001 Malat1 drives differentiation of memory CD8+ T cells
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Name CD8+ T cells to an infection can give rise to both effector cells that provide acute host defense and memory cells that provide long-lived immunity, but the transcriptional changes that regulate the differentiation of these distinct cell types remain unclear. Using single-cell RNA sequencing techniques applied serially to cells through the course of an in vivo infection, our lab has found that early differentiating cells exhibit striking transcriptional heterogeneity. Many molecular determinants of cell fate were identified, and we are now investigating the functional role of these regulators in the differentiation of memory or effector populations. We used a previously described in vitro assay to investigate candidate genes that were identified by our single-cell analysis. CD8+ T cells were isolated from LCMV-P14 T cell receptor transgenic mice. They were stimulated and then transduced with shRNA constructs that knock down candidate genes. The cells were incubated with cytokines known to drive them toward a memory or effector phenotypes. Cells were analyzed by flow cytometry for markers of effector and memory phenotypes (CD25 and CD69, respectively). When the cells were transduced with a shRNA construct targeting the transcription factor Malat1, a long non-coding RNA highly expressed in tumors, cells exhibited an effector-like phenotype (CD25hiCD69hi), raising the possibility that Malat1 may play a role in memory cell development. Thus, the in vitro assay used here may be a useful tool to screen candidate genes identified by single-cell RNA-seq analysis. Future studies will include single-cell analysis of the differentiation of tissue resident memory CD8+ T cells, a subset of memory cells recently identified in the skin that may play an important role in allergic contact dermatitis and eczema.

003 Peripheral blood transcriptomics combined with feature analysis reveals five novel potential therapeutic targets in vitiligo
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Significant transcriptional deregulation and alterations in the systemic environment relevant to vitiligo (VL) where loss of self-tolerance leads to the targeted killing of melanocytes. We undertook a genome-wide profiling approach to examine gene expression in peripheral blood of VL patients and healthy controls in the context of our previously published VL skin transcriptional profile. We used several in silico bioinformatics-based analyses to provide new insights into disease mechanisms. Unbiased clustering methods of the VL-blood gene expression dataset demonstrates a specific set of co-expressed genes driven by "disease-state". Ontology enrichment analysis of differentially expressed genes (DEGs) uncovers a down-regulation of both innate and adaptive immune/inflammatory response in vitiligo (VL-blood) as opposed to the activation of cytoskeletal remodeling, oxidative stress and apoptosis previously noted in VL-skin. Our data also showed differential expression of regulator genes that are implicated in melanocyte survival and drug resistance with tightly regulated mechanisms linked to disease-related pathways. There is evidence for both type I and II interferon (IFN) playing a role in VL pathogenesis. We used interactome analysis to identify several key blood associated transcriptional factors (TFs) from within (STAT1, STAT6 and NF-kB) as well as "hidden" (CREB1, MYC, IRF4, IRF7, and TPS3) from the dataset that potentially affect disease pathogenesis. The TFs overlap with our reported lesional-skin transcriptional circuitry, underscoring their potential importance to disease. We also identity a shared VL-blood and -skin transcriptional "hot spot" that maps to chromosome 6, and includes three VL-blood DEGs (PSMB8, PSMB9 and TAP1) previously reported as potential VL-associated genetic susceptibility loci. Finally, we provide bioinformatics-based support for prioritizing five dysregulated genes (STAT1, PRKCD, PTPN6, c-MYC, and FGFR2) in VL-blood/skin as potential therapeutic targets.

004 Autoantigens ADAMTS5 and LL-37 are significantly upregulated in active psoriasis and associated with dendritic cells and macrophages
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Psoriasis is a common immune-mediated disease that affects 2-4% of individuals in North America and Europe. In the past decade, advances in research has led to a better understanding of immune pathways involved in the pathogenesis of psoriasis and has spurred the development of targeted therapeutics. Recently, three auto-antigens have been discovered in psoriasis: (1) Cathelicidin (LL-37), (2) A Disintegrin and Metalloprotease Domain containing Thrombospondin Type 1 Motif-like 5 (ADAMTS5), and (3) Phospholipid A2 (PLA2G4D). The expression, regulation, and therapeutic modulation of these autoantigens is important to establish. In this study, we performed immunohistochemistry and two-color immunofluorescence on non-lesional and lesional skin to characterize ADAMTS5 and LL-37, and their co-expression with CD3+ T-cells, CD11c+ dendritic cells and CD163+ macrophages, which are the main immune cells that drive this disease. Our results showed that ADAMTS5+ and LL-37+ cells are significantly (p < 0.05) increased in lesional skin and are co-expressed by many dendritic cells, macrophages, and some T-cells in the dermis. Gene expression analysis showed significant (p < 0.05) upregulation of LL-37 in lesional skin and significant down-regulation with Etanercept treatment. ADAMTS5+ and LL-37+ cells are also significantly decreased by IL-17 or TNF blockade, suggesting feed forward induction of psoriasis auto-antigens by disease-related cytokines.

005 Fli1-deficient B cells induce scleroderma-like vascular disorganization via activating pro-angiogenic gene program in dermal microvascular endothelial cells – A possible role in sclerodema vasculopathy
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Aberant B cell activation is implicated in the development of systemic sclerosis (SSc), and B cell depletion is effective especially for SSc-related interstitial lung disease (SSc-ILD). Moreover, vascular injury is one of the three cardinal features of this disease, which is implicated in SSc-ILD as well as digital ulcers and telangiectasias. Actually, B cell depletion treatment for SSc-ILD decreased serum marker of vascular involvement, such as angiopoietin-2, which reflects vascular aspects in the development of SSc-ILD. Fli1 is a potential deducing factor of SSc, whose deficiency in fibroblasts and endothelial cells is involved in its fibrotic and vascular processes respectively. Given that B cells from K/BxN-Fli1+ mice, spontaneously developing SSc-like tissue fibrosis and vasculopathy, represent SSc-like features, Fli1 deficient B cells may play a role in the immunological and pathological features of SSc as well as B cell activation, which we confirmed decreased Fli1 expression in SSc-defined B cells and further explored the role of Fli1-deficient B cells especially in vascular involvement of SSc utilizing B cell-specific Fli1 knockdown B cells. Here, Fli1-deficient B cells were in an aberrantly activated status, such as higher IL-6 secretion and autoantibody production. Interestingly, SSc-like vascular changes were evident in the skin of Fli1 BcKO mice. In in vitro study, the possible direct interaction with Fli1-deficient B cells activated pro-angiogenic gene program in endothelial cells. These data support that Fli1-deficient B cells induce pro-angiogenic gene program in endothelial cells, which may be a possible role in sclerodema vasculopathy.