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Animal Symposia and Workshops

A-1

Establishment and Characterization of Disease-specific Human iPSCs in Serum-, Integration- and Feeder-free Cultures. ATSUKO HAMADA[1], Y. Nakase[1], F. Obayashi[1], T. Fukutani[1], H. Nakatao[1], E. Sakaue[1], S. Yamasaki[1], T. Kanda[1], K. Koizumi[2], Y. Yoshioka[2], R. Tani[1], S. Toratani[2], J. D. Sato, and T. Okamoto[1,2]. [1]Clinical Dept. of Oral and Maxillofacial Surgery, Hiroshima University Hospital, 1–2-3, Kasumi, Minami-ku, Hiroshima City, 734–8553, JAPAN and [2]Dept. of Molecular Oral Medicine and Maxillofacial Surgery, Graduate School of Biomedical & Health Sciences, Hiroshima University, Hiroshima, 1-2-3, Kasumi, Minami-ku, Hiroshima City, 734–8553, JAPAN. Email: hamaco@hiroshima-u.ac.jp

Human pluripotent stem cells hold great promise for their practical and scientific potential. To improve understanding self-renewal and differentiation, we have reported the successful generation and culture of hiPSCs from dental pulp cells (DPCs) and peripheral blood mononuclear cells (PBMCs) with a non-integrating replication-defective and persistent Sendai virus vector expressing four key reprogramming genes (SeVdp (KOSM)) using hESF9 serum-free medium in feeder-free culture conditions. We found that using this system pluripotent and self-renewing hiPSCs could be easily and stably generated and propagated. We successfully generated hiPSCs from the hereditary disease patients with abnormalities in oral-maxillofacial region, such as Cleidocranial dysplasia (CCD: *RUNX2* mutation), Cowden syndrome (*PTEN*), Noonan syndrome (*K-Ras*), von Recklinghausen's disease (*NF1*), and Nevoid basal cell carcinoma syndrome (*PTCH1*) . We have found that these patient-derived iPSCs retained disease-specific phenotypes during in vitro embryoid body formation assays and in vivo teratoma formation assays. These disease-specific iPSCs models will help to clarify the molecular mechanisms underlying these diseases, and they will be useful for screening therapeutic drug, and for developing medical applications of iPSCs.

A-2

Disease Modeling Using Disease-specific iPS Cell Collection in RIKEN Cell Bank. YOHEI HAYASHI. RIKEN Bioresourse Research Center, iPS Cell Advanced Characterization and Development Team, 3–1-1 Koyadai, Tsukuba, Ibaraki, JAPAN. Email: yohei.hayashi@riken.jp

RIKEN cell bank has released 3075 human disease-specific iPS cell lines from 744 patients of 231 disease types so far (as of January 31, 2019). Since national projects of establishing more disease-specific iPS cells are running now, these disease-specific iPS cell lines will be deposited in this bank. In the coming years, it is critical to utilize these cells for the research projects of intractable diseases and drug development. However, most of these cells have not been yet characterized in their mutations of responsible genes, pluripotency, self-renewal, or karyotype. Thus, I have established the "iPS cell advanced characterization and development team" from this year, and our team has characterized the disease-specific iPS cell lines in response to the need for their research and development. We have also started to generate mutation-corrected iPS cell lines from disease-specific iPS cell lines, mutation-introduced iPS cell lines from normal iPS cell lines from healthy donors, and fluorescent reporter iPS cell lines for cell type-specific marker proteins, by genome editing. Through these research, development, and dissemination, we would like to accelerate the utilization of these cells for the research projects of intractable diseases and drug development. I would like to introduce our recent research activities described above and also my previous research projects using disease-specific iPSCs.

A-3

Application of Neural Crest Stem-like Cells Induced from Human Gingiva-derived MSCs in Peripheral Nerve Regeneration. Q. Z. ZHANG[1], P. D. Nguyen[2], S. H. Shi[1], Q. L. Xu[1], and A. D. Le[1, 3]. [1]Department of Oral and Maxillofacial Surgery and Pharmacology, University of Pennsylvania School of Dental Medicine, Philadelphia, PA

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Even though the advancement of microsurgical techniques has greatly improved the management of peripheral nerve injury (PNI), the clinical outcome of current approaches for repair of PNI is still poor and then fully functional restoration of peripheral nerves following injuries remains a major challenge in the medical field. Currently, autografting of an intact nerve remains the gold standard for repairing the injured peripheral nerve with a large gap. However, several major limitations exist for the use of nerve autografts. Recently, there has been growing enthusiasm for the combined use of stem cell-based therapy and tissue engineering technologies for peripheral nerve regeneration. Schwann cells and neural stem or progenitor cells (NSCs) are considered as the ideal candidate cells for nerve regeneration, but the limited availability has significantly impeded their potential application in clinic. Nongenetic induction of somatic cells into neural stem- or progenitor-like cells has shown promises in cell-based therapies for PNI. Here, we report that human gingiva-derived mesenchymal stem cells (GMSCs), with a neural crest-origin, could be reproducibly and readily induced into neural crest stem-like cells (NCSCs) under defined nongenetic conditions. Compared to parental GMSCs, induced NCSCs showed increased expression of neural crest-related genes and displayed robust differentiation into neuronal and Schwann-like cells. Using both crush-injured sciatic nerve model and facial nerve defect model in rats, we showed that transplantation of GMSC-derived NCSCs or implantation of nerve guidance conduits laden with GMSC-derived NCSCs displayed superior therapeutic effects on functional recovery and regeneration of the injured nerve in comparison to parental GMSCs. These promising findings demonstrate that induced NCSCs derived from GMSCs represent an easily accessible and promising source of neural crest stem-like cells for peripheral nerve regeneration.

A-5

Cutaneous Models for Evaluating Pollution Induced Skin OxInflammation. GIUSEPPE VALACCHI, Alesandra Pecorelli, Francesca Ferrara, Brittany Woodby, and Mary Ann Lila. North Carolina State University, PHHI, Kannapolis Research Campus, 600 Laureate Way, Kannapolis, NC, 28081. Email: gvalacc@ncsu.edu

Ubiquitous exposure to environmental insults is one of the major global concern for health implications. Over the past 30 years, the damaging effect of pollutants on cutaneous tissue has become a growing research topic, also in view of the increase environmental pollution. As the interface between the environment and our body, the skin is the first defense line of the external boundaries and, therefore, along with eyes, lung, brain and digestive tract, one the main target organs for the harmful insults of air pollution. Indeed, evidences confirm a direct correlation between skin pathologies and air pollution exposure. Pollution is in fact a mix of many components that makes it difficult to know exactly how each of them acts on the skin. Several *in vitro* and *in vivo* models have been developed to assist researchers in investigating the molecular and cellular mechanisms underlying the pollution-related damaging effects on skin tissue. However, some of these models do not adequately mimic human cutaneous tissues. For example, the use of animal models has some scientific limitations due to morphological, physiological and biochemical differences respect to human skin. Some models such 2D dimensional and 3D dimensional cell cultures appear to be more useful alternatives to animals. In our studies, using different skin models including mice, 2D cell cultures, 3D reconstructed skin models and human skin explants, we have elucidated the processes implicated in the detrimental effects of pollutants on skin, with particular focus on ozone. Pollutants are able to induce a positive feedback between an altered redox balance and dysregulated immune responses that potentiate each other. The crosstalk between redox and inflammatory signaling pathways create a vicious circle that we define as cutaneous oxinflammation phenomenon.

A-6

Three Dimensional Test Systems for Predicting the Degree of Corneal Injury from Topical Exposure to Chemicals. J. W. HARBELL. JHarbell Consulting LLC, 16334 Sunset Valley Drive, Dallas, TX 75248. Email: johnharbell@sbcglobal.net

In vitro prediction of topical toxicity requires 1) a test system that mimics the essential features of the target tissue in cellular structure and exposure properties (e.g. penetration) and 2) endpoint measures that can quantitatively detect the known manifestations of toxicity to the tissue. Quantitative prediction of eye irritation has been a goal for developers of *in vitro* test systems for decades. While there are several tissues potentially exposed to chemicals in the anterior eye, the cornea has been the primary focus for these efforts as damage to this tissue can result in catastrophic loss of sight. The cornea is unique in being optically clear. It is comprised of three distinct layers. The stratified epithelium provides a partial barrier to water loss and chemical penetration. The avascular stroma accounts for the bulk of the corneal mass and provides structural integrity to the cornea. The stroma also contains a network of interconnected keratocytes that help maintain the

stromal integrity. Most posterior layer is the endothelium (one cell thick) which regulates the passage of water and nutrients from the aqueous humor to the stroma and epithelium. The physiological basis for prediction of the degree of corneal injury was elucidated through the work of Drs. Jester and Maurer in the 1990s. Using confocal microscopy and standard histology, they demonstrated that the depth of cell death (epithelium to endothelium) in the early stages of chemical exposure was predictive of the degree and duration of corneal injury. Mild injury restricted to the epithelium, moderate injury extending into the upper stroma and severe injury extending to the deep stroma and endothelium. This work has been the basis on which subsequent *in vitro* models systems (both the target tissues and endpoint measures) for predicting quantitative eye damage have been based. This presentation will provide an overview of corneal physiology and then focus on the application, strengths and limitations of current 3D models for the prediction of ocular damage including the underlying physiological basis for their selection.

A-7

Establishment of Insect Cell Lines in China to Address Agricultural Issues. YAO-FA LI and Jing-Jie An. Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, Baoding 071000, PR CHINA. Email: 13785266025@126.com

After the first report describing the culturing of silk moth sperm cells by Goldschmidt (1915), Professor Gaw (Wuhan University) reported on the establishment of cell lines from *Bombyx mori* ovaries in 1958. Since then, numerous Chinese researchers have focused on the establishment and use of insect cell lines to address agricultural issues. In this presentation, we will review progress made in insect cell line work in China, including their establishment, their use in research, and their application in insect control. 1. Establishment of cell lines Since 1958,approximately 70 cell lines have been established in China. Most of these lines are from tissues of noctuid or bombycid insects, including embryos, newly hatched larvae and ovaries. 2. Research using insect cell lines When working with insect cell lines, Chinese entomologists have focused on the use of baculovirus expression vector systems and the screening and optimization of the production of biological pesticides. Sf9 cells are the most frequently used cells for the expression of animal disease-related genes in vitro. Additionally, due to the similarity in genetic background between insect cells lines and pest insects, the efficacy of chemical and microbial insecticides have been evaluated using insect cell line bioassays, including the determination of the biological activities of organophosphorus, insect viruses, botanical pesticides, and δ-endotoxins. 3. Production of biopesticides With the development of serum-free insect cell culture media and the optimization of baculovirus expression systems, the large-scale propagation of insect cells for the production of insect viruses has been a major success. The resulting products have played an essential role in the control of pest insects in Chinese agriculture.

A-8

Specific Distribution of Glycan Motifs at the Surface of Midgut Cells in the Cotton Leafworm. GUY SMAGGHE. Department of Plants and Crops, Ghent University, Ghent, BELGIUM. Email: guy.smagghe@ugent.be

Glycans are involved in many biological phenomena, including signal transduction, cell adhesion, immune response or differentiation. Although a few papers have reported on the role of glycans in the development and proper functioning of the insect midgut, no data are available regarding the localization of the glycan structures on the surface of the cells in the gut of insects. For this purpose, we established in this project primary midgut cell cultures from larval stages of the pest insect, the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae). Then we probed these cells with a selection of lectins covering a range of different carbohydrate-binding specificities and visualized the interaction using confocal microscopy. Our analysis focused on the typical differentiated columnar cells with a microvillar brush border at their apical side, which are dominantly present in the Lepidopteran midgut and function in food digestion and absorption, and as well as on the undifferentiated stem cells that are important for midgut development and repair. Our results indicated that polarization of the midgut cells is also reflected by a specific distribution of glycans, especially between the basal and microvillar pole. The data are discussed in relation to the functioning and development of the insect midgut and potential future use of glycan moieties as novel insecticide targets and/or lectins as carriers for bioactives for agricultural applications.

A-9

Intracellular Cross-Talk in Insect Immune Signaling. DAVID STANLEY. USDA/ARS Biological Control of Insects Research Laboratory, 1503 S. Providence Road, Columbia MO. Email: David.Stanley@ars.usda.gov

Insects, like all invertebrates, express innate, but not adaptive, immunity. Insect immunity is typically assorted into two major mechanisms, cellular (hemocytic) and humoral. Hemocytic mechanisms include phagocytosis (the internalization and killing of bacteria), nodulation (melanized aggregates of hemocytes with adherent bacterial cells) and for larger invaders, encapsulation (invader surrounded by layers of

melanized hemocytes). These reactions are launched immediately infections are recognized by continuous surveillance systems. Humoral immune reactions involve activation of intracellular signal pathways, Toll and Immune Deficiency (Imd), both of which lead to induced expression of genes encoding anti-microbial peptides (AMPs), which directly kill bacterial cells by compromising cell membranes. AMPs appear in the hemolymph of infected insect 6–12 hours after an infection is recognized and serve mainly as a 'mop-up' operation to handle sustained and/or subsequent infections. Here, I will report that eicosanoid (prostaglandins [PGs and related compounds]) interactions with the Toll and Imd pathways are necessary for induction of AMPs. Insect hemocytic immune mechanisms are mediated by various signal compounds, biogenic amines (5-hydroxytryptamine [5-HT] and octopamine [OA]), insect cytokines (plasmatocyte spreading peptide; PSP) and eicosanoids. Treating hemocyte preparations separately with 5-HT, OA, PSP or PG leads to specific immune reactions. This finding led to the hypothesis of operational intracellular cross-talk among these immune mediators. Here I report that PSP, OA, 5-HT and PGs act through specific receptors. Each receptor leads to activation of a small intracellular G protein, Rac1, which leads to PG biosynthesis. PGs are the final mediator, which leads to hemocyte cytoskeleton rearrangements necessary for cellular immune reactions, such as hemocyte spreading. The various mediators of hemocytic immune mechanisms are coordinated by Rac1.

A-10

Genetic Approaches to Rapid Cell Line Generation in Drosophila. AMANDA SIMCOX and Shiva Raghuvanshi. Department of Molecular Genetics, Ohio State University, Columbus, OH. Email: simcox.1@osu.edu

Historically, Drosophila cell lines have been generated spontaneously from embryonic primary cultures. Presumably the lines resulted from random genetic events in a subset of cells, which went on to produce a continuously proliferating line. This latent period is a bottleneck. We found that expressing oncogenic Ras (Ras^{V12}) in primary cultures decreases the time to cell line production and increases the efficacy. This has enabled reliable production of lines, including those from embryos of different genotypes. Here we will present work adapting the Ras-method for the generation of tissue-specific cell lines. We are using the Gal4/UAS binary system to limit Ras expression to specific lineages. In this case, specific cell types gain a proliferation advantage and dominate the culture. We have generated lines of epithelial, neuronal, glial, and mesodermal lineages. Marker gene expression confirms cell type in a number of cases. We are currently defining conditions to induce differentiation. We expect the lines will be useful for basic biological research, the generation of disease models, and for high throughput genetic and small molecule screens.

A-11

The Benefits of Cell-based Seafood. MICHAEL J. SELDEN. Finless Foods, 6460 Hollis St. Suite B, Emeryville, CA 94608. Email: mike@finlessfoods.com

Seafood as it is produced today has a plethora of problematic elements. Mercury and plastic pose health hazards to consumers, while antibiotics and growth hormones cause herd immunity problems and toxic runoff. Mislabeling of fish can lead to allergic reactions or just baffled consumers. Distribution too poses problems, leaving low-quality product to be shipped around the globe racking up greenhouse gas emissions. The imminent collapse of ocean ecosystems means that the way we currently produce fish can't continue. Cell-based seafood can solve many of these problems by creating real fish meat on land without all of the inefficiencies tied up in raising an entire animal just to eat its meat.

A-12

Breaking Down a Fish, the Cellular Way! J. J. TUNG. Finless Foods, 6460 Hollis St, Suite B, Emeryville, CA 94608. Email: jennie@finlessfoods.com

Animal cell culturing has become routine and is a staple of biomedical and academic research labs. While the underlying cell culturing foundations established by such fields as cancer research, gene therapy, stem cell biology, and hormone production exist to encourage mass production of bioengineered *in vitro* meats, the application of those principles present a new set of problems. For instance, the use of animal serum, a commonly necessary component of cell culturing media, is antithetical to the mission of most cellular agriculture companies, and formulating a serum-free medium that promotes long-term proliferation has proven to be difficult. For fish cell agriculture, the hurdles are compounded by a comparative lack of basic cell and molecular biology research as well as an overwhelming diversity of fish types. This talk will overview the practical cell culturing hurdles involved in producing a cellular agriculture product with a focus on the more specific hurdles the fish cell agriculture industry faces.

A-13

Automation in Cell-based Meat Production. NICHOLAS LEGENDRE. New Age Meats, 479 Jessie St., San Francisco, CA 94103. Email: nick@newagemeats.com

Lab grown meat is a challenging endeavor that requires the optimization of hundreds of parameters in combination, not

isolation. For the industry to grow and prosper, leading to a new era of meat production encompassing new and unique flavors and textures, resources need to be used in a cost- and time-effective manner. Automation of experimental set-up during the R&D phase allows scientists to test many protocol variations in a short amount of time. Automation of data acquisition, processing and interpretation will lead to the creation of tools that speed up the readouts for experimental output. To close the loop, data science and potentially machine learning can be used to further interpret the data and gain insights which would otherwise be missed.

A-14

Creating Cellular Agriculture as an Interdiscipline. KATE KRUEGER. New Harvest, 316 Bergen St, Brooklyn, NY 11217. Email: kate@new-harvest.org

Founded in 2004, New Harvest is the world's longest standing organization dedicated to research in the field of cellular agriculture. Through the contributions of a diverse and rapidly expanding international community of donors, we have funded open cellular agriculture research. We fund and conduct critical open, public, collaborative research that effectively advances discoveries in cellular agriculture but is lacking support from conventional funding channels in industry or academia. We bring together the community that is building this field (scientists, academia, funders, industry, policy-makers, regulatory authorities, prospective consumers, etc.), fostering dialogs and collaboration. We educate and inform stakeholders and the public at large of what cellular agriculture research is, and why it is necessary, in an honest, transparent, science-based manner.

A-17

Data Analysis Techniques for Microbiome Research. SONNY T. M. LEE. University of Chicago, Chicago, IL. Email: sonny.lee.t.m@gmail.com

The advancement of culture-independent techniques and next-generation sequencing has led to a rise in the number of microbiome studies over the last decade. Communities should be studied as a whole because many micro-organisms have never been cultured independently. The advancement of next-generation DNA sequencing has allowed sophisticated analysis and sampling of these complex systems, revealing differences in community structure as well as microbial functions. Although it remains important to identify the taxa of microbes, the next stage of microbiome research will need to focus on uncovering the role of the microbiome rather than its mere composition. I will first briefly discuss the historical origins of microbiome studies and tools for determining the ecological diversity and identity of a microbial community. Next, I will introduce shotgun sequencing technologies such as metagenomics and metatranscriptomics, the computational challenges and methods associated with these data. Finally, I will conclude with examples of the functional genomics of the microbiome from the human gut and environmental model systems, and its influences upon health and disease.