MicroRNA-mediated Gene Expression in Sezary Syndrome: An Overview

C. Cristofoletti, PhD1, M. C. Picchio, PhD3 and G. Russo, MD1

Abstract— Sezary syndrome (SS) is a rare leukemic cutaneous T-cell lymphoma (CTCL) with an aggressive clinical course. It is characterized by erythroderma, generalized lymphadenopathy and neoplastic skin-homing CD4+ memory T cells (Sezary cells) in the skin, lymph nodes, and peripheral blood. Despite the availability of a number of active systemic therapeutic strategies, SS remains an incurable lymphoma. Recently, high throughput analyses have revealed a novel approach to identify the molecular process implicated in the development and tumorigenesis of SS, which is relevant to a pharmacological therapeutic strategy. The aim of this review is to describe some altered genes, starting from the main deregulated microRNAs discovered in SS.

Keywords — Cutaneous T Cell Lymphoma, Sezary syndrome, microRNA, oncogenes, tumor-suppressor genes.

INTRODUCTION

Traditionally, Sezary syndrome (SS) has been defined as a leukemic form of CTCL associated with erythroderma. A series of studies in the early to mid-20th century, beginning with Sezary’s initial landmark observation in 1938, identified a population of large lymphocytes in the peripheral blood with grooved, lobulated (i.e. “cerebriform”) nuclei in patients with Mycosis Fungoides (MF) or SS [1][2][3]. In SS, these clonal T cells are generally CD31, CD41 and CD82 by multicolor flow cytometry and are present in skin, lymph nodes and peripheral blood [4][5]. The aberrant loss of pan-T-cell antigens, including CD2, CD3, CD4, CD5, and CD7, is frequently observed [6][7]. Of these, the aberrant loss of CD7 expression is most common, being observed in approximately two-thirds of cases [7][8]. Loss of CD26 expression is also useful in the identification of Sezary cells, being observed in the majority of cases [6][8][9]. Molecular studies, including the detection of a clonal TCR gene rearrangement by PCR and the presence of a clonal cytogenetic abnormality, provide evidence of T-cell clonality. An alternative approach to demonstrate this clonality incorporates multicolor flow cytometry using a panel of antibodies that are specific for various TCR beta-chain variable region family members (TCR-Vβ) [10][11]. The currently criteria recommended for the diagnosis of SS by the International Society of Cutaneous Lymphomas include the following: absolute Sezary count ≥1000/ml, a CD4/CD8 ratio ≥10, aberrant expression of pan-T-cell antigens, demonstration of T-cell clonality by Southern blot or PCR-based methods, or cytogenetic demonstration of an abnormal clone [5]. In addition, the most recent WHO classification requires erythroderma, generalized lymphadenopathy, and clonally related T cells (Sezary cells) in the skin, peripheral blood and lymph nodes [12]. SS is generally considered incurable unless allogeneic stem cell transplantation is implemented [13]. Despite the availability of a number of active systemic therapeutic strategies, including biological therapy, cytotoxic chemotherapy and extracorporeal photopheresis, there is a need for targeted therapies, with favorable therapeutic indices, for the treatment of advanced and refractory SS, which often renders patients highly susceptible to infection. The management approach is truly multidisciplinary [14]; the various treatment options are outlined in Table 1. High throughput analyses have been recently performed on whole genome, gene expression and microRNA profiles to understand the molecular process implicated in the etiopathogenesis of SS [15][16][17][18]. In the past few decades, both conventional and molecular cytogenetics study have been used to analyze the pathogenesis and prognosis of Sezary syndrome. Chromosomal instability plays an important role in the pathogenesis and prognosis of this lymphoma and genetic instability has associated with a poor prognosis [19]. Recurrent regional gains are usually observed at 17q, 8q and 17p, whereas regional losses have been observed at 17p, 10q, 6q, 9q, 10p, 9p and 19p [20]. These regions are likely to contain genes involved in the development of SS. Gene expression analysis has revealed several genes that are significantly over-expressed in SS [21] (such as DNM3, cMyc, TWIST1 and TNFSF11) or down-regulated [4][22] (such as STAT4 and PTEN), that are correlated to microRNA expression (Table 2).

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By using bioinformatics and public databases of genomic regions that are prone to alteration in cancer, such as minimal regions of LOH, minimal regions of amplification, common breakpoint regions and fragile sites, Calin et al. [23] analyzed the location of, known that time, microRNAs in the human genome and found they frequently reside (> 50%) in such genomic regions [24]. Recent microRNA array studies indicate that a considerable number of miRs show aberrant expression in cutaneous T-cell lymphoma (CTCL), including SS [25]. MicroRNAs (miRNAs) are short non-coding RNA molecules involved in crucial biological processes, including development, immune function, proliferation, apoptosis, and the stress response through negative regulation of the stability and translation of target messenger RNAs (mRNAs) [26]. Although the total number of miRNA remains controversial and their roles are only beginning to be defined, miRNA expression analyses indicate that diverse tumors display miRNAs expression profiles (for mature and/or precursor miRNAs) significantly different from normal tissue. Furthermore, miRNAs are emerging as highly tissue-specific biomarkers with potential clinical applicability for defining the cancer origin of metastases. They have been appreciated in various fundamental biological processes such as cell proliferation, stem cell division and apoptosis. Recently, it has been revealed that altered expression of specific miRNA genes contributes to the initiation and progression of cancer [27]. A growing number of studies indicate that deregulated miRNAs play a key role in cancer development, acting both as oncogenes and as tumor suppressor genes [23]. It is becoming increasingly clear that specific miRNAs contribute to cancer initiation and progression, and that miRNAs may have either oncogenic or tumor suppressing properties depending on their target genes [28][29]. Moreover, there is increasing evidence pointing to a role of miRNAs in the pathogenesis of lymphoid malignancies such as lymphoma [30]. The purpose of this review was to describe, by considering the main deregulated miRNAs discovered in SS, the most crucially altered genes implicated in tumor pathogenesis and progression. A schematic representation is shown in Table 2.
miR-21 and PTEN in SS

miR-21 is a miRNA frequently up-regulated in human cancers such as breast cancer, glioma, colorectal cancer and hepatocellular carcinoma, and in hematological malignancies such as chronic lymphatic leukemia, acute myeloid leukemia, B-cell lymphoma, and Hodgkin’s lymphoma and previous observations have demonstrated its oncogenic role. The miR-21 gene is located on the long arm of chromosome 17 (17q23.2), the most frequently chromosomal gain region present in 84% of SS patients (17p11.2-q25.3) [17]. Van der Fits L et al. observed that the expression of miR-21 is increased in neoplastic CD4+ cells purified from Sezary patients, and demonstrated that STAT3 binds to the promoter of miR-21 in Sezary cells [31]. Increased miR-21 expression might well be a direct consequence of constitutive activation of STAT3 in Sezary cells [33][34], whereas miR-21 inhibition results in increased apoptosis of Sezary cells [31]. Aberrant expression of STAT signaling and gains of STAT3/STAT5 (17q21.31) have been previously observed in SS [34][35][37]. STAT3 is considered as an oncogene and its constitutive activation is reported in nearly 70% of solid and hematological tumors [37][38][39][40]. An important role for STAT3 in regulating immune-mediated survival of cancer cells has also recently emerged [41]. Among the thousands of miR-21 gene targets, one of the best characterized and involved in neoplastic transformation is PTEN. PTEN is a non-redundant lipid phosphatase whose main role is to antagonize the PI3K signaling [38]. PTEN/PI3K/AKT constitutes an important pathway regulating the signaling of multiple biological processes such as apoptosis, metabolism, cell proliferation and cell growth, and thus represents an important target for drug discovery [39]. PTEN plays a critical role in T-cell malignancies [40]. This tumor suppressor gene is development; in fact, conditional loss of PTEN in thymocytes one of the most frequently lost or mutated genes in human cancers [41], including T-cell malignancies such as T-cell acute lymphoblastic leukemia and CTCL [42]. In a previous paper, we demonstrated PTEN genetic depletion in a high percentage (36%) of the Sezary patients and PTEN down-regulation in almost all of the SS samples examined. All these data had indicated that PTEN levels have critical consequences on tumorigenesis, [44] and that its down-regulation represents a crucial step in SS pathogenesis [43] unveiling a potential elements of clinical utility in this malignancy.

**Table 2:** Schematic representation of principal miRNAs altered in SS. ↑: over-expressed miRNA in SS; ↓↓: down-regulated miRNA in SS; (+): Chromosomal gain region in SS; (-) Chromosomal loss region in SS.

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Chromosomal location</th>
<th>Target Genes</th>
<th>Effects in SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ miR-21</td>
<td>17q23.1 (+) STAT3</td>
<td>PTEN</td>
<td>Tumor-suppressor gene inhibition</td>
</tr>
<tr>
<td>↑ miR-155</td>
<td>21q21.3</td>
<td>STAT4</td>
<td>T helper lymphocytes mediator</td>
</tr>
<tr>
<td>↑ miR106b</td>
<td>7q22.1 lq24.3</td>
<td>PTEN</td>
<td>Tumor-suppressor gene inhibition</td>
</tr>
<tr>
<td>↑ miR-486</td>
<td>8p11.21 (+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ miR-199a2</td>
<td>1q24.3 DNmos</td>
<td>TWIST1</td>
<td>Transcription and signal transduction activation</td>
</tr>
<tr>
<td>↑ miR-214</td>
<td>9q22.32 EVL1</td>
<td>TNFSF11</td>
<td>Oncogene activation</td>
</tr>
<tr>
<td>↓ miR-342</td>
<td>9q22.32 (-) 22q13.31</td>
<td>c-Myc</td>
<td>Invasion and metastasis promotion</td>
</tr>
<tr>
<td>↓ miR-let7a</td>
<td>21q21.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ miR-let7b</td>
<td>9p21.3 (-)</td>
<td></td>
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<tr>
<td>↓ miR-let7c</td>
<td>9p21.3 (-)</td>
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The inverse correlation between STAT4 and miR155

Expression and function of STAT3, STAT4 and STAT5 have been extensively studied in this cancer. In particular, Nebozhyn M. et al. demonstrated that STAT4 protein levels in purified CD4+ cells from patients with SS were reduced or absent [45]. STAT4 activity, which is required for T helper (Th) 1 differentiation, is induced by IL-12 cytokine, which is profoundly deficient in CTCL patients [46]. STAT4 expression is subsequently lost at later clinical stages, when the disease predominantly acquires the Th2 phenotype [47]. Recent studies have shown that down-regulation of STAT4 may be indirectly driven by a constitutive activation of STAT5 [48]. Kopp KL et al. demonstrated that one of the critical functions of STAT5 in CTCL is to up-regulate oncogenic miR-155[48]. miR-155 has been dubbed as the “bridge between inflammation and cancer” because a transient increase in this miRNA leads to activation of several types of immune cells, while constitutive up-regulation of this gene results in the development of malignancies as a result of increased genomic instability [48][49]. In a recent study, Litvinov et al. demonstrated that STAT4 is one of the targets of miR-155[46]. In summary, STAT signaling appears to play a central role in the pathogenesis of this cancer where early in the disease STAT5 up-regulation drives the expression of miR-155 oncogene,
which targets STAT4 and contributes to a switch from the Th1 to Th2 phenotype, while during later stages activation of STAT3 leads to increased survival and resistance to apoptosis, expression of miR-21 oncogenic microRNA and up-regulation of IL-5 and IL-10 signaling, all working in concert to promote carcinogenesis. These findings indicate that loss of STAT4 expression is an important prognostic marker, prominent as therapeutic agents for the treatment of CTCL [47].

**miR106-B, miR214 AND miR486 REGULATE PTEN EXPRESSION IN SS**

Narducci et al. demonstrated that miR-21, miR-106b and miR-486 are co-overexpressed in SS [4]. Their results indicated that miR-214 and miR-486 were over-expressed in the majority of SS patients therefore more likely related to diagnosis, whereas miR-21 up-regulation is detected in a smaller number of patients thereby more probably associated to prognosis of SS. In addition, performing functional experiments, this authors observed that all these miRs are able to promote cell survival in CTCL cell line. Moreover, miR-214 over-expression has also been recently reported to promote proliferation of healthy T lymphocytes via PTEN targeting [50] whereas miR-486 is located on chromosome 8p11.21, a frequent gain region in this pathology [4]. We recently demonstrated that miR-21, miR-106b and miR-486 modulate PTEN levels in vitro [43]. In addition, we found a significant inverse relationship between PTEN abundance and miR-106b levels in vivo, indicating that this miRNA is deeply involved in SS pathobiology. Our study displayed that PTEN genomic loss, when accompanied by miRNA down-regulation, might be more effective at promoting tumorigenesis in SS cells [43]. In conclusion, miR-21, miR106b, miR-214 and miR-486 were proposed as novel diagnostic/prognostic biomarkers for this incurable variant of CTCL [4].

**TWIST1 ACTIVATION PROMOTES miR-214 AND miR199A OVER-EXPRESS IN SS**

Narducci et al. noted that miRNA 214/199a clusters are co-expressed in a large group of SS patients [4]. In addition, Y Qin et al. demonstrated the up-regulation and high expression of pre-miR-199a2 and pre-miR-214 in Sezary patients [51]. pre-miR-199a2 and pre-miR-214 are located as a tandem in a single transcript, the so-called dynamin 3 opposite strand (DNM3os), which is transcriptionally activated by TWIST1 (7p21) [52], a transcription factor known to be over-expressed in SS [15]. The TWIST1 gene functions as a regulator of mesodermal differentiation and is normally not expressed in lymphoid cells [53]. TWIST1 belongs to the basic helix-loop-helix family of transcription factors, whose members are known to include several T-cell oncproteins [54]. This gene has been shown to possess oncogenic properties because it can prevent c-myc-induced apoptosis by antagonizing the p53 pathway [55]. The antiapoptotic properties of TWIST1 have also been suggested by its interaction with components of the nuclear factor-kB (NFkB) pathway, which regulates susceptibility to TNF-α induced apoptosis [56]. Interestingly, the NFkB pathway, which both regulates and is regulated by TWIST1 activity in mammalian cells, has been reported to be constitutively activated in CTCL cells [57]. Yin G. et al., demonstrated that the increase in TWIST1 levels, during the in vitro differentiation process in epithelial ovarian cancer, is accompanied by an increase in miR-199a and miR-214, a decrease in IKKβ and PTEN, and an increase in AKT activity [58]. They described TWIST1 as a regulator of a unique miRNA cluster responsible for the regulation of the inhibitor of the NFkB kinase subunit beta (IKKβ) IKKβ/NFκB and PTEN/AKT pathways, and reported its association with ovarian cancer stem cell differentiation. In conclusion, the over-expression of TWIST1 down-regulates PTEN level indirectly through the transcription of miR-214 and miR-199a.

**THE INVERSE CORRELATION BETWEEN miR-342 AND miR-199A INHIBITS APOPTOSIS IN SEZARY CELLS**

Ballabio et al. elucidated the complete miRNome of purified T cells from 21 patients diagnosed with SS, demonstrating that the most discriminatory microRNA was miR-342 [25]. This microRNA is intronically encoded within the *EVL* gene. Both miR-342 and EVL were found to be down-regulated in SS patients [25]. One intriguing explanation for the down-regulation of miR-342 in SS is that the host gene, *EVL*, is itself a target of microRNA regulation. It is noteworthy that one of the micro-RNAs hypothesized to target *EVL* is miR-199a*(alias miR-199a2), which is up-regulated in SS, as previously described [25]. Ballabio et al. demonstrated an inverse relationship between miR-199a* and miR-342 levels in SS, with inhibition of the former resulting in increased miR-342 endogenous levels. As mentioned above, miR-199a* is also intronically encoded within the DNM3 gene, which is itself specifically expressed in SS [59]. Furthermore, miR-199a* is directly up-regulated by TWIST1 binding [60], which is in turn up-regulated in SS owing to the frequent gain of the encoding region. They also demonstrated that reduced miR-342 levels in SS resulted in a significant increase in apoptosis levels, but did not significantly affect cell proliferation levels. These findings suggest that miR-342 may play a tumor-suppressor role in these cancers. Using the miRGen database they identified a putative apoptosis-associated target genes TNFSF11 (also known as RANKL or TRANCE) [25], an antiapoptotic molecule that is up-regulated in SS patients [61]. This protein activates antiapoptotic kinase AKT/PKB through a signaling complex involving SRC kinase and tumor necrosis factor receptor-associated factor (TRAF), which indicates that this protein may play a role in the regulation of cell apoptosis [62]. Ballabio et al. found that TNFSF11 levels decreased in SS cells transfected with either miR-342 or anti-miR-199a* [25]. TNFSF11 is highly expressed in activated peripheral and memory T cells, but not in resting peripheral T cells [61]. This pattern is consistent with their previous observation that miR-342 is more highly expressed in resting than memory T cells [63]. It has been suggested that TNFSF11 expression can promote T-cell survival by interacting with dendritic cells, leading to the induction of IL-15 [61]. Interestingly, IL-15 has been shown to protect SS cells from apoptotic agents [64]. All
these data suggests that miR-342 down-regulation in SS mediates its anti-apoptotic affect via up-regulation of TNFSF11. All these data strongly suggest that miR-342 and miR-199a are important in the pathogenesis of SS and provides new possibilities for the diagnosis and treatment of this lymphoma.

LET-7A, LET-7B AND LET-7C ARE DOWN-REGULATED IN SS
Narducci et al. observed a reduced expression of let-7a, let-7b and let-7c in their group of SS patients [4]. Let-7 family members are widely viewed as tumor suppressor miRNAs targeting RAS, HMGA2 and c-Myc. These miRNAs are strongly down-regulated in many cancers as well as during tumor progression [65]. The human genome contains 12 loci that encode the let-7 miRNA family: let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i and miR-98. One of the transcription factors controlling LET-7 expression is Myc (8q24.3) [66][67][68], the well-known oncogene that is up-regulated in the majority of patients with SS [16]. Myc is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes. Mutations, over-expression, rearrangement and translocation of this gene have been associated with a range of hematopoietic tumors, leukemia and lymphomas, including Burkitt lymphoma. Heo et al. demonstrated an inverse correlation between Let-7 and c-Myc, and in particular Let-7 targeting down-regulation of c-Myc [69]. These findings provide an insight into mechanisms that contribute to miRNA repression in cancer and highlight an important role of let-7 repression in Myc-driven cellular phenotypes.

MIIR-31 IS DOWN-REGULATED IN RECURRENT DELETED CHROMOSOMAL REGION IN SS
Narducci et al. observed a down-regulation of miR-31 in 79% of SS patients. miR-31 is an anti-metastatic miRNA [4]. The down-regulation or deletion of its genomic locus at 9p21 promotes the invasion/metastasis of cancer. This locus encodes the well-known tumor suppressor genes CDKN2A and CDKN2B [70]. The entire CDKN2A–CDKN2B locus is frequently lost and/or mutated in a wide range of human primary solid and hematopoietic neoplasia [71][72]. The CDKN2A–CDKN2B deletion, which is a frequent feature of late-stage epidermotropic CTCL, occurs in 44% of patients with SS. This deletion can induce cell cycle arrest in the G1 and G2 phases. These genes bind to MDM2 and block its nucleocytoplasmic shuttling by sequestering it in the nucleolus. In addition, they inhibit the oncogenic action of MDM2 by blocking MDM2-induced degradation of p53 and enhancing p53-dependent transactivation and apoptosis. CDKN2A–CDKN2B also induce G2 arrest and apoptosis in a p53-independent manner by preventing the activation of cyclin B1/CDC2 complexes [73]. They bind to E2F1 and MYC, thereby blocking their transcriptional activator activity but with no effect on MYC transcriptional repression. These findings suggest that, like other cancers, miR-31 down-regulation might contribute to the atypical trafficking ability of these malignant lymphocytes and representing a potential diagnostic marker in SS.

CONCLUDING REMARKS
SS is a very aggressive and incurable form of CTCL. Scientists have been trying to elucidate the molecular mechanisms that cause cancer development and cancer prevention for a long time. miRNAs are critical downstream components of key oncogenic and tumor suppressor signaling pathways [74]. Gain or loss of function of these pathways in tumor cells directly affects miRNA expression, which in turn affects malignant cellular behavior. Moreover, more than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites, as occurs with miR-21, miR-486 and miR-31 in patients affected by SS [75]. Over-expressed miRNAs in cancers may function as oncogenes and promote cancer development by negatively regulating tumor suppressor genes and/or genes that control cell differentiation or apoptosis. Some of these miRNAs, which are over-expressed in SS, negatively modulate PTEN expression directly, such as miR-21, miR214, miR-106b and miR-486, whereas others modulate PTEN expression indirectly, such as miR199a. With regard to over-expressed miRNAs, worthy of note is the role played by miRNA-155 in promoting inflammation and cancer development through the STAT signaling pathway. On the other hand, some under-expressed miRNAs that function as tumor suppressor genes, such as miR-342, miR-7 family members and miR-31, may inhibit cancers by regulating oncogenes and/or genes that control apoptosis, such as TNFSF11 and CDKN2A-B. Lastly, we assessed the contribution made by the DNA copy number alteration in the aberrant expression of three miRNAs (miR-21, miR486 and miR-31), and two genes (PTEN and TWIST1) in this lymphoma. The close correlation between gene expression, microRNA alterations and genomic aberrations appears to trigger a feedback mechanism that might prove useful as a means of defining the molecular mechanism underlying tumor initiation and progression in SS. The continuing, intense activity being conducted in this broad field of research is highly likely to lead to further progress and, eventually, to success in the clinical setting.

COMPETING FINANCIAL INTERESTS STATEMENT
The Authors declare no competing financial interests.

REFERENCES


