DEPARTMENT OF BIOTECHNOLOGY

FACULTY OF ENGINEERING & TECHNOLOGY

LT 25: Molecular marker linked to human disorder and disease resistance gene

Content Outline

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Molecular Marker

➢**Molecular Marker** is a fragment of DNA that is associated with a certain location within the genome.

➢**Molecular markers** are used in **molecular** biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA

It is used to reveal following information:

•Reveals mutations/variations

•Detect polymorphism between different genotypes or alleles

• variations or polymorphisms that exist among individuals in the population for specific regions of DNA

➢Specific regions of the DNA (genetic markers) are used for diagnosing the autosomal recessive genetic disorder cystic fibrosis, taxonomic affinity (phylogenetics) and identity (DNA BarCoding).

List of Marker

List of Markers	Acronym
Restriction Fragment Length Polymorphism	RFLP
Random Amplified Polymorphic DNA	RAPD
Amplified Fragment Length Polymorphism	AFLP
Variable Number Tandem Repeat	VNTR
Oligonucleotide Polymorphism	OP
Single Nucleotide Polymorphism	SNP
Allele Specific Associated Primers	ASAP
Inverse Sequence-tagged Repeats	ISTR
Inter-retrotransposon Amplified Polymorphism	IRAP

Restriction fragment length polymorphism (RFLP)

The RFLP is defined by the existence of alternative alleles associated with restriction fragments that differ in size from each other. RFLP is the nucleotide base substitutions, insertions, deletions, duplications, and inversions within the whole genome, can remove or create new restriction sites. RFLP analysis is useful to find out where a specific gene for a disease lies on a chromosome.

Single-strand conformation polymorphism (SSCP)

The SSCP technique, discovered by Orita *et al*. has been found to be very useful for quick, sensitive and relatively inexpensive detection of differences in the nucleotide sequences of closely related genomes.

Simple sequence repeats (SSR)/microsatellites

SSR loci, referred as VNTRs and simple sequence length polymorphisms, are found throughout the nuclear genomes of most eukaryotes and to a lesser extent in prokaryotes. Microsatellites range from one to six nucleotides in length and are classified as mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats. They are tandemly repeated (usually 5-20 times) in the genome with a minimum repeat length of 12 base-pairs. The number of repeats is variable in populations of DNA and within the alleles of an individual.

SNP

In 1996, Lander proposed a new molecular marker technology named SNP. When a single nucleotide in the genome sequence is altered, this will represent the SNP. In other words, it refers to a sequence polymorphism caused by a single nucleotide mutation at a specific locus in the DNA sequence

Molecular marker associated with disease

Marker associated with cancer

Breast Cancer cell	Breast cancer cells commonly express several markers including cytokeratin (CK), CEA, Mucin 1, epidermal growth factor receptor (EGFR), EpCAM, human epidermal growth factor receptor 2, and BRCA1
Pancreatic cancer	K homology domain containing protein overexpressed in cancer (KOC). KOC is strongly overexpressed in pancreatic cancer, CA19-9
RCC	CAIX (carbonic anhydrase IX)
Bladder	CD44
CRC	CEA

Disease resistance gene

➢**Resistance genes** (R-**Genes**) are **genes** in **animal** genomes that convey **animal disease resistance** against pathogens by producing R proteins. The R protein encodes enzyme that degrades a toxin produced by a pathogen.

•It is thought that most *R* genes encode protein products that trigger intracellular signaling when engaged by pathogen molecules such as the *avr* products.

•Over 20 *R* genes have been identified whose products fall into five structurally distinct classes of proteins.

•Some *R* proteins are transmembrane molecules and interact with the pathogen outside the plant cell. Others are cytoplasmic such that the pathogen must actually penetrate the plant cell wall and secrete *avr* peptides in order to be recognized and trigger the HR. The various forms of *R* proteins do share some structural features, notably a region rich in leucine repeats and a nucleotide-binding site.

•This leucine-rich region is homologous to the LRR domain of Toll-related receptors and appears to be responsible for pathogen recognition. Some *R* proteins also have a serine-threonine kinase domain marking them as belonging to the SIIK group. In addition, the cytoplasmic domains of certain *R* proteins, such as the N protein in tobacco, the Cf-9 protein in tomato, and the Xa-21 protein in rice, contain regions similar to the TIR domain present in the cytoplasmic regions of *Drosophila* Toll and mammalian TLRs.

Malarial resistance gene

The malaria resistance genes ABO, G6PD, *HLA*, *α*-globin, and *β*-globin, are some of the most variable human genes. Such variation might be because disease resistance genes have high amounts of standing variation or because these genes have high mutation rates and produce new adaptive alleles very quickly.

CCR5; C-C motif chemokine receptor 5 (gene/pseudogene)

A **genetic** mutation known as **CCR5-delta 32** is responsible for the two types of HIV **resistance** that exist. **CCR5-delta 32** hampers HIV's ability to infiltrate immune cells. The mutation causes the **CCR5** co-receptor on the outside of cells to develop smaller than usual and no longer sit outside of the cell. It could be argued that RCD in the form of *CCR5-Δ32* editing does not actually represent a functional upgrade to immune activity the way vaccination does. It merely changes the structure of the CCR5 receptor in a way that limits HIV entry into host cells. Furthermore, this allele appears to be associated with a significant increase.

IRAK1 Gene

Interleukin 1 Receptor Associated Kinase 1 (IRAK1 Gene). This gene encodes the interleukin-1 receptor-associated kinase 1, one of two putative serine/threonine kinases that become associated with the interleukin-1 receptor (IL1R) upon stimulation. This gene is partially responsible for IL1-induced upregulation of the transcription factor NF-kappa B.

Test your understanding

Further reading

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