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Biology of Graft Rejection & Damage

For patients undergoing kidney transplantation, there are many risks from both within the body and from external threats that may result in rejection, injury, or failure of the graft and jeopardize patient outcomes. These risks relate to many different biological pathways and systems at play within our bodies. The immune system is the most well recognized risk. Because it is designed to monitor and attack invading foreign elements to protect the body, it is not able to distinguish that the transplanted kidney is there to help. Carrying on with its duties, the immune system will mount an attack on the graft, leading to an inflammatory response and eventual damage to the graft, unless blocked by medications to suppress the immune system and prevent this from happening. Knowing exactly what the right dose of these medications is for each patient is challenging. If not enough medication is used, the immune system will still be able to attack the graft.

Conversely, if too much medication is used, there is a significantly increased risk of infection and cancer development. Identifying the appropriate dose for each patient is further complicated by the fact that every patient is different. Some may have a very active immune system, which would require them to have a higher dose of immune-suppressing drugs or to be treated with them for longer durations. other patients may have immune systems that naturally have a reduced response and require less assistance. Aside from the immune system, other systems may also cause harm when they become imbalanced or malfunction. For example, when blood flow is disrupted, often is the case with transplants, oxygen and nutrient delivery to and from the graft is interrupted, which further introduces stress upon the graft.

Chronic inflammation also poses a significant long-term threat to graft survival. Injury to the graft recruits inflammatory immune cells to the injured sites, which promotes their secretion of pro-inflammatory molecules. These pro-inflammatory molecules further stimulate immune activation and immune cell infiltration and inflammation within the graft—a vicious cycle that can increase tissue damage. Importantly, there are also systems that can help reduce the risk of graft rejection, such as cellular and tissue repair pathways that strengthen the graft's ability to recover from damaging events. These repair mechanisms can support a return to balance by reducing inflammation and promoting recovery from damage. Patients with robust repair pathways and an immune system that is less likely to attack a graft may benefit from reduced doses of immunosuppressant therapies.

Although high-risk patients with acute graft rejection are generally easier to diagnose because patients are often symptomatic and warning signs are usually detected in post-procedure tests, these tests are late indicators of compromises on kidney health.

Additionally, patients with intermediate risk may have rejection occurring that goes undetected in post procedure tests due to their lack in sensitivity—posing a silent risk to their long-term outcomes. Altogether, graft tolerance is a complex process, and outcomes are governed by a continuum of delicate balancing acts between many integrated mechanisms and systems, ranging from cellular repair and regulation of inflammation to tissue regeneration and immune system suppression. There's relatively little understanding of the molecular mechanisms underlying the complex biological pathways and interconnected systems responsible for transplant success.

Leading-edge technologies have made possible a branch of biological studies collectively referred to as "omics". Omics include genomics, proteomics, metabolomics, and transcriptomics: the large-scale study of genes, proteins, small molecules, and RNA transcripts, respectively—all of which are being widely studied in clinical research in order to gain insight into the mechanics of complex biological processes. New technologies, such as nextgeneration sequencing, and artificial intelligence methods, such as deep learning and machine learning, are being used to investigate the molecular mechanisms underlying clinical outcomes in kidney transplantation. Importantly, next-generation sequencing technologies enable rapid and broad examination of the molecular mechanisms at play in an individual from just a blood sample, which can simply be shipped out for analysis Importance of RNA profiling. Each of our cells contains DNA, the genetic code that instructs the cells in our body and is responsible for aspects of health and disease. Individual instructions from our DNA are called genes, which are turned on or off— or "expressed"—at different levels of abundance depending on environmental conditions and the necessity to perform a process: for example, growth, digestion after meals, fighting an infection, repairing UV damage, etc..

When expressed, genes are converted— or "transcribed"—into a chemically modified version of the DNA instruction called RNA. Because they carry instructions from DNA, they are called messenger RNA and provide the code for making proteins—considered the main players in the functions of the cells and body. Additionally, RNAs may be non-protein coding and play important roles in regulating gene expression by directly controlling transcription-the process of making an RNA copy of a gene sequence—from DNA or by regulating the stability of other RNAs; when functioning in this capacity, they are referred to as regulatory RNAs. Regulatory RNAs are therefore critical for maintaining appropriate gene expression and may ultimately impact the activity of biological pathways.

Abnormal RNA expression has been implicated in numerous human diseases, including autoimmune diseases. Collectively, all the RNAs are referred to as the transcriptome. Given that genes are converted to RNAs when expressed, identification and quantification of RNAs in the transcriptome allows for the direct measurement and profiling of gene expression. Recent advances in genomic sequencing technology

led to RNA sequencing, which allows for the affordable, rapid, reproducible, and high-throughput identification and quantification of RNA levels and is currently being used in numerous clinical applications. The transcriptome is highly dynamic and sensitive to environmental conditions, and RNA profiling enables real-time profiling of the RNAs expressed at the time of sample collection. From RNA profiling, information on which biological pathways are active may be deduced by measuring the amount of RNAs corresponding to genes or regulatory RNAs known to be involved in the pathways.

RNA sequencing is a powerful technology that is highly sensitive and provides realtime information about the systems and cellular signaling affecting the transplant beyond just the immune system. This can be invaluable to physicians during their management of patients undergoing kidney transplantation. For example, performing RNA profiling of a patient prior to kidney transplantation may provide insights on the tendency for a patient's immune system to attack a graft and help guide immunosuppressant therapy dose levels and other related decisions. Profiling of a patient following a kidney transplant may offer realtime insights into their biological responses and signs of damage, such as inflammation, which gives clinicians actionable data to guide their patient's treatment plan ! Importantly, RNA profiling may serve as a more sensitive method of detecting damage that would have gone undetected otherwise and is important for both short- and long-term damage assessments. It is expected that RNA profiling of patients throughout their kidney transplant journey will provide important information that supports successful outcomes.

Because RNA sequencing allows for both the identification and relative quantification of RNAs for thousands of genes and regulatory RNAs, the resulting datasets are extremely large and complex. Therefore, powerful computing methods are required for the analysis and interpretation of the data. Employing deep learning techniques and machine learning algorithms—forms of artificial intelligence—in the data analysis enables meaningful and data interrogation and interpretation. Additionally, these techniques can learn from past data it encountered to enhance the accuracy of its data predictions ! In these RNA datasets, there will be differential expression of RNAs related to an outcome or biological activity of interest; however, there will also exist many RNA changes that are unrelated. Artificial intelligence methodologies can differentiate these by applying specific criteria and performing advanced mathematical analyses to discover patterns in the RNA sequencing dataset that are connected with various activities or outcomes.

Once these patterns are identified, machine learning algorithms are trained to recognize these patterns to identify unique RNA signatures that indicate particular outcomes or biological processes! Over time, as new data is included in the models, the algorithm is enhanced from this new learning and the RNA signatures become increasingly robust. Artificial intelligence can also be trained to incorporate additional clinical factors into the analysis. Deep learning techniques and machine learning algorithms are ideal for investigating RNA sequencing data because they perform analyses of the data.

And machine learning algorithms are currently being developed in other areas of the medical landscape to make prognoses or diagnoses. These computational methods make indepth data analysis easier and lend the opportunity to discover biological systems previously unknown to be involved in the graft rejection or tissue healing process. Artificial intelligence can offer greater insights into non–immune system-mediated mechanisms of graft tolerance, such as cellular stress and cell or tissue repair.

Defining RNA signatures associated with graft tolerance may also provide the opportunity to identify new biological markers or therapeutic targets in this process and allow for the personalization of immunosuppressant therapy Impact and clinical application. The combined power of high-throughput next-generation RNA sequencing and deep learning enables the gathering of molecular insights critical for understanding the mechanisms underlying kidney graft tolerance. With this knowledge, trained machine learning procedures can ultimately be utilized for making prognoses or diagnoses about kidney transplantations to improve patient outcomes—all from just the patient's blood sample.