WHITE PAPER

Assessing the epigenetics of T cell exhaustion using CRISPR screens

Introduction

A major challenge in therapeutic immuno-oncology today is the limited persistence of CAR-T cells. Many studies have focused on altering CAR structure and regulating CAR-T cell differentiation to optimize their clinical efficacy. Research is also focused on identifying new molecular targets to reduce T cell exhaustion.

T cell exhaustion emerges due to continuous antigen exposure in instances of cancer or chronic viral infection. The conditional state of T cell exhaustion results in altered transcriptional programming characterized by the inability to elicit normal immunological functioning including cytotoxicity and effector functioning. Although T cells are not at complete inertia, functional depletion renders T cells suboptimal in combating infection and tumorigenesis. Research has demonstrated that T cells in the exhausted state differ epigenetically from their non-exhausted T cell counterparts, and therefore may limit the efficacy of an immunotherapeutic intervention. These epigenetic mechanisms are therefore an important focus for the industry today.

Epigenetic mechanisms of interest include chromatin remodeling and DNA methylation, which are the molecular basis for cellular memory. By utilizing CRISPR editing, researchers can identify new molecular targets controlling the epigenetics of T Cell exhaustion, opening up opportunities to reinvigorate exhausted T cells and expand responses to checkpoint therapies.

We were fortunate to speak with Dr. Verena Brucklacher-Waldert of Revvity to learn more about the epigenetics of T cell exhaustion and practical techniques to assess immune cell epigenetic regulators using CRISPR screens.

What are the fundamental concepts behind the epigenetics of T cell exhaustion?

VBW: To understand the concepts related to the epigenetics of T cell exhaustion, one must first understand what is meant by T cell exhaustion and epigenetics. T cell exhaustion is a state that T cells can acquire in patients with cancer or chronic viral infection. It is defined by impaired T cell effector function, expression of inhibitory receptors, and a transcriptome characteristically distinct from non-exhausted cells. Exhausted T cells are incapable of optimally controlling tumors or infections.

Understanding the phenotypical and functional profile of exhausted T cells can aid immunotherapies, which are therapies that utilize the immune system to attack, for example, tumors. Recent studies have shown that exhausted T cells have an epigenome, a full range of chemical changes to the DNA and histone proteins, distinct from their non-exhausted T cell counterparts. These epigenetic changes modulate gene expression and could prevent T cell reinvigoration by immunotherapy. Therefore, identifying epigenetic regulators of T cell exhaustion could advance immunotherapies.

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Why is T cell exhaustion important in developing CAR-T cell therapies?

VBW: CAR-T cell therapies are immunotherapies that harness the capabilities of genetically engineered T cells. They have emerged as a cancer treatment with promising therapeutic effects on hematological malignancies. Therapeutic challenges such as relapse during long-term follow-up and limited effect on solid tumors may contribute to T cell exhaustion. This can therefore preclude therapeutic success. Hence, preventing or overcoming CAR-T cell exhaustion could maintain CAR-T cell effector function and provide better therapeutic outcomes.

How could T cell exhaustion and epigenetic changes be investigated?

VBW: To overcome the phenomenon of T cell exhaustion and to recognize the implications of epigenetic changes, it is essential that the underlying mechanisms are understood. Several experimental approaches may be applied, such as *in vivo* animal models or *in vitro* cell-based experimental techniques. By harnessing the power of human *in vitro* cell-based assays, these experiments use either immortalized (secondary) or un-immortalized *ex vivo* (primary) cells derived from human blood or tissue and thus recapitulate the human *in vivo* environment within a controlled laboratory setting. To study T cell exhaustion, we can use primary human T cells isolated from blood and repeatedly stimulate them in the lab to characterize their phenotype and function. This approach can be taken to the next level by knocking out genes and studying the resulting phenotype and biological function to elicit a more comprehensive understanding of the underlying mechanisms.

These loss-of-function cell-based assays are called functional genomic screens (FGS) and often utilize CRISPR-Cas9 technology. If the phenotype and function are altered by knocking out a gene, then the protein encoded by this gene most likely contributes to a phenotype or function investigated. In the case of studying the effect of epigenetics in T cell exhaustion, a CRISPR knockout (CRISPRko) library targeting genes encoding the epigenome (~829 genes) could be used in a CRISPRko screen with human primary T cells to identify genes involved in T cell exhaustion.

What are the challenges of working with human primary immune cells?

VBW: Traditionally, immortalized cell lines have been used for *in vitro* cell-based assays due to their stability, predictability, and ease of handling. However, their ability to proliferate indefinitely may affect their original physiological properties. Given this context, using immortalized cell lines for cell-based screening has its merits, but it may not provide the most biologically predictable results. By contrast, primary human cells are more physiologically relevant, and therefore, screens using these cells could be more effective at identifying clinically relevant targets. There are challenges associated with using primary human immune cells including low cell yield due to limited biological abundance, low cell viability due to limited lifespan, low gene editing efficiency due to limited susceptibility to CRISPR- Cas gene editing, assay variability due to heterogenous cell populations, donor-to-donor variability, and difficulty with scale up due to restricted technology for automation, data acquisition, and analysis.

Another challenge is validating the potential results identified in primary cells using other cell types. This testing is crucial as the human body consists of several cell types in close vicinity, carrying the same DNA and sharing many protein functions. Although *in vitro* cell-based assays model the human *in vivo* environment, these complex interactions are experimentally simplified by often investigating only one or two cell types at a time. For example, a protein encoded by a gene identified in an FGS screen in T cells might pose a potential therapeutic target for a drug, but it will be essential to assess what function this gene has in other cell types of the human body to predict what effect a drug might have when administered to a patient.

How do you overcome these challenges?

VBW: Experienced scientists in laboratories conducting cell-based assays with primary human cells have overcome important challenges. For example, low cell yield is overcome by sourcing appropriate blood products, expanding and polarising cells, pooling cells from various donors, and miniaturizing assays. Viability issues are overcome by specialized cell culture consumables and reagents and optimization of assay conditions and durations.

Gene editing efficiency can be overcome by using sophisticated algorithms to identify gene targeting sites, state-of-the-art gene editing reagents, and optimized gene editing approaches. Assay variability can be controlled by increasing technical and biological replicates, and even though increased variability might make data interpretation challenging, it provides insight into the spectrum of patient responses that can be expected in the clinic. Scale-up needs are met with automation and robotic liquid handling systems. For target validation, cell-based assays using various cell types can be used for validating gene interactions in FGS and drug interactions in cell panel screens, such as our recently released long-term assay to study agents modulating epigenetic regulators. Overcoming these challenges requires a robust understanding of the intricacies of assay development and can help to further advance drug discovery efforts.

How can primary immune cell-based assays help to create the next generation of therapies?

VBW: These assays can accelerate next-generation therapies along the entire drug discovery pipeline by offering physiological insights into immune system functioning. An example is using early drug discovery methods to define a therapeutic target. Here FGS can identify targets or pathways that play a role in a particular phenotype or cell function. They can also identify which genes play a role in tumor infiltration, as well as those that hinder or promote the effectiveness of immunotherapy. Later in the drug discovery pipeline, immune cell-based assays can help understand the potential effects of a particular drug, either in FGS to identify proteins targeted by the drug, or by determining the phenotypic effect of a drug on the function of immune cells. This approach might be important for two reasons: either safety by monitoring unwanted immune cell responses, or efficiency by ensuring effector functions.

What is next in the field of human *in vitro* immune cell-based assays?

VBW: The field is evolving by developing tools and solutions to overcome some of the challenges of working with primary human immune cells. We are looking at miniaturization of experiments such as organ-on-a-chip solutions, better *in vitro* culture reagents, and editing tools such as base editing. We are also exploring better automation and data analysis tools. This trend will lead to more disease-relevant physiological *in vitro* models, including 3D assays and co-culture setups. Another trend is readout quality and quantity, meaning reliable high-quality data and multiplexed read-outs to maximize biological insights.

Dr. Verena Brucklacher-Waldert
Dr. Verena Brucklacher-Waldert heads up immune cell-based Services at Revvity. With her team, she delivers drug discovery and development projects, such as compound and FGS with primary human immune cells, to customers worldwide across the healthcare sector. These assays and screens involve the use of state-of-the-art automation and gene-editing technologies. She also leads Services R&D at Revvity. She is a board member of The European Laboratory Research and Innovation Group (ELRIG). Prior to her current roles, Verena worked in the biotech industry and academia. She has a Ph.D. in Immunology and an MSc in Neurosciences and is currently studying toward her MBA.

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