By the name of ALLA

LECTURE NO# 13

This lecture include the slide from 43 to 74…so plz refer to them

Genome-wide association studies (GWAS) :

GWAS is the study of whole genome of the Pts to find mutation and genetic biomarker associated with certain disease but the limited factor of these studies is the number of samples .

For example Multiple sclerosis MS which is a multifactorial disease and many genes are associated with it and we weren't able to know these genes until we studied the whole genome of 10000 Pts.

To calculate the statistical significance we use certain formula to know the significance of certain mutation .

Guilt by association:

It's not necessary for the genetic biomarker to be a mutation but we can use it to detect the presence of mutation ……meaning we can discover genetic bio markers that are associated with certain mutation (if the mutation is rare) so I can find what the mutation is by knowing the biomarker associated with it and that's what is known as Guilt by association.

GWAS approaches:

How GWAS is done?

I. next generation sequencing:

Briefly I sequence the whole genome of people , it used to take along

time to sequence a gene ,but now with new technology its much faster and efficient and I can sequence genomes quickly .

II. SNP micro array …we'll talk about it later in this lec

<u>Manhattan plot :</u>

When I sequence to genome I use the mathematical and the statistical formulas to connect a SNP(for example) with a disease ..to see which SNP is associated with the disease of concern

X axis: is the SNP in different chromosomes ((each small dot represent a SNP in a chromosome and we have half million SNPs in our DNA

Y axis: is the statistical significance meaning How much these SNPs are significant in association with a disease

The SNP must pass the threshold liner (the red line in the slide) if they (I mean the SNPs) above the line then they are associated with a disease .

Ex. STAT4 disease is associated with a SNP chromosome 2

This plot is called the Manhattan plot

<u>How do different cells appear?</u>

The answer is they appear because they express different genes and this is what is called differential gene expression

<u>Differential gene expression</u> :

We mean by this :if we take muscle cell, nerve cell , stomach cell, skin cell all of them have the same genome and the same SNPs but they differ in what gene they express ….all cells have 20000 gene but not all of them expressed .

Some genes are common like the actin gene which is important for all cells but other gene are important for the function of the cell …(i.e. a gene is important for the function of glandular function but it's not for the muscle cell will be expressed in the 1st cell but not in the 2nd one).

<u>Important terms:</u>

Genomics: is the study of DNA

Transcriptome: the study of gene expression at the level of RNA

Proteomic: is the study of protein ..their expression ,,modification .. phosphorylation …..etc

<u>Northern blotting</u>:

Its one of the old method to study gene expression

What we did in this method is as the following :

We take RNA sample →run it in a gel →we get different RNA separated on the size→ transfer the RNA from the gel to a membrane →add the probe to the membrane .

Can we have hybridization between RNA and some thing else?

The answer is: of course yes ,we can ……remember the microRNA .

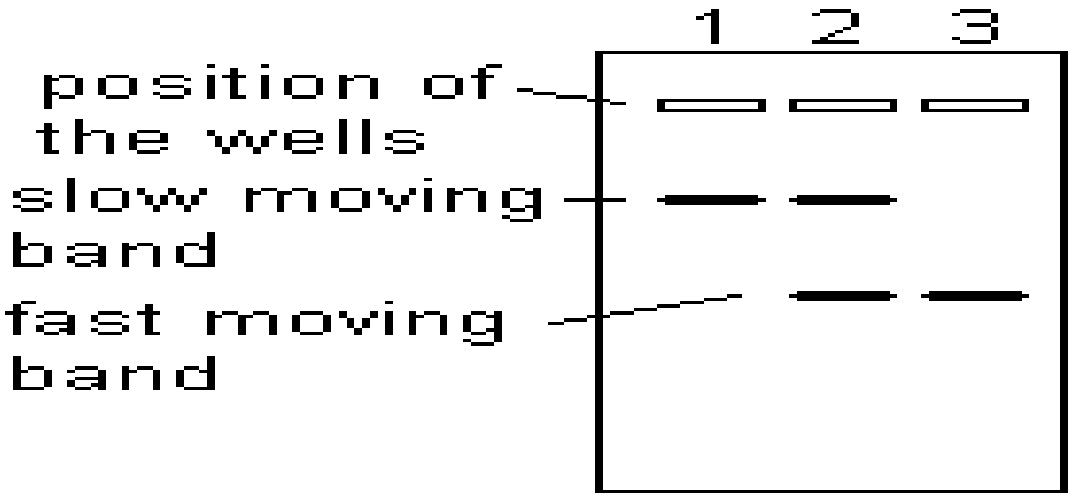
Northern blotting can give us 3 information

I. If the gene expressed or not (if there is signal so there is expression).

II. How active the gene expression is (high or low depending on the signal)

III. The size of the mRNA (short or long).

Here we have 3 samples 1,2 and 3



Why we have in sample 2 a lower Molecular weight mRNA (Unlike sample 1)????

That could be a result of :

a- mutation (deletion or insertion) in one allele

b- polyadenlylation

c-may be 2 gene of the same family and the probe complementary for both .

d- alternative splicing

Also here we have 3 samples

Notice that if we use a probe complementary to the gene AMG we have higher signal in the sample 3 meaning that it's the most active in the sample 3 (((may be somebody is studying the effect of a cytokine on the gene expression and he add different concentration of the cytokine, so may be the sample 3 contain the higher conc. of the cytokine and so on….

<u>In situ hybridization</u>:

If I have a tissue section,, and I take all the mRNA from this section ….and this section contain different type of cells(epithelial,, inflammatory …etc) the question is :from which type of cell did this mRNA arise ?

We do in situ hybridization…I take the tissue section then I add the probe that will bind to the mRNA

The immunohistochemistry and the in situ hybridization is the same except that in the 1st we add Antibody and the in the 2nd we add probe and then we can know which cell expresses the gene

***We can study the gene expression in the drosophila embryo**

Look at the figure in slide 55

St.5= the gene is expressed on the both side

St.10 = the gene is expressed in the periphery

……..in our body we have right and left ..but during development how the body determine the right and left …….they are genes that determine the symmetry (right and left) ….and we studied these gene in the drosophila embryo ….. they did a nice experiment …..they took the gene that is responsible for the production of the legs and put it in

the head region ….amazingly the legs appear in the head region and this allow us to know the function of the gene

DNA LIBRARY :

It's away to facilitate the study of the DNA

YOU have a piece of the DNA that you want to study from the genome and this piece is carried on a plasmid of a bacteria that is kept in a freezer in an exact place .

How to make a DNA library?

I take the whole genome ….cut it into segments by endonucleases ….then I insert each segment into a plasmid …then I put one plasmid per bacterial cell ……and then I put the bacteria in the freezer … and I can refer to any segment at any time .

The genomic DNA library contain everything in the GENOME

If I create A DNA LIBRARY from a muscle cell it will be the same if I create it from a nerve cell …since it the same genome ..it will contain exons,, entrons,, promoter ,coding or non coding sequence…etc

cDNA library :

it's a DNA LIBRARY that represents the genes only (coding regions that are transcribed into mRNA)

How to make IT

I take the mRNA from the a cell then I convert the mRNA into cDNA (complementary DNA) by reverse transcriptase enzyme that present in the retroviruses then I inset the DNA into a plasmid then into a bacteria .so I have a library of expressed gene …only the exons ..no

entros or promoter or any thing else and this is a difference between cDNA and genomic DNA library.

So if I create a cDNA library from a muscle cell it will be different from a cDNA library created from a nerve cell because they express different genes .

<u>The science of –omics</u>:

If I study the genome →then I am studying what can happen(if I have a mutation or a SNP THEN I can predict what can happen)

if I study the transcriptome →what appear to be happen (the expression of mRNA whether it's high or low)

if I study the proteome →what makes it happen(Cancer is a proteomic disease because what makes cancer cancer is a protein) .

if I study the metabolites →then I am studying what actually happen (the end result that affect the phenotype).

<u>Studying the transcriptome as a whole:</u>

There is a new technology called DNA microarrays (small chip 2x2 cm that contain arrays of genes .

In this method we have the probe and we add the DNA to it ,,,,remember that in the northern blotting that we have the DNA and we add the probe .

<u>Using a DNA microarray:</u>

I. mRNA from the cells being studied is first extracted and converted to cDNA

II. The cDNA is labeled with a radioactive probe

III. The microarray is incubated with the labeled cDNA sample for hybridization to occur

If a gene is expressed, then the cDNA will exist and bind to a specific complementary DNA fragment on the microarray

Binding can be detected since the cDNA is labeled and expression is determined.

<u>Comparative expression:</u>

Comparing gene expression between different cells …i.e. I can use this technique to study gene expression in different stages of cancer of the same type …whether it's up regulated or down regulated.

In this method we do the following : (see the fig on slide 70)

I. We have bacterial cells ..we divide them into 2 groups …one is control ..and we add ethanol to the other to study the effect of ethanol on gene expression …

II. we isolate the mRNA

III. make cDNA and lable it with fluorescent dye(its green for the control and red it's red for the one that contain ethanol

IV. mix them

If the gene expression is the same then the intensity of green and red signal will be the same…

If the red signal is higher then the gene is up regulated by ethanol

If the green signal is higher then the gene is down regulated by ethanol

If no fluorescence is detected and it appear black spot then the gene is not expressed .

If we are studying a large no# of genes we use the computer to gather the information and analyze it as the following

Yellow means: equal fluorescence

Green means: more expression in one cell vs. the other

Red means: the opposite

Black means: no expression

Cancer is a heterogeneous disease ..the gene expression is very different even between different stages of the same type and within the same stage of the different Pts ..but there must be things important for the cancer cell to progress and in order for the cancer cell to be aggressive there must be up or down regulation of some genes……if a gene is up regulated in a cancer cell so this gene is responsible for making it aggressive and if the gene is down regulated then it's responsible for controlling the cell and it's down regulation allow the cell to become more aggressive .

They did experiment (slide 73) they took cancer cell from 10s of women and they studied 1000s of gene using microarray

Based on the gene expression profiling the computer determine that sample 1 and 3 are similar and sample 2 is different .

In the last slide 73 :here we have thousands of data points for many samples of beast cancer about 200 gene have been studied

In the upper part of the picture the red spots represent up regulated gene and this pattern is associated with poor outcome ….

In the lower part …the opposite is happened and this is associated with good outcome

We can use this technique to know the prognosis of other Pts and this help in choosing the proper treatment .

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