NUS Graduate School for Integrative Sciences and Engineering
Research Project Write-up

Title of Project : Derivation of progenitor and somatic lineages from human embryonic stem cells

Name of Supervisor : CAO Tong

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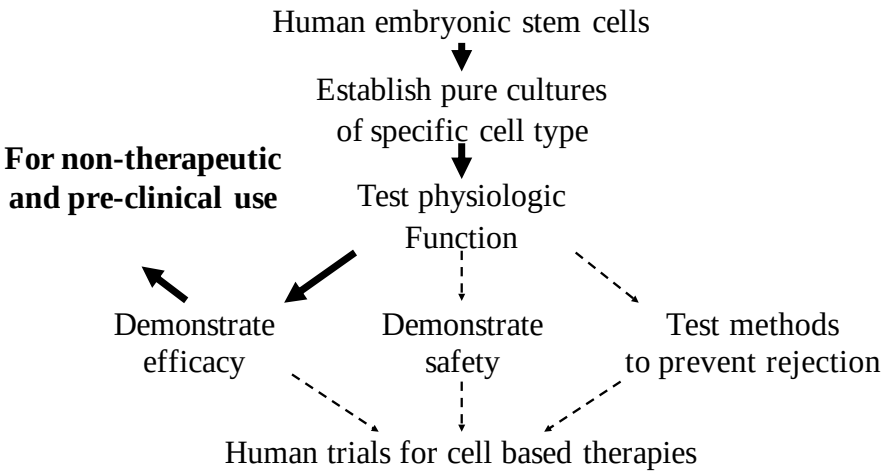
Short Description

Human embryonic stem cells and progenies are ideal and promising cell source for wide biomedical and biotech applications [1]:

- human relevant function and biosafety/toxicity assessment of therapies, drugs, cosmetics, food, chemicals, materials and techniques;
- Human relevant environment analysis of water, soil, air, and natural/artificial products and techniques;
- Gene/protein delivery to cure diseases;
- Cell based reconstruction therapy including regenerative medicine;
- Development and gene control of human tissue/body;
- Disease study.

Current work on human embryonic stem cells is at the first target: for non-therapeutic and pre-clinical applications:



Lineages of differentiation:

- Ectodermal — Keratogenic, Neurogenic and Amelogenic lineages
  Mesodermal — Mesenchymal, Fibrogenic, Osteogenic, Chondrogenic, Endothelial, Adipogenic, Myogenic, Odontogenic lineages
- Endodermal — Hepatocyte lineage (planed)

NUS Graduate School for Integrative Sciences and Engineering
Research Project Write-up

Title of Project :	Creation of vascularized and innervated tissue and organ from human ESC for wide applications
Name of Supervisor :	CAO Tong
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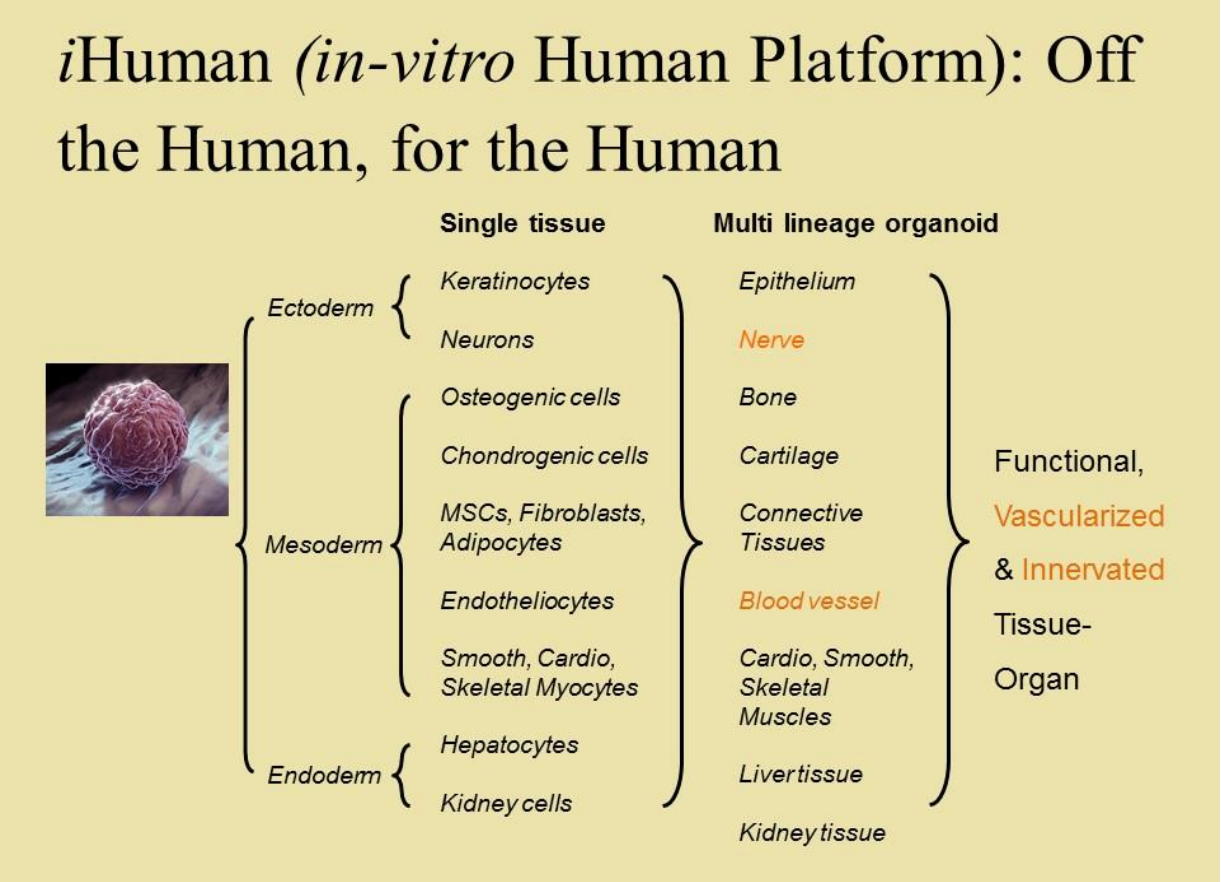
Short Description

Government authorities, academies, research institutes and the industries of health, drug, food, cosmetics, chemicals and environment are presently hindered by a lack of functional, healthy and standardized human platforms of cells, tissues and organs, and predominantly use costly live animal models in addition to the cells of low human relevance. Existing models of live animals or on immortalized cell lines of either animal or human origin, often poorly reflect human physiology. Primary human cell cultures are difficult to procure in sufficient quantity and can be prone to much inter-batch variability, depending on the cell source. By contrast, self-renewable, genetically-healthy and single-sourced human embryonic stem cells (ESC) exhibit enhanced biological relevance and stable predictivity over its more expansive counterparts. As genuine pluripotent stem cells, human ESC serves as an unlimited source potential to develop into all cell types of human body. Hence, global pioneers and governments like EU and UK endeavour to develop a technically-simple, cost-effective and replicable system of human ESC derived live platforms in last decade. This fast development is revolutionizing health sciences from animal-based platforms to much more accurate human-based platforms. The revolution will bring a new burgeoning industry of ESC human platforms of live cells, tissues, organs and systems in next decades.

Leading in the world, the US Congress, federal and local governments, investors and charities have been supporting 'promising' human ESC R&D through legislations, policies, guidelines and funds. US initiated the clinical trials of human ESC therapies for eye diseases and spinal cord injury since 2011. Besides various human ESC progenies, functional tissues with multiple cell lineages, unique vascularization and innervation by autologous human ESC progenies are currently being explored. The human ESC progenies, functional tissues and organs will offer ideal *in vitro* 'clinical' platforms of no-risk trials/tests for the basic, translational studies and applications of all human health related sciences including fundamental study of health, ageing, disease, prevention, diagnosis, therapy and transplant; drug and med-tech R&D. Moreover, those functional and standardized ***in vitro* human live platforms (iHuman)** of no-risk trials/tests will be widely adopted in much more areas beyond medicine and pharmaceutics. The major other applications will be the human function and safety evaluation of food; cosmetic; daily and general chemicals; organisms; nuclear, IT, communication, electromagnetic, radiating device and technique; environment (air, water, soil, daily living and working environment); other human-contact substance, products and techniques. The platforms of human ESC progenies, functional tissues and organs will be

ethically and gradually used at reasonable and practical pace, non-clinically, pre-clinically and clinically in all human health related industries, academies and authorities.



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Europe and United States have recently initiated the similar strategy. EU Embryonic Stem cell-based Novel Alternative Testing Strategies (ESNATS, EU) has since 2008 been developing a novel toxicity test platform based on human ESC to accelerate drug development, reduce related R&D costs and propose a powerful alternative to animal tests. In 2007**,** The UK Government decided to establish a public-private partnership to develop predictive toxicology tools for stem cell lines. Department of Health UK has started the program of Stem Cells for Safer Medicines (SC4SM) to enable the creation of a bank of stem cells, open protocols and standardised systems in stem cell technology that will enable consistent differentiation of stem cells into stable homogenous populations of particular cell types, with physiologically relevant phenotypes suitable for toxicology testing in high throughput platforms. In 2009, GE Health and Geron in US have jointly initiated the strategy to develop and commercialize cellular assay products derived from hESCs for use in drug discovery, development and toxicity screening.

Challenges

'Worldwide it is estimated that the number of vertebrate animals—from zebrafish to non-human primates—ranges from the tens of millions to more than 100 million used annually [3]. Invertebrates, mice, rats, birds, fish, frogs, and animals not yet weaned are not included in the

figures; one estimate of mice and rats used in the United States alone in 2001 was 80 million [4]. US government funded animal testing alone costs US taxpayers over US$31 billion in 2010 and increases yearly [5].

Despite the supposed stringency of animal tests on drugs deemed safe for human consumption and released onto the market, two million Americans become seriously ill and approximately 100,000 people die every year because of reactions to medicines they were prescribed [6]. This figure exceeds the number of deaths from all illegal drugs combined, at an annual cost to the public of more than US$136 billion in health care expenses [7]. In England, an estimated 70,000 deaths and cases of severe disability occur each year because of adverse reactions to prescription drugs, making this the third most common cause of death (after heart attack and stroke) [8]. The drug company Ciba-Geigy has estimated that only five per cent of chemicals found safe and effective in animal tests actually reach the market as prescription drugs after the R&D investment of at least 10 years and over $ 1billion on each single drug candidate [9]. Even so, during 1976 to 1985 the US Food and Drug Administration (FDA) approved 209 new compounds – 102 of which were either withdrawn or relabeled because of severe unpredicted side-effects including heart attacks, kidney failure, liver failure and stroke [10]. The fact that months or years of human studies are also required suggests health authorities do not trust the results. In 2004, the FDA reported that 92 out of every 100 drugs that successfully had passed animal trials subsequently failed human trials [11]. ’

Reference

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The Commitment of US, EU and UK to Human Embryonic Stem Cell Research

- EU Commission confirmed EU support to the funding from the Horizon 2020 EU Research and Innovation program is to be used for human embryonic stem cell research, on 28 May 2014 [1].

- NIH Director Dr Francis Collins made the Congressional Testimony for US government on 16 Sep 2010: *'The **Promise** of Human Embryonic Stem Cell Research'* [2].

- The NIH Statement by Dr. Francis Collins on the hESC Ruling: *'Today's ruling from the U.S. Court of Appeals is important news for patients. President Obama is committed to supporting responsible stem cell research and today's ruling was another step in the right direction. NIH will continue to move forward, conducting and funding research in this **very promising** area of science. The ruling affirms our commitment to the patients afflicted by diseases that may one day be treatable using the results of this research.'* [3]. The U.S. Court of Appeals for the District of Columbia upheld an earlier dismissal of Sherley v Sebelius, a court case that had challenged federal funding of hESC research on 24 Aug 2012 [4].

- The US government and Congress have been pushing hESC R&D through **policies**, **guidelines** and **legislations** since 2009 [5-13].

- There have been 243 lines in the **new** NIH **hESC Registry** eligible for use in US Federal funded research since Dec 2009 [14].

- US initiated the **clinical trials** of hESC therapies for eye diseases and spinal cord injuries since 2011 [15].

- European governments of **EU** and **UK** have been developing a technically-simple, cost-effective and standardized platforms of hESC derived progenies since 2008 [16-18].

- The International Society for Stem Cell Research officially stated *'Human Embryonic Stem Cells are the Prototype "**Gold Standard**" Pluripotent Cell'* in ISSCR statement on 11 Oct 2012 [19]. The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Dr Shinya Yamanaka "for the discovery that mature cells can be reprogrammed to become pluripotent" on 8 Oct 2012. Dr Shinya Yamanaka was ISSCR President during Jun 2012-Jun 2013.

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