\section{Discussion}

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

In this chapter, the use of the latest epigenetic methodologies has allowed the characterisation of the chromatin landscape in relevant immune cells isolated from blood of psoriasis and healthy individuals. Comparison of chromatin accessibility and H3K27ac histone modifications has revealed a low number of differential regions between patients and controls in the four cells types under study. Both epigenetic features revealed CD14$^+$ monocytes and tCD8$^+$ cells as those presenting the largest number of discrete changes. One of the regions found to be more enriched in H3K27ac in CD14$^+$ monocytes from patients compared to controls was nearby \textit{SLC15A2} and \textit{ILDR2} genes. SNPs harboured in that region are LD SNPs for a MS GWAS signal and eQTL data has linked this region to the putative regulation of the calmodulin-binding motif-containing protein \textit{IQCB1} gene, 186.4Kb up-stream of this peak, in monocytes and tCD4$^+$ cells \parencite{GTeX,Fairfax2014, Kasela2017}.

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, also overlapping FANTOM eRNA in the same cell type. Both genes are well known for being pro-inflammatory and involved in chronic inflammatory diseases. For example, \textit{TNFSF11} is downstream the lead SNPs of a CD risk locus the 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 an overlapping region at an intron of the \textit{DTD1} gene, described to play a role in the initiation of DNA replication and associated with aspirine-intolerance in asthmatics but with currently lacking evidence of involvement in chronic inflammation \parencite{Pasaje2011}. The lack of overlap between DARs and differentially H3K27ac modified regions could be expected given the fact that chromatin accessibility is driven by the interaction between a number of histone modifications, TFs binding to specific sites as well as structural proteins, such as CTCF \parencite{}?

Conversely to my observations, recent similar studies performing ATAC in primary cells and tissues isolated from clinical samples have revealed larger differences in the chromatin accessibility landcape of B cells from SLE patients and in retina and retinal pigmented epithelium from AMD patients \parencite{Scharer2016,Wang2018}. The investigation of the main cell type and tissue affected by inflammation in these two studies may partly explain the more profound changes observed in the chromatin landscape of patients compared to controls. Similarly, H3K27ac mapping in mCD4$^+$ cells isolated from juvenile idiopathic arthritis SF demonstrated approximately thousand differential enhancers when compared to healthy control circulating cells \parencite{Peeters2015}. Conversely only discrete differences were found when comparing mCD4$^+$ from PB of patients and controls, highlighting the location of specific disease signature at the site of inflammation. Moreover, the complex nature of disease, where different patients have different genetic backgrounds, also shared with control individuals may require studying differences in chromatin accessibility in the context of genotype and maybe under exogenous inflammatory stimuli that may manifest those differences \parencite{Alasoo2018,Calderon2018}

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

Comparison of the gene expression between psoriasis and healthy controls in a cell type specific manner identified larger number of DEGs compared to DARs or differential H3K27ac modifications. This may suggest a lack of lineal relationship between the transcriptomic changes captured in this cohort and the priming epigenetic modifications at regulatory regions \parencite{Alasoo2016,other paper}. The magnitude of changes in expression was moderate, with FCs not exceeding 1.6. Similarly to ATAC and ChIPm, CD14$^+$ monocytes and tCD8$^+$ cells showed the largest number of transcriptomic changes in disease (699 and 687 DEGs FDR$<$0.05, respectively). This may suggest the grater relevance of these two cell types in the systemic “footprint” of psoriasis. The more dysregulated gene expression response between patients and controls found in circulating psoriasis tCD8$^+$ when compared to the tCD4$^+$ may suggest the same hypothesis as in skin, where CD8$^+$ are considered the main effector upon induced-activation by CD4$^+$ cells \parencite{Nickoloff1999}. The importance of monocytes/macrophages in psoriasis has also been proven by their presence in psoriatic skin and TNF-$\alpha$ production contributing towards maintenance of inflammation \parencite{Nickoloff2000,Wang2006}. Similar numbers of DEGs in tCD4$^+$ and CD19$^+$ cells may reflect the relevance of B cells in psoriasis shown by recent studies \parencite{Yanaba2013}. The overlap of DEGs with previous studies comparing PBMCs from psoriasis patients was limited, probably due to many differences masked by the admixture of cells as well as the use of micro arrays instead of RNA-seq \parencite{Lee2009,Coda2012}.

Previous studies comparing PBMCs from patients and controls did not find specific enrichment for any pathway; however Coda \textit{et al.} found some of the genes to be associated with pathophysiological processes implicated with psoriasis such as immune response, oxidative stress or apoptosis. The cell type specific analysis conducted in my thesis identified significant enrichment of relevant biological processes for DEGs in the CD14$^+$ monocytes and tCD8$^+$ cells contrast between patients and controls. Enrichment for MAPK and IL-12 signalling was shared by both, CD14$^+$ monocytes and tCD8$^+$ cells. 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, which leads 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. Interestingly, another member of the STAT TF family, \textit{STAT2} was found to be down-regulated in psoriasis PBMCs when compared to controls by Coda \textit{et al.} and also in AS monocyte-derived macrophages \parencite{Smith2008}. STAT4 has mainly been described to have a role in mediating T cell activation through IL-23 and IL-12. Research has also demonstrated that monocytes fail to express \texit{STAT4} in basal conditions but up-regulation follows upon IFN-$\alpha$ stimulation \parencite{Frucht2000}. Similarly, STAT5 phosphorylation in monocytes is induced by granulocyte macrophage-colony stimulating factor (GM-CSF), which leads to activation of monocytes and differentiation into macrophages has been shown to be persistently increased in circulating monocytes isolated from T2D upon GM-CSF stimulation \parencite{Litherland2005}. Further investigation at the protein level would be require to determine if the the down-regulation of \textit{STAT4} and \textit{STAT5} observed in psoriasis CD14$^+$ monocytes is biologically relevant.

In tCD8$^+$, expression of \textit{IFNG}, one of the down-stream pro-inflammatory cytokines from the IL-12 signalling pathway, was also 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 as well as in a SpA rat model \parencite{Smith2008,Fert2014}. This down-regulation was accompanied by an overall “inverse” transcriptional response of IFN-regulated genes, which has not been found in my data. Nevertheless, IFN-$\gamma$ dysregulation has been broadly studied in SpA, where for example lower levels of IFN-$\gamma$ have been found in SF from SpA patients compared to RA \parencite{}. 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 have an resulting pro-inflammatory effect.

Only in tCD8$^+$ DEGs between psoriasis patients and controls 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 very well characterised negative immunoregulatory genes. This was the case of \textit{NFKBIA} and \textit{TNFAIP3}, also up-regulated in CD14$^+$ monocytes and CD4$^+$ cells, respectively, and both genes associated with psoriasis GWAS signals. \textit{NFKBIA} is an inhibitor of NF-$\kappa$B that binds to the NF-$\kappa$B subunits preventing it from translocation to the nucleus. Similarly, \textit{TNFAIP3} codes for the zinc finger protein and ubiqitin-editing enzyme A20 and its expression is up-regulated in the presence of inflammation and NF-$\kappa$B activation in order to inhibits both NF-$\kappa$B TNF-mediated response and return to homeostasis. Neither \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.}. Dysregulation of these two genes also failed to be shown in a study comparing PBMCs from PsA patients versus controls \parencite{Dolcino2015}. Polymorphisms within \textit{TNFAIP3} or in the vicinity have been associated with a number of chronic inflammatory diseases including MS, RA, SLE and T1D \parencite{Vereecke2011}. 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 controls PBMCs. This would be in line with my findings with the caveat that all patients from my cohort would be classified as severe according to Jian \textit{et al.}. Therefore, the sustained expression of \textit{TNFAIP3} may not be unexpected as it reflects persistent inflammatory stimuli in psoriasis PB and limitation of the inflammatory response to some extent by up-regulation of inflammatory inhibitors \parencite{Idel2003}.

Regarding the DEGs within the chemokine pathway, one of the more relevant was the up-regulation of \textit{CCR10} in tCD8$^+$ cells from psoriasis patients. Some studies have demonstrated an increase of CCR10$^+$ infiltrated T lymphocytes in psoriasis \parencite{Homey2002}. 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), which are preferentially recruited to cutaneous sites of inflammation where KCs express \textit{CCR10} ligand \textit{CCL27} \parencite{Hudak2002}. A study in psoriasis circulating cells revealed a correlation between the frequency of CTLA$^+$ CD8$^+$ cells and disease severity measured by PASI score \parencite{Sigmundsd\'{o}ttir2001}.

Altogether, this data has 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, the overall effect of those interactions in the inflammatory response is difficult to predict.

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

In this chapter, greater changes in gene expression compared to chromatin accessibility have been identified in circulating immune cells between patients and controls, with none or one DAR in CD14$^+$ monocytes, tCD4$^+$ and CD19$^+$ cells. Strikingly, in tCD8$^+$ cells, 687 genes were differentially expressed between psoriasis and healthy controls but only 53 regions showed differential chromatin accessibility when performing the same contrast. Correlation between chromatin accessibility measured by ATAC and gene expression has been reported to some extent in a number of studies, with the limitation of establishing relationship between enhancer regions and the regulated target \parencite{}. Out of the 687 DEGs between psoriasis and controls in tCD8$^+$ cells, only six were proximal to a DAR. This poor overlap could be the result of a strong manifestation of non-lineal changes in chromatin accessibility and gene expression in psoriasis circulating immune cells, a phenomenon that has been demonstrated for genotype-dependent variation across different stimuli \parencite{Alasoo2018}. Nevertheless, the discrepancies here observed between dysregulation of chromatin accessibility and gene expression are more likely to be due to lack of sensitivity of ATAC to identify changes in chromatin accessibility that have an impact in gene expression.

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}. Nevertheless, 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}

Study of differences in the transcriptomic profile between lesional and uninvolved skin was conducted for three of the psoriasis patients in my cohort. Most of the previous transcriptional studies in psoriasis have used full thickness skin biopsies, formed by 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 great differences in gene expression between whole biopsies and FACS-isolated KCs, which may be masking KCs pathophysiological relevant differences in a large number of previous studies using psoriasis skin biopsies \parencite{Ahn2016}. In this chapter, contrast of RNA-seq expression between paired lesional and uninvolved skin was conducted on epidermal sheets isolated from whole biopsies and a total of 1,227 DEGs were identified. Comparison with Tervaniemi \textit{et al.} study performing contrast in 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. Interestingly, the overlap with Tsoi \textit{et al.} study using whole biopsies was greater (505) and only 5 genes appeared opposite direction of change, in contrast with the 75 showing discrepancies with Tervaniemi’s study. The unexpected greater overlap with Tsoi study could be just the result of larger number of DEGs identified in this study, probably due to a much larger sample size compare to Tervaniemi and my cohort. Some of the genes consistently up-regulated across the three studies included \textit{STAT1} and genes from the \textit{S100A} family. The \textit{S100} family are located within the epidermal differentiation complex within ch1p21 acting as calcium sensors and maybe also having a chemotactic effect \parencite{}. Particularly, \textit{S100A9} and \textit{S100A12} undergo up-regulation in psoriasis \parencite{Broome2003}. Moreover, \textit{S100A12} has been characterised as one of the ligands for receptor for advanced glycation endproducts (RAGE), which in T cells is involved in their proliferative response and IFN-$\gamma$ and IL-2 production \parencite{Moser2007}. One interesting observation made across all three studies was the up-regulated expression of \textit{LCE3B} in lesional skin compared to uninvolved. \textit{LCE3B/C}\\_\textit{del}, ones of the psoriasis GWAS associations, is found in approximately 60 to 70\% of the European psoriasis patients \parencite{Cid2009}. As explained in Chapter \ref{ch:Intro}, \textit{LCE} genes expression is induced upon disruption of skin barrier and expression of \textit{LCE3B} and \textit{LCE3D} has been only detected in lesional but not uninvolved psoriatic skin of individuals harbouring the deletion in heterozygosis \parencite{Cid2009,Bergboer2011}. This may suggest the three psoriasis patients in the study being heterozygous.

Pathway enrichment analysis for the DEGs between lesional and uninvolved skin revealed a number of relevant biological processes in psoriasis pathophysiology. Some of the enriched pathways highlighted alterations in cell cycle and metabolic processes, including arginine and proline metabolisms, glycolysis, metabolisms of amino acids 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}.

A number of pathways were related to alterations in cell cycle and metabolic processes, including hypoxia-inducible factor 1 (HIF-1) signalling, arginine and proline metabolism,glycolysis/gluconeogenesis and metabolism of amino acids and derivatives. Dysregulation of similar functions have previously also been reported in other studies comparing lesional and uninvolved skin and genome-wide pathway analysis\parencite{Coda2012, Gudjonsson2010,Aterido2016, Tervaniemi2016}. Hypoxia and increased rate of cell proliferation are likely the cause of gene members of the HIF-I pathway. HIF-I pathways is also involved in regulating Th-17/Treg ratios and therefore in perpetuation and termination of the immune response \parencite{Dang2013}. Furthermore, up-regulated expression of the hypoxia-inducible TFs HIF-1$\alpha$ and HIF-2$\alpha$ has been found in lesional skin and co-related with the increase in \textit{VEGF} transcript levels, a target gene regulated by HIFs that mediates the pathological angiogenesis driving psoriasis \parencite{Rosenberg2007}. Nevertheless, no correlation was observed between \textit{HIFA} and \textit{VEGF} in my data, likely due to the small sample size.

Immune-related pathway enrichment was also found in my analysis, including Th-17, IL-12 and cytokine-cytokine and NOD-like signalling. Interestingly, NOD-like signalling was found to be enriched for DEGs between lesional and uninvolved skin in a contemporary study by Tervaniemi and colleagues \parencite{Tervaniemi2016}. When compared to Tsoi data, Tervaniemi and my study identified more genes from the NOD-like signalling pathway, which had not been previously identified as a dyregulated pathway in psoriatic lesional skin. Tervaniemi’s mainly attributed this new pathway to the greater sensitivity of RNA-seq compared to micro-arrays to detect changes in gene expression for genes involved in this pathway. The fact that Tsoi \textit{et al.} data has also been generated using RNA-seq and did not show enrichment for NOD-like signalling, suggest that the greater sensitivity to identified genes from this pathway is due to the study of epidermis instead of whole biopsies. NOD-like signalling involved 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 patway \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. Previous studies have also revealed dysregulation of synthesis of phospholipids and triglycerides genes in psoriasis and atopic dermatitis \parencite{Martel2016}. 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}. In KCs expression of the different PPAR members varies during differentiation \parencite{Rivier2018}. In psoriatic lesional skin, \textit{PPARD} has been found to be up-regulated 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 both, circulating immune cells and epidermis, have revealed dysregulation of a number of lncRNAs. The role of lncRNAs in disease, and particularly in chronic inflammatory diseases, have been studies RA, SLE and AS, amongst others \parencite{Muller2014,Shi2014,Zhang2017}. No study has been conducted to identify differentially lncRNAs in a cell type-specific manner in psoriasis PB. Recently, a study characterising the non-coding RNAs using micro-array technology in PsA PB has been published \parencite{Dolcino2017}. Conversely, several studies have been carried out to contrast 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. %A number of dysregulated lncRNAs in epidermis from my analysis were also identified to be dysregulated between lesional and healthy controls in other studies \parencite{Li2014, Gupta2016}. One of the

Characterisation of lncRNAs biological functions is a field in progress and the majority of dysregulated lncRNA transcripts lack biological meaning, which represents a limitation when interpreting these results \parencite{Uszczynska-Ratajczak2018}. Some of the well-characterised dysregulated lncRNAs had a role in the immune response. For example \textit{DYNLL1-AS1}, which has a role in down-regulation of ISGs through histone modifications upon viral infection, showed lower expression in psoriasis CD14$^+$ monocytes when compared to controls \parencite{Ouyang2014}. However, down-stream regulated genes such as \textit{IFITM3} and \textit{MxA} or any IFN genes were dysregulated in my data in this cell type. Conversely, for the up-regulation of \textit{HOTAIRM1} in psoriasis CD14$^+$ monocytes down-regulation of the predicted target gene \textit{USP1} was identified, opposite to the observation made by Dolcino \textit{et al.}, 2018 study. \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 mechanisms 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 and which knock-down demonstrated impairment of TLR4 signalling and down-regulation of inflammatory genes including IL-6 and CXCL10 \parencite{Zhang2016}. Nevertheless, none of those two genes has been found to be dysregulated in my study in this cell type.

Interestingly, \textit{MIR146A} was identified as 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 TLR-4 pathway through inhibition of \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 and subsequent transcriptional up-regulation of inflammatory genes. Interestingly, opposite direction of change was observed in each of the comparisons. 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 targests of miR-146a , the TF \textit{NFAT5} appeared 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 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.

Another dysregulated non-coding RNAs in lesional epidermis and with a relevant role in 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 study and are involved in KCs differentiation. Particularly, miR-31hg has been demonstrated to facilitate certain types of cancer progression through inducing expression of \textit{HIF-1A} and \textit{P300} \parencite{Wang2018}. Furthermore, silencing miR-31hg in KC immortal cell line HaCaT induced cell cycle arrest and inhibited cell proliferation, consistently with two characteristic aspects dysregulated in psoriatic KCs \parencite{Gao2018}.

Altogether, the lncRNAs differential analysis conducted in this chapter has presented an overview dysregulation in blood and skin of psoriasis patients. However, 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.

\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 differentially expressed genes in epidermis appeared to be larger than those found 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 those genes dysregulated between lesional and uninvolved epidermis (37 genes). However, almost half of them showed indeed opposite direction in the dysregulation across the two comparisons. This is in line with the finding of Coda and colleagues when comparing DEGS genes between psoriasis patients and controls PBMCs to those genes dysregulated between lesional and uninvolved skin biopsies. In fact, out of the 153 DEGs found in PBMCs only 30 genes overlapped the skin comparison and 17 showed opposite direction of change.

Genes showing opposite change in circulation and in skin included the GWAS hit \textit{TNFAIP3} and other genes such as \textit{EGR2} and \textit{EGR3}. As, previously commented for \textit{TNFAIP3}, its 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} have been described to have pivotal role for control of inflammation and antigen-induced proliferation. Importantly, loss of \textit{EGR2} and \textit{EGR3} expression led to hyperactive STAT1 and STAT3 signalling, associated to SLE pathophysiology \parencite{Li2012}. The down-regulation observed for \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 KCs proliferation as it has been shown in certain types of cancer \parencite{Wu2010}.

%Interestingly, genes coding for the three most widely used drug target inhibitors, TNF-$\alpha$, IL-17 and IL-6 did not appear to be dysregulated either in circulating immune cells or in lesional epidermis.

Differences in the dysregulated transcriptional profile in blood and skin may also be reflected the 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 in lesional skin of genes from the \textit{S100} family, such as \textit{S100A7}, \textit{S100A8}, \textit{S100A9}, contributed to enrichment of IL-17 signalling in this tissue 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 results of IL-17 antagonists in skin lesions than in the inflammation of the synovium \parencite{Belasco2015}.

\subsection{Fine-mapping and the chr2p15 locus}

Fine-mapping of GWAS loci is one of the strategies to further narrow down the putative functionally relevant variants identified by GWAS studies. In absence of genotyping data, methods have been developed in order to impute summary statistics from missing genetic variants and include them in the association analysis. Using summary stats 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 of the fine-mapped loci with the discrete number of DARs and differentially H3K27ac modified regions did not reveal any overlap. One of the particularly interesting psoriasis GWAS associations is the chr2p15 locus, which lead SNP is located at an intergenic regions 140Kb and 150Kb away from \textit{B3GNT2} and \textit{TMEM1}, respectively. Chr2p15 was also identified as a risk locus for AS and CD, independently, and was also identified in a cross-disease GWAS study as one of the multi-disease signal shared by the three phenotypes \parencite{Ellinghaus2016}.

Although psoriasis summary statistics fine-mapping at chr2p15 failed (probably due to lack of power) , fine-mapping analysis using genotyping AS Immunochip GWAS yielded a credible set of three SNPs where only rs4672505 overlapped a tCD8$^+$ ATAC peak not present in the other cell types in my data. At rs4672505 location, chromatin accessibility varied across individuals in a disease status unrelated way, with complete ablation of the ATAC peak in some of the individuals. Integration of genotyping data with ATAC revealed allele dependent-chromatin accessibility, with negative correlation for the risk allele. Similar approaches integrating fine-mapping SNPs and tissue-specific chromatin accessibility maps have also 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 FAIR-seq accessible chromatin, with the risk allele showing greater abundance at open chromatin and increased enhancer activity \parencite{Gaulton2010, Stefan2014}

Regarding the target gene which gene expression may be regulated by rs4672505, promoter capture-C data linked rs4672505 to the \textit{B3GNT2} promoter only in tCD8$^+$ cells \parencite{Javiere2016}. \textit{B3GNT2} gene 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 response 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}. The accessible chromatin at rs4672505 may be highlighting an enhancer element which is interacting with \textit{B3GNT2} promoter as a priming event. %This is supported by the presence of a promoter capture-C interaction in unstimulated cells \parencite{Javiere2016}.

This ATAC peak harbouring rs4672505 may be an enhancer that only regulates \textit{B3GNT2} expression under pro-inflammatory stimuli through recruitment of TFs such as STAT1 or RUNX3, found to be binding at this location in lymphocytic cell lines. 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 stimuli-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 more sophisticated mechanisms influencing 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. Future work will involve 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 a cell type and tissue specific manner in psoriasis, a number of limitations can be noted. Due to the impossibility of optimising ATAC protocols to yield good quality data, mapping chromatin accessibility in lesional and uninvolved KCs has not been achieved. This may have revealed larger changes in chromatin accessibility compared to the ones detected in circulating immune cells between psoriasis patients and controls, similarly to other studies performed in affected tissue \parencite{Scharer2016, Wang2018}. Additionally, chromatin and transcriptomic profiles from skin infiltrated cells should be generated using specific FACS approached 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 relatively small sample size, lack of genotyping data and skin biopsies only available for three patients in the cohort. These limitations are intrinsic to time and project budget constrain and will be addressed in the future. Availability of genotyping data will allow to perform 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 to explore changes in chromatin accessibility at GWAS loci in combination with fine-mapping, similarly to the proof of principle for 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 to better characterise the differences and similarities in regulatory elements specific of pro-inflammatory stimuli \textit{in vivo} and \textit{in vitro} between patients and controls \parencite{Peeters2015}.

Finally, improved in the analytical methods will also be required to ascribe chromatin accessibility changes in enhancer with target genes potentially regulated by these regions. That will involve a more systematic integration of available chromatin conformation data, eRNA FANTOM data and also analytical models and tools currently available or that will be further developed in the future \parencite{Wang2016,Cao2018}.

\subsection{Conclusions}

Nevertheless, this data has proven a systemic effect of gene expression dysregulation as the result of psoriasis pathophysiology that reflects alterations of relevant biological processes in the disease pathophysiology.

Refs for final discussion

-http://science.sciencemag.org/content/361/6409/1380.long

different strategies are being developed in order to address the limitation of linking enhancers to target genes.<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5501464/>

* Science paper bottom