\section{Discussion}

\subsection{Chromatin accessibility and H3K27ac landscape in psoriasis immune cells}

Comparison of chromatin accessibility and H3K27ac histone modifications has revealed a small number of differential regions between patients and controls in the four cells types under study. For both epigenetic features, CD14$^+$ monocytes and tCD8$^+$ cells had the largest number of discrete changes. In ATAC, greater accessibility in tCD8$^+$ cells from patients compared to controls was found at two regions proximal to \textit{IL7R} and \textit{TNFSF11}, respectively, that also overlap FANTOM eRNA in the same cell type. Both genes are well known for having a pro-inflammatory effect and be involved in chronic inflammatory diseases. For example, \textit{TNFSF11} is downstream the lead SNP of a CD risk locus, and its protein product RANKL was found to be overexpressed in epidermis from psoriasis patients, highlighting the role of this gene in the pathophysiology of psoriasis \parencite{Toberer2011}.

Integration of the ATAC and H3K27ac ChIPm differential analysis only found one overlapping region at an intron of the \textit{DTD1} gene, which participates in initiation of DNA replication and is associated with aspirine-intolerance in asthmatics\parencite{Pasaje2011}. However, evidence of \textit{DTD1} involvement in chronic inflammation has yet not been reported. The lack of overlap between DARs and differentially H3K27ac modified regions might be expected given that chromatin accessibility is driven by the interaction between a number of histone modifications, TFs, and structural proteins, such as CTCF.

The results in this chapter suggest that disease status does not involve global differences in chromatin accessibility and H3K27ac between patients and controls in the studied circulating immune cells. Recent similar studies performing ATAC in B cells from SLE patients and in AMD retina and retinal pigmented epithelium have revealed larger differences in the chromatin accessibility landscape between patients and controls \parencite{Scharer2016,Wang2018}. Similarly, H3K27ac mapping in mCD4$^+$ cells isolated from juvenile idiopathic arthritis SF found approximately one thousand differential enhancers when compared to healthy control circulating cells \parencite{Peeters2015}. Conversely only small differences were found when comparing mCD4$^+$ from PB of patients and controls, highlighting the specificity of the disease signature at the site of inflammation. Importantly, direct interrogation of the main cell type or tissue affected by inflammation in those studies would partly explain the more profound changes observed in the chromatin landscape compared to my study. Additionally, some differences will be driven by genotype, and by the nature of complex diseases different patients have different genetic backgrounds, with some variants also shared with control individuals. Thus it may be necessary to study changes in chromatin accessibility in the context of genotype and under exogenous inflammatory stimuli that may manifest those differences \parencite{Alasoo2018,Calderon2018}.

\subsection{Dysregulation of gene expression in psoriasis circulating immune cells}

Comparison of gene expression between psoriasis and healthy controls in a cell type specific manner identified larger numbers of DEGs compared to DARs or differential H3K27ac modifications. As for ATAC and ChIPm, CD14$^+$ monocytes and tCD8$^+$ cells showed the largest number of transcriptomic changes in disease. This may suggest greater relevance of these two cell types in the systemic “footprint” of psoriasis. The more dysregulated gene expression in tCD8$^+$ compared to tCD4$^+$ may suggest that, as in skin, CD8$^+$ are the main effector cells upon induced-activation by CD4$^+$ cells \parencite{Nickoloff1999}. The importance of monocytes/macrophages in psoriasis has also been demonstrated by their presence in psoriatic skin where TNF-$\alpha$ production contributes towards maintenance of inflammation \parencite{Nickoloff2000,Wang2006}.

The overlap of DEGs with previous studies comparing PBMCs from psoriasis patients was limited, probably due to many differences identified in my cell type specific analysis being masked by the admixture of cells as well as those studies using microarrays instead of RNA-seq \parencite{Lee2009,Coda2012}. Although those studies did not find specific enrichment for any pathway, Coda and colleagues identified some genes were associated with pathophysiological processes such as immune response, oxidative stress or apoptosis \parencite{Coda2012.}. The cell type specific analysis conducted in my thesis identified significant enrichment of relevant biological processes, including MAPK and IL-12 signalling, in the CD14$^+$ monocytes and tCD8$^+$ cells contrast. Interestingly, some of the well-known pro-inflammatory genes contributing to the enrichment of these pathways were down-regulated in psoriasis compared to controls. For example, \textit{MAP3K4} down-regulation in LPS stimulated PBMCs has been identified as an immune-suppressive feature in CD leading to reduced expression of the cytokine IL-1$\alpha$. In the IL-12 signalling pathway, leading to T cell proliferation and IFN-$\gamma$ production through activation of TFs from the STAT family, CD14$^+$ cells presented down-regulation of \textit{STAT4} and \textit{STAT5A} in psoriasis versus controls. Other member of the STAT family, such as \textit{STAT2}, was found to be down-regulated in psoriasis PBMCs and in AS monocyte-derived macrophages when compared to controls \parencite{Coda2012,Smith2008}. Monocytes do not express \texit{STAT4} in basal conditions but up-regulation follows IL-12, IL-18 and IFN-$\alpha$ stimulation \parencite{Frucht2000,Schindler2001}. STAT5 phosphorylation in monocytes is mainly induced by granulocyte macrophage-colony stimulating factor (GM-CSF) and promotes differentiation into macrophages. Interestingly, STAT5 downstream gene targets such as prostaglandin synthase 2 (\textit{COX2}) and \textit{IL-10} were not dysregulated in CD14$^+$ monocytes in my data. In other chronic inflammatory diseases such as T2D, persistent STAT5 phosphorylation has been found in circulating monocytes isolated from T2D upon GM-CSF \parencite{Litherland2005}. Further investigation to determine phosphorylated STAT4 and STAT5 protein abundance will be require to determine if the down-regulation at the transcript level observed in psoriasis CD14$^+$ monocytes is biologically relevant.

In tCD8$^+$, expression of \textit{IFNG} a gene activated by the IL-12 signalling pathway, was down-regulated when compared to healthy controls. Down-regulation of \textit{IFNG} has previously been reported in unstimulated and stimulated macrophages derived from AS patients, in SF from SpA patients compared to RA and in a SpA rat model \parencite{Smith2008,Fert2014, }. This down-regulation was accompanied by an overall “inverse” transcriptional response of IFN-regulated genes, which was not seen in my data. Moreover, the reduced expression of \textit{IFNG} in knock-out mice has been shown to increase activation of the IL-23/IL-17 axis, which is pivotal in psoriasis pathogenesis \parencite{Canete2000,Chu2007}. Therefore, down-regulation of \textit{IFNG} may actually result in a pro-inflammatory effect.

In tCD8$^+$ specifically, DEGs showed significant enrichment for three very relevant pathophysiological pathways in psoriasis: NF-$\kappa$B, TNF and chemokine signaling. Important cross-talk between the NF-$\kappa$B and TNF signaling pathway was observed, with a number of dysregulated genes contributing to both. Interestingly, the enrichment of these pathways involved up-regulation of pro-inflammatory genes (e.g \textit{ATF2}, \textit{ATF4}, \textit{RELA}, \textit{RELB}) but also increased expression of well-characterised immunoregulatory genes. These included \textit{NFKBIA} and \textit{TNFAIP3}, also up-regulated in CD14$^+$ monocytes and CD4$^+$ cells respectively, and both associated with psoriasis GWAS signals. Polymorphisms within \textit{TNFAIP3} or in its vicinity have also been associated with a number of chronic inflammatory diseases including MS, RA, SLE and T1D \parencite{Vereecke2011}. \textit{NFKBIA} codes for I$\kappa$B$\alpha$ which inhibits NF-$\kappa$B by binding it and preventing translocation to the nucleus. \textit{TNFAIP3} codes for the zinc finger protein and ubiqitin-editing enzyme A20, and is up-regulated in the presence of inflammation and NF-$\kappa$B activation in order both to inhibit the NF-$\kappa$B TNF-mediated response and promote return to homeostasis. Either \textit{NFKBIA} or \textit{TNFAIP3} were found to be dysregulated in psoriasis PBMCs by Coda \textit{et al.}, Lee \textit{et al.} and Mesko \textit{et al.} or in PBMCs from PsA patients versus controls \parencite{Dolcino2015}. Interestingly, qPCR analysis in PBMCs from mild (PASI$<$4.84) and severe (PASI$>$4.84) psoriasis vulgaris revealed a significant negative correlation between \textit{TNFAIP3} expression and disease severity \parencite{Jiang2012}. Furthermore, this study also demonstrated that in the mild group of patients but not in the severe \textit{TNFAIP3} expression was down-regulated when compared to healthy control PBMCs. This is in line with my findings with the caveat that all patients from my cohort would be classified as severe by Jian \textit{et al.}. Altogether, the up-regulated expression of \textit{TNFAIP3} and \textit{NFKBIA} compared to healthy controls may not be unexpected as it reflects a persistent inflammatory stimuli in psoriasis PB and a mechanism that limits the systemic inflammatory response to some extent \parencite{Idel2003}.

Of interest was also the up-regulation of the chemokine receptor \textit{CCR10} in tCD8$^+$ cells from psoriasis patients. In circulation, expression of \textit{CCR10} is restricted to a subset of circulating mCD4$^+$ and mCD8$^+$ T cells expressing the cutaneous lymphocyte-associated antigen (CLA), and preferentially recruited to cutaneous sites of inflammation \parencite{Hudak2002}. Indeed, an increase of CCR10$^+$ infiltrated T lymphocytes in psoriatic skin, where KCs express \textit{CCR10} ligand \textit{CCL27}, has been demonstrated \parencite{Homey2002}. The up-regulation of \textit{CCR10} in my data could potentially suggest an increase of mCD8$^+$ CCR10$^+$ cells ready to migrate into the skin lesions. Moreover, correlation between the frequency of CTLA$^+$ CD8$^+$ cells and disease severity measured by PASI score has been found \parencite{Sigmundsdottir2001}. Overall, these data have revealed dysregulation between psoriasis patients and controls for relevant immune genes showing pro- and anti-inflammatory effects in circulating immune cells. Although down-regulation of pro-inflammatory genes and up-regulation of anti-inflammatory genes has been detected, understanding the overall effect of those interactions in the inflammatory response requires further investigation.

\subsection{Correlation between changes in chromatin accessibility and gene expression}

In this chapter, greater changes in gene expression have been identified compared to chromatin accessibility. Strikingly, in tCD8$^+$ cells, 687 transcripts were differentially expressed between psoriasis and healthy controls but only 53 regions showed differential chromatin accessibility when performing the same contrast and only six of the 687 were proximal to a DAR. Correlation between chromatin accessibility measured by ATAC and gene expression has been reported to some extent in a number of studies, with limitations in establishing relationships between enhancer regions and the regulated target \parencite{}.

Chromatin accessibility shows the current functional landscape of the genome and will have some correlation with the current transcriptional state of the cell. The RNA transcripts however will be both a view of the current transcription and previous changes in transcription as well as other events related to RNA turnover and lncRNAs and microRNAs interactions and regulatory processes at the RNA level. As such, the larger changes in gene expression compared to chromatin accessibility here are likely due to a non-direct relationship between chromatin accessibility and transcripts. Also, the discrepancy may also be due to different sensitivities from the ATAC assay to identify changes in chromatin accessibility and that of the RNA-seq analysis.

An example of a relevant DEG nearby a DAR was \textit{TIAM1}, with increased chromatin accessibility and gene expression in psoriasis tCD8$^+$ cells compared to healthy controls. \textit{TIAM1} is involved in IL-17 expression and cell migration into the inflamed tissue \parencite{Kurdi2016, Gerard2009}. However, no eQTL or chromatin conformation data in this cell type has been found to formally establish a link between the region harbouring this DAR and \textit{TIAM1} expression.

\subsection{Trancriptomic profiles in lesional and uninvolved psoriatic epidermis}

Investigation of differences in the transcriptomic profile between paired lesional and uninvolved skin was conducted for three psoriasis patients in my cohort. Most previous transcriptional studies in psoriasis have used full thickness skin biopsies, formed of a mix of cell types including fibroblast, adipocytes, KCs from the epidermis and dermis and infiltrated immune cells. A study from Ahn and colleagues demonstrated large differences in gene expression between whole biopsies and FACS-isolated KCs, which may be masking KC-specific pathophysiological differences in many previous studies using psoriasis skin biopsies \parencite{Ahn2016}. In this chapter, RNA-seq was conducted on epidermal sheets isolated from whole biopsies and a total of 1,227 DEGs were identified.

Comparison with the Tervaniemi \textit{et al.} study contrasting gene expression between lesional and uninvolved epidermis split biopsies, mainly formed by epidermis, revealed an overlap of only 359 out of the 1,227 DEGs detected in my data (12.1\% of Tervaniemi \textit{et al.} DEGs). Interestingly, the overlap with the Tsoi \textit{et al.} study using whole biopsies was similar (505, 13.1\% of Tsoi \textit{et al.} DEGs) and only 5 genes had opposite direction of change, in contrast with the 75 showing discrepancies with Tervaniemi’s study. The similar percentage overlap with the Tsoi study despite the different source material could simply be the result of greater power in that study.

Genes consistently up-regulated across the three studies included genes from the \textit{S100A} family. The \textit{S100} family are located in the chr1p21 locus, which harbours genes involved in KCs differentiation, act as calcium sensors and may also have a chemotactic effect \parencite{Eckert2004}. In particular, \textit{S100A9} and \textit{S100A12} undergo up-regulation in psoriasis \parencite{Broome2003}, with the latter involved in the T cell proliferative response and IFN-$\gamma$ and IL-2 production \parencite{Moser2007}. \textit{LCE3B}, also at the chr1p21 locus, was also upregulated in lesional skin compared to uninvolved in all three studies. \textit{LCE3B/C}\\_\textit{del}, a psoriasis GWAS association, is found in approximately 60 to 70\% of European psoriasis patients \parencite{Cid2009}. As explained in Chapter \ref{ch:Intro}, \textit{LCE} gene expression is induced upon disruption of the skin barrier, and expression of \textit{LCE3B} and \textit{LCE3D} has been only detected in lesional but not uninvolved psoriatic skin of heterozygous individuals \parencite{Cid2009,Bergboer2011}. This suggests that the three psoriasis patients in the study are heterozygous for the deletion.

Pathway enrichment analysis for the DEGs between lesional and uninvolved skin revealed a number of relevant biological processes for psoriasis pathophysiology. These highlighted alterations in cell cycle and metabolic processes, including aminoacid metabolism, glycolysis, and hypoxia (HIF-I signalling), which had been identified in other studies performing DGE analysis between lesional and uninvolved skin or genome-wide pathway analysis \parencite{Coda2012, Gudjonsson2010,Aterido2016, Tervaniemi2016}. The enrichment of the HIF-I pathway in psoriasis is the result of an increased rate of cell proliferation leading to hypoxia and angiogenesis. Up-regulation of the hypoxia-inducible TFs HIF-1$\alpha$ and HIF-2$\alpha$ has been found in lesional skin, correlated with an increase in \textit{VEGF} transcript levels, a gene regulated by HIFs that mediates pathological angiogenesis also characteristic of psoriasis \parencite{Rosenberg2007}. No correlation was observed between \textit{HIFA} and \textit{VEGF} in my data, likely due to the small sample size. Moreover, HIF-I signalling is also involved in regulating Th-17/Treg ratios and therefore in perpetuation and termination of the immune response \parencite{Dang2013}.

Immune-related pathway enrichment were also found in my analysis, including Th-17, IL-12, cytokine-cytokine and NOD-like signalling. Interestingly, NOD-like signalling was found to be enriched in DEGs between lesional and uninvolved skin in a contemporary study by Tervaniemi and colleagues \parencite{Tervaniemi2016}. Tervaniemi mainly attributed this novel pathway to the greater sensitivity of RNA-seq compared to microarrays to detect changes in gene expression for genes involved in this pathway. The fact that Tsoi \textit{et al.} also used RNA-seq and had a larger sample size, yet did not show enrichment for NOD-like signalling is likely due to the type of biopsy, highlighting the value of studying epidermis instead of full thickness skin to uncover dysregulation of functional pathways in KCs. NOD-like signalling involves signal transduction by NOD-like receptors, a type of pattern-recognition receptors, which can recruit and activate caspases into the inflammasomes or trigger inflammation through NF-$\kappa$B and MAPK. Amongst the genes contributing to this pathway \textit{CARD6}, \textit{IFI16}, \textit{NOD2} and \textit{NLRX1} overlapped with Tervaniemi’s data and showed up-regulation in both. Notably, polymorphisms in \textit{NOD2} have been linked to inflammatory diseases such as CD, atopic eczema and arthritis and potentially with psoriasis and PsA \parencite{Zhong2013,Zhu2012}.

Lastly, PPAR signaling appeared as a pathway linking metabolic and innate immunity dysregulation in psoriasis. PPARs are TFs activated by fatty acid signaling with an anti-inflammatory role in the development of metabolic diseases and chronic inflammation such as RA \parencite{Straus2007,Ji2001}. Similar to my study, \textit{PPARD} has been found to be up-regulated in psoriatic lesional skin, and molecular studies have demonstrated a role of this PPAR in KC hyperproliferation through induction of heparin-binding EGF-like growth factor (HB-EGF) \parencite{Romanowska2008}.

\subsection{LncRNAs in psoriasis}

In addition to protein coding genes, the transcriptomic comparisons conducted in my study revealed dysregulation of a number of lncRNAs. The role of lncRNAs has been studied in RA, SLE, AS, and PsA \parencite{Muller2014,Shi2014,Zhang2017, Dolcino2018} but no study has been conducted to identify differentially lncRNAs in a cell type-specific manner in psoriasis PB. Conversely, several studies have contrasted lncRNAs in lesional compared to uninvolved or healthy skin \parencite{Li2014, Ahn2016, Gupta2016, Tsoi2015}. My analysis in circulating immune cells revealed the largest number of differentially expressed lncRNAs in CD14$^+$ monocytes and tCD8$^+$ (28 and 31, respectively for FDR$<$0.05) when comparing psoriasis patients to healthy controls. In skin, 46 lncRNAs showed dysregulated expression between lesional and uninvolved skin (FDR$<$0.05), and 24 had also been reported by Tsoi \textit{et al.}, 2015.

Characterisation of lncRNA biological function is a developing field, which represents a limitation when interpreting these results \parencite{Uszczynska-Ratajczak2018}. Some of the well-characterised dysregulated lncRNAs have a role in the immune response. For example, in psoriasis CD14$^+$ monocytes the up-regulation of \textit{HOTAIRM1} corresponded to down-regulation of the predicted target gene \textit{USP1}, unlike in Dolcino \textit{et al.} 2018. \textit{UPF1} is involved in nonsense-mediated decay and in partnership with the monocyte chemotactic protein-1-induced protein-1 (\textit{MCPIP1}) gene drives degradation of inflammation-related mRNAs to ensure maintenance of homeostasis \parencite{Mino2015}. Down-regulation of \textit{UPF1} in psoriasis CD14$^+$ monocytes may suggest impairment of this homeostatic mechanism, contributing to disease pathophysiology in this cell type. Another example of a relevant lncRNA up-regulated in CD14$^+$ monocytes was \textit{NEAT1}, which has also shown up-regulation in SLE CD14$^+$ monocytes. Knock-down demonstrated impairment of TLR4 signalling and down-regulation of inflammatory genes including IL-6 and CXCL10 \parencite{Zhang2016}. However, neither of those two genes was dysregulated in my study in this cell type.

Interestingly, \textit{MIR146A} was differentially expressed between lesional and uninvolved skin but also when comparing psoriasis tCD8$^+$ to healthy controls. Molecular studies have suggested a role for miR-146a as a negative regulator of the TLR4 pathway through inhibition of TNF associated factor 6 (\textit{TRAF6}) and IL-1 receptor-associated kinase 1 (\textit{IRAK1}) expression \parencite{Taganov2006}. TRAF6 and IRAK1 are adaptor molecules involved in the activation of kinases that eventually lead to translocation of NF-$\kappa$B and AP-1 into the nucleus. Opposite direction of change was observed in the two comparisons here. The up-regulation of \textit{MIR146A} in psoriasis tCD8$^+$ compared to controls is in line with findings in serum from SLE and early RA patients \parencite{Wang2012,Filkova}. In contrast, trancriptomic studies using PBMCs from plaque psoriasis and also similar studies in RA (including PBMCs, SFMCs and CD4$^+$ isolated from both tissues, amongst others) have reported increased levels of miR-146a in patients when compared to controls \parencite{Ele-Refaei2015,Churov2015}. Conversely, the up-regulation of \textit{MIR146A} expression in lesional epidermis compared to uninvolved has been observed in other studies, and was also shown to be increased in lesional skin versus healthy biopsies \parencite{Lerman2014, Tsoi2015,Li2014}. One of the predicted gene targets of miR-146a, the TF \textit{NFAT5} was down-regulated in lesional skin and showed significant negative correlation with \textit{MIR146A} expression. Interestingly, up-regulation of \textit{NFAT5} has been reported in RA SF and has a role in mediating angiogenesis and proliferation of synoviocytes \parencite{Han2017}. Although in disagreement with the observations in PBMCs, the down-regulation of miR-146a levels in tCD8$^+$ cells would support failure of one of the check-points controlling sustained inflammation and the subsequent pathophysiological implications. In contrast, the up-regulation observed in lesional skin would not fit directly with the dysregulated inflammatory response in skin.

Other dysregulated non-coding RNAs in lesional epidermis relevant to psoriasis pathophysiology were \textit{HG19} and \textit{MIR31HG}, down- and up-regulated respectively in my data. Both non-coding RNAs were also differentially expressed in Tsoi \textit{et al.}, 2015 and are involved in KC differentiation. In particular, silencing miR-31hg in the KC immortal cell line HaCaT induced cell cycle arrest and inhibited cell proliferation, consistent with two characteristic aspects dysregulated in psoriatic KCs \parencite{Gao2018}.

Overall, the lncRNA differential analysis conducted in this chapter gives an overview of dysregulation in blood and skin of psoriasis patients. A more comprehensive analysis could be performed to identify putative targets for all the identified lncRNAs. Those interactions could then be used to identify relevant biological processes through network and pathway analysis using only those dysregulated lncRNAs matching dysregulated target genes, similarly to the strategy used by Dolcino \textit{et al.,} 2018. However, such an analysis would likely require increased sample size to be appropriately powered.

\subsection{Differences in transcriptional dysregulation in blood and skin}

Comparison of the dysregulated genes in circulating immune cells and in psoriatic skin revealed very limited overlap. Overall, FCs in the epidermis appeared to be larger than in circulating immune cells, likely due to a more profound pro-inflammatory environment driving gene expression changes in skin. CD14$^+$ monocytes and tCD8$^+$ cells showed the greatest overlap of DEGs with genes dysregulated between lesional and uninvolved epidermis (37 genes). However, almost half showed opposite directionality across the two comparisons. This is in line with the finding of Coda and colleagues when comparing DEGS genes in psoriasis patients and controls PBMCs to genes dysregulated between lesional and uninvolved skin biopsies.

Genes showing opposite change in circulation and in skin included the GWAS gene \textit{TNFAIP3}, \textit{EGR2}, and \textit{EGR3}. As previously mentioned, \textit{TNFAIP3} down-regulation in lesional skin may reflect complete loss of an NF-$kappa$B pathway check-point to control and terminate the inflammatory response at the site of inflammation. Similarly, \textit{EGR2} and \textit{EGR3} are pivotal for control of inflammation and antigen-induced proliferation. Importantly, loss of \textit{EGR2} and \textit{EGR3} expression leads to hyperactive STAT1 and STAT3 signalling, associated with SLE pathophysiology \parencite{Li2012}. Down-regulation of \textit{EGR2} and \textit{EGR3} was not observed in Tsoi’s study, and in Tervaniemi’s data \textit{ERG3} appeared to be up-regulated in lesional skin. In addition to its role in regulating inflammation, down-regulation of \textit{EGR2} in skin may also increase KC proliferation as has been shown in certain types of cancer \parencite{Wu2010}.

Differences are also observed in the distinct enriched pathways. For example, DEGs in skin not only showed enrichment for immune-related functions but also highlighted a metabolic dysregulation that appears to be characteristic of this site of inflammation. Moreover, immune related pathway such as NOD-like signalling also seemed to be specific for the dysregulated gene signature in skin. Likewise, up-regulation of genes from the \textit{S100} family in lesional skin, such as \textit{S100A7}, \textit{S100A8}, \textit{S100A9}, contributed to enrichment of IL-17 signalling and appeared to be a feature of dysregulated inflammation only in skin. Notably, these genes had also been reported as a specific hallmark of skin inflammation when compared to inflamed synovium from matched PsA patients, supporting the better outcomes for IL-17 antagonists in skin lesions compared to the inflammation of the synovium \parencite{Belasco2015}.

\subsection{Fine-mapping and the chr2p15 locus}

Fine-mapping of GWAS loci is one strategy to narrow down putative functionally relevant variants identified by GWAS studies. Using summary statistics from the psoriasis Immunochip GWAS GAPC cohort, I performed fine mapping for x of the genome-wide significant loci. Integration of the credible set of SNPs from each fine-mapped loci with DARs and differentially H3K27ac modified regions did not reveal any overlap. I therefore considered overlap with all ATAC peaks. Similar approaches integrating fine-mapping SNPs and tissue-specific chromatin accessibility maps have led to successful prioritisation of putative causal variants in other diseases. For example, in T2D only one SNP from the credible set located at a \textit{TCF7L2} intron overlapped FAIRE-seq accessible chromatin, with the risk allele showing greater abundance at open chromatin and increased enhancer activity \parencite{Gaulton2010, Stefan2014}

One of the particularly interesting psoriasis GWAS associations is the chr2p15 locus, where the lead SNP is located in an intergenic region 140Kb and 150Kb away from \textit{B3GNT2} and \textit{TMEM1} respectively. Although fine-mapping at chr2p15 failed in psoriasis (probably due to lack of power), fine-mapping analysis using AS Immunochip GWAS genotyping data yielded a credible set of three SNPs, of which only rs4672505 overlapped a tCD8$^+$-specific ATAC peak. Chromatin accessibility varied across individuals unrelated to disease status, with complete ablation of the ATAC peak in some individuals. Integration of genotyping data with ATAC revealed allele-dependent chromatin accessibility, with ATAC reads negatively correlated with the risk allele.

Promoter capture-C data linked rs4672505 to the \textit{B3GNT2} promoter only in tCD8$^+$ cells, suggesting the accessible chromatin at rs4672505 may be highlighting an enhancer element interacting with \textit{B3GNT2} promoter as a priming event \parencite{Javiere2016}. This regulation may only occur under pro-inflammatory stimuli through recruitment of TFs such as STAT1 or RUNX3, found to be binding at this location in lymphocytic cell lines. \textit{B3GNT2} is a major polylactosamine synthase involved in the post-translational modifications of carbohydrate chains, which are essential for cell–cell, receptor–ligand and carbohydrate–carbohydrate interactions. Interestingly, \textit{B3GNT2} knock-out mice demonstrated more sensitive and strongly proliferating T cell and B cell responses to stimulation compared to wild-type \parencite{Togayachi2010}. In T cells, this effect was linked to a reduction of polylactosamine chains in co-stimulatory accessory molecules such as CD28, overall leading to enhanced initiation of the immune response \textit{in vitro}. Up-regulation of \textit{B3GNT2} in this context could be contributing to attenuation and modulation of tCD8$^+$ activation. Under this scenario, presence of the risk allele (A) at this stimulus-specific enhancer could increase risk of disease by reducing chromatin accessibility, in both homozygous and heterozygous individuals. In fact, the down-regulation of \textit{B3GNT2} expression in tCD8$^$ cells from psoriasis patients compared to controls may be the result of the majority of the patients being AA homozygous (1 out of 8) or heterozygous (6 out of 8). Nevertheless, the observation of heterozygous individuals with either presence or absence of the peak in both phenotypic groups may suggest additional mechanisms influence the epigenetic landscape at this location, such as other environmental cues interacting with the genotype. Formulation of a more comprehensive and accurate model to further explain the functional role of rs4672505 in psoriasis susceptibility will require additional work, such as increasing the sample size to acquire more homozygous individuals for the risk allele, studying chromatin accessibility and \textit{B3GNT2} expression in relation to rs4672505 genotype in stimulated tCD8$^+$ and performing EMSA for relevant TFs.

\subsection{Limitations in the approach and future work}

Although the work in this chapter has shed light on the chromatin landscape and gene expression in psoriasis in a cell type and tissue specific manner, a number of limitations are noted. Due to difficulties in optimising ATAC protocols to yield good quality data, mapping chromatin accessibility in lesional and uninvolved KCs was not achieved. This may have revealed larger differences in chromatin accessibility between psoriasis patients and controls compared to circulating immune cells, as in other studies performed in affected tissues \parencite{Scharer2016, Wang2018}. Additionally, chromatin and transcriptomic profiles from skin infiltrated cells could be generated using FACS or single-cell technologies to better understand the changes in chromatin accessibility and gene expression driven by the inflammatory stimuli at the site of inflammation. Moreover, generating this data would also allow comparison to the profiles obtained in blood to better understand disease pathophysiology.

Other limitations in this study include its relatively small sample size, lack of genotyping data and skin biopsies only being available for three patients in the cohort. These limitations are intrinsic to time and project budget constraints and will be addressed as the study continues. Recruitment of additional patients would allow validation of the findings described in this chapter. Genotyping data would permit the study of chromatin accessibility in a genotype-specific manner, using the current samples with prospective integration of chromatin conformation data \parencite{Kumasaka2018}. Importantly, this will enable exploration of changes in chromatin accessibility at GWAS loci in combination with fine-mapping, similarly to the chr2p15 locus analysed in this chapter. Furthermore, new sample recruitment could be used to study chromatin accessibility and gene expression in additional cell populations sorted by FACS and also to include \textit{in vitro} stimulations. Overall, this strategy would allow better characterisation of the differences and similarities between patients and controls in context-specific regulatory elements \textit{in vivo} and \textit{in vitro} \parencite{Peeters2015}.

Finally, improvements in analytical methods will also be required to ascribe chromatin accessibility changes in enhancers to target genes potentially regulated by these regions. These could involve a more systematic integration of available chromatin conformation data, eRNA FANTOM data and also use of analytical models and tools currently available or that may be further developed in the future to specifically address this challenge \parencite{Wang2016,Cao2018}.

\subsection{Conclusions}

In this chapter, use of the latest epigenetic methodologies (as established in the previous chapter) together with gene expression profiling has allowed characterisation of the regulatory landscape in relevant cell types isolated from psoriasis patients and healthy individuals. Minor differences in chromatin accessibility and H3K27ac modifications between psoriasis and healthy controls have been identified in circulating immune cells. Conversely, a number of relevant biological processes dysregulated in the context of psoriasis have been shown at the transcriptional level both, in circulating cells and in psoriatic epidermis. Moreover, this chapter illustrates how GWAS signals may be interpreted through integration of multiple data types. Overall, the protocols established and data generated in this chapter provide a valuable resource that may be built upon in future work.