

# Upregulation of miR-18a-5p contributes to epidermal necrolysis in severe drug eruptions

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**Background:** Toxic epidermal necrolysis (TEN) is a severe drug-induced cutaneous reaction. Although one of the primary histologic features of TEN is keratinocyte apoptosis, its exact mechanism remains unknown.

**Objectives:** We investigated the role of microRNAs (miRNAs) in the pathogenesis of severe drug eruptions and evaluated the possibility that miRNA can be a disease marker.

**Methods:** miRNAs were extracted from tissues and sera of patients. PCR array analyses were performed to identify pathogenic miRNAs. The results were confirmed with quantitative real-time PCR, *in situ* hybridization, transient transfection of small interfering RNAs or miRNA mimics into cultured keratinocytes, flow cytometry, immunoblotting, luciferase assay, and immunohistochemistry.

**Results:** PCR array analysis and real-time PCR using tissue miRNAs demonstrated that the miR-18a-5p level was increased in the skin of patients with TEN *in vivo*. Transfection of the miR-18a-5p mimic into keratinocytes *in vitro* resulted in increased apoptotic cell numbers and caspase-9 activity, which were also increased in the skin of patients with TEN. The miR-18a-5p mimic also downregulated the expression of B-cell lymphoma/leukemia-2-like protein 10 (BCL2L10), an anti-intrinsic apoptotic molecule. A luciferase assay with the BCL2L10 3' untranslated region showed BCL2L10 is directly targeted by miR-18a-5p. The protein and mRNA expressions of BCL2L10 were decreased in the skin of patients with TEN. Transfection with BCL2L10 small interfering RNA induced keratinocyte apoptosis and caspase activity. Furthermore, serum miR-18a-5p levels tended to be increased in patients with TEN and were correlated with areas of skin erythema or erosion in patients with drug eruptions.

**Conclusions:** Our results indicated that downregulated BCL2L10 caused by miR-18a-5p overexpression mediates intrinsic keratinocyte apoptosis in patients with TEN. Serum miR-18a-5p levels can be a useful disease marker for drug eruptions. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

**Key words:** MicroRNA, apoptosis, drug eruption, keratinocyte, serum

## Abbreviations used

|          |                                                                       |
|----------|-----------------------------------------------------------------------|
| BCL2L10: | B-cell lymphoma/leukemia-2-like protein 10                            |
| BSA:     | Body surface area                                                     |
| Ct:      | Cycle threshold                                                       |
| EM:      | Erythema multiforme                                                   |
| miRNA:   | MicroRNA                                                              |
| NHEK:    | Normal human epidermal keratinocyte                                   |
| siRNA:   | Small interfering RNA                                                 |
| SJS:     | Stevens-Johnson syndrome                                              |
| TEN:     | Toxic epidermal necrolysis                                            |
| TUNEL:   | Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling |
| UTR:     | Untranslated region                                                   |

Drug eruptions are adverse cutaneous reactions caused by drug administration. Not all drug eruptions have allergic mechanisms; some have nonallergic causes (eg, toxicity, overdose, or side effects). Allergic drug eruptions are divided into several clinical types, including maculopapular-type, lichenoid-type, and fixed drug eruptions. The erythema multiforme (EM) type is also known to be a common type of drug eruption; the minor form is mild and self-limited, whereas the major form is accompanied by systemic symptoms and sometimes becomes severe and life-threatening.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are characterized by targetoid erythematous lesions, with blistering and erosion of the skin and mucous membranes. SJS and TEN are considered 2 different severe forms of major EM. SJS involves less than 10% of the body surface area (BSA), whereas TEN affects more than 10% of the BSA. In addition, ocular, gastrointestinal, liver, renal, and respiratory involvement is sometimes observed in patients with TEN. Because of a high mortality rate, it is important to diagnose these diseases in the early stage, predict severity, and treat intensively. Histologically, the erosions of SJS and TEN result from keratinocyte apoptosis and epidermal detachment.<sup>1,2</sup> However, the exact mechanisms remain unknown.

MicroRNAs (miRNAs) are small noncoding RNAs only 22 nucleotides long on average.<sup>3</sup> miRNAs usually bind to complementary sequences in the 3' untranslated regions (UTRs) of target mRNAs and inhibit their expression.<sup>4,5</sup> Because more than a thousand miRNAs have been identified in human subjects,<sup>6</sup> miRNAs are thought to be the most abundant class of regulators. miRNAs have been implicated in the immune response, as well as in cell development, cell differentiation, organogenesis, growth control, and apoptosis. Accordingly, many publications have demonstrated that miRNAs are involved in the pathogenesis of various human diseases, such as immunologic disorders, neurologic

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diseases, cardiovascular diseases, metabolic disorders, and cancers.<sup>7-14</sup>

Recently, several studies have indicated an association between drug eruptions and genetic factors (eg, HLA typing).<sup>15</sup> However, no reports have investigated the contribution of epigenetics, including miRNAs, to the pathogenesis of drug eruptions. Our study is the first to evaluate the possibility that miRNAs play a role in the pathogenesis of drug eruptions, especially TEN.

## METHODS

### Patient materials

Serum samples were obtained from 8 patients with TEN, 10 patients with SJS, 15 patients with EM minor, and 22 healthy volunteers. Patients with other chronic inflammatory or malignant diseases were excluded from this study. In 2 of the 8 patients with TEN, the cause of the disease was identified to be allopurinol or carbamazepine administration. In the remaining 6 patients, multiple drugs were suspected, and we were unable to identify the exact cause. In 3 of the 10 patients with SJS, phenobarbital, fosfomycin calcium, or lamotrigine administration was identified as the cause. Meanwhile, the causative drugs were unknown in the remaining 7 patients. In addition, candesartan cilexetil, carbamazepine, creosote, or interferon administration was thought to be the cause in 4 of the 15 patients with EM. Skin specimens were also obtained from 4 patients with TEN, 3 patients with SJS, 8 patients with EM minor, 4 patients with psoriasis, 4 patients with atopic dermatitis, and 6 control subjects. These samples were stored at  $-80^{\circ}\text{C}$  or fixed in formaldehyde after resection. Institutional review board approval and written informed consent were obtained before the patients and healthy volunteers were entered into the study, according to the Declaration of Helsinki.

### Cell culture

Normal human epidermal keratinocytes (NHEKs; Lonza, Walkersville, Md) were cultured in KBM-Gold Basal Medium with KGM-Gold Single-Quots at  $37^{\circ}\text{C}$  in 5%  $\text{CO}_2$ .

### RNA isolation and quantitative real-time PCR

Total RNA isolation from paraffin-embedded tissue sections was performed with the RNeasy FFPE kit (Qiagen, Valencia, Calif). Total RNA was extracted from cultured cells by using ISOGEN (Nippon Gene, Tokyo, Japan). cDNA synthesis and real-time PCR were performed, as described previously.<sup>16</sup> Primers for B-cell lymphoma/leukemia-2-like protein 10 (*BCL2L10*) and glyceraldehyde-3-phosphate dehydrogenase were purchased from Takara and SABioscience (Frederick, Md), respectively.

### miRNA extraction from skin tissues, cultured cells, and sera

Small RNAs were extracted from tissue sections by using the miRNeasy FFPE kit (Qiagen). miRNAs were obtained from the total RNA of cultured cells by using the RT<sup>2</sup> qPCR-Grade miRNA Isolation Kit (SABioscience, Qiagen). miRNA isolation from serum samples was performed with the miRNeasy RNA isolation kit (Qiagen) and synthetic nonhuman miRNA (*Caenorhabditis elegans* miR-39; Takara, Otsu, Japan), as described previously.<sup>17-21</sup>

### miRNA PCR array

miRNAs were reverse transcribed into cDNA by using the miScript II RT Kit (Qiagen). The cDNA was mixed with QuantiTect SYBR Green PCR Master Mix, and the mixture was added to Human miFinder 384 Array (Qiagen). PCR was performed on the Bio-Rad CFX384 (Bio-Rad Laboratories, Hercules, Calif).

### Real-time PCR of miRNAs

cDNA was synthesized from the miRNA by using the Mir-X miRNA First Strand Synthesis and SYBR qRT-PCR Kit (Takara). The sequences of the

primers were designed based on miRBase (<http://www.mirbase.org>). PCR was performed, as previously described.<sup>16</sup> The transcript level of each miRNA was normalized to that of cel-miR-39 in serum samples or to that of U6 in other samples.

### In situ hybridization

*In situ* hybridization was performed with 5'-locked digoxigenin-labeled nucleic acid probes complementary to human mature miR-18a-5p (Exiqon, Vedbaek, Denmark) at  $59^{\circ}\text{C}$  overnight.<sup>16</sup>

### Transient transfection

Small interfering RNA (siRNA) against *BCL2L10* was purchased from Thermo Scientific Dharmacon (Rockford, Ill). miRNA mimics, inhibitors, and miScript Target protectors were purchased from Qiagen.

For reverse transfection, miRNA mimics, inhibitors, protectors, or siRNAs were mixed with Lipofectamine RNAiMAX (Invitrogen, Carlsbad, Calif) and then added when the cells were plated, followed by incubation at  $37^{\circ}\text{C}$  in 5%  $\text{CO}_2$ .

### Luciferase reporter assay

A luciferase construct containing the *BCL2L10* 3' UTR was purchased from GeneCopoeia (Rockville, Md). Substitution mutations of the miR-18a-5p seed match were introduced by using Quick Change site-directed mutagenesis kits (Stratagene, La Jolla, Calif) and verified by means of sequencing. miRNA mimics and reporter plasmids mixed with Lipofectamine 2000 (Invitrogen) were added when the cells were plated, followed by incubation at  $37^{\circ}\text{C}$ . The Luc-Pair miR luciferase assay (GeneCopoeia) and the FilterMax F5 microplate reader (Molecular Devices, Sunnyvale, Calif) were used to analyze luciferase expression.

### Apoptosis assay

Cellular apoptosis was measured by using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay and the Annexin V assay with the Guava TUNEL Kit and FlowCollect Annexin Red Kit (Millipore, Temecula, Calif), respectively.<sup>22,23</sup>

### Caspase assay

Caspase activity was determined by using the Caspase-3 Colorimetric Assay Kit and Caspase-9 Colorimetric Assay Kit (R&D Systems, Minneapolis, Minn).

### Immunohistochemical staining

Immunohistochemical staining was performed with antibodies for BCL2L10 or active caspase-9 (Abcam, Cambridge, United Kingdom) overnight at  $4^{\circ}\text{C}$ , as described previously.<sup>16</sup>

### Cell lysis and immunoblotting

NHEKs or tissue samples were washed with PBS twice and lysed in Denaturing Cell Extraction Buffer (Biosource International, Camarillo, Calif). Immunoblotting was performed with antibodies for BCL2L10, active caspase-9 (IMGEX, San Diego, Calif), active caspase-3 (BD Bioscience, Bedford, Mass), or  $\beta$ -actin (Santa Cruz Biotechnology, Santa Cruz, Calif).<sup>16</sup>

### Statistical analysis

Statistical analysis was performed with the Mann-Whitney *U* test for comparison of medians. Correlations were assessed by using Pearson correlation coefficients. *P* values of less than .05 were considered significant. In Table I, *P* values determined by using the Mann-Whitney *U* test in comparison with the values in normal skin were transformed into *q* values by using the *q* value method.<sup>24</sup>

**TABLE I.** Fifteen miRNAs significantly upregulated or downregulated in the skin of patients with TEN compared with healthy skin, as measured by using a PCR array

|                 | Healthy skin,<br>mean $\pm$ SD |         | Patients with TEN,<br>mean $\pm$ SD |         | Patients with<br>psoriasis, mean $\pm$ SD |         | Patients with atopic<br>dermatitis, mean $\pm$ SD |         |
|-----------------|--------------------------------|---------|-------------------------------------|---------|-------------------------------------------|---------|---------------------------------------------------|---------|
|                 | P value                        | q Value | P value                             | q Value | P value                                   | q Value | P value                                           | q Value |
| hsa-miR-9-5p    | 1.00 $\pm$ 1.49                | —       | 0.00 $\pm$ 0.00                     | —       | 0.02 $\pm$ 0.03                           | —       | 2.14 $\pm$ 2.83                                   | —       |
| hsa-miR-18a-5p  | 1.00 $\pm$ 1.08                | —       | 7.93 $\pm$ 1.11                     | 0.0369  | 1.75 $\pm$ 3.04                           | 0.0463  | 2.07 $\pm$ 3.58                                   | 0.8273  |
| hsa-miR-29a-5p  | 1.00 $\pm$ 0.88                | —       | 0.05 $\pm$ 0.01                     | 0.0495  | 0.11 $\pm$ 0.18                           | 0.5066  | 0.94 $\pm$ 1.16                                   | 0.8208  |
| hsa-miR-33a-3p  | 0.00 $\pm$ 0.00                | —       | 1.00 $\pm$ 0.75                     | 0.0495  | 0.01 $\pm$ 0.01                           | 0.1266  | 0.04 $\pm$ 0.07                                   | 0.8273  |
| hsa-miR-191-3p  | 1.00 $\pm$ 0.69                | —       | 0.20 $\pm$ 0.23                     | 0.0369  | 1.14 $\pm$ 1.25                           | 0.1213  | 0.75 $\pm$ 0.56                                   | 0.3173  |
| hsa-miR-210     | 1.00 $\pm$ 0.30                | —       | 3.09 $\pm$ 1.05                     | 0.0495  | 1.22 $\pm$ 1.06                           | 0.8273  | 0.92 $\pm$ 0.82                                   | 0.5127  |
| hsa-miR-223-3p  | 1.00 $\pm$ 0.23                | —       | 2.53 $\pm$ 1.37                     | 0.0495  | 3.40 $\pm$ 3.24                           | 0.5127  | 3.65 $\pm$ 0.68                                   | 0.8273  |
| hsa-miR-298     | 1.00 $\pm$ 0.79                | —       | 0.02 $\pm$ 0.03                     | 0.0495  | 0.29 $\pm$ 0.17                           | 0.5127  | 0.01 $\pm$ 0.01                                   | 0.0495  |
| hsa-miR-346     | 1.00 $\pm$ 0.19                | —       | 8.79 $\pm$ 6.19                     | 0.0495  | 7.92 $\pm$ 11.80                          | 0.1266  | 9.64 $\pm$ 5.12                                   | 0.0495  |
| hsa-miR-378a-5p | 1.00 $\pm$ 1.68                | —       | 0.00 $\pm$ 0.00                     | 0.0463  | 0.00 $\pm$ 0.00                           | 0.0463  | 1.07 $\pm$ 1.23                                   | 0.5127  |
| hsa-miR-383     | 1.00 $\pm$ 0.75                | —       | 0.00 $\pm$ 0.00                     | 0.0369  | 0.14 $\pm$ 0.24                           | 0.1212  | 0.00 $\pm$ 0.00                                   | 0.0463  |
| hsa-miR-424-3p  | 1.00 $\pm$ 1.23                | —       | 0.00 $\pm$ 0.00                     | 0.0369  | 1.81 $\pm$ 2.78                           | 0.8273  | 0.05 $\pm$ 0.09                                   | 0.1212  |
| hsa-miR-495-3p  | 1.00 $\pm$ 1.05                | —       | 0.07 $\pm$ 0.01                     | 0.0495  | 11.34 $\pm$ 14.22                         | 0.5127  | 5.25 $\pm$ 6.92                                   | 0.5127  |
| hsa-miR-605     | 1.00 $\pm$ 0.62                | —       | 0.18 $\pm$ 0.12                     | 0.0495  | 2.78 $\pm$ 3.39                           | 0.5127  | 4.17 $\pm$ 6.28                                   | 0.8273  |
| hsa-miR-1908    | 1.00 $\pm$ 1.28                | —       | 0.04 $\pm$ 0.05                     | 0.0495  | 0.17 $\pm$ 0.19                           | 0.2752  | 1.42 $\pm$ 1.25                                   | 0.8273  |
|                 | —                              | —       | —                                   | 0.0495  | —                                         | 0.6477  | —                                                 | 0.8446  |

The miRNA expression profile in each disease *in vivo* was evaluated by using a PCR array. The fold change was calculated as follows:  $1/2^{(\text{Raw Ct of each miRNA} - \text{Mean Ct of small RNA housekeeping genes})}$ .

The mean fold change (mean) and SD of each miRNA is shown. The mean value of the healthy skin samples was set at 1. When the value of healthy skin was 0, the mean value of another sample was set at 1. P values and q values compared with the values in healthy skin samples are indicated.

## RESULTS

### miRNA expression profile in drug eruptions

As an initial experiment, to determine which miRNAs are involved in the pathogenesis of drug eruptions, miRNAs were obtained from the skin tissues of 3 patients with TEN, and the global miRNA expression profile of skin of patients with TEN was compared with that of 3 samples of healthy skin by using a PCR array consisting of 372 miRNAs (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Three samples of the skin of patients with psoriasis and atopic skin were also included in this experiment as disease control specimens.

There were 15 miRNAs significantly upregulated or downregulated in the skin of patients with TEN compared with healthy skin, according to the array (Table I). Among them, the expression of miR-18a-5p, miR-33a-3p, and miR-210 was increased and expression of miR-495-3p was decreased in the tissues of patients with TEN significantly and specifically when compared with that seen in the skin of patients with psoriasis and atopic skin, as well as healthy skin. We confirmed the array results using quantitative real-time PCR analysis with a specific primer for each miRNA and increased number of samples (4 from patients with TEN, 3 from patients with SJS, 8 from patients with EM minor, 4 from

patients with psoriasis, 4 from patients with atopic dermatitis, and 5 from healthy skin). Among the 3 upregulated or 1 downregulated miRNAs in patients with TEN, only the expression of miR-18a-5p (UAAGGUGCAUCUAGUGCAGAUAG) was still significantly upregulated in the TEN samples compared with that observed in the other patient groups, according to the real-time PCR analysis (Fig 1, A). Furthermore, miR-18a-5p levels in the affected skin of the patients with EM and those with SJS were increased slightly compared with those in healthy skin and lower than those in the skin of patients with TEN. These results suggest that miR-18a-5p levels are correlated with the severity of drug eruptions. Therefore we focused on miR-18a-5p, one of the miR-17-92 miRNA cluster at 13q31.3, which was previously called miR-18a and recently renamed miR-18a-5p based on miRBase. On the other hand, levels of miR-17-5p, miR-19b-3p, and miR-20a-5p, other miR-17-92 cluster members, also tended to be increased in the samples from patients with TEN compared with healthy skin according to the array, although the findings were not statistically significant (see Table E1). miR-19a-3p and miR-92a-3p were similarly expressed in healthy skin and the skin of patients with TEN. Consistently, real-time PCR revealed expression of these 5 miRNAs, and

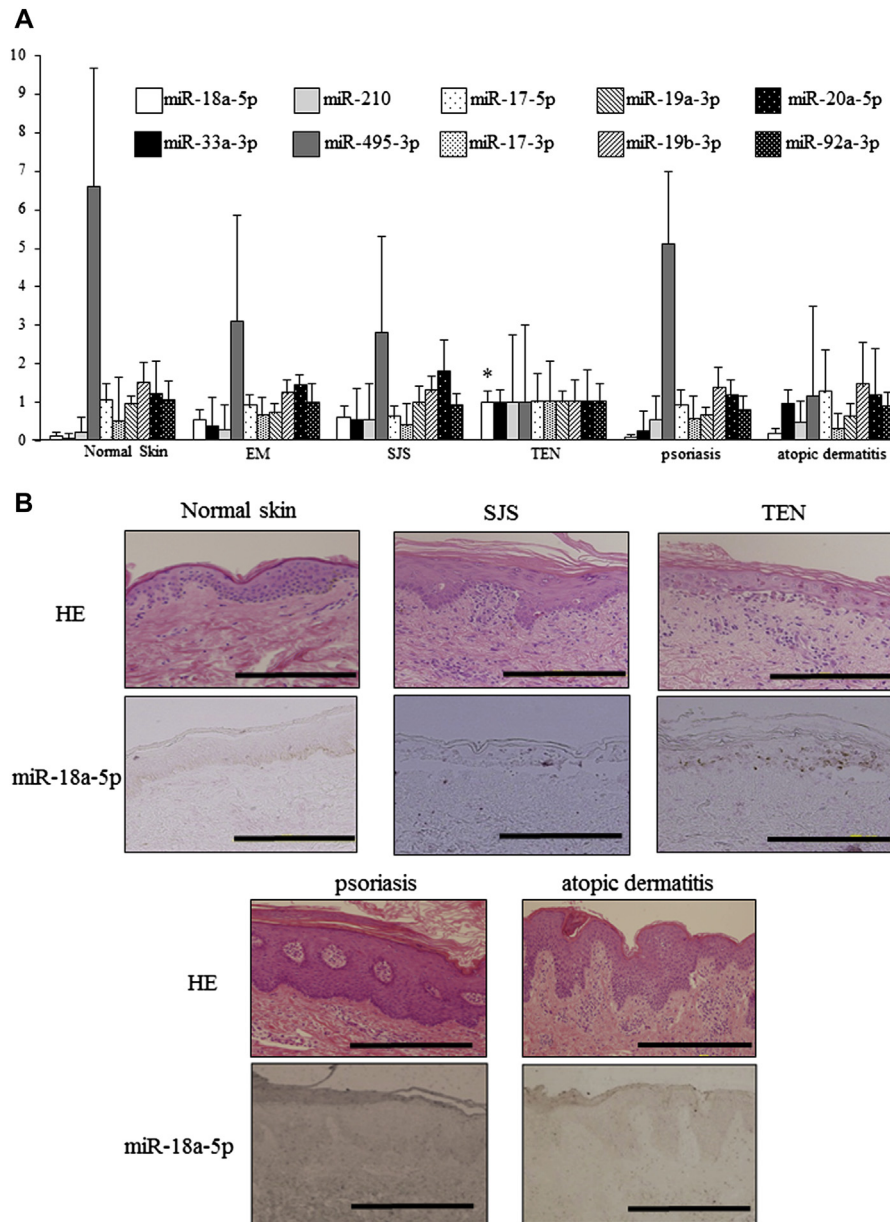


**FIG 1.** Expression of miR-18a-5p in cutaneous drug eruptions *in vivo*. **A**, Relative miRNA levels in tissues of 6 subjects with healthy skin, 8 patients with EM, 3 patients with SJS, 4 patients with TEN, 4 patients with psoriasis, and 4 patients with atopic dermatitis. Error bars represent SDs. \* $P < .05$  compared with values in the other disease samples. **B**, Upper panels, Hematoxylin and eosin (HE) staining. Bars = 200  $\mu$ m. Lower panels, *In situ* detection of miR-18a-5p. miR-18a-5p stained brown.

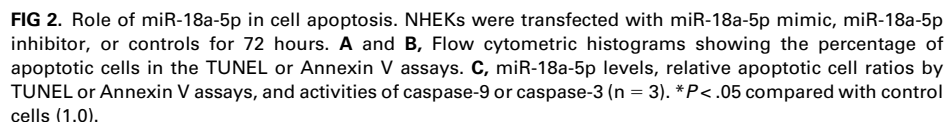
expression of the remaining miR-17-92 cluster member, miR-17-3p, was not significantly altered in the skin of patients with TEN compared with that seen in other patient groups (Fig 1, A). In addition, the array indicated expression of miR-18b-5p (UAAGGUG CAUCUAGUGCAGUUAG), which has a similar sequence to miR-18a-5p, was not different between healthy skin and skin of patients with TEN (see Table E1). Therefore the skin of patients with TEN was likely to overexpress miR-18a-5p specifically.

*In situ* hybridization showed that signals for miR-18a-5p were evident in the epidermis of patients with SJS and those with TEN but not in healthy skin samples (Fig 1, B). Of note, the miR-18a-5p signals were most apparent in the epidermal apoptotic cells of

the skin of patients with SJS and skin of patients with TEN, suggesting that miR-18a-5p might contribute to keratinocyte apoptosis. miR-18a-5p expression was not found in either the skin of patients with psoriasis or atopic skin.

### Downregulated BCL2L10 expression caused by miR-18a-5p overexpression is involved in keratinocyte apoptosis

We expected that miR-18a-5p might play a role in the pathogenesis of drug eruptions, especially TEN. One of the primary histologic features of TEN is keratinocyte apoptosis.<sup>1,2</sup>



of the miR-18a-5p mimic *in vitro* resulted in an increased percentage of apoptotic cells according to the 2 different apoptosis assays (Fig 2, A and B). The increase was statistically significant (Fig 2, C). On the other hand, miR-18a-5p inhibitor reduced

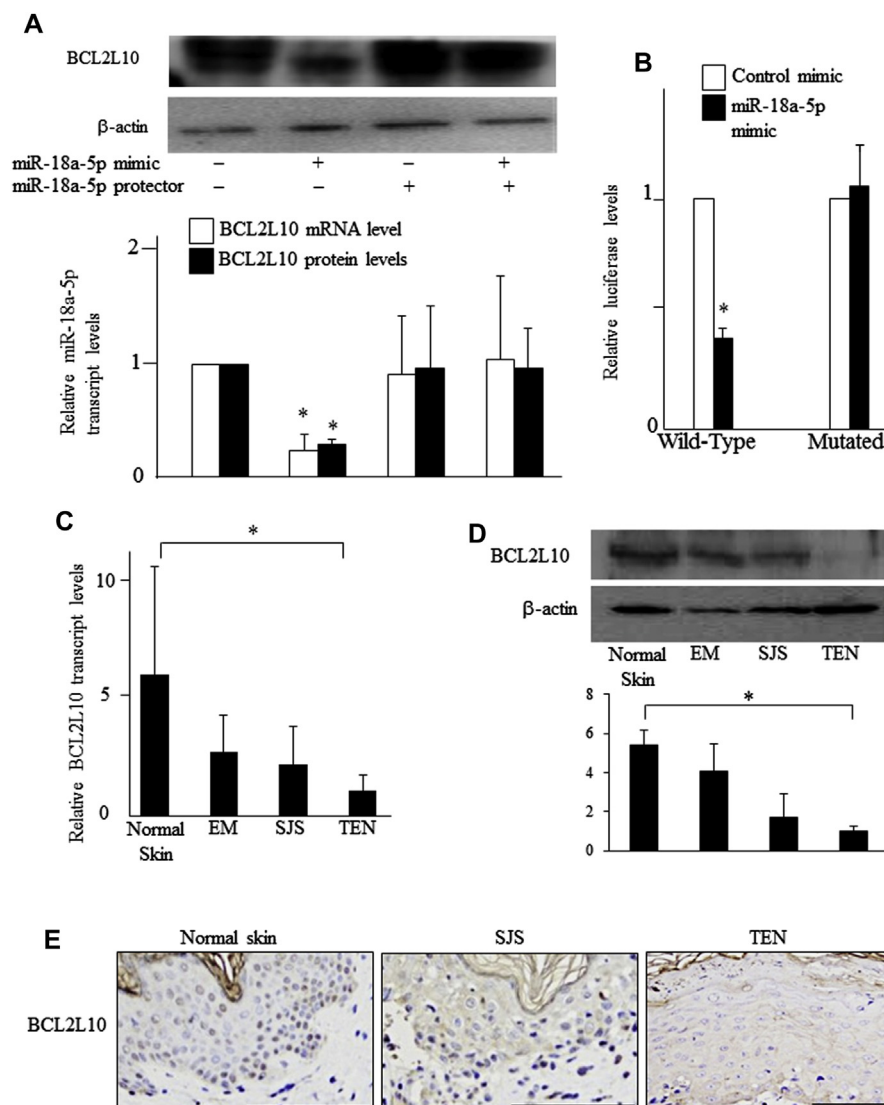


**FIG 3.** BCL2L10 expression *in vitro* and *in vivo*. **A**, BCL2L10 protein or mRNA levels in NHEKs transfected with miRNA mimics, protectors, or both ( $n = 3$ ). **B**, NHEKs were transfected with wild-type or mutated BCL2L10 3' UTR luciferase constructs together with control or miR-18a-5p mimic. The bar graph shows luciferase activities ( $n = 3$ ). **C** and **D**, mRNA (Fig 3, C) or protein (Fig 3, D) levels of BCL2L10 in skin samples. **E**, Immunostaining of BCL2L10. Bars = 100  $\mu$ m.

miR-18a-5p expression (Fig 2, C) but did not increase the apoptosis of NHEKs (Fig 2). This might be because the miR-18a-5p expression level was already low enough in NHEKs.

We searched for potential targets of miR-18a-5p by using the miRNA target gene prediction database. According to MicroCosm Targets (version 5, <http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/>) and TargetScan (version 6.0, <http://www.targetscan.org/>), we focused on *BCL2L10*, a new member of the BCL-2 family, as an apoptosis-associated putative target gene of miR-18a-5p. To confirm the association between miR-18a-5p and *BCL2L10*, NHEKs were transfected with the miR-18a-5p mimic and/or the miScript Target Protector (Qiagen), single-stranded RNA complementary to the miR-18a-5p binding site on the *BCL2L10* mRNA 3' UTR; the protector covers the flanking region of the binding site and specifically interferes

with direct interaction between miRNA and mRNA.<sup>25</sup> As shown in Fig 3, A, in the presence of the control protector, the miR-18a-5p mimic significantly reduced the expression of BCL2L10 protein and mRNA, indicating *BCL2L10* is a target of miR-18a-5p. On the other hand, in the presence of the miR-18a-5p-specific protector, the suppressive effects of the miR-18a-5p mimic on BCL2L10 expression were attenuated. To further assess the direct binding of miR-18a-5p to the *BCL2L10* 3' UTR, we performed a luciferase reporter gene assay using a luciferase construct containing wild-type *BCL2L10* 3' UTR and that with point mutations, changing gcacctt into gcattcc to mutate the miR-18a-5p seed match.<sup>26</sup> The luciferase activity of the wild-type construct was reduced by the miR-18a-5p mimic, whereas that of the mutated construct was not (Fig 3, B). These results indicate that miR-18a-5p binds to the *BCL2L10* 3' UTR and directly regulates BCL2L10 expression.

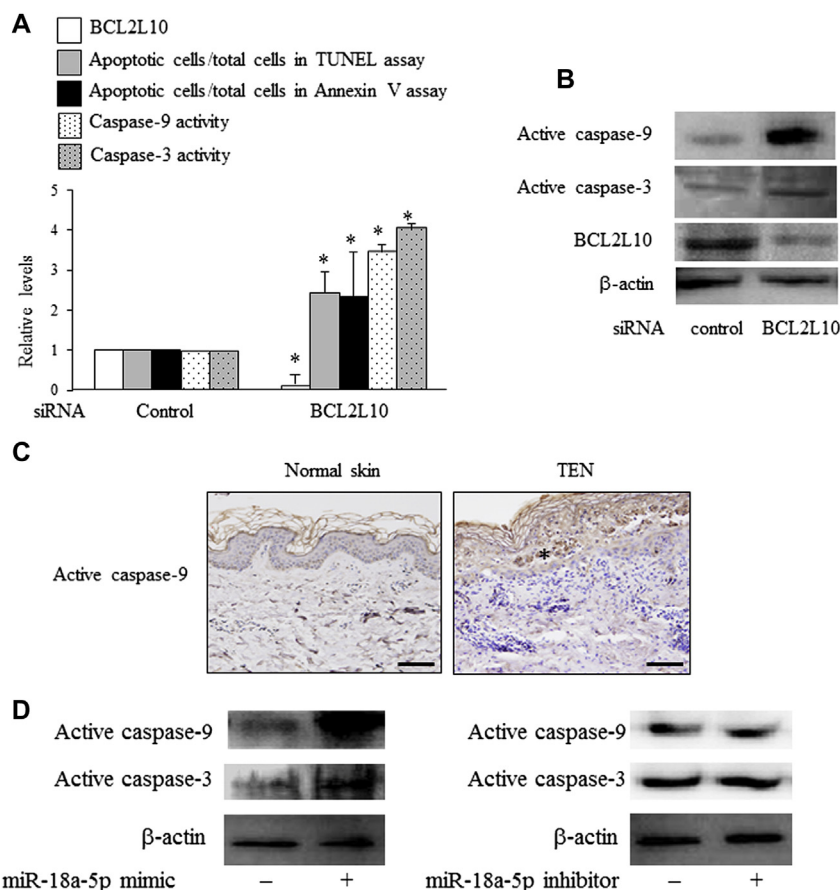


**FIG 4.** Function of BCL2L10 in patients with TEN. **A**, NHEKs were transfected with BCL2L10 siRNA for 72 hours. BCL2L10 mRNA levels, apoptotic cell ratios, and caspase activities are shown ( $n = 3$ ).  $*P < .05$ . **B**, Immunoblotting with NHEKs transfected with BCL2L10 siRNA. **C**, Immunostaining of active caspase-9. Bars = 100  $\mu$ m. Asterisks indicate apoptotic cells. **D**, NHEKs were transfected with miRNA mimics or inhibitors. Active caspases were determined by using immunoblotting.

Then we examined the expression levels of BCL2L10 in the skin tissues of patients with drug eruptions. There were significant decreases in the levels of *BCL2L10* mRNA (Fig 3, C) and protein (Fig 3, D) in the skin of patients with TEN compared with those seen in healthy skin. BCL2L10 levels were also decreased in patients with SJS and those with EM, although not significantly, indicating that the BCL2L10 expression tended to be inversely correlated with disease severity and the miR-18a-5p expression pattern, as shown in Fig 1, A.

Immunohistochemistry revealed that the protein expression of BCL2L10 was observed in the nuclei of keratinocytes in the normal epidermis, whereas its expression in the epidermis of patients with TEN was decreased (Fig 3, E). BCL2L10 expression was also reduced in patients with SJS slightly, which is consistent with the results shown in Fig 3, C and D.

The role of BCL2L10 in the skin has not yet been reported. When BCL2L10 expression was knocked down by siRNA (Fig 4, A), we observed a significant increase in apoptotic cell numbers using the TUNEL and Annexin V assays. Consistent with the notion that BCL2L10 is a member of the BCL-2 family and is known to inhibit the intrinsic apoptosis pathway (see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), the protein expressions of active caspase-9, the key caspase of intrinsic

apoptosis (Fig 4, B), and its activity (Fig 4, A) were also upregulated by the siRNA.

Taken together, our results showed that miR-18a-5p is overexpressed in the skin of patients with TEN, that miR-18a-5p is a suppressor of BCL2L10, and that BCL2L10 is a negative regulator of intrinsic keratinocyte apoptosis. We hypothesized that the downregulated BCL2L10 level caused by miR-18a-5p overexpression induces intrinsic apoptosis of keratinocytes in patients with TEN. Consistent with this hypothesis, caspase-9 was activated in the epidermis of patients with TEN compared with healthy skin (Fig 4, C). Of note, the apoptotic cells in the skin of patients with TEN strongly expressed active caspase-9. Furthermore, the miR-18a-5p mimic also induced the expression (Fig 4, D) and activity (Fig 2, C) of active caspase-9, as well as caspase-3, indicating that miR-18a-5p regulates intrinsic apoptosis. Consistent with the results of the apoptosis assay shown in Fig 2, miR-18a-5p inhibitor did not affect the expression or activity of caspases.

### Serum levels of miR-18a-5p in patients with drug eruptions and their correlations with clinical features

We also compared the serum concentration of miR-18a-5p between patients with drug eruptions and healthy subjects. To

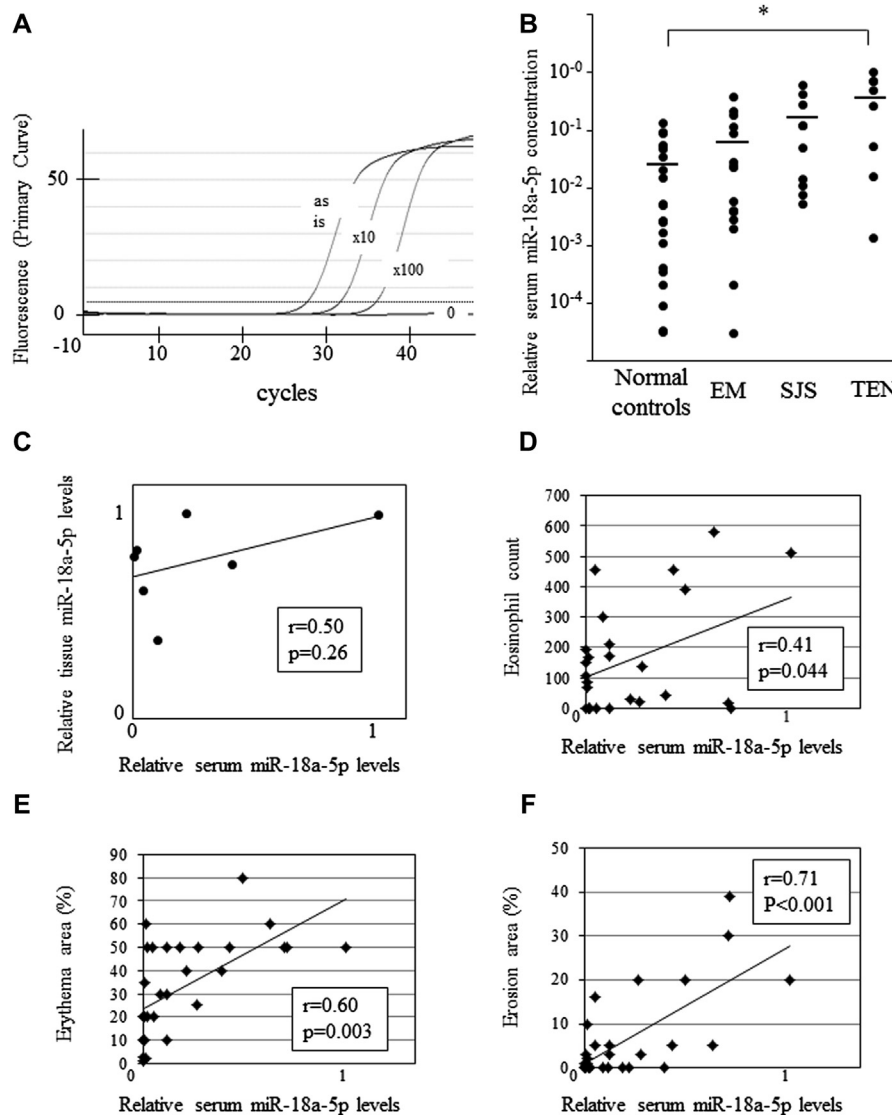


**FIG 5.** Serum concentrations of miR-18a-5p in patients with drug eruptions. **A**, Serial dilutions of cDNA (as is, 10-fold dilution, 100-fold dilution, and 0) synthesized from serum miRNA were used as templates for real-time PCR. **B**, Serum miR-18a-5p levels. Bars show means. \* $P < .05$ . **C**, Correlation between serum and skin miR-18a-5p level of each patient. **D-F**, Correlation between serum miR-18a-5p levels and clinical features of patients.

validate that the miRNA is indeed detectable in human serum, miRNA was extracted from sera of healthy subjects, and the level of miR-18a-5p was determined by using real-time PCR with miR-18a-5p primer (Fig 5, A).<sup>17-20</sup> Amplification of miR-18a-5p was observed. The cycle threshold (Ct) value for miR-18a-5p in the undiluted miRNA sample was 27.8, and the values were increased by serial dilution of the template. Therefore miR-18a-5p is thought to be detectable and quantitative in the serum by using our method. We also verify the presence of miR-18a-5p in human sera by sequencing the miR-18a-5p PCR product.

Serum miR-18a-5p levels were significantly increased in patients with TEN compared with those seen in healthy subjects, indicating they are useful for the diagnosis of TEN. Levels in patients with EM and those with SJS were slightly higher than those in healthy control subjects, although the differences were not statistically significant (Fig 5, B), which is consistent with the

finding that the miR-18a-5p expression in the skin tended to be correlated with the severity of the drug eruption (Fig 1, A). Furthermore, as shown in Fig 5, C, we found a positive correlation ( $r = 0.50$ ) between the serum miR-18a-5p level and miR-18a-5p expression in the skin tissue of each patient, although it was not statistically significant.

Therefore we next analyzed the associations between serum miR-18a-5p levels and the clinical features of drug eruptions. Serum miR-18a-5p levels exhibited a poor correlation with eosinophil counts ( $r = 0.48$ ,  $P = .010$ ; Fig 5, D). On the other hand, serum miR-18a-5p levels were correlated with the BSA of erythema ( $r = 0.60$ ,  $P = .003$ ; Fig 5, E) and the BSA of erosion ( $r = 0.71$ ,  $P < .001$ ; Fig 5, F) more strongly. Taken together, the serum level of miR-18a-5p can also be used as a disease activity marker for drug eruptions, reflecting the severity of keratinocyte apoptosis.

## DISCUSSION

This study demonstrated the role of the miR-18a-5p–BCL2L10 pathway in keratinocyte apoptosis and its contribution to the pathogenesis of TEN based on 3 major findings.

First, we found that the upregulation of miR-18a-5p can be highly specific to TEN. In addition, the miR-18a-5p expression was correlated with the severity of drug eruptions. According to recent reports, miR-18a-5p is involved in the pathogenesis of basal cell carcinoma, ataxia-telangiectasia, and pancreatic cancer.<sup>27–29</sup> This is the first study to investigate miR-18a-5p expression in patients with allergic skin diseases. According to the array, miR-18a-5p and miR-18b-5p exhibited different expression patterns in various skin diseases. miR-18a-5p and miR-18b-5p have a similar sequence; however, their source is chromosome 13 and chromosome X, respectively. Therefore they are likely regulated independently. The regulatory mechanism of miR-18a-5p overexpression in patients with TEN remains unclear. miRNA expression can be regulated by various stimuli, including activation of transcription factors and methylation in general. However, investigation of abnormalities in these factors in patients with TEN has just begun. Further studies are needed to clarify the mechanism.

miR-18a-5p overexpression resulted in an increase in cell apoptosis. miR-17-3p and miR-92a-3p, other members of the miR-17-92 cluster, are reported to be more important for the regulation of apoptosis in thyroid cancer cells or colon cancer cells than miR-18a-5p.<sup>30,31</sup> However, their expression was not significantly increased in the skin of patients with TEN in our study. Our results suggest that miR-18a-5p is also involved in the apoptosis pathway and that its effects on apoptosis might be cell type dependent.

Second, we also found new miRNA-mRNA target interactions in this study; upregulated miR-18a-5p led to decreased expression of BCL2L10 in keratinocytes. Immunohistochemical staining showed that BCL2L10 proteins were expressed in the epidermal layers of healthy skin. In addition, BCL2L10 siRNA and miR-18a-5p mimic upregulated keratinocyte apoptosis, indicating that BCL2L10 acts as an antiapoptotic regulator in the skin. There are several reports about the relationships between BCL2L10 and gastric cancer, breast cancer, and leukemia.<sup>32–34</sup> However, the role of BCL2L10 in skin diseases remains unknown. This study is the first to clarify the function and localization of BCL2L10 in the skin. BCL2L10 is one of the 6 antiapoptotic members of the BCL-2 family, the key regulators of apoptosis,<sup>35</sup> and is involved in the intrinsic apoptosis pathway. There are both extrinsic and intrinsic pathways in apoptosis (see Fig E1). The extrinsic pathway is activated by the binding of Fas ligand or TNF to the death receptor. The receptor contains an intracellular death domain and activates caspase-8 and subsequently caspase-3. On the other hand, in the intrinsic pathway proapoptotic proteins (BAX and BAK) cause mitochondrial outer membrane permeabilization, resulting in the release of cytochrome C. This stimulates the activation of caspase-9 and caspase-3 and then induces cell death.<sup>36</sup> The antiapoptotic members of the BCL-2 family (BCL2, BCL2L1, BCL2L2, BCL2A1, MCL1, and BCL2L10) inhibit the activity of BAX or BAK to lead cells toward survival.<sup>37–39</sup> According to previous reports, extrinsic pathways, such as Fas, are implicated in the mechanisms underlying the apoptosis observed in patients with TEN.<sup>1,2</sup> The present study demonstrated caspase-9 activation in the epidermis of patients

with TEN and suggested that the intrinsic pathway is also activated. The miR-18a-5p–BCL2L10 pathway might contribute to this process.

The inhibition of endogenous miR-18a-5p expression in NHEKs did not influence apoptosis and expression of BCL2L10. Thus we could not show the therapeutic potential of miR-18a-5p inhibitors for the treatment of TEN. To further evaluate therapeutic values, miR-18a-5p inhibition needs to be tested by using an *in vitro* or *in vivo* model of TEN. Widely accepted models should be developed in the future.

Lastly, we first investigated serum miRNA levels in patients with drug eruptions. There is only 1 report showing that miR-18a-5p is detectable and quantitative in body fluid. In that study miR-18a-5p was found to be increased in the plasma of patients with pancreatic cancer.<sup>28</sup> Our results indicate that serum miR-18a-5p concentrations were significantly increased in patients with TEN and correlated with the severity of drug eruptions, which is consistent with the expression pattern of miR-18a-5p in the skin. We often experience patients with TEN whose diagnoses are incorrect and who are not treated intensively in the early stage, which results in permanent damage to the eyes or esophagus and even death. Therefore it is urgent to develop reliable diagnostic markers and disease activity markers for TEN. Soluble Fas ligand has become such a candidate diagnostic marker, and recently, Fujita et al<sup>40</sup> had a rapid immunochromatographic assay for the detection of increased serum levels of granulysin, a cytolytic proapoptotic protein, found in patients with TEN. Our study suggests that the serum miR-18a-5p level might also be a useful disease marker. Although we did not find any differences in miR-18a levels between patients induced by different drugs, this might be due to the small number of patients. Larger studies are needed to evaluate the future clinical use of this marker.

In summary, miR-18a-5p can play a key role in the keratinocyte apoptosis observed in patients with drug eruptions, especially in patients with TEN. Investigating the regulatory mechanisms of keratinocyte apoptosis induced by miRNAs might lead to the development of new disease markers.

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### Key messages

- miR-18a-5p expression is upregulated in the skin of patients with TEN.
- Increased miR-18a-5p expression and subsequently decreased BCL2L10 expression might play key roles in the intrinsic keratinocyte apoptosis observed in patients with TEN.
- Serum miR-18a-5p levels are significantly increased in patients with TEN.

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**Supplemental Table E1. The expression profiles of miRNAs in the skin of TEN patients, as measured using a PCR array**

|                | normal skin |      | TEN    |        | psoriais |        | atopic dermatitis |      |
|----------------|-------------|------|--------|--------|----------|--------|-------------------|------|
|                | mean        | SD   | mean   | SD     | mean     | SD     | mean              | SD   |
| hsa-let-7a-5p  | 1.00        | 0.72 | 1.00   | 0.53   | 1.37     | 0.61   | 1.04              | 0.64 |
| hsa-let-7b-5p  | 1.00        | 0.40 | 0.96   | 0.28   | 1.18     | 0.19   | 0.99              | 0.47 |
| hsa-let-7c     | 1.00        | 0.50 | 1.03   | 0.54   | 1.28     | 0.38   | 0.96              | 0.57 |
| hsa-let-7d-5p  | 1.00        | 0.53 | 0.61   | 0.63   | 1.11     | 0.27   | 0.98              | 0.44 |
| hsa-let-7d-3p  | 1.00        | 0.10 | 0.95   | 0.46   | 0.96     | 0.11   | 1.10              | 0.55 |
| hsa-let-7e-5p  | 1.00        | 0.71 | 1.18   | 0.85   | 0.94     | 0.21   | 0.80              | 0.35 |
| hsa-let-7f-5p  | 1.00        | 0.67 | 0.76   | 0.37   | 1.38     | 0.46   | 0.85              | 0.66 |
| hsa-let-7g-5p  | 1.00        | 0.20 | 1.04   | 0.25   | 1.47     | 0.47   | 1.29              | 0.45 |
| hsa-let-7g-3p  | 1.00        | 1.28 | 0.73   | 0.62   | 1.64     | 2.53   | 2.57              | 1.72 |
| hsa-let-7i-5p  | 1.00        | 0.59 | 1.45   | 0.73   | 1.60     | 0.47   | 1.05              | 0.03 |
| hsa-miR-1      | 1.00        | 0.98 | 2.99   | 4.41   | 2.69     | 4.62   | 0.01              | 0.02 |
| hsa-miR-7-5p   | 1.00        | 0.88 | 1.73   | 1.53   | 2.91     | 2.93   | 2.49              | 1.54 |
| hsa-miR-9-5p   | 1.00        | 1.49 | 0.00   | 0.00   | 0.02     | 0.03   | 2.14              | 2.83 |
| hsa-miR-9-3p   | 0.00        | 0.00 | 0.00   | 0.00   | 0.00     | 0.00   | 0.00              | 0.00 |
| hsa-miR-10a-5p | 1.00        | 0.85 | 1.50   | 0.54   | 2.03     | 1.18   | 0.47              | 0.35 |
| hsa-miR-10a-3p | 1.00        | 0.93 | 0.52   | 0.91   | 1.77     | 2.82   | 0.58              | 1.00 |
| hsa-miR-10b-5p | 1.00        | 0.72 | 1.14   | 0.81   | 2.38     | 0.84   | 1.09              | 0.66 |
| hsa-miR-10b-3p | 1.00        | 1.73 | 0.48   | 0.78   | 0.39     | 0.67   | 2.72              | 3.86 |
| hsa-miR-15a-5p | 1.00        | 0.69 | 0.89   | 0.76   | 0.88     | 0.77   | 0.34              | 0.20 |
| hsa-miR-15b-5p | 1.00        | 0.56 | 1.31   | 0.67   | 1.17     | 0.93   | 1.23              | 0.15 |
| hsa-miR-15b-3p | 1.00        | 1.73 | 6.19   | 5.38   | 21.30    | 19.95  | 6.54              | 7.22 |
| hsa-miR-16-5p  | 1.00        | 1.26 | 1.59   | 1.24   | 0.55     | 0.10   | 1.07              | 0.52 |
| hsa-miR-17-5p  | 1.00        | 0.55 | 1.93   | 1.34   | 0.50     | 0.47   | 1.20              | 0.75 |
| hsa-miR-18a-5p | 1.00        | 1.08 | 7.93   | 1.11   | 1.75     | 3.04   | 2.07              | 3.58 |
| hsa-miR-18a-3p | 0.00        | 0.00 | 1.00   | 1.72   | 0.00     | 0.00   | 0.00              | 0.00 |
| hsa-miR-18b-5p | 1.00        | 0.61 | 0.82   | 0.86   | 0.64     | 0.64   | 0.67              | 0.53 |
| hsa-miR-18b-3p | 1.00        | 1.71 | 0.31   | 0.41   | 4.30     | 6.94   | 0.58              | 0.75 |
| hsa-miR-19a-3p | 1.00        | 1.13 | 0.88   | 0.61   | 0.65     | 0.36   | 0.61              | 0.44 |
| hsa-miR-19a-5p | 1.00        | 1.70 | 167.01 | 287.98 | 285.12   | 487.67 | 0.88              | 0.95 |
| hsa-miR-19b-3p | 1.00        | 0.20 | 2.74   | 2.67   | 1.23     | 0.45   | 0.99              | 0.54 |
| hsa-miR-20a-5p | 1.00        | 1.16 | 1.25   | 0.48   | 0.63     | 0.39   | 0.86              | 0.51 |
| hsa-miR-20a-3p | 0.00        | 0.00 | 1.00   | 1.73   | 0.00     | 0.00   | 0.98              | 1.67 |
| hsa-miR-20b-5p | 1.00        | 0.99 | 1.38   | 0.71   | 0.46     | 0.47   | 1.21              | 0.69 |
| hsa-miR-20b-3p | 1.00        | 1.24 | 1.27   | 1.65   | 0.53     | 0.74   | 0.11              | 0.09 |
| hsa-miR-21-5p  | 1.00        | 1.21 | 0.89   | 0.56   | 3.28     | 1.68   | 0.68              | 0.34 |
| hsa-miR-21-3p  | 1.00        | 1.34 | 2.34   | 2.91   | 0.07     | 0.07   | 0.29              | 0.25 |
| hsa-miR-22-3p  | 1.00        | 1.31 | 2.22   | 2.87   | 0.93     | 0.46   | 0.92              | 0.33 |
| hsa-miR-22-5p  | 1.00        | 0.51 | 1.37   | 1.92   | 0.12     | 0.20   | 0.96              | 0.64 |
| hsa-miR-23a-3p | 1.00        | 0.47 | 1.41   | 0.77   | 2.24     | 0.82   | 1.98              | 0.97 |
| hsa-miR-23a-5p | 1.00        | 0.45 | 1.45   | 1.09   | 0.31     | 0.54   | 1.53              | 0.50 |
| hsa-miR-23b-3p | 1.00        | 0.60 | 0.96   | 0.47   | 1.53     | 0.55   | 1.39              | 0.62 |
| hsa-miR-23b-5p | 1.00        | 0.84 | 0.42   | 0.71   | 0.44     | 0.73   | 0.86              | 1.03 |
| hsa-miR-24-3p  | 1.00        | 0.56 | 1.64   | 1.16   | 1.11     | 0.54   | 1.21              | 0.35 |
| hsa-miR-25-3p  | 1.00        | 0.43 | 0.82   | 0.43   | 1.23     | 0.37   | 1.10              | 0.55 |
| hsa-miR-25-5p  | 1.00        | 1.47 | 0.89   | 1.50   | 0.87     | 0.95   | 0.54              | 0.27 |
| hsa-miR-26a-5p | 1.00        | 0.29 | 1.29   | 0.04   | 1.79     | 0.77   | 1.09              | 0.36 |

|                  |      |      |        |        |       |       |        |        |
|------------------|------|------|--------|--------|-------|-------|--------|--------|
| hsa-miR-26b-5p   | 1.00 | 0.44 | 0.90   | 0.57   | 1.46  | 0.27  | 1.08   | 1.01   |
| hsa-miR-27a-3p   | 1.00 | 1.06 | 1.19   | 0.84   | 0.44  | 0.29  | 0.88   | 0.90   |
| hsa-miR-27a-5p   | 1.00 | 1.73 | 2.90   | 3.30   | 4.98  | 4.51  | 1.34   | 2.19   |
| hsa-miR-27b-3p   | 1.00 | 0.46 | 1.02   | 0.33   | 0.73  | 0.31  | 0.76   | 0.18   |
| hsa-miR-27b-5p   | 1.00 | 1.73 | 7.83   | 6.90   | 1.31  | 2.28  | 6.11   | 9.49   |
| hsa-miR-28-3p    | 1.00 | 0.53 | 2.10   | 1.26   | 3.03  | 0.43  | 0.60   | 0.28   |
| hsa-miR-28-5p    | 1.00 | 0.40 | 1.86   | 2.17   | 1.00  | 0.90  | 1.38   | 1.52   |
| hsa-miR-29a-3p   | 1.00 | 1.18 | 0.89   | 0.38   | 0.44  | 0.05  | 0.63   | 0.18   |
| hsa-miR-29a-5p   | 1.00 | 0.88 | 0.05   | 0.01   | 0.11  | 0.18  | 0.94   | 1.16   |
| hsa-miR-29b-3p   | 1.00 | 1.22 | 0.73   | 0.28   | 0.66  | 0.30  | 0.44   | 0.14   |
| hsa-miR-29b-2-5p | 1.00 | 0.95 | 0.90   | 0.84   | 0.01  | 0.01  | 1.02   | 1.47   |
| hsa-miR-29c-3p   | 1.00 | 0.86 | 0.81   | 0.63   | 0.75  | 0.22  | 0.85   | 0.49   |
| hsa-miR-30a-5p   | 1.00 | 0.64 | 0.90   | 0.92   | 0.66  | 0.27  | 0.78   | 0.38   |
| hsa-miR-30b-5p   | 1.00 | 0.50 | 1.23   | 0.10   | 0.75  | 0.30  | 1.12   | 0.22   |
| hsa-miR-30c-5p   | 1.00 | 0.23 | 1.10   | 0.36   | 2.09  | 0.93  | 1.16   | 0.09   |
| hsa-miR-30c-1-3p | 1.00 | 0.99 | 0.52   | 0.53   | 0.00  | 0.00  | 0.33   | 0.54   |
| hsa-miR-30d-5p   | 1.00 | 0.67 | 0.90   | 0.34   | 0.58  | 0.39  | 0.48   | 0.22   |
| hsa-miR-30d-3p   | 1.00 | 0.85 | 8.36   | 12.90  | 0.03  | 0.04  | 1.76   | 2.14   |
| hsa-miR-30e-5p   | 1.00 | 1.01 | 0.60   | 0.50   | 0.41  | 0.19  | 0.46   | 0.27   |
| hsa-miR-30e-3p   | 1.00 | 0.59 | 1.71   | 0.85   | 2.15  | 0.64  | 1.00   | 0.49   |
| hsa-miR-31-5p    | 1.00 | 1.00 | 0.16   | 0.14   | 2.24  | 0.82  | 0.73   | 0.32   |
| hsa-miR-31-3p    | 0.00 | 0.00 | 1.00   | 1.73   | 0.00  | 0.00  | 0.00   | 0.00   |
| hsa-miR-32-5p    | 1.00 | 1.34 | 1.71   | 1.50   | 2.14  | 3.24  | 0.67   | 1.16   |
| hsa-miR-32-3p    | 0.00 | 0.00 | 0.00   | 0.00   | 1.00  | 1.73  | 1.72   | 2.97   |
| hsa-miR-33a-5p   | 1.00 | 1.15 | 6.47   | 9.68   | 47.76 | 82.72 | 69.73  | 39.62  |
| hsa-miR-33a-3p   | 0.00 | 0.00 | 1.00   | 0.75   | 0.01  | 0.01  | 0.04   | 0.07   |
| hsa-miR-33b-5p   | 1.00 | 1.16 | 4.63   | 4.20   | 2.65  | 3.96  | 1.46   | 1.07   |
| hsa-miR-34a-5p   | 1.00 | 0.53 | 1.38   | 0.73   | 0.73  | 1.07  | 0.62   | 0.12   |
| hsa-miR-34a-3p   | 1.00 | 1.35 | 0.23   | 0.27   | 0.01  | 0.01  | 0.13   | 0.23   |
| hsa-miR-34b-3p   | 1.00 | 0.79 | 1.05   | 0.88   | 0.64  | 0.81  | 2.42   | 2.64   |
| hsa-miR-34b-5p   | 0.00 | 0.00 | 0.00   | 0.00   | 1.00  | 1.73  | 0.00   | 0.00   |
| hsa-miR-34c-3p   | 1.00 | 0.78 | 0.79   | 1.18   | 1.47  | 2.00  | 0.99   | 1.17   |
| hsa-miR-34c-5p   | 1.00 | 1.73 | 1.24   | 2.15   | 0.00  | 0.00  | 0.00   | 0.01   |
| hsa-miR-92a-3p   | 1.00 | 0.27 | 1.10   | 0.31   | 1.76  | 0.57  | 1.48   | 0.66   |
| hsa-miR-92a-1-5p | 1.00 | 1.30 | 1.30   | 1.59   | 0.33  | 0.56  | 0.51   | 0.87   |
| hsa-miR-92b-3p   | 1.00 | 0.53 | 1.82   | 1.57   | 1.40  | 0.87  | 1.72   | 0.60   |
| hsa-miR-92b-5p   | 1.00 | 1.08 | 2.48   | 1.97   | 1.17  | 1.61  | 2.30   | 1.42   |
| hsa-miR-93-5p    | 1.00 | 0.63 | 1.09   | 0.54   | 1.61  | 0.52  | 1.58   | 1.46   |
| hsa-miR-93-3p    | 1.00 | 1.26 | 0.31   | 0.43   | 0.19  | 0.18  | 0.87   | 1.50   |
| hsa-miR-95       | 1.00 | 1.08 | 2.43   | 1.17   | 1.14  | 1.05  | 2.52   | 2.23   |
| hsa-miR-96-5p    | 1.00 | 1.73 | 0.00   | 0.00   | 0.00  | 0.00  | 0.00   | 0.01   |
| hsa-miR-98-5p    | 1.00 | 0.60 | 1.12   | 0.78   | 2.24  | 0.47  | 1.05   | 0.65   |
| hsa-miR-99a-3p   | 1.00 | 1.73 | 223.35 | 386.86 | 0.00  | 0.00  | 100.49 | 171.91 |
| hsa-miR-99a-5p   | 1.00 | 1.49 | 0.87   | 1.10   | 0.13  | 0.04  | 0.23   | 0.06   |
| hsa-miR-99b-5p   | 1.00 | 0.70 | 2.66   | 1.74   | 2.00  | 0.68  | 1.82   | 0.60   |
| hsa-miR-100-5p   | 1.00 | 0.44 | 1.40   | 0.43   | 1.74  | 1.54  | 0.48   | 0.23   |
| hsa-miR-101-3p   | 1.00 | 1.10 | 1.46   | 1.25   | 0.55  | 0.09  | 0.33   | 0.09   |
| hsa-miR-101-5p   | 1.00 | 0.96 | 1.15   | 1.33   | 5.26  | 8.15  | 0.06   | 0.10   |
| hsa-miR-103a-3p  | 1.00 | 0.78 | 1.43   | 0.74   | 0.69  | 0.15  | 0.83   | 0.26   |
| hsa-miR-105-5p   | 1.00 | 1.73 | 0.00   | 0.00   | 0.00  | 0.00  | 0.00   | 0.00   |
| hsa-miR-106a-3p  | 0.00 | 0.00 | 0.00   | 0.00   | 0.00  | 0.00  | 0.00   | 0.00   |
| hsa-miR-106b-5p  | 1.00 | 0.94 | 1.80   | 1.30   | 1.24  | 0.36  | 2.06   | 0.60   |
| hsa-miR-106b-3p  | 1.00 | 0.94 | 0.98   | 1.65   | 1.35  | 1.20  | 0.79   | 0.15   |
| hsa-miR-107      | 1.00 | 1.63 | 0.69   | 1.19   | 0.10  | 0.18  | 0.70   | 0.68   |

|                  |      |      |        |        |      |       |       |       |
|------------------|------|------|--------|--------|------|-------|-------|-------|
| hsa-miR-122-5p   | 1.00 | 1.72 | 3.11   | 4.13   | 0.40 | 0.69  | 0.82  | 1.42  |
| hsa-miR-122-3p   | 0.00 | 0.00 | 0.00   | 0.00   | 0.00 | 0.00  | 0.00  | 0.00  |
| hsa-miR-124-3p   | 1.00 | 0.71 | 0.80   | 0.93   | 0.48 | 0.56  | 0.20  | 0.15  |
| hsa-miR-124-5p   | 1.00 | 0.91 | 0.80   | 1.34   | 2.52 | 3.81  | 0.33  | 0.31  |
| hsa-miR-125a-3p  | 1.00 | 1.02 | 1.31   | 0.91   | 0.01 | 0.01  | 1.94  | 1.85  |
| hsa-miR-125a-5p  | 1.00 | 0.32 | 0.85   | 0.21   | 1.36 | 0.74  | 1.07  | 0.38  |
| hsa-miR-125b-5p  | 1.00 | 0.79 | 1.41   | 1.45   | 0.95 | 0.26  | 0.60  | 0.09  |
| hsa-miR-126-3p   | 1.00 | 0.46 | 0.97   | 0.70   | 0.74 | 0.27  | 0.55  | 0.15  |
| hsa-miR-126-5p   | 1.00 | 0.84 | 1.11   | 0.93   | 1.76 | 0.74  | 0.91  | 0.51  |
| hsa-miR-127-3p   | 1.00 | 0.68 | 1.66   | 1.76   | 1.00 | 0.51  | 0.91  | 0.38  |
| hsa-miR-127-5p   | 1.00 | 1.66 | 2.34   | 4.06   | 0.13 | 0.23  | 0.51  | 0.79  |
| hsa-miR-128      | 1.00 | 0.57 | 2.02   | 1.77   | 0.62 | 0.58  | 2.13  | 2.02  |
| hsa-miR-129-1-3p | 0.00 | 0.00 | 0.00   | 0.00   | 0.00 | 0.00  | 0.00  | 0.00  |
| hsa-miR-129-2-3p | 1.00 | 1.51 | 6.02   | 6.24   | 2.67 | 3.15  | 3.08  | 2.98  |
| hsa-miR-129-5p   | 1.00 | 0.87 | 13.07  | 21.85  | 1.17 | 2.00  | 1.23  | 2.11  |
| hsa-miR-130a-3p  | 1.00 | 0.87 | 0.46   | 0.44   | 0.56 | 0.26  | 0.72  | 0.33  |
| hsa-miR-130b-3p  | 1.00 | 0.66 | 0.73   | 1.27   | 2.29 | 3.81  | 2.52  | 2.85  |
| hsa-miR-132-3p   | 1.00 | 0.61 | 0.94   | 1.30   | 2.60 | 1.14  | 1.34  | 1.25  |
| hsa-miR-132-5p   | 0.00 | 0.00 | 0.00   | 0.00   | 0.00 | 0.00  | 1.00  | 1.73  |
| hsa-miR-133a     | 1.00 | 1.73 | 241.55 | 415.15 | 0.00 | 0.00  | 0.86  | 1.50  |
| hsa-miR-133b     | 0.00 | 0.00 | 1.00   | 1.73   | 8.77 | 15.18 | 0.00  | 0.00  |
| hsa-miR-134      | 1.00 | 0.48 | 0.51   | 0.30   | 1.41 | 1.05  | 0.86  | 0.66  |
| hsa-miR-135a-5p  | 0.00 | 0.00 | 0.00   | 0.00   | 0.00 | 0.00  | 1.00  | 1.73  |
| hsa-miR-135b-5p  | 1.00 | 1.62 | 0.00   | 0.00   | 0.00 | 0.00  | 21.40 | 21.40 |
| hsa-miR-136-5p   | 1.00 | 1.26 | 0.14   | 0.23   | 0.70 | 0.79  | 0.00  | 0.00  |
| hsa-miR-137      | 1.00 | 1.26 | 0.14   | 0.23   | 0.70 | 0.79  | 0.00  | 0.00  |
| hsa-miR-138-5p   | 0.00 | 0.00 | 0.00   | 0.00   | 1.00 | 1.73  | 0.00  | 0.00  |
| hsa-miR-139-3p   | 1.00 | 0.97 | 0.82   | 0.72   | 1.19 | 1.34  | 0.61  | 0.54  |
| hsa-miR-139-5p   | 1.00 | 0.65 | 1.67   | 0.64   | 1.66 | 0.38  | 1.36  | 0.90  |
| hsa-miR-140-3p   | 1.00 | 0.79 | 1.31   | 0.85   | 0.92 | 0.27  | 1.26  | 0.69  |
| hsa-miR-140-5p   | 1.00 | 1.69 | 1.12   | 1.93   | 0.63 | 1.08  | 2.07  | 1.69  |
| hsa-miR-141-3p   | 1.00 | 1.06 | 0.68   | 0.41   | 0.98 | 1.02  | 0.32  | 0.26  |
| hsa-miR-142-3p   | 1.00 | 0.89 | 0.82   | 0.83   | 0.66 | 0.22  | 1.58  | 1.32  |
| hsa-miR-142-5p   | 1.00 | 1.73 | 1.37   | 1.28   | 2.97 | 3.97  | 1.28  | 2.21  |
| hsa-miR-143-3p   | 1.00 | 1.55 | 0.88   | 1.03   | 0.10 | 0.11  | 0.16  | 0.07  |
| hsa-miR-143-5p   | 0.00 | 0.00 | 1.00   | 1.73   | 0.16 | 0.28  | 0.42  | 0.73  |
| hsa-miR-144-3p   | 1.00 | 1.40 | 1.47   | 2.54   | 0.00 | 0.00  | 0.05  | 0.08  |
| hsa-miR-144-5p   | 0.00 | 0.00 | 0.00   | 0.00   | 0.00 | 0.00  | 1.00  | 1.73  |
| hsa-miR-145-5p   | 1.00 | 1.27 | 2.85   | 3.74   | 0.39 | 0.10  | 0.38  | 0.17  |
| hsa-miR-145-3p   | 1.00 | 1.22 | 2.54   | 4.39   | 2.06 | 2.48  | 0.68  | 1.11  |
| hsa-miR-146a-5p  | 1.00 | 0.37 | 0.56   | 0.75   | 0.96 | 0.15  | 1.24  | 0.53  |
| hsa-miR-146b-5p  | 1.00 | 0.50 | 0.59   | 0.78   | 2.99 | 3.00  | 1.88  | 0.15  |
| hsa-miR-147a     | 0.00 | 0.00 | 1.00   | 1.73   | 0.91 | 1.58  | 0.00  | 0.00  |
| hsa-miR-148a-3p  | 1.00 | 0.63 | 1.48   | 0.75   | 1.56 | 0.48  | 0.95  | 0.20  |
| hsa-miR-148b-3p  | 1.00 | 0.61 | 0.72   | 0.45   | 0.84 | 0.54  | 0.51  | 0.21  |
| hsa-miR-149-5p   | 1.00 | 0.94 | 2.05   | 1.18   | 0.47 | 0.44  | 0.20  | 0.19  |
| hsa-miR-150-5p   | 1.00 | 0.59 | 0.77   | 0.60   | 1.61 | 0.52  | 1.42  | 0.64  |
| hsa-miR-151a-3p  | 1.00 | 0.44 | 1.32   | 0.28   | 0.94 | 0.44  | 2.29  | 2.11  |
| hsa-miR-151a-5p  | 1.00 | 0.53 | 1.12   | 0.48   | 1.33 | 0.98  | 1.05  | 0.45  |
| hsa-miR-152      | 1.00 | 0.41 | 1.27   | 0.29   | 1.52 | 0.25  | 1.07  | 0.08  |
| hsa-miR-153      | 0.00 | 0.00 | 0.00   | 0.00   | 1.00 | 1.73  | 0.00  | 0.00  |
| hsa-miR-154-5p   | 1.00 | 1.61 | 3.82   | 5.70   | 1.20 | 1.49  | 0.95  | 0.98  |
| hsa-miR-155-5p   | 1.00 | 0.79 | 1.91   | 2.36   | 2.17 | 0.57  | 1.68  | 1.87  |
| hsa-miR-155-3p   | 1.00 | 1.73 | 0.00   | 0.01   | 0.00 | 0.00  | 0.00  | 0.00  |

|                 |      |      |         |         |       |       |       |       |
|-----------------|------|------|---------|---------|-------|-------|-------|-------|
| hsa-miR-181a-5p | 1.00 | 0.90 | 1.11    | 1.04    | 0.74  | 0.14  | 1.01  | 0.38  |
| hsa-miR-181a-3p | 0.00 | 0.00 | 1.00    | 1.44    | 7.29  | 10.29 | 0.66  | 1.14  |
| hsa-miR-181b-5p | 1.00 | 0.86 | 1.06    | 0.79    | 1.37  | 0.33  | 1.04  | 0.66  |
| hsa-miR-181c-5p | 1.00 | 0.59 | 1.40    | 0.62    | 0.37  | 0.18  | 0.66  | 0.29  |
| hsa-miR-181c-3p | 1.00 | 0.36 | 2.17    | 2.60    | 1.58  | 0.95  | 0.87  | 0.31  |
| hsa-miR-181d    | 1.00 | 0.59 | 0.56    | 0.48    | 1.43  | 0.20  | 1.09  | 1.03  |
| hsa-miR-182-5p  | 1.00 | 0.59 | 2.33    | 1.56    | 2.48  | 0.92  | 2.75  | 1.78  |
| hsa-miR-182-3p  | 0.00 | 0.00 | 0.00    | 0.00    | 0.00  | 0.00  | 0.00  | 0.00  |
| hsa-miR-183-5p  | 1.00 | 0.47 | 0.38    | 0.65    | 2.49  | 2.58  | 0.86  | 0.61  |
| hsa-miR-183-3p  | 1.00 | 1.00 | 5.25    | 4.80    | 2.28  | 1.31  | 1.68  | 1.71  |
| hsa-miR-184     | 0.00 | 0.00 | 0.00    | 0.00    | 1.00  | 1.73  | 0.01  | 0.02  |
| hsa-miR-185-5p  | 1.00 | 0.42 | 4.05    | 2.68    | 3.14  | 0.71  | 4.61  | 3.31  |
| hsa-miR-186-5p  | 1.00 | 0.78 | 1.30    | 2.05    | 3.17  | 0.32  | 1.48  | 1.30  |
| hsa-miR-186-3p  | 0.00 | 0.00 | 1.00    | 1.73    | 0.00  | 0.00  | 0.00  | 0.00  |
| hsa-miR-187-3p  | 1.00 | 0.75 | 1.53    | 1.62    | 2.18  | 2.72  | 3.15  | 4.91  |
| hsa-miR-187-5p  | 1.00 | 0.84 | 1.21    | 1.32    | 0.92  | 0.82  | 1.42  | 1.27  |
| hsa-miR-190a    | 1.00 | 1.73 | 11.72   | 12.18   | 0.00  | 0.00  | 64.37 | 69.61 |
| hsa-miR-191-5p  | 1.00 | 0.49 | 1.50    | 0.57    | 1.29  | 0.46  | 1.80  | 0.74  |
| hsa-miR-191-3p  | 1.00 | 0.69 | 0.20    | 0.23    | 1.14  | 1.25  | 0.75  | 0.56  |
| hsa-miR-192-5p  | 1.00 | 1.04 | 2.98    | 3.89    | 1.14  | 1.68  | 1.94  | 0.57  |
| hsa-miR-192-3p  | 0.00 | 0.00 | 0.00    | 0.00    | 1.00  | 1.73  | 1.68  | 2.90  |
| hsa-miR-193a-3p | 1.00 | 1.43 | 0.90    | 0.61    | 0.03  | 0.06  | 0.30  | 0.42  |
| hsa-miR-193a-5p | 1.00 | 0.69 | 1.34    | 0.79    | 1.38  | 0.41  | 0.94  | 0.61  |
| hsa-miR-193b-3p | 1.00 | 0.95 | 1.55    | 0.85    | 0.72  | 0.27  | 0.58  | 0.39  |
| hsa-miR-193b-5p | 1.00 | 0.70 | 2.10    | 1.47    | 2.43  | 1.23  | 1.19  | 0.85  |
| hsa-miR-194-5p  | 1.00 | 1.73 | 1.01    | 1.75    | 0.04  | 0.07  | 2.02  | 3.35  |
| hsa-miR-195-5p  | 1.00 | 0.87 | 2.50    | 1.86    | 0.99  | 0.46  | 0.95  | 0.50  |
| hsa-miR-195-3p  | 1.00 | 0.98 | 2.43    | 3.40    | 4.30  | 2.39  | 2.55  | 0.70  |
| hsa-miR-196a-5p | 1.00 | 0.61 | 1.53    | 1.46    | 1.30  | 0.89  | 1.09  | 0.73  |
| hsa-miR-196a-3p | 1.00 | 1.14 | 3.76    | 4.03    | 2.74  | 4.69  | 0.79  | 1.35  |
| hsa-miR-196b-5p | 1.00 | 1.65 | 1.85    | 2.54    | 4.16  | 3.60  | 8.94  | 12.62 |
| hsa-miR-197-3p  | 1.00 | 0.69 | 1.56    | 0.83    | 1.28  | 0.89  | 1.03  | 0.95  |
| hsa-miR-199a-3p | 1.00 | 0.41 | 1.11    | 0.15    | 1.28  | 0.52  | 1.03  | 0.31  |
| hsa-miR-199a-5p | 1.00 | 1.00 | 1.46    | 1.40    | 0.52  | 0.20  | 0.40  | 0.13  |
| hsa-miR-199b-5p | 1.00 | 0.82 | 1.05    | 0.82    | 0.24  | 0.11  | 0.35  | 0.17  |
| hsa-miR-200a-3p | 1.00 | 1.43 | 0.50    | 0.43    | 0.10  | 0.17  | 0.64  | 0.39  |
| hsa-miR-200a-5p | 1.00 | 1.55 | 0.00    | 0.00    | 7.93  | 13.74 | 27.80 | 38.85 |
| hsa-miR-200b-3p | 1.00 | 0.74 | 0.71    | 0.38    | 1.14  | 0.10  | 0.97  | 0.65  |
| hsa-miR-200c-3p | 1.00 | 0.64 | 1.08    | 0.75    | 1.20  | 0.18  | 1.25  | 1.17  |
| hsa-miR-200c-5p | 1.00 | 1.62 | 3.75    | 3.10    | 0.74  | 0.66  | 1.04  | 1.68  |
| hsa-miR-202-3p  | 1.00 | 0.21 | 1.55    | 1.28    | 6.46  | 4.65  | 1.16  | 0.86  |
| hsa-miR-203a    | 1.00 | 0.42 | 1.03    | 0.11    | 1.53  | 0.38  | 1.16  | 0.35  |
| hsa-miR-204-5p  | 1.00 | 0.80 | 1.72    | 2.62    | 5.51  | 4.99  | 2.00  | 3.24  |
| hsa-miR-205-5p  | 1.00 | 0.80 | 1.04    | 0.86    | 0.48  | 0.11  | 0.72  | 0.21  |
| hsa-miR-205-3p  | 1.00 | 1.27 | 1.15    | 1.13    | 4.02  | 4.31  | 3.27  | 2.81  |
| hsa-miR-206     | 1.00 | 1.73 | 2213.63 | 3834.13 | 0.00  | 0.00  | 1.13  | 1.96  |
| hsa-miR-208a    | 0.00 | 0.00 | 0.00    | 0.00    | 1.00  | 1.70  | 0.00  | 0.00  |
| hsa-miR-208b    | 1.00 | 1.73 | 2.38    | 4.13    | 55.11 | 95.46 | 0.00  | 0.00  |
| hsa-miR-210     | 1.00 | 0.30 | 3.09    | 1.05    | 1.22  | 1.06  | 0.92  | 0.82  |
| hsa-miR-211-5p  | 1.00 | 0.44 | 0.93    | 0.86    | 1.48  | 0.53  | 1.17  | 0.34  |
| hsa-miR-212-3p  | 1.00 | 0.81 | 0.86    | 1.46    | 1.14  | 0.56  | 1.14  | 0.93  |
| hsa-miR-214-3p  | 1.00 | 0.27 | 2.57    | 2.32    | 1.44  | 0.43  | 0.98  | 0.48  |
| hsa-miR-215     | 1.00 | 1.72 | 0.89    | 1.54    | 5.54  | 9.60  | 1.67  | 2.89  |
| hsa-miR-216a-5p | 0.00 | 0.00 | 1.00    | 1.73    | 0.15  | 0.25  | 0.26  | 0.44  |

|                  |      |      |       |       |       |       |        |         |      |
|------------------|------|------|-------|-------|-------|-------|--------|---------|------|
| hsa-miR-217      | 0.00 | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00   | 0.00    | 0.00 |
| hsa-miR-218-5p   | 1.00 | 0.68 | 1.11  | 0.85  | 1.66  | 0.42  | 1.24   | 0.85    |      |
| hsa-miR-218-1-3p | 1.00 | 1.49 | 13.46 | 23.31 | 0.00  | 0.00  | 0.02   | 0.02    |      |
| hsa-miR-219-5p   | 1.00 | 1.73 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00   | 0.00    |      |
| hsa-miR-221-3p   | 1.00 | 0.40 | 0.71  | 0.22  | 0.88  | 0.41  | 1.09   | 0.28    |      |
| hsa-miR-221-5p   | 0.00 | 0.00 | 1.00  | 1.73  | 0.03  | 0.05  | 0.82   | 0.76    |      |
| hsa-miR-222-3p   | 1.00 | 0.24 | 1.18  | 0.44  | 1.44  | 0.26  | 1.12   | 0.23    |      |
| hsa-miR-222-5p   | 1.00 | 0.76 | 11.85 | 16.99 | 7.87  | 11.50 | 2.03   | 1.44    |      |
| hsa-miR-223-3p   | 1.00 | 0.23 | 2.53  | 1.37  | 3.40  | 3.24  | 3.65   | 0.68    |      |
| hsa-miR-223-5p   | 1.00 | 1.73 | 0.01  | 0.02  | 0.00  | 0.00  | 2.94   | 5.09    |      |
| hsa-miR-224-5p   | 1.00 | 0.22 | 0.60  | 0.54  | 1.28  | 0.94  | 1.80   | 1.47    |      |
| hsa-miR-224-3p   | 1.00 | 0.91 | 3.34  | 2.63  | 2.92  | 2.61  | 1.71   | 1.62    |      |
| hsa-miR-296-3p   | 1.00 | 1.60 | 5.14  | 8.89  | 7.66  | 13.25 | 3.62   | 3.20    |      |
| hsa-miR-298      | 1.00 | 0.79 | 0.02  | 0.03  | 0.29  | 0.17  | 0.01   | 0.01    |      |
| hsa-miR-299-5p   | 1.00 | 1.73 | 0.36  | 0.63  | 1.38  | 2.38  | 0.00   | 0.00    |      |
| hsa-miR-300      | 1.00 | 1.73 | 3.80  | 6.58  | 12.71 | 22.01 | 0.72   | 1.25    |      |
| hsa-miR-301a-3p  | 1.00 | 1.68 | 17.45 | 29.16 | 0.00  | 0.00  | 19.76  | 33.86   |      |
| hsa-miR-302a-3p  | 1.00 | 1.73 | 19.36 | 17.96 | 1.34  | 2.32  | 3.92   | 5.15    |      |
| hsa-miR-302a-5p  | 1.00 | 1.73 | 0.00  | 0.00  | 0.11  | 0.19  | 0.00   | 0.00    |      |
| hsa-miR-302b-3p  | 0.00 | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00   | 0.00    |      |
| hsa-miR-302b-5p  | 1.00 | 1.73 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00   | 0.00    |      |
| hsa-miR-302c-3p  | 0.00 | 0.00 | 1.00  | 1.73  | 0.00  | 0.00  | 0.04   | 0.07    |      |
| hsa-miR-320a     | 1.00 | 0.42 | 1.23  | 0.92  | 1.07  | 0.31  | 1.17   | 0.77    |      |
| hsa-miR-320b     | 1.00 | 0.65 | 1.26  | 1.11  | 1.11  | 0.66  | 0.60   | 0.23    |      |
| hsa-miR-323b-5p  | 1.00 | 0.32 | 3.79  | 4.76  | 1.10  | 0.53  | 2.34   | 1.44    |      |
| hsa-miR-324-3p   | 1.00 | 0.93 | 0.73  | 0.59  | 0.71  | 0.76  | 0.76   | 0.60    |      |
| hsa-miR-324-5p   | 1.00 | 1.62 | 0.67  | 0.78  | 0.57  | 0.54  | 1.10   | 0.52    |      |
| hsa-miR-325      | 0.00 | 0.00 | 1.00  | 1.70  | 0.13  | 0.20  | 0.25   | 0.41    |      |
| hsa-miR-326      | 1.00 | 0.66 | 1.73  | 2.24  | 0.37  | 0.22  | 0.28   | 0.32    |      |
| hsa-miR-328      | 1.00 | 0.19 | 0.83  | 0.87  | 0.73  | 0.44  | 0.80   | 0.53    |      |
| hsa-miR-330-3p   | 1.00 | 1.54 | 0.21  | 0.17  | 1.48  | 1.78  | 0.94   | 0.82    |      |
| hsa-miR-331-5p   | 1.00 | 1.17 | 0.25  | 0.15  | 0.11  | 0.08  | 3.14   | 2.13    |      |
| hsa-miR-335-5p   | 1.00 | 0.42 | 0.65  | 0.55  | 0.02  | 0.02  | 0.28   | 0.28    |      |
| hsa-miR-335-3p   | 1.00 | 0.86 | 1.02  | 0.99  | 1.95  | 2.86  | 0.07   | 0.12    |      |
| hsa-miR-337-5p   | 0.00 | 0.00 | 1.00  | 1.66  | 0.17  | 0.29  | 0.00   | 0.00    |      |
| hsa-miR-338-3p   | 1.00 | 0.81 | 1.29  | 1.49  | 0.35  | 0.61  | 0.61   | 0.57    |      |
| hsa-miR-338-5p   | 1.00 | 0.82 | 4.22  | 6.46  | 10.48 | 18.16 | 38.97  | 37.68   |      |
| hsa-miR-339-3p   | 1.00 | 0.86 | 4.86  | 5.88  | 1.67  | 2.03  | 2.44   | 3.10    |      |
| hsa-miR-340-5p   | 1.00 | 1.51 | 0.01  | 0.01  | 1.36  | 2.36  | 0.88   | 0.80    |      |
| hsa-miR-340-3p   | 0.00 | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 1.00   | 0.96    |      |
| hsa-miR-342-3p   | 1.00 | 0.11 | 1.17  | 0.32  | 1.93  | 0.49  | 2.08   | 1.29    |      |
| hsa-miR-342-5p   | 1.00 | 0.91 | 1.16  | 1.03  | 1.12  | 0.49  | 1.80   | 2.03    |      |
| hsa-miR-345-5p   | 1.00 | 1.13 | 2.85  | 4.94  | 0.94  | 1.04  | 0.58   | 0.68    |      |
| hsa-miR-346      | 1.00 | 0.19 | 8.79  | 6.19  | 7.92  | 11.80 | 9.64   | 5.12    |      |
| hsa-miR-361-5p   | 1.00 | 0.52 | 1.84  | 2.00  | 1.72  | 0.34  | 1.37   | 0.95    |      |
| hsa-miR-363-3p   | 1.00 | 1.73 | 1.09  | 1.88  | 0.00  | 0.00  | 0.00   | 0.00    |      |
| hsa-miR-363-5p   | 0.00 | 0.00 | 0.00  | 0.00  | 1.00  | 1.66  | 0.44   | 0.75    |      |
| hsa-miR-365a-3p  | 1.00 | 0.45 | 0.76  | 0.42  | 0.64  | 0.28  | 0.78   | 0.57    |      |
| hsa-miR-370      | 1.00 | 1.23 | 1.16  | 0.82  | 0.42  | 0.07  | 1.34   | 1.35    |      |
| hsa-miR-372      | 0.00 | 0.00 | 1.00  | 1.73  | 16.53 | 28.63 | 66.80  | 115.70  |      |
| hsa-miR-373-3p   | 0.00 | 0.00 | 1.00  | 1.73  | 0.00  | 0.00  | 632.06 | 1089.62 |      |
| hsa-miR-373-5p   | 1.00 | 0.26 | 0.94  | 0.26  | 0.86  | 0.16  | 1.21   | 0.35    |      |
| hsa-miR-374a-5p  | 1.00 | 0.24 | 1.31  | 0.51  | 1.34  | 0.43  | 1.27   | 0.82    |      |
| hsa-miR-374b-5p  | 1.00 | 0.40 | 0.99  | 0.24  | 1.37  | 0.55  | 1.29   | 0.76    |      |

|                 |      |      |       |       |       |       |       |        |
|-----------------|------|------|-------|-------|-------|-------|-------|--------|
| hsa-miR-375     | 1.00 | 0.58 | 1.12  | 1.35  | 0.69  | 0.37  | 0.81  | 0.57   |
| hsa-miR-376a-3p | 1.00 | 1.73 | 11.11 | 18.80 | 6.17  | 9.66  | 0.71  | 1.10   |
| hsa-miR-376b-3p | 1.00 | 1.62 | 1.24  | 1.68  | 0.18  | 0.14  | 0.10  | 0.09   |
| hsa-miR-376c-3p | 1.00 | 1.50 | 0.75  | 1.25  | 0.00  | 0.00  | 0.34  | 0.46   |
| hsa-miR-377-3p  | 1.00 | 1.11 | 6.79  | 9.74  | 0.00  | 0.00  | 0.38  | 0.45   |
| hsa-miR-377-5p  | 0.00 | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 1.00  | 1.73   |
| hsa-miR-378a-3p | 1.00 | 0.60 | 0.78  | 0.14  | 0.45  | 0.13  | 0.99  | 0.76   |
| hsa-miR-378a-5p | 1.00 | 1.68 | 0.00  | 0.00  | 0.00  | 0.00  | 1.07  | 1.23   |
| hsa-miR-379-5p  | 1.00 | 1.73 | 7.59  | 8.01  | 0.00  | 0.00  | 2.42  | 2.16   |
| hsa-miR-381-3p  | 1.00 | 1.73 | 0.38  | 0.61  | 4.71  | 8.16  | 0.12  | 0.19   |
| hsa-miR-382-5p  | 1.00 | 0.18 | 1.75  | 2.02  | 2.43  | 1.19  | 0.85  | 0.28   |
| hsa-miR-383     | 1.00 | 0.75 | 0.00  | 0.00  | 0.14  | 0.24  | 0.00  | 0.00   |
| hsa-miR-409-3p  | 1.00 | 1.57 | 0.18  | 0.16  | 1.13  | 1.93  | 1.95  | 2.29   |
| hsa-miR-410     | 1.00 | 1.05 | 11.34 | 19.20 | 1.03  | 1.37  | 1.00  | 1.60   |
| hsa-miR-411-5p  | 1.00 | 0.53 | 2.22  | 3.33  | 1.10  | 0.96  | 1.43  | 1.35   |
| hsa-miR-421     | 1.00 | 0.33 | 0.90  | 0.47  | 0.77  | 0.22  | 1.76  | 0.64   |
| hsa-miR-423-3p  | 1.00 | 0.39 | 1.07  | 0.59  | 0.78  | 0.83  | 1.19  | 0.71   |
| hsa-miR-423-5p  | 1.00 | 0.04 | 1.63  | 1.13  | 1.81  | 0.86  | 1.23  | 0.44   |
| hsa-miR-424-5p  | 1.00 | 1.53 | 0.52  | 0.45  | 0.08  | 0.09  | 0.69  | 0.14   |
| hsa-miR-424-3p  | 1.00 | 1.23 | 0.00  | 0.00  | 1.81  | 2.78  | 0.05  | 0.09   |
| hsa-miR-425-5p  | 1.00 | 0.97 | 0.67  | 1.16  | 0.00  | 0.00  | 1.93  | 0.60   |
| hsa-miR-425-3p  | 1.00 | 0.39 | 0.54  | 0.22  | 0.61  | 0.38  | 0.34  | 0.23   |
| hsa-miR-429     | 1.00 | 1.73 | 3.74  | 5.96  | 0.05  | 0.08  | 1.09  | 1.88   |
| hsa-miR-431-5p  | 1.00 | 0.26 | 2.24  | 2.83  | 1.57  | 0.92  | 1.89  | 1.31   |
| hsa-miR-432-5p  | 1.00 | 1.73 | 0.06  | 0.10  | 0.54  | 0.75  | 0.18  | 0.31   |
| hsa-miR-433     | 1.00 | 0.20 | 1.71  | 2.41  | 0.89  | 0.27  | 0.97  | 0.80   |
| hsa-miR-449a    | 1.00 | 0.67 | 5.44  | 4.41  | 4.81  | 7.66  | 1.74  | 1.62   |
| hsa-miR-451a    | 1.00 | 1.09 | 2.87  | 4.03  | 1.54  | 1.09  | 2.33  | 2.10   |
| hsa-miR-454-3p  | 1.00 | 1.17 | 1.71  | 2.79  | 4.78  | 4.13  | 3.54  | 4.33   |
| hsa-miR-455-5p  | 1.00 | 1.62 | 0.02  | 0.03  | 1.45  | 2.51  | 0.22  | 0.37   |
| hsa-miR-483-5p  | 1.00 | 1.73 | 3.72  | 6.05  | 0.00  | 0.00  | 30.94 | 24.68  |
| hsa-miR-484     | 1.00 | 0.54 | 1.36  | 1.44  | 1.91  | 0.84  | 1.60  | 0.59   |
| hsa-miR-485-5p  | 1.00 | 1.54 | 0.63  | 1.08  | 2.78  | 4.81  | 3.06  | 5.30   |
| hsa-miR-486-3p  | 1.00 | 1.73 | 1.72  | 2.98  | 0.00  | 0.00  | 0.01  | 0.01   |
| hsa-miR-486-5p  | 1.00 | 0.37 | 4.38  | 6.01  | 1.85  | 1.78  | 8.66  | 12.73  |
| hsa-miR-488-3p  | 0.00 | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00   |
| hsa-miR-489     | 1.00 | 0.24 | 2.05  | 2.53  | 1.75  | 1.20  | 1.11  | 0.74   |
| hsa-miR-490-3p  | 1.00 | 0.10 | 1.96  | 2.04  | 1.08  | 0.63  | 1.14  | 0.81   |
| hsa-miR-491-5p  | 1.00 | 0.87 | 3.23  | 3.67  | 0.38  | 0.09  | 3.23  | 2.06   |
| hsa-miR-493-3p  | 1.00 | 1.51 | 2.63  | 3.43  | 2.27  | 3.59  | 0.64  | 0.40   |
| hsa-miR-495-3p  | 1.00 | 1.05 | 0.07  | 0.01  | 11.34 | 14.22 | 5.25  | 6.92   |
| hsa-miR-497-5p  | 1.00 | 1.43 | 0.79  | 1.07  | 0.36  | 0.32  | 0.35  | 0.20   |
| hsa-miR-498     | 0.00 | 0.00 | 0.00  | 0.00  | 1.00  | 1.73  | 0.00  | 0.00   |
| hsa-miR-499a-3p | 1.00 | 1.73 | 0.76  | 1.31  | 0.01  | 0.01  | 0.08  | 0.14   |
| hsa-miR-499a-5p | 0.00 | 0.00 | 0.00  | 0.00  | 1.00  | 1.73  | 0.04  | 0.03   |
| hsa-miR-500a-5p | 1.00 | 0.78 | 4.13  | 4.85  | 3.93  | 4.24  | 4.06  | 0.28   |
| hsa-miR-504     | 1.00 | 1.73 | 5.87  | 10.17 | 1.08  | 1.88  | 64.10 | 103.43 |
| hsa-miR-505-3p  | 1.00 | 0.42 | 0.89  | 0.21  | 0.62  | 0.89  | 0.80  | 0.70   |
| hsa-miR-506-3p  | 0.00 | 0.00 | 1.00  | 1.73  | 0.08  | 0.09  | 0.68  | 0.76   |
| hsa-miR-508-5p  | 1.00 | 1.73 | 12.55 | 12.14 | 26.95 | 35.65 | 2.45  | 1.61   |
| hsa-miR-509-3p  | 1.00 | 1.73 | 0.77  | 1.28  | 6.04  | 5.28  | 0.38  | 0.57   |
| hsa-miR-511     | 1.00 | 1.73 | 16.27 | 28.19 | 0.17  | 0.17  | 4.94  | 8.56   |
| hsa-miR-512-5p  | 1.00 | 1.73 | 0.13  | 0.22  | 0.00  | 0.00  | 0.00  | 0.00   |
| hsa-miR-513a-5p | 1.00 | 1.20 | 0.36  | 0.61  | 1.36  | 0.73  | 0.55  | 0.42   |

|                 |      |      |       |       |         |         |        |        |
|-----------------|------|------|-------|-------|---------|---------|--------|--------|
| hsa-miR-514a-3p | 1.00 | 1.71 | 0.00  | 0.00  | 0.00    | 0.00    | 0.02   | 0.03   |
| hsa-miR-517a-3p | 0.00 | 0.00 | 0.00  | 0.00  | 0.00    | 0.00    | 1.00   | 1.73   |
| hsa-miR-518b    | 0.00 | 0.00 | 1.00  | 1.73  | 1.17    | 1.17    | 0.95   | 1.64   |
| hsa-miR-519d    | 0.00 | 0.00 | 0.00  | 0.00  | 1.00    | 1.73    | 0.00   | 0.00   |
| hsa-miR-520c-3p | 1.00 | 1.73 | 1.33  | 1.17  | 1.85    | 2.08    | 0.00   | 0.00   |
| hsa-miR-520g    | 0.00 | 0.00 | 0.00  | 0.00  | 1.00    | 1.73    | 0.00   | 0.00   |
| hsa-miR-522-3p  | 0.00 | 0.00 | 0.00  | 0.00  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-524-5p  | 1.00 | 1.73 | 28.16 | 46.86 | 0.00    | 0.00    | 292.38 | 500.96 |
| hsa-miR-532-5p  | 1.00 | 1.73 | 48.10 | 41.66 | 76.20   | 131.99  | 36.70  | 38.13  |
| hsa-miR-539-5p  | 0.00 | 0.00 | 0.00  | 0.00  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-542-3p  | 1.00 | 1.26 | 0.00  | 0.00  | 7.86    | 7.16    | 0.37   | 0.06   |
| hsa-miR-542-5p  | 1.00 | 1.66 | 1.40  | 2.42  | 0.00    | 0.00    | 0.26   | 0.31   |
| hsa-miR-549a    | 1.00 | 1.73 | 0.00  | 0.00  | 0.00    | 0.00    | 5.71   | 7.50   |
| hsa-miR-551b-3p | 1.00 | 1.73 | 0.00  | 0.00  | 0.00    | 0.00    | 0.55   | 0.95   |
| hsa-miR-567     | 1.00 | 1.73 | 0.26  | 0.45  | 0.37    | 0.36    | 7.52   | 6.59   |
| hsa-miR-570-3p  | 1.00 | 1.73 | 0.92  | 1.59  | 0.00    | 0.00    | 6.66   | 11.53  |
| hsa-miR-574-3p  | 1.00 | 0.67 | 2.31  | 1.49  | 2.03    | 1.87    | 1.21   | 0.45   |
| hsa-miR-575     | 1.00 | 0.88 | 1.23  | 1.80  | 0.20    | 0.22    | 9.32   | 5.86   |
| hsa-miR-580     | 1.00 | 1.73 | 0.00  | 0.00  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-581     | 0.00 | 0.00 | 0.00  | 0.00  | 1.00    | 1.73    | 0.00   | 0.00   |
| hsa-miR-583     | 0.00 | 0.00 | 0.00  | 0.00  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-588     | 0.00 | 0.00 | 1.00  | 1.73  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-589-3p  | 1.00 | 1.73 | 0.00  | 0.00  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-600     | 1.00 | 1.73 | 5.66  | 7.31  | 0.00    | 0.00    | 0.01   | 0.01   |
| hsa-miR-605     | 1.00 | 0.62 | 0.18  | 0.12  | 2.78    | 3.39    | 4.17   | 6.28   |
| hsa-miR-606     | 0.00 | 0.00 | 0.00  | 0.00  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-608     | 1.00 | 0.86 | 5.01  | 7.79  | 0.22    | 0.30    | 1.08   | 0.88   |
| hsa-miR-622     | 1.00 | 1.16 | 6.09  | 10.44 | 0.00    | 0.00    | 2.25   | 2.95   |
| hsa-miR-626     | 1.00 | 1.73 | 0.09  | 0.16  | 2.21    | 3.83    | 0.13   | 0.19   |
| hsa-miR-639     | 1.00 | 1.48 | 1.50  | 2.56  | 0.00    | 0.00    | 2.41   | 3.99   |
| hsa-miR-643     | 1.00 | 1.73 | 0.00  | 0.00  | 2330.12 | 4035.89 | 0.00   | 0.00   |
| hsa-miR-649     | 0.00 | 0.00 | 1.00  | 1.73  | 7.30    | 12.65   | 0.00   | 0.00   |
| hsa-miR-652-3p  | 1.00 | 0.63 | 2.33  | 2.53  | 3.10    | 1.39    | 2.02   | 0.76   |
| hsa-miR-661     | 1.00 | 0.31 | 2.34  | 2.74  | 1.10    | 0.51    | 1.10   | 0.70   |
| hsa-miR-708-5p  | 1.00 | 0.47 | 1.23  | 1.05  | 1.96    | 0.95    | 1.72   | 1.25   |
| hsa-miR-708-3p  | 0.00 | 0.00 | 0.00  | 0.00  | 1.00    | 1.73    | 273.73 | 316.17 |
| hsa-miR-720     | 1.00 | 0.37 | 1.10  | 0.48  | 1.38    | 0.14    | 2.12   | 0.68   |
| hsa-miR-744-5p  | 1.00 | 0.66 | 2.91  | 4.43  | 1.78    | 0.65    | 2.25   | 2.13   |
| hsa-miR-765     | 1.00 | 1.23 | 1.39  | 2.41  | 0.12    | 0.20    | 1.49   | 2.55   |
| hsa-miR-770-5p  | 1.00 | 0.19 | 1.88  | 1.01  | 1.08    | 0.24    | 1.92   | 0.84   |
| hsa-miR-802     | 0.00 | 0.00 | 0.00  | 0.00  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-885-5p  | 1.00 | 0.66 | 4.44  | 4.14  | 0.81    | 0.86    | 2.18   | 1.66   |
| hsa-miR-888-5p  | 0.00 | 0.00 | 1.00  | 1.73  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-920     | 1.00 | 1.71 | 10.84 | 15.55 | 0.20    | 0.25    | 5.41   | 9.23   |
| hsa-miR-924     | 1.00 | 1.73 | 0.34  | 0.58  | 5.02    | 8.70    | 0.09   | 0.15   |
| hsa-miR-1180    | 1.00 | 0.86 | 0.74  | 1.27  | 1.44    | 1.24    | 2.55   | 3.65   |
| hsa-miR-1207-5p | 1.00 | 0.77 | 2.98  | 2.61  | 1.88    | 1.44    | 3.23   | 2.41   |
| hsa-miR-1224-3p | 1.00 | 0.99 | 1.10  | 1.67  | 0.01    | 0.02    | 0.05   | 0.05   |
| hsa-miR-1224-5p | 1.00 | 0.52 | 0.69  | 0.14  | 2.68    | 2.20    | 1.51   | 2.16   |
| hsa-miR-1265    | 0.00 | 0.00 | 1.00  | 1.71  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-1284    | 1.00 | 1.73 | 0.10  | 0.14  | 0.25    | 0.33    | 0.00   | 0.00   |
| hsa-miR-1290    | 1.00 | 1.10 | 1.69  | 1.65  | 1.24    | 0.54    | 1.12   | 0.76   |
| hsa-miR-1324    | 1.00 | 1.73 | 2.88  | 4.99  | 0.00    | 0.00    | 0.00   | 0.00   |
| hsa-miR-1908    | 1.00 | 1.28 | 0.04  | 0.05  | 0.17    | 0.19    | 1.42   | 1.25   |

|                 |      |      |      |      |       |       |      |      |
|-----------------|------|------|------|------|-------|-------|------|------|
| hsa-miR-1914-3p | 0.00 | 0.00 | 1.00 | 1.54 | 13.78 | 16.16 | 1.49 | 2.57 |
| hsa-miR-4258    | 1.00 | 0.82 | 0.24 | 0.16 | 0.13  | 0.11  | 1.53 | 1.13 |

miRNAs from three normal skin samples, three TEN skin samples, three psoriasis skin samples, and three atopic dermatitis skin samples were prepared, and the miRNA expression profile in each disease in vivo was evaluated using a PCR array. The fold-change was calculated as  $1/2^{(\text{raw Ct of each miRNA} - \text{mean Ct of small RNA housekeeping genes})}$ . The mean fold-change (mean) and standard deviation (SD) of each miRNA is shown. The mean value of the normal skin samples was set at 1. When the value of normal skin was 0, the mean value of another sample was set at 1.

### **Fig E1. Regulation of apoptosis by BCL2L10 and miR-18a-5p**

Apoptosis is regulated by two major intracellular pathways; the extrinsic pathway and the intrinsic pathway. The extrinsic pathway is activated by ligation of FasL or TNF to their receptors on the cell membrane. These receptors contain an intracellular death domain and activate caspase-8, followed by caspase-3 activation.

In the intrinsic pathway, cell stress including growth factor deprivation or DNA damage yields death signals. Proapoptotic proteins (BAX and BAK) cause mitochondrial outer membrane permeabilization, followed by the release of cytochrome C. Cytochrome C activates caspase-9 and subsequently caspase-3, then induces apoptosis. The intrinsic pathway is regulated by the BCL2 family; BCL2L10 inhibits the activity of BAX and BAK, resulting in the prevention of the cell from apoptosis. The extrinsic pathway is known to be activated in the epidermis of TEN. We found that caspase-9, a molecule in the intrinsic pathway, is also activated. The miR-18a-5p-BCL2L10 pathway may contribute to the activation of intrinsic apoptosis.