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WORKSHOPS

OP-108

BamQuery: A new proteogenomic tool to explore the immunopeptidome and validate tumor-specific antigens

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MHC class I–associated peptides (MAPs), collectively referred to as the immunopeptidome, have a pivotal role in cancer immunosurveillance. While MAPs were long thought to be solely generated by the degradation of canonical proteins, recent advances in the field of proteogenomics (genomically-informed proteomics) evidenced that ~10% of MAPs originate from allegedly noncoding genomic sequences. Among these sequences, the endogenous retroelements (EREs) are under intense scrutiny as a possible source of cancer-specific antigens (TSAs). With the increasing number of cancer-oriented immunopeptidomic and proteogenomic studies comes the need to accurately attribute an RNA expression level to each MAP identified by mass-spectrometry. Here, we introduce BamQuery (BQ), a computational tool to count all reads able to code for any MAP in any RNA-seq data chosen by the user, and to annotate each MAP with all available biological features. Using BQ, we found that most canonical MAPs can derive from an average of two different genomic regions, whereas most tested ERE-derived MAPs can be generated by numerous (median of 210) different genomic regions and RNA transcripts. We show that published ERE MAPs considered as TSA candidates can be coded by numerous other genomic regions than those previously studied, resulting in high undetected expression in normal tissues. We also show that some mutated neoantigens previously published as presumably specific anti-cancer targets can in fact be generated by other non-mutated, non-coding, widely expressed RNA-seq reads in normal tissues. We therefore conclude that BQ could become an essential tool in any TSA-identification/validation pipelines in the near future.

Keywords: Anti-cancer vaccine, big data, cancer immunopeptidome, immunological techniques, immunotherapy, omics technologies

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Discovery of citrullination of MMP-9 in rheumatoid arthritis synovial fluid

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Matrix metalloproteinase-9 (MMP-9) or gelatinase B is a zinc-dependent protease that modulates the extracellular matrix (ECM) in health and disease. In rheumatoid arthritis (RA), matrix metalloproteases have been associated with the breakdown of cartilage and joint inflammation. Evidently, elevated MMP-9 levels were found in RA patients at both mRNA and protein levels. However, since MMP activities greatly depend on posttranslational modifications, such as proteolysis and glycosylation, increased expression of MMP-9 cannot be directly extrapolated to enhanced activity. In light of this, we decided to perform in-depth characterisation of MMP-9 in synovial fluids of arthritis patients. We combined classic immunohistochemical methods (ELISA and Western blot) with orthogonal methods (zymographic analysis) to perform unbiased and detailed MMP-9 profiling. Moreover, we produced a monoclonal antibody specific for modified citrulline, which drastically improved detection sensitivity of citrullinated peptides compared to commercially available anti-citrulline antibodies. proMMP-9 was consistently found in all arthropathies, whereas the active form was observed only in a limited fraction of our patient cohort. Proteolytic fragments of MMP-9 were also detected in specific patients and these included the hemopexin-less form (57 kDa) and a MMP-9 peptide (25 kDa). Importantly, citrullinated MMP-9 peptides of 57 kDa and 25 kDa were detected in RA patients, as opposed to post-trauma samples. We describe, for the first time, the discovery of citrullinated MMP-9 proteoforms in synovial fluid and their apparent unique expression in RA.

Keywords: Autoimmunity, immunological techniques, rheumatoid arthritis

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Foxp3-specific deletion of CREB generates Th2 biased ST-2 positive regulatory T-cells with enhanced IL-10 production and suppressive capacity

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Regulatory T-cells (Tregs) are characterized by the expression of Foxp3, a master regulator involved in the development and function of Tregs. Foxp3 expression is balanced by the transcriptional activator CREB. We aimed to find out how CREB is regulated in Tregs under Foxp3 specific CREB deficient conditions. Tregs were analyzed by flow cytometric analysis. Cytokine analysis was performed by ELISA and RT-qPCR. Gene expression analysis was performed by ATAC-sequencing, Transcriptome and Methylation analysis. Foxp3creCREBfl/ fl mice showed increased frequencies of Tregs (CD25+/Foxp3+) in Thymus, spleen and peripheral lymph nodes, but decreased Foxp3 expression at the single cell level. In addition, bone marrow chimera mice experiments revealed down regulation of Tregs in CREB-/- CD45.2 Tregs indicating a cell intrinsic mechanism of Foxp3 downregulation by CREB. Despite decreased Foxp3 expression, T-cell suppression assays revealed increased suppressive capacity of CREB deficient Tregs. Upregulation of IL-10, IL-13, IL-4 and IL1RL1(ST2) among other genes was observed in CREB deficient Tregs indicating the induction of a Th2-skewed phenotype in Tregs by CREB deletion. Experimental transfer colitis showed decreased intestinal inflammation and reduced IL-17 and IFN-γ expression in Rag2-/- mice that received Foxp3creCREBfl/fl T-cells. On the other hand, Ova-induced asthma model revealed enhanced levels of IgE in serum and ST2+ Tregs in lungs and BAL of Foxp3creCREBfl/fl mice indicating increased type 2 immune responses. Collectively, our data suggest that CREB expression in Foxp3 cells is of importance in maintaining the balance of Th1 and Th2 responses.

Keywords: Cytokines and mediators, molecular immunology, regulatory cells

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