LATEST ADVANCES IN CORD BLOOD APPLICATIONS & COMMERCIALIZATION

SPOTLIGHT

INTERVIEW

Advances in the use of cord blood-derived iPSCs

Mahendra Rao received his MD from Bombay University in India and his PhD in Developmental Neurobiology from the California Institute of Technology. He is widely known for his research involving hESCs, iPSCs, and other somatic stem cells, having worked in the stem cell field for more than 20 years with stints in academia, government, regulatory affairs and industry. Dr Rao has published more than 300 papers on stem cell research and is the co-founder of a neural stem cell company, Q therapeutics, based in Salt lake City (Utah). He continues to work with the NIH, FDA and other regulatory authorities on ESC related issues, most recently as the CIRM and ISSCR liaison to the ISCT. Dr Rao is currently the Vice president of Research in Regenerative Medicine at Q therapeutics and its subsidiary Neuro Q. He currently serves on the Board of CESCA, XCell and Stempeutics and on the SABs and as a consultant of various stem cell companies including the New York Stem Cell foundation. He continues to maintain an active research program in neural development and in evaluating cell-based screening and therapy to treat disorders of the nervous system.

Q We've advanced our understanding of induced pluripotent stem cells (iPSCs) since the early work of Shinya Yamanaka. What do you see as the stand out developments in the utility of iPSCs over the last decade?

Several things have happened that have built on the work of Shinya Yamanaka. Perhaps the simplest change that has occurred in advancing their utility is that instead of using integrating viruses to perform the iPSC procedure, we are now able to use non-integrating reprogramming methods and actually bypass using DNA. Nowadays, one can reduce the chance of integration by using mRNA, synthetic mRNA, minicircle plasmids and now even protein.

Since that first step, there have been additional developments in the field of iPSC research that are really important. The first is that we no longer need to go back to the iPSC stage, meaning you may be able to go back to an intermediate earlier progenitor stage of the cell. This development has been pushed forward by a number of research groups and offers two advantages: 1) you can take a fully differentiated cell and make either a neural stem cell, T-cell precursor or red blood cell precursor using a combination of factors and reprogram the cell at this intermediate stage and 2) at the same time this allows for maturation to occur quicker.

The other major advance is that we can now ask, if we can do all of this *in vitro*, can we extend to doing it *in vivo*? If we can manage that, we will create a short cut to the whole process of having to culture. You can simply introduce these cells, the reprogramming vectors, mRNA, proteins and minicircles, that can be delivered by adenovirus. A few research groups have already begun these efforts and have published exciting results.

Q What makes iPSCs preferable for regenerative medicine compared with alternative cell types such as embryonic stem cells (ESCs)?

There have been three major kinds of stem cells that are used: adult stem cells, ESCs and iPSCs. Both ESCSs and iPSCs offer a huge advantage over adult stem cells. Adult stem cells differ from pluripotent cells such as ESCs and iPSCs because they have limited expansion potential and a more restricted differentiation ability.

When comparing ESCs and iPSCs, iPSCs offer one advantage and one disadvantage. The disadvantage is the fact you're required to carry out one further step in a process that is not totally natural and so there may be differences we don't anticipate. Any time we introduce an additional step, there is a larger risk.

The major advantage of iPSCs over ESCs is the fact that ESCs have been cloaked in a multitude of ethical and political issues. The legality of ESCs differs between regions and so this can be detrimental to the commercial side of things where some ESCs lines can be used in some countries but can't in others. This makes it very difficult to choose an ethically derived cell line and still make it a commercial product. Unfortunately, this is a big disadvantage of ESCs, even though scientifically, in my opinion, they're a better choice.

From the technical point of view, there are two huge advantages to iPSCs. One is that we can prospectively identify the cell type we need, including its HLA type and make an iPSC cell from it. This is a huge advantage because being able to select the cell type and immune matched cells means we can use it for a particular indication.

The second advantage is that personalized medicine is possible with iPSCs and harder with ESCs. Because we can make the iPSC from an adult cell, we have the clinical history of that patient. This allows us to make a truly personalized iPSC from an individual and use it for therapy.

DOI: 10.18609/cgti.2017.047

Q How did the idea to use cord blood as a source of iPSCs emerge and what are the advantages of this approach?

When I was working at the NIH we were tasked with trying to provide a generalized way of producing HLA-typed cells that matched the patient so that there would be no immune response to the transplanted cells.

On a practical level, what it meant was that we had to search large data banks such as the bone marrow registry and cord blood registry. The bone marrow registry is different from the cord blood bank in that it's a living registry and therefore you have to contact people and get consent to make sure they are willing to send you a sample. On the other hand, cord blood is already stored so it's much quicker to get the sample. In the USA, the Food and Drug Administration (FDA) had also created new criteria that ruled cord blood banks had to be licenced as a provider of a biological product. This was critical because it meant that we would not only get a starting sample that was easy to access but also had been collected in an appropriate way so that when manufacturing a secondary product from it, the regulatory criteria were already met. The upshot of this was that cord blood became a reasonable option.

There are also scientific advantages to using cord blood as a source. We found that making iPSCs from cord blood was much more efficient. The process was simpler, we got a larger number of clones and we could do it with a much smaller volume of cells. They were pristine cells, collected appropriately and divided much better than a lot of adult cells.

Q Are there any disadvantages of using cord blood as a source of iPSCs?

There are two practical disadvantages of using cord blood. The first one is the fact that there's something called the donor consent rule that requires consent for certain types of testing to be carried out. The problem is that when it comes to cord blood, there is the question of who this sample really belongs to. Does it belong to the parents or the child? When the child turns 18 does that change? So to move forward with this option, we had to get clarification from the institutional review boards and registers on the ownership and donor consent exemption rules required by the FDA.

The second disadvantage is the expense. Because cord blood is a therapeutic product, companies are keen to only provide that at the same price as using it for a patient. And the average cost of a cord blood unit, at least in the USA, is around $30,000. This means the cost of using cord blood as a source of iPSCs is potentially huge. To overcome this, we had to develop an alternative method where we didn't use the entire sample but only use a smaller sample that is collected in parallel (pig tail) and is usually intended for genetic testing. Obtaining this is much cheaper and preserves the parent sample for future use.

Q What are some of the challenges in deriving GMP-grade human iPSCs from cord blood?

There used to be a myth that there was an epigenetic history if you derived iPSCs from cord blood versus from skin or keratinocytes, and that the cells would be more biased to that fate due to epigenetic history. Several papers were written saying that was indeed the case.

However, it was soon discovered that once we developed integration-free iPSCs and maintained them as stable cells, this epigenetic memory did not last. Several groups ultimately showed that a good quality cell line does not have epigenetic memory and there was no bias and no difference in ability to differentiate into any of the phenotypes from cell types made. Just as is the case with ESCs, if it's a good ESC line it doesn't have a bias and works well whereas if you have a bad ESC because of a chromosomal abnormality or mutation in a key gene or whatever, it's biased.

A good iPSC line behaves just like a good ESC line.

Nevertheless, there is one practical disadvantage of using cord blood that people have reported, and we also found this in our research. Some of the vectors that are used in electroporation techniques don't work with high efficiency in these cells. For example, even though Sendai virus works well, certain adenoviruses don't show good results. This means that with cord blood iPSCs, you are somewhat limited by which technique you can use.

We have also found that because cord blood-derived cells are suspension cells, some of the automation procedures that involve washing and handling of iPSCs are difficult to implement with cord blood-derived cells.

Q Can you tell us about the development of the Haplo Bank initiative and its objectives?

The Haplo Bank initiative was proposed by several groups but the person who championed it the most was Sir Ian Wilmut in Scotland. He attempted to get global consensus from several groups including mine and Shina Yamanaka's group as well as others in Korea and India. The idea being that if you can have a bone marrow registry and an HLA bank, then why can you not have an iPSC bank of all HLA types?

We also know that we can make the process cost effective by looking for super donors. The best analogy to explain the concept of super donors is the ABO blood group system. If you have AB type blood, you can receive blood of all types and if you have type O, you can donate to all other blood groups. HLA super donors are the equivalent to type O blood group. If you can identify the right HLA types that could act as super donors, then you can create a small number of lines that can be used to treat a wide range of people.

The first study to establish whether this was possible was carried out in the UK and they showed on a theoretical basis that to match the UK population, you might need as few as 50 lines and 25 would be enough to cover 50% of the population.

DOI: 10.18609/cgti.2017.047

Other groups then did a similar analysis and were able to identify the right HLA combination, which would help the majority of the population. In Japan, it was found that between 30 and 50 lines were needed. In the USA, which is much more heterogeneous, something like 300 lines were going to be needed to cover 50% of the population.

Based on the research it was posited that we can prospectively identify super donors and get blood samples from them to create a bank of iPSC lines.

Q Looking forward, which developments on the horizon of iPSC research are you most excited about?

iPSC research lends itself to rapid discovery like never before. Before stem cell research, a huge hurdle in getting drugs to market was that a number of patients always develop adverse events. That means even if a drug is beneficial to 70% of people, we withdraw the drug from the market because of the risk that a patient may be adversely affected. By being able to make iPSCs from the individuals who have an adverse event, we can now very quickly start understanding the mechanism of action. This means we can change the way we run our clinical trials so we can now subdivide how we use the drug, put appropriate label warnings, and come up with diagnostic tests for who should be using a small molecule drug or not.

The same thing applies to toxicology. We've never before been able to conduct effective toxicology screens for side effects because we've always used animal models and these have been largely inaccurate. With iPSCs we have the potential to make reference material for toxicology screens. We've already seen that this is being put into practice with cardiac stem cells being used as toxicology screens for small molecule drugs.

Another key consideration is that iPSC research is moving forward incredibly rapidly. We need to start planning for this. It's now reached the stage where the rules and regulations are not keeping pace with the speed of discovery and this might hinder things.

It's also true we might be doing things that require ethical or moral debate but there's been no discussion on a societal scale of what's acceptable or not. For example, we can now make both sperm cells and oocytes from an iPSC. This means we can now make gametes in culture but the issue is whether this is right to do so and how do we regulate this? The societal discussion needs to move forward to keep pace with technology.

AFFILIATION

Mahendra Rao, Founder and CSO, Mahendra Rao LLC