001 Malat1 drives differentiation of memory CD8+ T cells

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In vitro addition to an in vivo model, 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 in vitro, we have shown lineage and immunophenotypic differences between effector cells that were identified by our single-cell analysis. CD8+ T cells were isolated from VL-p14 T cell receptor transgenic mice. They were stimulated and then transduced with shRNA constructs that knock down candidate genes. These 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 CD62L), respectively. When the cells were transduced with a shRNA construct targeting the transcription factor T-bet, which plays a central role in the differentiation of effector cells, they expressed more CD62L and less CD25, consistent with a memory-like phenotype. When cells were transduced with a shRNA construct targeting the candidate gene Malat1, a long non-coding RNA highly expressed in tumors, cells exhibited an effector-like phenotype (CD25+/CD62L-), 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 interactome analysis reveals five novel potential therapeutic targets in vitiligo

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Peripheral blood transcriptomics and interactome analysis 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 demonstrate a specific set of co-expressed genes driven by “disease-state.” Ortholog 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 in VL-skin. Our data deliver insight into disease-specific changes associated 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 TPS1) from the dataset that potentially affect disease pathogenesis. The Tfs overlap with our reported lesion-skin transcriptional circuitry, underscoring their potential importance to disease. We also identify a shared VL-blood and -skin transcriptional “hot spot” that maps to chromosome 6, and includes three VL-blood DEGPs (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, TAP1, c-MYC, and FGFR2) in VL-blood/skin as potential therapeutic targets.

004 Autoantigens ADAMTS1 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 therapies. 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 (ADAMTS1), and (3) Phospholipid A2 (PLA2G4D). The expression, regulation, and therapeutic modulation of these auto-antigens is important to establish. In this study, we performed immunohistochemistry and two-color immunofluorescence on non-lesional and lesional skin to characterize ADAMTS1 and LL-37, and their co-expression with CD3+ T-cells, CD11c+ dendritic cells and CD68+ macrophages, which are the main immune cells that drive this disease. Our results showed that ADAMTS1+ 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. ADAMTS1+ 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 sclerodema-like vascular disorganization via activating pro-angiogenic gene program in dermal microvascular endothelial cells – A possible role in scleroderma vasculopathy

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Aberrant B cell activation is implicated in the development of systemic sclerosis (SSc), and B cell depletion is effective specifically 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 scleroderma. 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 predilusory factor of SSc, whose deficiency in fibroblasts and endothelial cells is involved in its fibrotic vascular processes respectively. Given that B cells from K5-Fli1−/− mice, spontaneously developing SSc-like tissue fibrosis and vasculopathy, represent SSc-like features, Fli1 deficiency may also contribute to its immunological aspect, such as B cell activation. Here, we confirmed decreased Fli1 expression in SSc-derived B cells and further explored the role of Fli1-deficient B cells especially in vascular involvement of SSc utilizing B cell-specific Fli1 knockdown. Moreover, we found 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−/− mice. In in vitro study, the possible direct interaction with Fli1-deficient B cells activated pro-angiogenic gene program in endothelial cells. These data suggest that Fli1 deficiency in B cells and SSc-like vasculopathy, indicating a pivotal contribution of Fli1 deficiency in B cells to the development of SSc.

006 Anti-thyroid peroxidase reactivity in pemphigus is driven by HLA status and the absence of desmoglein reactivity

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We have recently shown that the autoimmune blistering skin disease Pemphigus vulgaris (PV) belongs to an established autoimmune disease cluster that includes autoimmune thyroid disease (AITD), rheumatoid arthritis and type 1 diabetes, supporting the hypothesis for common genetic elements across clinically distinct entities. Additionally, we have found that PV patients exhibit significant reactivity to the AITD-related auto-antibody thyroid peroxidase (TPO), and that patient derived anti-TPO antibodies affect signaling pathways in keratinocytes similar to anti-desmoglein (Dsg) antibodies. To extend our studies, we measured serum anti-TPO antibody levels by ELISA in 280 PV and 66 control samples stratified by genetic and clinical parameters. We confirm that PV patients have significantly higher activity rates for anti-TPO than healthy controls. Interestingly, patients that carry both the PV-associated HLA alleles DRB1*0402 and DQB1*0503, or DQB1*0503 alone exhibit low levels of anti-TPO (activity rate (A.R.) 9.5% and 4.8%, respectively); patients that type for neither allele, or DRB1*0402 and DQB1*0503 alone exhibit low levels of anti-TPO (A.R. 23.1% and 15.8%, respectively). Thus, the absence of DQB1*0503, regardless of the presence of DRB1*0402, is associated with the development of anti-TPO antibodies. Similarly, we observe a significant association between anti-TPO and anti-Dsg profiles, where anti-Dsg1/3-patients have a higher activity rate (26.9%) than anti-Dsg1/3-patients (18.8%), anti-Dsg1/3- (14.3%) and anti-Dsg1/3+ (3.9%). Our data suggest that specific combinations of established PV HLA-susceptibility alleles and perhaps those in linkage disequilibrium with them drive the selection of a common genetic background of TPO. These findings raise the possibility that PV patients may have common autoantibodies, with anti-TPO seemingly filling the gap in active patients that do not carry the established PV-associated autoantibodies and/or lacking the established PV-HLA-susceptibility alleles.