

Nanotechnology Symposium

1-May-06 - 8:30 am - 10:15 am

Man: Before we open the floor up for questions, if Professor Konig is here could he please come forward so we can load the slides for your talk? Are there any questions for Professor Richards? You know I think everybody under thirty in this room ought to be crowding the microphones. Don't be self-conscious this is the place to be. If I were 25-years-old again, I'd think of nothing but nanotechnology. And don't be self-conscious about asking questions. I used to be and then somebody else would ask it and the speaker would say, "Great question", so I got over it. Let's encourage.

The next speaker is Professor Muna Naash who is going to speak about application of nanotechnology to gene delivery in ophthalmology.

Naash: I would like to thank the organizers for putting this symposium together and I want to tell you guys out there you look like you are undercover with your glasses and your hat. Okay the nanotechnology we are using in our lab is to try and rescue diseases of the eye. So before I start, I would like to thank the funding agency, which is National Eye Institute for giving us an RO3 for our work and The Foundation Fighting Blindness.

The particles, the nanoparticles, that we are working with is self-assembling DNA nanoparticle. The way it gets synthesized is by combining interacting polyethylenic _____ maleimide with polylysine to form polycation complex that can compact DNA into small particles. The shape of the particles is depending on the lycine counter ion that uses to neutralize the particles and so far, we have about 37 different particles. And we have tested two of these particles and that is by using trifluoracetate or acetate. The triluoracetate gave us a particle that is ellipsoid in nature and it's about less than 25 nanometers in diameter. The – do you mind if I lower this? Okay, thank you because my laser is in the way – the acetate on the other hand gave us a rod-shaped type of particle with a minimum diameter of about 12 nanometers. These particles are nucleus resistant. They mimic the native DNA complex in the cell and they are resistant to cellular degradation. We found them to enter the cell surface through a cell surface receptor and the entered the nucleus within 15 minutes. And then most importantly they stay episomal so we don't have any mutagenesis coming from site of integration and it has been shown to transfect postmitotic cells at high levels.

The trafficking of the particles have been studied by Pamela Davis in primary airway epithelial cells and we are in the process of testing the trafficking of the particles in photoreceptor cells and in the RPE cells. And she has shown that these particles have the capability to bind the receptor on the cell surface that has a nucleolin as a member of the receptor and then get targeted to the nucleus within about 15 minutes. And the gene in the particle gets expressed about 30 minutes from the time that the cells have been incubated with the particles. And it has shown that this targeting machinery is non-degradative darkening machinery. It bypasses the lysosomal machinery. So we got interested in these particles to – I mean in this system – so we first wanted to test it in ocular tissues. So the very first particle we used is to express GFP as a reporter gene targeted by the CMV promoter and the idea is to test all the ocular tissues that can express this particle. And we tested the acetate formula and the trifluoracetate and we used the naked plasmid as a control. And we injected in the subretinal space of the BALB/c mice or in the vitreous. And the size of the volume we injected in these animals is one microliter and then because we were using CMV promoter we know it gets shut

down very quickly so we did our analysis at two days post injection by looking at it by a real time PCR and by fluorescent microscopy and immunohistochemistry.

First, I'm showing you here that the real time PCR showing the expression of GFP by intravitreal injection of one microliter that has 0.6 microgram of the GFP particle. And as you can see here, both particles work very well in the lens. Gave several hundred-fold expression when you compare it to the naked DNA. Both the pigment epithelium and choroid and the retina show very little expression to the lens, of course because this is in the vitreous. When you magnify this at higher scale you can see both particles gave comparable levels of expression in the pigment epithelium and the choroid in comparison three-fold in comparison to the naked DNA.

When we looked at the retina we see a lot more expression from trifluoroacetate in comparison to the acetate and when we looked at the fluorescein most of this expression coming from the ganglion cells. Subretinal injections however gave us different patterns. The lens gave us a lot less level of expression from both particles while the pigment epithelium and the retina gave us several fold expression in comparison to the naked DNA. But in both cases we see the acetate gave us higher levels of expression from when we compared it trifluoroacetate.

So in the next slide, I will show you the work that we've further we've done on the acetate particles. So here we looked at the dose effect on the retinal expression of the acetate particles. We started from 0.04 microgram to 4 microgram and we've seen an increase in the level of expression of GFP as you increase the amount of DNA you deliver it in the subretinal space. To put this in prospective we compared it to the photoreceptor specific gene as you can see here in the same tissue--this is the expression off rodopsin. And with 4 microgram of the particle we can see about 80 percent expression in comparison to rhodopsin which is the highest expressing gene in the retina. By loading the amount of particle delivered in the subretinal space one can modulate the level of expression to be comparable to, for example, to S-antigen or to transducent or even to RDS.

So this became very valuable to us to work with the amount of particle as well as with the promoter, how powerful the promoter we use in constructing these particles. So here to show you the immunohistochemistry looking at GFP here by anti-GFP antibody the mock injection showed no immunofluorescent but when you look at the naked DNA – I don't know if you can see that- you can see a little bit of expression in the pigment epithelium and a few cells also expressing the GFP. But when you deliver the particles in the subretinal space we could see tremendous levels of expression almost 100 percent of photoreceptor cells. And if you look carefully you could see the RPE cells, all the RPE cells are expressing it and some of the inner retinal cells because here we are dealing with the CMV promoter. So next we looked at--here from subretinal injection we see tremendous expression in the optic nerve cells and this is good for glaucoma and also in the ganglion cell layer we see quite a bit of expression. So now we felt very comfortable with this particle. We want to test it with cell specific genes. And of course being working with peripherin/rds for several years and because we have several models for diseases that causes retinitis pigmentosa and macular dystrophy with the mutations and RDS so the first particle we generated is to express the normal mouse peripherin/rds and here we call it NMP.

We used, we did three particles based on the promoter. We used the IRPP promoter, here is the human IRPP promoter, to insure expression both in rods and cones and here

we used the rod opsin promoter which is 221 base pair and in this case we used the chicken beta-actin promoter to make sure, you know, expressing it at high levels.

Now we use these promoters to direct the expression of the full length cDNA but what we did in here we put C-terminus modifications that would allow us to recognize the transferred gene from the endogenous. And by doing that we took advantage of the 3B6 antibody that was generated by Molde (?) that has shown to see the bovine, human, and rat RDS, but not the mouse. When we compared the sequence of this region the only difference is the proline acquisition 341 to glutamic acid. So we went ahead first and tested the expression of this protein and the specificity of intergenic mice. And here you can see with the IRPP promoter we can see quite a bit of expression in photoreceptor cells that can be seen by 3B6 but 3B6 can not see the endogenous mouse RDS. And here is antibody that is polyclonal antibody to the C-terminus.

So we went ahead and tested the efficacy of this particle's ___ photoreceptor cells. But before we wanted to do that we asked the question what happened to these particles if we delivered at early time point meaning at postnatal day two. So we went ahead and established the injection in young animals. And here's an example that I want to give you of animals they were injected with saline at two days of age and survived up to 30 days and we did functional analysis and in our hand we get about 80 percent survival, meaning the eye developed fully. And we could see the injected eye recovered the scotopic and photopic ERG fully in comparison to the uninjected eye. So we were excited about this and we went ahead and injected the particle--this is very, very preliminary data that was done almost two weeks ago of the three particles in here and these are two examples of injected eyes and the fellow eye was left uninjected and here's we're showing you the total RDS in these retinas and in comparison to the wild type--the uninjected eye. As you can see all the three particles gave us several fold of total RDS that was generated in comparison to the endogenous, except in this case probably the particle either got degraded or - I'm almost done.

Here we did immunohistochemistry to show you the 3B6 at postnatal day five does not recognize, also the wild type RDS is not there, can not be seen. But when we inject the ___ particle we see expression in the outer plexiform layer - or I don't know what this area is - probably at the bottom of the photoreceptor cells. But when we use the IRPP promoter we see a nice expression on the tip of the outer segments of the photoreceptor cells. The chicken beta-actin particles with the promoter shows expression everywhere at high level.

So in summary, we feel that these particles are very promising particles and I think we can do a lot with them. We are in the process of testing the other formulas for toxicity and inflammation - by the way I want to point out in here - we looked for any toxicity with these two particles and we saw no toxicity, no induction of inflammatory response. And we are very, very excited about it and we are in the process of testing it and rescuing animal models. And before I finish, I would like to acknowledge that our members who participated in this work, and my collaborator, Mark Cooper, and the funding agency. Thank you very much.

Man: For people standing in the back, there are a lot of seats up in front here.

Man: Are there any questions from the floor for Professor Naash?

Yes, please.

Q: When you were mixing the DNA with the nanoparticles what ratio to DNA to nanoparticles were you using? And also, do you have any degradation rate of the nanoparticles?

A: No. First of all, the compaction procedure is patent so I'm not allowed to talk about it. And if there is any degradation one of the testing for these particles that we put it with the DNA and we look for degradation and we test it on gel electrophoresis and it shows no degradation whatsoever.

Q: I'm talking about degradation of the nanoparticles, _____ plasmoids?

A: I'm sorry. Once it gets in the, okay the particles are staying in the nucleus and we have tested the presence of the particles up to 14 days in ocular tissues - and maybe longer than that – but in airway of epithelial cells it has been shown these particles stay up to eight months in the long epithelial cells after inhalation. And I have to point out here that these particles are in Phase II clinical trials for cystic fibrosis, so it has a lot of promise.

Man: Yes.

Q: Excuse me. Can you use these particles to express RNAi's?

A: Yes. We are in the process of testing it for RNAi. It has been used for other systems with RNAi and showed efficiency. In other words, you can compact a linear DNA and also you can compact a circular DNA.

Man: Are there any other questions? I'm sorry I can't see, that's why I'm asking if anyone's up there. Thank you, Professor Naash.

Naash: Thank you.