Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 30 : Proteomic Techniques – Protein Microarray

Namaskar. Hello students. Welcome back to our lecture series on Comprehension Molecular Diagnostics and Advanced Gene Expression Analysis. We are in our module 6, where we are discussing the various basic techniques for basic proteomic techniques for molecular diagnosis. And we are we have already discussed we are discussing this workflow, where we are discussing the very basic steps for proteomic workflow. And we have discussed in our last class till differential in gel electrophoresis.

And therefore, as you have guessed right way the today's discussion will be regarding protein microarrays ok. Now, the concepts that will be covering today are what is microarray you have already gone through DNA microarray right. So, we will be extending that concept of the histone development of microarray the principle of protein microarray what is the procedure how it is done, what are the various types, what are the various detection methods in protein microarray and ultimately where we can apply this technique all right. So, to start with what is microarray since you have already studied in brief the DNA microarray, you already know microarray technology refers to the miniaturization of thousands of assay in one small plate in a solid platform right arrays are the arrangements all right and micro scale very small multiple thousands of assays procedures in one small solid plate that is basically the microarray as you can see in the diagram each and every section has got one test or assay that is going on over here.

So, regarding the history of microarray the thing is it was originally it has been developed from the term or the concept of ambient analysis of analyte amino acid very early as early as 1989 all right . Then it was later transformed into DNA microarray it is one of the high throughput processes of molecular diagnostics. However, you have already learned DNA microarray still there are some limitations that is a very wonderful novel procedure where we can do the genetic molecular diagnosis of large scale of sample in a very small platform, but still when it comes to functional proteomics and understanding the protein function DNA microarray has got it is limitation all right mainly because of the fact that we need to understand the gene expression levels right not all DNA will transform into RNA not all RNA will transform into protein. So, you

know the central dogma. So, it is best if you want to study the functional analysis of protein the disease process best is to study the protein not just the parental DNA that is why the need of development of protein the similar such proteomic acid involves small platform was there and there that was the impetus that was the innovation or need of innovation for which the protein microarray was developed.

Now as I told you basically mRNA messenger RNA quantity may not accurately reflect the amount of protein expression level in cells this you already know it is very clear right the amount of translation it may so happen that they are are this is not 1 is to 1 reflection of function from the mRNA level to the protein level and specially from the DNA level to the protein level from the genes to the proteins. Moreover proteins rather than mRNA play a functional role in cell response ultimately the functional effector or the functional body is the proteins all right and moreover even after a protein has been translated from the mRNA there are some post translational modification which simply will be overlooked if we are only relying on DNA microarray. So, you understand why there is a need of protein microarray in understanding the cellular function in understanding the disease process disease modification compared to a DNA microarray and again the there are already existing high throughput processes in proteomics high throughput techniques. For example, we studied in detail 2D gel electrophoresis differential gel electrophoresis EAS those are very good, but still those are time consuming HPLC yes very high throughput. However we are still not being able to achieve thousands of assays at one single platform in this much size and tiny small size right.

So, therefore, protein microarray surpasses all of them all right in applicability. So, when the sample is more the quantity is low sample quantity is low requirement of sample quantity is low, but the sample amount number of sample is very high protein microarray are one of the most sort out techniques in modern day proteomics all right. So, what is protein microarray I mean if you have understood the concept of DNA microarray this is very easy. So, regarding the alternative names protein microarray also known as protein array a protein biochip or protein chip all right this is very commonly used protein chip same thing protein microarray involves immobilization. So, it is immobilize the protein is immobilized of several proteins on to a solid surface it is fixed on the solid surface all right this allows the use of miniaturized experiment to study and explain protein interaction protein function so many things about proteins all right.

So, immobilization of several proteins on to a solid surface these are the keywords which you need to mention which you need to note whenever you are understanding or describing protein microarray to anyone. So, what is the principle the principle if you have understood again I am repeatedly saying this if you have followed the lecture of DNA microarray this is actually very easy to understand just substitute the DNA probe

with the help of a functional protein or receptor antibody all right. So, protein chip or protein microarray the chip on which the array is arranged or fabricated or made mind it this term is very important fabrication we will also be discussing it later fabrication or manufacturing of the chips engineering discipline all right, but since it is a course from IIT Kharagpur definitely we are a multidisciplinary institute and we also be discussing a bit about fabrication even if it is not so much core may not be a core area of interest for biological students, but there are many stakeholders of this course. So, we extend it so that everyone finds the comfort zone anyway. So, protein chip consists of a support surface very important such as glass fly nitrocellulose bead or microtiter plate all right to which an array capture of protein is bound.

We will again be discussing what are the support materials what are its function in a future slide in this very lecture, but for now just note there is a solid phase or a support which is made of these material on which a capture protein is bound all right or immobilized. So, what happens we already have got some machinery or mechanism to capture our protein of interest all right. So, one bound protein on a surface and that will capture another protein from our sample of interest ok. Then when there is a capture we then send another probe which is typically a fluorescent dye to the array which will react with this whole assembly wherever there has been a capture all right. So, you can see if you just follow along the steps antibodies or proteins or peptide are fixed on the support slide all right.

It is incubated with a biological sample it may be serum sample it may be cell lysate or anything incubated means the serum samples are exposed or poured over the plate it is kept for some time may be at 37 degree centigrade or depending the standardization procedure of the microarray thereafter and which allows if sufficient time for the antigen antibody reaction to occur if it is an antigen antibody type of microarray assembly all right. So, basically in DNA microarray complementary DNA was being hybridized in protein microarray or complementary protein will be bound right then antigen antibody I mean attachment has occurred and then antigen antibody detection reaction is happening which is done by a typical fluorescent dye which is added to the array. Then what happens this gives the signal wherever their proteins were immobilized that has captured an antigen or antibody or a protein of interest that will give a fluorescent signal that is acquired by the laser scanner that is read by a laser scanner and the signal is interpreted in form of a graph which is then analyzed. So, basically the laser scanner goes to every small area small slide just like droplet PCR it is very important and it is very easy for you to understand just like droplet PCR each and every just in drop dd PCR where each and every droplet had one reaction it emitted one fluorescent signal. Similarly in protein micro each and every small area has got its own fluorescent signal depending on the antigen antibody reaction or protein protein interaction that has happened mind it is not only protein protein it can be protein DNA as well we will be discussing that in the types

of microarray, but whatever the ligand analyte reaction that will emit a fluorescent signal and that will be captured and analyzed by the data analysis system to give us quantitative information all right.

So, what are the types of protein microarray? Basically the protein microarrays are divided into these three conceptual types or the functional types that depending on the mechanism of reaction all right number on analytical microarray protein microarray mind everything is protein micro analytical protein microarray reverse phase protein microarray functional protein microarray we can include the word protein all right since we are discussing protein microarray. So, we will be discussing them one by one it might appear very overwhelming, but please try to pause the video if you are finding it too difficult just go through slowly it is actually very very very easy to understand and follow all right. If you have got a basic understanding of immunology works it is very very easy for you. See analytical microarrays also known as capture arrays very important multiple choice question right what are the other names. So, what happens here a library of antibodies are arrayed on the support surface very important.

So, an antibody which will capture our target protein of interest. So, this is an antibody antibodies the immunoglobulins are all have got a structure like this. So, they are denoted by a Y symbol or in the diagram right. So, antibodies are fixed on the surface all right. So, whenever they are I mean whenever this array is exposed or is poured or is treated with a mixture of complex protein for example, a cell lysate which has got multiple such proteins, but we have only attached antibodies to a specific protein on the surface.

So, what will happen only our target protein of interest will bind to the antibody right and as from the principle you already know the next step being a fluorophore with a tag which will only interact with the area immobilized antibody where antigen has been captured and that will emit the signal. So, there are multiple version of diagram the concept is actually same whenever you see an antibody that is on the surface. So, whenever this type of Y or immunoglobulin is on the surface this is analytical microarray capture microarray all right. So, what happens? So, we will definitely get a situation where from two different samples suppose we expose the microarray two different samples one array or one sample may have more of one protein and the second sample may have less of that protein. So, definitely they will interact differently the amount of fluorescence signal will be different the amount of binding will be different all right because the sample have got their different affinity for the proteins all right.

So, therefore, we can have I mean we can have direct information about the expression of proteins because the amount of protein if it is expressed more will bind more if it is binding less means there is a less protein expression. For example, we are treating I

mean taking two cell culture plates all right or lysate or cell lysates one which has been treated with some drug another which has not been treated for example, a control right. If we see the amount of signal fluorescence signal is more then definitely we will have the idea that due to the treatment of the drug the expression has been more. This concept is absolutely the same we can do the same via western blot we can do the same via ELISA, but the beauty here is we can do thousands of such assay in one simple plate the basic concept is just like a enzyme linked immunosorbent assay which is also a non high throughput proteomic techniques are traditional proteomic technique ELISA western blots where we I mean expose the single samples or maximum two through three samples to one gel electrophoresis then we blot them or we can run an ELISA on 96 well plates to get which is time taking to get the idea about the expression of proteins, but protein micro since we are discussing various high throughput techniques we are discussing advanced proteomics over here this has got basically exponential or manifold for example, elison steroids right. So, very very useful in capturing protein expression identifying and profiling of protein from disease tissue basically same thing that we do while ELISA western blot we can do manifold in much shorter time and just like ELISA we can have a sandwich version for example, the protein is sandwiched between a capture antibody and the secondary antibody the capture antibody is the primary antibody in case of sandwich LAS it is a secondary antibody mind it in case of the sandwich LAS it is a secondary antibody which has got a fluorescent tag and in case of the this one I mean non sandwich version directly immunocapture version the itself the proteins of interest is tagged with a fluorophore.

So, whenever it is binded it is bound they will emit a signal all right. So, this is analytical micro the concept is very easy for fundamental understanding just note on the solid surface antibody is bound and this antibody captures our protein of interest. Once captured the antigen antibody reaction is done we are tagging it with a fluorophore which emits a signal. So, this is analytical microarray also known as normal phase unless it is mentioned normal phase we do not mention it. So, why do we need to know whether it is normal phase just like HPLC right there is also reverse phase.

So, this is the operational chemistry is just the opposite of this here antibody was bound and we are capturing the antigen protein of interest what happens in reverse phase just the opposite. Here all the proteins from the cell for example, a tissue sample tissue lysate are bound or immobilized on the sample for right cells isolated from various tissues of interest are lysed ok and then this tissue lysate is arid on the microarray surface for example, on the microarray surface I have got a tissue lysate from bone from periosteum from skin just I am given a hypothetical example and then I treat it with an antibody which is tagged with a fluorophore which has got a I mean which will only bind to our protein of interest right. So, in this case since protein is I mean our protein of interest POI stands for protein of interest. So, here in the earlier normal phase antibody was on

the surface protein of interest is being captured here protein of interest is already on the surface we are designing an antibody in such a way. So, that only if it binds to our protein of interest then it will emit a signal all right.

So, now, this also can be done with the help of two antibodies or a single antibody. So, primary antibody whenever there is one single antibody which is tagged with a fluorophore or if you are using two antibodies generally the second antibody which binds to the first antibody. So, antibody to the first antibody. So, that is secondary antibody then the secondary antibody is tagged with a fluorophore. So, basic difference in the normal phase antibody was bound in the reverse phase antigen is bound right.

Now, these antibodies are actually can be detected by multiple methods for example, chemiluminescent method, fluorometric method or cholerimetric methods all right. And reference peptides are printed on the slides to allow for protein quantification of sample lysates. For example, I am I have lysed two different tissues all right. I just want to see what are the amount of proteins total proteins that have been expressed by the tissue. So, I will assay the entire I mean array the entire protein on the cell surface of both the samples in two different array chambers.

And if I now target the treat them or incubate them with an antibody the array chamber or array slot which gives more signal will definitely give us the information that the amount of protein or the total amount of protein is much more in that array. So, mind it quantification of sample protein quantification very important is done by reverse phase microarray ok. Reverse phase microarray also allow for determination of the presence of disease causing altered proteins. For example, post translational modification if we design an antibody which will only bind to the normal protein and we can design the such a way. So, that the diseased sample the proteins will not be bound or we can design in other way also.

If you already know the nature of the post translational modified protein we can design the antibody in such a way. So, that only the antibody will bound to the diseased sample array slot all right. So, there are multiple opportunities in this case just mind it the fundamental concept is we are immobilizing our protein of interest and we are treating it with an antibody. So, basically depending on the nature of antigen antibody reaction this is the first type of classification where we are either doing it by analytical normal phase or the reverse phase protein microarray right. So, you can see this these are the 4 diagrams I have compiled together and this is basically the conceptual diagram in case of analytical micro the normal phase the bound one is the antibody and we are detecting the cell all right or detecting the protein and in reverse phase the protein is immobilized on the surface and we are detecting the I mean this being detected by the antibody which is tagged with the fluorophore.

Here the protein of interest is tagged with the fluorophore all right and both the cases can be designed with either one or two antibody. If it is two antibody the one is known as primary antibody that directly binds with the protein of interest and the one that is binding with the primary antibodies in a secondary antibody and in general the secondary antibody has got the and in not in general in all cases the secondary antibody is tagged with the fluorophore if we are devising the microarray with two antibody mechanism all right. So, what is functional microarray? Basically functional microarray again the name target protein microarray. So, target protein microarray are basically designed to by immobilizing a large number of purified protein ok. So, what do we need to I mean why do we need this or how it is different all right.

Mind it in case of the reverse phase or analytical we are only detecting protein-protein interaction or detecting any unknown protein of a cell by designing an antibody all right. Here this is not only limited to protein-protein since we are having a large number of purified proteins all right in the sample over there mind it the protein we are not purifying we are just using cell lysates whether as a sample or as a array fabricating material. Here purified proteins are immobilized in the surface in order to study protein-protein interaction, protein-DNA interaction, protein-RNA interaction, protein-phospholipid interaction, protein small molecule, protein glycan for example, protein-glucose interaction. Any other molecule we are interested in studying their interaction with these purified proteins our method of choice is basically functional microarray. So, in here we are not treating the microarray with cell lysate.

So, for your for the purpose of this course and as a overview of functional microarray since this is a topic on its own and it can take up a whole text book all right. So, I am just making things very simple for you just understand functional protein microarray is a variety of protein microarray where we are immobilizing purified proteins on the surface right and we are using we can treat it with any non-protein ligand all right. For example, protein-protein-protein interaction definitely immunoassay is the type of functional protein microarray definitely right. However we are also extending the immune not the immunoassay antigen antibody reaction is not only we are not only limiting the protein microarray to antigen antibody reaction, but also to interaction between other protein and non-protein molecules all right. So, as to say enzymatic activity and detect antibodies demonstrate the specificity fabrication of monoclonal I mean profiling monoclonal antibodies there are much much much utility in this.

They differ from analytical protein microarray by containing full length functional proteins or protein domains very important. Whenever you see in multiple choice question the whenever you are making an array we are mentioning full length functional word might not be mentioned, but if it is mentioned full length functional protein we are

referring to functional protein microarray not analytical or capture microarray. So, why they are used? They are used to study the biochemical activities of an entire proteome in a single experiment very important. We already know we have already discussed what is proteome when we are introducing the term proteomics right. So, if we can understand the whole function of a entire protein in one single experiment we can do that with the help of functional protein microarray, but one thing to note is these proteins must retain their native or original structure right because if some of the proteins denatured while fabricating the array it will not be very helpful.

So, this fabrication of functional protein microarray chips are costly and they require expertise right. So, otherwise functional integration will not take place, but we always take care so that the original structure native structure the functional domain is maintained on the microarray chip. So, that the functional interaction on the array surface can be studied to understand the nature of the entire proteome. Now, as I discussed earlier we will be briefly touching the fabrication surfaces again purely speaking this is a I mean concerned or a special your expertise of material science all right. However, since we are discussing interdisciplinary stuff.

So, now fabrication is also done by a nanotechnologist and electrical engineering department electronic engineering department and this is not limited to any biology or any chemical engineering or material science or electronics or nanotechnology right in our institute all work hand in hand to fabricate these type of amazing devices. So, just for safe of studying let us see covalent immobilization. So, these are the systems these are the phenomena biological phenomena which are being used while designing or fabricating the chip all right. So, for covalent immobilization we are using aldehyde epoxy material carboxylic ester etcetera all right. For adsorption we are using PVDF polyvinylidene fluoride PVDF membrane again very important it is also used in western blot nitrocellulose membrane polystyrene agarose polyacrylamide gel in case of diffusion based microarray fabrication affinity all right nickel nitrolysisic acid NTA streptavidin glutathione based metal based there are multiple use of metal based I mean heavy metals and also inert metals for example, gold silver steel etcetera which are also used to design the surface or the base all right.

So, what are the special properties? For example, whenever you are using covalent immobilization very important it results in irreversible protein immobilization good for covalent reaction and long immobilization reaction right. Adsorption in order to negate the high background signal in certain assays these type of materials are used all right. When do we use agarose or gel based? Good for preservation of protein conformation and weak it leads to weak protein immobilization. So, can you I mean apply your brains for telling me which is better to device a functional protein microarray where we need to make sure the protein retains their conformation definitely diffusion or gel based right.

Affinity capture using streptavidin and glutathione it offers a possibility to control protein orientation very important the way how the protein is oriented in vitro it will remain it will not change in experiment.

Again conductive surface compatible with surface plasmon resonance or mass spectrometry detection all right. So, whenever we are using these detection methods novel detection methods this is SPR is surface plasmon resonance. We will be again discussing or naming the detection methods, but whenever we are using that we need to make sure what detection method we are using must be compatible with the surface not every surface is compatible with every detection method. So, metal based chips are used whenever we plan to detect with help of these type of detection method. So, this is in brief about the fabrication question may come up, but it is actually very difficult to memorize all of that.

So, I suggest you should just apply I mean study it lightly and if you are really interested in microchip fabrication micro fabrication then this will become your bread and butter and you need to study a lot more whatever we have discussed here all right. So, now we come to the detection system all right. So, detection was the last part after each and every microarray slots are being scanned using a laser scanner they will be detected. Now these detection can be done by labeled methods and label free methods all right there are two types of detection method. You already know about dyes like psi 3 and psi 5 cyanine based dyes we have discussed in last class in our DIGE class right differential in gel electrophoresis.

protein enzymes like HRP, radio isotopes like 32 and 33 potassium, 14 carbon, liposomes all can be used and are classified under label dependent detection. Detection means the signal what fluorophore or what tag we are attaching so that they will make a reaction they will produce some color or signal that will be detected by the detector in order to give us information about the protein expression or interaction. Again when the sample is low low abundance protein then there are two methods that have been published in multiple journals one is rolling circle amplification RCA and one is tyramide signal amplification TSA. These two names you need to remember because these are the method of choice whenever the amount of protein is very very very low these are costly, but these are much more sensitive and effective ok compared to these methods. And when we are discussing about label free methods these are now being adopted because the risk of I mean whatever it is whenever using label compounds it is costly it is dangerous to the it has got some deleterious effect due to radiation other issues right.

So, label dependent may not only that this is very important label dependence may affect the protein activity all right. So, we are completely I mean we are not using any

chemical based method basically we are using optics and physical physics based methods in order to detect them. So, they also emit their characteristic signal and those signals are interpreted and they give us information about the proteins and the microarray of the anti antibody reaction. So, what are them what are they to name few surface plasmon resonance spectroscopy SPRS right optical ellipsometry OE reflectometric interference spectrometry RIFS ok oblique incidence reflectivity difference OI-RD it is often written as that OI-RD atomic force microscopy AFM Seldi and Maldi Tof MS all right. Now Maldi Tof MS you already know we have discussed in details.

So, what is this is a new term right everything is new right, but these are all what I can say these are all specialized branches of spectroscopy and optics and microscopy which is beyond the domain of this course to be explained, but definitely I will take I will digress from the discussion and I will discuss in brief about Seldi which is actually surface enhanced laser desorption ionization it is very similar to Maldi what you have what we have learnt in previous lectures. Basically it is a variation of matrix assisted laser desorption ionization Maldi. You know in Maldi the sample is mixed with the matrix material right and it was applied to a metal plate it was sample was mixed with matrix crystallized and then added to a metal plate before irradiation by laser whereas, in Seldi what happens the proteins of interest in a sample become bound to a surface before MS analysis. So, basically the first we bound the sample the protein sample on to the surface and then after which it after it is immobilized then we are treating them with a matrix you get the difference in MALGI protein was first mixed with a matrix and the protein matrix mixture was being immobilized or treated or dried on the surface of the reflector. In case of surface and as a cell GACLGI what is happening here we are applying the sample to the surface modified surface and then we can wash it after the actual sample has bound to the surface and only our protein of interest will remain.

So, unbound proteins and contaminants are washed away and then we are treating with the help of matrix then the rest of the procedure is same we treat with a laser beam it sample gets ionized and then if the sample the ions are being targeted to a time of flight mass spectrometer all right. So, basically it is a combination of solid phase chromatography and time of flight mass spectrometry right very important the surface sample sample surface the key component in the purification all right mind it we are purifying it on the surface itself right we are designing the surface in such a way it is a special type of surface in which only our target protein will bind right and the detection method in case of cell D I mean the since it is a variant of mass spectrometry the analyzer is time of flight analyzer which we already discussed in detail in our mass spectrometry class. So, where do we use this this technique to detect proteins into sample blood urine any other clinical sample and basically we can modify the surface in such a way that we can actually with the technical innovation with the beauty of engineering

material science we can detect any sample of interest by modifying the surface ok by modifying surface we can potentially we use it for any type of diagnosis. So, it is got a huge promise and it will definitely be used in future proteomic and other many other techniques. So, coming back to conclude so, what are the applications of protein microarray all right.

So, broadly we can sub classify into diagnostic application protein functional analysis application basically the core proteomic application antibody characterization treatment development. So, let us briefly touch these points. So, in case of diagnostics definitely detection in antigen antibody reaction blood sample multiple blood sample in a small area everything can be detected together in one go. To discover new disease biomarker monitoring of the disease state response to therapy a very important term is coming up digital bioassay just like digital PCR since it is done in a small chip and electronics is involved to get the signal very important it might. So, happen the all the in very soon it is already have started happening dry chemistry is a phenomena, but in case of proteo high through proteomic technique we may be soon in a era where all the wet chemicals antigen antibody everything liquid based things are totally gone from the laboratory all right.

So, digital bioassay. Antibody characterization characterization of the cross reactivity specificity mapping epitopes basically whenever new monoclonal antibodies are diagnosed monoclonal antibody profiling very important profiling of monoclonal antibody we use we prefer protein microarray because huge amount of information can be obtained with much less effort all right in a very small area in a very you short amount of time. When we are discussing protein functional analysis expression profiling very important which proteins are expressed in a particular cell and as we have discussed just to name of you protein protein interaction protein phospholipid interaction enzymatic substrate receptor ligand protein DNA protein RNA protein glycan everything that we discussed in functional microarray basically is the function or as the application of protein microarray in general right . Last, but not the least development of new drug whenever we are discovering a new drug we need to know what antibodies are what are the proteins what are the various molecules of the body which will interact with the drug how they are done right. So, we can do a dry simulation with the help of molecular dynamic simulation which will again be discussing in protein protein interaction the bio informatics part in this course will briefly be touching it and after we are have got some result we can actually take those information apply them using this protein microarray again functional protein microarray where proteins are being exposed with many other ligands. So, they will give some antigen specificity for the therapy for cancer therapy allergic therapy any autoimmune diseases and so many things all right .

So, identification of all small small molecules that could be potentially used as new

drugs and how they are interacting with the body this information can also be achieved or studied using protein microarray lot of things can be done tested together with the help in a single experiment all right . So, to summarize what we have learned today we have learned what is microarray how microarray is been developed and what is the history what is the principle of microarray technology what is the principle of protein microarray basic principle of protein microarray what are the procedure what are the basic steps of protein microarray how it is done what is the various types and detection method we also discussed about surface enhanced laser desorption ionization cells as an extension of a previous topic that is MALDI all right and we have also discussed the applications of protein microarray. So, these are my references very important this is a very important journal those who have been interested in this class and those who want to know more can specially go and study this journals a free journal available the full text journal is available from which I have include some of my very important information and that is it for today I thank you for your kind attention.