7

General discussion

Studies in this thesis have been aimed to characterize the molecular mechanisms involved in the development and progression of different types of cutaneous T-cell lymphomas (CTCL), namely mycosis fungoides (MF), Sézary syndrome (Sz), primary cutaneous anaplastic large cell lymphoma (C-ALCL) and primary cutaneous peripheral T-cell lymphoma not otherwise specified (C-PTCL-NOS). In this final chapter, the results of these studies, together with those from recent literature, will be summarized and discussed. We will first discuss the results of array-based comparative genomic hybridization (aCGH) and gene expression profiling in tumor-stage MF (T-MF) and Sz. Since the relationship between both conditions is a matter of ongoing debate, we will focus on the similarities and differences between the two disease entities. In the second part, we will discuss the results of our studies on miRNAs in T-MF and Sz and the possible implication of miRNAs in the pathogenesis of these lymphomas. The third part of this chapter addresses two other types of CTCL, C-ALCL and C-PTCL-NOS. We performed aCGH and gene expression profiling to gain insight into the possible mechanisms underlying the different clinical behavior of these types of CTCL. The discussion will conclude with future perspectives.

Mycosis fungoides & Sézary syndrome

MF is the most common type of CTCL and generally has an indolent course with slow progression from patches to more infiltrated plaques and eventually tumors.[1] Sz is a malignant disease characterized by a triad of erythroderma, generalized lymphadenopathy and the presence of neoplastic T cells in the skin, lymph nodes and peripheral blood.[1] Sz has often been considered to represent a leukemic phase or variant of MF, with both malignancies originating from activated, skin-homing, memory T cells with cerebriform nuclei. For this reason, MF and Sz share the same classification and staging system and patients with these conditions are often included in the same clinical trials. However, in the recent WHO-EORTC classification and in the WHO classification of 2008 MF and Sz are included as separate disease entities based on their distinct clinical features and disease behavior (see Table 1, **Chapter 1**).[1,2] There are also differences in the histopathologic findings of involved skin and lymph nodes in the two conditions. However, whether Sz syndrome should indeed be regarded as a separate type of CTCL or represents a leukemic phase of MF is still a matter of debate. We approached this issue by performing a detailed analysis of numerical chromosomal alterations present in the genomes of MF and Sz. The genomic architecture, especially the occurrence of highly recurrent pathogenic genetic alterations, can be characteristic of a tumor type. To further examine the notion that Sz

and MF might be distinct disease entities, we carried out a series of genomic analyses described in **Chapter 2**. We started with the delineation of recurrent numerical chromosomal alterations in malignant T cells from tumor-stage MF (T-MF) samples using aCGH. We subsequently evaluated whether this pattern corresponded to the highly recurrent gains and losses previously observed in Sz.[3] An additional goal was to identify chromosomal regions that may have prognostic value. Finally, we sought to identify candidate oncogenes and tumor suppressor genes residing in chromosomal regions with recurrent copy number alteration by integration with gene expression data.

The detailed genomic profiles of chromosomal imbalances of MF tumor cells displayed marked differences with those previously identified in Sz cells using identical methods (See Table 2, **Chapter 2**). Numerical chromosomal alterations most frequently observed in T-MF include gain of 7q21-36 and 1p36.2 as well as loss of 5q13 and 9p21, whereas Sz is characterized by gain of 17q22-25 and 8q22-24, and loss of 17p13 and 10q25. Notably, several aberrations commonly observed in T-MF, such as 7q11.2, 7q21-7q22, 7q32-7q35 and 7q36 are not or infrequently seen in Sz, arguing against the notion that Sz represents an advanced stage of MF. Conversely gains involving 17q23, 17q22-17q23, 17q24-17q25 and 8q24.1-8q24.2 (harboring *MYC*) are most common in Sz and less frequent in T-MF (see Table 2, **Chapter 2**). Subsequently, we investigated the possible relationship between chromosomal alterations and clinical behavior in T-MF. We identified three chromosomal regions with a prognostic value, namely: loss of 9p21 harboring the *CDKN2A* tumor suppressor gene, gain of 8q24.3 and gain of 1q21-1q22.

Consecutive studies by others largely confirmed our results on gross chromosomal alterations in T-MF in independent patient cohorts[4,5] supporting the validity of our findings (see Table 1). In addition, Salgado *et al*. confirmed the correlation of 9p21 loss and 8q24.3 gain with a poor prognosis in a larger group of T-MF patients.[5] Laharanne and colleagues also described prognostic value for 9p21 loss and 8q gain. However, this was only significant for a large group of CTCL patients studied (including T-MF, Sz and C-ALCL), while similar correlations could not be made for separate entities, possibly due to subgroup size.[4] Additional studies may reveal whether it will be possible to identify patients prone to disease progression by determining 9p21 loss and/or 8q24.3 gain in early-stage MF.

Integration of DNA copy number alterations with gene expression data of 22 T-MF cases by investigating regions of DNA copy number gain for up-regulated genes and regions of loss for down-regulated genes revealed 253 transcripts up- or down-regulated in respective regions of gain or loss. Of these 253 transcripts, 23 are established cancer-associated genes reported in the literature. The most frequently altered minimal common region (MCR) of DNA copy number alteration (CNA), gain of 7q36 (Table 1), contains the anti-apoptotic gene *FASTK*,[6] which is associated with increased expression.

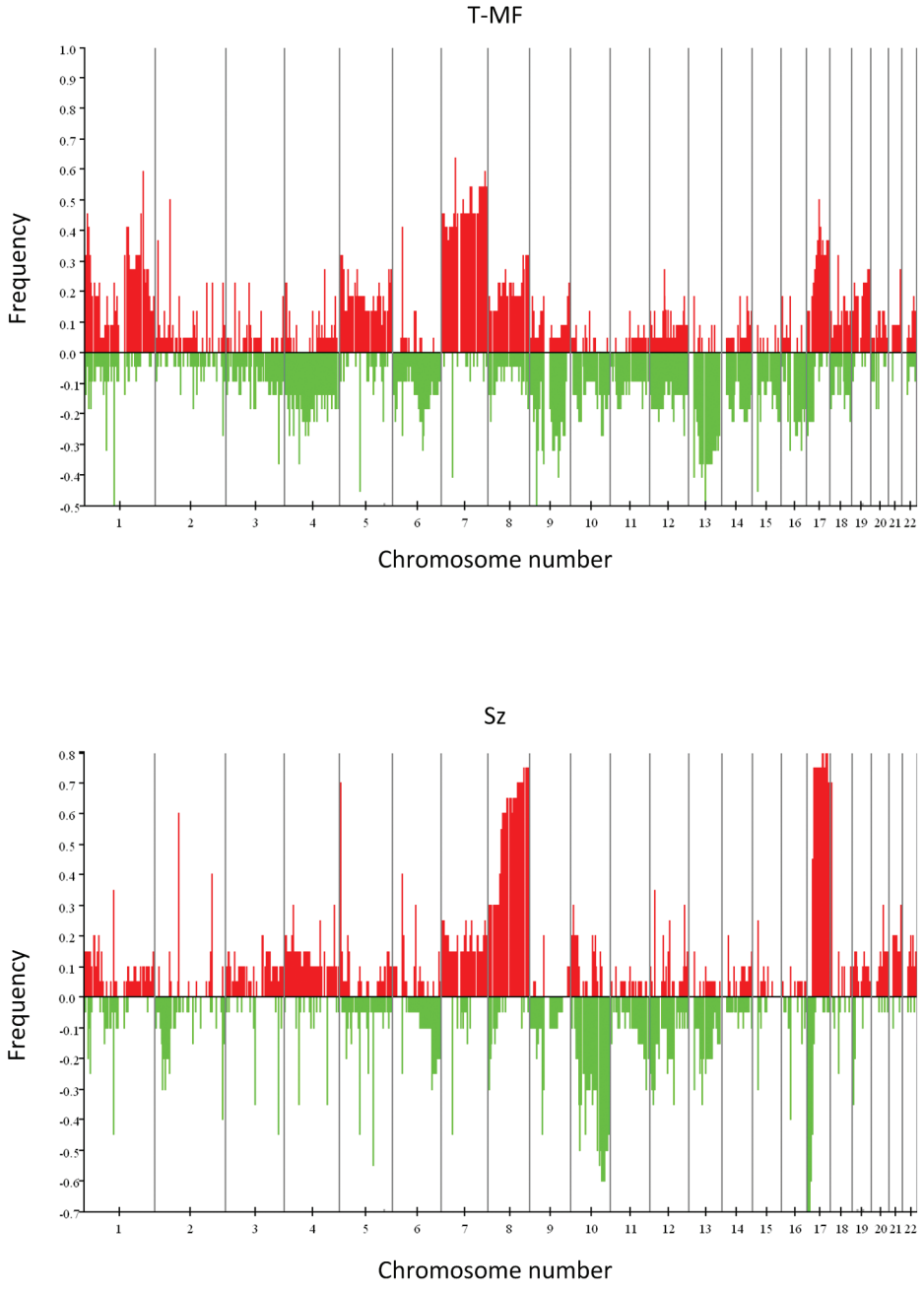
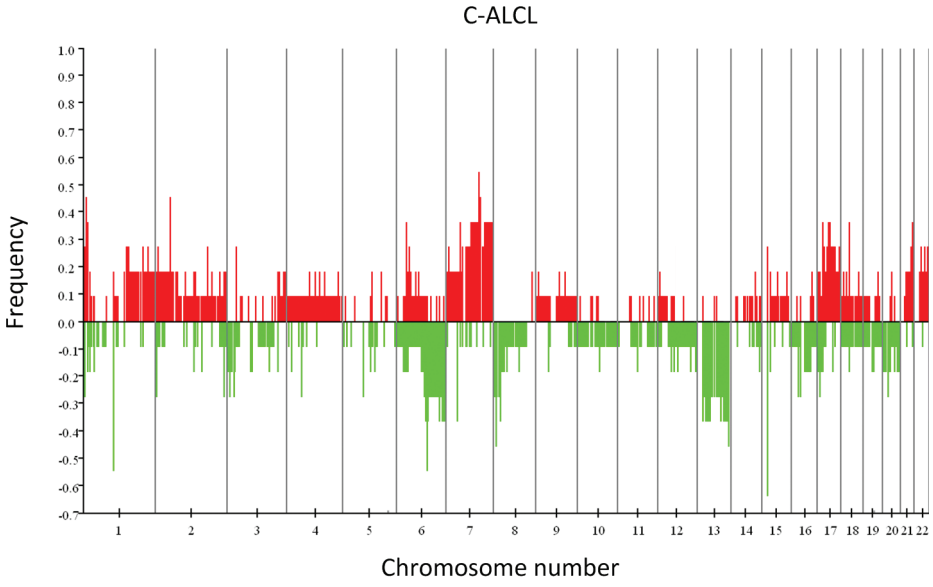
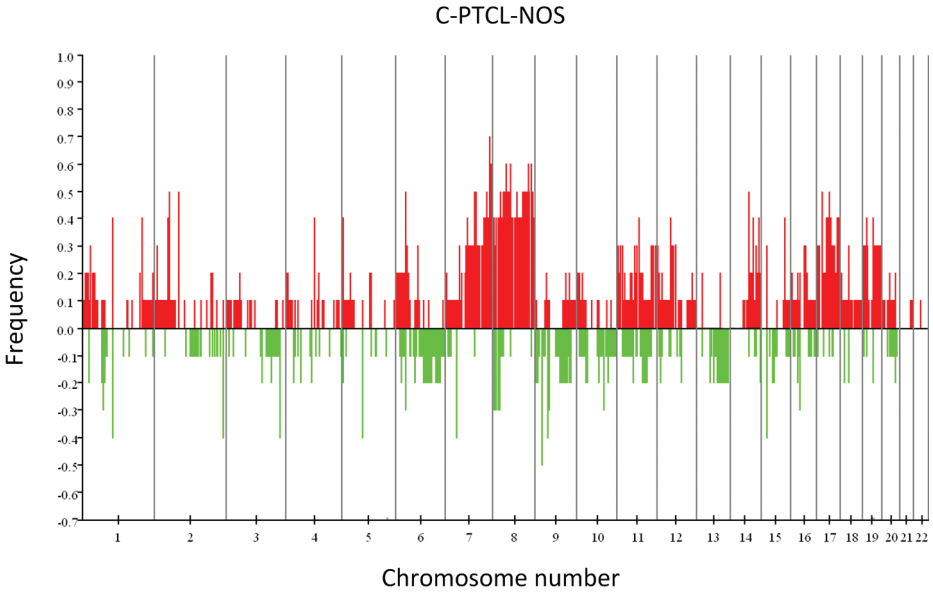


Figure 1 Comparison of Frequency of Amplicon, Gain, and Loss plots of Sz, T-MF, C-ALCL and C-PTCL-NOS


C-ALCL
Frequency
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Chromosome number


C-PTCL-NOS
Frequency
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Chromosome number

The tumor suppressor gene *CDKN2A*, located in a CNA with prognostic value (9p21), showed decreased expression consistent with previous and subsequent studies.[7-9] Also consistent with previous reports, we found recurrent loss of 13q14 and diminished expression of the tumor suppressor gene *RB1*,[10] which is located in this region.

In **Chapter 3** we generated a molecular signature of T-MF based on gene expression data. We employed a bioinformatic approach involving meta-analysis of publicly available gene expression data sets combined with gene expression data described in **Chapter 2**. Results for a selection of genes were further refined and validated by quantitative PCR and inclusion of additional controls. With this approach we identified a profile specific for T-MF consisting of 989 aberrantly expressed genes, the majority of which (718 genes) were higher expressed in T-MF compared to normal skin, inflamed skin, and normal T cells. As expected, the signature contains genes reflecting the proliferative character of this T-cell malignancy including altered expression of cell cycle and kinetochore regulators. Moreover, we found reduced expression of *NFKBIZ*, an inhibitor of the NF-κB signaling pathway, possibly explaining enhanced activity of NF-κB characteristic for CTCL,[11] and up-regulation of NF-κB target genes. Furthermore, the MF tumor profile provided novel insights in the immunophenotype and skin-homing properties of this lymphoma, and revealed expression of possible therapeutic targets. Comparing these data with the Sz expression profile as determined by van Doorn and colleagues[12] shows that only 4 overlapping genes (*ACP2, ARPC4, ATP5J2, PTPRN2*) are up-regulated in T-MF and Sz. Likewise, a minimal overlap (6 out of 53) was observed between T-MF and the list of differentially expressed genes in Sz versus normal $CD4^+$ T cells determined by Booken and colleagues (*CRIP1, KIR3DL2, CHN1, IL32, TNFSF11* and *CDCA7*)[13] or Sz versus skewed Th2 cells (an overlap of only 12 genes out of 135 identified as being differentially expressed).[14] Although these differences would support the notion that Sz and MF are different disease entities, these results should be considered to represent not more than an indication, as a similar lack in overlap was found when comparing our results with earlier gene expression studies in MF.[15-17] This lack of consistency between results of MF gene expression studies is most likely due to the different platforms used to measure gene expression levels, dissimilar statistical methods employed as well as choice of controls for comparisons in the different studies. Recently, Campbell and colleagues proposed that Sz and MF are distinct diseases because they arise from different T-cell subsets.[18] Whereas MF derives from the effector memory T cells, Sz arises from the central memory subset of $CD4^+$ T cells.
To conclude, the results of studies identifying DNA copy number alterations in Sz and T-MF support the notion that they are separate disease entities. Gene expression studies

also indicate differences between Sz and T-MF, however due to differences in experimental design solid conclusions regarding this matter cannot be drawn.

miRNA expression in Sz and T-MF

In **Chapter 4** we studied the miRNA expression profile of Sz by miRNA microarrays, identifying 114 differentially expressed miRNAs compared to normal $CD4^+$ T cells. The majority (104 out of 114) of Sz-associated miRNAs were down-regulated and their expression pattern was generally consistent with previously reported genomic copy number abnormalities. However, similar to other studies,[19] this correlation does not always hold true, implying the existence mechanisms of miRNA expression regulation other than copy number effect. To examine the gene regulatory function of dysregulated miRNAs, the previously identified list of up-regulated genes[12] was correlated with the down-regulated miRNAs by assessing which up-regulated genes were predicted targets of down-regulated miRNAs. Almost all (97 out of 104) of the down-regulated miRNAs were predicted to target one or more of these genes. Down-regulation of miR-342 for example coincides with over expression of its putative target *TNFSF11*, a gene encoding an anti-apoptotic protein.[12,13] Transfection with miR-342 decreased the levels of *TNFSF11* and induced apoptosis in Seax cells, suggesting that down-regulation of miRNA-342 prevents apoptosis by up-regulation of *TNFSF11*. Reintroduction of miR-17-5p, part of the miR-17-92 cluster often described for its oncomir function,[20-22] in Seax cells resulted in increased apoptosis, and decreased proliferation implying a tumor suppressive role for miR-17-5p. Taken together these results suggest that altered miRNA expression plays a role in the pathogenesis of Sz.

To investigate the role of miRNAs in tumor-stage MF we initially determined the miRNA expression pattern of MF tumors by comparison with benign inflammatory dermatoses. Accordingly, we extracted a miRNA signature characteristic for T-MF, presented in **Chapter 5**. In contrast to Sz, we found that for T-MF that the majority (30 out of 49) of the differentially expressed miRNAs are up-regulated compared to the chosen benign controls. For most of the identified dysregulated miRNAs a role in cancer is described and several up-regulated miRNAs (miR-93, miR-155 and miR-17-92) have been validated functionally as oncomirs.[20,22-25] In order to gain more insight into the correlation between gene expression and miRNA expression in T-MF, we subsequently searched for enrichment for miRNAs regulating genes that belong to the T-MF expression signature and identified 13 up-regulated miRNAs such as miR-93, miR-21 and miR-92a.

Although the same arrays were used to investigate miRNA profiles in Sz (**Chapter 4**) and

T-MF (**Chapter 5**), a direct comparison between miRNA expression patterns in these two diseases was not possible because different reference RNAs (activated tonsils versus synthetic RNA) and different miRNA sources ($CD4^+$ T cells versus skin biopsies) were used. A preliminary screening comparing the lists of differentially expressed genes for both diseases showed minimal overlap, which favors the hypothesis that the two are different disease entities. However, due to the differences in control groups solid conclusions cannot be drawn.

In a recent study of our group using deep sequence technology, we not only confirmed increased expression of miR-214 and miR-199a* in Sézary cells compared to $CD4^+$ T cells from healthy controls, but also compared to $CD4^+$ T cells isolated from patients with erythroderma secondary to atopic dermatitis,[26] further suggesting a potential role for these miRNAs as diagnostic classifiers. Narducci *et al*. also identified miR-214 and miR-199a* up-regulation using a commercial assay-based miRNA expression detection platform. In this study, they also confirmed up-regulation of miR-7 and decreased expression of miR-342, miR-223, miR-92, miR-181a, and miR-191 in Sz.[27] Ralfkiaer *et al*. studied a heterogeneous group of CTCL in search of a classifier between CTCL and benign control samples. Due to the use of such a combined group, an overall comparison of these results with our data is not possible. Nevertheless, they did demonstrate high miR-155 expression in MF samples by miRNA-Q-PCR.[28] MiR-155 over expression in MF compared to normal skin samples was recently confirmed by Maj *et al*. also using miRNA-Q-PCR.[29]

C-ALCL and C-PTCL-NOS

Few molecular genetic studies have been performed on CTCL types other than MF and Sz. In **Chapter 6** we describe the results of aCGH and gene expression profiling of C-ALCL and C-PTCL-NOS. Although both CTCLs present with skin tumors, the two lymphomas display a markedly different clinical course. C-ALCL shows a tendency towards spontaneous regression, uncommonly disseminate to extracutaneous sites and have an excellent prognosis with a 5-year survival exceeding 90%.[30-32] In contrast, C-PTCL-NOS quickly disseminates to extracutaneous sites, and has a poor prognosis with a 5-year survival of less than 15%.[33-35] The aim of these studies was to find possible explanations for the different clinical behavior of these two entities.

C-ALCL and C-PTCL-NOS showed distinct patterns of DNA copy number alterations. C-ALCL was characterized by gains on chromosome 7q and 17q and losses on 6q and 13q. C-PTCL-NOS similarly demonstrated gains on 7q and 17q, but was distinguished by

gains on chromosome 8 and loss of minimal region on 9p21 (harboring the *CDKN2A* tumor suppressor gene). Detailed analysis of CNAs in C-ALCL showed that the most highly recurrent MCR with gain was located on 7q31 (harboring the *MET* oncogene) and loss on 6q16-6q21 (harboring transcription factor *PRDM1/BLIMP-1* implicated in T-cell homeostasis and differentiation) and 13q34, each affecting 45% of the patients. In C-PTCL-NOS the most frequently affected MCR with gain was 7q36 (harboring the anti-apoptotic gene *FASTK*); other frequent MCRs were 7q21-7q22, 8p12-8q12, 8p21.1-8q21.3 and 8q22-8q24.2. When comparing all CTCLs, one finds there was overlap between T-MF and C-PTCL-NOS tumors as both shared gains on 7q36, 7q21-7q22, 17q21, 17q22-17q23 and loss of 9p21 and in similar frequencies; however large discrepancies were shown on chromosome 8 and 13 (see Figure 1 and Table 1). Losses on chromosome 13 were frequently found in T-MF but rarely in C-PTCL-NOS. Gains on chromosome 8 were demonstrated at high frequencies in C-PTCL-NOS and Sézary syndrome, but at low frequencies in T-MF. Interestingly, although both C-PTCL-NOS and T-MF showed frequent loss of 9p21 with an established correlation with poor prognosis in T-MF (**Chapter 2**), C-ALCL lacked this loss, which might provide an explanation for the relatively good prognosis of C-ALCL.

Subsequent studies investigating C-ALCL with aCGH describe similar aberrations,[4,36] but discrepancies were also found including gains on 7q and losses on 16q[36](see Table 1). When comparing the minimal common regions of C-ALCL with those identified in systemic ALK$^-$ ALCL by conventional CGH, overlap is found at 6q16-q21 and 17q12-q21, but differences are found at locations such as 1q41-qter, 6q21-6q22, 13q21-13q22, 13q32-q33.[37,38] Systemic ALK$^+$ ALCL shows characteristic losses of chromosome 4 and 11[37,38] not identified in C-ALCL and in low frequencies in ALK$^-$ ALCL. In summary, different types of ALCL show different DNA copy number alterations.

C-PTCL-NOS

To date no other studies investigated the genomic profile of C-PTCL-NOS. Nodal PTCL-NOS show a heterogeneous pattern of alterations possibly reflecting the heterogeneous character of the group.[39] ACGH shows recurrent gains in chromosome 7q, 8q, 17q and losses in chromosome 5q, 6q, 9p, 10q, 12q and 13q.[38,40-42] Similar to C-PTCL-NOS, gains on chromosome 8q, including 8q24 containing the MYC locus, were also described for nodal PTCL-NOS.[38,42] Gain of chromosome 8q was described previously to indicate a shorter survival in CTCL.[43,44] Besides loss of 9p21, gain of 8q could explain the more aggressive character of C-PTCL-NOS. However, the implication of other DNA copy number alterations on the pathogenesis requires further investigation.

Subsequently we studied the gene expression profile of C-ALCL and C-PTCL-NOS.

The lymphoma types C-ALCL and C-PTCL-NOS in particular differed in expression of gene clusters with regards to a role in chemokine receptor activity, apoptosis and lymphocyte proliferation. C-ALCL showed increased expression of the T-cell-homing receptors CCR10 and CCR8, which might explain their higher affinity for the skin and lower tendency to disseminate to extracutaneous sites. C-ALCL and C-PTCL-NOS lymphoid cells are both assumed to demonstrate apoptosis impairment compared to benign $CD4^+$ T cells from which these lymphomas are derived. The occurrence of spontaneous tumor regression in a subset of patients with C-ALCL and the higher sensitivity of C-ALCL to therapy suggest that apoptosis impairment in this lymphoma type is less pronounced than in C-PTCL-NOS. C-PTCL-NOS is characterized by diminished expression of pro-apoptotic genes *FAS* and *Caspase 10*, which may contribute to the more clinically aggressive behavior of C-PTCL-NOS. Another class of genes dysregulated in C-PTCL-NOS possibly contributing to aggressiveness are those involved in proliferation. An example of this class is PRKCQ, exclusively expressed by T cells and a down-stream target of the T-cell receptor, transducing signals required for activation and survival.[45-47] Moreover PRKCQ could be a therapeutic target using protein kinase inhibitors.[48] With gene expression analysis we confirmed high CD30 expression and showed high expression of IRF4 and TRAF1 mRNA in C-ALCL, which is in agreement with increased protein expression.[49] Wozniak and Piris noted that CD30/IRF4/TRAF1 all act through the NF-κB axis,[50] which is impaired in other types of CTCL. We also compared the gene expression profile of C-PTCL-NOS with that of MF tumors, and noticed that they were highly similar.

Conclusion and future perspectives

Our studies of DNA copy number alterations and gene expression in CTCL support the notion that MF and Sz should be considered as separate diseases. Therefore MF and Sz cases should be stratified accordingly in clinical trials. Our studies provide clues regarding the molecular pathogenesis of the different types of CTCL underlying the clinical behavior and prognosis. Interestingly, we found that T-MF and C-PTCL-NOS tumors share several chromosomal alterations and show highly similar gene expression profiles. Our miRNA array analysis identified many aberrantly expressed miRNAs in Sz and T-MF. Though our genome-wide studies provided many new insights, exact mechanisms explaining aberrant expression and the functional consequences of altered gene and miRNA expression in CTCL remain to be revealed. Therefore the following lines of inquiry for future research are proposed.

Table 1 Schematic overview of results of aCGH studies investigating Sz[3,4], T-MF[4,5], C-ALCL[5,36] and C-PTCL-NOS

	T-MF			Sz		C-ALCL			C-PTCL-NOS
Number of cases	This thesis n=22	Salgado et al n=41	Laharanne et al n=24	Vermeer et al n=20	Laharanne et al n=16	This thesis n=11	Laharanne et al n=18	Sánchez-S. et al n=19	This thesis n=10
1p gain	1p36.2 (45%)					1p36.2 (36%)		1p36.32 (16%) 1p36.31 (16%)	
1q gain	1q21-22 (41%) 1q31-32 (45%)	1q31.2-q32.2 (15%)	1q25-31 (35%)			1q21-23 (27%)			1q32 (30%)
1p loss						1p36.3 (27%)	1p36.3 (30%)		
2q loss				2q37 (40%)					
3p loss						3p26-p25 (27%)			
5q loss	5q13 (45%)			5q13 (45%)					
6p gain						6p21.3 (27%)			6p21.3 (30%)
6q loss		6q21.3 (17%)				6q16-q21 (45%) 6q25-q27 (36%)	6q27 (40%)		
7p gain	7p22-p21 (45%) 7p15-p14 (41%) 7p14-p13 (45%)		7p22.1-11.2 (50%)			7p22-p21 (27%)	7p11.1-q11.2 (45%)		
7q gain	7q11.2 (50%) 7q21-22 (55%) 7q32-35 (55%) 7q36 (59%)	7q33.3-q35 (55%)	7q21 (60%) 7q31 (50%)			7q21-22 (36%) 7q31 (45%) 7q32-34 (36%) 7q35-36 (36%)			7q11.2 (40%) 7q21-q22 (50%) 7q32 (40%) 7q36 (60%)
7p loss				7p14 (45%)					
8p gain				8p11.2-p11.1 (40%)					8p23-8p22 (40%) 8p12-8q12 (50%)
8q gain	8q24.2 (32%) 8q24.3 (36%)	8q24.21 (32%)		8q11.2-q12 (60%) 8q12-21.1 (65%) 8q22-23 (70%) 8q24.1-24.2 (75%) 8q24.2-24.3 (75%)	8q23-q24.3 (41%)				8q21.1-21.3 (50%) 8q22-24.2 (50%)

follow up table 1

	T-MF			Sz		C-ALCL			C-PTCL-NOS
Number of cases	This thesis n=22	Salgado et al n=41	Laharanne et al n=24	Vermeer et al n=20	Laharanne et al n=16	This thesis n=11	Laharanne et al n=18	Sánchez-S. et al n=19	This thesis n=10
8p loss						8p23-8p22 (27%) 8p22-8p21 (36%)	8p23 (45%)		8p23 (30%) 8p22-21 (30%)
9q gain		9q34 (17%)							
9p loss	9p21 (41%) 9p13-p11.1 (32%)	9p21.3 (42%)	9p21 (30%)	9p13.1-p12 (45%)					9p21 (30%) 9p13-p12 (40%)
9q loss	9q21 (32%) 9q22-q31 (32%)	9q31.2 (30%)	9q21-q22 (35%)						
10p gain		10p14 (17%)							
10p loss		10p11.22 (17%)	10p11.2 (30%)	10p11.2 (40%)	10p12-p11.2 (41%)				
10q loss			10q26 (40%)	10q22 (40%) 10q23.31 (50%) 10q23.32 (50%) 10q24-q25 (60%) 10q25.2 (60%) 10q25-q26 (50%)	10q22-q24 (47%) 10q25-q26 (41%)				
11p gain									11p15 (30%)
11q gain									11q13 (30%) 11q23-q25 (30%)
11q loss					11q22-q23 (30%)				
12p gain									12p13 (30%)
12q gain									12q13 (40%)
13q loss	13q14-q31 (36%)	13q14.11 (20%)	13q34 (40%)	13q14 (35%)		13q12-q14 (36%) 13q34 (45%)	13q34 (45%)	13q14.3 (21%) 13q21.32 (21%) 13q33.3 (26%)	
14q gain									14q31-14q32 (30%)

follow up table 1

	T-MF			Sz		C-ALCL			C-PTCL-NOS
Number of cases	This thesis n=22	Salgado et al n=41	Laharanne et al n=24	Vermeer et al n=20	Laharanne et al n=16	This thesis n=11	Laharanne et al n=18	Sánchez-S. et al n=19	This thesis n=10
16p gain								16p13.3 (21%)	
16p loss						16p11.2-q11.2 (27%)	16p11.2-16p11.1 (45%)	16p13.13 (21%) 16p13.12 (21%)	
16q loss		16q23.2 (17%) 16q24.3 (17%)	16q21-q22 (30%) 16q23-q24 (35%)					16q11.2 (32%) 16q12.1 (37%) 16q21 (32%) 16q22.1 (37%) 16q24.3 (37%)	16q12.1 (30%)
17p gain				17p11.2 (45%)		17p11.2 (27%)			17p11.2 (40%)
17q gain	17q21 (41%) 17q22-q23 (32%) 17q25 (36%)	17q21.1 (37%)	17q12 (30%)	17q21.31 (70%) 17q23 (85%) 17q24-q25 (80%)	17q23-q24 (35%)	17q12-q21 (36%) 17q21-q22 (27%) 17q23 (27%) 17q25 (27%)			17q21 (40%) 17q22 (30%) 17q22-q23 (30%) 17q23-q24 (30%) 17q25 (40%)
17p loss		17p13.1 (27.5%)		17p13.3-p13.1(55%) 17p13.1 (75%) 17p12 (70%)	17p13-q11.1 (47%)	17p13 (27%)		17p13.1 (21%)	
19p gain									19p13.3-p13.1 (30%)
19q gain									19q12-q13.4 (30%)
20p loss							20p11.1-20q11.2 (30%)		
20q loss								20q13.13 (21%)	
21q gain						21q22 (36%)			

In addition to more detailed identification of genetic alterations (e.g. using exome, whole genome or RNA deep sequence analysis), confirmation of identified dysregulated genes and miRNAs has proven to be essential and further studies confirming abnormal expression of single genes and miRNAs are recommended to be followed by functional studies. Functional studies, either the down-regulation of specific genes or miRNAs with inhibitors, or transfection of cells with the gene or miRNA of interest, followed by investigation of the effects on target gene expression, apoptosis and proliferation could elucidate the specific role genetic alterations play in the molecular pathogenesis of CTCL. It is recommended to study the effects of *NFKBIZ* transfection in Myla cells on other members NF-κB pathway and on proliferation and apoptosis. Likewise, it would be interesting to study the effect of inhibition of *FASTK* in MF and *PRKCQ* in C-PTCL-NOS. Primary miRNA candidates would be miR-17, being up-regulated in MF and down-regulated in Sz, whether inhibition induces apoptosis and diminishes proliferation in Myla cells. Other primary candidates for inhibition would be miR-214 in Sz and miR-155 in MF. These *in vitro* studies could show the validity of the therapeutic targets and be the subsequent step to *in vivo* studies. Further research assessing the miRNA expression levels, for example miR-16, miR-17 and miR-93, between Sz and T-MF directly by investigating isolated tumor cells, as well as identification and examination of (the expression levels of) target genes could provide clues regarding the different mechanisms of action in Sz and T-MF. Furthermore, studying dysregulated genes and miRNAs in patch and plaque-stage MF could potentially teach us more about disease progression. In **Chapter 3** a start was made for some genes, but a more thorough validation is recommended, such as an independent patient group, with additional controls, and immunohistochemistry permitting evaluation of protein expression in tumor cells within a background of tumor infiltration lymphocytes.

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