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SPOTLIGHT

From naïve pluripotency to chimeras: a new ethical challenge?

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ABSTRACT

In recent years, there has been much interest in the prospect of generating and using human stem cells that exhibit a state of naïve pluripotency. Such a pluripotent state might be functionally confirmed by assessing the chimeric contribution of these cells to non-human blastocysts. Furthermore, the generation of naïve human pluripotent stem cells *in vitro* could lead to the creation of chimeric animal models that can facilitate the study of human development and disease. However, these lines of research raise thorny ethical concerns about the moral status of such chimeric animals. Here, I call attention to these ethical barbs and suggest a way in which to proceed cautiously.

In pursuit of naïve pluripotency

It has become a prevailing view among stem cell researchers that pluripotency is not a singular state but rather a spectrum that ranges from 'naïve' to more developmentally 'primed' states (Hackett and Surani, 2014). Stem cells are considered to be 'naïve' if they are free of developmental biases, with 'ground state' stem cells being the least developmentally and epigenetically constrained. By contrast, 'primed' pluripotent stem cells are more committed toward lineage-specific developmental programs and are epigenetically restricted (Nichols and Smith, 2009). These distinctions underlie the differences between human embryonic stem cells (ESCs) and those derived from mouse blastocysts; human ESCs correspond more closely to primed mouse epiblast stem cells (EpiSCs), which are derived from the epiblast of the postimplantation stage mouse embryo (Brons et al., 2007; Tesar et al., 2007). An important research question is thus whether human pluripotent stem cells, including ESCs and induced pluripotent stem cells (iPSCs), can be converted to a naïve state under culture conditions, and recent studies (Gafni et al., 2013; Ware et al., 2014; Takashima et al., 2014; Theunissen et al., 2014) suggest that this is indeed possible. The generation and maintenance of such naïve human pluripotent stem cells *in vitro* could lead to the creation of better chimeric animal models that would facilitate the study of human development and disease and possibly allow purer populations of specialized cells to be developed for regenerative therapies.

However, one of the major challenges standing in the way of these goals is that there is not yet a universal test for defining and confirming the naïve pluripotent state of human stem cells (Hackett and Surani, 2014). Whereas molecular benchmarks, such as global gene expression profiles and global DNA hypomethylation, are undoubtedly important, researchers are keen to find a functional basis for designating naïve status to human stem cells. In the case of mouse ESCs, naïve pluripotency is functionally confirmed by generating intraspecies chimeras in which the ESCs are marked/tagged and injected into viable mouse blastocysts that are then implanted back into the mouse uterus. If the resulting mouse pups contain derivatives of these tagged cells in all their organ systems, the injected stem cells are confirmed as being truly naïve. Could a chimeric murine-host test offer a defining standard for naïve pluripotency in human cells?

The answer appears to be no. Although one research group reported that interspecies chimeras could be generated by injecting their derived naïve pluripotent human stem cells into mouse morulae (Gafni et al., 2013), another group attempted to repeat this chimeric assay but were not able to succeed (Theunissen et al., 2014). Alternative approaches using animal species closer to humans might thus have to be attempted in order to assess whether lab-generated naïve pluripotent human stem cells can further differentiate and contribute to all tissues *in vivo*. Whereas naïve iPSCs were recently reported to have been derived from rhesus monkey fibroblasts and were used to create chimeric rhesus-mouse preimplantation embryos (Fang et al., 2014), researchers will undoubtedly want go beyond non-human primate cells to test the functional capabilities of naïve pluripotent human stem cells *in vivo* through the gestation of interspecies chimeras. But what are the appropriate ethical considerations for these kinds of human-to-non-human experiments?

Concerns about naïve pluripotent stem cell-generated chimeras

The ethics of human-to-non-human chimera research has received much attention in recent years, driven in large part by the imagined possibilities of extreme interspecies chimerism that stem cell-based approaches might afford over other, less developmentally based types of chimeras, such as human tumor grafting in adult nude mice (Behringer, 2007). Up to now, people's fears about human/non-human chimerism were confined by the fact that researchers had only 'primed' human pluripotent stem cells at their disposal. The advent of naïve pluripotent human stem cells, however, could tilt the possibilities far more toward the realization of extreme human/non-human chimerism. The scientific prospect of the latter is, after all, one of the anticipated research benefits of using naïve pluripotent human stem cells to create better (i.e. more biologically humanized) chimeric animal models of human development and disease, and possibly even to generate transplantable human organs in large animal species, as discussed by Göran Hermerén elsewhere in this issue (Rashid et al., 2014; Hermerén, 2015).

As long as researchers pursue their interest in naïve pluripotency, the prospect of extreme interspecies chimerism looms on the horizon. Some members of the lay public might worry that the radical biological humanization of animals could lead to their moral humanization, thus elevating these beings to a level of protected moral status that is not possessed by other types of laboratory animals (Streiffer, 2005). One chimera study has already raised eyebrows in the media: mice containing human glial cells in their brains were reported to perform memory and learning tests much faster than control mice, raising the specter that such 'humanized' mice might be cognitively enhanced (Han et al., 2013). If moral

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humanization were to accompany biological humanization in research chimeras, would we have reasons to prohibit either the development of new naïve human pluripotency assays in non-human hosts or the generation of extreme chimeras for research?

This question is not readily accommodated by existing institutional regulations and stem cell research guidelines. Whereas all *in vivo* chimera studies are overseen by institutional animal research committees, these committees are tasked with focusing on animal welfare and study design issues, not with questions about the moral status of the generated chimeras (National Research Council, 1996). Furthermore, stem cell-specific professional guidelines issued by the International Society for Stem Cell Research (ISSCR) only address the impermissibility of transferring any products of research involving human pluripotent or totipotent cells into a human or non-human primate uterus (ISSCR, 2006; http://www.isscr.org/docs/default-source/hesc-guidelines/isscrhescguidelines2006.pdf). Aside from gestation efforts in human and non-human primate surrogates, all other chimera research is potentially allowable, subject to review by the appropriate animal research and stem cell committees.

Recommending a path forward

The ISSCR Ethics and Public Policy Committee has issued an advisory report that provides a framework for overseeing chimera research (Hyun et al., 2007). The Committee's approach attempts to build on current animal research welfare principles, but incorporates stem cell-specific expertise to consider the developmental effects of human stem cell-based chimerism on animal welfare. This stepwise approach is consistent with Hermerén's recommendation that regulating bodies ought to avoid constructing entirely new ethical frameworks for every new area of science (Hermerén, 2015). Critics might complain, however, that the Committee's report does nothing to address the worry that naïve human pluripotent stem cells could be used to create extreme human/non-human chimerism, particularly in the central nervous systems, of animal hosts that are evolutionarily closer to humans. What if researchers propose to create extreme neurological chimeras that subsequently could end up with a moral status that approximates or is equal to ours? Merely extending animal welfare principles to these types of neurological chimeras seems insufficient to address people's ethical concerns. In response to this worry, two key issues must be addressed and clarified.

First, we should seriously question the underlying assumption that the biological humanization of animals could lead to their moral humanization. The important issue here is not whether chimerism could produce the appearance of human-like characteristics in animal hosts *tout court*, but rather whether these human-like characteristics serve as the basis for our moral status as human beings. Perhaps a leading candidate for such a status-conferring characteristic is 'self-consciousness', defined as one's ability to perceive oneself as a temporally extended being who is the subject of one's own experiences. Philosophers typically believe that this type of awareness requires subjects to be language users, as self-consciousness must involve recursive thinking (i.e. thinking about thinking) or at least having a higher-order mental awareness of one's own mental states (Allen and Bekoff, 1997). No animals to date, however, not even non-human primates, have been proven to be language users that can express their beliefs propositionally (Chomsky, 1980; Pinker, 1994). Added to these formidable limitations is the fact that self-consciousness is a complex mental faculty that takes years to emerge in human infants under the nurturing conditions of our everyday socialization processes. Chimeric research animals will not be raised within the "bosom of society" (to use Rousseau's memorable phrase) and thus, regardless of their level or nature of chimerism, will not have the supporting conditions necessary to facilitate the emergence of self-consciousness, as I have argued extensively elsewhere (Hyun, 2013). Others might object that the response above misses an important point. That is, as long as acute neurological chimerism were to confer enough of the structural features of a humanized brain to biologically support self-consciousness in host animals, then it would be wrong to deny these animals this potential by failing to rear them in conditions that would support the eventual emergence of self-consciousness. This hypothetical complaint brings me to the second point that requires clarification.

In chimera ethics debates, it is very common to try to identify one or more human-like traits that are believed to be necessary and sufficient to confer a threshold level of protected moral status. We can call this the 'mathematical classical set' approach, whereby membership in a set is defined by the possession of one or more characteristic properties that are common to all members of the set and are not shared by any entities that are not members. The problem with taking a classical set approach to moral status is that it is bound to leave out individuals whom we would otherwise want to include as having protected moral status – be they newborns, the cognitively disabled or the senile elderly who might lack self-consciousness (to use the sample trait discussed above). A better approach, in my opinion, is to view moral membership as a 'fuzzy set', in which moral status is not absolutely dependent on having a single characteristic or set of characteristics, but rather is a category with membership that is dependent on any of a large group of attributes. This is the way people tend to conceptualize social categories such as parenthood, and biological entities such as immunoglobulins (Greenspan, 2001), and I propose, without having the space to defend the point fully here, that questions of moral status should be approached in a similar manner. In summary, people's concerns about the possible emergent higher moral status of chimeric animals presuppose a simple reductionist view of what it takes to be a member of our common moral community. These concerns disregard the richer array of attributes we actually draw upon when recognizing the moral status of those around us.

With these two clarifying points in mind, I recommend that the best path forward with regards to naïve pluripotent stem cell-based chimera research is to keep the regulatory and ethical focus firmly on animal welfare considerations, with the degree of animal welfare at stake being dependent on the physical and mental attributes of the animal; we should be far less concerned about the emergence of moral humanity in chimeric animals.

Competing interests

The author declares no competing financial interests.

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