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TITLE: Immunoglobulin and T Cell Receptor Genetics

PRESENTER: Bing Melody Zhang

Slide 1:

Hello, my name is **Bing Melody Zhang**. I am an **Assistant professor (Clinical) of Pathology at Stanford University**. Welcome to this Pearl of Laboratory Medicine on "**Immunoglobulin and T Cell Receptor Genetics**."

Slide 2:

Here is the outline of this talk. I will cover the structure and genetics of Ig and TCR, T/B cell development and TCR/Ig gene rearrangements, molecular testing and clinical utility of TCR/Ig rearrangement analysis.

Slide 3:

Let's first review the structure of Ig and TCR.

B and T lymphocytes are characterized by their antigen-specific receptors, which are the primary effector molecules of the adaptive immune system. For B cells, there are the membrane-bound form of the immunoglobulin as main component of the B cell receptor (BCR), and the soluble form of Ig secreted by plasma cells. Both forms of Ig have the identical Y-shaped structure, composed of 2 heavy and 2 light chains.

On the other hand, T cell receptors are heterodimeric cell surface molecules responsible for recognizing antigens presented to T cells in context of MHC molecules. In the circulation, majority (95%) of T cells express alpha beta chains and a minority of T cells express gamma-delta chains, as part of the TCR complex. Gamma-delta T cells are dominant subtype of T cells found in epithelial tissues.

Both TCR and Ig molecules consist of variable and constant regions. Each lymphocyte receptor has a unique antigen-specificity determined by the structure of antigen-binding site primarily from the variable amino acid sequences.

Slide 4:

At the genetic level, there is a complex and elegant mechanism to generate the remarkably diverse lymphocyte antigen receptor repertoire needed to respond to a great variety of antigens. Each receptor chain variant cannot be encoded in full in the genome due to the extremely high level of diversity. Instead, the receptor chains are encoded in the germline genome in several pieces—as variable (V), diversity (D), and joining (J) gene segments, with each type of gene segment present in multiple copies.

These gene segments are assembled in the developing lymphocyte by random somatic DNA recombination to form a complete variable region sequence, a mechanism known as gene rearrangement. V and J segments are present in all antigen receptor genes, with D segment present only in some. The large number of possible combinations of different segments accounts for much of receptor repertoire diversity.

Besides, there is non-templated insertion and deletion of random nucleotides at the junctional regions by the enzyme terminal deoxynucleotidyl transferase. This occurs early in lymphoid ontogeny in both B and and T cells, essentially in tandem with the rearrangement process, and further increases repertoire diversity.

Mature B cells, upon encountering and activated by antigens, can extend the Ig repertoire through somatic hypermutation, which is high rate of point mutations in the V regions of Ig genes.

Slide 5:

As depicted here, the rearrangement process for *IGH* genes usually starts with a D to J rearrangement followed by a V to D–J rearrangement. The same process applies to TCR Beta (*TRB*) and TCR delta (*TRD*) genes.

Ig light chain (*IGK, IGL*), TCR alpha (*TRA*), and TCR gamma (*TRG*) genes do not contain D gene segments, and their rearrangements involve direct V to J recombinations.

Slide 6:

The rearrangement process of Ig usually starts with a D to J joining followed by a V to D–J joining in case of heavy chain (*IGH*) gene, or direct V to J joining in case of light chain kappa (*IGK*) and lambda (*IGL*) genes. The sequences between rearranging gene segments are usually deleted.

Antigen receptor gene rearrangement is an error-prone process and some rearrangements result in nonfunctional genes. In the case of *IGH*, if rearrangement of the first allele fails, the second allele is rearranged. A single B cell may have two different *IGH* rearrangements. The *IGK* genes rearrange in a similar way only after the successful rearrangement of the *IGH* gene. In most cases, the *IGL* genes rearrange only if rearrangements of the *IGK* genes fail and both alleles are deleted. Normal B cells that fail to produce a functional *IGH* or light chain rearrangement would usually undergo apoptosis.

Slide 7:

T cells develop from common lymphoid progenitor cells that seed the thymus from the fetal bone marrow or liver. The differentiating T cells in the thymus are either double negative (DN), double positive (DP), or single positive (SP) for the expression of CD4 and CD8 cell surface antigens. During the DN stage, T cells can be subdivided into four subsets (DN1-4), based on rearrangement of their TCR genes and the expression of surface antigens.

TCR gene rearrangements are more complex and generally follow a hierarchical order. First the TCRD chain (*TRD*) genes rearrange, then TCRG chain (*TRG*) genes, potentially resulting in TCRγδ expression; or followed by further TCRB chain (*TRB*) gene rearrangement and *TRD* deletion with subsequent TCRA chain (*TRA*) gene rearrangement, potentially followed by TCRαβ expression.

Slide 8:
The estimated numbers of functional and rearrangeable human V, D and J gene segments involved in TCR/Ig gene rearrangements are shown in the table.

The many different combinations of V, D, and J gene segments represent the so-called combinatorial repertoire, which is estimated to be $2x10^6$ for Ig molecules, $3x10^6$ for TCRαβ molecules, and $5x10^3$ for TCRγδ molecules.

Slide 9:
We have talked about the genetics of lymphocyte antigen receptor rearrangements. How would this information be useful in the diagnosis of lymphoid neoplasms?

Neoplasms are populations of cells that are in theory derived from the proliferation of a single common precursor cell, and therefore would be expected to contain identical DNA sequences, which could be used as specific tumor markers. Clonal rearrangements of *IGH* and *IGK* can be detected in essentially all malignancies of mature B cells, but many precursor B-cell malignancies will have only *IGH*

rearrangements, since malignant transformation occurs before rearrangement of the light chain genes.
Rearrangements of the TCRB and/or TCRG chains have been observed in almost all types of T lymphoproliferative disorders. However, there are rare T-cell neoplasms that lack *TRB* or *TRG* gene rearrangements, and in these cases, the evaluation of *TRD* could be helpful.

Although the analysis of antigen receptor gene rearrangements can provide useful diagnostic laboratory tool for lineage determination, they are not necessarily lineage-specific and cross lineage rearrangements can occur. Also, clonality does not equal malignancy—benign and reactive conditions can produce clonal patterns due to Ag-driven lymphocyte expansions. Therefore, results should always be interpreted within appropriate clinical context and in conjunction with morphologic findings.

Slide 10:
So what molecular diagnostic methods can we use to test for TCR/Ig rearrangements in the laboratory?

Southern blotting was used in the past, and the method currently used by majority of laboratories is PCR and capillary electrophoresis-based. With the advancements in sequencing technology, some labs have transitioned to next-generation sequencing-based method. I will introduce each of these methods in more details in the next few slides.

The applications of TCR/Ig rearrangement testing include clonality analysis and minimal residual disease assessments.

Slide 11:
In the past, clonality was primarily studied using Southern blotting analyses, which used to be considered as gold standard for such testing. This method generally takes

advantage of the combinatorial repertoires of TCR/Ig genes to detect genetic rearrangements based on reproducible set of DNA fragments generated by a specific restriction enzyme.

To perform this type of testing, high-molecular-weight DNA is needed to be digested with restriction endonuclease enzymes and electrophoresed overnight in agarose gel to separate DNA fragments. These fragments are then transferred and hybridized with labeled DNA probes homologous to the target genes. A clearly visible labeled band is detected when clonal gene rearrangement is present.

This method generates fewer false-positive results compared to PCR. However, there are disadvantages inherent to Southern blotting, such as: limited sensitivity, significant demands in time and technical skill, the need for large quantities of high-quality DNA, longer turnaround time and not suitable for tracking clones.

Slide 12:
Compared to Southern blotting, PCR-based method is faster, more accurate, and requires only small amounts of DNA as template.

In addition, PCR can also amplify partially degraded DNA. Therefore, PCR-based techniques can be used to analyze small biopsies (e.g. skin biopsies) or formalin-fixed paraffin-embedded samples (FFPE), which generally yield low-quality DNA.

Two different types of PCR analysis are used for TCR/Ig gene rearrangement assessments: qualitative and quantitative. Qualitative assays are suitable for clonality assessment and do not allow for precise MRD analysis. The PCR amplicons generated using TCR/Ig specific primers then undergo capillary electrophoresis to be separated by size. The interpretation of clonality is based on the fragment size distribution pattern. Representative monoclonal and polyclonal patterns are shown on this slide.

Quantitative PCR-based approaches are important for assessing treatment responses and monitoring MRD, prior to availability of NGS-based assays.

Slide 13:

TCR/Ig gene rearrangement analysis using next-generation sequencing (NGS) technology started to become available in clinical molecular diagnostic laboratories in recent years. Compared to PCR/capillary electrophoresis (CE)-based method, NGS-based method can provide the fingerprint characterization of each clonotype, namely the specific rearranged sequence, as well as quantitative sequencing count information. It can improve the test sensitivity and specificity compared to PCR-based method.

The test can be readily performed on a benchtop sequencers with capacity to multiplex several samples in the same run.

Slide 14:

Some major clinical utilities of molecular TCR/Ig gene rearrangement analysis in lymphoid malignancies are listed here.

It can aid diagnosis of challenging or inconclusive cases of lymphoproliferative disorders, evaluation of clonal relationship between two or more lesions in the same patient, discrimination between a relapse and a second malignancy, lymphoma staging, and monitoring/evaluation of treatment effectiveness.

Slide 15:

To summarize the main points of this pearl--

TCR/Ig genes are encoded in the germline genome as different gene segments. During T/B cell development, the V, (D), and J gene segments undergo random recombination to form complete sequence, known as TCR/Ig gene rearrangement.

Additional non-templated nucleotide insertions and deletions at the junctional regions during gene rearrangements, as well as somatic hypermutations in Ig genes further increase TCR/Ig repertoire diversity.

Molecular analysis of TCR/Ig gene rearrangements has important clinical utility in diagnosis and monitoring of lymphoid malignancies.

Slide 16: Figure/Table Titles

Slide 17: References

Slide 18: Disclosures

Slide 19: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Immunoglobulin and T Cell Receptor Genetics.”