

Nanotechnology Symposium

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

Zarbin: Thank you very much. The next speaker is Professor Julia Richards who's going to speak about the role of nanotechnology in genetics and genomics.

Richards: (Inaudible) Okay, I'm here to talk today in a rather different capacity than many of the other speakers. I'm not here as someone who creates nanotechnology products, I'm here as one of the potential users who's very eager to arrive at solving particular problems that I deal with on a regular basis, who ended up taking a look at some of the capabilities that are out there, and I want to present some of that to you today. This is rather more a thought piece than an experimental piece. And most of what I'm going to talk about today is going to be in the context of glaucoma because that's the problem that I struggle with on a daily basis in my search for genes that are responsible for glaucoma.

One of the problems that we deal with in genetics is comparable to many of the other problems that the people working on nanotechnology deal with which is an issue of scale. And Francis Collins and others have described the problem of trying to find a gene in the human genome as being rather like starting out from beyond the moon and trying to find one individual on the face of the planet. And in most cases we get to do this through a series of yes-no questions--Is it here? Is it there? Is it the other place?--with almost all of the answers coming out to be "no." Once we have those genes we face comparable problems of scale as we try to go after solutions to the problems as well as approaches to monitoring what's going on in cases where we attempt interventions or even trying to detect who has the genetic differences that we're trying to study.

And so what we have here is perhaps the ophthalmic geneticist's nanotechnology wish list. which is to say a list for genotyping in real time in clinical field situations without having to run back to the lab each time we want to figure out whether a particular individual or a particular population has the genotypes that we're trying to study. Nanoscale devices for assaying levels of gene expression in situ so we can evaluate what's happening when we begin attempting interventions and methods to deliver either gene therapy or the products of genes that we've identified to cells that are in need of remediating a problem.

In the case of diagnostics, genotyping in real time field situations has a series of associated problems. We need to be able to do this very rapidly. The expense can't be large. We need to be able to do it in very small scale. We really can't do this if we're going to have to drag around an 18-wheeler truck behind us. Portability goes along with the scale issue. So one of the items I want to present to you today is something going on at the University of Michigan: David Burke and colleagues are developing a handheld device. Certainly they're not the only ones in the field who are working on this – and while the device is a microscale device, the critical thing for us is that it deals with nanoscale volumes. And that starting from very tiny amounts of material that they're able to carry out PCR reactions on the spot in very short periods of time.

Why would this make such a difference for us? Let me offer a couple of examples. One of the studies that Bob Ritch and I have done involves a family with 857 individuals in it. In trying to study a family like that, especially with this family being spread around the

world, you end up with the issue of having to go out and study a branch of the family and come back, go out and study a branch of the family. As we're ending up with new findings on this family and we're wanting to look at genotype/phenotype correlations we'd actually like to be able to trace particular branches of the family on the spot without having to actually have every single person in this family--all 857 individuals all over the planet—go in and be seen by ophthalmologist, give blood samples and undergo extensive testing. If we can follow them in the field and basically go trace down the paths that we want to be dealing with, we can carry out these studies with much greater efficiency.

One of the other areas in which this makes a tremendous difference to us, we have field work going on in places like Ghana and some cases eight hours inland from the capitol and the high technology centers where a lot of the people have not even experienced electricity, things like electric lights, computers, and so forth, don't exist in some of these villages. And again, we are working with some large families, wanting to trace particular branches of families, being able to take something like this into the field would make a huge difference in our ability to study other families and, in some cases, populations.

In the case of gene therapy, sometimes once we've found a gene we're actually going to want to do gene therapy. In other cases we're rather going to want to be able to deliver a particular protein product that's been identified or even a medication.

And again we face particular problems. One of the biggest problems being faced in gene therapy is this problem of the immune reactions against the viral vector systems and so we'd really like to be able to improve upon the success rates for gene therapy or surrogates for gene therapy. We'd like to be able to bypass immune responses. We'd like to be able to target things to specific cell types. And issues that are perhaps a little farther away at this point involve targeting specific sites of integration for DNA once you manage to get things into the cell. And one of the other problems is because so many people are now investigating siRNA approaches to suppression of expression of genes, siRNA in many situations ends up becoming a chronic long term problem that is perhaps farther a field than we are ready to tackle by these approaches. But I wanted to offer here—and this builds upon the talk that we just had—James Baker and scientists at the University of Michigan and the Nanotechnology Institute for Medicine and Biological Sciences are working with the dendrimer system. And this dendrimer system offers the ability to attach other molecules to the dendrimer system and they use it in this particular case in targeting certain kinds of treatments. I wanted to take a look at what would happen if we were trying to target this same thing relative to glaucoma. And this goes to the question that I asked at the end of the last talk, one of the problems that we have from many cell types is, "What if you need to get the treatment into the cell rather than having it on the surface of the cell or in the surrounding spaces?" Trabecular meshwork cells have phagocytic properties and could potentially be induced to take up dendrimer structures that might not be taken up by some of the other cell types that people are trying to work with.

One of the things that we want to be able to do is to be able to target things to the particular cell types that we want to reach. And so in this case I hypothesize the use of a latrotoxin analog to be able to direct the dendrimer to the latrotoxin receptors on the surface of the trabecular meshwork cells. One of the other things that we'd like if we're going to get this inside of the cell potentially whatever we have stuck onto the dendrimer is something that we might want to be able to remove by building in specific sites for proteolysis. In this case gene expression studies we've done tell us that the protease PCS K1 is present in trabecular meshwork cells. Now you can potentially engineer such

sites out of the rest of the protein but into a point at the base of the protein to allow it to be cleaved off of the dendrimer.

Okay, and so issues still to be addressed are targeting this to the cell, getting it into the cell, and in some cases getting things across the nuclear membrane, and then the problem with the most popular therapeutic approaches at this point—the siRNA approaches where RNA is fragile and potentially you would need chronic delivery systems that might exceed what this could do.

Okay and then nanoscale devices for assaying levels of gene expression in situ. In some cases you can do this kind of thing with patch clamp approaches, but there is another set of approaches being taken with what's called FRET Technology: Fluorescence Resonance Energy Transfer Technology that allows us to overcome some of the issues. Telemetry, mobility, continuity of monitoring. One of the things we'd like to be able to do is to monitor things *in vivo* and so if we end up with a device like this. So Wolf Frommer and colleagues at Stanford University have devised a variety of molecules that have components of a fluorescent system that sit on a molecule spaced at a distance such that if the molecule binds a small metabolite the conformation of the protein changes—it brings the two fluorescent items closer together and it changes the fluorescence emissions of the protein in question. And at the result of this FRET Technology, especially in trying to look at something like glutamate in vitreous in the back of the eye, would potentially allow for someone to be able to do monitoring *in vivo* of things like glutamate levels, glucose levels, in the case of people studying diabetes, which is another one of the molecules that he's developed this type of assay for. Okay so these are just three examples. A lot of people in the field have other variations on these going on and I've been here at ARVO, I've been talking to other people who are saying, "Oh yes, I'm starting to use FRET Technology", so it's clearly coming into the field. Thank you.