Reference Genes for qPCR-Based miRNA Expression Profiling in 14 Human Tissues

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Highlights of the Study

- Different studies use different normalization strategies to report miRNA expression data and there is no universal reference gene.
- A complex normalizer, which is the geometric mean of miRNA-16-5p, miRNA-103a-3p, miRNA-191-5p, may be used as a universal reference gene.
- Numerous studies have shown that small nuclear RNAs have variability in expression and that miRNAs are preferable for use as reference genes.

Abstract

MicroRNAs (miRNAs) are promising biomarkers for the diagnosis and prognosis of various diseases. Quantitative PCR is the most frequently used method of measuring expression levels of miRNA. However, the lack of validated reference genes represents the main source of potential bias in results. It is normal practice to use small nuclear RNAs as reference genes; however, they often have variable expression. Researchers tend to prefer the most stable reference genes in each experiment. The review includes reference genes for the following tissue types: gliomas, lung cancer, melanoma, gastric cancer, liver cancer, prostate cancer, breast cancer, thyroid cancer, ovarian cancer, cervical cancer, endometrial cancer, rectal cancer, blood tumors, and placental tissues.

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Introduction

MicroRNAs (miRNAs) are small noncoding RNAs. They regulate gene expression post-transcriptionally [1]. Ludwig et al. [2] measured miRNA profiles for tissues in various organs and showed that each tissue expresses a unique set of more than 1,000 miRNAs, of which 143 were found in all tissues studied. There is little doubt that aberrant miRNA expression may entail the initiation and progression of various diseases [3]; thus, miRNAs are viewed as promising diagnostic and prognostic biomarkers [4].

Basically, there are two strategies in use for exploring the role played by miRNAs and various diseases: one by analyzing circulating miRNAs and the other one, by analyzing miRNAs in solid tumors [5, 6]. The material used includes fresh frozen (FF) tissue, formalin-fixed paraffin-embedded (FFPE) sections, biopsy samples on glass slides, and cell cultures [7–9]. Undoubtedly, RNAs expression depends on the stabilization methods used [10].

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Expression levels of miRNA were shown to depend on the storage time of the FFPE block and that U6B, the most frequently used reference gene, had higher degradation rates compared to those of miRNA-141-3p or miRNA-221-3p [11]. Although fixation with formalin was reported to reduce the expression levels of miRNAs, their expression profiles in FF and FFPE samples from the same tumor were normally still strongly correlated, with miR-103a-3p being the most stable [12]. There is no doubt that any comparative analysis of miRNA expression levels is worthwhile if it is performed for the samples stabilized using an identical method.

The accuracy of measuring variation in expression is an important part of the answer to the question regarding the roles played by miRNAs in biological processes. Normalization of expression levels is an important step for ensuring accurate quantitative assessment of qPCR data. Normalization is performed to differentiate true variation in the expression levels of the target in the samples from the one induced experimentally. Factors affecting the results include sampling protocols, the quantity and quality of material introduced, and RNA extraction methods, to name a few [13]. Apart from technical factors, one of the major problems with interpretation of qPCR data is the one related to proper choice of reference genes. A good reference gene should display the qualities of least variation in expression between the subgroups being compared and stable expression regardless of RNA isolation methods or storage conditions [14]. Wong et al. [15] demonstrated that the reference gene used and the study target should belong to the same class of RNA.

Several mathematical approaches such as geNorm, NormFinder, and BestKeeper have been developed to help identify suitable reference genes (i.e., the genes with the least variation and high stability in biological samples) [16–18]. However, none of them is the gold standard, and the researchers make their choices on a case-to-case basis.

It should be noted that a number of studies have shown that U6, which is the most commonly used reference gene, has variable expression and therefore cannot be the optimal reference gene for miRNA analysis [19, 20]. Meanwhile, in 300 studies randomly selected from PubMed publications retrieved using the search criteria "microRNA" + "qPCR" + "cancer", we observed the following preferences for reference genes: 84% used the small nuclear RNA (snRNA) U6, 10% used U48 or U58, and only as few as 6% chose from miRNAs with the most stable expression in each particular experiment. Despite many thousands of publications exploring the effects of miRNAs on human diseases, there exists no single stan-dard for normalizing qPCR data; hence, the expression levels of candidate reference genes need to be checked for stability in each experiment. However, in some studies, expression levels of many miRNAs cannot be analyzed due to limited sample amounts or funds for developing miRNA systems for analysis. In such cases, the only solution for this technical problem is the selection of reference genes based on analysis of published data. Systematization of the data on reference genes in various human tissues, which has been performed in this review, will help researchers choose the most optimal reference genes for their studies. The aim of our work is to systematize the data on reference genes used for analyzing expression levels of miRNA in tumors of different origin by qPCR.

Search Strategy

We searched for studies exploring the expression levels of miRNAs in various tissues by qPCR with a view to ascertain which reference genes are tissue-specific and which ones are universal. The tissues of interest were the malignancies most often referred to in the literature: gliomas, lung cancer (LC), melanoma, gastric cancer, liver cancer, prostate cancer (PC), breast cancer (BC), thyroid cancer, ovarian cancer (OC), cervical cancer (CC), endometrial cancer, rectal cancer (RC), and blood tumors. Additionally, we considered placental tissues. To exclude review articles, electronic searches were conducted using the following search criteria: "microRNA" and "qPCR" and "human" and "Journal Article". We have looked into 2,950 articles in the PubMed database. Articles were reviewed in the third quarter of 2021. To exclude articles related to circulating miRNAs, the variable search criteria used were the name of the disease, the name of the tissue type, and the type of material. Next, at least 50% of the articles with each tissue type in them were randomly selected for analysis.

Tissue Types

The reference genes for the analysis of miRNAs in different tissues are summarized in Table 1.

Brain Tumors
snRNAs are most often used as reference genes for profiling miRNA expression in glioblastomas in both FF and FFPE samples [21–23].

Table 1. Reference genes for qPCR-based miRNA expression profiling in 14 human tissues

Tissue	Sample type	Reference genes	References
Brain tumors	FF/FFPE	miR-103a-3p, U49, U54	[7]
	FF	U6	[21]
	FF	U48	[22]
	FFPE	U6	[23]
	FFPE	miR-103a-3p, U49, U54	[24]
	FFPE	U48	[25]
	Cell lines/FFPE	U6	[26]
LC	FF	miR-103a-3p, miR-191-5p	[19]
	FF	U48	[27]
	FF/FFPE	miR-26a-5p, miR-140-5p, miR-195-5p, miR-30b-5p	[28]
	FFPE	miR-103a-3p, miR-191-5p	[29]
	FF	miR-16-5p	[30]
	FFPE	miR-16-5p	[31]
	FF/FFPE	U6	[32]
	Sputum	U6	[33]
	Cell line	U6	[34]
Melanoma	Cell line	miR-191-5p	[35]
	FFPE	let-7e-5p, miR-30d-5p, miR-423-3p	[36]
	FF	U6	[37]
	FFPE	U6	[38]
	Cell line	U44	[39]
	Cell line	U6	[9, 40]
	Cell line	U43, U48, U49	[41]
Placenta	FF	miR-103a-3p	[42]
	FF	U48	[43]
	FF	U44	[44]
	FFPE	U18	[45]
	FFPE/FF	U6	[46]
GC	FF	miR-101-3p, miR-140-3p	[47]
	Cell line/FF	U6	[48]
	FF	U48	[49]
	FFPE	U6	[50]
	FFPE	Human gastric reference RNA	[51]
	FF	miR-127-3p	[52]
LC	Cell line	miR-24-3p, miR-151a-5p, miR-425-5p	[20]
	FF	miR-30c-5p/miR-30b-5p, miR-30c-5p/miR-126-3p	[53]
	FF	miR-152-3p, miR-23b-3p	[54]
	FF + Euro-Collins solution	miR-152-3p, miR-16-5p	[55]
	FFPE	miR-103a-3p, miR-191-5p	[56]
	FF	U6	[57]
	Cell line	U6	[58]
	Cell line	β-Actin	[59]
	FFPE/FF	miR-23a-3p	[60]
	FFPE	U6, U48	[61]
BC	FF	let-7a-5p, miR-16-5p	[62]
	FFPE	let-7a-5p	[63]
	FFPE	miR-16-5p, miR-29a-3p	[64]
	Tissue imprinted on slides	miR-100-5p, miR-143-3p	[65]
	FF	U6	[66]
	FF	U44, U48	[67]
	FF	Global median normalization	[68]
	FFPE	U44, U48, U6	[69]

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Table 1 (continued)

Tissue	Sample type	Reference genes	References
	FFPE	miR-191-5p, U6b	[70]
TC	Cytological specimens	miR-197-3p, miR-23a-3p, miR-29b-3p	[8]
	FF	U6	[71]
	FF	U48	[72]
	FFPE	let-7a-5p	[73]
	Cytological specimens	miR-197-3p, miR-99a-5p, miR-151a-3p, miR-214-3p	[74]
	FFPE/FF	U44, U48	[75]
	FFPE	U44, U6	[76]
	FFPE/FF	miR-10b-5p, miR-191-5p	[77]
	FFPE	U48, miR-16-5p	[78]
	Cell line	U6	[79]
	Cytological specimens	let-7a-5p, miR-103a-3p, miR-125a-5p, let-7b-5p, miR-145-5p, U48	[80]
OC	FFPE	let-7e-5p, miR-423-3p	[81]
	FF	U48, miR-191-5p	[82]
	FF	miR-27a-3p	[83]
	FFPE	miR-27a-3p	[84]
	Cell line/FF	U6	[85]
	FFPE	cel-miR-39, U68, U95, U96A, U6	[86]
	FFPE	U48	[87]
CC	FF	miR-151-5p, miR-152-3p, miR-423-3p	[88]
	Cervical scrapes	U43, miR-423-3p	[89]
	FF	miR-423-3p, U24	[89]
	Self-collected cervicovaginal specimens	miR-423-3p, U43, miR-30b-5p	[89]
	FF	miR-191-5p, miR-23a-3p	[90, 91]
	Liquid-based cervical cytology samples	U6, U49	[92]
	FFPE	U44, U48, U6	[93]
	Cell line/FF	U6	[94]
PC	FFPE	U44, U48	[95]
	FFPE	U6	[96]
	FF	miR-130b-3p, U6-2	[97]
	Cell line	miR-16-5p, miR-1228-3p	[98]
	FF	miR-423-5p	[99]
	FF	U24	[100]
	FF/cell line	U6	[101]
	FFPE	U48, U24, U6B	[102]
	FFPE	miR-151-5p	[103]
	FFPE	let-7f-5p	[104]
	FFPE	18s rRNA	[105]
	Cell line	U44	[106]
EC	FF/cell line	U6	[107]
	Cell line	U44	[108]
	FFPE	U68, U6	[109]
	Cell line	miR-423-5p	[110]
	Cell line	U48	[111]
	Cell line	U6	[112]
	FF/FFPE	U48, U44, U75	[113]
Bone marrow	Cell line	miR-16-5p, miR-25-3p, let-7a-5p	[114]
	FFPE	miR-16-5p, miR-26b-5p	[115]
	Cell line	miR-191-5p	[116]
	Cytological samples	miR-103a-3p, miR-191-5p, miR-378-3p	[117]
	FF	U24	[118]
	FF/cell line	U6	[119]

Table 1 (continued)

Tissue	Sample type	Reference genes	References
	Cell line	U61, U68, U72, U95, U96A	[120]
Colorectal cancer	FFPE	miR-103a-3p	[12]
	FF	miR-520d-3p, miR-345-5p, miR-1228-3p	[121]
	FFPE	miR-16-5p, miR-345-5p	[122]
RC	FFPE	miR-193a-5p, miR-27a-3p, let-7g-5p	[123]
	FF	U66, U44, U48	[124]
	FF	U6, U47	[125]

FF, fresh frozen; FFPE, formalin-fixed paraffin-embedded; GC, gastric cancer; LC, liver cancer; TC, thyroid cancer; EC, endometrial cancer.

Lung Cancer

U6 and U48 are the most common choices for profiling miRNA expression in LC samples [27, 32], although geNorm and NormFinder suggest other options (Table 1). miR-103a-3p or miR-191-5p were shown to be optimal reference genes for the analysis in LC tissue samples [19, 34].

Melanoma

miRNA-191-5p was found to be the most stable miRNA in melanoma cells and levels of expression U6 depend on hormone levels; thus, it is better not to regard U6 as a candidate reference gene [35].

Placenta

Today, snRNAs (predominantly U6) are most commonly used as reference genes when analyzing the miRNA expression levels in placental tissue.

Gastric Cancer

Anauate et al. [47] assessed the stability of potential reference genes (U6, U44, U48, let-7a-5p, miR-28-5p, miR-101-3p, miR-140-3p, miR-152-3p, and miR-374a-3p) profiling miRNA expression in gastric tissues and demonstrated that the best reference gene is a combination of miR-101-3p and miR-140-3p, while U6, U44, and U48 were not suitable options.

Liver Cancer

Jacobsen et al. [20] proposed the use of a combination of miR-151a-5p, miR-425-5p, and miR-24-3p as reference genes for hepatocytes, pointing out that U6 which is most often used as a reference gene had the lowest stability among all the potential reference genes. Levels of expression miR-30c-5p, miR-30b-5p, and miR-126-3p were demonstrated to be stable in liver tissue, while U6, U48, and U44 had low stability and thus were not suitable for use as reference genes [53].

Breast Cancer

Davoren et al. [62] reported that let-7a-5p and miR-16-5p were the top two miRNAs most stably expressed in FF samples of BC tissue and Chen et al. [63] confirmed that let-7a-5p was the most stable reference gene for the analysis of BC samples using FFPE tissue. In addition, Rinnerthaler et al. [64] confirmed that miRNA-16-5p is expressed at invariable levels in FFPE samples of BC tissue, but noted that this reference gene is suitable for metastatic BC, while the primary tumor should be analyzed using miR-29a-3p.

Thyroid Cancer

miRNA expression profiling in thyroid samples often involves the use of snRNAs (U6, U48, and U44) as reference genes [71, 72, 75]. Titov et al. [74] proposed a fundamentally different approach regarding choosing reference genes: they selected their reference genes based on the NanoString outputs. For example, miR-151a-3p, -197-3p, -99a-5p, and -214-3p had a relatively low variation in expression among the 800 miRNAs analyzed.

Ovarian Cancer

Azzalini et al. [81] used the geometric mean of let-7e-5p and miR-423-3p as a reference gene for miRNA expression profiling in FFPE samples of OC tissue. U48 and miR-191-5p were noted as being among the most stable reference genes [82]. MiRNA-27a-3p was found to be appropriated as a reference gene for both FF and FFPE samples of OC tissue [83, 84].
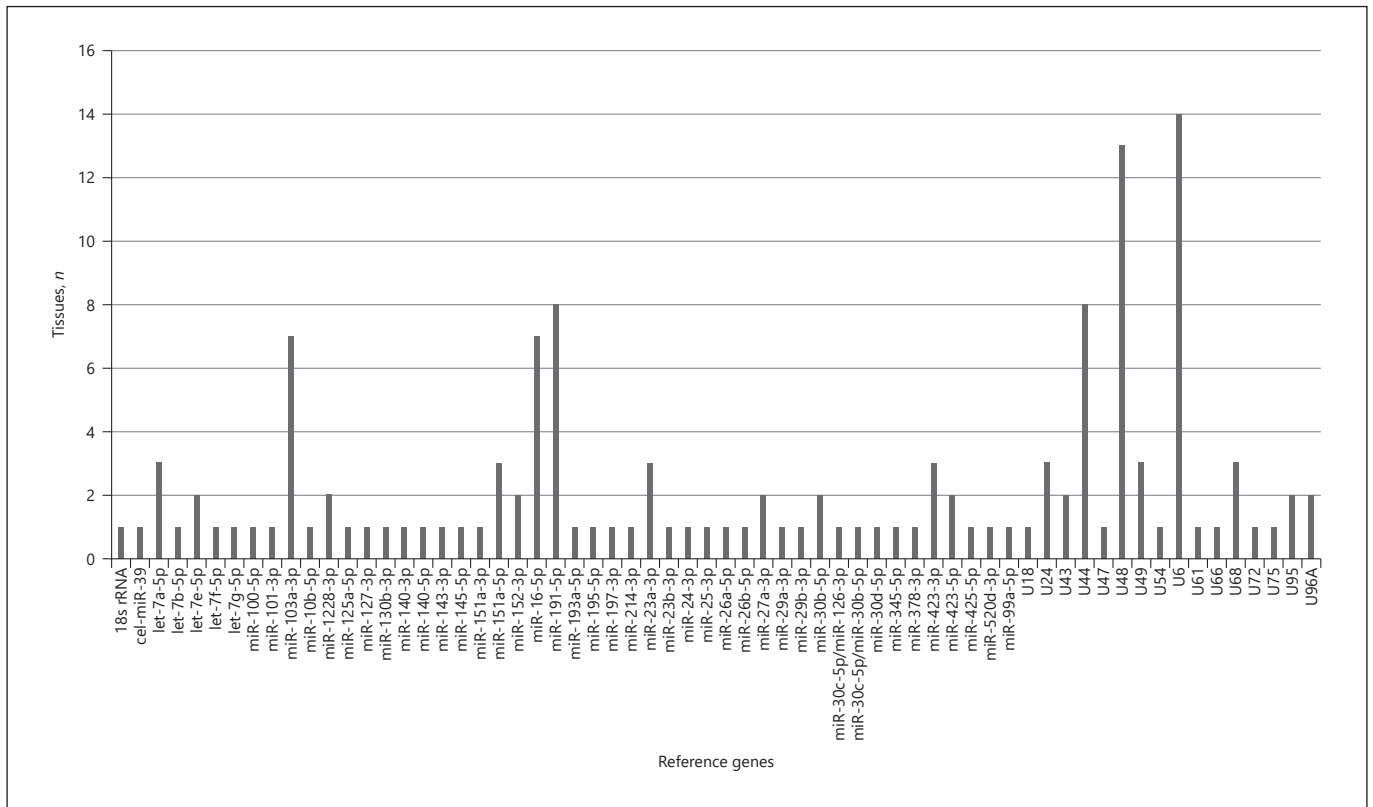
Fig. 1. Frequency of using reference genes in analysis of miRNA expression levels in various human tissues.

Cervical Cancer

Cervical cytology is an efficient approach for reducing the mortality from CC worldwide. However, the sensitivity of the standard cervical cytology test may be too low to detect cervical intraepithelial neoplasia; hence, more sensitive molecular diagnostic tests that could substantially improve detection rates and diagnostic accuracy need to be developed. miRNA expression profiling in cell smear samples obtained in a minimally invasive way by liquid-based biopsy cytology has shown promise for early diagnosis of CC. U6 and U49 have been described as being the most stable reference genes for miRNA expression profiling in LBC samples from patients who had undergone CC screening [92].

Prostate Cancer

When choosing a reference gene for miRNA expression profiling in PC tissue samples, it is preferred to normalize data by snRNAs [95, 96]. However, Schaefer et al. [97] recommend the use of miR-130b-3p or the geometric mean of miR-130b-3p and U6-2 for the purposes of

normalization in PC tissue samples because this combination is subject to much lower variation than U6-2 alone.

Endometrial Cancer

miRNA expression profiling in endometrial cancer tissue samples commonly involves the use of snRNAs, either alone or as combinations [107–109].

Bone Marrow

Complex reference genes are the most common choices for profiling miRNA expression in bone marrow samples [114–117].

Colorectal Cancer

Various combinations of miR-520d-3p, miR-1228-3p, and miR-345-5p have been shown to be suitable reference genes for miRNA expression profiling in FF samplers of colorectal cancer tissue [121]. Chang et al. [122] identified a combination of miR-16-5p and miR-345-5p as the most stable reference gene for the analysis in FFPE samples of colorectal cancer tissue.

Rectal Cancer

The mean expression of miR-27a-3p, miR-193a-5p, and let-7g-5p has been reported to be best for qPCR-based miRNA expression profiling in RC tissue [123]. Thus, miRNA-16-5p, miRNA-103a-3p, and miRNA-191-5p as well as snRNAs U6, U44, and U48 are commonly used reference genes in 14 human tissue types (Fig. 1). However, numerous studies have demonstrated that snRNAs have variability in expression and that miRNAs are preferable for use as reference genes. Unfortunately, it is difficult to identify the trend of using individual miRNAs as reference genes for each tissue type. We have listed all miRNAs found in publications as reference genes in 14 tissues and decided that the conclusion about the possibility of using a universal reference gene is more important. Thus, we think that a complex reference gene, which is the geometric mean of threshold fluorescence cycles of miRNA-16-5p, miRNA-103a-3p, and miRNA-191-5p, may be used as a universal reference gene to study miRNA expression levels.

Discussion

In search of suitable reference genes, researchers normally follow three strategies: (1) searching through literature sources for the most frequently used reference gene, (2) using mathematical algorithms to choose the most stably expressed RNAs among the ones expressed in a particular experiment, and (3) choosing reference genes among the miRNAs identified by NanoString nCounter as occurring in the most stable quantities. Small noncoding RNAs, and snRNAs in particular, are most commonly used as reference genes. However, snRNAs are not miRNAs and may therefore have different extraction efficiencies, reverse transcription efficiencies, and PCR amplification efficiencies. Also, the efficiencies of their extraction, reverse transcription, and PCR amplification may differ from those of miRNAs.

A large number of papers showing variation in snRNA expression levels in different tumor tissues have been published. Torres et al. [126] considered a selection of candidate reference genes (miR-16-5p, miR-26b-5p, miR-92a-3p, U44, U48, U75, U54, U6, U49, U6B, U38B, and U18A) and found U48, U75, and U44 to be most stable in FFRE endometrial carcinoma tissues. Their study confirmed the possibility of using snRNAs as reference genes; however, they obviously have different levels of stability. The use of combinations of snRNAs (and a combination of U6 and U47 [or U66, U44, and U48] in particular) is preferred for the analysis in RC [124, 125]. A complex reference gene composed of U44, U48, and U6 has been used for the anal-

ysis of FFPE samples of BC tissue and lymph nodes [69]. The geometric mean of as many as five snRNAs (U61, U68, U72, U95, and U96A) was used for the analysis of miRNA expression levels in human bone marrow-derived multipotent stromal cells [120]. The use of multiple reference genes was shown to improve the quantification accuracy achievable with a single reference gene for miRNA expression profiling [16]. Although various mathematical algorithms for the identification of the most stable genes in individual experiments are becoming increasingly popular when reference genes need to be chosen, U6 is the most commonly used reference gene.

A review of publications has shown that the same miRNA can be successfully used as a reference gene in one tissue and have aberrant expression in another one. Liang et al. [127] analyzed the expression levels of 345 miRNAs in 40 normal tissues and found miR-30e-5p, miR-92a-3p, and miR-423-3p to have the least variable expression. Although our study has identified promising reference genes that can be universally used in different tissues, literature analysis has demonstrated that miRNA-16-5p, miRNA-103a-3p, and miRNA-191-5p are most commonly used as universal reference genes [29, 31, 35, 42, 89, 99, 110].

miRNA expression levels can be affected by the heterogeneity of tumor tissue depending on the sampling site and account for the bias in the results obtained by different laboratory teams [65]. In addition, the results can be affected by the dimensions of the samples. The test samples being handled can often be very small, thus leading to biomarker misidentification [128].

Conclusions

We conclude that it is most appropriate to use a complex reference gene represented by several miRNAs with stable expression in each experiment. We suggest that a complex normalizer, which is the geometric mean of threshold fluorescence cycles of miRNA-16-5p, miRNA-103a-3p, and miRNA-191-5p, may be used as a universal reference gene to study miRNA expression levels.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Conflict of Interest Statement

The authors have no competing interests.

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Author Contributions

References

1 Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014 Aug; 15:509–24.

2 Ludwig N, Leidinger P, Becker K, Backes C, Fehlmann T, Pallasch C, et al. Distribution of miRNA expression across human tissues. Nucleic Acids Res. 2016 May;44(8):3865–77.

3 Ardekani AM, Naeini MM. The role of microRNAs in human diseases. Avicenna J Med Biotechnol. 2010 Oct;2(4):161–79.

4 Wang J, Chen J, Sen S. MicroRNA as biomarkers and diagnostics. J Cell Physiol. 2016 Jan;231(1):25–30.

5 Cui M, Wang H, Yao X, Zhang D, Xie Y, Cui R, et al. Circulating microRNAs in cancer: potential and challenge. Front Genet. 2019 Jul; 10:626.

6 Dillhoff M, Wojcik SE, Bloomston M. MicroRNAs in solid tumors. J Surg Res. 2009 Jun;154(2):349–54.

7 de Biase D, Visani M, Morandi L, Marucci G, Taccioli C, Cerasoli S, et al. miRNAs expression analysis in paired fresh/frozen and dissected formalin fixed and paraffin embedded glioblastoma using real-time pCR. PLoS One. 2012 Apr;7(4):e35596.

8 Titov SE, Ivanov MK, Demenkov PS, Katanyan GA, Kozorezova ES, Malek AV, et al. Combined quantitation of HMGA2 mRNA, microRNAs, and mitochondrial-DNA content enables the identification and typing of thyroid tumors in fine-needle aspiration smears. BMC Cancer. 2019 Oct;19(1):1010.

9 Zhao G, Wei Z, Guo Y. MicroRNA-107 is a novel tumor suppressor targeting POU3F2 in melanoma. Biol Res. 2020 Mar;53(1):11.

10 Esteva-Socias M, Gómez-Romano F, Carrillo-Ávila JA, Sánchez-Navarro AL, Villena C. Impact of different stabilization methods on RT-qPCR results using human lung tissue samples. Sci Rep. 2020 Feb;10:3579.

11 Peskoe SB, Barber JR, Zheng Q, Meeker AK, De Marzo AM, Platz EA, et al. Differential long-term stability of microRNAs and RNU6B snRNA in 12–20 year old archived formalin-fixed paraffin-embedded specimens. BMC Cancer. 2017 Jan;17:32.

12 Boisen MK, Dehlendorff C, Linnemann D, Schultz NA, Jensen BV, Hogdall EVS, et al. MicroRNA Expres-sion in formalin-fixed paraffin-embedded cancer tissue: identifying reference microRNAs and variability. BMC Cancer. 2015 Dec;15:1024.

13 Bustin SA, Nolan T. Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. J Biomol Tech. 2004 Sep; 15(3):155–66.

14 Kozera B, Rapacz M. Reference genes in real-time PCR. J Appl Genet. 2013 Nov;54(4):391–406.

15 Wong L, Lee K, Russell I, Chen C. Endogenous controls for real-time quantitation of miRNA using TaqMan microRNA assays. Applied Biosystems Application Note. 2007 Aug. 127AP11-01.

16 Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002 Jun;3:research0034.

17 Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: bestKeeper – excel-based tool using pair-wise correlations. Biotechnol Lett. 2004 Mar;26:509–15.

18 Andersen CL, Jensen JL, Orntoft TF. Normalization of real-time quantitative reverse transcription-PCR dNilsenata: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res. 2004 Aug;64:5245–50.

19 Peltier HJ, Latham GJ. Normalization of microRNA expression levels in quantitative RT-PCR assays: identification of suitable reference RNA targets in normal and cancerous human solid tissues. RNA. 2008 May;14(5):844–52.

20 Jacobsen KS, Nielsen KO, Winther TN, Glebe D, Pociot F, Hogh B. Identification of valid reference genes for microRNA expression studies in a hepatitis B virus replicating liver cell line. BMC Res Notes. 2016 Jan;9:38.

21 Xiong X, Deng J, Zeng C, Jiang Y, Tang S, Sun X. MicroRNA-141 is a tumor regulator and prognostic biomarker in human glioblastoma. Oncol Lett. 2017 Oct;14(4):4455–60.

22 Han IB, Kim M, Lee SH, Kim JK, Kim SH, Chang JH, et al. Down-regulation of microRNA-126 in glioblastoma and its correlation with patient prognosis: a Pilot Study. Anticancer Res. 2016 Dec;36(12):6691–7.

23 Zhao S, Yang G, Mu Y, Han D, Shi C, Chen X, et al. MiR-106a is an independent prognostic marker in patients with glioblastoma. Neuro Oncol. 2013 Jun;15(6):707–17.

24 Visani M, de Biase D, Marucci G, Cerasoli S, Nigrisoli E, Bacchi Reggiani ML, et al. Expression of 19 microRNAs in glioblastoma and comparison with other brain neoplasia of grades I–III. Mol Oncol. 2014 Mar;8(2):417–30.

25 Rivera-Díaz M, Miranda-Román MA, Soto D, Quintero-Aguilo M, Ortiz-Zuazaga H, Marcos-Martinez MJ, et al. MicroRNA-27a distinguishes glioblastoma multiforme from diffuse and anaplastic astrocytomas and has prognostic value. Am J Cancer Res. 2014 Dec; 5(1):201–18.

26 Lin J, Ding S, Xie C, Yi R, Wu Z, Luo J, et al. MicroRNA-4476 promotes glioma progression through a miR-4476/APC/β-catenin/c-Jun positive feedback loop. Cell Death Dis. 2020 Apr;11(4):269.

27 Molina-Pinelo S, Gutiérrez G, Pastor MD, Hergueta M, Moreno-Bueno G, García-Carbonero R, et al. MicroRNA-dependent regulation of transcription in non-small cell lung cancer. PLoS One. 2014 Mar;9(3):e90524.

28 Bediaga NG, Davies MP, Acha-Sagredo A, Hyde R, Raji OY, Page R, et al. A microRNA-based prediction algorithm for diagnosis of non-small lung cell carcinoma in minimal biopsy material. Br J Cancer. 2013 Oct;109(9):2404–11.

29 Zhang X, Li P, Rong M, He R, Hou X, Xie Y, et al. MicroRNA-141 is a biomarker for progression of squamous cell carcinoma and adenocarcinoma of the lung: clinical analysis of 125 patients. Tohoku J Exp Med. 2015 Mar; 235(3):161–9.

30 Wang W, Li W, Ding M, Yuan H, Yang J, Meng W, et al. Identification of miRNAs as non-invasive biomarkers for early diagnosis of lung cancers. Tumour Biol. 2016.

31 Begum S, Hayashi M, Ogawa T, Jabboure FJ, Brait M, Izumchenko E, et al. An integrated genome-wide approach to discover deregulated microRNAs in non-small cell lung cancer: clinical significance of miR-23b-3p deregulation. Sci Rep. 2015 Aug;5:13236.

32 Wang Y, Chen J, Lin Z, Cao J, Huang H, Jiang Y, et al. Role of deregulated microRNAs in non-small cell lung cancer progression using fresh-frozen and formalin-fixed, paraffin-embedded samples. Oncol Lett. 2016 Nov; 11(1):801–8.

33 Razzak R, Bédard EL, Kim JO, Gazala S, Guo L, Ghosh S, et al. MicroRNA expression profiling of sputum for the detection of early and locally advanced non-small-cell lung cancer: a Prospective Case-Control Study. Curr Oncol. 2016 Apr;23(2):e86–94.

34 Wang Q, Zhang L. Possible molecular mechanisms for the roles of microRNA-21 played in lung cancer. Technol Cancer Res Treat. 2019 Jan;18:1533033819875130.

35 Fochi S, Orlandi E, Ceccuzzi L, Rodolfo M, Vergani E, Turco A, et al. Identification of suitable mRNAs and microRNAs as reference genes for expression analyses in skin cells under sex hormone exposure. Gene. 2021 Feb; 769:145336.

36 Hanniford D, Zhong J, Koetz L, Gaziel-Sovran A, Lackaye DJ, Shang S, et al. A miRNA-based signature detected in primary melanoma tissue predicts development of brain metastasis. Clin Cancer Res. 2015 Nov;21(21): 4903–12.

37 Yang H, Shen C. MicroRNA-29c induces G1 arrest of melanoma by targeting CDK6. J BUON. 2019 Mar-Apr;24(2):819–25.

38 Aksenenko M, Palkina N, Komina A, Tashireva L, Ruksha T. Differences in microRNA expression between melanoma and healthy adjacent skin. BMC Dermatol. 2019 Jan; 19(1):1.

39 Díaz-Martínez M, Benito-Jardón L, Alonso L, Koetz-Ploch L, Hernando E, Teixidó J. miR-204-5p and miR-211-5p contribute to BRAF inhibitor resistance in melanoma. Cancer Res. 2018 Feb;78(4):1017–30.

40 Zhang K, Wu L, Zhang P, Luo M, Du J, Gao T, et al. miR-9 regulates ferroptosis by targeting glutamic-oxaloacetic transaminase GOT1 in melanoma. Mol Carcinog. 2018 Nov; 57(11):1566–76.

41 Dynoodt P, Speeckaert R, De Wever O, Chevolet I, Brochez L, Lambert J, et al. miR-145 overexpression sup-presses the migration and invasion of metastatic melanoma cells. Int J Oncol. 2013 Feb;42(4):1443–51.

42 Daddali R, Ojaniemi M, Hallman M, Rämet M, Haapalainen AM. CPPED1-targeting microRNA-371a-5p expression in human placenta associates with spontaneous delivery. PLoS One. 2020 Jun;15(6):e0234403.

43 Thamotharan S, Chu A, Kempf K, Janzen C, Grogan T, Elashoff DA, et al. Differential microRNA expression in human placentas of term intra-uterine growth restriction that regulates target genes mediating angiogenesis and amino acid transport. PLoS One. 2017 May;12(5):e0176493.

44 Hiura H, Hattori H, Kobayashi N, Okae H, Chiba H, Miyauchi N, et al. Genome-wide microRNA expression profiling in placentae from frozen-thawed blastocyst transfer. Clin Epigenetics. 2017 Aug;9:79.

45 Mong EF, Yang Y, Akat KM, Canfield J, Van-Wye J, Lockhart J, et al. Chromosome 19 microRNA cluster enhances cell reprogramming by inhibiting epithelial-to-mesenchymal transition. Sci Rep. 2020 Feb;10(1):3029.

46 Gottlieb A, Flor I, Nimzyk R, Burchardt L, Helmke B, Langenbuch M, et al. The expression of miRNA encoded by C19MC and miR-371-3 strongly varies among individual placentas but does not differ between spontaneous and induced abortions. Protoplasma. 2021 Oct;258(1):209–18.

47 Anauate AC, Leal MF, Wisnieski F, Santos LC, Gigek CO, Chen ES, et al. Identification of suitable reference genes for miRNA expression normalization in gastric cancer. Gene. 2017 Jul;621:59–68.

48 Tao Y, Zhao Y, Peng Y, Ma X, Sun C, Xu K. MicroRNA-621 inhibits the growth of gastric cancer cells by targeting SYF2. Arch Biochem Biophys. 2020 Jul;688:108406.

49 Zhang J, Ding F, Jiao D, Li Q, Ma H. The aberrant expression of microRNA-125a-5p/IGF2BP3 axis in advanced gastric cancer and its clinical relevance. Technol Cancer Res Treat. 2020 Jan-Dec;19:1533033820917332.

50 Bibi F, Naseer MI, Alvi SA, Yasir M, Jiman-Fatani AA, Sawan A, et al. microRNA analysis of gastric cancer patients from Saudi Arabian population. BMC Genomics. 2016 Oct; 17(Suppl 9):51.

51 Osawa S, Shimada Y, Sekine S, Okumura T, Nagata T, Fukuoka J, et al. MicroRNA profiling of gastric cancer patients from formalin-fixed paraffin-embedded samples. Oncol Lett. 2011 May;2(4):613–9.

52 Juzėnas S, Saltenienė V, Kupcinskas J, Link A, Kiudelis G, Jonaitis L, et al. Analysis of deregulated microRNAs and their target genes in gastric cancer. PLoS One. 2015 Jul;10(7): e0132327.

53 Shen J, Wang Q, Gurvich I, Remotti H, Santella RM. Evaluating normalization approaches for the better identification of aberrant microRNAs associated with hepatocellular carcinoma. Hepatoma Res. 2016 Nov;2: 305–15.

54 Lamba V, Ghodke-Puranik Y, Guan W, Lamba JK. Identification of suitable reference genes for hepatic microRNA quantitation. BMC Res Notes. 2014 Mar;7:129.

55 Zárybnický T, Matoušková P, Ambrož M, Šubrt Z, Skálová L, Boušová I. The selection and validation of reference genes for mRNA and microRNA expression studies in human liver slices using RT-qPCR. Genes. 2019 Sep; 10(10):763.

56 Zhang HF, Wang YC, Han YD. MicroRNA-34a inhibits liver cancer cell growth by reprogramming glucose metabolism. Mol Med Rep. 2018 Mar;17(3):4483–9.

57 Yang Y, Zhao Z, Hou N, Li Y, Wang X, Wu F, et al. MicroRNA-214 targets Wnt3a to suppress liver cancer cell proliferation. Mol Med Rep. 2017 Sep;16(5):6920–7.

58 Zhang A, Lakshmanan J, Motameni A, Harbrecht BG. MicroRNA-203 suppresses proliferation in liver cancer associated with PIK-3CA, p38 MAPK, c-Jun, and GSK3 signaling. Mol Cell Biochem. 2018 Apr;441(1–2):89–98.

59 Zhu HT, Dong QZ, Sheng YY, Wei JW, Wang G, Zhou HJ, et al. MicroRNA-29a-5p is a novel predictor for early recurrence of hepatitis B virus-related hepatocellular carcinoma after surgical resection. PLoS One. 2012 Dec;7(12): e52393.

60 Toffanin S, Hoshida Y, Lachenmayer A, Villanueva A, Cabellos L, Minguez B, et al. MicroRNA-based classification of hepatocellular carcinoma and oncogenic role of miR-517a. Gastroenterology. 2011 May;140(5): 1618–28.

61 He RQ, Yang X, Liang L, Chen G, Ma J. MicroRNA-124-3p expression and its prospective functional pathways in hepatocellular carcinoma: a Quantitative Polymerase Chain Reaction, Gene Expression Omnibus and Bioinformatics Study. Oncol Lett. 2018 Apr; 15(4):5517–32.

62 Davoren PA, McNeill RE, Lowery AJ, Kerin MJ, Miller N. Identification of suitable endogenous control genes for microRNA gene expression analysis in human breast cancer. BMC Mol Biol. 2008 Aug;9:76.

63 Chen L, Li Y, Fu Y, Peng J, Mo MH, Stamatakos M, et al. Role of deregulated microRNAs in breast cancer progression using FFPE tissue. PLoS One. 2013 Jan;8(1):e54213.

64 Rinnerthaler G, Hackl H, Gampenrieder SP, Hamacher F, Hufnagl C, Hauser-Kronberger C, et al. miR-16-5p is a stably-expressed housekeeping microRNA in breast cancer tissues from primary tumors and from metastatic sites. Int J Mol Sci. 2016 Feb;17(2):156.

65 Veryaskina YA, Titov SE, Kometova VV, Rodionov VV, Zhimulev IF. Intratumoral heterogeneity of expression of 16 miRNA in luminal cancer of the mammary gland. Noncoding RNA. 2020 May;6:16.

66 Wang Y, Zhang Z, Wang J. MicroRNA-384 inhibits the progression of breast cancer by targeting ACVR1. Oncol Rep. 2018 Jun;39(6): 2563–74.

67 Rothé F, Ignatiadis M, Chaboteaux C, Haibe-Kains B, Kheddoumi N, Majjaj S, et al. Global microRNA expression profiling identifies MiR-210 associated with tumor proliferation, invasion and poor clinical outcome in breast cancer. PLoS One. 2011 Jun;6(6):e20980.

68 Tsai HP, Huang SF, Li CF, Chien HT, Chen SC. Differential microRNA expression in breast cancer with different onset age. PLoS One. 2018 Jan;13(1):e0191195.

69 Elango R, Alsaleh KA, Vishnubalaji R, Manikandan M, Ali AM, Abd El-Aziz N, et al. MicroRNA expression profiling on paired primary and lymph node metastatic breast cancer revealed distinct microRNA profile associated with LNM. Front Oncol. 2020 May;10:756.

70 Rohan T, Ye K, Wang Y, Glass AG, Ginsberg M, Loudig O. MicroRNA expression in benign breast tissue and risk of subsequent invasive breast cancer. PLoS One. 2018 Feb; 13(2):e0191814.

71 Luo Y, Hao T, Zhang J, Zhang M, Sun P, Wu L. MicroRNA-592 suppresses the malignant phenotypes of thyroid cancer by regulating lncRNA NEAT1 and downregulating NOVA1. Int J Mol Med. 2019 Jul;44(3):1172–82.

72 Zhang J, Liu Y, Liu Z, Wang XM, Yin DT, Zheng LL, et al. Differential expression profiling and functional analysis of microRNAs through stage I–III papillary thyroid carcinoma. Int J Med Sci. 2013 Mar;10(5):585–92.

73 Pamedytyte D, Simanaviciene V, Dauksiene D, Leipute E, Zvirbliene A, Sarauskas V, et al. Association of microRNA expression and BRAFV600E mutation with recurrence of thyroid cancer. Biomolecules. 2020 Apr; 10(4):625.

74 Titov SE, Demenkov PS, Ivanov MK, Malakhina ES, Poloz TL, Tsivlikova EV, et al. Selection and validation of miRNAs as normalizers for profiling expression of microRNAs isolated from thyroid fine needle aspiration smears. Oncol Rep. 2016 Sep;36(5):2501–10.

75 Dettmer M, Vogetseder A, Durso MB, Moch H, Komminoth P, Perren A, et al. MicroRNA expression array identifies novel diagnostic markers for conventional and oncocytic follicular thyroid carcinomas. J Clin Endocrinol Metab. 2013 Jan;98(1):E1–7.

76 Dettmer MS, Perren A, Moch H, Komminoth P, Nikiforov YE, Nikiforova MN. MicroRNA profile of poorly differentiated thyroid carcinomas: new diagnostic and prognostic insights. J Mol Endocrinol. 2014 Mar;52(2): 181–9.

77 Yusof AM, Jamal R, Muhammad R, Abdullah Suhaimi SN, Mohamed Rose I, Saidin S, et al. Integrated characterization of microRNA and mRNA transcriptome in papillary thyroid carcinoma. Front Endocrinol. 2018 Apr;9: 158.

78 Pamedytyte D, Leipute E, Zilaitiene B, Sarauskas V, Dauksiene D, Dauksa A, et al. Different stability of miRNAs and endogenous control genes in archival specimens of papillary thyroid carcinoma. Mol Med. 2020 Nov;26(1): 100.

79 Wang J, Yang H, Si Y, Hu D, Yu Y, Zhang Y, et al. Iodine promotes tumorigenesis of thyroid cancer by suppressing Mir-422a and upregulating MAPK1. Cell Physiol Biochem. 2017 Oct;43(4):1325–36.

80 Santos MTD, Buzolin AL, Gama RR, Silva ECAD, Dufloth RM, Figueiredo DLA, et al. Molecular classification of thyroid nodules with indeterminate cytology: development and validation of a highly sensitive and specific new miRNA-based classifier test using fine-needle aspiration smear slides. Thyroid. 2018 Dec;28(12):1618–26.

81 Azzalini E, De Martino E, Fattorini P, Canzonieri V, Stanta G, Bonin S. Reliability of miRNA analysis from fixed and paraffin-embedded tissues. Int J Mol Sci. 2019 Sep;20(19): 4819.

82 Bignotti E, Calza S, Tassi RA, Zanotti L, Bandiera E, Sartori E, et al. Identification of stably expressed reference small non-coding RNAs for microRNA quantification in high-grade serous ovarian carcinoma tissues. J Cell Mol Med. 2016 Dec;20(12):2341–8.

83 Wahab NA, Othman Z, Nasri NWM, Mokhtar MH, Ibrahim SF, Hamid AA, et al. Inhibition of miR-141 and miR-200a increase DLC-1 and ZEB2 expression, enhance migration and invasion in metastatic serous ovarian cancer. Int J Environ Res Public Health. 2020 Apr; 17(8):2766.

84 Ibrahim FF, Jamal R, Syafruddin SE, Mutalib NSA, Saidin S, MdZin RR, et al. MicroRNA-200c and microRNA-31 regulate proliferation, colony formation, migration and invasion in serous ovarian cancer. J Ovarian Res. 2015 Aug;8:56.

85 Xie HH, Huan WT, Han JQ, Ren WR, Yang LH. MicroRNA-663 facilitates the growth, migration and invasion of ovarian cancer cell by inhibiting TUSC2. Biol Res. 2019 Apr; 52(1):18.

86 Braicu OL, Budisan L, Buiga R, Jurj A, Achimas-Cadariu P, Pop LA, et al. miRNA expression profiling in formalin-fixed paraffin-embedded endometriosis and ovarian cancer samples. Onco Targets Ther. 2017 Aug;10: 4225–38.

87 Wu RL, Ali S, Bandyopadhyay S, Alosh B, Hayek K, Daaboul MF, et al. Comparative analysis of differentially expressed miRNAs and their downstream mRNAs in ovarian cancer and its associated endometriosis. J Cancer Sci Ther. 2015 Jan;7(7):258–65.

88 Nilsen A, Jonsson M, Aarnes EK, Kristensen GB, Lyng H. Reference microRNAs for RT-qPCR assays in cervical cancer patients and their application to studies of HPV16 and hypoxia biomarkers. Transl Oncol. 2019 Mar; 12(3):576–84.

89 Babion I, Snoek BC, van de Wiel MA, Wilting SM, Steenbergen RDM. A strategy to find suitable reference genes for miRNA quantitative PCR analysis and its application to cervical specimens. J Mol Diagn. 2017 Sep;19(5): 625–37.

90 Leitão Mda C, Coimbra EC, de Lima Rde C, Guimarães Mde L, Heráclio Sde A, Silva Neto Jda C, et al. Quantifying mRNA and microRNA with qPCR in cervical carcinogenesis: a validation of reference genes to ensure accurate data. PLoS One. 2014 Nov;9(11):e111021.

91 Shen Y, Li Y, Ye F, Wang F, Wan X, Lu W, et al. Identification of miR-23a as a novel microRNA normalizer for relative quantification in human uterine cervical tissues. Exp Mol Med. 2011 Jun;43:358–66.

92 Causin RL, Pessôa-Pereira D, Souza KCB, Evangelista AF, Reis RMV, Fregnani JHTG, et al. Identification and performance evaluation of housekeeping genes for microRNA expression normalization by reverse transcription-quantitative PCR using liquid-based cervical cytology samples. Oncol Lett. 2019 Nov; 18(5):4753–61.

93 How C, Pintilie M, Bruce JP, Hui AB, Clarke BA, Wong P, et al. Developing a prognostic micro-RNA signature for human cervical carcinoma. PLoS One. 2015 Apr;10(4): e0123946.

94 Zhang W, Zhang M, Liu L, Jin D, Wang P, Hu J. MicroRNA-183-5p inhibits aggressiveness of cervical cancer cells by targeting integrin subunit beta 1 (ITGB1). Med Sci Monit. 2018 Oct;24:7137–45.

95 Nonn L, Vaishnav A, Gallagher L, Gann PH. mRNA and micro-RNA expression analysis in laser-capture microdissected prostate biopsies: valuable tool for risk assessment and prevention trials. Exp Mol Pathol. 2010 Feb; 88(1):45–51.

96 Kumar B, Rosenberg AZ, Choi SM, Fox-Talbot K, De Marzo AM, Nonn L, et al. Cell-type specific expression of oncogenic and tumor suppressive microRNAs in the human prostate and prostate cancer. Sci Rep. 2018 May;8(1):7189.

97 Schaefer A, Jung M, Miller K, Lein M, Kristiansen G, Erbersdobler A, et al. Suitable reference genes for relative quantification of miRNA expression in prostate cancer. Exp Mol Med. 2010 Nov;42(11):749–58.

98 Zhao H, Ma TF, Lin J, Liu L, Sun W, Guo L, et al. Identification of valid reference genes for mRNA and microRNA normalisation in prostate cancer cell lines. Sci Rep. 2018 Jan; 8:1949.

99 Costa-Pinheiro P, Ramalho-Carvalho J, Vieira FQ, Torres-Ferreira J, Oliveira J, Gonçalves CS, et al. MicroRNA-375 plays a dual role in prostate carcinogenesis. Clin Epigenetics. 2015 Apr;7(1):42.

100 Carlsson J, Helenius G, Karlsson M, Lubovac Z, Andrén O, Olsson B, et al. Validation of suitable endogenous control genes for expression studies of miRNA in prostate cancer tissues. Cancer Genet Cytogenet. 2010 Oct;202(2):71–5.

101 Wang J, Li X, Xiao Z, Wang Y, Han Y, Li J, et al. MicroRNA-488 inhibits proliferation and glycolysis in human prostate cancer cells by regulating PFKFB3. FEBS Open Bio. 2019 Oct;9(10):1798–807.

102 Bucay N, Shahryari V, Majid S, Yamamura S, Mitsui Y, Tabatabai ZL, et al. miRNA expression analyses in prostate cancer clinical tissues. J Vis Exp. 2015 Sep;(103):53123.

103 Kristensen H, Thomsen AR, Haldrup C, Dyrskjot L, Hoyer S, Borre M, et al. Novel diagnostic and prognostic classifiers for prostate cancer identified by genome-wide microRNA profiling. Oncotarget. 2016 May; 7(21):30760–71.

104 Zaman MS, Chen Y, Deng G, Shahryari V, Suh SO, Saini S, et al. The functional significance of microRNA-145 in prostate cancer. Br J Cancer. 2010 Jul;103(2):256–64.

105 Shan J, Al-Rumaihi K, Chouchane K, Al-Bozom I, Rabah D, Farhat K, et al. Prostate cancer small non-coding RNA transcriptome in Arabs. J Transl Med. 2017 Dec;15(1):260.

106 Cagle P, Niture S, Srivastava A, Ramalinga M, Aqeel R, Rios-Colon L, et al. MicroR-NA-214 targets PTK6 to inhibit tumorigenic potential and increase drug sensitivity of prostate cancer cells. Sci Rep. 2019 Jul;9: 9776.

107 Sun X, Dongol S, Qiu C, Xu Y, Sun C, Zhang Z, et al. miR-652 promotes tumor proliferation and metastasis by targeting RORA in endometrial cancer. Mol Cancer Res. 2018 Dec;16(12):1927–39.

108 Van Sinderen M, Griffiths M, Menkhorst E, Niven K, Dimitriadis E. Restoration of mi-croRNA-29c in type I endometrioid cancer reduced endometrial cancer cell growth. Oncol Lett. 2019 Sep;18(3):2684–93.

109 Canlorbe G, Wang Z, Laas E, Bendifallah S, Castela M, Lefevre M, et al. Identification of microRNA expression profile related to lymph node status in women with early-stage grade 1–2 endometrial cancer. Mod Pathol. 2016 Feb;29:391–401.

110 Yanokura M, Banno K, Aoki D. MicroRNA-34b expression enhances chemosensitivity of endometrial cancer cells to paclitaxel. Int J Oncol. 2020 Nov;57(5):1145–56.

111 Yang C, Ota-Kurogi N, Ikeda K, Okumura T, Horie-Inoue K, Takeda S, et al. Micro-RNA-191 regulates endometrial cancer cell growth via TET1-mediated epigenetic modulation of APC. J Biochem. 2020 Jul;168(1): 7–14.

112 Wang J, Zhang L, Jiang W, Zhang R, Zhang B, Silayiding A, et al. MicroRNA-135a promotes proliferation, migration, invasion and induces chemoresistance of endometrial cancer cells. Eur J Obstet Gynecol Reprod Biol X. 2019 Nov;5:100103.

113 Torres A, Torres K, Pesci A, Ceccaroni M, Paszkowski T, Cassandrini P, et al. Diagnostic and prognostic significance of miRNA signatures in tissues and plasma of endometrioid endometrial carcinoma patients. Int J Cancer. 2013 Apr;132(7):1633–45.

114 Drobna M, Szarzyńska-Zawadzka B, Daca-Roszak P, Kosmalska M, Jaksik R, Witt M, et al. Identification of endogenous control miRNAs for RT-qPCR in T-cell acute lymphoblastic leukemia. Int J Mol Sci. 2018 Sep; 19(10):2858.

115 Morenos L, Saffery R, Mechinaud F, Ashley D, Elwood N, Craig JM, et al. Evaluation of microRNA expression in patient bone marrow aspirate slides. PLoS One. 2012 Aug; 7(8):e42951.

116 Costé E, Rouleux-Bonnin F. The crucial choice of reference genes: identification of miR-191-5p for normalization of miRNAs expression in bone marrow mesenchymal stromal cell and HS27a/HS5 cell lines. Sci Rep. 2020 Oct;10:17728.

117 Kovynev IB, Titov SE, Ruzankin PS, Aga-kishiev MM, Veryaskina YA, Nedel'ko VM, et al. Profiling 25 bone marrow microRNAs in acute leukemias and secondary nonleukemic hematopoietic conditions. Biomedicines. 2020 Dec;8:607.

118 Verboon LJ, Obulkasim A, de Rooij JD, Katsman-Kuipers JE, Sonneveld E, Baruchel A, et al. MicroRNA-106b~25 cluster is upregulated in relapsed MLL-rearranged pediatric acute myeloid leukemia. Oncotarget. 2016 Jul;7(30):48412–22.

119 Zhou JD, Li XX, Zhang TJ, Xu ZJ, Zhang ZH, Gu Y, et al. MicroRNA-335/ID4 dysregulation predicts clinical outcome and facilitates leukemogenesis by activating PI3K/Akt signaling pathway in acute myeloid leukemia. Aging. 2019 May;11(10):3376–91.

120 Bellayr IH, Kumar A, Puri RK. MicroRNA expression in bone marrow-derived human multipotent stromal cells. BMC Genomics. 2017 Aug;18:605.

121 Danese E, Minicozzi AM, Benati M, Paviati E, Lima-Oliveira G, Gusella M, et al. Reference miRNAs for colorectal cancer: analysis and verification of current data. Sci Rep. 2017 Aug;7(1):8413.

122 Chang KH, Mestdagh P, Vandesompele J, Kerin MJ, Miller N. MicroRNA expression profiling to identify and validate reference genes for relative quantification in colorectal cancer. BMC Cancer. 2010 Apr;10:173.

123 Eriksen AH, Andersen RF, Pallisgaard N, Sørensen FB, Jakobsen A, Hansen TF. Mi-croRNA expression profiling to identify and validate reference genes for the relative quantification of microRNA in rectal cancer. PLoS One. 2016 Mar;11(3):e0150593.

124 Gaedcke J, Grade M, Camps J, Sokilde R, Kaczkowski B, Schetter AJ, et al. The rectal cancer microRNAome-microRNA expression in rectal cancer and matched normal mucosa. Clin Cancer Res. 2012 Sep;18(18): 4919–30.

125 Yang Y, Tang T, Peng W, Xia L, Wang X, Duan B, et al. The comparison of miR-155 with computed tomography and computed tomography plus serum amyloid A protein in staging rectal cancer. J Surg Res. 2015 Feb; 193(2):764–71.

126 Torres A, Torres K, Wdowiak P, Paszkowski T, Maciejewski R. Selection and validation of endogenous controls for microRNA expression studies in endometrioid endometrial cancer tissues. Gynecol Oncol. 2013 Sep; 130(3):588–94.

127 Liang Y, Ridzon D, Wong L, Chen C. Characterization of microRNA expression profiles in normal human tissues. BMC Genomics. 2007 Jun;8:166.

128 Schwarzenbach H, da Silva AM, Calin G, Pantel K. Data normalization strategies for microRNA quantification. Clin Chem. 2015 Nov;61(11):1333–42.