Journal of Alzheimer's Disease 8 (2005) 75–80
IOS Press

Discussion

Alzheimer: Stem Cell Therapies for Neurodegenerative Disease: How Should We Push Ahead?[1]

http://www.alzforum.org/res/for/journal/rao/rao_transcript.asp

Dr. Mahendra Rao led this live discussion with co-moderator George Martin on 30 March 2004.

Participants: Mahendra Rao (*National Institutes of Health, Baltimore, Maryland*), *George Martin* (*University of Washington, Seattle*), *Joy Snider* (*Washington University School of Medicine, St. Louis, Missouri*), *Jeanne Loring* (*ARCGEN, San Diego, California*), *Dan Freudenberger* (*Neurobiology Council, Harvard Medical School*), *June Kinoshita* (*Alzheimer Research Forum*), *Greg Brewer* (*Southern Illinois University*), *Kirk Townsend* (*University of South Florida*), *Franz-Josef Mueller* (*Burnham Institute in La Jolla, CA*), *Angela Biggs* (*independent researcher*).

Mahendra Rao: I want to point out that while stem cells offer hope, there are several important issues that must be considered when assessing the utility of stem cells, but which have been ignored.

George Martin: Dr. Rao, yes, indeed. It is time for reality testing. I wonder if you could begin with your definitions of a stem cell, a progenitor cell?

Mahendra Rao: A stem cell is a cell that has the capability of self-renewing over a prolonged time period and can generate multiple phenotypes in response to an exogenous signal. A progenitor cell is more restricted in its differentiation capability and undergoes only limited self-renewal (perhaps by symmetric division). The most important question, of course, is are there stem cells in the adult brain and is their number reasonable to consider clinical therapy of whatever form.

George Martin: Dr. Rao, thanks for these clear definitions. I could perhaps add some further definitions that sometimes cause confusion. No stem cell seems to be truly totipotent, as they cannot make a trophoblast. Embryonic stem cells are pluripotent, while I regard embryonic carcinoma cells as merely multipotent (which includes capability of some neural differentiation).

Mahendra Rao: Agreed. Indeed, stem cells should be classified based on their differentiation bias. ES, tissue specific, etc. Does anyone have a comment on the statement that in the adult, neural stem cells are restricted to limited regions of the brain?

Joy Snider: The evidence that stem cells are present in the adult brain seems solid, and they can differentiate into neurons and other cell types. But what is the status of the data suggesting that they can play a functional role, or potentially replace cells damaged in neurodegenerative disorders?

Mahendra Rao: Joy, the evidence is solid. It is the numbers I am concerned about. I also am concerned about the transdifferentiation potential of hematopoietic stem cells (HSCs). The number of cells in the human brain that appear to be stem cells is small. In many

[1] Note: The transcript has been edited for clarity and accuracy.

ISSN 1387-2877/05/$17.00

regions, when stem cells are transplanted, they do not become neurons, and it appears that the hippocampus is perhaps the only really neurogenic region.

Joy Snider: I was just wondering about the status of studies on functional integration of the endogenous stem cells – I know there is some data out there, but not much last time I checked.

George Martin: Joy, you are correct. We need a lot more information on that point. Dr. Rao, are you as depressed as I am about those two Nature papers that just came out showing a failure of bone marrow hematopoietic progenitor cells to differentiate into myocardial cells in ischemic hearts? One was from my colleague Chuck Murry [1], and the other involved Irv Weissman [2].

Mahendra Rao: As Dr. Martin pointed out, the functional integration data that looks really good is mostly from neural cells, and generally only in regions of ongoing neurogenesis as far as neuronal integration is concerned.

June Kinoshita: Mahendra, does your comment suggest that there is some kind of region-specific signal that enables functional integration to occur? If so, what might that be?

Mahendra Rao: Yes. Multiple lines of evidence suggest that cell-intrinsic and cell-extrinsic signals coordinate to direct site-specific integration. These vary in different regions.

Greg Brewer: In light of the Parkinson's graft trials [3], what do you think we need to do to control expression or overexpansion of the graft?

Mahendra Rao: Greg, I am not quite sure which problem you are referring to-perhaps the Freed results? But the general answer, I think, is that we need to know how to control cells, and this knowledge is rapidly being acquired.

Kirk Townsend: Yes, if neural stem cells can be introduced into the brain of Alzheimer's disease patients, what are its prospects in the context of the underlying pathology-amyloid deposition, inflammation, etc.?

Mahendra Rao: This is an unknown, Kirk, but in general, cells, particularly healthy cells, seem to survive in an inflamed environment. The best data has come from stroke and injury models.

George Martin: Kirk, I am not optimistic about the clinical applications of the introduction of stem cells into AD brains because the pathology is so diffuse-there are even plaques in the hypothalamus. But Greg's interest in PD may be an earlier target, although there are concerns about the underlying premise that we might discuss.

Kirk Townsend: Well, I know the literature can be contradictory, but the recent article in science by Monje et al. [4] suggests that CNS inflammation blocks adult hippocampal neurogenesis . . .

Mahendra Rao: Endogenous neurogenesis is reduced in multiple situations but its rate can be altered even in the inflamed environment.

George Martin: Kirk and others, there is a table in a recent review by X. Zhao et al. (Research and Perspectives in Neurosciences, 2003) that lists some 30 variables moderating in vivo proliferation and neurogenesis in adult hippocampus and olfactory bulb.

Mahendra Rao: Likewise, Forest laboratories has just gotten approval for memantine (a glutamate regulator) for palliative treatment.

June Kinoshita: Mahendra, what are you thinking about with regard to memantine? Do you think there might be effects on neurogenesis or other regenerative processes?

Mahendra Rao: June, yes, there was a paper by Rakic and colleagues [5] which showed that glutamate had specific direct effect on stem cell proliferation. All, I am wondering if anyone has an opinion on selective serotonin-reuptake inhibitors (SSRIs) and their effect on stem cells.

Dan Freudenberger: Mahendra, would you care to comment (from your personal perspective as opposed to NIH's) about the migration of stem cell research to privately funded laboratories and offshore, because of the policies of the Bush Administration? Is it likely that this research can reach critical mass with the threat of retaliation from the Right lurking in the national background?

Mahendra Rao: This is a difficult question. On the one hand, the technological expertise required to follow on is really best in the United States. However, the number of cell lines available here is small. I see really only collaborations being the best way to go forward in the current situation of rules and regulations. The biggest problem is really that people do not share results and that makes it hard to move fast.

Jeanne Loring: Mahendra, I agree. We should collaborate.

Mahendra Rao: Reprogramming: yes, another contentious topic. In a review, Liu and Rao [6] point out exactly what Dr. Martin said. We need to understand the mechanisms, and there is data on this from work done in the cancer field.

George Martin: Anyone wish to add further comments about the question raised by Dan?

June Kinoshita: I worry that privately funded research will result in important reagents and intellectual property getting into the hands of only a few parties.

Mahendra Rao: I cannot emphasize collaborations enough. Look at the genome project and other large-scale attempts. They are simply impossible without pooling expertise and skills. June's worry is real. The patent landscape is quite depressing.

George Martin: I agree with June. I think, however, we should move on to another major topic. Who has one to suggest?

Franz-Josef Mueller: Most human neural cell lines are beyond passage 10, which might mean that they are transformed, so how should one get around this problem, if you want to see real biology and/or have a well-characterized system?

Mahendra Rao: Hi, Franz. That is an important question. Basically, there are no stable human lines that one can get off the shelf. They must be harvested and carefully checked for karyotypic stability.

Jeanne Loring: I will comment: I have worked with both somatic and embryonic stem (ES) cells (as have many of you). Only ES are immortal and diploid. And, most importantly, all of us start in more or less the same place with ES cells – which is very much not true with somatic cells.

Mahendra Rao: I think that there are two possibilities and both require going back to tissue. Taking either ES cells or harvested adult stem cells from fetal tissue or brain biopsies (temporal), characterizing them carefully and making sure results are repeatable.

George Martin: So the question is the maintenance of genomic integrity of cultured cells?

Mahendra Rao: Yes, absolutely. There are other issues as well – epigenetic modulation and alteration in cell surface properties.

Joy Snider: Another point to consider: ES cells are immortal and diploid, but their karyotype varies over time in culture, also.

George Martin: Franz, there will always be strong selection for cells with slight replicative advantages and these could result from minor or major genetic changes.

Mahendra Rao: Both Joy and George make important points. The solution currently is to carefully monitor and discard abnormal cells. We need good pathologists.

Jeanne Loring: George, I was trying to say that as well: With mouse ES, trisomies often arise because they divide faster. They have to be routinely karyotyped and recloned . . . the same goes for human embryonic stem (HES) cells.

Franz-Josef Mueller: So, an approach would be to test biology on a long-term culture and try to replicate the results in a "new" culture harvested from "fresh" tissue?

Mahendra Rao: Absolutely, dead-on, Franz. We did this by making immortalized cell lines, but went back and rechecked everything with primary cells. Many things change, others stay the same, and the trick is knowing which is which.

George Martin: Can I change the topic to the issue of intervention, via stem cells, in Parkinson's disease? Who wishes to comment?

Mahendra Rao: Stem cells are a viable therapy in Parkinson's disease. Just like islets in diabetes and skeletal muscle in heart disease. The issue in all of these has been a source of verifiable, usable cells.

Jeanne Loring: Is there any way that we can all be working on the same neural stem cells-a common set of conditions, a common set of characteristics that are used to define the cells? This has a lot of relevance to Parkinson's disease – lots of variation in cells means lots of variation among laboratories. And, I might add, what good does it do if only one laboratory can get a technique to work?

Joy Snider: This is a critical question in stem cells. We work with neurally differentiated murine ES cells, and transferring the technique between laboratories, even when using the same cells and reagents, is not trivial.

Mahendra Rao: Jeanne, my strategy has been to try and define markers and characteristics of whichever population I work with so that other people can replicate the results. The publishing of techniques should then make it useful to all laboratories. The NIH realized this, Joy, and has tried, at least in the case of ES cells, to sponsor training courses.

Mahendra Rao: I think it is useful to do both. Many techniques are conceptually similar, but differ in details.

June Kinoshita: We are starting to develop a Web page for the Alzforum on NS cells. The idea is to have a central information resource where essential data about different cell lines and methods can be posted. We are inviting input from everyone with an interest in this area to contribute advice and data.

Greg Brewer: I agree about maybe a vote for the top 10 markers that we should commonly examine, but focusing on one cell line/type seems too restrictive until we can find one that works well. There will always be the arguments to work first with mouse or get to the crux of the issue with human cells.

Mahendra Rao: Greg, the marker issue thread is true, and I think perhaps even microarrays with 50–100 genes might be a good way to go.

Joy Snider: Sounds like a good plan. Regarding the Parkinson's disease question: Is everyone convinced that the stem cell transplantation of dopaminergic neurons was effective? As opposed to nonspecific effects of surgery, a lesion, etc.?

Greg Brewer: I agree with Joy. There is a large literature on injury-induced regeneration.

Mahendra Rao: The Swedish groups have really good data on human transplants and correlating improvement with dopamine release.

George Martin: Back to my question on PD. I was impressed by a paper by John Haycock et al. [7] in which there was evidence that the dopaminergic neuropil is reasonably intact during aging, despite dopamine being diminished.

Mahendra Rao: For Parkinson's disease: There is another strategy that may be exciting, and that is delivery via stem cells or glial cells of molecules and growth factors.

George Martin: Would someone now wish to switch to a new subtopic on neural or other stem cells?

Angela Biggs: What about the research of using the patient's own bone stem cells, assuming they will adapt to the injury at hand . . . and become neuron stem cells.

Jeanne Loring: Do we really think that bone marrow stem cells become neurons?

George Martin: Angela, earlier in this chat session, I was bemoaning the negative results of two Nature papers that tried this with ischemic myocardium.

Mahendra Rao: The idea of hematopoietic stem cells (HSCs) is seductive. They are already used for therapy, they are an autologous source, and there has been tantalizing evidence of transdifferentiation. However, as George pointed out, the numbers are not great.

Angela Biggs: I see.

Mahendra Rao: However, it is possible to consider them as sources of growth factors – a rich source that may be useful.

Greg Brewer: Mahendra, back to the array issue. Is there some way to poll workers in the field so that everyone's favorite marker will arrive in a consensus array, made available to all?

Mahendra Rao: Regarding array: Yes, the array people have formed consortia for this purpose and some private companies are trying to make focused arrays available cheaply. It is like information; I think if sufficient numbers of reagents are available, people will

choose the best one and converge on a standard. We cannot mandate it.

Jeanne Loring: How about fusion of transplanted cells?

Kirk Townsend: What about human umbilical cord blood as an alternative source?

Jeanne Loring: I have heard that umbilical cord has too few cells for anything but restoring blood cells. Am I right?

Mahendra Rao: The immune issues for cord blood remain unresolved and the companies apparently do not find it a profitable market. New York and Duke are the two active cord blood banks available.

Kirk Townsend: Well, actually, there is a company right here in Tampa, Florida.

June Kinoshita: I have heard that astrocytes can be induced to transform to a neuronal phenotype. Mahendra, can you comment?

Mahendra Rao: Transdifferentiation: The problem is numbers, reliability, and heritability. If you transdifferentiate, will it stay transdifferentiated?

Joy Snider: Back to Dr. Rao's topic of other roles for stem cells and relevant to the astrocyte question. Given the issues surrounding making new functional neurons, stem cells as a source of growth factors are a possibility, or what about using stem cells differentiated into astrocytes or microglia to promote clearance of Aβ?

Mahendra Rao: Joy, I think this is a good idea and an example of thinking outside of the box, while most researchers in the field have fallen into it.

Jeanne Loring: Regarding astrocytes and markers: Is it not true that neural stem cells express glial fibrillary acidic protein (GFAP), and that we are finding more and more "differentiated" markers in pluripotent cells? We could use stem cells to target plaques because of the inflammation – and have them deliver an Aβ cleaving enzyme like neprilysin. Ideas?

Mahendra Rao: Jeanne, there is also data, particularly from Steindler's group [8], about authentic astrocytes dedifferentiating into stem cells and independent work from Martin Raff on glial progenitors [9]. While I am not a fan of transdifferentiation on a practical level, I have to give credence to the published work from solid labs.

George Martin: Jeanne, cultures of pluripotent cells may include mixtures of cells in various stages of differentiation.

Jeanne Loring: George, I think "may" is inaccurate – always do, I think.

Mahendra Rao: To follow up on George's comment: Does anybody know of any experiments where pure stem cell populations have been transplanted into the brain as opposed to a mixture?

Jeanne Loring: What is pure? Fluorescence-activated cell sorting (FACS)? I think the NT2s are the closest anyone is done.

Mahendra Rao: I agree with Jeanne, which suggests to me at least a major lacuna in our studies.

June Kinoshita: I have heard some scientists suggest (a few years ago) that if you transplant undifferentiated NS cells into an injured brain, the cells will migrate to the injury site and differentiate into the appropriate cell type(s), and become functional. Others advocate making the correct cell type *in vitro* and then transplanting. Does the weight of evidence currently favor one approach over the other as being more likely to succeed?

George Martin: Dr. Rao, while waiting for an answer, I want to bring up the dangers of using feeder layers from other species, or even from different individuals when trying to maintain stem cells in culture (i.e., viral transfer and viral recombinations).

Angela Biggs: Helen Blau and colleagues from Stanford University injected bone marrow from adult mice that express a marker called green fluorescent protein (GFP) into adult mice that had been irradiated to eliminate their bone marrow. They found that bone marrow-derived cells migrated into several regions of the brain, including the olfactory bulb, the cortex, the hippocampus, and the cerebellum [10]. Some of the marrow-derived neuronal cells also grew long fibers and produced a protein that indicates cell activity. These re-

sults suggest that the marrow-derived neurons not only entered the brain, but also responded to their environment and began to function like the native ones. They are not pure, but they migrated there.

Mahendra Rao: Angela, see the fusion results from the same laboratory [11].

Joy Snider: Would "pure" stem cells necessarily be better? Less differentiated cells can make their own support cells, vasculature, etc. Of course, they can also have unregulated growth.

Mahendra Rao: Feeders and xenocells: I think they are a clear concern, as is fetal bovine serum. However, the FDA has allowed xeno-organ transplants, and perhaps they know more than we do.

George Martin: I want to thank all of the participants, and especially Dr. Rao for this chat session.

References

[1] C.E. Murry, M.H. Soonpaa, H. Reinecke, H. Nakajima, H.O. Nakajima, M. Rubart, K.B. Pasumarthi, J.I. Virag, S.H. Bartelmez, V. Poppa, G. Bradford, J.D. Dowell, D.A. Williams and L.J. Field, Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts, *Nature* **428** (2004), 664–668.

[2] L.B. Balsam, A.J. Wagers, J.L. Christensen, T. Kofidis, I.L. Weissman and R.C. Robbins, Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium, *Nature* **428** (2004), 668–673.

[3] C.R. Freed, M.A. Leehey, M. Zawada, K. Bjugstad, L. Thompson and R.E. Breeze, Do patients with Parkinson's disease benefit from embryonic dopamine cell transplantation? *J Neurol* **250**(Suppl 3) (2003), III44–III46.

[4] M.L. Monje, H. Toda and T.D. Palmer, Inflammatory blockade restores adult hippocampal neurogenesis, *Science* **302** (2003), 1760–1765.

[5] T.F. Haydar, F. Wang, M.L. Schwartz and P. Rakic, Differential modulation of proliferation in the neocortical ventricular and subventricular zones, *J Neurosci* **20** (2000), 5764–5774.

[6] Y. Liu and M.S. Rao, Transdifferentiation – fact or artifact, *J Cell Biochem* **88** (2003), 29–40.

[7] J.W. Haycock, L. Becker, L. Ang, Y. Furukawa, O. Hornykiewicz and S.J. Kish, Marked disparity between age-related changes in dopamine and other presynaptic dopaminergic markers in human striatum, *J Neurochem* **87** (2003), 574–585.

[8] E.D. Laywell, P. Rakic, V.G. Kukekov, E.C. Holland and D.A. Steindler, Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain, *Proc Natl Acad Sci USA* **97** (2000), 13883–13888.

[9] T. Kondo and M. Raff, Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells, *Science* **289** (2000), 1754–1757.

[10] M.A. LaBarge and H.M. Blau, Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury, *Cell* **111** (2002), 589–601.

[11] J.M. Weimann, C.B. Johansson, A. Trejo and H.M. Blau, Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant, *Nat Cell Biol* **5** (2003), 959–966.