SoCal Flow Summit 2015 Poster Oral Presentation Winners

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Abstract:

Genetic engineering of hematopoietic stem cells to generate invariant natural killer T cells

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Invariant natural killer T (iNKT) cells comprise a small population of αβ T lymphocytes. They bridge the innate and adaptive immune systems and mediate strong and rapid responses to many diseases, including cancer, infections, allergies, and autoimmunity. However, the study of iNKT cell biology and the therapeutic applications of these cells are greatly limited by their small numbers in vivo (~0.01–1% in mouse and human blood). Here, we report a new method to generate large numbers of iNKT cells in mice through T-cell receptor (TCR) gene engineering of hematopoietic stem cells (HSCs). We showed that iNKT TCR-engineered HSCs could generate a clonal population of iNKT cells. These HSC-engineered iNKT cells displayed the typical iNKT cell phenotype and functionality. They followed a two-stage developmental path, first in thymus and then in the periphery, resembling that of endogenous iNKT cells. When tested in a mouse melanoma lung metastasis model, the HSC-engineered iNKT cells effectively protected mice from tumor metastasis. This method provides a powerful and high-throughput tool to investigate the in vivo development and functionality of clonal iNKT cells in mice. More importantly, this method takes advantage of the self-renewal and longevity of HSCs to generate a long-term supply of engineered iNKT cells, thus opening up a new avenue for iNKT cell-based immunotherapy.

Wei-Le Wang, MS
Graduate Student, Dr. Mark Boldin Laboratory
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Abstract:

Control of mammalian hematopoiesis and immune response by microRNA-142

Wei-Le Wang[1], Nicholas Kramer[1], Estefany Reyes[1], Bijender Kumar[2], Ramakrishna Chandran[3],Edouard Cantin[3], Ching-Cheng Chen[2], Nelson Chau[4], and Mark P. Boldin[1]

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MicroRNAs (miRNAs) are a class of small (~22 nucleotide) noncoding RNAs that regulate gene expression at the post-transcriptional level and control hematopoiesis and immune response. miR-142 gene is broadly and abundantly expressed in hematopoietic lineages. To define the biological functions of miR-142 gene, we have created a loss-of-function mouse model by targeted deletion of miR-142 locus in embryonic stem cells. Our results demonstrated that miR-142 knockout (KO) mice develop splenomegaly and display marked expansion of both myeloid and B2 B cell populations. In contrast, the number of T and B1 B cells in the periphery is dramatically reduced. Analysis of mixed bone marrow chimeras suggests that miR-142 plays a cell-autonomous role in the regulation of these hematopoietic defects. miR-142 KO mice develop hypoimmunoglobulinemia and fail to mount a productive antibody response upon challenges with either T cell-dependent or -independent antigens. In addition, the KO mice are also highly susceptible to HSV-1 infection in comparison to wild-type animals, strongly suggesting that deletion of miR-142 results in immunodeficiency.

Expression profiling of miR-142 null B cells revealed genes that play key roles in shaping the adaptive immune response, like BAFF-R, RAG1 and WASL. We established that miR-142 could directly bind and silence expression of these three genes *in vitro*. BAFF-R is a member of the TNF receptor superfamily that is essential for normal mature B cell homeostasis and plasma cell development. miR-142 null B cells have elevated levels of BAFF-R on the cell surface and as the result proliferate and activate noncanonical NF-κB signaling more robustly in response to BAFF stimulation. Lowering the BAFF-R gene dose in miR-142 KO mice resulted in rescue of several immune defects, including splenomegaly and B cell expansion, suggesting that BAFF-R is a *bona fide* miR-142 target through which it controls some aspects of B cell development.

Alborz Karimzadeh, BS

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Abstract:

CD11a and EPCR as high-resolution markers to identify long-term hematopoietic stem cells

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Hematopoietic stem cells (HSCs) are multipotent progenitors with self-renewal capacity that give rise to all downstream progenitor and effector cells of the blood system. Molecular characterization of HSCs would enhance our basic understanding of HSC biology and also elucidate governing pathways for generation of patient-specific HSCs (from iPSCs/ESCs) for treatment of virtually any disease inherent to defects in the blood system. However, current markers used for identification of long-term HSCs are limited to purification of a functionally heterogeneous population. Furthermore, currently used HSC markers (such as Sca and CD34) show inconsistent expression in different mouse models and in different conditions, and therefore cannot be used in a number of relevant scenarios. Our preliminary data identified CD11a as a novel marker for purification of long-term HSCs. CD11a (integrin alpha L) is highly expressed on downstream progenitor and effector blood cells, however it is absent on a subset of HSCs. *In vitro* and *in vivo*, CD11a- HSCs show higher multipotency potential, and higher engraftment and self-renewal capacity compared to their CD11a+ counterpart. EPCR (CD201) expression is also correlated with higher HSC activity. Our data suggests CD11a and EPCR are consistently expressed in a number of mouse models. Therefore, we hypothesize that CD11a and EPCR together can be used to highly enrich for HSCs without the need for any other aforementioned inconsistent markers, potentially simplifying the purification procedure and making HSC sorting more accessible in different contexts. Inconsistent expression of markers is also true in the developing embryo. However, we have determined that CD11a and EPCR can be used to identify embryonic populations equivalent to HSCs across multiple timepoints and tissues.

Moreover, we have shown that CD11a-EPCR+ HSCs strongly outcompete HSC activity of CD11a+ HSCs in a competitive setting *in vivo*, and therefore can be used for high-resolution characterization of “true” HSCs.

Alexandra Lee, MS

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Abstract:

High-Dimensional Single Cell Data Analysis: Methods and Cyberinfrastructure

Alexandra Lee[1], Hyunsoo Kim[1], Rick Stanton[1], Shweta Purawat[2], Jianwu Wang[2], Holden Maecker[3], Ilkay Altintas[2], Robert Sinkovits[2], Yu Qian[1], Richard H. Scheuermann[1,4]

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While polychromatic flow cytometry (FCM), mass cytometry (e.g. CyTOF), and imaging flow cytometry (IFC) have generated enormous opportunities for understanding cellular heterogeneity at the single cell level, they have also generated huge challenges in data analysis due to the large number of cellular characteristics measured. For experimentalists, it has become both unreliable and intractable to identify cell-based biomarkers using manual gating analysis on 2D projections of high-dimensional cytometry data because the process is very subjective and labor-intensive. For bioinformaticians, it remains challenging to develop computational methods to exhaustively explore the high-dimensional data space and identify patterns. Here we introduce CyTOFLOCK, a density-based data clustering method for CyTOF. We have applied CyTOFLOCK to process and analyze multiple CyTOF datasets, including a public dataset containing 22 peripheral blood NK cell samples with 35 parameters measured, which we will present in this talk. Compared with CyTOF, state-of-the-art IFC data contains even more features derived from both fluorescence channel and microscopy image processing. In order to tackle the increasing data volumes and data dimensionality, we developed a cyberinfrastructure – FlowGate – that integrates graphical user interfaces, analytical workflow engines, and parallel computing resources for efficient and reproducible identification of cell populations. The FCM analysis workflow supported by FlowGate includes the ability to filter data using a novel Directed Automated Gating (DAG) method, and identify cell populations using FLOCK. DAG is a supervised method that performs hierarchal gating to select out target cell populations for input into FLOCK. These target populations are identified by selecting the densest region defined by the contour levels that stay within user-defined boundary gates. The performance and utility of the infrastructure has been demonstrated through the computational analysis of ~10,000 FCS files from a published clinical study for quantifying immune responses to tolerance-inducing immunotherapy for seasonal allergies.