Human Cell Atlas - Asia Meeting
Nov 30 - Dec 1, 2017
Okinawa, Japan

Mission

Human Cell Atlas (HCA) is an international consortium aiming to create comprehensive reference maps of all human cells - the fundamental units of life - as a basis for both understanding human health and diagnosing, monitoring, and treating disease. To embody the global nature of this project, the HCA aims for a greater representation of human diversity - defined by gender, genetics, geography, environment and age. This first HCA Asia meeting in Okinawa aimed to bring together leading scientists in the field of single cell genomics to discuss and define human diversity, and establish a coordinated framework to represent Asia in the global HCA.

Executive summary

A group of 51 scientists from Japan, South Korea, Singapore, and India along with national and international funders convened to share, discuss, and devise strategies to establish a regional framework of HCA. The scientists were represented from 23 universities and research institutes. The two day meeting was divided into six thematic sessions ranging from imaging, technology platforms, stem cells, cancer and coordination. Each session followed ample time for discussions (avg. over 50 minutes) around sample sourcing, diseases, ethics, genomic platforms, data sharing, and pilot projects. The participating members recognize the importance of synergizing our efforts to the global HCA while maintaining visible representation and leadership in the region. The members also sought **common interest in building a reference dataset from the Asian population** with the **emphasis on targeting prevalent diseases in the region** such as liver, colon, gastric cancer and proposed the **creation of an interim organizing committee** to devise concrete pilot projects to jumpstart collaboration and to explore local ethics regulation and funding opportunities.

The document herein highlights key discussion points and follow up items.

Session summary

Session 1 : Commencement
Chaired by Jong Hoon Park

Chan Zuckerberg Initiative Update (Kevin Moses)

- 1st round of RFA funded 38 projects from 9 different countries (however, none from Asia) https://www.humancellatlas.org/news/12
- 2nd round of RFA has 200-300 application now related to computation that are under review.
- Possible 3rd round of RFA next year (2018).

CZI is largely funding structure and operation.
CZI does not want to inhibit any existing projects funded by governments, institutes (e.g. Human Brain Initiative, TCGA), but want to be a good interface and fill the gaps (e.g. international projects, pilot projects).

CZI will also fund computation (e.g. data coordinated platform for HCA) and administrative operation (e.g. executive offices in HCA including in Japan)

There are other fundings that are not related to HCA.

Session 2 : Imaging technologies
chaired by Shyam Prabhakar

What is the primary focus of HCA?
"Skydive" approach to obtain a broad understanding of cellular diversity.
And then, stratify cells for deeper exploration into molecular makeup and gene regulation.

A. Technology platform
Standarizing technology platform is essential.
Okada Yasushi's group is establishing an imaging platform together with a company to create consistency in data and standardization.

Ideally use commonly agreed platform and protocol but this causes 'monopoly' effect and inhibits competition. How to incorporate new technology and stay flexible?

Similar to ENCODE, we can suggest *data* and *technology* freezes (Shyam)

How can we *combine* genomics and imaging? The current single cell genomics technology can capture only 20-30% of RNA molecules. But we can combine it with imaging techniques (e.g. FISH) to extract biomarkers and label tissues to acquire spatial information (Jay)

Nuclear is more stable than RNA. ATAC seq and other similar techniques (scMethyl-seq) can define cell types (Okada)

B. Sampling
Should we focus on Asia specific disease and normal samples? (Aki)

"Normal" is difficult to define (Partha)
Should consider the molecular depth and variation in the population genetics, which is even greater in disease (Yeon-su).

Let's consider exchanging samples to each other; keep in mind ethics (Piero)

Each country have to decide which sample should be taking care of - but is there a common problem we can address together?

Korean scientists are interested in cancer related area, HBV. Liver, colon, gastric cancer samples. Have access to these samples. It's a prevalent disease in Asian countries.

How to differentiate genetics and environment such as diet?

Need to have a scientific coordinator in each country and in the region (Piero)
Creating "general catalog" of healthy cells is also a mission.

Session 3 : Single cell genomics technologies
chaired by Partha Majumder

"Many isn't always better" (Yutaka)
At current rate, more cells lead to less detectable genes. This hampers our understanding of molecular composition and its regulation. It may be sufficient to define cell types but less efficient to build a gene network.

A. Computation
How do you deal with length normalization when working with 3' end based sequencing? (Sanghyuk).

Normalization remains an issue. Unique Molecular Identifier (UMI) is popularized to normalized read counts; the real challenge is biological variation even within the same platform (Tarjei)

Generating a lot of data will help solve normalization issues - *ad hoc* - approach to resolve variations in data (Seita)

Sample collection leads to more variation than technical variation. Should agree on the platform while seeking 'transitionalable' platforms - fine balancing act. Computational is another strategy to overcome this (Jay)

B. Scope of HCA
How 'biological' is looking at 3' end? Is looking at expression sufficient? How about transcript variation? (Sanghyuk)

Nx1-seq can pull down poly-dT transcripts and sequence using Oxford Nanopore Technology (ONT) to get the full length.

There are two domains in this discussion (Jay):
1) What is a cell type?
2) What is the molecular composition of a cell and how are they regulated?

The first draft of HCA aims to define cell type. Defining gene regulation is most interesting but will require improved technology.

Session 4 : Cancer biology
chaired by Woong Yang Park

Intrinsic noise from scRNA-seq may hamper interpretation of cell type/transition but DNA methylation is more straightforward (Fujibuchi)

C1 CAGE based method can detect as many genes as C1 scRNA-seq and enhancer RNAs (Erik)

A. Sample and Data Coordination
Which cancer should we focus? What is the common interest?
Can we exchange samples across countries? Can we share patient data?

Currently, each country hosts their own data centers.
Centralized database will be more valuable (stronger reference) and benefits small labs.
DCP is free, storable, scalable, engineered by CZI.

Deposition into both local and global data centers should be considered. Helps to address different biological questions (with different reference datasets).

What are the policies behind data sharing in each country?

1. Japan: the IRB regulation in Japan is changing. Treatment of RNA and DNA is different. FANTOM has received samples from outside and shared data under ethics.
2. Korea: As long as seq data have patients' consent it can be public. Population study data is easier than individual data.
3. Singapore: Data can be shared as long as one agrees not to use it for commercial use (only for academic purposes). The committee is still maturing.
4. India: samples cannot be shared outside, but data can be shared as long as it is anonymized.

Sharing mapped reads can also circumvent ethics around raw genomic data (Piero).

How to coordinate and combine clinical data from different countries?
Global HCA should provide guidelines while accommodating different rules in each country.

Homework: find out local regulation around ethics for data and sample sharing

Plan: Local projects should start initially with own funding and build regional network with support of government (and CZI?).

Session 5 : Stem cells/aging
chaired by Akira Watanabe

What is the role of HCA in iPS cells and stem cell research?
The Human Cell Atlas reference dataset will be critical for regenerative medicine. The reference dataset can be widely used to evaluate transplantation in patients.

A. Developmental biology
Do we really know the history of cells? (Kevin)
We need to understand how they change cell state to better understand disease (Fujiwara)
HCA is also focusing on fetal tissues and also model organisms. (Jay)

Human embryos: It is possible in US (e.g. California), some countries in Europe (e.g. UK, Sweden). The samples can be acquired from UK.

Organoids will also be a good model to study the history of cells (Wataru)

B. Environmental
Aging and diet. Mouse has a lot of data but data is lacking in human subjects (Aki)
How can we define (tissue resident) immune cells? (hair)
Infectiuous disease in Asia (including developing nations) - Pathogenic atlas.

C. RNA biology
Allelic expression variation is observed at single cell resolution.
Conventional RNA-seq may not be able to detect them.

- Nanopore will help - including repeat elements such as LINE (Piero) but has high error rate (Yutaka)
- PacBio can amplify but amplification may lead to shortening of reads. May be a good platform to catalog full length RNA but not for expression analysis (Yutaka)

D. Unique technologies/samples
- Japan: RIKEN has been leading in the ncRNA field and FANTOM projects. Technologies such as Nx-seq (nano-wells), RamDA-seq (single cell full-length total RNA seq), C1 CAGE (5'-end) are accessible. CiRA in Kyoto is leading in the iPS cell technology.
- Singapore: A*Star leads in the MER-FISH (spatial) technology along with wider access to clinical samples. Multiethic country access to wide range of genetic background.
- Korea: Samples from brain-dead patients can be obtained; many cancer samples have already been profiled using single cell technologies. Multiomics technologies (e.g. SIDR) for simultaneous DNA and RNA seq is also accessible.

Session 6 part I: Establishing HCA Asia Network
chaired by Roger Foo, Yutaka Suzuki

In the global HCA, major organs are listed and anyone can join the working groups. Rather than having one group focusing on one organ, multiple (international) labs working on the same organ is ideal - and generates a more comprehensive reference.

HCA aims to catalog normal tissues as a primary purpose. But need to include disease to attract funders (in almost all countries).

How can we ‘lead’ from this region?
What is necessary? What is unique?

A. Tissues
Skin may be good for pilot project and also attracts cosmetic companies (big in Asia).
Muscle and gut are country specific (hard vs soft tissues)
Should explore ethics and rules behind samples from brain-dead patients
Cancer is good source but disease is too heterogeneous. Maybe difficult to make a reference (Yutaka) but still useful to have one. DNA methylation should also be considered to minimize cell state variation.

B. HCA Asia
Need to make a HCA reference for Asia - for disease and regenerative medicine
Need to be synergistic with the global HCA to complete the reference [see Appendix II for how to connect to the global HCA].

To complete the “Human” aspect of HCA, we need Asia and other regions like Africa and South America (Jay).

[added] 56% of world’s population is from this region.
We need to incorporate other nations in the region for the collective effort (e.g. sequencing, sampling) including China, HK, Taiwan, Vietnam, Thailand, Malaysia, Indonesia, etc.

C. Platform
Using Spike in controls to benchmark platforms and lab-effects.
IPSC is not ideal; sensitive to culturing condition.
Broad Institute is working on this effort (Kevin)

D. Coordination
1. Tissue sampling (Liver, Gastric, Colon centric) - tumor and normal tissues, also from brain-dead individuals
2. Single cell RNA-seq (e.g. 10x) combined with spatial techniques like MER-FISH
3. Exchanging samples to bench mark and data sharing
4. Inquire ethics

Session 6 part II: Establishing HCA Asia Network
chaired by Seon-Young Kim, Jay Shin

A. Funding situation
1. India: No funding plan for HCA at the moment. The government expects phenotype. Making a case for healthy reference is challenging but regional support will be helpful.
2. Japan: MEXT (ministry in Japan) is aware of HCA activities. New phase of single cell program in RIKEN from April 2018 will support this effort but need greater promotion to acquire national funding.

3. Korea: National Research Foundation (NRF) on behalf of ministry is already funding projects and reasonably confident that funding will increase - to several million dollars per year from 2019-2020. Making healthy reference and ethical regulation need to be carefully reviewed. We need compelling arguments to persuade NRF and ministry to support HCA Asia.
4. Singapore: Could not invite funders due to short notice. Single cell research is A*Star's priority focusing on disease and applied research (e.g. diagnostics). But there is interest in healthy atlas but may need to tackle simultaneously with disease such as cancer, cardiovascular, immunity and technology development in the view to turn it into diagnostics.
5. CZI: Confident to commit to HCA - especially for data coordinate platform (DCP). Aiming to support by central facilities (e.g. storage, help desk) with partnership with other funders. Provide funding to set up centralized ethic and also support executive offices. Support international efforts through multiple rounds of RFAs.

B. Organization

Plan: Establish an interim (preparatory) organization committee consisting of key leaders (1-2) from each country. The committee members are the first point of contact related to global HCA and regional HCA. They are also responsible for communicating and promoting HCA activities to local research community and funders.

C. Summary

1. Biology:
    a. immunity (in aging, tumor, geographic/environment diversity, tissue-specific)
    b. stem cells (ES/iPSC, tissue stem cells, organoids, cancer stem cells)
    c. cancer (liver, colon, gastric) - most prominent in Asian countries
    - Strong relevance and funding opportunity around liver, colon, gastric cancer in Asia; possible to extract both healthy and disease samples

2. Technology:
    a. 10x genomics, C1 CAGE, Nx1-seq (nano-wells), RamDA-seq
    b. MER-FISH, super-resolution microscopy
    c. Epigenome: scMethyl-seq, ATAC-seq
    d. Multiomics
    e. Laser Capture Microdissection (LCM)
    - 10x is commonly supported but should stay flexible (e.g. Nadia, iCell8). Make efforts to benchmark across platforms and utilize cell spike in controls when possible

3. Computational
    a. Local data centers
    b. HCA/CZI Data Coordinated Platform
        i. Follow the common standards for sample and data preparation.
        ii. Promote Open Data Policy in own country
    c. ShoGin Database from Kyoto University

- Start with local data centers but integrate to the global DCP for a stronger reference

Conclusion and action items

The members recognize strong outreach efforts to local funders is needed. In general, funders are seeking disease phenotypes (and/or economical returns such as diagnostics). At the same time, we as a HCA community emphasize the importance and relevance of building a human reference - especially from the Asian population - to enrich our understanding of diseases, its intervention and regenerative medicine.

The members recognize there are universal challenges such as sampling techniques, data normalization, platform comparisons. It is sensible to engage with the global HCA community to solve these issues together, while addressing regional challenges.

It became apparent that each country has their own terms and conditions around ethics (e.g. data and sample sharing). It will be imperative for each country's representatives to engage with the local ethics committee and promote open sample/data policies to "accelerate science".

In order to execute concrete plans going forward, the members discussed and agreed to establish an interim (preparatory) organizing committee to define targets, draft documents for funders, and initiate pilot studies.

For any inquiries
HCA-secretariat@ml.riken.jp

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Local reprentatives
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Appendix I: Program

Day 1: Thursday, November 30, 2017

13:00-14:00 Registration

14:00-14:45 Session I: Commencement

Session chair: Jong Hoon Park

Welcome	Piero Carninci
Human Cell Atlas	Jay Shin
Chan Zuckerberg Initiative	Kevin Moses

14:45-16:00 Session II: Imaging technologies

Session chair: Shyam Prabhakar

Imaging Technologies for single cell analysis
Yasushi Okada /RIKEN
Super-resolution microscopy
Sang-Hee Shim/Korea University
MER-FISH
Kok Hao Chen/Genome Institute of Singapore A*Star
Single cell seq based on histological locations
Kyeung Min Joo/SungKyunKwan University School of Medicine

16:00-16:30 Coffee break

16:30-17:45 Session III: Single cell genomics technologies

Session chair: Partha Majumder

Single cell analytical platform
Yutaka Suzuki/University of Tokyo
Simultaneous gDNA and total RNA
Woong-Yang Park/Samsung Medical Center
Tumor microenvironment by Nx1-seq
Shinichi Hashimoto/Kanazawa University
10x Genomics (sponsor talk)
Tarjei Mikkelsen/10x

Discussion item: Common Coordinated platform

17:45-18:15 Transport to Rizzan Sea Park Hotel

18:30-20:00 Dinner reception @ RIZZAN

Day 2: Friday, December 1, 2017

9:00 Session start

9:00-10:30 Session IV: Cancer biology
Session chair: Woong Yang Park

Detecting somatic mutations in single cells
Young Seok Ju/KAIST
Drug resistance and Intra-tumor heterogeneity
Ramanuj DasGupta/Genome Institute of Singapore, A*Star
Drug-induced cellular response
Erik Arner/RIKEN

Discussion items: Asia Tumor Cell Atlas

10:30-11:00 Coffee break

11:00-12:30 Session V: Stem cells/ageing
Session chair: Akira Watanabe

Hair follicle stem cells
Hironobu Fujiwara/RIKEN
Ageing
Aki Minoda/RIKEN
SHOGoiN database
Wataru Fujibuchi/Kyoto University

Discussion items: Stem Cell Atlas, Immune Cell Atlas
(Photo time after the session)

12:40-14:00 Lunch @ OIST cafeteria

14:00-15:30 Session VI: Establishing HCA Asia Network - Part I
Session chairs: Roger Foo, Yutaka Suzuki
Topic 1: Tissues, disease, sampling
Topic 2: Profiling platforms

15:30-16:00 Coffee break

16:00-17:15 Session VI: Establishing HCA Asia Network - Part II
Session chairs: Seon-Young Kim, Jay Shin
Topic 3: Funding
Topic 4: Coordination

17:15-17:30 Closing remarks Young Joon Kim

Appendix II HCA materials

To join the global HCA
https://www.humancellatlas.org/joinHCA

HCA SLACK channels
https://humancellatlas.slack.com

White paper
https://www.humancellatlas.org/files/HCA_WhitePaper_18Oct2017.pdf

Publications
http://www.nature.com/news/the-human-cell-atlas-from-vision-to-reality-1.22854
https://elifesciences.org/articles/27041

News articles (press releases)
https://www.humancellatlas.org/news