Host-Pathogen Interaction (Immunology)
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Module No # 08
Lecture No # 37
Pattern-Recognition Receptors- RLR

Hi so in previous session we have learned about the TLR toe like receptor we have learned about the various ligands. We have learned about the signaling pathway and we have also learned about how the mutation or single nucleotide polymorphism or single nucleotide variants result to the development of some or other disease. And in some cases it plays an important role in protection against disease and we have also discussed in a great length that there are some molecules which we the researcher made.

And these molecules are acting as agonist or antagonist and these molecules are used in treatment of cancer. And they are also used in bacterial and viral infection and they are also used for autoimmune disease, asthma so and so. So in this session we will discuss about one very important pattern recognition receptor which is expressing in the cytoplasm cell. So the basic question is when TÐLR was already discovered then there is a few basic questions or there in the field.

As you may aware that most of virus they replicate in the cell they use the host cellular machinery and there are some RNA species are present in the cytoplasm. And these RNA species which is present in the cytoplasm there must be some sensor which can sense these RNA molecules in order to elicitate or activate or induce appropriate innate immune response. So this was a basic rational in the field when a lot of TÐLR work was going on.

So there was a kind of need that so how these species of RNA are sensed and there is development of appropriate innate immunity. So, when I say innate immunity and virus infection then one obvious responses the type 1 interferon production. This is a quite obvious so various RNA species present in the cytoplasm and how this is inducing a response our innate immune response or an induction of type 1 interferon.

So there was a discovery of some molecule before discovery of RLR so these molecules are protein kinase if you remember there is some interferon inducible gene. This interferon inducible gene one is protein kinase and another is if you remember there is 2 prime 5 prime oligoadenylate synthase enzyme is there. This molecule which is playing important role in antiviral immunity and this 2 prime 5 prime oligoadenylate synthase basically their end product activates RNase is L and that checks the viral replication.

However these molecules are not able to induce type 1 interferon so that was the basis for the discovery of RLR or cytosolic sensor. In 2004 group of Takeshi Fujita in Japan he extensively perform a screening experiment and he found out that there is a some molecule which can sense this RNA molecule and this molecule can induce the synthesis of type 1 interferon.

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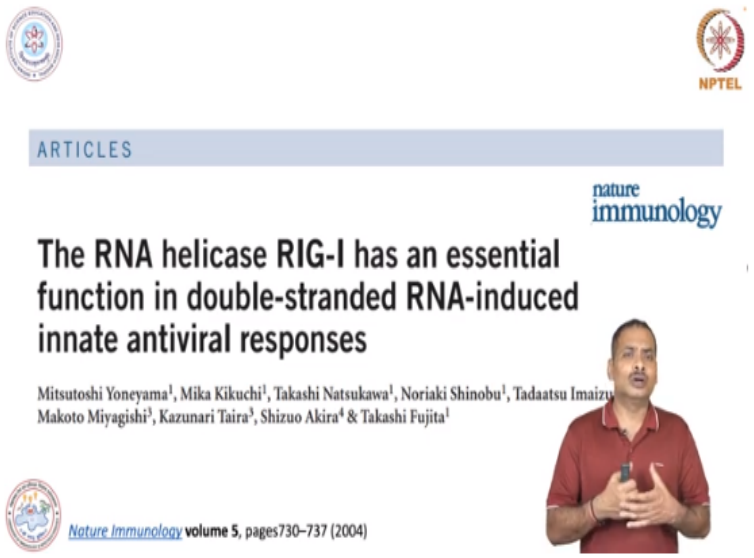

This is the original paper if you see this paper and this was published in 2004 in nature immunology and this was the first report or discovery paper for the identification and characterization of cytosolic sensor that is RIG-I. And after that this field expanded a lot there was a discovery of other members of RLR family protein and so and so. There was a discovery of the adapter molecule for this RLR sensores was taken place and then all signaling pathway was dissected out and then this field was very well established now I will tell what are the members of RLR family sensors or PRRS.

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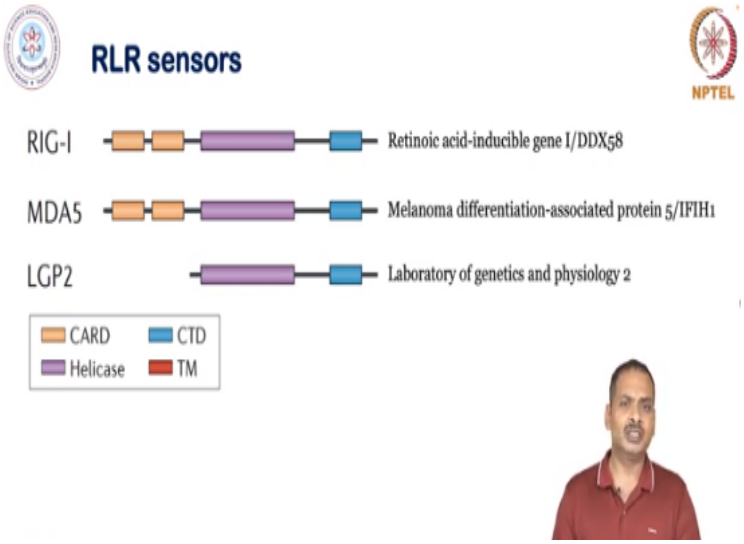

So here you can see there are RIG-I which I have discussed the RIG-I is basically the Retinoic acid induced gene 1. If you see this protein this has a card there are 2 domain which we call it as a card so card is basically stand for I have seen the literature people has a 2 kind of full form one is caspase recruitment domain which is originally given in Takeshi fujita's paper. And in recent several articles it is also written that caspaserd space activation and recruitment domain.

So do not worry about that you should you should know that there are 2 similar kind of full form of card and this as the name suggests RIG-I it is a retinoic acid induced gene. It is another name is DDX-58 now another member is MDA5 stand for Melanoma differentiation associated protein 5 and this is also known as IFIH-1. And this protein also has a 2 card domain and there is a RNA helicase domain. In both cases RIG 1 and MDA5 here it is shown in purple color.

So, this RNA helicase domain is very much essential in order to bind with the ligand you will see a I have a schematic for how this ligandke and the RNA molecule is binding with this sensor. So these are 2 major sensors and there is one more sensor which we call it as a LGP2. LGP2 is a little different name if you see it is a laboratory of genetics and physiology 2 the name of the gene. So this gene or this protein does not have a card they have only a helicase domain or RNA helicase domain and there is a CTD, it is a Carboxy terminal domain.

So towards Nn terminal there is a CARD domain in case of RIG-I and MDA5 and there is towards C terminal there is a RNA helicase domain in both RIG-I and MDA5 and there is a

carboxy terminal domain is there and this domain is also playing important role in sensing. Very interestingly, this LGP2 does not have a CARD the CARD domain is not there this has only RNA Helicase~~arley case~~ and CTD carboxy terminal domain.

So, when this molecule was identified people thought that this molecule is a kind of a negative regulator of RLR pathway signaling because this can bind with the ligand but this cannot transduce the signal. Please note the CARD domain is needed for the activation of downstream signaling so since this LGP2 does not have a CARD.

So, people thought that this is maybe a negative regulator later on the in vitro studies suggested a similar observation that this molecule is a kind of negative regulator but genetic studies means the knockout mice created for the LGP2 does not show that phenotype. So this is little debatable that LGP2 is a positive modulator or positive or it is a sensor or it is a negative regulator.

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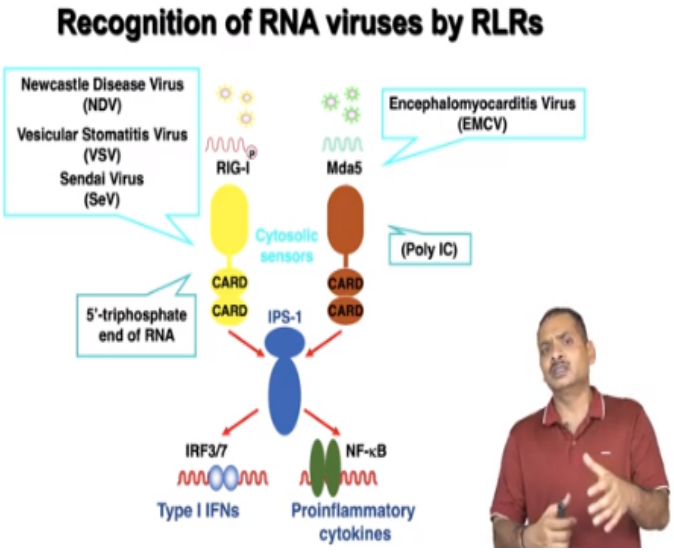

So how this or what are the ligands recognized by RIG-I and MDA5? So originally there are several viruses are reported to recognize in these studies like Dr. Fujita's studies and subsequent studies they extensively use these viruses. So RIG-I basically recognizes if you see in this slide there is a new-castle disease virus so Newcastle disease virus is not pathogenic to the human they are infect the birds and this virus we can easily use it in the lab as they are not affecting the human.

So NDV is one virus another is vesicular stomatitis virus VSV you are probably very well aware about the VSV. There is a Sendai virus, sendai virus is also not pathogenic to the normal human so these viruses we use it for the study of our RLR or RIG-I pathway. In addition, this RIG-I also recognizes a chemically synthesized ligand that is 5-prime triphosphate. The RNA molecule having this 5 prime triphosphate later on it was shown that in addition to 5-prime triphosphate they also recognize as diphosphate or to some extent monophosphate as well.

So these are the ligands for RIG-I and for MDA5 people used this EMCV virus encephalo-myocarditis virus. And in addition to EMCV this also recognizes chemically synthesized molecule which we call it as a Poly IC. Poly IC is a polymer of ionosine and cytidine and when this polymer is introduced inside the cell then this mimic like a virus infection. So this Poly IC is extensively used in this field in RLR signal in investigation of RLR signaling pathway.

So there are a variety of Poly IC low molecular weight, high molecular weight and it is considered that the MyD~~mighty~~88 recognizes a high molecular weight Poly IC a low molecular weight or short stretches of Poly IC is also recognized by RIG-I. So, after that this once you introduce these ligands then this RIG-I and MD5 pathway activates and then it induces type 1 interferon and pro-inflammatory cytokines.

So I will talk more about the signaling in next session and over there we will discuss what are the signaling pathways? How this types 1 interferon's as well as inflammatory cytokines are induced in the cell?

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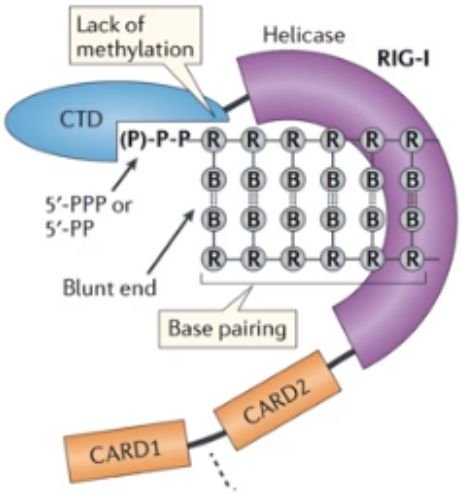

So this is a very interesting schematic here you can see that this RNA molecule which is having the triphosphate is binding with the RIG-I. Please note RIG-I is extensively studied it is a crystal structure and everything post translational modification so and so. So this is a schematic representation that how; the RNA molecule is sensed by the RIG-I means how RNA molecule bind with this RIG-I.

Here you can see there is a CTD this is carboxy terminal domain this is basically binding with this triphosphate it is holding the triphosphate and it could be a diphosphate also. And here you can see the ribose sugar and there are bases in RNA also there you remember the structure of RNA. So that has a nitrogenous basis and this is a ribose sugar so basically this is a recognized or it is binding with the helicase domain.

And it is considered that this when there is no ligand then the CTD mask this helicase domain and stop the activation of RLR signaling pathway and once ligand approaches to the RIG-I in the inside the cell. Then this helicase domain is basically unmasked by the ligand and then this is binding with CTD and helicase domain the ligand is not binding with card of this protein. This is a domain it is not binding with card so card is basically involved in activation of downstream signaling.

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Table 1 | **Selected viral infections detected by RLRs**

Baltimore classification	Virus family (examples)	RLR
I dsDNA	Herpesviridae (herpes simplex virus type 1; Kaposi's sarcoma-associated herpesvirus; Epstein–Barr virus)	RIG-I and MDA5
	Poxviridae (vaccinia virus)	RIG-I and MDA5
	Adenoviridae (adenovirus)	RIG-I
II ssDNA	No known examples that activate RLRs	NA
III dsRNA	Reoviridae (rotavirus)	RIG-I and MDA5
IV ssRNA (+)	Picornaviridae (encephalomyocarditis virus; rhinovirus; coxsackie B virus)	RIG-I and MDA5
	Flaviviridae (West Nile virus; hepatitis C virus; Zika virus)	RIG-I and MDA5
	Coronaviridae (SARS coronavirus)	RIG-I and MDA5
V ssRNA (–)	Orthomyxoviridae (influenza A virus)	RIG-I
	Paramyxoviridae (measles virus)	RIG-I and MDA5
	Filoviridae (Ebola virus, Marburg virus)	RIG-I and MDA5
VI ssRNA (RT)	Retroviridae (human immunodeficiency virus)	RIG-I and MDA5
VII dsDNA (RT)	Hepadnaviridae (hepatitis B virus)	RIG-I and MDA5

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So now I will discuss about this what the various viruses which is recognized by this are RIG-I and or ~~moiety~~ Myd88. Here you can see this is a Baltimore classification probably you might be aware that what is Baltimore classification. It is basically a type of classification system which places viruses into 1 of 7 classes. Here you can see there are 7 classes 1, 2, 3, 4, 5, 6, 7 and our group 7 classes or group based on combination of its nucleic acid that is nucleic acid could be a RNA molecule it could be a DNA molecule and the strand they possess.

For example there is a double stranded DNA there is a single standard DNA, double standard RNA, single standard RNA with positive sense. Single standard RNA negative sense and so on. So this is the basis of this Baltimore classification. So as you can see in this Baltimore classification so RIG-I and MDA5 can recognize all kinds of viruses except if you see there is these RLR sensors they do not recognize or it is not known at the moment that they recognizes. Some single standard DNA viruses so we are not aware let me take you with each class.

So first is that double standard DNA this is basically in this class there is a herpesviridae family viruses are there this is basically consists of a herpes simplex virus which cause a Kaposi sarcoma and the same class there is a ~~abstinent~~Epstein-~~-~~bar virus. If you remember or if you have studied this cause the bucket's lymphoma so this viruses are recognized by RIG-I and MDA5 they also recognizes Poxviridae a family member which is vaccinia virus and this is also recognized by both RIG-I and MDA5.

Adenoviruses~~idae~~ are basically recognized by only RIG-I so this is a class 1, class 2 single standard DNA they it is not known that any of these RLR members recognizes single stranded DNA molecule. The third class is double stranded RNA viruses which; is basically a Reoviridae family members and the best example is Rotavirus and this is recognized by both RIG-I and MDA5. The fourth class is single stranded RNA which is positive sense RNA viruses which basically consists of the pi~~C~~coronaviridae family viruses and the best example is EMCV you have seen in previous slide.

So EMCV is recognized by MDA5 but this is also recognized by RIG-I the rhinoviruses are also recognized by RIG-I and MDA5 Coxsackie B virus is also recognized by RIG-I and MDA5 viruses which is again the class 4 single standard positive sense RNA viruses and this is a quite in disease point of view this this family the flavi~~or~~viridae family is quite dangerous. If you see carefully you know that all these viruses like a West Nile Virus; hepatitis C virus; zika virus.

So all are quite dangerous or pathogenic right and they are recognized by RIG-I and MDA5 and the most important virus probably all of you are extremely well aware about this virus is Corona virus. So this coronavirus which is belongs to the Coronaviridae family member or Corona virus is basically SARS Cov~~id A~~2 or cov1. So this virus is a sensed by both RIG-I and MDA5 and there is a class 5 single standard RNA negative sense RNA virus.

And the most again fatal virus is Orthomyxoviridae family member and in that one of the key member and very famous member is influenza A virus. So this influenza virus is exclusively recognized by a RIG-I, there is a Paramyxoviridae which is a family member and this virus is basically a ~~missile~~ measles virus and this is also recognized by both RIG-I and MDA5. Filoviridae family member which is consists of again very dangerous virus that is Ebola virus you probably know that Ebola is causing a massive Hemorrhage and people die in very short duration.

So Ebola virus and Marburg is also equally fatal this is also recognized by RIG-I and MDA5. Single standard RNA with a reverse transcriptase activity so this is Retroviridae the best example is HIV this is also recognized by RIG-I and MDA5. Double standard DNA virus this is a Hepadnaviridae which is basically Hepatitis B virus is also recognized by RIG-I and MDA5. So

here you can see most of the viruses it irrespective to the DNA or RNA they are recognized by the RIG-I and MDA5 the key member of RLR family protein.

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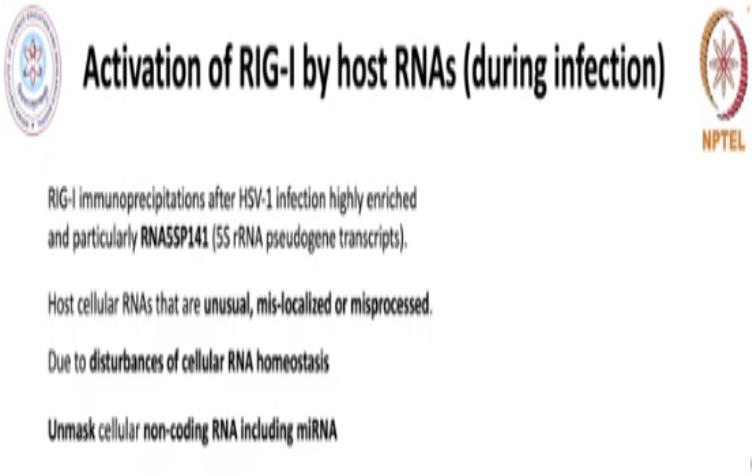

Now I will discuss about its not RIG-I and MDA5 is not only is sensing this viral origin nucleic acid this can be activated by host RNA and this host RNA which is sensed by RIG-I or MDA5 is activated during the infection. Again I will take your attention to this fact that when the virus infect the cells? At that time they basically make their own copy and how they make their own copy by hijacking the host cellular machinery.

They hijack the host cellular machinery and then they use this machinery to make their own copies either nucleic acid or protein and finally they assemble and then there will be ~~aggression~~egression of virus or release of virus. And this newly found virus or progeny of virus they again infect another healthy cells. So this is a very simple life cycle of a virus when they hijack then these cells are under stress the host cells are under stress.

This stress can also result to the dy~~i~~sregulation of a particularly if you see carefully the nucleic acid metabolism will be quite severely dys~~e~~regulated. And over there is a possibility that our sensors which is basically expressing for protection may start recognizing our own RNA molecule or nucleic acid. Then that will cause the problem so here you can see that after Herpes~~arper's~~ simplex virus infection people found out that this RIG-I is strongly associated with our own RNA molecule and this RNA molecule is RNA5SP141.

This is basically a 5S ribosomal RNA and this 5S ribosomal RNA is a basically a pseudogene transcript. So you can understand due to this virus infection this RIG-I started binding or recognizing the host 5S RNA and that will cause a activation of signaling. So when this is happening then there will be a mis-localization of this RNA unusual it was which is unusual in normal scenario this is not happening the mis-localization or misprocessing.

So this infection basically results to this mis-localization and misprocessed overall. If you see very carefully there is a disturbance of this nucleic acid metabolism or nucleic acid homeostasis. Particularly in this case the RNA homeostasis is severely skewed and this is skewed process basically result to the Binding of RIG-I with our own RNA molecule. That is this infection also cause unmasking of non-coding RNA and so probably you might be aware that now there are various species of RNA molecule in the cells

And all these species of RNA molecule basically play a very important role in gene regulation. They are kind of fine tuner and they act at transcription level so this fine tuning is basically caused by a variety of RNA species which we call it as a non-coding RNA this is a quiteck chunk of RNA non coding RNA is a quitearter chunk. If you see the genome so only 2% of genome makes a protein and rest of genome we were not aware but now we are having evidences that they encode for is various species of RNA.

So if you see this various species of RNA they are not just expressing like that they play a very important role in transcriptional regulation. And this transcriptional regulation is basically taken by various non-coding RNA. For example there is long non-coding RNA there are short long non-coding RNA there are circular RNA. So all this plays a very important role in regulation and among this; short non-coding RNA one micro RNA rnask are also there.

So micro RNA, rnask these days there is huge evidences; of evidences of this micro RNA play a very crucial role in regulation of any gene. So basically, here you can see the cell is loaded with a variety of RNA and if there is some disturbance in homeostasis of RNA metabolism then that may result to the activation of a RLR pathway.

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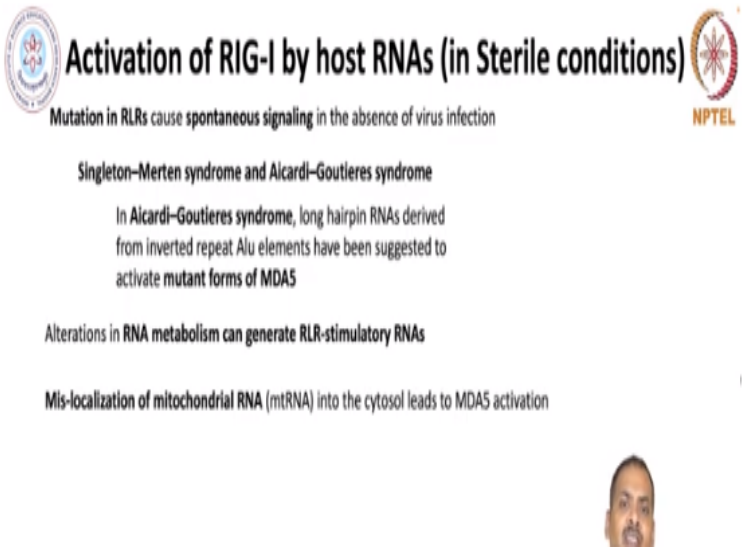

So this in previous slide you have seen there is some disturbance in cellular homeostasis by virus infection. Now there are also evidences that this RIG-I can get activated by itself and without any perturbation which means without any virus infection. ~~If you~~ This is quite obvious and common that there must be some individual who is having some mutation in the members of RLR protein. So mutation in RLR cause the spontaneous; signaling in absence of any virus infection.

For example, if there is some mutation in CTD which will keep it in unmasked form of helicase then probably that will keep on activating and that is a point of concern. So, there are 2 diseases which are known as Singleton-Merten syndrome and aicardi-goutières syndrome~~Alcardi-Goutleres syndrome~~. So these 2 syndromes are basically in these individual there is a mutation in the member of RLR pathway protein or RIG-I or MDA5 sensors.

So in case of this a aicardi-goutières syndrome ~~Alcardi-Goutleres syndrome~~ I am speaking in convenience because the long name is little complicated. In case of a Alcardi syndrome there is a long hair pin RNA derived from inverted repeat you know there are Alu element with a lot of Alu~~olive~~ element. So repeated Alu element has been suggested to activate mutant form of MDA5 so; this mutant form of MDA5 basically activated by this inverted repeat of Alu element and that will cause activation of RLR pathway.

Alteration in the RNA metabolism can generate the RLR stimulatory RNA so if there is some alteration in RNA metabolism. For example some enzyme lacking or expressing more so that will that can generate the stimulatory RNA and that stimulatory MDAs activate this RLR pathway. There could be a possibility that there is a mis-localization of a mitochondrial RNA into cytoplasm and that may lead to the MDA5 activation.

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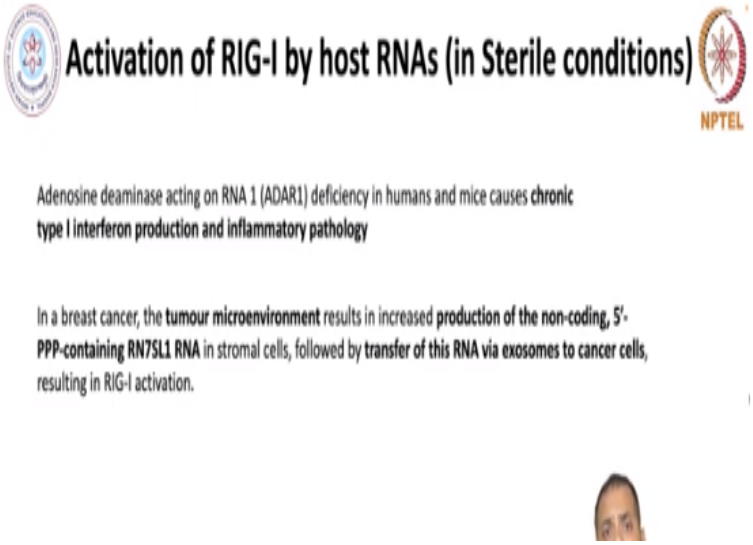

So ~~The~~there are more situation in which there is activation of a RLR signaling pathway that is a Adenosine deaminasse is acting on RNA 1 deficiency. If there is a enzyme deficiency will be there in human or mice that result to the chronic type 1 interferon production and inflammatory and that chronic production of type 1 interferon result to the inflammatory pathology. There is evidences that in breast cancer the tumor micro environment result in increased production of non-coding 5 prime triphosphate containing RNA that is RN7S~~5~~L1 RNA in stromal cell and that followed by transfer of this RNA via exosomes.

So exosomes are a vesicle which is released from the cells and this exosomes is loaded with a variety of molecule and basically these exosome, the molecules which is present in the exosome they tell about the health of cell or eventually the health of host. So in many scenario these exosomes are used for future diagnostic so transfer of this RNA via exosomes to cancer cell and that result in activation of RLR pathway particularly RIG-I.

So now in this session you have learned that this how the RIG-I pathway was discovered or how RIG-I is discovered and what are the various ligand for this RIG-I and MDA5? And here you also learned that this RIG-I or RLR pathway is not only playing or getting activated during virus infection it is also activated by host various RNA species can activate this RLR pathway. In this field the RIG-I is quite extensively studied but MDA5 was not that extensively studied so this is just for your note.

So with this I will stop this session and in next session I will discuss about the RLR signaling pathway. What are the signaling things are there and I will also discuss this RIG-I and MDA5 they are undergoing lot of post translational modification or there is a micro RNA mediated regulation. So I will just show you the complexity that how this RIG-I and MDA5 how complex the regulation of these proteins are there?

Finally I will discuss about the disease associated with RLR signaling pathway and there are some molecules which is used for used as a therapeutic in order to activate or activate the RLR pathway. And there are some molecules which damp this RLR pathway thank you we will see you in next session.