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# MicroRNA-93 Targets p21 and Promotes Proliferation in Mycosis Fungoides T Cells

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**Keywords**
Cutaneous T-cell lymphoma · Mycosis fungoides · microRNA-93 · p21 · Tumor progression · SAHA/Vorinostat

**Abstract**
***Background:*** Mycosis fungoides (MF), the most common form of cutaneous T-cell lymphoma (CTCL), is a lymphoproliferative disorder characterized by proliferation of malignant T cells in a chronic inflammatory environment in the skin. The nature of MF is still not fully understood, but aberrant microRNA (miR) expression and function seem to play an important role in the pathogenesis and disease progression and have been proposed as a putative disease marker. Recent studies have reported aberrant expression of miR-93 in situ in MF lesions and linked dysregulated miR-93 expression to advanced stages of MF. However, the pathophysiological role of miR-93 in MF is unknown. ***Objective:*** Here, we provide the first evidence that miR-93 targets the cell cycle regulator cyclin-dependent kinase inhibitor p21 and promotes growth of malignant T cells in MF. ***Methods/Results:*** Thus, inhibition of miR-93 in MF patient-derived malignant T-cell lines increases expression of p21 and inhibition of malignant proliferation. Notably, treatment with the histone deacetylase inhibitor Vorinostat (SAHA) reduces miR-93 expression and enhances p21 expression in the malignant T cells. Importantly, transfection with an miR-93 mimic partly blocks SAHA-induced p21 expression. ***Conclusions:*** we provide evidence that enhanced expression of the putative oncogenic miR, miR-93, represses the cell cycle inhibitor p21 and promotes proliferation of malignant T cells. Moreover, we demonstrate that SAHA triggers p21 expression – at least partly – through an inhibition of miR-93.



## Introduction

Cutaneous T-cell lymphoma (CTCL) represents a heterogeneous group of extranodal non-Hodgkin lymphoproliferative disorders arising from abnormal T cells and is characterized by cutaneous infiltrates of malignant T cells [1, 2]. The most common variant mycosis fungoides (MF) arises from skin-resident memory T cells [3]. The etiology remains unknown, and genetic, epigenetic,

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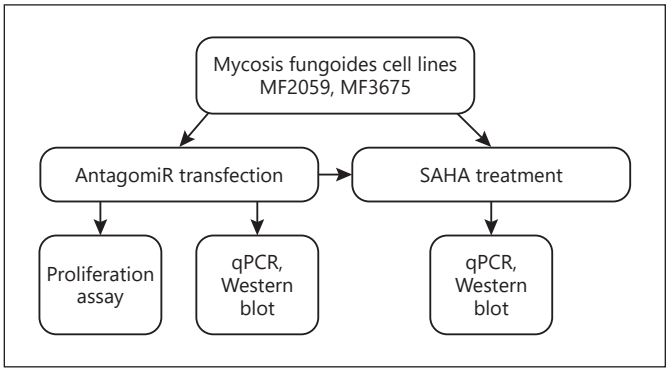


**Fig. 1.** Flowchart of Materials and Methods.

and environmental factors have all been implicated in CTCL and probably contribute to the high level of disease heterogeneity [4–6]. Notably, a recent study on CTCL in a Danish cohort of twins showed no evidence for heredity playing a role in CTCL [7]. In parallel, emerging evidence suggests that environmental factors may have both an etiological and a pathogenic role in CTCL [8–11]. Importantly, the pathogenesis of CTCL including Sézary syndrome (SS) is closely associated with chronic inflammation and aberrant activation of the NF-κB pathway, NFAT pathway, and JAK/STAT pathway in lesional skin and blood [12–15]. Dysregulation of microRNA molecules (miRs) has also been implicated in the pathogenesis of CTCL [2, 16–19]. miRs are small noncoding RNAs consisting of 20–22 nucleotides, which interfere with gene regulation at the post-transcriptional level and regulate crucial cellular processes including development, proliferation, and survival [20]. miR profiles in CTCL have been extensively studied and demonstrate unique profiles with diagnostic and prognostic potential, including the ability to discriminate between CTCL and benign skin diseases as well as different stages of CTCL [17, 19, 21, 22]. miR-155 is one of the best-characterized miRs in CTCL and is classified as an oncogenic miR (onco-miR). The high expression of miR-155 mediates inhibition of the tumor suppressor SATB1 and induction of proliferation in malignant T cells [16, 23]. In contrast, miR-22, miR-203, and miR-205 are significantly downregulated and fail to execute their putative tumor suppressive functions in CTCL [22, 24]. Interestingly, several studies investigating miR profiles have demonstrated that miR-93 is highly overexpressed in MF [17, 19, 25] but not in SS [26]. miR-93 is clustered with miR-25 and miR-106b and located in intron 13 hosted by the gene encoding MCM7, which expression has been reported to be elevated in MF [27, 28]. Studies have additionally shown that miR-93 is upregulated in advanced stages of MF, thus suggesting a role for this miR in disease progression [19]. Furthermore, miR-93 has previously been described in a range of cancers as an onco-miR preventing apoptosis and promoting tumor survival [29]. Despite its apparent dysregulation in CTCL, the role of miR-93 remains to be elucidated. Deregulation of numerous tumor suppressors, including the important cell cycle inhibitor p21, has been described in CTCL, and tumor suppressors may serve as potential targets for miR regulation [30]. In this study, we sought to gain further insights into the miR-93-mediated effects on tumor progression.

## Materials and Methods

For further details, see online supplementary material (see www.karger.com/doi/10.1159/000505743 for all online suppl. material) [31, 32] (Fig. 1).

## Results

In order to determine the role of miR-93, which is hypothesized to be involved in the progression of MF [19], the tumor cell lines MF2059 and MF3675 were transfected with an miR-93-5p inhibitor to reduce the activity of the miR. Following 24 h of incubation with the inhibitor, a 50% increase in p21 mRNA in MF2059 cells and a profound increase in the p21 protein expression in MF2059 and MF3675 cell lines were observed (Fig. 2a–c). However, the SS cell line SeAx did not show any inhibition of p21 protein expression following mir-93 inhibition (online suppl. Fig. S1). To determine the effect on proliferation, thymidine incorporation was measured following transfection experiments with miR-93 inhibitors. Inhibition of miR-93 resulted in a 20–30% reduced proliferation of the malignant T-cell lines (Fig. 2d). This reduced proliferation is comparable to the effects observed following inhibition of miR-155, which is a well-established miR known to play an important role in MF [16] (online suppl. Fig. S2).

The histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA/Vorinostat), which modulates the epigenetic landscape of malignant T cells, is an approved treatment option for CTCL [33, 34]. In order to test the hypothesis that HDAC inhibitors exert their functions through regulation of miRs, SAHA was

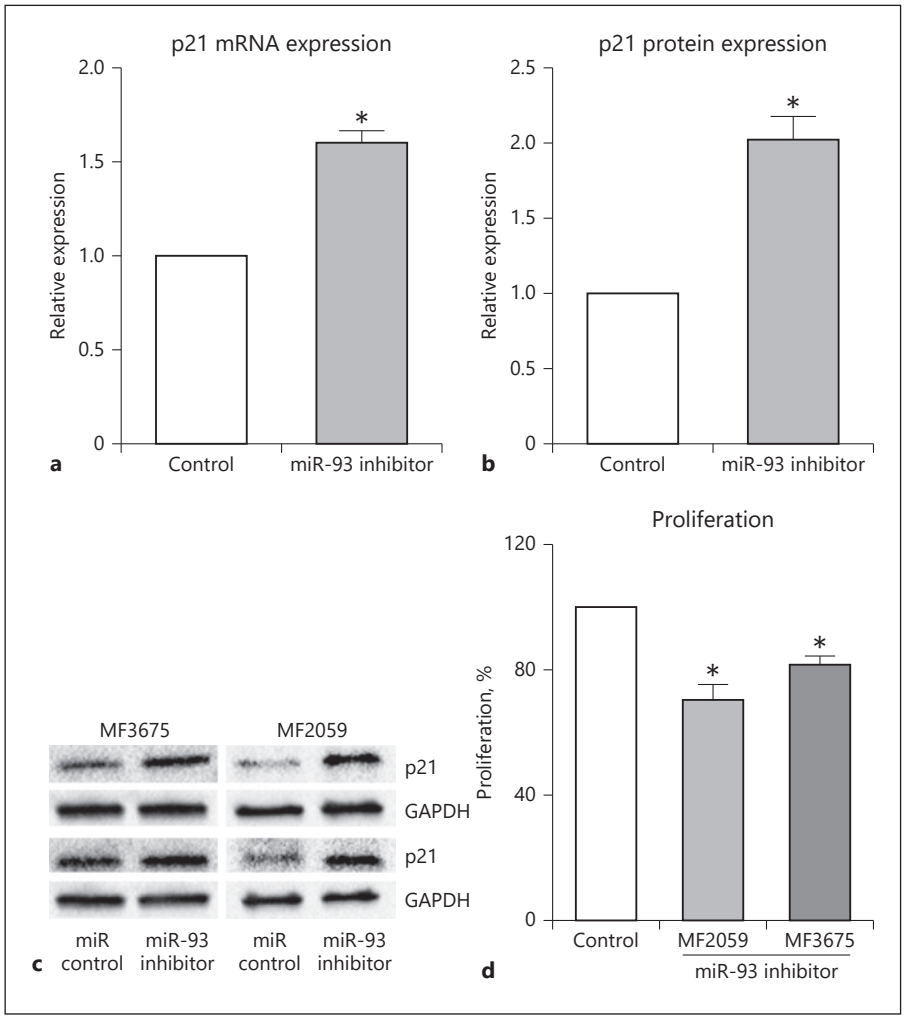


**Fig. 2.** miR-93 downregulates the expression of p21 and reduces proliferation in malignant T cells. The tumor cell lines MF2059 and MF3675 were transfected with miR-93 inhibitors and cultured for 24 h prior to analysis. **a** mRNA expression of p21 was measured in MF2059 cells by qPCR ($n = 3$) using GAPDH as a reference gene. **b** Western blotting was used to determine the protein expression of p21 in MF2059 and the intensity of the bands were quantified ($n = 4$). **c** Western blotting analysis was evaluated for MF2059 and MF3675 cells ($n = 2$). GAPDH was applied as a loading control. **d** The MF2059 and MF3675 cells were transfected with miR-93 inhibitors and cultured for 24 h in the presence of $^3$H-thymidine. Incorporation of $^3$H-thymidine was analyzed to determine the proliferation of malignant T cells ($n = 4$). * $p < 0.05$, indicates statistical significance.

added in concentrations ranging from 0.6 to 5 μM to the culture of the malignant T-cell line MF2059. SAHA reduced the expression of primary miR-93 by up to 72% in a concentration-dependent manner (Fig. 3a). Furthermore, the addition of SAHA induced the expression of the tumor suppressor miR-22 primary transcript and the expression of p21 mRNA, consistent with previous reports (Fig. 3b, c) [35, 36]. Protein expression analyzed by Western blotting confirmed that SAHA enhanced p21 protein expression and that the reduction of miR-93 activity and SAHA additively induced the expression of p21 protein (Fig. 3d). As illustrated in Figure 3e, transfection experiments applying miR-93 mimics reduced the upregulation of p21 observed as a response to SAHA treatment (Fig. 3e), indicating that SAHA-induced p21 expression is mediated, at least partly, through an inhibition of miR-93.

## Discussion/Conclusion

miRs play a key role in the regulation of T-cell development, differentiation, and proliferation [20]. Abnormal miR expression profiles have been strongly implicated in a broad range of cancers, and patients suffering from CTCL show distinct miR expression profiles, which may act to promote tumor progression [19, 21]. miR-93 has been reported to be significantly upregulated in advanced stages of MF, which suggests a role in the progression of the disease [17, 19]. However, miR-93 did not serve as a prognostic marker in a large cohort of early-stage MF patients [21]. Here we report that miR-93 interferes with p21 expression in MF tumor T-cell lines but not in an SS cell line. p21, a known cell cycle inhibitor and tumor suppressor, is one of many dysregulated tumor suppressors reported in CTCL [30]. We furthermore demonstrate

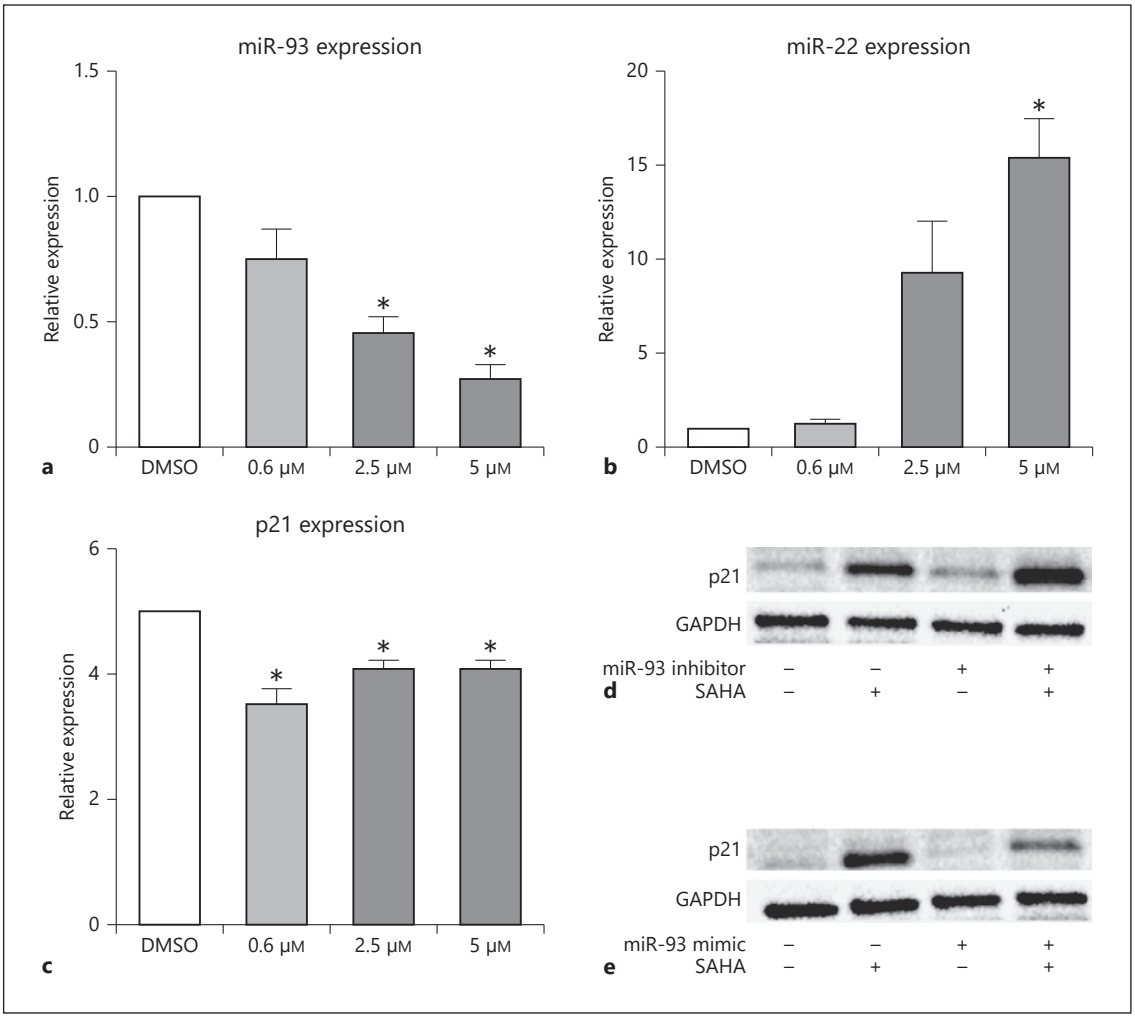


**Fig. 3.** SAHA treatment inhibits miR-93 and induces miR-22 and p21 in malignant T cells. The tumor cell line MF2059 was cultured for 24 h with concentrations of SAHA ranging from 0.6 to 5 μM prior to analysis. Primary miR levels were measured in MF2059 cells by qPCR for miR-93 (**a**) ($n = 3$), miR-22 (**b**) ($n = 3$), and mRNA expression for p21 (**c**) ($n = 3$) using GAPDH as a reference gene. Western blotting was used to evaluate the protein expression of p21 in MF2059 cells transfected with either miR-93 inhibitors and controls (**d**) or miR-93 mimics and controls (**e**). Cells were transfected and incubated for 24 h prior to being cultured for 24 h in the presence of SAHA or DMSO. GAPDH was applied as a loading control. Statistical significance was determined using one-way ANOVA, with multiple comparison tests. * $p < 0.05$, indicates statistical significance.

that miR-93 inhibition decreases the proliferation of malignant T cells, suggesting that the aberrant expression of miR-93 seen in MF may accelerate proliferation of malignant T cells. The effects on proliferation were comparable to those observed for miR-155 inhibition, a well-characterized onco-miR in CTCL, which currently is being investigated as a therapeutic target in clinical trials [37]. miRs regulate many genes post-transcriptionally, thus numerous miRs may act through multiple mechanisms to promote proliferation of malignant T cells [20]. A recent study describes that miR-16 indirectly targets p21 in CTCL, thus contributing to the diminished expression of p21 [35]. This indicates that repression of p21 may be a key event in disease progression in MF and that multiple miRs, directly or indirectly, orchestrate an inhibition of p21 expression at the protein level.

Deciphering the molecular mechanisms leading to cancer progression is crucial in the search for new therapeutic targets as well as in the understanding of the underlying mechanisms of already existing therapeutics.

The mode of action of SAHA remains to be completely elucidated [34]. Studies have shown that SAHA upregulates p21, inhibits cell growth of malignant T cells, and induces apoptosis [38]. In the present study, we provide evidence of a possible mechanism by which SAHA exerts part of its effects, as SAHA reduces miR-93 expression which in turn influences the regulation of p21. Our findings are in concordance with previous studies which indicate that SAHA regulates numerous miRs [24, 35], downregulates tumor-promoting miRs, and induces important tumor suppressor miRs, such as miR-22. In conclusion, we provide novel insights into the role of miR-93 as a potential tumor-promoting factor in MF. Further characterization of potential mechanisms of action in relation to tumor promotion could advance our understanding of miR-93 and contribute to the establishment of miR-93 as an important factor in tumor progression. Thus, miR-93 remains a target of great interest, and further studies are required to determine the potential of miR-93 as a treatment target in MF.

## Key Message

The highly expressed miR-93 decreases p21 expression and promotes proliferation in mycosis fungoides T cells.

## Statement of Ethics

In accordance with the Declaration of Helsinki, samples were obtained with written consent from the patients after approval by the Committee on Health Research.

## Disclosure Statement

The authors have no conflicts of interest to declare.


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## Author Contributions

M.G. performed the experiments. M.G., S.F., E.B., A.W.-O., T.B.B., C.N., and T.K. analyzed the data and made the figures. M.G., A.W., N.Ø., L.M.L., C.M.B., C.G., T.L., and L.I. designed the research. M.G. wrote the original draft of the manuscript. All authors reviewed and edited the manuscript. N.Ø. supervised the project and acquired the funding.

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