Host-Pathogen Interaction (Immunology)
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Module No # 09
Lecture No # 43
Pattern-Recognition Receptors-DNA Sensor & Signaling Pathway

Hi, in previous session you have learned about the discovery of this various kind of DNA sensors and their adapter molecule more precisely that discovery of a sting. And you have also learned that this DNA sensing is basically dependent on TBK 1 and IRF 3 and the signaling axis~~ccess~~ basically induces the type 1 interferons. So in this session we will discuss about the various DNA sensors and their signaling pathways.

Because it is very interesting that how various signaling pathway converge to the T~~V~~BK 1 and that basically induces type 1 interferon in addition this also activate NF-Kappa B to induce the pro-inflammatory cytokines.

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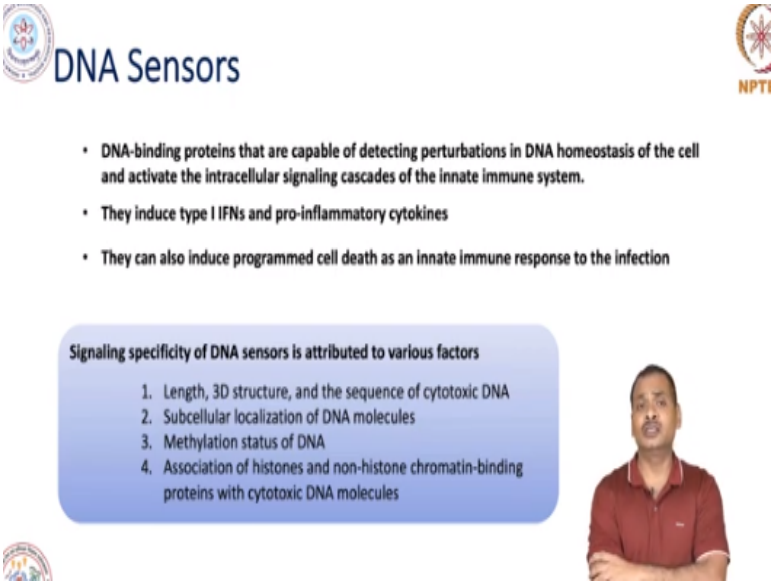

So the DNA sensors basically and there are some obvious property of DNA sensors. So DNA sensor basically binds with DNA and when the; DNA goes inside the cell basically this perturb the DNA homeostasis. And basically this will activate the cascades of a signaling pathway in

order to induce innate immune response. This innate immune response is basically the production of type 1 interferon and pro-inflammatory cytokines.

They can in addition to the production of type 1 interferon and inflammatory cytokine they can also induce the cell death and in technical term we call it as apoptosis. This is if you see the apoptoasis is also very important defense mechanism here I will just elaborate a bit more why because? Generally when we see the cell apoptosis we may think that it is not good for the host. But in case of virus infection cell death is a very important defense mechanism why?

Because when virus infects the cells they hijack the cell and then they use this host cell machinery in order to make their copy. So if there is no other way to stop the virus rather than using a programmed cell death. If the cell triggered the program cell death then the whole cell will die and along with the virus will be virus replication or the metabolic activity which is used by which is skewed towards or skewed for the replication of a virus will be stopped.

So this is the best way to stop the viral replication to kill the virally infected cell and in that way the progeny will be not produced more and then eventually the viral load will be reduced in the host. So it is something like that you know that in order to so our body are our host basically in order to protect the whole host they kill some cell. So it is something like that it is a very old saying probably you may aware that in order to protect the pond you need to take out that dead fish.

So something like that so similar strategy is adopted by our body so this signaling specificity of DNA sensors is attributed to various factor and that includes the length of DNA molecule length in terms of base pair the 3 dimensional structure. So the 3 dimensional structure is very important and you know that mainly RNA makes a various kind of 3 dimensional complicated structure but DNA do make some or various kind of three dimensional structure.

If you remember the various kind of DNA are existing although in physiological condition B form of DNA is a most predominant structure so in some cellular processes may be these structures are changing. Then this may be sensedassessed by the DNA sensor more precisely the DNA from another microbial pathogen or which includes bacteria as well as viruses probably

they have a much more diverse structure and that can be sensed by our DNA sensors and sequence of this foreign DNA.

So length as well as sequence and 3 dimensional structures these are the key point in order to sense the DNA, subcellular localization of a DNA molecule is also very important. For example in general the DNA molecule is not present in cytoplasm if you under normal circumstances. So if the DNA is localized in cytoplasm then it will be sensed by the DNA sensor or it will be considered as a foreign.

Another is a methylation status as I have explained you in previous session that CPG region or CPG Motif DNA they are hypeor methylated in case of or unmethylated in case of microbial pathogen and viruses. However in case of mammalian or host cell DNA this DNA is a highly methylated and that is a kind of distinguishing feature in order to induce the appropriate innate immune response against the foreign DNA association with histone and non-histone chromatin binding protein with cytotoxic DNA molecule.

So you know that this bacterial DNA or viral DNA they are not associated with histone protein however in our case in mammalian cell this is the DNA is present in bound form with the histone although in bacteria some histone-like protein is there. But it is not a stone and those distinguishing feature are basically sensed by the DNA sensors.

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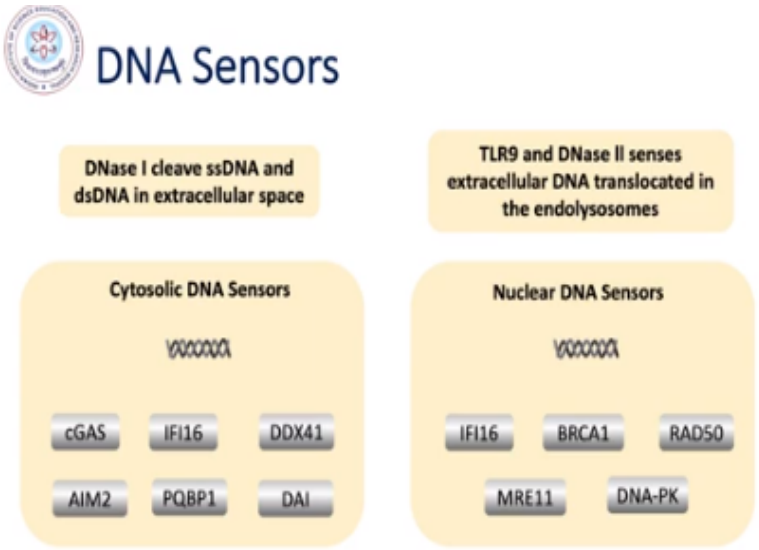

So DNA sensors could be a DNAase~~'s~~ 1 which can be leave the single stranded DNA and double stranded DNA in extracellular space so this is also protective response try to understand. So DNA's are which is present extracellular space so if some viral DNA will be there or some bacterial DNA will be there this will be cleaved by the DNAase~~'s~~ 1 this can be also cleaved by DNA's 2 in extracellular space and if the DNA is translocated in the endolysosome.

So you know very well this will be sensed by the TLR 9 inside cell there is various kinds of sensors and we categorize these sensors in 2 major part. One is cytosolic DNA sensors as you can see these are the cytosolic DNA sensor that is cGAS, IFI 16, DDX 41, AIM 2, PQBP 1 and DAI. So these are the basically a cytosolic sensor they sense in cytosol outside the nucleus. In addition the DNA sensors are also reported in nucleus or ~~nuclear~~ there are nuclear DNA sensor and they are IFI 16.

If you see IFI 16 and this basically senses the DNA molecule in cytosol as well as in nucleus. I will I show you in subsequent slide one of our work that DNA this IFI 16 which is sensing DNA can also sense influenza virus RNA this is our work and this is inducing the kind of cell death which we call it as a pyroptosis. In addition to IFI 16 there are various other nuclear DNA sensors like BRCA 1, RAD 50, MRE 11, DNA-PK.

So here you can see in all or in all situations the DNA can be sensed outside the cell in endosome, in cytosol and in nucleus. If you see our cell is fully guarded against foreign DNA and if foreign DNA will come then it will be either digested by enzymes or it will be basically sensed and there will be a production of inflammatory cytokine~~,~~. Type 1 interferon and one more very important response will be that this cell will undergo apoptosis this is another possibility. So the cell or outside cell and the cell is fully guarded against the foreign DNA.

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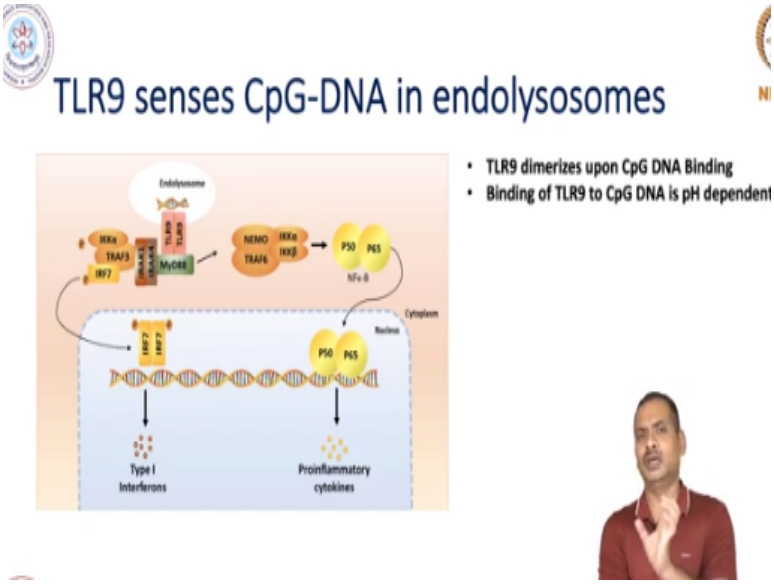

Now I will take up each sensor the first is TLR 9 and you are very well aware about the TLR 9 as I have discussed in previous session when I have discussed a toll like receptors anyway I will again explain. So this TLR 9 basically since the CpG-DNA inside the endolysosome and please note it is not depicted in this image. But here there is one important note that this DNA molecule is sensed by a post-translationally modified TLR 9.

There are some enzymes known as a cathepsin family protein they are protease. So these protease basically cleave this native receptor TLR 9 and up after cleavage they this protein basically sense~~ays~~ the CpG-DNA and this is an active form of TLR 9. The whole receptor itself the ~~knife~~nive receptor whole is not able to sense the CpG-DNA. So before sensing it undergoes cleavage by cathepsin family proteases.

And upon sensing you know that this will activate NF Kappa B through IKK pathway. IKK kinase will be activated and then this will activate the NF Kappa B to induce the pro-inflammatory cytokine. On another hand basically upon sensing this will be also recruit the MYD 88. And this MYD 88 activates IRF 7 I have told you this is a transcription factor so MYD 88 directly activate IRF 7 via various protein complexes here you can see there is a IRAK 1, IRAK 4, IKK Alpha.

So all these ~~draft~~ TRAF3 all these guys basically activate the IRF 7 and that will induce the type 1 interferon. So this TLR 9 basically after cleavage they get dimerized and then they this will bind to the TLR 9 in pH dependent manner and then all the signaling are induced.

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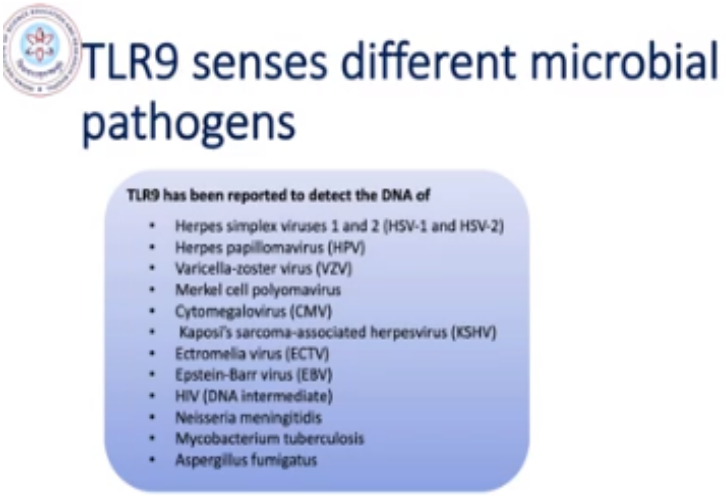

There are various TLR 9 ligands here you can see that there is a herpes simplex virus there are 2 types of herpes simplex virus and the DNA is just the TLR 9 sense the DNA from these 2 viruses. Herpes papilloma virus, Varicella-zoster virus, Merkel cell Polyomavirus, Cytomegalovirus, Kaposi's sarcoma-associated herpesvirus and Ectromelia virus Epstein-Barr virus, HIV the DNA intermediate.

So when HIV in fact then there is a stage they make a DNA intermediate and after making this DNA intermediate this DNA is integrated into the genome of a host cell and we call it as a pro virus. So that intermediate is also sensed by the TLR 9 the DNA molecule from various bacteria and Neisseria meningitis~~des~~, Mycobacteruium tuberculosis and fungus that is a Aspergillus fumigatus. So the DNA from viruses, bacteria and fungus is sensed by TLR 9 and that induces the type 1 interferon and inflammatory cytokine.

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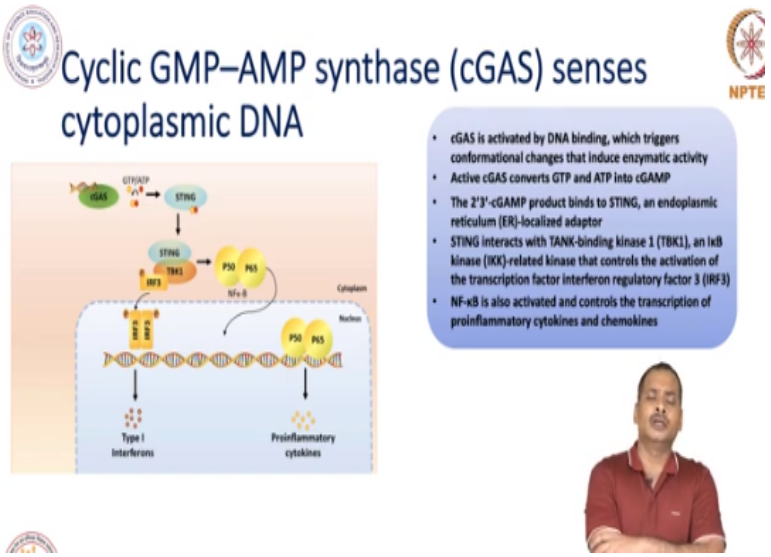

Another very important DNSA sensor which directly interacts with DNA molecule is a cyclic GMTP-AMP synthase. And this cyclic GMTP-AMP and AMP synthase we call it in short cGAS. So this cGAS also senses the cytoplasmic DNA here you can see that the cGAS basically binding with a DNA molecule and this trigger the conformational change that induces the enzymatic activity. So you can understand this is an enzyme and this enzymatic activity converts GTP and ATP into cCGAMP.

This cCGAMP which is basically 2 prime 3 prime cCGAMP product basically bind with the STING which is a present you remember the STING molecule which is act as an adapter and this a STING molecule is basically present on endoplasmic reticulum. And this STING interact with TBK 1 and then this TBK 1 or IKKI basically phosphorylate this interferon regulatory factor or IRF particularly IRF 3.

And this will induce the type 1 interferon this also activate IKK complex and subsequently activate NF-Kappa B. This NF Kappa B basically produce a pro-inflammatory cytokines and chemokines as well. So in that way the cGAS is sensing the DNA molecule here you can see it is quite interesting the cGAS is an enzyme and upon binding with the DNA molecule this activate this molecule basically produce an intermediate which you can see that this is a 2 prime 3 prime CcGAMP.

So this intermediate basically activate the STING molecule and indirectly and this is since the DNA is sensed indirectly and it induces the innate immune response.

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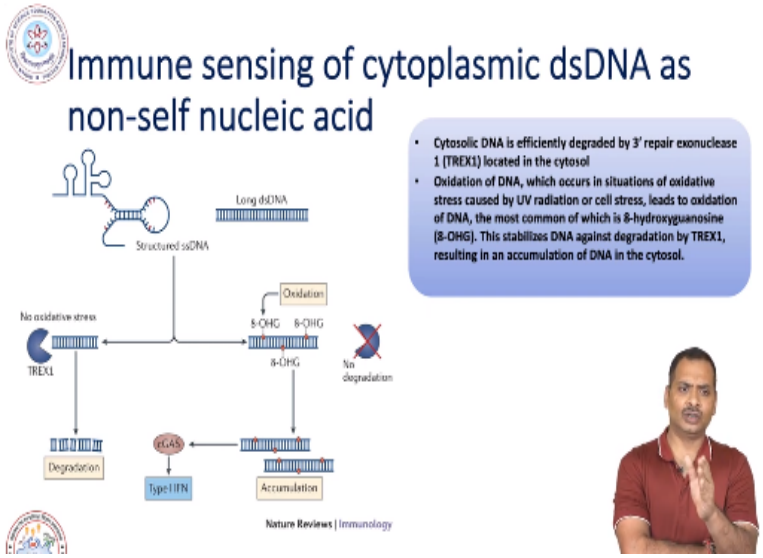

Another is there are immune sensing of cytoplasm double stranded DNA as a non-self nucleic acid so here you can see that there are unstructured single stranded DNA long double stranded DNA molecule. And there is a one molecule known as TREX 1. So this TREX1 is also plays an important role in sensing DNA molecule. Basically this cytoplasmic DNA is an efficiently degraded by 3 prime repair endonuclease which is a TREX located in the cytosol. And oxidation of DNA here you can see there is a oxidation of a Non oxidative stress.

So here you can see that oxidation of DNA which occurs in situation of oxidative stress caused by ultraviolet radiation or some kind of cell stress result to the oxidation of DNA molecule. So this is quite interesting there is a oxidation of DNA molecule you have studied various redox reaction in various biochemical pathway like NADPH is involved in this kind of reaction. But here you can see the DNA also undergo the oxidation the most common of which is a 8-hydroxyguanosine.

This oxidation product is 8-hydroxyguanosine and this stabilizes the DNA against degradation by TREX 1 resulting in accumulation of DNA in the cytoplasm. So this DNA will be accumulated in the cytoplasm and eventually you have studied the cGAS so this oxidized DNA is basically recognized by cGAS. And once it is recognized by cGAS then you note this will

make an intermediate. And this intermediate stimulate the STING and then the system will activate downstream is signaling in order to produce the type 1 interferon and inflammatory cytokines.

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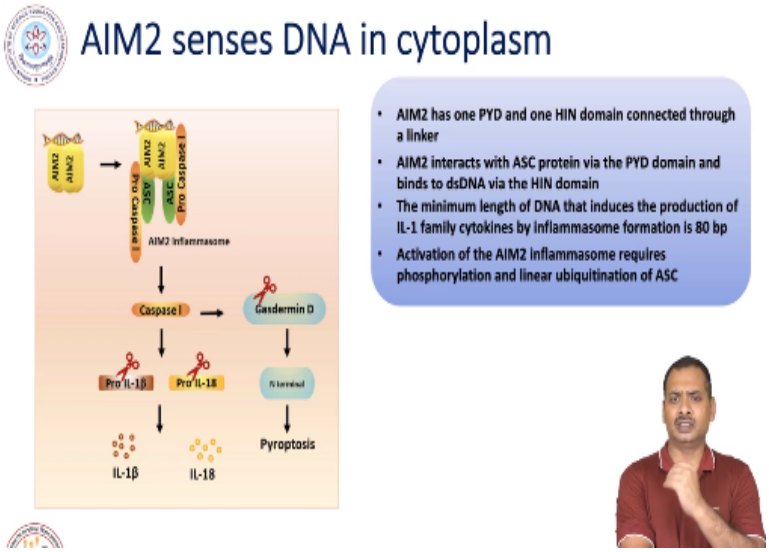

There is another sensor which plays a very important role in DNA sensing but it is not inducing a conventional cytokine that is a pro-inflammatory cytokine or I can say that this induces a very potent pro-inflammatory cytokine. But it is not inducing the type 1 interferon and this sensor is known as AIM 2 and this makes an AIM 2 inflammasomet~~ion~~. So here you can see that there is AIM 2 Inflammasome and this is basically consists of ASC molecule as I have discussed in N~~K~~LR when I was discussing NLR signaling pathway.

And it is also associated with Pro caspase and what happens once this complex forms? Then there will be activation of pro caspase into the caspase and this caspase is basically trigger the production or this caspase is basically cleave the inactive form of IL-1 family cytokine which is present in Proform and then this proform will be converted into the active IL-1 family cytokine. So here you can see that Pro IL-1 beta is cleaved and then there will be a production of Pro production of IL-1 beta.

And pro IL-18 is cleaved and there will be a production of IL-18 which is active form and this is secretory. In addition, this pathway also triggered the fire of tosses and pyroptosis is basically triggered by Gasdermin D and this will trigger pyroptosis it is a kind of cell program cell death.

So AIM 2 has a PYD domain and that is why this can interact with ASC. ASC has also PYD domain and CARD domain so there will be a homotypic interaction.

And the DNA is sensed by one HIN domain there is a HIN domain in AIM 2 and this is basically HIN domain and PYD domain and there are some amino acid which is basically linking these two domains. So I have already explained to interact with ASC protein or a PYD domain and double stranded DNA is binding with the HIN domain the minimum length of DNA that induced the production of IL-1 family cytokine by inflammasome formation is basically 80 base pair.

If there is a shorter stretch of DNA then it will not induce the IL-1 beta production so activation of this AIM 2 inflammasome basically require the phosphorylation and linear ubiquitination of ASC so this is one additional point

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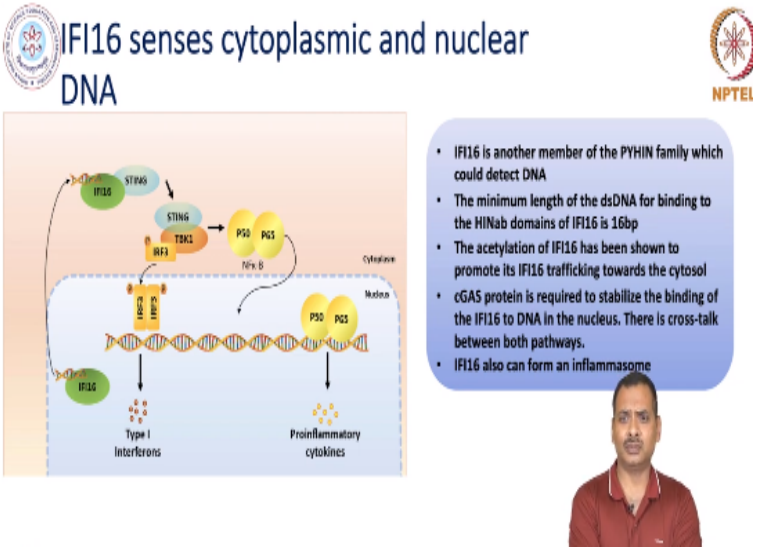

Now I will talk about the IFI 16 how IFI 16 basically senses cytoplasmic and nuclear DNA here you can see that this IFI 16 is basically localized in cytoplasm as well as in nucleus. And when DNA approaches to this sensor it interact with a STING and then you know the downstream path testing will activate TBK 1, IKK complex and there will be a production of type 1 interferon and inflammatory cytokines.

So IFI 16 is another member of PYHIN family protein and this could detect the DNA and minimum length of double stranded DNA for binding in domain there are 2 ~~in~~HIN domain HIN

1 and HINab domain is a 16 base pair. So here you can see that previously you have seen that AIM 2 can bind with approximately 80 base pair. And if there is a shorter stretch of DNA then IFI 16 can bind and induce the immune response the acetylation of this IFI 16 has been shown.

To promote the IFI 16 trafficking towards the cytosol and cGAS protein is required to stabilize the binding of this IFI 16 to DNA in the nucleus and there is a crosstalk between both pathway cGAS. And IFI 16 at some time at some places they do have a crosstalk and IFI 16 can also form an inflammasometion since this has a PYHIN domain it is involved in making the inflammasometion

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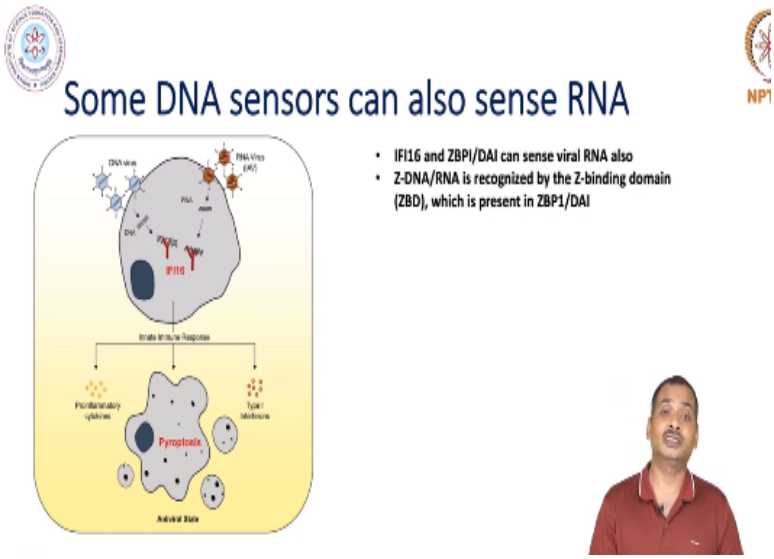

So this is our study here we have discovered that this IFI 16 is not only playing important role in sensing DNA molecule it also senses RNA. And RNA is basically from influenza virus so here you can see that IFI 16 can sense viral RNA. Viral RNA is basically from influenza virus they do not sense from other viruses at least what we have tested and that is NDV Newcastle disease virus they do not sense NDV RNA. And basically this upon sensing they trigger the as you can see in this slide this pyroptosis besides the production of inflammatory cytokine and type 1 interferon they also induce the pyroptosis.

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DNA ligand structures that indicate non-self

Structure	Recognized structure	Receptor or effector molecule	Origin
>40-80 bp	Base-paired DNA ≥40-80 bp (≥25 bp in mouse cells)	cGAS, AIM2 (human); cGAS (mouse)	Eukaryotic genomic DNA, bacterial plasmid DNA, dsDNA viruses and lentivirus dsDNA
>12 bp	G-rich Y-form DNA: base paired DNA ≥12 bp plus unpaired G in adjacent ssDNA (G indicated in red)	cGAS	Long ssDNA with secondary structures: reverse transcripts (cDNA) of lentiviruses, endogenous retroviruses and retroelements
...CG...	CpG-rich DNA. CpG methylation blocks TLR9 recognition	TLR9	Unmethylated DNA from bacteria; DNA from viruses and host
...ATATATATATATATATATATATATATA...	AT-rich 'ssDNA'*	RNA polymerase III and RIG-I	Unclear
>400 bp?	Long DNA–RNA hybrids	cGAS	Reverse transcript of cDNA–RNA template hybrids of lentiviruses, endogenous retroviruses and retroelements
	Short DNA–RNA hybrid	TLR9	Hybrids of RNA transcripts plus DNA template (RNase H deficiency)

So now I will talk about a kind of I will summarize various DNA sensor and here you can see that about more than 40 to 80 base pair DNA double stranded DNA can be sensed by cGAS, AIM 2 in human and in mice. It is sensed by the cGAS another is that there is a G-rich Y-form DNA; base paired DNA is more than 12 base pair. And this unpaired G is adjacent in a single stranded DNA and this is sensed by cGAS. There is a CpG motif DNA you know that this CpG motife DNA is sensed by TLR 9.

AT-rich DNA or AT-rich which is present in DNA that makes AT or AU-rich RNA and this is basically sensed by the RNA polymerase 3. And basically RNA polymerase makes this AUu-rich stretch of RNA and this stretch of RNA is sensed by the RIG-I and in that way this RNA polymerase 3 indirectly sendse the DNA molecule. So more than 400 base pair can be also sensed which is a basically a long DNA-RNA hybrid it is in the case of HIV infection.

So this is also sensed by the cGAS and there is a short DNA-RNA hybrid and this short DNA-RNA hybrid can be sensed by the TLR 9. So with this I will stop here and in next session I will discuss about DNA sensor and disease and therapeutic and with this we will complete the pattern recognition receptor and we will also complete the sixth week of this course thank you.