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MiRNAs in primary cutaneous lymphomas

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Abstract

Primary cutaneous lymphomas (PCL) compose a heterogeneous disease with still unknown aetiology and mechanisms of development. MicroRNAs (miRNAs) have recently been discovered as one of the crucial players in PCL carcinogenesis through post-transcriptional regulation of gene expression. miRNAs have been reported to be frequently deregulated in PCLs and their biological significance has been further confirmed in multiple functional experiments. Such studies help us understand molecular pathogenesis of PCL. In this review, we summarize expression of miRNAs and their corresponding roles in different subtypes of PCL. With expression and functional role of miRNAs revealed, investigation of their possible clinical use as biomarkers for diagnosis, prediction of prognosis and target for therapies, will be a promising area in the future.

Introduction

Primary cutaneous lymphomas (PCL) are a heterogeneous group of neoplasias characterized to be clonal proliferations of neoplastic T or B lymphocytes, in the skin, with no evidence of extracutaneous disease at the time of diagnosis (1). Primary cutaneous lymphomas is the second most common type of extranodal lymphoma, with an estimated annual incidence of 0.5–1 per 100 000 people. They are considered to be separate entities from nodal or systemic malignant lymphomas involving the skin secondarily, since they have distinct clinical behaviour and prognosis (2). Primary cutaneous lymphomas can be classified into cutaneous T-cell lymphoma (CTCL) and cutaneous B-cell lymphoma (CBCL). In CTCL, there are indolent subtypes such as mycosis fungoides (MF) and lymphomatoid papulosis, whereas other CTCL subtypes have poor prognosis such as Sézary syndrome (SS) and CD30-lymphoma (3). Classification of CBCL distinguishes indolent subtypes such as primary cutaneous marginal zone B-cell lymphoma and primary cutaneous follicle centre lymphoma, and aggressive subtypes, such as primary cutaneous diffuse large B-cell lymphoma, leg type.

To date, aetiology and molecular mechanisms underlying pathogenesis of PCL remain largely unknown (4), although understanding them is important for better clinical diagnosis and management. Its pathogenesis is a multistep process involving genetic and epigenetic alteration of protein-coding proto-oncogenes and tumour -suppressor genes. Recent data have indicated that miRNAs are differentially expressed in (and possibly also involved in) pathogenesis of the disease (5).

miRNAs are small (approximately 22 nucleotides) non-coding RNAs that regulate approximately 30% of protein-coding genes in the human genome, at the post-transcriptional level (6). They make up the most important epigenetic factor in regulation of protein expression, and play important roles in numbers of biological functions including tumourigenesis, cell differentiation, metabolism and apoptosis (7). This review will summarize recent publications concerning miRNAs in different PCL subtypes, including their targets and effects on development of PCL, and reveal their potential use clinically as diagnostic, prognostic and treatment strategies.

MicroRNA expression in PCL

Accumulating evidence has revealed miRNA expression in different PCL subtypes, using various gene expression profiling approaches. Differentially expressed miRNAs are summarized in Tables 1 and 2. Below, we summarize this research individually.

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Table 1. MicroRNA profiling in PCL

	Ralfkiaer *et al.*	Kester *et al.*	Benner *et al.*	Koens *et al.*	Narducci *et al.*	Qin *et al.*
Year	2011	2011	2011	2013	2011	2012
Cancer type	CTCL	Tumour-stage MF	C-ALCL	PCLBCL-LT	SS	SS
Control type	Benign skin diseases[a] as well as healthy volunteers.	Benign inflammatory dermatoses[b]	Benign inflammatory dermatoses	PCFCL	Healthy controls	Atopic dermatitis(EAD) and healthy donors
Number of PCL cases	63	19	14	19	28	12
Number of control cases	85	12	13	4	6	4
Cut-off criteria	$P < 0.001$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P \leq 0.01$	$P < 0.05$
Up		30	12	6	21	4[c] 6[d]
Down		19		10	24	7[c] 11[d]
Array validation method	qRT-PCR	qRT-PCR	qRT-PCR	qRT-PCR	qRT-PCR	Deep-sequencing analysis
Functional analysis	No	No	No	No	Yes	No
Clinical implication	Diagnosis	Unknown	Unknown	Unknown	Prognosis/diagnosis	Diagnostic/prognostic or therapeutic targets

[a]Benign skin diseases, including psoriasis, atopic dermatitis, contact dermatitis and unspecified dermatitis.
[b]Benign inflammatory dermatoses: eczema and lichen planus.
[c]Differentially expressed miRs in SS patients compared to EAD patients.
[d]Differentially expressed miRs in SS patients compared to healthy donors.

Table 2. Differentially expressed miRNAs in PCL

miRNA	Expression	Function	Clinical significance	Target gene	Reference
miR-125b-5p	Up in earlier-stage MF; down in advanced MF (T3, tumors)	Increase proliferation and cell resistance	Poor survival	MAD4	Manfe *et al.*
miR-122	Up	Decrease apoptosis	Not mentioned	cyclin G1?	Manfe *et al.*
miR-150	Down	Reduce metastasis	Prognostic	CCR6	Ito *et al.*
miR-223	Down	Decrease proliferation	Diagnostic	E2F1, MEF2C and TOX	McGirt *et al.*
miR-21	Up	Contribute to apoptotic resistance	Unfavourable outcome	PTEN?	Van der Fits *et al.*
miR-486	Up	Contribute to apoptotic resistance in SS	Unfavourable outcome	Not mentioned	Narducci *et al.*
miR-214	Up	Contribute to apoptotic resistance in SS	Unfavourable outcome	PTEN?	Van der Fits *et al.*
miR-155	Up	Promote proliferation	Diagnostic/prognostic	Not mentioned	Kopp *et al.*

MicroRNA expression in cutaneous T-cell lymphoma

cutaneous T-cell lymphoma is a heterogeneous family of PCLs that includes many subtypes, such as MF, SS and CD30+ T-cell lymphoproliferative disorders of the skin (8). As it is sometimes difficult to classify CTCLs into specific subtypes, some authors haven't specified them exactly (MF, SS or other) in their investigations. Thus, we summarize miRNA expression in CTCLs as a whole here. In one study to elucidate a diagnostic tool for CTCL, Ralfkiaer and colleagues showed they are powerful new tools for distinguishing CTCL from benign dermatoses, using microRNA microarray analysis of a large patient sample. Through systematic statistical analysis of the 27 most deregulated miRs, the 3 most up-regulated (miR-326, miR-663b and miR- 711) and the 2 most down-regulated (miR-203 and miR-205) were identified as successful candidates to distinguish CTCL from benign skin diseases, with >90% accuracy, in a training

set of 90 samples and a test set of 58 samples examined blind. They further identified a minimal miRNA classifier of only three miRNAs (miR-155, miR-203 and miR-205) that distinguished patients with CTCL from those with benign skin diseases with 95% classification accuracy and high sensitivity and specificity, in 103 patients. Moreover, the majority miRNAs were not affected by drug treatment (9). miR-150 has previously been reported to function as a tumour suppressor in acute leukemia and in lymphoma (10). One recent study by Ito *et al.* indicated that miR-150 was down-regulated, and that this was strongly associated with tumour metastasis (11).

MicroRNA expression in mycosis fungoides

Mycosis fungoides is the most common type of CTCL and has an indolent clinical behaviour, progressing from patches to plaques then on to tumours (12). miRNAs have been widely studied in MF. miR-155 and miR-92a are significantly up-regulated in tumour-stage MF compared to benign inflammatory dermatoses (eczema and lichen planus), as shown using microarray analysis of 19 patients with tumour stage MF and 12 patients with benign inflammatory dermatoses (13). Kopp *et al.* demonstrated that miR-155 was expressed in both malignant and non-malignant T cells in the vast majority of MF patients (14). However, expression of miR-223 was found (using quantitative real-time reverse transcriptase–PCR (qRT-PCR)) to be significantly lower in MF lesions versus normal controls and BID (15). In addition, peripheral blood mononuclear cells (PBMC) collected from patients with leukemic MF and SS also had lower miR-223 levels compared to a pooled collection of PBMCs from Red Cross donors. A further investigation revealed that miR-122 was expressed in the malignant T-cell infiltrate of MF, but not in quiescent T-cells (16). In addition, expression of miR-122 increased with progression of MF from patch- to advanced-stage. MiR-125b-5p, previously shown to act as an oncomiR in leukemogenesis (17), inversely correlated with cMyc expression in lesions of MF. At earlier stages (T2, plaques), cMyc expression was low in 72% cases (8/11), whereas high cMyc signalling was detected in advanced MF (T3, tumours) (17).

MicroRNA expression in Sézary syndrome

Sézary syndrome is a rare aggressive form of CTCL, with estimated 5 year survival of only 24% (18). SS is characterized by presence of neoplastic cerebriform nucleate CD4+ T cells in peripheral blood. Several pieces of work have defined the predictive value of miRNA profiling in SS. Using microRNA profiles of CD4+ T cells from 21 patients with SS and 6 healthy controls, Ballabio *et al.* (19) demonstrated that of 114 microRNAs identified, only 10 were up-regulated in SS samples. These 10 most discriminatory are: miR-145, miR-574–5p, miR-200c, miR-199a, miR-143, miR-214, miR-98, miR-518a-3p, miR-7 and miR-152. The 10 most discriminatory down-regulated miRNAs were: miR-342, miR-223, miR-150, miR-189, miR-186, miR-423-3p, miR-92, miR-181a, miR-191 and miR-376a. The 10 most discriminatory up- and down-regulated miRNAs could serve as diagnostic biomarker for SS, discriminate SS and control samples and correctly predict diagnosis in 26 of 27 samples (96%). Further investigation conducted by Narducci *et al.* (20) profiled expression of 470 microRNAs in a cohort of 22 SS patients, and identified 45 miRNAs differentially expressed between SS and controls. Significantly higher expression of miR-214, miR-199a* and miR-7, along with lower miR-342, miR-223, miR-92, miR-181a and miR-191 were observed in those patients. Moreover, using deep-sequencing analysis, Qin *et al.* found that 11 miRs were identified to be statistically differentially expressed in SS; up-regulation of miR-214/214* and miR-199a/199a* were the most prominent distinction when compared to atopic dermatitis (EAD). When healthy donors were used as additional control, 17 miRNAs were found to be significantly differentially expressed in SS patients, including upregulation of miR-214/214* and miR-199a/199a* as most profound differences. In addition, higher expression of pre-miR-199a2 and pre-miR-214 were observed in SS, while expression levels of pre-miR-199a1 and pre-miR-199b did not differ significantly between SS and controls. Pre-miR-199a2 and pre-miR-214 are located as a tandem in a single determinant, dynamin 3 opposite strand (DNM3os). Discrepancies found between differently expressed miRNAs in SS might result from different technologies used in the alternative laboratories, as well as from differences in control materials (21). A crucial role of miR-21 in SS has recently been defined. van der Fits *et al.* showed that miR-21 expression are high in SS patients compared to healthy controls and patients with BE (22).

MicroRNA expression in primary cutaneous anaplastic large cell lymphoma

Primary cutaneous anaplastic large cell lymphoma (C-ALCL), accounting for approximately 8% of C-ALCLs, and lymphomatoid papulosis (LyP) are included in primary cutaneous CD30+ lympho proliferative disorder (3). C-ALCL is usually limited to the skin and typically

has excellent prognosis. In an miRNA expression profile of skin biopsies from 14 C-ALCL patients, Benner *et al.* (23) found 13 differentially expressed miRNAs between C-ALCL and BID using miRNA microarrays. Up-regulation of miR-155, miR-27b, miR-30c and miR-29b was also validated by miRNA-Q-PCR.

MicroRNA expression in primary cutaneous large B-cell lymphomas

Primary cutaneous large B-cell lymphomas (PCLBCLs) include two subtypes, primary cutaneous follicle center lymphoma (PCFCL) and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL-LT) (24). PCFCL is considered to be an indolent lymphoma (5 year overall survival 95%), whereas PCLBCL-LT has a more aggressive clinical course (5 year OS approximately 40%) (25). MicroRNA expression between PCFCL and PCLBCL-LT were statistically differentially significant. Higher expression of miR-129-2-3p, miR-214-3p, miR-31-5p and miR-9-5p in PCFCL was observed compared to PCLBCL-LT. In addition, microRNA profiles of PCFCL and PCLBCL-LT were different from their nodal counterparts, GCB- and ABC-type nodal DLBCL, although they showed strong resemblance at gene expression level with nodal counterparts. MicroRNAs previously proved to be up-regulated in ABC-type compared to GCB-type nodal DLBCL, were not differentially expressed between PCFCL and PCLBCL-LT, suggesting distinct pathogenic mechanisms (26).

MicroRNA expression in primary cutaneous marginal zone B-cell lymphoma and primary cutaneous centrofollicular lymphoma

Primary cutaneous marginal zone B-cell lymphoma and primary cutaneous centrofollicular lymphoma are indolent CBCLs. Recently, miRNA expression analysis was revealed to be a potential tool for diagnosis and outcome prognosis in indolent primary CBCL. Expression of 11 miRNAs in 68 patients with primary CBCLs (30 cutaneous marginal-zone B-cell lymphomas and 38 primary cutaneous centrofollicular lymphomas) was profiled using real-time PCR (27). Only miR-150 differed significantly between the two groups, significantly upregulated in cutaneous marginal zone B-cell lymphomas compared to primary cutaneous centrofollicular lymphoma samples. In addition, association between miRNA expression and clinical outcome was also analyzed in the set of 57 patient cases. Low expression levels of miR-155 and miR-150 were both associated with shorter progression-free survival only in primary marginal-zone B-cell lymphomas cases.

Mechanisms of miRNA deregulation in PCL

Mechanisms of miRNA deregulation in cancer are complex. Genes encoding miRNAs are regulated in similar ways to those of coding genes. Recent studies have provided new insights to explain miRNA deregulation in PCL, including epigenetic alteration and deregulated transcription. p53 induces miR-122 expression in MF and SS cells, while iR-21, high in Sézary cells, is a direct target of STAT3 in them. Stimulation of Sézary cells with IL-21 results in strong activation of STAT3, and subsequent upregulation of miR-21 expression. Similar induction of miR-21 expression by STAT3 has previously been shown in multiple myeloma cells (28), thus, increased miR-21 expression in Sézary cells might well be a direct consequence of constitutive STAT3 activation. The c-Myc proto-oncogene is a hallmark of aggressive, poorly differentiated tumours (29,30). Previous study has shown that c-Myc accumulation is associated with poor clinical outcome of advanced stages of MF and SS (31,32). Overexpression of c-Myc repressed miR-125b-5p transcription and sensitized lymphoma cells to bortezomib, a further possible mechanism linking cMyc to apoptosis. STAT5 is a downstream effector of the IL-2R/JAK3 complex and previous work has demonstrated that it is aberrantly activated in CTCL and involved in survival of malignant T cells *via* induction of Bcl-2 (33,34). However, alternative investigation indicated that STAT5 is truncated and functions as a transcriptional repressor in malignant T cells in SS (35). Recently, Kopp and colleagues showed that STAT5 drives expression of oncogenic BIC/miR-155 in CTCL and the STAT5/BIC/miR-155 pathway promotes proliferation of malignant t cells (36). In SS, high expression of miR-199 and miR-214 can result from a single tandem in DNM3os, transcriptionally activated by TWIST1 (21,37), a transcription factor overexpressed in SS (38).

Functional consequences of aberrantly expressed miRNAs

Little progress has been made in understanding functional consequences of aberrantly expressed miRNAs in PCL; this is due to difficulties in obtaining and culturing appropriate cell lines. Ectopic miR-17-5p expression has been reported to increase apoptosis and reduce proliferation in SS cells (19); silencing miR-21 increases apoptosis in them (22) and overexpression of miR-122 reduces sensitivity to chemotherapy-induced apoptosis (16). Ectopic miR-125b-5p expression increases tumourigenic potential and cell resistance to bortezomib in MyLa cells (17) and xenotransplantation of human CTCL cells overexpressing miR-125b-5p promotes tumour growth and

results in shorter median survival in immunodeficient mice. Overexpression of miR-223 reduces growth and clonogenic potential of some CTCL cell lines (15) and overexpression of miR-150 has been reported to reduce tumour metastasis (11).

MiRNA targets and pathways in PCL

Research on miRNA targets relevant to PCL is relatively limited. MAD4, a cMyc antagonist, has been shown to be a direct target of miR-125b-5p in CTCL while miR-125b-5p increases Myla cell resistance to proteasome inhibitors *via* modulation of MAD4 (17). Previous study has also shown that miR-223 targets oncogenic transcription factors *E2F1* and *MEF2C* in acute myeloid leukemia cells (39). Recently, it was demonstrated that expression of miR-223 was significantly reduced in MF lesions and might target *E2F1, MEF2C*, and *TOX* (15); E2F1 functions as an oncogene and is elevated in various malignancies (40). MEF2C is also a transcription factor overexpressed in a subset of T-cell malignancies (41) and it has been concluded that miR-223 seemed to reduce proliferation of CTCL by targeting pro-proliferative genes such as *E2F1* and *MEF2C*. MiR-122 is overexpressed in CTCL cell lines, including MF and SS. Akt signalling is an essential survival pathway for maintenance of many kinds of malignant cell clones, including those of CTCL. MiR-122 overexpression has been proven to reduce sensitivity to chemotherapy-induced apoptosis *via* a signaling circuit involving activation of Akt and inhibition of p53. Similarly, the Akt-p53 regulatory circuit has also been demonstrated in other neoplasms, including T-ALL (42,43), suggesting the significance of p53 for outcomes for the lymphoma. Cyclin G1 has been suggested to be a direct target of miR-122 in CTCL (16). Overexpression of miR-150 substantially reduces CTCL metastasis by targeting chemokine receptor 6 (CCR6), a specific receptor for chemokine CCL20 (11). Activation of CCR6 by interleukin-22 (IL-22) results in cell proliferation and migration. Tumour-suppressor gene phoshatase and tensin homo- log (*PTEN*) is one of the best investigated targets of miR-21 in many malignancies (44,45). Thus, it is supposed that over-expression of miR-21 and miR-214 increases SS cell survival, through targeting PTEN (22).

MiRNAs as diagnostic or prognostic biomarkers

Diagnosis of PCL is a major challenge for dermatologists. The main cause is lack of specific cell or molecular markers that can reliably differentiate PCL from benign inflammatory dermotoses (2); incidence and prevalence rates are underestimated due to insensitive diagnostic criteria. Prognosis of PCL depends largely on its staging, thus, development of an efficient diagnostic tool is crucial for early diagnosis. Both tumour samples and body fluids can be used to detect changes in miRNAs. Based on miRNA alterations, multiple studies have evaluated their potential use as PCL's molecular markers. Since extraction of miRNAs from formalin-fixed, paraffin-embedded (FFPE) skin samples is well established, their use as biomarkers is perfectly feasible. In addition, as expression of several miRNAs has been proven to be associated with disease outcome or survival of PCL patients, miRNA levels also can be used as prognostic markers.

MiR-155 combined with miR-203 and miR-205 has been shown to successfully distinguish patients with CTCL from benign skin diseases, with 95% classification accuracy and high sensitivity and specificity, in an independent cohort of 78 patients, using standard TaqMan qRT-PCR assay (9). Also, the majority of miRNAs are not affected by drug treatment. MiR-223 is significantly low in MF and SS lesions. Thus its levels distinguished SS samples from healthy controls and patients with MF in more than 90% of samples with a specificity of 87% and sensitivity of 92% (15). Expression of miR-150 is significantly lower in advanced-stage CTCL with extensive nodal or visceral involvement, and its down-regulation is strongly associated with tumour metastasis (46). In addition, miR-150 and miR-155 have been proposed to be markers in primary cutaneous marginal zone B-cell lymphoma cases as their low levels are correlated with increased risk of cutaneous progression (47). However, clinical use of miRNAs as diagnostic or prognostic markers or therapeutic targets needs further validation in larger patient cohorts.

MiRNAs as therapeutic targets

Elevated expression of miR-223 in CTCL lines reduces population expansion and clonogenic potential of tumour cell (15) while ectopic miR-17-5p expression increases apoptosis and reduces cell proliferation in SS cells (19). Overexpression of miR-150 substantially reduces tumour metastasis by directly down-regulating CCR6, a specific receptor for chemokine CCL20 (11) and silencing miR-21 increases apoptosis in Sézary cells (22). Either by pharmacologic inhibitors that target miRNA repression or by miRNA replacement, restoration of miRNA expression in malignant cells provides a promising therapy for PCL treatment.

High miR-125b-5p expression increases tumourigenic potential and cell resistance to bortezomib in MyLa cells (17) and xenotransplantation of human CTCL cells overexpressing miR-125b-5p promotes tumour growth,

resulting in shorter median survival, of immunodeficient mice. Overexpression of miR-122 reduced sensitivity to chemotherapy-induced apoptosis, serving as an amplifier of the antiapoptotic Akt/p53 circuit (16). Targeting of miR-122 may serve to be a chemotherapy sensitizer in therapy-resistant CTCL, improving its outcome.

Conclusions

A distinct miRNA profile has been reported in many subtypes of PCL, providing considerable information regarding its pathogenesis. In this review, we have summarized publications of expression and roles of miRNA in PCLs. At present, miRNAs have been detected in most body fluids such as blood, faeces, peritoneal and cerebrospinal fluids. Alterations of miRNA in cutaneous samples or body fluids provide us a potential target for diagnosis and estimation of PCL prognosis. Clinical use of miRs as diagnostic or prognostic markers or therapeutic targets needs further validation in larger patient cohorts and relevant control samples, however, functional roles of miRNAs revealed in previous experiments presents promising therapeutic strategies for the future.

Future perspectives

Deep sequencing analysis can improve miRNA landscape in PCL, since it has major advances in robustness, resolution and inter-laboratory portability. In addition, numbers and types of relevant controls need also been added and uniformed of. Furthermore, due to difficulties in obtaining and culturing the cell lines, little has been known of functional consequences of aberrantly expressed miRNAs in PCL. Future work should focus on this in different types of PCL cell line (48).

While large amounts of evidence proposed diagnostic value of miRNAs in PCL, potential of miRNAs as diagnostic or predictive biomarkers has not been practiced clinically. Larger clinical trials with samples of different geographical areas are needed. In addition, relatively limited work has been carried out to identify relevant targets of specific miRNAs in PCLs. With functional roles of miRNAs revealed and functional targets identified, investigation of these molecules as therapeutic targets will become a promising area.

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