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W5L28_Studying Human Genetic Disorders using Transgenic Animals - Research talk (Guest lecture)

Hello, welcome back to I Think Biology lecture series on genetics. My name is Antara Das. Today we will be talking about studying human genetic disorders with transgenic animals.The main objective today would be to Define a transgenic animal model, Evaluate the use of transgenic animals to study epilepsy and we will talk about Animal rights and ethics towards the end. So let's talk about what are transgenic animals. A transgenic organism carries a foreign DNA sequence from another organism.

Now the foreign DNA sequence is inserted into the cell using genetic engineering and a variation of this could be that the DNA from the organism is modified and then it is put back into the organism and hence now the organism would have a modified DNA of its own genome. Even then it will be referred to as genetically modified organism. Some of the examples of transgenic organism could be a bacteria expressing human insulin protein, a tomato plant expressing a bacterial pest resistant gene, a Drosophila which is a fruit fly expressing GFP which is isolated from jellyfish. Here you can see a larva of a Drosophila and the brain of this larva is expressing GFP.

We could have a mouse which expresses a human ion channel or a mutated human ion channel gene and all these organisms which carry a foreign DNA from some other organism are referred to as transgenic organisms. So what are transgenic model organisms? Now any of these transgenic models which are frequently used in research can include bacteria, yeast, C. elegans which is a nematode worm, Drosophila, melanogaster, mosquitoes, rodents includes mouse and rat models and then you can have cell lines and even organoids which are half differentiated rudimentary forms of certain tissues. So brain organoids or some tissue organoids can be used to study different aspects of the cell lines or those tissues and can be used to study some diseases related to those tissues. Research areas which use transgenic models heavily include cellular

functions and development.

For example Drosophila models have been used extensively to figure out the circadian clock circuitry, disease morphology and cure. Example there are models of mouse models of Alzheimer's Parkinson, Epilepsy, and various forms of cancer and they can be used for preclinical drug or vaccine testing and this has several examples. So the use of transgenic model organisms to study human genetic disorders. Let's see some examples of that. So what is the approach that one takes when they want to use a transgenic model organism to study a human genetic disorder? So these are the basic steps.

A patient with a genetic disorder presents themselves to a clinic or to a doctor. The patient is evaluated for his or her symptoms and then often a family pedigree is constructed to figure out the pattern of inheritance whether it follows an autosomal dominant, autosomal recessive or an x-linked or y-linked inheritance pattern. And then the DNA of the patient and several family members, either affected family members or non-affected family members is evaluated, the gene is sequenced to figure out which genes in the patient is unique and might be responsible for the disease phenotype. Once we know the sequence of the disease or of the gene then we can go to the next step. This is the creation of a transgenic model organism.

Now depending on the studies that would be required to figure out the mechanism of the disease and what kind of drugs can be used to cure, we can use several different kinds of model organisms. Some of the frequent ones are Drosophila, mouse and patient derived stem cells. And then once we have a model system established, then we can look at the cellular mechanism of the disease development, we can study the affected cells and the tissue function and we can also use the model system for drug screening and finding out novel therapeutics. Next we're going to talk about how we use transgenic animals to study human epilepsy. So what is epilepsy and why there is no cure? Epilepsy is a neurological disorder which is characterized by seizures.

Epilepsy affects about 65 million people worldwide, that includes about 12 lakh people in India with active epilepsy. Epilepsy can affect people of all ages, it can begin in infants as young as three months old, it can present itself in adolescent, in adults and in elderly populations. Among a lot of the epilepsy burden falls on low and mid income countries where due to lack of awareness, there is not much health care that can be provided. There is no absolute cure for epilepsy. However, the seizure disorders can be managed with different strategies.

Many patients become refractory or resistant to anti epileptic drugs. And then depending on the seizure phenotype, and which areas in the brain is affected, there could be surgical

interventions or brain stimulation devices that could be implanted that can help manage the symptoms. However, there is no absolute cure for epilepsy. According to a survey from WHO, it was found that almost 75% of patients do not receive proper treatment, and most of them belong to low or mid income countries. Hence, an approach towards removing the taboos and removing the social stigma attached with epilepsy can help bring in awareness and bring in more public health care services that can help the patients manage their symptoms better.

The stigma and discrimination could be at home, could be at work, could be among social gatherings. And even in popular culture, there often seems to be a misrepresentation of epilepsy. Here, I have two examples. One is a movie that came out in 1970s. Here, the protagonist played by Sharmila Tagore has epilepsy, and we see how that affects her life.

The next is an example from a stand-up comedian. He recounts his childhood experiences of having seizures and how people around him thought that he was either possessed or he was faking it, or he was making up stories. And sometimes these incidences can have a great impact on somebody's health. So what are the causes of epilepsy? Broadly, it can be classified into two kinds of causes. It could be symptomatic, which means that there is some symptom attached to it, and that accounts for about 40% of the cases.

The symptoms could include brain trauma, brain injury, infection, or stroke. But 60% of the cases were known to have idiopathic causes, which means that the cause was not known. But because of the recent advances in genomic studies and gene sequencing, we have now been able to identify that most of these idiopathic cases have a genetic component or gene mutations attached to it. That means that most of these 60% of the cases arise because of genetic mutations that cause epilepsy. Now, among the genes that can result in an epilepsy mutation, sodium ion channel gene is a hotspot.

Now, this is a channel, a 2D representation of the sodium channel, and there are four domains. This is domain 1, 2, 3, and 4. The transmembrane segments 1 through 4 are considered the voltage sensing domain, and the black ones here, segment 5 and 6, they form the pore forming regions. And here are some of the mutations that have been identified, and the transgenic models have been created. However, there are over 1300 epilepsy causing mutations that are known in SCN1A gene alone, and that covers almost all the domains and most of the segments.

So why would a mutation in sodium ion channel gene cause seizures? For that, we need to look at what is the function of a sodium ion channel gene. So sodium ion channels,

they are heavily expressed in neurons, and here they are represented by these purple cylinders. And if we zoom it, we can see that there is an ion channel, and these ion channels regulate the flow of sodium ions into the neuronal cell body. Now, because they regulate ionic flow, they play an important role in generation of an action potential. Action potentials are nothing but electrical signals that are produced by neurons in our brain.

And here is an example of an action potential firing from a mouse brain. So why does mutations in SCN1A gene cause seizures? We can think that there can be increased neuronal firing, and this hyper excitation could lead to seizures. Now, why would that happen? So suppose there is a normal brain and there is normal kind of action potential firing going on, then broadly in the brain we have two kinds of neurons. There are inhibitory neurons and excitatory neurons. If it is a normal brain, it's a disease-free brain, then there is a balance between the excitatory neurons and the inhibitory neurons, and the person is unlikely to experience any kind of seizures.

However, in an epileptic brain, there are these hyper excitable signals that are often seen. As a result, the firing of the neurons is heavily increased, and most often either the inhibitory neurons are firing less or the excitatory neurons are firing more, or a combination of these. This would tilt the balance, and now there will be more excitation in the brain, and this hyper excitability is thought to cause seizures in the brain. Epilepsy, specifically genetic epilepsy, is a spectrum disorder. What do I mean by that? So if we look at the mutation type that causes seizures, and specifically in SCN1A, which is the sodium ion channel gene, we could have Missense mutations, and they give rise to mild disorders like Febrile seizures.

Febrile seizures are fever-induced seizures. If there is a missense mutation, it could also give rise to something called as GEFS+, which is a Generalized epilepsy, genetic epilepsy with febrile seizure plus, and that has a moderate seizure phenotype. In-frame deletion or truncation mutations generally tend to have more severe phenotypes, and that phenotype is referred to as Dravet syndrome, and here the patient has a very early onset of the disease, has febrile seizures, has generalized seizures, also has developmental delays. So depending on the kind of mutation, the seizure phenotypes could vary largely in SCN1A mutation. We are going to talk about GEFS+, which is a moderate seizure phenotype for the rest of our talk.

GEFS+, as I mentioned, is a moderate level disorder. GEFS+, stands for Genetic epilepsy with febrile seizure plus. This is caused mainly by missense mutations and follows an autosomal dominant inheritance pattern. Homozygous patients are generally not seen in humans. It will have an early childhood onset, and the seizures can persist

even in adulthood.

If the patient has only febrile seizures, which means fever-induced seizures, whenever there is a fever, the patient is likely to undergo epileptic seizures. If they only have febrile seizures, then those seizures go away by the age of six to seven years. It's self-limiting. But if it is febrile seizure plus, then the patient continues to experience febrile seizures even in adulthood, and because of early life trauma induced by these febrile seizures, if they are not controlled with drugs, then the patient can even develop generalized seizures in their adulthood. So patients with GEFS+, they show pronounced clinical variation in seizure phenotype, and sometimes family members having the same gene mutation can also show different seizure phenotypes, and they also respond to drugs differently.

Febrile seizures are considered a hallmark of GEFS+, epilepsy. Febrile seizures are presented generally when the patient has fever or high body temperatures. Now, according to a survey, almost 70% of GEFS+, patients have febrile seizures. It could be a mix of febrile seizures and febrile seizure plus, but fever-induced seizures seem to be a hallmark and has been seen in all families and all patients with GEFS+. Now, in Professor Diane O'Dowd's lab at UC Irvine, we took an approach where we created several transgenic models, and we looked at epilepsy, and in this case, we looked at a particular Gefs+, mutation, and we created a fly model, a transgenic fly model using CRISPR, and then we created a mouse model using CRISPR, and we also had stem cells which were derived from patients who carried the same mutation, and we had a control from a non-seizure sibling, and their stem cells were used as a control line.

The aim of our study was to find out conserved cellular mechanism that would give rise to GEFS+ seizures, and then try and see if we can come up with a novel therapy or novel drugs that could cure or control these GEFS+ seizures. So what kind of studies can be done using different kinds of transgenic animals? If we look at Drosophila, we could look at the Neuronal firing in the brain, we could study the ion channel function, we could do drug screenings on adult flies or even larvae, and we can study the seizure behavior in adult flies. If we look at a mouse model, we can pretty much do all the things that I just said. If we look at the stem cells, we can look at the neuronal firing, we can study the ion channel function, we can even do drug screening, but of course we cannot look at behavior. So from a behavioral point of view and a neurological disorder like epilepsy which has a very very different seizure phenotypes depending on the mutation, studying behavior in a live organism becomes very very important.

So in that case, the Drosophila and the mouse model have an advantage over the stem cells that you can study the seizure behavior and map it to the human patients. Drosophila flies can have seizures too and I wanted to show this video where we can see

that we have a control vial and these vials are dipped in a hot water bath and there are flies in the vials. So we have a control vial and we have an epileptic mutant vial, then we have a control vial and another epileptic mutant vial. So what you will see is when I play the video that the epilepsy flies they tend to fall down and they have these seizure-like movements so they vortex around at the bottom of the tube whereas the control flies they continue to fly around. So you can see that the control flies are moving here and some of the flies in the mutant vial are already falling and here you can see more flies falling down and by the end of the video most of the flies in the epileptic mutant vials they would fall down but the controls will continue to fly around.

So when I was a postdoc in Diane O'Dowd's lab my work involved generating a transgenic mouse model to study a GAFS plus epilepsy. So like I mentioned the approach was that you first identify a mutation in a human patient this was done by some other group and then what we did is we took the exact we mapped the SCN1A mutation in the human patient and then we created a mouse model using CRISPR. CRISPR is a precise gene editing so it allows us to change one or few base pairs in a gene in a targeted manner. So we could introduce the same mutation from the human patient into the mouse model. So the human GAFS plus patient had a change in a amino acid sequence in the SCN1A gene where a lysine residue got modified to a threonine residue.

So even in the mouse in the SCN1A gene we introduced the same change from a lysine residue to a threonine residue at an equivalent position in the mouse genome. So how did we do that? So very briefly we could introduce a micro-inject all the components of the CRISPR into a pseudo pregnant dam and then when she gave birth we screened the progenies for the one mutant which had this particular CRISPR edited gene SCN1A and then we mated that founder male with two different strains of mice which have been used in epilepsy research so that we could study the genetic background effects of the mutation. But for most part of my talk from now I will focus on only one strain which is the B6N strain. So to confirm that the mutation that was introduced using the CRISPR strategy had actually worked we sequenced the mouse the heterozygous mouse and compared it with the wild type mouse. So on the top we have a wild type mouse here the gene in the SCN1A codes for lysine at the expected position but in the heterozygous mouse which has one mutant copy of the gene we can see that the lysine has been changed to threonine in one of the homologous chromosomes.

So we found that the mutant mouse had identical missense mutation as the human patients and that the mutation was the lysine to threonine change which was what was expected out of the CRISPR strategy. So the requisite for a good transgenic model is that the model should phenocopy the human patient and so we wanted to check if the mutant mouse model that we had made had the seizure phenotypes just like in the GAFS plus

patients. So we had three mouse groups under study we had the control mouse which have two copies of the normal gene the heterozygous mutant mouse which has one mutant copy and one normal copy of the gene and the homozygous mutant mouse which has two copies of the mutant gene. So we first found that the homozygous mutant mice have a very short lifespan. So here I there is a percentage survival on the y-axis and postnatal age of the mouse pups on the x-axis and we can see that the homozygous mouse shown here in red they tend to die by day 25 whereas a heterozygous or a wild-type mouse they live up to six months of age.

They live beyond that in this study they were monitored only up to six months and what we found that the homozygous mouse they were dying after having violent seizure episodes. So here is a video to show that a small homozygous mouse is having a seizure episode here and then it goes into tonic-clonic seizures and we found that if a mouse is having several of these seizures one of the seizures would result in a death and this is known as SUDEP or sudden unexpected death due to epilepsy. So homozygous mice were dying after having repeated spontaneous seizure episodes. We also found that GEFs plus mice have a spontaneous seizures. In order to monitor if a freely moving mouse was having seizures which were not induced by high body temperature or any other means we implanted an EEG device on the mouse head and we recorded the activity the brain activity of the mouse when it was freely moving in the cage.

So here is what an EEG pattern would look like for a control mouse which does not have any mutati on. In contrast we found that heterozygous mouse have these seizure signatures in their EEG and they were found randomly during their freely movement behavior and these were classified as spontaneous seizures. But since we know that GEFs plus patients have febrile seizures we wanted to see if the mouse were exposed to high body temperatures would they undergo seizures. In order to do that we constructed this heat chamber in which we could control the heat inside the chamber and we could place a mouse and monitor the body temperature of the mouse with a rectal thermometer. So what we're going to see over here is that if the mouse is wild type when the body temperature goes up because the chamber is slowly getting heated up the wild type mice do not undergo seizures whereas if we have a mutant mouse which has the SCN1A mutation then at a certain body temperature it will undergo seizure.

So here we have a wild type mouse and you can see the body temperature on the thermometer. Here we have a mutant mouse and now it is undergoing a seizure. So heat induced seizures were found to be a hallmark even for the GEFs plus mouse. Next we wanted to look at the neuronal firing patterns in these mice and so here we have a mouse brain and it would be cut into coronal sections and we looked at the hippocampus. We wanted to specifically look at certain kinds of inhibitory neurons so we label them with

certain tags and that helped us to identify the inhibitory neurons that we were interested in.

So when we look at the PV inhibitory neurons they are a kind of GABAergic neurons in the mouse brain we found that the action potential amplitude was reduced in these mice in the heterozygous mice. Compared to the control mice which are shown here in black so the amplitude of the action potential firing was reduced in the mutant mice. We wanted to explore this further so we looked at different parameters in the inhibitory neurons. So we had already seen that the amplitude was low so we also measured the action potential threshold and we found that there were differences there. We also looked at the amplitude and we had already seen from the traces that the amplitude was reduced.

We also looked at the half width which is this distance between the the area under the curve of these action potentials and we found that there were differences there as well. The mutant mice had a broader half width so these indicated that the properties of action potentials in the inhibitory neurons were altered in the GEFS+ mouse. But when we looked at the excitatory neuronal firing there was no change between the GEFS+ mouse and their control mouse. So we looked at the same parameters the AP threshold, the AP amplitude and the AP half width and they were found to be exactly same and similar to their wild type controls.

So the excitatory neuronal firing did not seem to undergo any change because of this particular mutation. So to summarize we created a CRISPR mouse of GEFS+ epilepsy and it recapitulated the key features of the human epilepsy patients. We learned that the homozygous patients must be dying prenatally because the homozygous mice they were dying very early on and in humans we did not see any patients who have a homozygous mutation. The cellular mechanisms of GEFS+ epilepsy could be elucidated with the mouse model. We found that the neuronal firing is selectively impaired in the inhibitory neurons whereas the excitatory neurons are not affected in this GEFS+ model.

So when we compare the mouse model with the human patients we see that the mutation is not present in the wild type and it is not present in the human patient as well. They have a normal lifespan, they do not show any spontaneous seizures or heat induced seizures. Heterozygous patient and heterozygous mice they have a one copy of the mutation, they have normal lifespan, they both show spontaneous seizures and heat induced seizures. Homozygous mice we do not have a human counterpart, it has the mutation, it has two copies of the mutation, it has a shortened lifespan and it undergoes spontaneous seizures very early on in life. We have not done any heat induced seizures on these young mice because they die very young and the heat seizures were done on little older mice otherwise it will become really traumatic for these young pups to undergo heat

induced seizures so those experiments were not done.

So remember I said that we were looking at different kinds of transgenic models in the Diane O Dowd's lab so what did we learn from the other transgenic models? So for the same GEFS+ mutation we found that the transgenic fly, the mouse model and the stem cells they all had defects in the inhibitory neurons. So it seemed like the inhibitory neurons in all these models were either not firing properly or had some defects whereas the excitatory neurons were more or less remained unchanged compared to their controls. So the mechanism of seizures in GEFS+ and particularly in this mutation where a lysine is changed to threonine we found that the impaired neuronal activity of inhibitory neurons lead to seizures and this hyperexcitability is happening because of impaired firing in the inhibitory neurons. And this is a corroborative of the interneuron hypothesis where other people, other researchers who have studied mechanism of seizure generation have also proposed a similar thing where neuronal impaired firing in inhibitory neurons can lead to seizures. So in summary we saw that transgenic animal models can be successfully generated using gene editing techniques like CRISPR.

Drosophila and mouse models of SCN1A epilepsy have helped to elucidate cellular mechanism of seizure. Conserved pathways of seizure generations are likely to provide therapeutic targets. So now that we know that all the three model organisms have shown the same kind of cellular impairment then now testing the drugs becomes quite easy and we have confidence that the mechanism that has been seen in one organism is also conserved in another organism which has the same mutation. This makes the model system very robust for drug screening programs. Now the use of animals in research always raises an ethical question.

So while transgenic animal research has advanced our understanding of several human diseases not just epilepsy and the use of transgenic animals is heavily regulated. There are strict laws governing the number of animals that you can use and what kind of experiments you can use, what conditions you can expose the animal to and especially when we are looking at seizures or any kind of discomfort we have to be extremely mindful of the trauma or of the pain the animal is going through. We have to sometimes perform surgeries on the animals and then we have to make sure that the animal is completely anesthetized and cannot feel any of the surgical procedures and even post-op we have to monitor their health and give them pain relieving medicines when required. So there are a lot of animal ethics and there is an animal committee which guides lot of these research initiatives yet even if we take care of all of this the question remains whether we have the rights to manipulate the genome of these organisms and use them for studies related to human diseases.

I leave the question up to you. And here is a statue of a mouse weaving a DNA. This is at the Russian Academy of Sciences in Siberia and this is to remind this the statue is dedicated to all the mice that have been used in genetic studies for biological mechanisms of diseases and drug discovery. So I leave you with this thought. With that I would like to thank all the members of the O'Dowd lab and all the current members in my research team at APU and all our collaborators and our funding bodies. If you are interested in epilepsy you might want to look up these resources where you can learn more about the statistics of epilepsy patients all over the world first aid that can be given to epilepsy patients and all kinds of resources about patients and their success stories and what kind of health care is available.

With that I would like to conclude today's session and thank you for joining us today.