Repertoire Genesis
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REPERTOIRE GENESIS

Repertoire analysis: a key to super-personalized medicine

Repertoire Genesis is revealing new possibilities for personalized diagnosis and treatment with its technology platform for unbiased next-generation T and B cell receptor repertoire analysis.

The human immune system is capable of generating a diversity of lymphocyte receptor molecules in order to respond to antigens. The full range of T cell receptors (TCRs) and B cell receptors (BCRs) found in an individual—known as the TCR/BCR repertoire—changes over time in response to disease development, progression or treatment. Analysis of the TCR/BCR repertoire could therefore provide valuable information for many personalized medicine applications.

Repertoire Genesis has developed a proprietary, unbiased, next-generation TCR/BCR repertoire analysis technology. By overcoming some of the limitations of other immune-repertoire analytic technologies, Repertoire Genesis has been able to obtain precise data that accurately reflect immune cell distribution *in vivo*.

Founded in 2014 and based in Osaka, Japan, the company is already well established in Japan and has links with research institutions, including investment from the University of Tokyo Edge Capital and a joint research program with the National Institute of Infectious Diseases. Repertoire Genesis is now expanding its analytical service to research institutions and pharmaceutical companies worldwide.

"We can also provide a joint development service for new drugs or diagnostic devices, leveraging immune genetic information," said Masuo Ichikawa, chief commercial officer at the company.

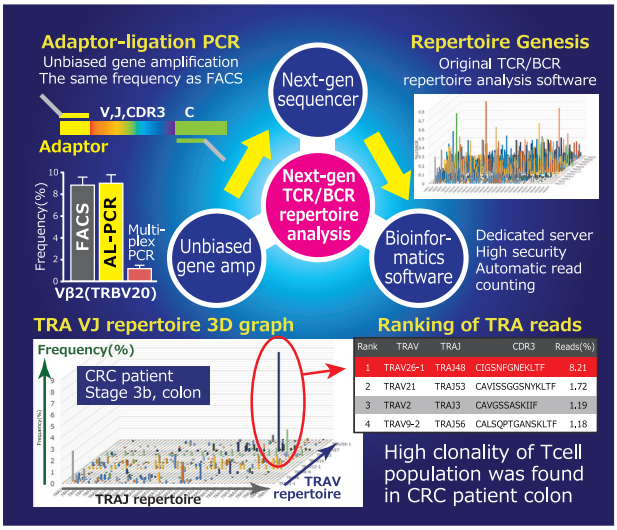

Figure 1: Next-generation TCR/BCR repertoire analysis platform technology and results of the analysis. 3D, three dimensional; amp, amplification; C, complementary region gene segment; CRC, colorectal cancer; FACS, fluorescence-activated cell sorting; J, joining region gene segment; TRA, T cell receptor alpha; TRBV20, T cell receptor beta variable 20; V, variable region gene segment.

Repertoire of immune cells

The theoretical diversity of lymphocyte receptor molecules is estimated to be as high as 10^{18} for TCRs and 10^{14} for BCRs. The receptors are generated during maturation of the T or B cells via a process of gene rearrangement, whereby one of many available options is selected for each type of gene segment, including the variable, diversity and joining region gene segments. Gene segment junctions, including complementarity determining region 3 (CDR3), may also be altered via nucleotide additions or deletions.

The Repertoire Genesis platform technology enables quantitative genetic analysis of these variable regions of TCRs and BCRs based on total RNA extracted from a sample of blood or tissue containing lymphocytes. It comprises three technologies, integrating over 25 years of experience in immune analysis with the power of advanced next-generation sequencing (NGS) technology and proprietary bioinformatics software.

The first stage involves a proprietary unbiased gene-amplification technology based on adaptor-ligation polymerase chain reaction (AL-PCR). Unlike multiplex PCR, which requires a mixture of primers that can introduce amplification biases, the AL-PCR method adds an adaptor sequence at the 5′ end of double-stranded DNA, which is then subjected to PCR amplification using a pair of primer sets (an adaptor primer and a complementary region–specific primer). A more accurate quantitative analysis can therefore be carried out as all genes encoding TCRs (TCRα, TCRβ, TCRγ and TCRδ) and BCRs (IgA, IgD, IgE, IgG, IgM, IgL and IgK) in humans and mice are amplified without bias.

NGS is carried out with relatively long-read sequencers, such as MiSeq (Illumina), as the analysis requires a read sequence of 400–600 base pairs. The data output from NGS is processed at high speed and accuracy with a proprietary repertoire analysis program. Data analysis includes a homology search in each sequence read with a dedicated database of variable, diversity, joining and complementary reference sequences.

Results of the analysis include three-dimensional graphs that offer a bird's-eye view of the entire TCR or BCR repertoire by plotting the percentage of usage for each possible variable and joining gene combination (**Fig. 1**). A ranking analysis of all clones with specific CDR3 sequences can also be generated.

Super-personalized medicine

Next-generation TCR/BCR repertoire analysis can enable super-personalized medicine, as doctors can use the results to guide therapy decisions or make reliable predictions of cancer recurrence. For example, if the TCRs or BCRs of tumor cells are identified before treatment of leukemia or malignant lymphoma, it is possible to detect minimal residual disease after therapy with a higher degree of sensitivity than possible with conventional methods such as flow cytometric analysis and immunohistochemical staining. The technology is also useful for evaluating the recovery of immune function after bone marrow transplantation.

The technology can be used to more precisely evaluate the efficacy of immune checkpoint inhibitors and cancer immunotherapies. For example, TCR repertoire analysis could be used to assess qualitative and quantitative changes in tumor-infiltrating T lymphocytes. "We strongly believe that our technology could play an important role as a novel immunity-analysis method in the validation of new products or therapies," said Ichikawa.

The genetic information about TCRs and BCRs obtained using next-generation repertoire analysis techniques could also help develop advanced medical technologies. For example, neoantigen-specific TCRs adapted to an individual patient's tumor obtained with repertoire analysis could create effective TCR gene therapies, whereas next-generation BCR repertoire analysis could aid the development of antibody drugs and chimeric antigen receptor T cell therapies.

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