Host-Pathogen Interaction (Immunology)
Prof. Himanshu Kumar
Department of Biological Sciences
Indian Institute of Science Education and Research, Bhopal

Module No # 09
Lecture No # 42
Pattern – Recognition Receptors – Discovery of DNA Sensor

Hi so we have studied lot of pattern recognition receptor and if you see your previous session we have started this pattern recognition with discovery component. We have learnt how the pattern recognition receptor concept was emerged and there after we have learnt how this pattern recognition receptor was discovered the first pattern recognition receptor was tolle-like receptor. And we have extensively studied like tolle-like receptor.

And we have studied discovery of RIG-I receptor and then we have also studied various signaling pathway the disease associated with RLR pathway. And we have also studied like the node line receptors and we have again studied various ligand the signaling pathway and disease associated with nod like receptors. And this pattern recognition receptor is a DNA sensor and this DNAS sensor if you see it is very important in sensing various DNA viruses.

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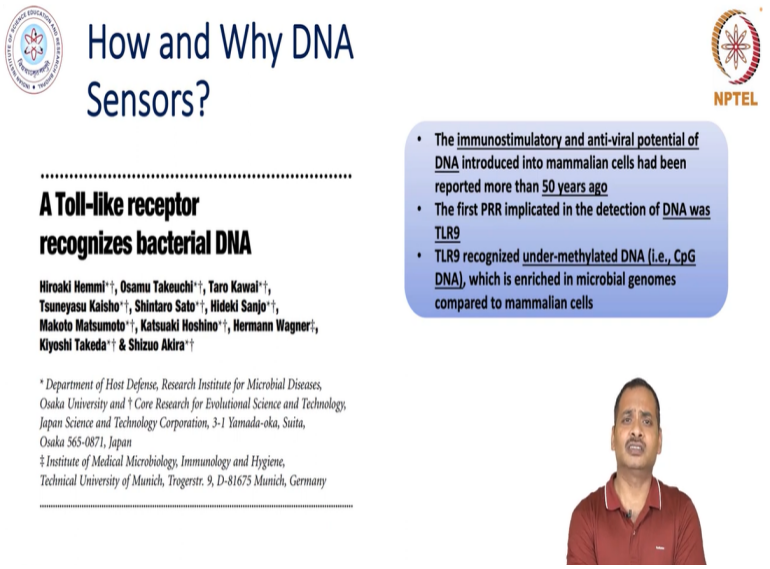

So let us begin with discovery of DNA sensor and if you see this is slide you will understand that this immune-stimulatory property of DNA in order to induce anti-viral response over the kind of discovered or noticed by the scientist about 50 years ago. And this is basically started with one simple observation when mammalian cells are when we introduced the DNA in mammalian cells then that induces some kind of immune-stimulatory responses.

So if you see carefully the pattern recognition receptor toell like receptor so there is a one TLR which is sensing the DNA molecule and that TLR is TLR 9. And this TLR 9 basically TLR 9 is also a DNA sensor and this TLR 9 basically senses hypomethylatedry ~~related~~ DNA molecule particularly CpG region in DNA which is quiet abundant in viruses and microbial cells irrespective of pathogen or non-pathogen.

Compared to the mammalian DNA so in general the mammalian DNA is a quite heavily methylated and that is why they are not sensed by the TLR 9. So this is the original discovery of TLR 9 here you can see that this toell receptor basically recognizes the bacterial DNA. And if you just carefully see Professor Shizuo Akira is a person who as I told you in when I discussed the discovery of this pattern recognition receptor.

So Professor Shizuo Akira basically he characterized most of these TLR sensors various adopters and signaling molecules. So this is a one of very good example he basically characterized he and his group characterize this TLR 9.

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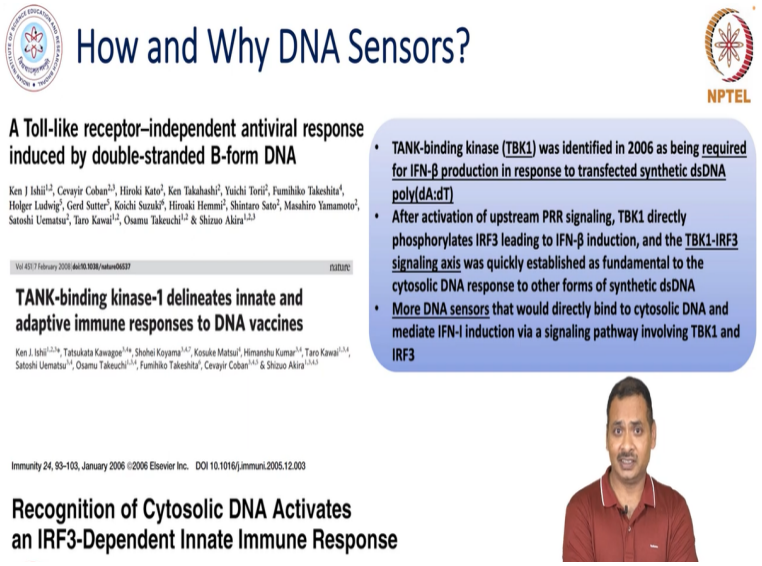

So people observed that this TBK1 if you remember the TBK1 in TRIF~~drift~~ dependent signaling. So TBK1 is associated with IKKI or IKK Epsilon this kinase basically phosphorylate IRF 3 or IRFs and then that induces the production of type 1 interferon. So people observed that the induction of type 1 interferon is basically dependent on TBK1 and this suggest that the signaling axis ~~eccss~~ the TBK1 IRF3 axis~~eccss~~ is same as TRIF~~drift~~ dependent signaling or RLR signaling is or this signaling access this TBK1 IKKI, IRF3 signaling axis~~eccss~~ is also needed for the DNA dependent induction of type 1 interferon.

So it is a very simple experiment you can very easily prove it if you have a TBK1 knockout mice. You simply introduce this poly dA dT or double standard DNA poly dA-dT is a synthetic ligand which commonly used for activation of DNA dependent signaling. So if you introduce this poly dA-dT inside the cell by chemical mean or by electro-chemical mean. Then you will observe that there will be induction of type 1 interferon but this production of type 1 interferon will be completely abolished if you use the TBk1 knockout mice.

So this observation was a quite well taken by the scientific community and then this is studies suggest that there are DNA senses which is upstream to the TBK1 and that DNA senses basically bind with DNA molecule and induces TBK1 and IRF3 dependent type 1 interferon. So this simple experiment also creates a question among innateer immune-biologists and that result to the discovery of DNA sensors.

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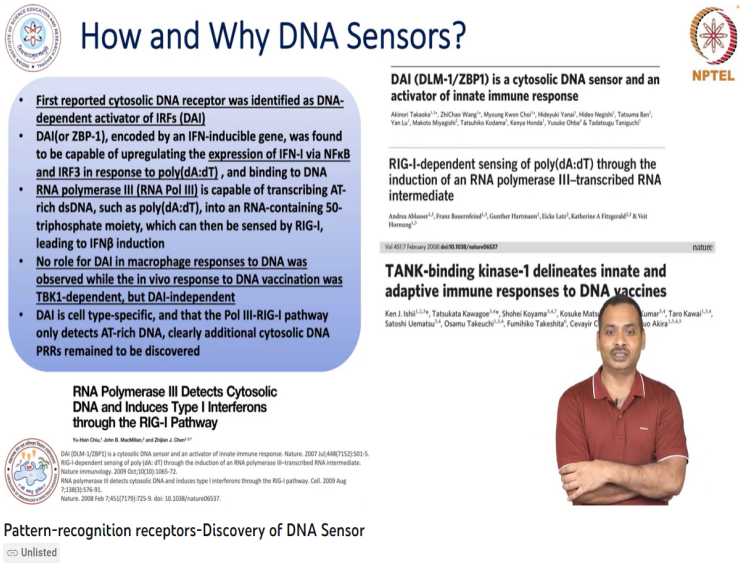

So the first DNA senses which was reported in around if you see carefully it is therefore the first DNA sensor was reported in 2007 and this is the DNA sensor. So this group this is again Japanese group this is Taniguchi group discovered or reported first cytosolic DNA sensor or receptor. And they gave this name DAI, DNA dependent activator of IRFs so this name was given by this group to this DNA sensor this is also known as DLM and ZBP1.

And this work was quite well established that, there is a some DNA sensor and this DNA sensor can sense and induce TBK1 dependent and IRF3 dependent induction of type 1 interferon. So this DAI a sensor is also inducing NF kappacup or B activation in order to induced inflammatory cytokine after binding with the DNA. This was a first discovery of

DAI another concept was emerged that you know that our genome is having a 8AT~~0~~ rich sequences.

And it was suggested that RNA polymerase 3 is capable of transcribing this ~~80~~AT rich double stranded DNA. And this basically creates RNA molecule and that 50 around base pair RNA molecule is having this triphosphate moiety and this triphosphate moiety. If you remember can activate the RIG-I so this work basically suggested that DNA is sensed but it is indirectly sensed by RIG-I so there will be a poly dA-dT.

And this dA-dT is a first transcribed to the RNA molecule which will have this triphosphate moiety~~motive~~ and this RNA molecule with this triphosphate moiety will be sensed by RIG-I and then there will be a TBK1 IRF3 dependent production of type 1 interferon. This was a basically supported by 2 research paper if you can see the middle panel as well as the bottom panel this which suggested that RNA polymerase 3 plays a very important role in DNA sensing indirectly the DNA sensing.

But we have there is a report that showed that this DAI is not playing important role in Vivo condition. In vivo condition they do not sense that DNA vaccine and they are not important in production of type 1 interferon so this can be very easily achieved this there is a simple experiment if you have a DAI knockout mice. And if you stimulate with DNA molecule then if it is a still inducing the production of type 1 interferon then that will suggest that.

So DAI is not very important maybe in some cell type it is maybe playing an important role but in general in vivo~~VIVO~~ under physiological condition DAI is not very crucial. So here you can see that there is a work that tank binding kinase one delineate innate and adaptive immune responses to DNA vaccine. So this work basically suggested the same so DAI is a cell type specific and that RNA polymerase 3 RIG-I pathway is only deducted ~~80~~AT rich DNA and all this work clearly suggest that there mus~~t~~eh be another or additional cytosolic DNA sensors and that need to be discovered.

So in research is giving a question is also very important or giving a direction to the research is also very important so this all this work gave a direction for the discovery of DNA sensor.

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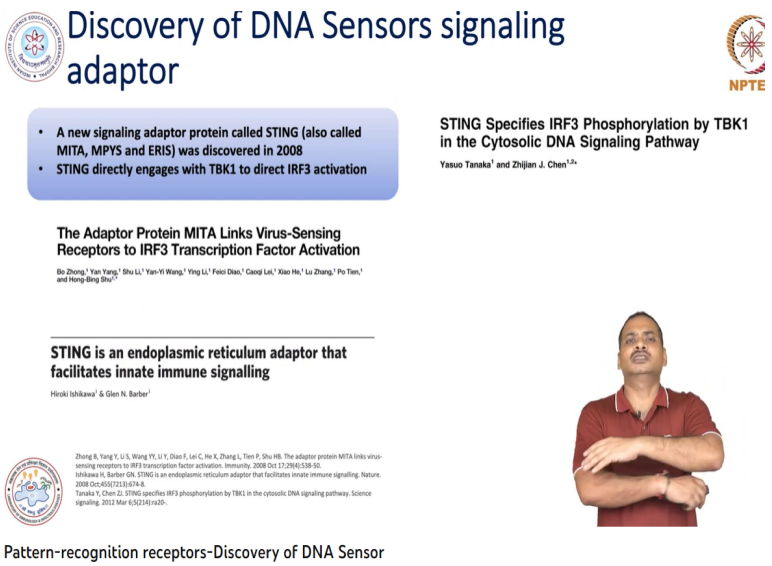

So after that there was a discovery of one molecule which is basically adaptor protein called as a sting and this sting when this sting was discovered simultaneously this molecule was reported by several other groups and they gave different name like MITA, MPYS and ERIS. So all this names where given independently when string was reported simultaneously the same molecule was reported by other group with a different name and people showed that this sting directly engage or activate TBK1 upon DNA stimulation.

So this suggested that this system is playing very important role in activation of TBK1 and subsequent activation of IRF3. However, this string is not directly binding to the DNA molecule suggesting that this is not a sensor maybe this is a one of signal transducer of this DNA sensing pathway and people suggested it as a adapter. So there are several papers which was published almost together here you can see and all this work suggest that a sting is playing an important role in DNA sensing however it is not directly interacting with DNA molecule and it is inducing the type 1 interferon depend on TBK1 and IRF3.

So that basically is the very important or very key finding after DAI was not successful in vivo~~VIVO~~ condition sting was a quite correct discovery and this plays a very important role and this is playing an important role upstream to the TBK1.

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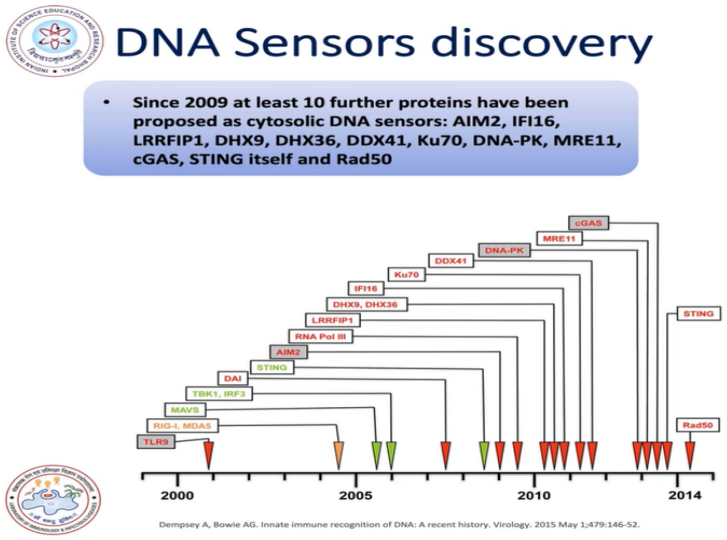

Pattern-recognition receptors-Discovery of DNA Sensor

So since then lot of work was profound and there was a discovery of various DNA sensors and here you can see that since 2009 at least 10 protein have been proposed as a cytosolic DNA sensors. Here this is very nice schematic also and those notice molecules are AIM2~~aimed too~~ if you remember into in NLR. So in to basically bind with the DNA molecule and it induces IL1 family cytokine. IFI 16 is also playing a very important role in induction of type of 1 interferon and inflammatory cytokines upon stimulation with DNA.

And other molecule like LRRF, IP1, DHX9, DHX36, DDX41, Ku70, DNA-PK MRE11 and cGAS, STING and Rad50. So all these are several DNA sensor and here you can see ~~the chronal~~ the discovery of these molecule in chronological order. If you see this graphical representation the first DNA sensor was which was discovered and well documented is TLR9. After that there is a discovery of DAI however it was suggested that in ~~VIVO~~ vivo they may not play a very important role.

After that AIM2 was discovered involvement of RNA polymerase 3 was reported and subsequently various DNA sensor was reported. And all this DNA sensors basically play a very important role in induction of pro-inflammatory cytokine which is NF kappa~~copper~~ B dependent and production of type 1 interferon. In next session I will talk about the DNA sensors and what is the ligands and what kind of signalling pathway they activate thank you very much.