Comprehensive miRNA expression analysis for histological subtypes of soft tissue sarcoma

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(Received December 25, 2020; Accepted February 25, 2021)

SUMMARY

Sarcoma is a rare mesenchymal malignancy that comprises more than 50 histological subtypes. Because of the rarity and diversity of sarcomas, their differential diagnosis is difficult, and there is still a need for biomarkers to support pathological diagnoses. Micro RNAs (miRNAs) are small noncoding RNAs that regulate the behavior of tumors, such as invasion and metastasis. The expression patterns of miRNAs reflect the origin of malignancy and are considered to be candidate biomarkers. To understand the molecular background of those histological subtypes, we investigated the miRNA expression in 89 tumor tissues of eight subtypes. The correlation coefficients between each sarcoma subtype on the basis of miRNA expression values were mostly higher than 0.7, reflecting the common mesenchymal origin.

By contrast, hierarchical clustering and principal component analysis showed that three types of sarcoma with chromosomal translocation (i.e., dermatofibrosarcoma protuberans, myxoid liposarcoma, and synovial sarcoma) were grouped according to their histological subtypes, whereas five types with complex karyotypes (i.e., myxofibrosarcoma, malignant peripheral nerve sheath tumor, undifferentiated pleomorphic sarcoma, dedifferentiated liposarcoma, and pleomorphic liposarcoma) were not. Notably, the number of miRNAs whose expression pattern was unique to histological subtypes with statistical significance was higher in sarcomas with chromosome translocation than in those with complex karyotypes. Hence, it can be concluded that the miRNAs unique to histological subtypes are candidate biomarkers for the differential diagnosis of sarcomas, particularly in those with chromosomal translocation.

Key words: sarcoma, microRNA, microarray

INTRODUCTION

Soft tissue sarcomas are heterogeneous mesenchymal tumors that comprise over 50 histological subtypes. Despite their diversity, the frequency of individual sarcomas is quite low, and they are considered to be a rare cancer. Although they are generally divided into two main groups (i.e., sarcomas with characteristic chromosomal translocation and sarcomas with complex karyotypes), their detailed biology remains to be explored. Moreover, because of their rarity and diversity, diagnosing sarcomas remains to be challenging. Diagnostic discrepancies can lead to differences in the treatment strategies, explaining the need for biomarkers to support pathological diagnoses.

MicroRNAs (miRNAs) are small noncoding RNAs that are 20–25 nucleotides long. These miRNAs are involved in various biological processes, such as cell proliferation, differentiation, and apoptosis. To date, a number of miRNAs have been reported to be involved in several clinical events, such as tumorigenesis and metastasis. Particularly, miRNA expression signatures are considered to be different in each tumor subtype, hence reflecting its origin. Therefore, miRNAs are considered to be useful in diagnoses. However, the current knowledge regarding the role of miRNAs in sarcomas is far less than that in cancer, with approximately 40,000 publications about miRNAs in cancer but less than 500 about miRNAs in sarcomas. A small number of
microarray-based comprehensive miRNA expression analyses have been reported in sarcomas, and several miRNAs have been reported to be of diagnostic aid\(^{(2-15)}\). However, such histological subtypes are limited considering the diversity of sarcomas. In addition, the number of newly identified miRNAs has increased over the years, with more than 2,500 being identified\(^{(16)}\).

In this study, we performed a large-scale analysis of soft tissue sarcomas and determined their characteristic miRNA expression patterns using miRNA array with more than 2,500 probes. The results showed that miRNA expression profiles specific to histological subtypes can support the diagnosis of sarcomas.

**MATERIALS AND METHODS**

1. **Clinical specimens**

Sarcoma samples were obtained from 89 patients who were treated at the National Cancer Center Hospital, Tokyo, Japan, between 2014 and 2018. All samples were frozen and stored at \(-80^\circ\text{C}\) before use. The tumor tissues examined in this study included 23 myxofibrosarcoma (MFS), 12 malignant peripheral nerve sheath tumor (MPNST), 12 undifferentiated pleomorphic sarcoma (UPS), 10 myxoid liposarcoma (MLPS), 9 dedifferentiated liposarcoma (DDLPS), 9 dermatofibrosarcoma protuberans (DFSP), 8 pleomorphic liposarcoma (PLPS), and 6 synovial sarcoma (SS). Table 1 summarizes the age, site of occurrence, and presence of neoadjuvant therapy, as well as whether the tumor is primary or recurrent, for these samples. This study was approved by the ethical committee of the National Cancer Center, and written informed consent was obtained from all donor patients.

2. **Preparation of total RNA**

Tumor tissues were mixed with 750 \(\mu\)L of TRIzol LS reagent (Invitrogen, Carlsbad, CA, USA), and the aqueous phase was then collected by adding chloroform. After adding ethanol to the aqueous phase, total RNA was purified using RNeasy Mini Spin Columns (Qiagen, Hilden, Germany). The RNA concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Quant-iT RiboGreen RNA Assay Kit (Invitrogen). The RNA quality was analyzed using an Agilent 2100 Bioanalyzer and small RNA chips (Agilent Technologies, Santa Clara, CA, USA). RNAs with RIN values of \(>7.0\) were used in the miRNA array experiment.

3. **miRNA expression array of tumor tissue samples**

Total RNA extracted from tumor tissues was prepared using a 3D-Gene miRNA labeling kit (Toray Industries, Inc., Tokyo, Japan) and analyzed using a 3D-Gene Human miRNA Oligo chip according to the manufacturer’s protocol. The microarray chip was designed to detect 2,555 miRNA sequences registered in miRBase release 20 or 2,565 miRNA sequences registered in miRBase release 21 (http://www.mirbase.org/).

4. **Statistical analysis**

All miRNA expression levels were normalized together using global normalization. Four outlier miRNAs (i.e., miR-4454, miR-5100, miR-7975, and miR-7977) with signal values of \(>60,000\) across most samples were removed (Supplementary Fig. 1). Then, data were log\(_2\)-transformed and only miRNAs with a signal value of \(>6\) in more than 50% of the samples were kept. The correlation coefficients between each sample were also calculated and described in figures using these identified miRNAs. A heatmap was also described using these data. The distance between each sample was calculated using the maximum distance method, and each cluster was linked using the ward.D2 method.

Analysis of variance (ANOVA) was performed for each sarcoma subtype for each miRNA, and 289 miRNAs with a \(p\)-value of \(<0.05\) were included in the subsequent analysis. Then, Student’s \(t\)-test was performed and the false discovery rate (FDR) was calculated using the Benjamini–Hochberg method\(^{(17)}\). Moreover, unsupervised hierarchical clustering analysis and principal component analysis were performed on only MLPS, DFSP, and SS, which are sarcomas with characteristic chromosomal translocation.

All miRNA array data were analyzed using R version 3.6.3 (R Foundation for Statistical Computing, http://www.R-project.org), genefilter package version 1.68.0 (Bioconductor, https://bioconductor.org/), heatmap.plus package version 1.3 (https://CRAN.R-project.org/package=heatmap.plus), corplot package version 0.84 (https://github.com/taiyun/corplot), and ggplot2 package version 3.3.0 (https://ggplot2.tidyverse.org).

**RESULTS**

1. **Overall expression of miRNAs in each sarcoma subtype**

We calculated the correlation coefficients between each sarcoma subtype on the basis of miRNA expression values and examined the similarity of miRNA expression between the different subtypes (Fig. 1A, B). The correlation coefficients among the same subtypes were higher than 0.75 for both the median and the mean, indicating a strong similarity, whereas UPS and PLPS showed broader ranges than those of the other types. Moreover, the correlation coefficients between different sarcoma subtypes were mostly higher than 0.7. On the other hand, UPS showed relatively low correlation coefficients between the other subtypes. In particular, the combination of UPS versus MLPS, UPS versus DFSP, and UPS versus SS presented low values. However, the mean and median correlation coefficients were still over 0.6 for those combinations, offering moderate correlations.

We described a heatmap of 89 tumor tissues (Fig. 1C, Supplementary Fig. 2) with the primary/recurrent status...
| Case No. | Diagnosis | Age (years) | Sex (M/F) | Primary/Recurrence | neoadjuvant therapy | Site note |
|---------|-----------|-------------|-----------|--------------------|---------------------|-----------|
| 1       | MFS       | 93          | F         | Recurrence         | No                  | Lower limb |
| 2       | MFS       | 73          | M         | Primary            | No                  | Body trunk |
| 3       | MFS       | 65          | M         | Recurrence         | No                  | Body trunk |
| 4       | MFS       | 69          | F         | Primary            | No                  | Lower limb |
| 5       | MFS       | 69          | M         | Primary            | No                  | Lower limb |
| 6       | MFS       | 86          | F         | Primary            | No                  | Body trunk |
| 7       | MFS       | 68          | M         | Primary            | No                  | Body trunk |
| 8       | MFS       | 56          | F         | Primary            | No                  | Lower limb |
| 9       | MFS       | 73          | F         | Recurrence         | No                  | Lower limb |
| 10      | MFS       | 77          | M         | Primary            | No                  | Lower limb |
| 11      | MFS       | 67          | M         | Primary            | No                  | Lower limb |
| 12      | MFS       | 82          | M         | Primary            | No                  | Upper limb |
| 13      | MFS       | 64          | F         | Recurrence         | CTx                 | Lower limb |
| 14      | MFS       | 55          | M         | Primary            | No                  | Lower limb |
| 15      | MFS       | 72          | F         | Primary            | No                  | Upper limb |
| 16      | MFS       | 72          | F         | Primary            | No                  | Retroperitoneum |
| 17      | MFS       | 80          | F         | Recurrence         | No                  | Upper limb |
| 18      | MFS       | 56          | M         | Primary            | No                  | Lower limb |
| 19      | MFS       | 90          | F         | Recurrence         | No                  | Lower limb |
| 20      | MFS       | 78          | M         | Recurrence         | No                  | Body trunk |
| 21      | MFS       | 40          | M         | Primary            | No                  | Lower limb |
| 22      | MFS       | 57          | M         | Primary            | CTx                 | Lower limb |
| 23      | MFS       | 69          | M         | Primary            | No                  | Lower limb |
| 24      | MPNST     | 38          | M         | Recurrence         | CTx                 | Body trunk |
| 25      | MPNST     | 37          | F         | Primary            | No                  | Body trunk |
| 26      | MPNST     | 42          | F         | Primary            | No                  | NF1 patient |
| 27      | MPNST     | 28          | F         | Primary            | CTx                 | Retroperitoneum |
| 28      | MPNST     | 72          | M         | Recurrence         | No                  | Body trunk |
| 29      | MPNST     | 19          | F         | Primary            | No                  | Retroperitoneum |
| 30      | MPNST     | 35          | M         | Recurrence         | No                  | Body trunk |
| 31      | MPNST     | 48          | M         | Recurrence         | No                  | Lower limb |
| 32      | MPNST     | 40          | M         | Recurrence         | CTx                 | Body trunk |
| 33      | MPNST     | 9           | F         | Recurrence         | No                  | Lower limb |
| 34      | MPNST     | 9           | F         | Recurrence         | No                  | Lower limb |
| 35      | MPNST     | 68          | F         | Primary            | No                  | Body trunk |
| 36      | UPS       | 70          | F         | Primary            | No                  | Lower limb |
| 37      | UPS       | 83          | M         | Primary            | No                  | Upper limb |
| 38      | UPS       | 64          | M         | Primary            | No                  | Lower limb |
| 39      | UPS       | 84          | F         | Primary            | No                  | Lower limb |
| 40      | UPS       | 79          | M         | Primary            | No                  | Lower limb |
| 41      | UPS       | 70          | F         | Primary            | No                  | Lower limb |
| 42      | UPS       | 79          | M         | Recurrence         | No                  | Body trunk |
| 43      | UPS       | 77          | F         | Recurrence         | CTx                 | Body trunk |
| 44      | UPS       | 79          | M         | Recurrence         | No                  | Lower limb |
| 45      | UPS       | 75          | M         | Primary            | No                  | Upper limb |
| 46      | UPS       | 60          | M         | Primary            | No                  | Body trunk |
| 47      | UPS       | 79          | F         | Recurrence         | No                  | Lower limb |
| 48      | MLFS      | 33          | M         | Recurrence         | No                  | Retroperitoneum |
| 49      | MLFS      | 49          | M         | Recurrence         | No                  | Body trunk |
| 50      | MLFS      | 40          | M         | Recurrence         | No                  | Body trunk |
| 51      | MLFS      | 46          | M         | Primary            | CTx                 | Lower limb |
| 52      | MLFS      | 59          | M         | Primary            | No                  | Body trunk |
| 53      | MLFS      | 43          | F         | Primary            | No                  | Lower limb |
| 54      | MLFS      | 31          | M         | Primary            | No                  | Lower limb |
| 55      | MLFS      | 65          | M         | Primary            | No                  | Lower limb |
| 56      | MLFS      | 43          | F         | Primary            | No                  | Lower limb |
| 57      | MLFS      | 36          | M         | Primary            | CTx, RTx            | Lower limb |
| 58      | DDLPS     | 59          | M         | Recurrence         | CTx                 | Retroperitoneum |
| 59      | DDLPS     | 76          | F         | Primary            | No                  | Retroperitoneum |
| 60      | DDLPS     | 75          | M         | Primary            | No                  | Retroperitoneum |
| 61      | DDLPS     | 73          | M         | Primary            | No                  | Lower limb |
| 62      | DDLPS     | 58          | M         | Recurrence         | No                  | Retroperitoneum |
| 63      | DDLPS     | 55          | M         | Recurrence         | No                  | Retroperitoneum |
| 64      | DDLPS     | 69          | M         | Primary            | No                  | Lower limb |
| 65      | DDLPS     | 85          | M         | Recurrence         | No                  | Body trunk |
| 66      | DDLPS     | 61          | M         | Primary            | No                  | Body trunk |
| 67      | DFSP      | 67          | M         | Primary            | No                  | Body trunk |
| 68      | DFSP      | 46          | M         | Primary            | No                  | Body trunk |
| 69      | DFSP      | 52          | M         | Primary            | No                  | Body trunk |
| 70      | DFSP      | 36          | F         | Primary            | No                  | Body trunk |
were identified; for UPS, no miRNAs were identified; for MLPS, nine overexpressed and one underexpressed miRNA were identified; for DDLPS, one overexpressed and no underexpressed miRNAs were identified; for DFSP, 53 overexpressed and two underexpressed miRNAs were identified; for PLPS, no miRNAs were identified; for SS, 13 overexpressed and 142 underexpressed miRNAs were identified. More characteristic miRNAs were identified in MLPS, DFSP, and SS, which formed subgroups by unsupervised hierarchical clustering, than in other sarcoma subtypes.

**DISCUSSION**

In this study, we performed a large-scale miRNA expression analysis of 89 soft tissue sarcoma samples. Sarcomas were diagnosed on the basis of their morphology and cytogenetic features. However, because of their diversity and rarity, accurately diagnosing sarcomas is considered to be difficult. Therefore, novel biomarkers are needed to support the pathological diagnosis of sarcomas. Although miRNAs have been reported to be useful in diagnosis because they reflect the origin of the tumor, the knowledge regarding the role of miRNAs in sarcomas is still limited. Thus, we report the miRNA profiles of soft tissue sarcomas.

We calculated the correlation coefficients to examine the similarity between miRNAs and found that the correlation coefficients of the overall miRNA values were generally larger than 0.7, not only between the same subtypes of sarcoma, but also in combination with other sarcomas. Notably, all sarcomas are derived from mesenchymal cells and are likely to exhibit many similarities, even though they are of different subtypes. On the other hand, sarcomas with complex karyotypes did not show clustering based on miRNA expression profiles.

**2. miRNA expression in sarcomas with characteristic chromosomal translocation**

As MLPS, DFSP, and SS exhibit characteristic chromosomal translocation, unlike other sarcomas, and formed a characteristic subgroup in the previous analysis, we performed unsupervised hierarchical clustering and principal component analysis for only these three sarcomas. It was observed that 7/10 cases of MLPS, 7/9 cases of DFSP, and 5/6 cases of SS formed a subgroup. These three sarcoma subtypes could not be subdivided according to their primary/recurrent or neoadjuvant therapy status. On the other hand, sarcomas with complex karyotypes did not show clustering based on miRNA expression profiles.

**3. Characteristic miRNAs of each sarcoma subtype**

Using ANOVA and t-test, we identified characteristic miRNAs that were either overexpressed or underexpressed in each sarcoma subtype. Up to the top 10 miRNAs with a fold change of > 2, p-value of <0.05, and FDR of <0.10 are listed in Table 2, and whole identified miRNAs satisfying the same criteria are listed in Supplementary Table 1. The numbers of overexpressed and underexpressed miRNAs that met the criteria are as follows: for MPNST, three overexpressed and no underexpressed miRNAs were identified; for UPS, no miRNAs were identified; for MLPS, nine overexpressed and one underexpressed miRNA were identified; for DDLPS, one overexpressed and no underexpressed miRNAs were identified; for DFSP, 53 overexpressed and two underexpressed miRNAs were identified; for PLPS, no miRNAs was identified; for SS, 13 overexpressed and 142 underexpressed miRNAs were identified. More characteristic miRNAs were identified in MLPS, DFSP, and SS, which formed subgroups by unsupervised hierarchical clustering, than in other sarcoma subtypes.
However, DFSP, which exhibits characteristic translocation, was included in the same subgroup at a lower rate than those of MLPS and SS in this study. DFSP generally has multiple subtypes. Particularly, fibrosarcomatous DFSP is more malignant than other DFSP subtypes. Two of the DFSP samples (Cases 72 and 75) were associated with fibrosarcomatous changes and clustered into the same subgroup, indicating that differences in the DFSP subtype affected clustering.

We carried out unsupervised hierarchical clustering and principal component analysis using only MLPS, DFSP, and SS to avoid the influence of other heterogeneous sarcomas. Although clustering showed that these three types of sarcoma did not form a completely independent group, principal coefficients.

Heatmap analysis showed that no subgroups formed on the basis of the primary/recurrent status and the presence of neoadjuvant therapy, whereas certain histological subtypes (MLPS, DFSP, and SS) formed subgroups. The expression of miRNA reflects the tumor origin, suggesting that the histological difference is more dominant than the primary/recurrent status and the presence of neoadjuvant therapy in miRNA expression. While the correlation coefficients between samples were high, sarcomas with characteristic chromosomal translocation were divided into subgroups. In a previous comprehensive study, sarcomas with chromosomal translocation were also tightly clustered, whereas those with complex karyotypes tended to show dispersed clustering. However, DFSP, which exhibits characteristic translocation, was included in the same subgroup at a lower rate than those of MLPS and SS in this study. DFSP generally has multiple subtypes. Particularly, fibrosarcomatous DFSP is more malignant than other DFSP subtypes. Two of the DFSP samples (Cases 72 and 75) were associated with fibrosarcomatous changes and clustered into the same subgroup, indicating that differences in the DFSP subtype affected clustering.
number of identified characteristic miRNAs was higher in sarcomas with chromosomal translocation than in those with complex karyotypes. Sarcomas with chromosomal translocation have a homogeneous genomic background, and exhibit lower copy number changes and mutations than those of sarcomas with complex karyotypes\(^\text{21}\). Therefore, only specific miRNAs are considered to be over or under expressed, resulting in the identification of many characteristic miRNAs.

In MFS, miR-142-5p was found to be overexpressed. A previous study has shown that miR-142-5p is overexpressed in malignant fibrous histiocytoma (i.e., a concept in the pre-

Fig. 2. Unsupervised hierarchical clustering and principal component analysis of sarcoma with chromosomal translocation. (A) Sarcomas with chromosomal translocation are mostly subdivided by unsupervised hierarchical clustering. (B) Principal component analysis more clearly reflected these sarcoma subtypes. Case 72 and Case 75 indicate the DFSPs with fibrosarcomatous change.

component analysis strongly reflected the histological sarcoma subtype. In addition, although two of the DFSP samples with fibrosarcomatous change did not clustered into the same subgroup unlike the heatmap analysis which includes all samples, these DFSP samples were located closely in principal component analysis. While previous reports have assessed the differences in each histological type only by clustering\(^\text{13–15}\), principal component analysis may reflect these subtypes more, even if they seemed to belong to different subgroups in clustering.

ANOVA and Student’s \(t\)-test were performed to identify the characteristic miRNAs of each sarcoma subtype. The number of identified characteristic miRNAs was higher in sarcomas with chromosomal translocation than in those with complex karyotypes. Sarcomas with chromosomal translocation have a homogeneous genomic background, and exhibit lower copy number changes and mutations than those of sarcomas with complex karyotypes\(^\text{21}\). Therefore, only specific miRNAs are considered to be over or under expressed, resulting in the identification of many characteristic miRNAs.

In MFS, miR-142-5p was found to be overexpressed. A previous study has shown that miR-142-5p is overexpressed in malignant fibrous histiocytoma (i.e., a concept in the pre-
Table 2. Top 10 characteristic miRNA expressions for each type of sarcoma.

| miRNA ID                      | Fold change | p-value | FDR  |
|-------------------------------|-------------|---------|------|
| **(1) MFS vs other sarcoma subtypes** |             |         |      |
| over expressed miRNA          |             |         |      |
| hsa-miR-142-5p                | 3.737       | 0.000   | 0.024|
| under expressed miRNA         |             |         |      |
| hsa-miR-181a-5p               | −2.663      | 0.000   | 0.019|
| **(2) MPNST vs other sarcoma subtypes** |         |         |      |
| over expressed miRNAs         |             |         |      |
| hsa-miR-210-3p                | 2.905       | 0.000   | 0.074|
| hsa-miR-4732-5p               | 2.653       | 0.001   | 0.074|
| hsa-miR-8073                  | 2.065       | 0.001   | 0.074|
| **(3) UPS vs other sarcoma subtypes** |         |         |      |
| no over expressed or under expressed miRNA |         |         |      |
| **(4) MLPS vs other sarcoma subtypes** |         |         |      |
| over expressed miRNAs         |             |         |      |
| hsa-miR-193a-3p               | 12.744      | 0.000   | 0.000|
| hsa-miR-365a-3p, hsa-miR-365b-3p | 9.172      | 0.000   | 0.000|
| hsa-miR-20a-5p                | 4.412       | 0.006   | 0.097|
| hsa-miR-20b-5p                | 4.175       | 0.006   | 0.097|
| hsa-miR-17-5p                 | 4.036       | 0.004   | 0.089|
| hsa-miR-108a-5p               | 3.678       | 0.004   | 0.084|
| hsa-miR-193b-3p               | 3.343       | 0.001   | 0.029|
| hsa-miR-93-5p                 | 2.940       | 0.002   | 0.054|
| under expressed miRNAs        |             |         |      |
| hsa-miR-21-3p                 | −5.726      | 0.000   | 0.000|
| hsa-miR-221-3p                | −3.207      | 0.001   | 0.038|
| hsa-miR-29a-3p                | −3.165      | 0.003   | 0.079|
| hsa-miR-7150                  | −2.061      | 0.003   | 0.079|
| **(5) DDLPS vs other sarcoma subtypes** |         |         |      |
| over expressed miRNA          |             |         |      |
| hsa-miR-26a-5p                | 6.470       | 0.000   | 0.021|
| **(6) DFSP vs other sarcoma subtypes** |         |         |      |
| over expressed miRNAs         |             |         |      |
| hsa-miR-497-5p                | 13.124      | 0.000   | 0.000|
| hsa-miR-195-5p                | 9.911       | 0.000   | 0.007|
| hsa-miR-23c                   | 9.580       | 0.000   | 0.006|
| hsa-miR-130a-3p               | 8.825       | 0.000   | 0.004|
| hsa-miR-376a-3p               | 8.002       | 0.007   | 0.045|
| hsa-miR-136-5p                | 7.716       | 0.003   | 0.022|
| hsa-miR-125b-5p               | 6.926       | 0.000   | 0.004|
| hsa-miR-4324                  | 6.771       | 0.000   | 0.004|
| hsa-miR-127-3p                | 6.508       | 0.009   | 0.051|
| hsa-miR-100-5p                | 6.490       | 0.001   | 0.012|
| under expressed miRNA         |             |         |      |
| hsa-miR-6771-5p               | −3.098      | 0.003   | 0.021|
| hsa-miR-128-2-5p              | −2.158      | 0.007   | 0.046|
| **(7) PLPS vs other sarcoma subtypes** |         |         |      |
| no over expressed or under expressed miRNA |         |         |      |
| **(8) SS vs other sarcoma subtypes** |         |         |      |
| over expressed miRNAs         |             |         |      |
| hsa-miR-127-3p                | 10.884      | 0.005   | 0.020|
| hsa-miR-136-5p                | 10.853      | 0.004   | 0.020|
| hsa-miR-376c-3p               | 10.750      | 0.012   | 0.027|
| hsa-miR-376a-3p               | 9.942       | 0.014   | 0.028|
| hsa-miR-377-3p                | 6.463       | 0.034   | 0.063|
| hsa-miR-130a-3p               | 4.058       | 0.043   | 0.053|
| hsa-miR-199a-5p               | 3.781       | 0.034   | 0.053|
| hsa-miR-125b-5p               | 3.771       | 0.029   | 0.047|
| hsa-miR-214-3p                | 3.694       | 0.005   | 0.020|
| hsa-miR-4324                  | 3.516       | 0.034   | 0.053|
| under expressed miRNAs        |             |         |      |
| hsa-miR-424-5p                | −17.743     | 0.000   | 0.019|
| hsa-miR-122-3p                | −8.050      | 0.000   | 0.020|
| hsa-miR-142-5p                | −7.786      | 0.002   | 0.020|
Cell proliferation and apoptosis. MiR-365 is regarded as overexpressed in esophageal cancer and is involved in proliferation of breast cancer. MiR-193a-3p is one of several miRNAs, including miR-193a-3p, miR-365a-3p, and miR-365b-3p, with a high fold-change ratio. MiR-193a-3p is involved in colorectal cancer and breast cancer, and is considered to be a tumor suppressor in lung cancer and hepatocellular carcinoma. MiR-365b-3p, with a high fold-change ratio and significantly low FDR. Interestingly, in many tumors (e.g., gastric cancer and angiosarcoma), miR-497-5p acts as a tumor suppressor. In the melanoma cell line, both miR-497-5p and miR-195-5p related to proliferation, apoptosis, and cell cycle. However, the functions of miRNAs are highly complex and diverse. Thus, such miRNAs seem to have another mechanism in DFSP.

In SS, several overexpressed miRNAs, including miR-183, miR-125a-3p, and miR-200b, have been reported. In our study, miR-127-3p, miR-136-5p, and miR-376c-3p were newly identified as miRNAs with high fold changes. MiR-127-3p promotes glioblastoma cell migration and invasion. Bioinformatics research also revealed that miR-136-5p is involved in lung adenocarcinoma. The under expression of miR-143, miR-34b, miR-142-5p, and miR-34c-3p has also been reported, partially overlapping our results. Additionally, miR-424-5p has been identified with a particularly high fold-change ratio. In a previous study, miR-424-5p promoted the proliferation of gastric cancer.

In UPS and PLPS, no characteristic miRNAs were identified in this study. These sarcomas are generally highly heterogeneous compared to other sarcomas, which reflects the lack of subgroups in clustering and relatively low correlation coefficients.

We identified multiple novel miRNAs, particularly in sarcomas with characteristic chromosomal translocation. Although sarcomas are often difficult to diagnose because of their diversity, the miRNAs identified in this study may serve as a diagnostic aid. However, considering the diversity of sarcomas, we need to examine the miRNA expression profiles of more sarcoma samples. The number of newly identified miRNAs is increasing compared to the past, and there may be miRNAs that can serve as a biomarker specific to particular types of sarcoma. We concluded that miRNAs unique to histological subtypes may be candidate biomarkers for the differential diagnosis of sarcomas, particularly sarcomas with chromosomal translocation.

**ABBREVIATIONS**

MFS, myxofibrosarcoma; MPNST, malignant peripheral nerve sheath tumor; UPS, undifferentiated pleomorphic sarcoma; MLPS, myxoid liposarcoma; DDLPS, dedifferentiated liposarcoma; DFSP, dermatofibrosarcoma protuberans; PLPS, pleomorphic liposarcoma; SS, synovial sarcoma; WDLPS, well-differentiated liposarcoma; CTx, chemotherapy; RTx, radiotherapy; NF1, neurofibromatosis type 1;
FDR, false discovery rate.

ACKNOWLEDGEMENTS

This research was financially supported by the National Cancer Center Research and Development Fund (Grant no. 20-A-2), the Japan Agency for Medical Research and Development (Grant no. 20ck0106537h0001), and New Energy and Industrial Technology Development Organization, Japan.

ETICS APPROVAL

This study was approved by the ethical committee of the National Cancer Center (approval no. 2004-050).

CONFLICT OF INTERESTS

This research was financially supported by the National Cancer Center Research and Development Fund (Grant no. 20-A-2), the Japan Agency for Medical Research and Development (Grant no. 20ck0106537h0001), and New Energy and Industrial Technology Development Organization, Japan.

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