RNA Biology
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Lecture - 59
Epitranscriptome and Protein Synthesis: Readers Writes and Erasers

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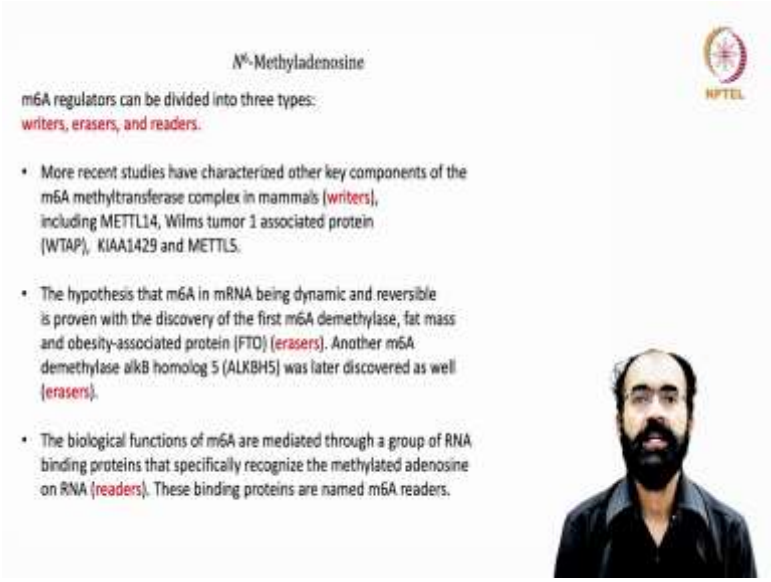

Hello everyone. Welcome back to another session of RNA Biology. So, we were here learning about the epitranscriptome modifiers in three categories, writers, erasers and readers. And we will see their importance with some examples in the coming sections.

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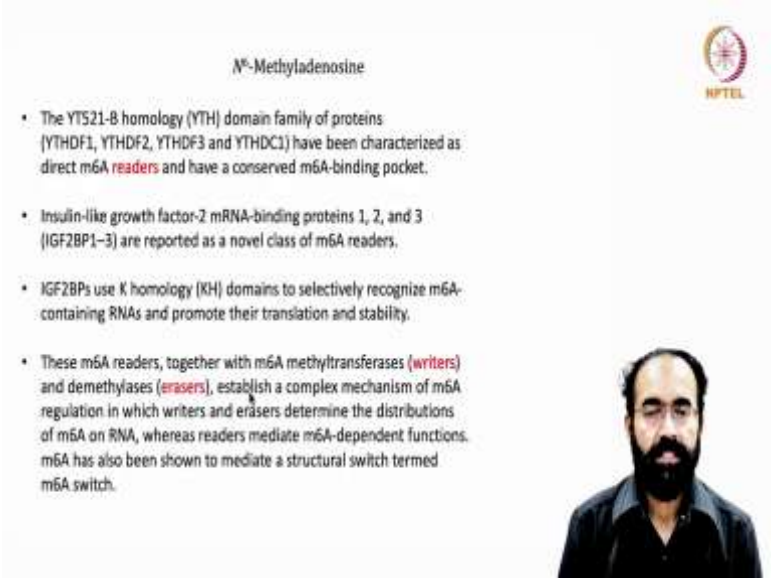

The N6-methyladenosine and one of the protein factors that recognize the modified adenosines, they are basically the readers. So, the YT521-B homology and usually call them as YTH domain family of proteins and they are there are different members like YTHDF1, YTHDF2, YTHDF3, YTHDC1 and many more will be discovered in future years.

And they have been characterized as the direct m6A that is modified adenosine bearing RNA. And these proteins recognize these modified adenosine and we call them as readers and have a conserved m6A binding blocks.

All proteins have a specific domain in their sequence, in their structure that will recognize this modified adenosine or methylated adenosine. One example is insulin like growth factor 2, mRNA-binding proteins, 1, 2, 3 that is IGF2BP1 to 3 are reported as a normal class of m6 readers. They are joining the group. Say for example, many proteins in a system have a bona fide function and where they are supposed to do that particular function.

However, many proteins will have an altered functions also, we usually call it as promiscuous function or promiscuity. So, a given enzyme or a given protein or a given receptor will have a defined function. However, given a particular circumstance, it may perform a different task and one such example is the IGF2 mRNA binding proteins.

So, they have their own dedicated function. However, they can recognize some specific modified RNAs as well. IGF2BPs used a K homology, that is KH domain, that is K is stands for lysine, H stands for histidine; domains to selectively recognize m6A containing RNAs and promote their translation and stability.

In general, we can say as a thumb rule, if an RNA have got this m6A, adenosine residue, they will facilitate, they will be marked for a translation and they will be marked for their stability. Means if an RNA has modifications, this methylation of the adenosine residues, they will be marked for their stability, means you can simply say prosperity of an mRNA.

What is the function of an mRNA? It has to be stable and it should be available for translation. Both it is doing. It is almost like giving a tuition or special class to a student. That means it will boost up the RNAs function. It may sound ok; it may sound great,

however, if this RNA or the product of this RNA that is a given protein is not so welcome or not so much needed in a given scenario that can lead to problematic situations.

Just like obesity, you think about it. Like if you are eating a lot and your body is not wasting anything, it is depositing in your body. Is not it a fantastic thing? But is it really so that you have lot of fat deposited in your body? Is it a good thing? It is definitely not a good thing because food need not necessarily be stored in your body forever.

It has to be stored for managing an emergency. Say you did not eat 1 day you did not could not eat at a time. So, your body's glucose should not take. So, it should simply be able to manage maybe for 1 day, but some people will have food stored in their body for 2 months or even 6 months. So, that is not a healthy sign.

Similarly, RNA facilitated by this kind of external factors. In some circumstances it is beneficial, but in some circumstances it will accelerate, it will boost up. So, it is like pouring oil in a fire. So, this KH domain is selectively meant for recognizing the modified RNAs.

So, these m6A readers together with m6A methyltransferase; that means, they are the writers and dimethylases, erasers establish a complex mechanism of m6A regulation in which the writers and erasers determine the distributions of m6A on a given RNA which are the A should be modified, which how much dense a m6A should be present in a given RNA has to be decided by these three that is readers, writers, and erasers.

Whereas the readers mediate m6A dependent functions in general. So, m6A also has been shown to mediate a structural switch termed as m6A switch; that means, it can influence the stability of the RNA due to its unique structural properties.

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So, the differential m6A profile in an is an emerging hallmark of cancer. So, that is why this modification becomes very important. You can predict, you can detect whether this particular tissue is vulnerable to get cancerous or not purely by looking at the m6A profiling of certain RNAs. Just like you may have heard about measuring your glucose, fasting glucose level or glycated hemoglobin levels etcetera as an indicator of diabetes. There is also possibility of predicting a potential cancer, cancer in future.

So, aberrant m6A deposition plays a key role in tumor genesis in various cancers by broadly altering the gene expression. So, by and large, the tumor genesis is occurring because of an accelerated stability and accelerated translation. So, this can just like you think about it, none of the humans are dying from the planet. Every human, some human beings are 500 years or the 1000 years or the 2000 years.

You can imagine how much crowded the planet will be because clearing every species after certain age is a must for the propagation of the species, not propagation of an individual or propagation of a one particular person is not necessary because maintaining a body or maintaining a particular individual is not desirable for the planet or as a species.

It is always good to contribute to the next generation. Why? Because a person who leaves longer just like an RNA living longer can do lots of undesirable effect because none of this molecules like an RNA or a none of the organism is perfect. It is always

required to change. So, dynamics nature of RNA dynamics nature of individual members of a species is a must.

So, m6A methylation plays an important role in a wide variety of biological and pathological process and including the formation of cancer. One example, one of the m6A methyltransferase METTL3 and it is a m6 methyltransferase is up-regulated in gastric, colorectal and liver cancers.

So, a lot of studies have found that they can undergo solid up regulation. A drastic up regulation means if you have an elevated action of this methyltransferase, it can definitely lead to cancer and it is detected so, in a variety of cancerous condition. And the presence of this methyltransferase will naturally increase the methylation levels of various RNAs including non-coding RNAs.

The m6A modification frequently involve key components of oncogenic pathways such as WNT beta-catenin pathway, PI3K, Akt, mTOR signaling pathway. So, these are all pathways, say for example, the WNT beta catenin pathway is a must like in order to repair, your lost cells, like your hair is lost, the WNT signaling is important to rejuvenate your new hair growth.

Say your skin is torn or you got a cut in your hand or you got a mosquito bite and your tissue is damage, you got a pimple and you make some tissue is damage. So, you need to repair that damage. So, starting from the MIU test damage occurring in your body to persistent damage like a big wound, a blister, a wound that formed because of a fire accident or even your intestinal epithelium constantly you know because of the movement of post digested food particle, your intestinal epithelium is constantly doing a lot of wear and tear.

So, all this repair required WNT signaling. So, in a nutshell you can say without WNT beta-catenin signaling, no organ substance can survive, organism’s chance of living is 0. So, it is so important. However, the same signaling is super up-regulated during cancer. That means, there is a fine balance between not having WNT signaling and excessive WNT signaling. Both are bad. Not having WNT signaling also is bad, too much of WNT signaling also is bad. So, you have to leave in between.

So, m6A modification accelerates the WNT signaling because it stabilizes each and every component of the WNT signaling. So, naturally, the WNT signaling gets deregulated into a positive side; that means, accelerated side. So, this can naturally leads to cancer.

Same way you have the this PI3, Akt, mTOR pathway. So, which is basically a cell proliferation associated pathway; that means, if you have Akt up-regulated you will end up having too much of cell proliferation. It is meant for maintaining the homeostasis. Basically, system has to decide, system has to sense, whether a given tissue or a given tissue should proliferate or should not proliferate. Both these things are decided by the WNT beta-catenin pathway and the Pi3, Akt pathway.

So, both these things are up-regulated drastically when it comes to the m6A superactivation or m6A methyltransferases up-regulated. And both these pathways when they are super active that eventually contribute to their apparent activation leading to cancer.

So, understanding the function and molecular mechanism of m6A mediated epitranscriptome may thus unravel the novel therapeutic targets and biomarkers for the cancers in early detection. So, it is important for us to know whether a given tissue is vulnerable to get cancer in future. That is one way.

These days we have lots of ways of detecting it by you know genotyping. Like many people you may have heard about it some famous celebrities who are vulnerable to get you know cancer. They get rid of their tissue, amputated much before that, so that they can escape from the cancer. That is a proactive measurement.

And some other similar approach is although your genes do not have any mutation, then how are you going to detect it? Because genes are perfectly fine. The RNAs that is formed is now undergoing this kind of changes.

Then, you should have a ways of detecting or ways of measuring such an altered RNA expression or methylated adenosine bearing RNA’s are present. So, this is also helpful in advising person or a subject whether they are vulnerable for cancer in a given tissue. So, lot of applications can be attributed to such kind of studies.

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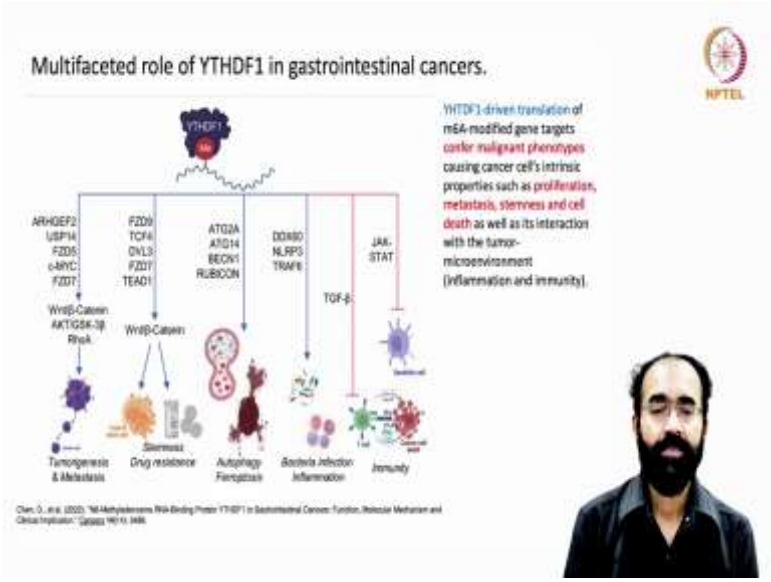

So, let us think about an example. Multifaceted role of YTHDF1 in gastrointestinal cancer. Gastrointestinal cancer is very complex and many a times it is extremely invasive and hardly any treatment can be done because they are so aggressive. By the time you detected the gastrointestinal cancer, it the time may be almost over. Means you detected once the cancer has established itself.

That is why it becomes so, aggressive. There remain silent. They do not give any clue. They do not give any indication. By the time you detected it, it is way too late and no possibilities of recovering. So, that is why gastrointestinal cancer becomes so pivotal. So, let us see. You have a methylated RNA. It is sensed by the YTHDF1 because it is a reader and it can work along with some of the signaling pathway like we have mentioned here, WNT beta-catenin signaling pathway.

That is WNT beta-catenin, I will briefly mention WNT is the ligand and the beta-catenin is the product and the product is normally being formed, that is the beta-catenin in a given cell. However, the product is not stabilized. The product is marked for degradation. However, if the WNT is present in the system, in the environment, it binds onto it receptor that is the frizzled receptor. It binds onto it and helps in stabilizing the beta-catenin.

The stabilized beta-catenin goes to the nucleus and help with the transcription pathway, which can be usually the cell proliferation and new cells are formed and it can repair the tissue. It is pretty simple.

Many components contribute to it. We will not go into the details because there are like GSK 3 beta is there like (Refer Time: 15:52) Many proteins are there. But idea is quite clear that if you have a stabilizing beta-catenin in your system, it can facilitate proliferation. It can be in regular proliferation or cancerous proliferation. So, lot of proteins in this mRNA, lot of proteins comes in this category like you can see ARHGEF2 and USP14, FZD5, c-MYC, FZD7 so on and so forth.

Many of them are components of the WNT beta-catenin signaling pathway and eventually you will see an accelerated WNT beta-catenin, Akt, GSK3 beta, RhoA, pathways and it will lead to tumorigenesis and metastasis. Metastasis means we already kind of discussed that this cancer cell originated from a given tissue established as secondary or tertiary quality in some other organ and damaging that organ also.

So, that becomes a (Refer Time: 16:49) Stage 1 is the beginning stage, stage 2, stage 3, stage 4. Stage 4 is the last stage, so when the secondary has spread all over the body. Another example is that FZD-9, TCF4, DVL3, FZD7 and this proteins have this methylation, and it is detected by the YTH domain family containing protein, that is YTHDF means YTH domain family protein.

So, they can stabilize the WNT beta-catenin and can lead to stemness and also drug resistance in case of various cancers. Stemness care what we are referring, to the like cancer stem cell. So, normally when you do chemotherapy in case of a cancer, many a times cancer will completely disappear everything is perfectly fine, cancer cell, the tissue damaged everything is fine.

So, after few months, then again, they will start showing the signs. So, because that is because of the presence of cancer stem cell. Why they did not die earlier? Because they were not showing any characteristics of cancer. So, it is just like it is a like a normal cell. So, not even proliferating.

Normally, all chemotherapy targets those cells which are proliferating. If non-proliferating cells also dead then the organism will be dead. After chemotherapy, the

patient has to be cremated. So, that you do not want to happen. So, chemotherapy when you are taking, always targets proliferating cells. But these cancer stem cell still remain dormant, they do nothing.

So, these drugs can do nothing on to them. They will come back to cancer stage later. So, when they come second time, they are look much more experienced with the chemicals and they are more fit than the old cancer condition. So, these stem cells when they come back, they will completely dominate and the patient will be dead in no time.

So, that is why cancer stem cells also a problematic situation and that lead to drug resistance because they survive the earlier drug treatment. Next time you cannot use the same drug. If you use that, the cells will be very resistant even while they are proliferating.

So, another list of genes like ATG2A, ATG14, BECN. So, you do not worry too much about the names of the gene. These list are included mainly because to understand how diverse the number of mRNA's we have and how they are contributing to it. Like, these pathway genes they lead to autophagy and ferroptosis. So, this happens because there will be an elevated metabolism and running short of resources, and this will lead to autophagy.

Autophagy means self-eating. Like simplistic way you are very hungry, no one is giving you food. So, you are eating your own finger or you are eating your own body's flesh. How does it sounds like? Like you have no food, no one is giving you food. So, you are biting your own body and eating.

So, this is what cells will do and they want resources and they do not have resources available. Not available from outside because they are utilizing too much of resources. So, they will do eating up themselves. And our body does always like you do not realize it.

Like, if you have not done a long running, if you have not done lots of you know exercise in the gym or something, after 6 months of gap or after 2 years of gap you are going to gym and you are doing some exercise. So, what will happen? After 2 days you will have all of your body will have pain.

Earlier you used to do same exercise you had no problem. And then, you gave a gap and then you are going and next day you will have lot of and that is called we will say sore muscle. Why muscles turn sore? Because muscle was able to handle the show 6 months ago when you were actively doing, now it is not able to handle.

What happened to the muscle? Because body ate it up. Because lot of, ate the muscle does not mean that the muscle went missing. Muscles are made of cells and the body did not make fresh muscle fibers in your body. The existing muscle, the number of protein, the amount of protein that contributes to the bulk. That is why Bodybuilders have got very fat, real bulky body whereas, regular person have got less.

This does not mean that they have more cells; they have the same number of cells. But the amount of protein deposited per muscle fiber is much much high. They also we have say 1000 muscle fiber, you also have same 1000 muscle fiber. But theirs is looking much bulkier and stronger because individual muscle fibers have got much more protein.

So, when you are not doing exercise, your body is utilizing those protein for body's own survival. Body do not want to waste unnecessarily build the protein. That is why first time when you do this existing muscle is not strong enough and you create micro cracks, micro tears in your muscle and it takes time to repair.

And after 1 week, you have a new muscle, you have new muscle in the sense, new proteins in the muscle and it will be stronger. And if you keep doing it, it will be stronger and stronger and you keep continue to do; it will become bulkier and bulkier. And this is how normally every body functions.

Any job, when you are do within limit. This does not mean that 24 hours you are in gym, you will become very bulky. You will be hospitalized. That is what it is going to happen. So, there is a limit, your body need a recovery time. So, your body automatically does the autophagy.

But it should not be doing simply because of an apparent mechanism or apparent. Then what will happen, some of the crucial tissues will go missing. So, your body will start suffering really badly. So, that is why autophagy and ferroptosis.

That means, the elevated metabolism will create a little bit more metabolic temperature and that also lead to apoptosis. Because beyond certain limit, if the cells temperature goes up, then lot of proteins go back. Then, that will be marked for apoptosis. So, this is what happens because of this accelerated methylation.

And then, lot of other proteins are there I am not going to read the names of each of them because it is not required. And it is helpful in preventing the bacterial infection and inflammation. But these proteins when they are hyperactive because of their methylation, you will be vulnerable for bacterial infection and even inflammation.

Lot of gastro intestinal cancers come because of your intestinal bacteria is producing some of the toxins and your body is inflamed. Inflammation is a trigger to cause cancer. So, your body should not get inflamed just because of having bacteria. But it do happen, it can happen because of some of the altered expression rate of some of the mRNA because of this kind of modification.

Another example is the TGF-beta, transformation growth factor beta. So, this is important in various pathways. Normally, it is a negative regulator of cell proliferation, but this also influential in inhibiting some of the unwanted activation of your immune system, immune cells. So, the lack of proper regulation of the TGF-beta in the desired level that can even lead to some of the autoimmune disorders.

Then, comes the famous JAK-STATs JAK-STAT pathway which is normally a contact based a recognition system. In the sense, if JAK is activated it will phosphorylate the stat and the phosphorylated stat goes to the nuclease and it is a transcription factor. It can create the cell proliferation environment.

The JAK-STAT and TGF-beta pathway are kind of negative regulators on when it comes to the immune system, maturation, immune system. So, the over action, over action of these pathway genes you will have a compromised immunity and it can lead to lot of complication.

So, let us see the YTH domain containing readers. And then, these facilitate the translation of the modified target genes. And this will confer malignant phenotypes and causing cancer cells intrinsic properties such as proliferation, metastasis and stemness and cell death.

So, these all these things are attributed together because of one common factor that is accelerated methylation as well as the interaction with the tumor microenvironment such as inflammation and immunity. That means cancer is already facilitated because of the over action of many of these genes. On top of that, the body lacks the ability to interact or counter the bacterial toxins, which will facilitate the inflammation and compromise the immunity.

In that situation, it is just like someone is about to fall and you are giving a push from to that person in the same direction where he is falling. So, falling is assured. So, everything is working together to cause a cancerous condition in several actively methylated mRNA transcript. So, we will learn more about this RNA modification and their impact in next class.

Thank you.