APPLICATION FOR NEW CELL LINES AWARDS: RFA 07-05

RL1-00642-1: Derivation of New ICM-stage hESCs

Recommendation: Recommended for Funding
Scientific Score: 80
First Year Funds Requested: $573,156   Total Funds Requested: $1,719,468

Public Abstract (provided by applicant)

Recent studies in the derivation of rodent pluripotent epiblast stem cells and their molecular characterizations have provided strong evidence that the conventional human embryonic stem cells may represent a distinct, later developmental stage, i.e. late epiblast stage, than the conventional murine embryonic stem cells, which is a "capture" of the ICM stage. Those two stages (i.e. ICM/pre-implantation stage vs. epiblast/post-implantation stage) of pluripotent stem cells are typically maintained in their self-renewal state by different sets of exogenous signaling molecules. Meanwhile, other studies have suggested that rather than exogenously activating multiple additional pathways to achieve a fine balanced self-renewal state, a more fundamental approach to main self-renewal of stem cells is to inhibit endogenously expressed differentiation-inducing protein activity. In addition, cell-permeable small molecules have the unique advantage of acting intracellularly to inhibit differentiation without requirement of expression of the desirable membrane receptors by cells for transducing differentiation-inhibiting signals by the desirable exogenous growth factors in the culture media. Those studies together suggested the possibility that an earlier stage (i.e. ICM-stage) of human pluripotent stem cells than the conventional human embryonic stem cells, which would represent an equivalent counterpart of the conventional murine embryonic stem cells, could be derived with helps of small molecules that could block further differentiation and capture the state of human ICM-stage of pluripotent stem cells. Here we propose to screen chemical libraries for small molecules that can facilitate derivation of the above hypothesized, new, earlier developmental state of human pluripotent stem cells from donated human IVF blastocysts. Such new human pluripotent stem cells may have better properties than the conventional human embryonic stem cells (e.g. ease of culture and manipulation), facilitate ready transfer of knowledge/techniques learn from murine embryonic stem cells to human pluripotent stem cells, and perhaps provide a new cell type for studying fundamental biology.

Statement of Benefit to California (provided by applicant)

The putative human pluripotent stem cells proposed here may have better properties than the conventional human embryonic stem cells (e.g. ease of culture and manipulation), facilitate ready transfer of knowledge/techniques learn from murine embryonic stem cells to human pluripotent stem cells, and perhaps provide a new cell type for studying fundamental biology. In addition, small molecules have been more useful than genetic approaches in the treatment of human disease. The demonstration that one can systematically identify, optimize and characterize the mechanism of action of small drug-like molecules that selectively control cell fate and reprogramming will: (1) provide important tools to manipulate cell fate in the lab; (2) provide new insights into the complex biology that regulates (stem) cell fate; and (3) provide an important first step which may ultimately lead to drugs that facilitate the clinical application of stem cells.

Review

It is well known that mouse and human embryonic stem cells (ESC) differ in a variety of properties, including the general growth factor conditions used for their self-renewal in vitro. This application is based

on the central hypothesis that conventional human ESCs (hESC) represent a later epiblast stage of development than mouse ESCs (mESC) which represent the earlier inner cell mass (ICM) stage. The principal investigator (PI) proposes to derive new hESC lines using small molecule conditions that should promote a pluripotent state more similar to that of mESC. Three aims are proposed. Aim 1 is to derive new hESC lines from human blastocysts in the presence of inhibitors of differentiation pathways that are known to maintain self-renewal in mESC or in the presence of compounds newly discovered in Aim 2. In Aim 2, the PI intends to develop a large high throughput screen to identify small molecules that transition existing hESC lines to a phenotype more reminiscent of mESC. Aim 3 is to determine if the newly derived cell lines are pluripotent using a variety of cellular, biochemical and molecular methods.

Reviewers agreed that this proposal is based on an interesting concept. Although they were uncertain whether the newly derived cell lines would prove superior to existing hESC lines, reviewers did appreciate that derivation of hESC with properties similar to mESC may facilitate translation of mESC data to hESC research. While the technologies and methodologies are not novel and not particularly innovative, the integration of chemistry, high throughput screening and biology is laudable and has significant potential. If successful this project would provide valuable reagents to the field.

Reviewers judged aims 1 and 3 to be feasible and straightforward, with the potential to deliver new high quality hESC lines. The proposed characterization of the newly derived hESC is adequately comprehensive. However, one reviewer pointed out that the comparison of the transcriptome of the newly isolated hESCs with conventional hESCs will be complex while the PI provides little guidance as to how this complexity will be handled. Similarly, the PI did not provide criteria for the number of human blastocysts to be used.

Reviewers expressed mixed opinions about Aim 2. One reviewer felt that Aim 2 should be prioritized over Aim 1, since molecules identified and optimized for their activity on existing hESC lines (Aim 2) should be employed when using human blastocysts to derive new hESC (Aim 1). By contrast, another reviewer argued that Aim 2 is not of high priority, since a screen for additional compounds should be postponed until it has been determined, in Aim 1, if the molecules already known to support self-renewal in mESC are effective in deriving ICM-stage-like hESC from human blastocysts. Furthermore, although the proposed read-outs were judged to be suitable for large scale screening, a reviewer questioned whether the proposed screen can be developed into a robust high throughput assay, since it is currently unknown if simply changing culture conditions on existing hESCs can turn them into mESC equivalents. The PI presents preliminary data that this is the case, at least at the morphological level, but provides no details on the conditions or molecules involved, making this observation difficult to evaluate. Finally, a reviewer pointed out that the number of compounds to be screened is reasonable, but that the description of the compound libraries was not detailed enough to be able to evaluate whether they are adequate and appropriate. Overall, reviewers judged Aim 2 to be high risk, but nevertheless thought it had a good chance of success, based on convincing preliminary data and the qualifications of the research team.

The PI is in an ideal position to conduct the proposed studies. He/she has access to high throughput screening tools and has demonstrated experience with the discovery of biologically active small molecules that have been validated by independent laboratories. The collaborators provide appropriate expertise in stem cell biology. Although the PI is a well-trained chemical biologist, one reviewer recommended to involve a medicinal chemist once the project moves closer to clinical application.

In conclusion, reviewers thought that Aims 1 and 3 are likely to generate high quality new hESC lines but suffer from a lack of innovation, which is balanced by the high risk screen proposed in Aim 2. Reviewers felt confident that the PI is in the right environment and has the right tools and experience to deliver small molecule approaches to hESC research.