarcoma tumor tissues and normal fat samples, normalized to RNU6.2, plotted as % of maximum value. C. Spearman correlation of miRNA-155 levels detected in plasma and in corresponding tumor tissue extracts of patients with adipocytic tumors, r-correlation coefficient. D. Spearman correlation of miRNA-155 levels detected in plasma and tumor tissue extracts of patients with malignant liposarcoma (CON-healthy subjects, NF-fat tissue from healthy subjects, MLS-myxoid liposarcoma, DDLS-dedifferentiated liposarcoma, ALT-atypic lipomatous tumors, L-lipomas; *-p < 0.05, **-p < 0.001).

miRNA-181a, miRNA-210, miRNA-214, miRNA-221, and miRNA-593. All of these miRNAs were detectable by qRT-PCR in the plasma samples investigated here except for miRNA-221 that remained undetectable. The fold-changes (up or down) in the levels of the selected miRNAs in the plasma of LS patients compared to healthy controls are summarized in the **Table 2**. For the vast majority of the miRNAs analyzed, only minor differences were observed between plasma levels in the different LS subtypes and in the healthy controls. However, the mean levels of circulating miRNA-21 and miRNA-593 in MLS were significantly (p < 0.05) decreased compared to control. In addition, plasma levels of miRNA181a in patients with DDLS were 3.7-times lower than in control subjects (p < 0.05). In contrast and most interestingly, patients with DDLS exhibited significantly (p < 0.05; 3.9-

times) higher levels of miRNA-155 in the circulation than control subjects; importantly, all other subgroups of LS patients had plasma levels of miRNA-155 indistinguishable from those detected in normal individuals. Consequently, we decided to further investigate circulating miRNA-155 as a novel diagnostic marker in DDLS.

Elevated circulating miRNA-155, a specific non-invasive indicator for dedifferentiated liposarcoma, correlates with an upregulated expression in tumor tissue

The results illustrated in **Figure 1B** demonstrate a significantly (p < 0.05) higher expression of miRNA-155 in DDLS tumor tissue than in normal fat tissue. Moreover and in perfect agreement with the findings described for the
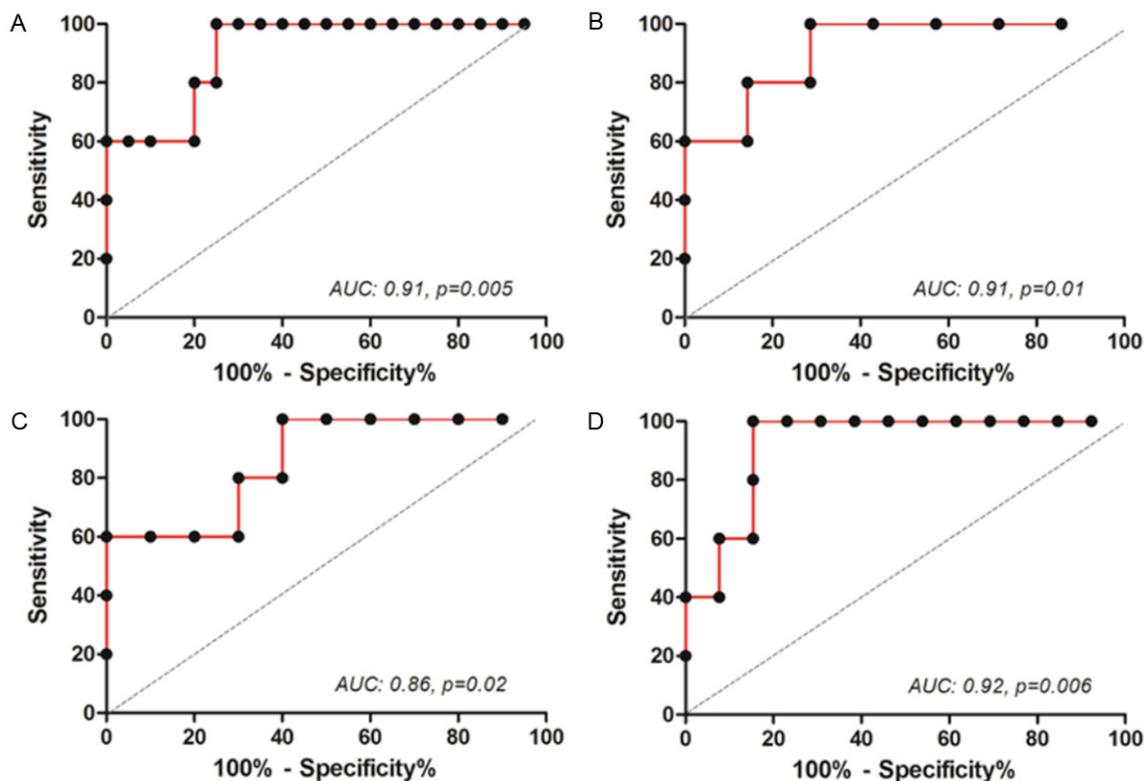
Figure 2. Diagnostic accuracy of miRNA-155 levels detected in plasma of liposarcoma patients. The ROC curve plotted to distinguish DDLS patients from healthy subjects (A), from ALT patients (B), from lipoma patients (C) and from MLS patients (D). AUC-area under the curve.

plasma samples, the levels of miRNA-155 expression in DDLS tumors were significantly higher than those detected in any other LS tumor subtype (**Figure 1A**, **1B**). Of most important diagnostic and even prognostic relevance are the significantly elevated plasma and tumor tissue miRNA-155 levels in DDLS compared to those found in patients with ALT, since DDLS develops from ALT. A Spearman Correlation analysis of the plasma and tumor tissue levels measured in individual patients of all LS subtypes revealed a statistically significant (r=0.41, p=0.02) correlation between the two parameters (**Figure 1C**). This correlation was even stronger (r=0.51, p=0.01) when only malignant lipomatous tumors were included in the analysis (**Figure 1D**).

Diagnostic accuracy of plasma miRNA-155 in liposarcoma

We performed a ROC curve analysis to assess the potential usefulness of plasma miRNA-155 levels as a noninvasive diagnostic marker for LS. As shown in **Figure 2A**, an analysis designed to distinguish patients with DDLS from healthy

controls yielded an AUC value of 0.910 (95% CI, 0.783-1.037). We further explored whether plasma miRNA-155 can distinguish between DDLS and other LS subtypes. The analysis performed with plasma miRNA-155 levels of patients with DDLS or ALT revealed an AUC of 0.914 (95% CI, 0.751-1.077) (**Figure 2B**). miRNA-155 proved to be also useful to distinguish DDLS from lipomas (AUC 0.860 (95% CI, 0.659-1.061)) (**Figure 2C**) and from MLS (AUC 0.923 (95% CI, 0.798-1.048)) (**Figure 2D**). Taken together, these results indicate that the levels of circulating miRNA-155 in LS patients are a useful noninvasive biomarker with high diagnostic power for DDLS.

Discussion

In many tumor types, miRNA expression correlates with various biological and clinical properties [16]. Sarcomas are no exception, since miRNA profiling was shown to be useful for tumor subtype classification and a strong correlation with aggressiveness, response to therapy and tumor differentiation was observed [17, 18]. It is also well known that miRNAs are

actively secreted by the tumor cells into the circulation and can therefore be used as circulating tumor biomarkers [26]. Based on these observations, we investigated such circulating miRNAs as biomarkers in LS.

Concerning LS, there exist four studies on miRNA profiling in tumor tissue [18, 20-22]. These reports describe a differential analysis between normal fat and LS subtypes that revealed specific miRNA signatures for each LS subtype. Following these studies only few miRNAs were studied in depth for their biological role in LS and a secondary validation of their expression was rarely included. In the study by Gits et al. the authors observed distinct miRNA expression patterns for each LS subtype. Furthermore, miRNA-145 and miRNA-451, which were found down-regulated in DDLS compared to normal fat, were validated as tumor suppressors [20]. This work also confirmed previous findings with miRNA-143 as a tumor suppressor miRNA in LS [22]. Zhang et al. focused on differential miRNA expression in DDLS/ALT and normal fat. This study revealed 4 up-regulated and 31 down-regulated miRNAs in diseased compared to normal tissue. Among these they validated miRNA-10, miRNA-126 and miRNA-155 in subsequent experiments. Strikingly, there was little overlap between the four studies investigating miRNA expression profiles in LS. When we only compared up-regulated miRNAs in DDLS over normal fat in the four published expression profiles, we found not a single miRNA appearing in all four lists. Nevertheless, we selected 9 miRNAs that were found significantly upregulated more than 2-fold in LS compared to normal fat in at least two of the four studies, for further evaluation in plasma of our LS patient cohort. We focused only on upregulated miRNAs in our meta-analysis since we assumed a higher probability for the release of up- rather than down-regulated miRNA from tumor tissue into the circulation. Interestingly, we were able to detect all but one miRNA (miRNA-221) in plasma of LS patients as summarized in **Table 2**. Out of 8 miRNAs, only three miRNAs showed a significantly different change in plasma levels of patients with a particular LS subtype compared to the levels detected in patients with any other LS subtypes. However, in the case of miRNA-21 and miRNA-181a we detected decreased levels in MLS and DDLS patients respectively, in comparison to healthy control subjects. This finding contrasts with an upregulated expression of these miRNAs in LS tumors reported by Gits et al. [20]. In the light of this discrepancy, we decided to analyze the expression of miRNA-21 and miRNA-181a in LS tumor samples. We did not observe any LS subtype related change in expression in tumor tissue that would correlate with the levels found in plasma (data not shown). The discovery of an almost 4-fold higher expression of miRNA-155 in plasma of DDLS patients compared to healthy individuals prompted us to further focus on this miRNA. Nevertheless, it is striking that only one miRNA showed significant increase in plasma out of 9 we expected from their elevation in tumor tissue, which further emphasizes that presence of tumor originating miRNAs in the circulation is a result of active excretion of only some miRNAs thus not always reflecting the expression in tumor cells.

It has recently been shown by Zhang et al. that miRNA-155 is overexpressed in DDLS tumors [21]. Furthermore, they were able to show a DDLS tumor growth driving activity of miRNA-155 that targets Casein kinase 1α and thereby interferes with the Wnt/b-catenin pathway [21]. A very recent study by Vincenzi et al. confirmed miRNA-155 overexpression in DDLS tumors [27]. Since miRNA-155 was shown to play an important role in DDLS tumors, we hypothesized that tumor and plasma levels of miRNA-155 may correlate in DDLS patients. The results of the present study demonstrate that the plasma levels of miRNA-155 measured in individual LS patients correlated significantly with the levels detected in corresponding tumor extracts, providing strong evidence for the postulated tumor origin of elevated circulating miRNA-155 in LS patients including those with DDLS.

ROC curve analysis confirmed that increased levels of miRNA-155 in plasma are a reliable non-invasive parameter to distinguish DDLS patients from both healthy controls, as well as patients with other LS subtypes including benign lipomas. Even more important in view of the difficult and laborious diagnostic procedures needed for a meaningful differential diagnosis of ALT and DDLS is the fact that miRNA-155 can be used as a noninvasive biomarker that allows to distinguish ALT and DDLS

patients with high accuracy reflected by a sensitivity of 80% and a specificity of 80% (AUC=0.914, 95% CI, 0.751-1.077). The diagnostic power of miRNA-155 is of limited importance in distinguishing easy-to-diagnose cases due to for example classical localization of DDLS in retroperitoneum. However, the relevance of miRNA-155 as a marker is as an additional, non-invasive marker in difficult cases such as DDLS located in extremities and misleading histology finding based on lipogenic component of DDLS tumor. Exactly those kinds of cases have been included in this study and they provide proof of principle.

However, miRNA-155 was shown to be also overexpressed in several other cancers including thyroid carcinoma, breast cancer, colon cancer, cervical cancer, pancreatic ductal adenocarcinoma, and lung cancer, and it was also found to be an indicator for a poor prognosis in all these cancers [28]. Interestingly, in lung cancer and in breast cancer in particular, circulating miRNA-155 was shown to be a powerful diagnostic marker with a sensitivity and specificity similar to that observed in the present study [26, 28]. Thus, these and our own findings show that miRNA-155 alone is not a unique circulating biomarker for the diagnosis of DDLS or any other type of cancer, but deserves further validation as a first valuable, circulating biomarker in LS diagnostics to differentiate between DDLS and ALT and to monitor DDLS treatment.

Disclosure of conflict of interest

None.

Address correspondence to: Aleksandar Boro, Laboratory for Orthopedic Research, Department of Orthopedics, Balgrist University Hospital, University of Zurich, Forchstrasse 340, 8008 Zurich, Switzerland. Tel: +41443863775; Fax: +41443861666; E-mail: aboro@research.balgrist.ch

References

[1] Gadgeel SM, Harlan LC, Zeruto CA, Osswald M and Schwartz AG. Patterns of care in a population-based sample of soft tissue sarcoma patients in the United States. Cancer 2009; 115: 2744-2754.

[2] Mack TM. Sarcomas and other malignancies of soft tissue, retroperitoneum, peritoneum, pleura, heart, mediastinum, and spleen. Cancer 1995; 75: 211-244.

[3] Toro JR, Travis LB, Wu HJ, Zhu K, Fletcher CD and Devesa SS. Incidence patterns of soft tissue sarcomas, regardless of primary site, in the surveillance, epidemiology and end results program, 1978-2001: An analysis of 26,758 cases. International journal of cancer. Int J Cancer 2006; 119: 2922-2930.

[4] Fletcher CDM BJ, Hogendoorn PCW, Mertens F. WHO Classification of Tumors of Soft Tissue and Bone. World Health Organization, 2013.

[5] Fletcher CD, Akerman M, Dal Cin P, de Wever I, Mandahl N, Mertens F, Mitelman F, Rosai J, Rydholm A, Sciot R, Tallini G, van den Berghe H, van de Ven W, Vanni R and Willen H. Correlation between clinicopathological features and karyotype in lipomatous tumors. A report of 178 cases from the Chromosomes and Morphology (CHAMP) Collaborative Study Group. Am J Pathol 1996; 148: 623-630.

[6] Pedeutour F, Forus A, Coindre JM, Berner JM, Nicolo G, Michiels JF, Terrier P, Ranchere-Vince D, Collin F, Myklebost O and Turc-Carel C. Structure of the supernumerary ring and giant rod chromosomes in adipose tissue tumors. Genes Chromosomes Cancer 1999; 24: 30-41.

[7] Henricks WH, Chu YC, Goldblum JR and Weiss SW. Dedifferentiated liposarcoma: a clinicopathological analysis of 155 cases with a proposal for an expanded definition of dedifferentiation. Am J Surg Pathol 1997; 21: 271-281.

[8] Weiss SW and Rao VK. Well-differentiated liposarcoma (atypical lipoma) of deep soft tissue of the extremities, retroperitoneum, and miscellaneous sites. A follow-up study of 92 cases with analysis of the incidence of "dedifferentiation". Am J Surg Pathol 1992; 16: 1051-1058.

[9] Kilpatrick SE, Doyon J, Choong PF, Sim FH and Nascimento AG. The clinicopathologic spectrum of myxoid and round cell liposarcoma. A study of 95 cases. Cancer 1996; 77: 1450-1458.

[10] Aman P, Ron D, Mandahl N, Fioretos T, Heim S, Arheden K, Willen H, Rydholm A and Mitelman F. Rearrangement of the transcription factor

gene CHOP in myxoid liposarcomas with t(12;16)(q13;p11). Genes Chromosomes Cancer 1992; 5: 278-285.

[11] Antonescu CR, Tschernyavsky SJ, Decuseara R, Leung DH, Woodruff JM, Brennan MF, Bridge JA, Neff JR, Goldblum JR and Ladanyi M. Prognostic impact of P53 status, TLS-CHOP fusion transcript structure, and histological grade in myxoid liposarcoma: a molecular and clinicopathologic study of 82 cases. Clin Cancer Res 2001; 7: 3977-3987.

[12] Knight JC, Renwick PJ, Dal Cin P, Van den Berghe H and Fletcher CD. Translocation t(12;16)(q13;p11) in myxoid liposarcoma and round cell liposarcoma: molecular and cytogenetic analysis. Cancer Res 1995; 55: 24-27.

[13] Gebhard S, Coindre JM, Michels JJ, Terrier P, Bertrand G, Trassard M, Taylor S, Chateau MC, Marques B, Picot V and Guillou L. Pleomorphic liposarcoma: clinicopathologic, immunohistochemical, and follow-up analysis of 63 cases: a study from the French Federation of Cancer Centers Sarcoma Group. Am J Surg Pathol 2002; 26: 601-616.

[14] Mertens F, Fletcher CD, Dal Cin P, De Wever I, Mandahl N, Mitelman F, Rosai J, Rydholm A, Sciot R, Tallini G, Van den Berghe H, Vanni R and Willen H. Cytogenetic analysis of 46 pleomorphic soft tissue sarcomas and correlation with morphologic and clinical features: a report of the CHAMP Study Group. Chromosomes and MorPhology. Genes Chromosomes Cancer 1998; 22: 16-25.

[15] Jones RL, Fisher C, Al-Muderis O and Judson IR. Differential sensitivity of liposarcoma subtypes to chemotherapy. Eur J Cancer 2005; 41: 2853-2860.

[16] Calin GA and Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.

[17] Subramanian S, Lui WO, Lee CH, Espinosa I, Nielsen TO, Heinrich MC, Corless CL, Fire AZ and van de Rijn M. MicroRNA expression signature of human sarcomas. Oncogene 2008; 27: 2015-2026.

[18] Renner M, Czwan E, Hartmann W, Penzel R, Brors B, Eils R, Wardelmann E, Buttner R, Lichter P, Schirmacher P and Mechtersheimer G. MicroRNA profiling of primary high-grade soft tissue sarcomas. Genes Chromosomes Cancer 2012; 51: 982-996.

[19] Tap WD, Eilber FC, Ginther C, Dry SM, Reese N, Barzan-Smith K, Chen HW, Wu H, Eilber FR, Slamon DJ and Anderson L. Evaluation of well-differentiated/de-differentiated liposarcomas by high-resolution oligonucleotide array-based comparative genomic hybridization. Genes Chromosomes Cancer 2011; 50: 95-112.

[20] Gits CM, van Kuijk PF, Jonkers MB, Boersma AW, Smid M, van Ijcken WF, Coindre JM, Chibon F, Verhoef C, Mathijssen RH, den Bakker MA, Verweij J, Sleijfer S and Wiemer EA. MicroRNA expression profiles distinguish liposarcoma subtypes and implicate miR-145 and miR-451 as tumor suppressors. International journal of cancer. Int J Cancer 2014; 135: 348-361.

[21] Zhang P, Bill K, Liu J, Young E, Peng T, Bolshakov S, Hoffman A, Song Y, Demicco EG, Terrada DL, Creighton CJ, Anderson ML, Lazar AJ, Calin GG, Pollock RE and Lev D. MiR-155 is a liposarcoma oncogene that targets casein kinase-1alpha and enhances beta-catenin signaling. Cancer Res 2012; 72: 1751-1762.

[22] Ugras S, Brill E, Jacobsen A, Hafner M, Socci ND, Decarolis PL, Khanin R, O'Connor R, Mihailovic A, Taylor BS, Sheridan R, Gimble JM, Viale A, Crago A, Antonescu CR, Sander C, Tuschl T and Singer S. Small RNA sequencing and functional characterization reveals MicroRNA-143 tumor suppressor activity in liposarcoma. Cancer Res 2011; 71: 5659-5669.

[23] Fletcher CD HP, Mertens F, Bridge J. WHO Classification of Tumours of Soft Tissue and Bone. Lyon, France: IARC Press, 2013.

[24] Andersen CL, Jensen JL and Orntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res 2004; 64: 5245-5250.

[25] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.

[26] Khoury S and Tran N. Circulating microRNAs: potential biomarkers for common malignancies. Biomark Med 2015; 9: 131-151.

[27] Vincenzi B, Iuliani M, Zoccoli A, Pantano F, Fioramonti M, De Lisi D, Frezza AM, Rabitti C, Perrone G, Onetti Muda A, Russo A, Giordano A, Santini D, Dei Tos AP and Tonini G. Deregulation of dicer and mir-155 expression in liposarcoma. Oncotarget 2015; 6: 10586-10591.

[28] Faraoni I, Antonetti FR, Cardone J and Bonmassar E. miR-155 gene: a typical multifunctional microRNA. Biochim Biophys Acta 2009; 1792: 497-505.