WEBVTT

00:00:00.030 --> 00:00:30.960 The pulling up her slides our second speaker is Doctor Grace Chen. Grace is a assistant professor in the Department of Immunobiology and a member of the cancer menology research program. She received your PhD at Harvard, where she worked on characterizing RNA modifications to post a post doctoral work at Stanford and now I think is her lab is really advancing an understanding of the intersection between the function of regulation RNA in MENA biology and an beyond so thank you.

00:00:32.850 --> 00:01:00.520 Thank you Charlie for the introduction for the opportunity to present some of our work that we're doing to understand the landscape of circular rnas so I chose this map. That's a little bit older because you might see that the major continents are there, but there are key landforms like California and part of Australia that's missing and so similarly. I think in our in a biology. There's still a lot to discover an uncover in this area.

00:01:02.450 --> 00:01:34.980 We typically think of Rnas as being spliced to yield consecutive exons in a linear fashion typically with the 3 Prime Poly, a tail. The 5 prime cap and often the first step in RNA sequencing involves selection of all the rnas with this Poly a tail and then we sequence them and we characterize them and that actually means that we miss all the other rnas that lack this Poly a tail and so technology has been a really strong promoter of biology.

00:01:34.980 --> 00:02:06.750 Basic science discovery and I think you know, even though we've had next generation sequencing for awhile. It took quite some time in order to discover this whole new class of rnas so about 7 or 8 years ago. Investigators total RNA depleted ribosomal RNA and then sequenced everything that was remaining and they found evidence of back splice junctions where for example, Exxon 3 is upstream of Exxon 2 and they found that this could only take place from templates of circular rnas and this really launched.

00:02:06.750 --> 00:02:14.880 The entire new field where people are trying to understand what circular rnas are doing and you know first identify what they are.

00:02:15.570 --> 00:02:45.660 So this shows the phylogeny tree where species and red had been found to contain circular rnas and I've labeled the metazoans on the also contained a circular Arnason Gray and actually since 2014, essentially every eukaryotic that's been looked at has encoded circular rnas so some of the individual circular rnas have been found in metazoans are labels up here and they?

NOTE Confidence: 0.916589796543121

00:02:45.660 --> 00:03:18.240 People have shown that they contribute to specific hallmarks of cancer and given that circular rnas are classified as a group based on their topology and not based on say function you know, people wondered how might they actually work and so this shows a few different categories of circular rnas function. The first one, that was discovered. In came into sort of public knowledge. Prominence was acting as a micro RNA sponge, so an individual circular RNA encoded 70 copies that was.

NOTE Confidence: 0.858238279819489

00:03:18.240 --> 00:03:25.910 Anti sense to a specific micro RNA and it was able to effectively sponge up that micro RNA from acting on its micro RNA targets.

NOTE Confidence: 0.90470016002655

00:03:26.630 --> 00:03:53.980 So now I've just explained that you know, essentially all eukaryotes contain these circular rnas and there can be quite abundant. There have been maybe up to 10s of thousands of different circular names that have been identified and sometimes they are say tenfold the copy number as their linear Arnie counterparts. Generally speaking, though they are predominantly less than their linear Arnie counterparts.

NOTE Confidence: 0.904128670692444

00:03:54.650 --> 00:04:21.300 Now you know in this context of circular rnas people have taken patient samples with different different types of diseases and found the circular. Rnas are dysregulated. That means either. The types of circular, Arnie's expressed change or the levels change and now there's a lot of active research to understand how did these circular rnas actually contribute to the initiation and progression of these different types of diseases?

NOTE Confidence: 0.883337736129761

00:04:22.520 --> 00:04:53.630 Circular rnas are actually also encoded in viruses, so the hepatitis Delta virus was the I guess original RNA encodes circular RNA virus because it has a circular RNA genome and this electron microscopy image. She was one of the first examples that circular rnas actually existed in nature. Last year 2 different groups found the Epstein Barr virus as well as the capozzi sarcoma associated herpes viruses also.

NOTE Confidence: 0.859540224075317

00:04:53.630 --> 00:04:58.830 Encode circularize so these are 2 DNA viruses that can lead to the progression of cancer.

00:04:59.620 --> 00:05:31.490 So given that a healthy cell needs to maintain this vital balance between recognizing its own endogenous. RNA is to prevent autoimmunity and recognize pathogenic arnese to prevent infection. We had several different questions essentially circling around how to cells do this for circular rnas as our 1st question had his cells distinguish between self and nonself circular. Rnas then we wondered if they have this distinction and disability to distinguish between self and Nonself.

00:05:31.490 --> 00:05:35.620 Can we harness that for specific therapies specifically related to cancer?

00:05:36.180 --> 00:05:57.600 We also wanted to understand what are the molecular mechanisms to sense foreign circular nezan self circular rnas and finally given all of these 10s of thousands of circular rnas both virally and indigenously encoded that become dysregulated in disease? What are the functions of these circular rnas it might we be able to target them for specific therapies.

00:05:58.400 --> 00:06:28.460 So to answer this, 1st question, we needed to design a system that would model of foreign circular RNA and to do that. We used a TD gene from T4 bacteriophage, which contains a Group One intron so this intron typically will auto catalytically splice itself into a lariat in order to join Exxon one to Exxon. Two we found that if you take this group went into Ronan permute the order you take the 3 prime end and put it down upstream.

00:06:28.460 --> 00:06:43.590 About genome interest and the 5 prime and downstream of a gene of interest. Whatever is in between. These 2 pieces will AutoCAD analytically slice itself into a circle during in vitro transcription without the addition of any other protein components.

00:06:45.010 --> 00:07:01.760 And so we can form this circular RNA and we designed the sequence such that in the linear form an internal rivals am entry site is downstream over open reading frame and during circular ization. the IRS becomes upstream, enabling for translation of this open reading frame.

00:07:02.320 --> 00:07:11.130 This process during in vitro transcription also generate some linear arnej byproducts, which becomes substrates for an extra

nucleus called our knees are.

NOTE Confidence: 0.860509514808655

00:07:11.830 --> 00:07:42.090 So we were able to generate the circular RNA which I'll call Cirque foreign for the rest of the talk and um characterize it. NPR Vita homogeneity, so the first experiment that we did was we took the circular RNA and we transfected it into HeLa cells and then we also chose a linear RNA with the 5 prime hydroxyl group because that should not be immune stipulatory and also transfected it because we wanted to compare the sequence of the IRS in the GFP.

NOTE Confidence: 0.856906473636627

00:07:42.090 --> 00:08:13.490 In between these 2 different topologies. We performed RNA sequencing where each dot is one of the jeans. That's found in Richton. Either Sir Karni transaction compared to unlock transfected cells or linear arnej transaction compared to mock transfected and then the color of the dot represents the more read the dot is the more enriched it incident on the circular RNA transfected cells compared to linear our nations like themselves so I've labeled some of the jeans that we found up here and you may notice they fall into a specific.

NOTE Confidence: 0.896166324615479

00:08:13.490 --> 00:08:26.390 Pathway and indeed when we performed gene ontology enrichment. We found that circular RNA transfected cells had strong induction of antiviral response an inflammatory response.

NOTE Confidence: 0.910531044006348

00:08:26.960 --> 00:08:57.560 And so given that sells respond and sense. These foreign circular rnas? What prevents self circular, rnas from also being sensed and inducing this strong inflammatory response and we hypothesize that potentially the Arnie binding proteins that are associated with self. Circular nays are distinct from the ones associated with foreign circular rnas and so to test this hypothesis. We performed an experiment using the comprehensive.

NOTE Confidence: 0.866519212722778

00:08:57.560 --> 00:09:30.090 Identification of RNA binding proteins by Mass Spectrometry, where we take live cells. We cross link them. We use bite slated tiling oligos to Enrich Bar, Arnie's of interest. We can isolate that RNA with all of the proteins bound to it, and that identify the RNA binding proteins by mass spectrometry. So we took several different arnese. Through this process. We took our foreign circular an linear RNA. Through it, and then we also took a self endogenous circular RNA through this process.

NOTE Confidence: 0.864007115364075

00:09:30.090 --> 00:09:36.340 But we made one change, so the ZK scan is a gene that encodes an endogenous circular RNA.

4

NOTE Confidence: 0.863567531108856

00:09:36.900 --> 00:10:00.490 And we wanted to test the Biogenesis difference. And so we swapped out the endogenous. ZK scan for RGFIRESE. Woods so right now. These Cirque foreign and Cirque self sequences are identical there. Just made through 2 different processes mimicking of foreign circular RNA and a self circular RNA.

NOTE Confidence: 0.901064157485962

00:10:01.270 --> 00:10:18.260 And we found that a specific category of proteins came up as highly differentially expressed or binding to Cirque self and Cirque born and these are proteins associated with the RNA modification and 6 methyl adenosine.

NOTE Confidence: 0.900035321712494

00:10:19.320 --> 00:10:34.330 So we find that there are M6A riders that install the modification their readers that recognize the modification and their erasers that remove this modification and so here's the actual sort of catalytic cycle of the RNA modification.

NOTE Confidence: 0.893028974533081

00:10:34.910 --> 00:11:07.770 So given that potentially M6A is a molecular marker for self circular, rnas compared to non self circular rnas. We wondered maybe there's a differential modification of these different circular rnas and so to test this hypothesis. We used a method called R clip with M6 say where we use an antibody that recognises the RNA modification. We can cross link it so that the antibodies covalently attached to the Arnie's that's modified.

NOTE Confidence: 0.889086067676544

00:11:07.770 --> 00:11:34.560 We can perform limited pro T8 Nysca digestion, leaving a scar of the protein attached the army modification. This car is large enough such that during reverse transcription. The polymerase will hit this car and stop their meaning that using this method with sequencing. We can have nucleotide specific level of identification of exactly where the RNA modification exam exists.

NOTE Confidence: 0.890452742576599

00:11:35.730 --> 00:12:06.660 And so we took both the Cirque self ansark foreign through this process and we found that right after the Spice Junction. There's a significant peak of M68 density only on Cirque self compared to serve wine and so we potentially wondered maybe Cirque self contains this Arnie modification in order for the cells to sent it as self and so then. That means that we should be able to take this arty modification incorporated into circle.

NOTE Confidence: 0.831230163574219

00:12:06.660 --> 00:12:10.980 Born and that should mask the foreignness of the circular RNA.

NOTE Confidence: 0.892929017543793

00:12:11.590 --> 00:12:40.050 And so we try that experiment that's exactly what we found so if we take unmodified circular. Arnie we get strong induction of this panel of innate immune jeans. However, when we add in 1% of 6, eight into the Cirque foreign where out of 100 days. One of them is replaced with an M6A. We get about 50% reduction in the immunogenicity. So 1% is just about endogenous levels that we would find in the cell.

NOTE Confidence: 0.880522668361664

00:12:40.610 --> 00:13:11.340 And so this was really exciting to us and then we wondered you know is there a way that given the cells can distinguish between self circular, Arnesen Nonself. Secular nays might that be useful in some context. And so 1st. We took cells in vitro and we transfected them with either linear RNA or circular RNA that's labeled with the floor for them. We treated those cells and subjected them with a positive single stranded RNA virus.

NOTE Confidence: 0.896167933940887

00:13:11.340 --> 00:13:26.400 The Venezuelan ECB wine encephalitis virus that's tagged with GFP so we would be able to tell whether or not the cells were infected and we find that indeed the cells that were first transfected with circular may were protected from subsequent viral infection.

NOTE Confidence: 0.899058163166046

00:13:27.140 --> 00:13:57.770 And we quantify the data here where the cells with circular Arnie had significant lowers levels of VV infection. We found interesting Lee that even the cells that did not themselves receive the circular RNA but we're in the same neighborhood or you know, especially well of the circular. RNA also were protected. So then we wondered could we extend this to an in vivo model and see if we can get systemic protection.

NOTE Confidence: 0.851800739765167

00:13:57.770 --> 00:13:59.240 Of an animal.

NOTE Confidence: 0.861901760101318

00:14:00.940 --> 00:14:13.240 And so we design an experiment where we wondered if we could use circular foreign circular rnas as a vaccine adjutant and we used over albumen Asmodel antigen here.

NOTE Confidence: 0.849020957946777

00:14:13.870 --> 00:14:22.410 And so we immunize the mice twice and then we collected T cell and probably antibody titer for both of them.

NOTE Confidence: 0.893760561943054

00:14:23.010 --> 00:14:55.400 And here we see this is over specific CD 8, plus T cells that both foreign circular RNA with a transfection reagent an without a transaction region was they were equally affected as our positive control, Polly. I see at inducing the same levels of CVA plus T cell response and we were really puzzled by the fact that you know whether we did not need an enviable transfection reagent and this is something that we're trying to understand mechanistically a molecular.

NOTE Confidence: 0.719964623451233

00:14:55.400 --> 00:14:56.190 As well.

NOTE Confidence: 0.879692494869232

00:14:56.700 --> 00:15:19.950 When we looked at the antibody response. We found that similarly both circular rnas with the in vivo transfection reagent as the one without also produced similar levels as a positive control, Polly. I see so then we wondered would circular arte be a method as a vaccination and could we use this to prevent overexpressing tumors.

NOTE Confidence: 0.902415990829468

00:15:20.620 --> 00:15:50.780 And so here we wanted to vaccinate the mice 1st and then 2 weeks later, established be 16 of expressing Melanoma tumors and measure the tumors and image them because the cells are tagged with the surprise so here are images of the mice treated with either PBS or search for and and we find that there's a dramatic difference in the rate of the tumor growth between the 2 different sets of mice and this translates into a doubling of survival.

NOTE Confidence: 0.822662174701691

00:15:50.780 --> 00:15:57.070 Or the mice treated with surfboard compared with with negative control PBS.

NOTE Confidence: 0.897458970546722

00:15:57.920 --> 00:16:30.110 So this is with unmodified Cirque foreign and we wondered maybe there are some context where we want to be able to modulate and tune. The level of immune response and so we wondered could we introduce M6A into our Cirque born to change an adjust that level of induction and indeed that's what we see here where again in Vivo. We model similar results that we saw in vitro where 1% of M6A introduced in the foreign circular RNA.

NOTE Confidence: 0.88734358549118

00:16:30.110 --> 00:16:43.170 Decreases the munition ecity by about 50% so we're really excited by these different findings. And we're hoping to find some specific enviable models to continue and probe Sir Garnas therapeutic purposes.

NOTE Confidence: 0.912950754165649

00:16:44.040 --> 00:17:15.050 So we think that some of these experiments should go hand in hand with a deeper understanding of the molecular mechanism because if we can understand that side potentially we can also give insight into the therapies as well. And so we had found previously that rig. I which is a cytoplasmic nucleic acid sensor is necessary for detecting the presence of orange circular. Rnas now rig. I typically is used to find viral rnas with the 5 prime triphosphate.

NOTE Confidence: 0.868144690990448

00:17:15.050 --> 00:17:47.960 And so in the inactive state, the rig. I has these card domains that fold up. However, in the presence of an agonist rig eyes card domains are now revealed and then their stabilized, either because there are other rig. I elements nearby or in the presence of these unanchored. Poly big written chains. And so we wondered you know does circular RNA induce rig. I in a similar way that these viral other viral rnas would induce and does the immune signaling sort of continue?

NOTE Confidence: 0.884445190429688

00:17:47.960 --> 00:17:51.610 Through this Canonical pathway all the way to interferon production.

NOTE Confidence: 0.875173568725586

00:17:52.230 --> 00:18:23.000 So 1st we tested whether or not circular RNA itself was able to induce regai filamentation and we find that the answer is no so here are electron microscopy images and with our known agonist a double stranded RNA. We can see these nice white filament switches regai forming these filaments. However, both in the presence of a 5 prime hydroxyl linear RNA as well as with Cirque foreign RNA we were not able to.

NOTE Confidence: 0.880446553230286

00:18:23.000 --> 00:18:53.930 Induce these redeyef elements, then we wondered maybe if we add these unanchored. Poly ubiquitin chains. That would be enough to stabilize the card domains and so to probe that we looked at whether or not. We could activate regai into a confirmation that was able to trigger the next step, which is to trigger Mads monomers to form these filaments. So now on. These electron microscopy images. We would be looking for mass filamentation, so we did this in an?

NOTE Confidence: 0.881479740142822

00:18:53.930 --> 00:19:25.080 In vitro system where we combined all of these different components an and then we modulated the concentration of the different RNA agonist and looked for presence of match filamentation. We find indeed as we increase sort of circular RNA concentration. We get strong mass filamentation, but we don't with our negative control. Our linear RNA and then we can also modulate by the addition of M6 side and so we took these samples and we made.

NOTE Confidence: 0.88305652141571

00:19:25.080 --> 00:19:52.380 M grids and you can see just by the number of filaments that unmodified Cirque born strongly induced mass filamentation and the addition of Mxe, Arnie modification decrease that and when we count the number of filaments and quantify them again observe this trend where just 1% of M6A is enough to decrease the immune response by about 50%.

NOTE Confidence: 0.867586255073547

00:19:53.260 --> 00:20:14.800 So after mouses formed the next step is dimerization of IR 3 and when we probed whether or not circular. RNA was able to make this induction again. We saw our three dimerization was forming specifically in the presence of circular RNA and so our model right now is that?

NOTE Confidence: 0.875869274139404

00:20:15.370 --> 00:20:45.710 Regai is inactive until in the presence of unmodified circular RNA joined with these Poly ubiquitin chains. We can activate regai to trigger mags. Filamentation are 3 dimerization and subsequently interferon production. However, if there is RNA modification of M6A on the circular RNA that inhibits this process, so rig. I may bind but it doesn't become into this active stage in order to trigger the rest of this immune signaling.

NOTE Confidence: 0.917195618152618

00:20:46.830 --> 00:21:16.980 So, in the last minute. I wanted to comment on you know how can we study the different functions of circular rnas and can we break that up into some smaller questions to ask more specifically. What are circular Arnie's doing in certain contexts so right now the field is really nascent and there are a lot of different unanswered questions. It's really exciting to be in this area because there's so many things that we don't know for example, how does a cell distinguish?

NOTE Confidence: 0.881887078285217

00:21:16.980 --> 00:21:43.290 And choose when to make a linear RNA and went to make a circular RNA. There's been some bioinformatics studies showing that when the introns that Hlinka circular RNA are a little bit longer than usual and base pair. There are more tends to be circular. RNA produced but that's not always the case and so there is a decision in the nucleus during transcription, splicing to choose whether or not so forward supplies to make linear RNA orbax twice to make circular.

NOTE Confidence: 0.912601590156555

00:21:43.900 --> 00:22:02.500 We're also interested in understanding the structures of circular the secondary structures of circular RNA. We hypothesize that they are quite different than their linear RNA counterparts because of the unique back splice junction, but exactly how they change and what functions they might have when they change are still unknown.

00:22:03.650 --> 00:22:20.430 We're interested in all the other chemical modifications. There's more than 160 they have been discovered. I just talked about 1:00. So far and all the RNA binding proteins that associated with them and how that might contribute either to sensing of self nonself or other cellular processes.

00:22:21.620 --> 00:22:39.290 Circular rnas endogenously are made in the nucleus, but they predominantly localized to the cytoplasm. Now there's so many organelles in the cytoplasm that potentially if we can gain insight into organelle specific localization of these circular. Rnas we might be able to understand what those securities doing there.

00:22:40.950 --> 00:23:11.760 And circular rnas are also packaged in extracellular vesicles, so could this be one way given their high stability to be able to communicate between sort of just you know, Dispur parts of the body and what types of decisions are made to select which types of circular rnas for these packaging and release and how does cells actually receive them and interpret the signal are still unknown and finally? What are the circular rnas on these?

00:23:11.760 --> 00:23:17.920 Organs and systems levels and you sort of more importantly, what happens when this becomes dysregulated.

00:23:18.480 --> 00:23:49.970 I mentioned at the beginning that technology is really a key component to these types of investigations and there's a strong need to develop new types of approaches to study circular rnas because for the most part, many RNA protocols start with selection of Poly, a tail Darn Azan that automatically sort of eliminates all of the circular days that would be present. In this sample and then can we take a deeper understanding on this area in order to apply circular rnas.

00:23:49.970 --> 00:24:10.370 To develop some useful therapies so thank you very much for your attention. This is my small and hopefully growing group at Yale and then I also did some of this work at during my postdoc where I was with Howard Chang and names of some of our collaborators in our funding sources. Thank you again.

00:24:12.680 --> 00:24:16.120 Thank you questions.

00:24:17.760 --> 00:24:48.150 Genetic way to prevent the formation of circular rnas and if so, how does that gene been found to be altered in disease processes? Yeah, so there is not 1 single way to prevent there are a couple of different ways to induce formation of an there have been a couple proteins have been found that if you put their recognition signal, you can get circularization, an then if you change?

NOTE Confidence: 0.922539174556732

00:24:48.150 --> 00:24:59.980 The splicing machinery, you can also promote circular rnas so this is a really big question I think there's a lot of people sort of working hard to try and answer it.

NOTE Confidence: 0.928369343280792

00:25:00.570 --> 00:25:04.750 I don't know that there's going to be like really clean answer.

NOTE Confidence: 0.818566262722015

00:25:05.450 --> 00:25:07.580 The 2 questions 1st.

NOTE Confidence: 0.772043406963348

00:25:08.210 --> 00:25:11.880 I assume these are virtually never translate.

NOTE Confidence: 0.893852472305298

00:25:12.440 --> 00:25:42.630 Yeah, so that's also a big question at the beginning say you know 656 years ago. People are doing a lot of different rebel zone profiling palisson fractionation and they concluded that there was no translation 2 years ago, 2 papers came out, saying they found evidence of small proteins that could only come from translation across the back splice junction.

NOTE Confidence: 0.91338062286377

00:25:42.630 --> 00:25:48.560 They haven't shown active translation on these circular rnas, but they have sort of like a correlation.

NOTE Confidence: 0.749692142009735

00:25:50.030 --> 00:25:53.860 And also, if you look on we sequence tumors.

NOTE Confidence: 0.78016072511673

00:25:54.570 --> 00:26:02.560 I must say seems to be increasing phenomenon for some reason, it's like living in DC mutations in spy stores this place accepted.

NOTE Confidence: 0.404963493347168

00:26:03.150 --> 00:26:03.830 Play free.

NOTE Confidence: 0.849218547344208

00:26:04.380 --> 00:26:14.000 Have you looked in those specific kinds of tumors? Yeah. So um many, many groups are taking just patient samples just sequencing?

00:26:14.500 --> 00:26:25.160 Them, as much as they can say the circular rnas exist. They do change compared to sort of you know normal tissue.

00:26:25.680 --> 00:26:34.170 What their functions are and are the change is enough to drive something specific is still unknown so I'm not sure.

00:26:34.960 --> 00:26:39.750 So we can give you those great that be fantastic. Yeah.

00:26:40.580 --> 00:26:56.130 Bobby Dobby Fantastic I mean, I'm really interested in getting samples. We have pipelines that we worked really hard to be able to find circular rnas from sequencing so I'd love to hear more on chat more.

00:26:56.710 --> 00:26:57.410 Yeah.

00:26:57.990 --> 00:27:28.780 He showed that these connectors and attribute it suppressed, yeah, so we were using a 5 prime hydroxyl linear RNA, which we know is not supposed to be immune system in Latore, where we started doing some different types of experiments to see you know does the increased ability from circular rnas yield additional benefits compared to these linear knees.

00:27:30.940 --> 00:27:35.890 Great thank you. That's wonderful work terrific grand rounds, yeah, thank you.

00:27:38.790 --> 00:27:45.070 Really interesting certainly timely related to obviously he could share.