Module:
Biological Foundations of Mental Health

Week 2
Building blocks of the brain

Topic 3
Exploring mental health using stem cells: What are iPSCs? – part 1 of 3

Professor Jack Price
Department of Basic and Clinical Neuroscience

Lecture transcript

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OK. So, as I said in my introduction, what we're going to talk about today is stem cells and how they're relevant to the study of diversity in brain and diversity that leads to neurodevelopmental disorders. And the stem cells I'm going to talk about are stem cells that we call iPSCs. So, iPSC stands for induced Pluripotent Stem Cells, and we're going to describe what iPSCs are and how you make them.

So the key word, really, in the title is pluripotent. So what does pluripotent mean, and why is it important?

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So pluripotentiality-- to understand pluripotentiality, we really have to go back right to the start of embryonic development in mammals, including humans. So to remind you of some sort of basic embryology, life starts with a fertilised egg-- sperm fertilising the oocyte.

This fertilised egg grows, divides, forms a morula, and eventually it forms the structure we call a blastocyst. And a blastocyst is the first stage in development where you can see two distinct populations of cells. So there are these outer cells here, the trophoblast cells, shown in yellow in this diagram.

But the cells we're interested in are the blue ones. And these are the cells that we call the inner cell mass. And it's these blue inner cell mass cells that we're interested in, because these are the cells that are pluripotent. And what pluripotent means is that these cells have the ability-- the capacity-- to generate all the different cell types that make up both the foetal and then, later, the adult body.

So, they can generate the immune system, the nervous system, the heart and circulatory system, muscles, and all the rest. So, that's the definition of pluripotent. These are cells that can make everything that makes up the body.

Now, the other point about this idea of pluripotency-- although it's the most important concept you can imagine, really, in embryology, the property itself is incredibly ephemeral. So, these inner cell mass cells have this property of pluripotency, but they only have it for a few days, and then they lose it as these cells give rise to derivatives that are going to go on and make these different lineages of cells that make up the body.

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So, it's a very ephemeral property. It's there for a few days, then it disappears. And as far as we know, it never really reappears again during the entire life cycle of the organism. There's some dispute about that-- there might be some populations of pluripotent cells crop up in other circumstances later. But, by and large, it looks as if these inner cell mass cells, for these few days, are the only cells that are ever pluripotent.

Now, that makes it a very interesting property, needless to say, for an embryologist. But it also makes it a very difficult property to study, because if a property is only held by a handful of cells for a very short period of time in development, how on earth are you going to start to understand what it means in biological terms.

And the real key to progress in this area has been the development of what we now call ES cells-- Embryonic Stem cells. Embryonic stem cells were first derived from mouse, but have subsequently been derived from lots of different species, including, now, human.

And the key here is-- what ES cells are is a population of stem cells derived directly from the inner cell mass. And the key was the generation of culture properties that assured that the ES cells retained this pluripotency.

So, whereas for the inner cell mass cells, pluripotency is this ephemeral property that only exists for a few hours or days, in the ES cell lines derived from the inner cell mass cells, it's a permanent property. ES cells are permanent cell lines that have this property of pluripotency forever. So, you can grow up as many of these cells as you like, and keep growing them, keep referring back to them.

But these cells are pluripotent in exactly the same way as inner cell mass cells are, and that means, of course, just like the inner cell mass cells, they can give rise to all these different cell types that make up the body.

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So, what's the biological basis for pluripotency? Well the answer is that until very, very recently, we had a very poor grasp of that. But the experiment that gave us the first clue as how to go about looking for the biological basis for pluripotency came from an experiment done by this gentleman, John Gurdon-- then at Oxford, now in Cambridge.

And what he did was this experiment. He was working on frogs, and he took eggs from frogs-- so, an egg being the pluripotent cell of a frog, just equivalent, as it were, to the inner cell mass that we were just discussing.

But what he did was he destroyed the nucleus of those egg cells. So, he used radiation, which would destroy the DNA. And sure enough, he was able to generate what he called enucleated eggs. So, these are pluripotent egg cells missing a nucleus, now.

What he does then is he takes cells from fully differentiated tissue-- in this case, the skin. So, he takes skin fibroblasts-- also from frogs-- and he does the reverse experiment. So, what he does now is he takes the nucleus from those skin cells, and then the experiment is to combine this nucleus with this enucleated egg.

So, what he's got now is an egg with a nucleus transplanted from this fully differentiated skin cell. And the question, obviously, is, can this egg-- this egg with a transplanted nucleus-- go on and develop into a tadpole. In other words, is this cell-- this constructed cell-- is it pluripotent?

And the answer was that it was. So, he was able to show in a convincing number of these circumstances where he transplanted the nucleus into the egg-- he was able to show that in fact that cell could go on and form tadpoles. So it was, indeed, pluripotent.

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Now, what do we conclude from that? There was a number of different conclusions to do with the fact that it shows that this nucleus, even though it's from a fully differentiated cell, nonetheless still has all the genes-- all the information required to generate an entire organism. So, that was actually the primary reason he did the experiment.

But we can actually take another conclusion from this. What we can conclude is the following. If we ask, where does pluripotency reside, it's clear that it's coming with the enucleated egg. In other words, the pluripotency is captured here, in the cytoplasm of this enucleated egg. And we can conclude that because this cell has been able to instruct this nucleus-- that was derived from the differentiated cell-- to act in a pluripotent manner.

So, in other words, we can conclude that there must be factors in this enucleated egg that tell this nucleus-- once the two become combined-- that tell this nucleus that it's got to start turning on the genes, behaving in a way appropriate for a pluripotent cell. So, we can conclude there are factors in the cytoplasm of pluripotent cells that dictate pluripotency. That's a very important conclusion, and it allows us to beg the next question, which is, what are those factors?

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So, the Gurdon experiment told us there must be factors in the cytoplasm of that pluripotent cell that capture the essence of pluripotency. So the question is what are those factors. So, the real breakthrough in understanding those factors comes from the work of this gentleman, Shinya Yamanaka, in work that he published in 2006 and then again in 2007. And his approach to this problem was the following.

He thought that the factors-- first off, they must be, themselves, gene products. In other words, they must be proteins. And he looked at the literature, looking at what had been published about pluripotent cells, and he came up with a list of 24 factors that seemed-- from the research of others and from himself-- that seemed to be associated with pluripotency.

So, these are genes that seem to always be expressed, or always be around, when pluripotency was being studied. So, he figured that probably somewhere in this list of 24 factors must be the ones-- the really important ones-- that are dictating pluripotency in pluripotent cells.

The problem, of course, is how is he going to show that the 24 factors do indeed include the important ones, and secondly, how is he going to find out which of those 24 really are important, and which, perhaps, are less important. So, this was the experiment that he set out to do.

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So, like all good biochemists, he realised that step number one-- he had to have an assay. He had to have a way of recognising if he'd produced pluripotency in cells that weren't pluripotent. And just like John Gurdon, he chose to start with fibroblasts-- so, again, skin fibroblasts. And the challenge was, could he make those skin fibroblasts become pluripotent. And if he did, his assay would have to be, how would he recognise that had happened.

And the key for him was to use this construct. So, what this is is a gene, Fbx15. And the actual nature of the gene doesn't really concern as very much today. What's important is, he recognised that pluripotent cells always seem to have this gene active.

So, he figured that if he could switch on this gene in fibroblasts, then maybe he'd actually come up with a strategy that made them pluripotent. In other words, he was using this Fbx15 locus as a reporter, as we would term it, for pluripotency.

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So, he made this construct whereby if this gene became active, it would turn on this reporter construct that we call beta-geo. And as a consequence of that, if you stain the cells appropriately, they turn blue. So the experiment, now, is can he-- by putting those 24 factors into the cells, into fibroblasts-- can he turn on this beta-geo construct? Can he turn the cells blue?

So, what he next did is actually a real tour de force-- so, a really impressive piece of molecular biology, because first off, he had to make retroviral constructs of all of those 24 genes. He had to have a way of getting all 24 genes into these fibroblasts. So he made 24 different retroviral constructs.

He then had to engineer a situation where he could infect the cells with all 24 genes simultaneously. And what he found was the following-- that if he was able to introduce all 24 retroviruses into a population of fibroblasts, then some of them did indeed turn blue.

And that's shown over here. So, this is a plate of fibroblasts at very, very low power, so you can't see individual cells. And these are what's called the mock-infected. So, these are the control cells. But this is the plate of cells that were infected with all 24 retroviral vectors. And what you can immediately see-- and what Yamanaka immediately saw-- was that there are individual colonies that have started to emerge, and they're stained-- they're stained blue.

So, in other words, by transducing in all 24 factors, he'd been able to show that some of those transfected fibroblasts turned on the Fbx15 locus, and therefore, putatively, the idea would be that maybe they had become pluripotent. So, just that step is an amazing step forward, because what he's shown is that his first bet is correct. Somewhere in that 24 factors are the important ones that induce pluripotency in otherwise non-pluripotent fibroblasts.

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So, so far so good. But now he's got another problem. How is he going to work out which of the 24 are really necessary, and which are perhaps not necessary? So, what he takes on now is an enormous experiment. So, what he's going to do now is he's going to repeat the experiment I've just shown you.

He's going to repeat the infection of these fibroblasts. But what he's going to do-- instead of infecting with 24 factors, he's going to infect with 23 factors. And he's going to do it over and over, each time leaving a factor out.

And the argument is that if it leaves out a factor that really isn't necessary, then he should still see blue colonies, whereas if he leaves out a factor that's important, now he won't be able to generate the colonies. So, now what you're looking at here is a whole series of experiments where he's used the 24 factors minus one. So, each one of these experiments, he's used 23 factors-- the 24 minus one.

And what you can see, indicated by the green arrows, are circumstances where leaving out a factor made no difference. What you're looking at here is the number of colonies he was able to observe-- the number of blue colonies that emerged in these fibroblasts.

And you can see leaving out factor 2, you still get lots of colonies. So factor 2 clearly isn't very important. And similarly, these other factors, indicated with green arrows-- he could leave those out and the experiment still worked. So those weren't necessary to induce pluripotency.

But you can also see that there are some factors that, if he leaves them out, the experiment no longer works. So if he leaves those out, he doesn't get any blue colonies. So, the conclusion is that those factors are probably necessary to induce pluripotency.

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As a consequence of that experiment, he takes-- he's able to narrow down these 24 factors down to 10 factors. These are the 10 most promising-looking factors that emerged from this experiment. So, what does he do now? Well, he repeats the experiment that I've just shown you, but this time using 10 factors.

So, first off he confirms here that by adding all 10 factors he does indeed induce pluripotency-- he gets colonies. So, what he does now is he leaves out one of the factors again. So, each of these experiments is one of the 10-- is the 10 factors with one factor missing. And again, what you can see is that in some cases he can leave the factors out, and it makes no difference.

But there were four factors-- four factors that, if he left factor out, either there was no reprogramming to blue cells, or-- in the case of this one here-- there was some, but it was very much less efficient. So, a much reduced efficiency.

So, now he's down to these four factors. And these are the four factors we've now come to call the Yamanaka factors. So, Oct3/4, Klf4, Sox2, and c-Myc. And it seems like these are the four that really matter.

And to confirm that, he does this experiment. So, now what he shows is that just those four factors still give him lots of colonies. So, if he uses just the four, he still gets conversion of the cells into blue cells-- so, putatively, pluripotent cells. And now, if he leaves out any one of those four, it works much less efficiently. And any two factors really don't work at all.

So, he concludes from that that these four factors are all necessary to generate pluripotent cells, and the four factors together are sufficient on their own. He doesn't need any other of the 24 factors, and nothing else is required to generate pluripotent cells.

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So, this is a remarkable breakthrough, because he's been able to identify four factors that seem to carry this property of pluripotency. But hold on-- all he's shown so far is that those four factors can turn fibroblasts blue using that reporter construct. It's still only a hypothesis that those blue cells-- those cells that turn on the blue gene-- truly are pluripotent.

To convince himself and other scientists that those cells really were pluripotent, he has to really show that they really can do the job of generating all the different cell types that make up the body. So he does that, first of all, by generating what we call embryoid bodies.

So, if you take pluripotent cells-- for example, embryonic stem cells-- and you get them to grow in clusters, and treat them in an appropriate way, they'll start to differentiate into little clusters of differentiated cells. And amongst these clusters, you will find all the different lineages that make up the body.

In particular, you'll find derivatives from what we call each of the three major germ layers-- that's the endoderm, the ectoderm, and the mesoderm. And this is generally taken to be a good in vitro assay of pluripotency.

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So the question was, then, could he take these fibroblasts that have been transduced with the four factors, grow them as embryoid bodies, and show that each of the three germ layers were represented within the embryoid bodies. And what you can see here, in this figure, is the evidence

that he can do exactly that.

So, these are histological sections through embryoid bodies derived entirely from those transduced fibroblasts. And what you can see is that there are mesoderm derivatives, like muscles and smooth muscle, and cartilage, there was epithelial tissue, there was brain tissue, there was adipose tissue. In other words, these cells had managed to go on and make all of these different cell lineages.

So, that was remarkable. It seemed to show that those cells were indeed pluripotent. Nonetheless, Yamanaka wanted to go one step further, because if those cells were really pluripotent, then he should be able to use them to contribute to the cells actually in a living mouse.

So, ES cells, the other pluripotent cell type we've talked about-- if you inject those into a growing mouse blastocyst, they will contribute to the development of the mouse. And they will contribute not just to the embryoid body formation you're of seeing here, but actually to cells in a mouse as it develops.

So the question was, could he use these induced pluripotent fibroblasts-- could he use those to contribute to mouse development in the same way? Well, what this figure shows is in fact that he could.

So, here you're looking at two mouse foetuses. The one on the right is mock-transfected-- so, it didn't receive the four factors. But the one on the left is a mouse that, as a blastocyst, was injected with those fibroblasts that had been transduced with the four factors-- and also they'd been labelled green so that we could see what happened to them.

And what you can see in the fluorescent image over here is that this mouse has had green cells contribute to lots of the different tissues of the body. So, those fibroblasts that had been transduced with the four factors and are putatively pluripotent can indeed contribute to lots of different cell types if you inject them into a mouse blastocyst.

So, he was able to confirm that these green cells had indeed contributed to all the different tissues of the body by taking histological sections of mice, like this one. And you can see the stained cells are in lots of different tissues-- so, in the neural tube, in the liver, the heart, the gastrointestinal tract, gonads, and so on and so forth. So, this confirmed that the cells that he'd injected into the blastocyst of this embryo had indeed contributed to all the different tissues of the body.

Now, importantly, they contribute also to the germ cells. So, you can see cells here, resident in the gonad. And what that meant was that they contributed-- the fibroblasts that had been injected into this blastocyst contributed to the germ line such that, when mice like this were grown up to adulthood and bred, he was able to produce mice that were entirely derived from these injected fibroblasts.

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Let me spell that out again. The fibroblasts that have been transduced with the four factors are introduced into the blastocyst at this very, very early stage. The embryo grows up.

Some of those blastocysts-- injected fibroblasts contribute to the germ line of this animal, such that when this animal is bred downstream, those germ cells contribute to the next generation of mice. Such that you're able to generate a whole line of mice derived entirely from those transduced fibroblasts.

So, you can see that this is real evidence that those cells-- those skin fibroblasts that have been transduced with those four factors-- really were pluripotent. They weren't just turning on the reporter gene in vitro. They weren't just giving rise to differentiated cells in embryoid bodies. They were truly able to contribute to the generation of an animal in exactly the same way as the inner cell

mass cells would.

So, Yamanaka had really found the biological basis for pluripotency. He'd found the four factors that were both necessary and sufficient to generate pluripotent cells, starting from just common-or-garden fibroblasts.

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So, Yamanaka reported the work that I've just told you about in 2006. In 2007, he published a paper showing that you could do essentially exactly the same thing, starting with human cells. So, the original publication had been with mouse fibroblasts. The following year, he was able to show exactly the same process starting with human fibroblasts.

Now, in the years since 2006, 2007, a number of changes have been made to the protocol. It's been made slightly more sophisticated. We can deliver the genes in slightly different, slightly better ways. But, in essence, the procedure that Yamanaka demonstrated to us has now been broadly adopted, really, worldwide. So, many, many labs across the world now are generating these induced pluripotent stem cells-- these iPS cells, as Yamanaka christened them-- by using essentially his protocol.

So, one of the various things we've learned during that period is that Yamanaka started with skin fibroblasts. In fact, you can start with essentially any cell type in the body. So, for example, several groups now have shown that you can make iPS cells starting from blood cells.

A couple of colleagues that I know about have made iPS cells starting simply with the cells that you're able to centrifuge out of urine-- so, sloughed off bladder cells that can be isolated from urine.

In our lab and a number of other labs, we've started from a slightly different sample. So, we start with hair. And the reason for that is we're quite interested-- as I'm going to go on to tell you about a little later-- in disorders of childhood. So, we're interested to be able to collect biological samples from children. And we weren't really keen on trying to take skin biopsies, or even blood, from autistic children.

But what we can take really quite easily are hair samples. So, we pluck a hair, or a small number of scalp hairs, just from the head of a child. And from the bulb at the end of the hair, we can grow a population of cells-- of so-called hair keratinocytes.

We can then use Yamanaka's four factors-- engineered now into a different kind of vector, but fundamentally the same as the way he did it-- and from these keratinocytes, we can grow colonies of iPS cells. We can grow these up, expand them, freeze them down, and generate really enormous quantities of these iPS cells. And they're the substrate for the experiments that I'm going to go on and tell you about in the next section.