

Report on the 17th JAACT Meeting

“Industrial Applications of Animal Cell Technology”

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The 17th Annual and International Meeting of the Japanese Association for Animal Cell Technology, JAACT 2004, was held in Nagoya, from November 15th to 18th in Nagoya. The meeting was chaired by Prof. S. Iijima, and gathered about 200 delegates from Japan and about 70 from countries abroad Japan. The main programme was organised in eight symposia, with 40 oral presentations and 3 plenary lectures. In addition, five workshops with shorter oral presentations were also organised, in a parallel session to the Symposia, and more than 80 contributions were discussed in the poster format, with specific short presentations and poster discussion session. The programme also included luncheon seminars, where lunch was combined with presentations from companies. On the whole, it was a very intensive and complete meeting. The complete programme was developed in three days, in a very tight schedule and efficient organisation. ESACT contributed to the programme by organising an specific ESACT Lecture, consisting in three oral communications from T. Noll (Jülich, Germany), P. Umana (Zürich, Switzerland) and F. Gòdia (Barcelona, Spain). In addition, other ESACT members contributed to specific seminars with their oral presentations. In the following part of this report, the main trends of the sessions are briefly described.

Murakami Memorial Lecture

This special lecture was presented as a memory to Prof. Hiroki Murakami. Prof. Murakami, passed away 4 years ago, was the founder of Animal Cell Technology activities, as considered nowadays, in Japan, and was founder of JAACT. In the JAACT meeting series, he was a key person in introducing science and ACT among young researchers and specially in using English as the language for the meetings, in a clear sign for the need of international communication in Science.

The lecture was given by Prof. Gordon H. Sato, director of the Manzanar Project in US. Prof. Sato discussed some of the highlights of his long scientific career in US, where he was born, like the discussions on selection versus differentiation in the evolution of cells in culture or the development of defined media. He made especial emphasis on his views on the importance of tissue engineering as the science for the future, and its implications in many fields, such as endocrinology and oncology, in the way that the experimentation with cells in culture is providing tools for the understanding at the level of the tissue and animal physiology.

Symposium 1. Recent development in the small RNA world

A complete symposium of the meeting was dedicated to this recent breakthrough in the biological sciences, including one of the Plenary lectures, a very comprehensive presentation by Prof. K. Taira, presenting the generation of libraries of hammerhead ribozymes with randomised binding arms and/or of U6 and t-RNA-driven siRNAs. The transfection of cells with these libraries showed to be a powerful tool to identify relevant genes involved in important cellular processes such as apoptosis, cancer metastasis, and cell differentiation pathways. The session also included presentation on

the methodology to introduce RNA-i in *C. elegans*, and the connection between RNA-i and the fragile X syndrome. Finally, a presentation on the complex RNA-i patent situation was also given, with analysis of some specific cases.

Symposium 2. Stem Cells Technology

The second symposium of the meeting was also dedicated to a very relevant topic, such as stem cells technology, and also included a Plenary Lecture, that was given by Prof. Ch. S. Pottem, presenting a very detailed description of the adult epithelial stem cells, particularly those present in the intestine, discussing on their number, characteristics and genome protective mechanisms, particularly those involved in the protection against the risk of replication induced errors. The rest of the session presented contributions on the generation of neural stem cells genetically modified for brain repair, the study of adhesion on microcarriers of mesenchymal stem cells, and the development of low-serum culture for the expansion of stem cells populations.

Symposium 3. Food Allergies – Current and Perspectives

The topic of food allergies has received a lot of interest in the JAACT meeting, with five oral presentations. They were focussed on two main topics. In one side, the development of new methodologies for the detection of allergenic substances in processed food, particularly based on ELISA concepts was discussed. The role of animal cell technology as a supporting technology for the developments of these tests was made evident. On the other side, the discussion was concentrated in the legal and regulatory developments around this topic, particularly with implications in the food labelling.

Symposium 4. Advanced functional water for the prevention of diseases

An specific and innovative session, with three oral contributions, was dedicated to the concept of treated water as a vector for enhancing population health. It is the observation made at the physiological level that originate such waters treatments. Specific examples of this approach were given by the positive effects demonstrated in a clinical trial on the use of potable electrolysed alkaline water on the prevention of gastrointestinal symptoms. Electrolysed reduced water also received the attention regarding its effects in the reduction of oxidative stress, particularly in that induced by haemodialysis in final-stage renal disease. Also its effects on the reduction of oxidative species as active oxygen donor was discussed.

Plenary Lecture. Prof. N. Hirokawa.

Prof. Hirokawa plenary lecture was one of the highlights of this JAACT meeting. In a very educational presentation, he discussed on intracellular transport and molecular motors, KIFs structure, dynamics function and diseases, summarizing a vast research effort carried out by his team at the University of Tokyo. Using advanced tools, the characteristics and mechanisms, the role of kinesine superfamily proteins, KIFs, in the transport of functional molecules in neuron cells is elucidated and its relationship with various cell functions and fundamental development events were presented. Finally, the identification the mechanisms of how these motor proteins KIFs move along the

microtubules of the cells was elucidated using molecular biophysics, cryoelectron microscopy, X-ray crystallography and optical trapping nanometry.

Workshop on Cell culture engineering and production of biologicals

This workshop was celebrated partially in parallel to the main sessions, in a format of short oral presentations, covering contributions from different groups in the more mature areas of cell culture technology. It combined presentations from academia and industry covering a broad spectrum of topics ranging from cell line development/engineering, functional genomics and medium development up to primary cell culture.

Duk Jae Oh from the Sejong University, Korea, described the development and use of a depth filter technology for long-term perfusion culture of antibody producing CHO cells. Cell densities up to $3 \times 10^7 \text{ mL}^{-1}$ were maintained for up to 1800 h. The antibody productivity was more than 70fold higher than that of a simple batch fermentation.

Danny Wong from the Bioprocessing Technology Institute in Singapur presented interesting results on the transcriptional investigation of apoptotic events in CHO batch and fed-batch fermentations using their in-house developed CHO cDNA array. Time-dependent upregulation of early and late pro-apoptotic effector genes could be observed. While receptor-mediated apoptosis induction is mainly mediated by Fas, FADD and Daxx, the mitochondrial-mediated apoptosis is dominated by members of the Bcl-2 family (Bad, Bax, Bid). Several other genes also appear to be upregulated during apoptosis induction but their exact role still has to be elucidated.

Philip Sass (COO of Morphotek, Pennsylvania) gave a keynote lecture the company's Morphogenics technology as a tool for drug discovery. Several examples were presented showing impressive improvements in cell specific productivity or antibody binding affinity.

From a completely different topic Akira Mori, university of Tokyo, described the development of a migration assay to investigate the intestinal epithelial cells and immune cells.

Philip Offin, (The Automation Partnership) presented the company's SelecT system, an automated cell culture processing system for robotic handling of up to 400 tissue flasks which was developed in collaboration with major pharmaceutical companies. In the discussion Dr Offin mentioned that a system for automated handling under controlled conditions is under construction.

Satoshi Terada, (University of Fukui) suggested sericin, a major component of raw silk, as cell culture supplement for serum replacement with several potential applications from cryoprotection to growth and production enhancement. The results being presented were comparable to an FBS containing control culture but a comparison to state-of-the-art chemically defined culture media or media containing hydrolysates was missing. Furthermore it remains unclear whether there are batch to batch variations in the sericin. Alistair Irvine (ML laboratories, UK) presented an interesting approach for the generation of high production cell lines in short time. When linking the transgene to UCOEs (ubiquitous chromatin opening elements) a larger number of transgene expressing clones showing higher and more stable expression can be generated

compared to transgenes not being linked to UCOEs. The production of 1g of antibody from 10 liters of cell culture within about three weeks after transfection was described.

Symposium 5. Metabolomics as a powerful tool in post-genome era.

Within this symposium six presentations were given and the term metabolomics is still be used very differently. The papers covered the identification and quantification of cellular peptides, lipids and proteins as well as metabolites from the central metabolism. In the discussions another problem in animal cell metabolomics became obvious. There is still no reliable technology available for rapid quenching of the cells metabolism, making the quantification of intracellular pools with a rapid turnover very difficult. Strong improvements have been made regarding the identification and quantification of metabolites using LC-MS and MS/MS techniques. The presentations covered quite diverse topics from the effect of broccoli on oxidative stress, inflammation and hypertension (Dayan Goodenowe, Phenomenome Discoveries Inc), the establishment of cellular peptide databases (Naoto Minamino, Osaka) and metabolic flux analysis in plant cell culture (Ei-ichiro Fukusaki, Osaka) up to comparative evaluation of lipids, proteins or central metabolites in various tissues and diseases (Hiromasa Toja, Osaka; Toshihide Nishimura, Tokyo and Chris Beecher, Metabolon).

Symposium 7. Process Development and Manufacturing of Biologicals

Highlights of this Symposium on Process Development and Manufacturing of Biologicals were the characterization of glyco-engineered therapeutic anti-cancer antibodies and their manufacturing process, as well as the continued development of extremely high-yielding fed-batch processes based on CHO and NSO recombinant cell lines generated with glutamine synthase (GS) selection.

Researchers from Kyowa Hakko described the generation of a fucosyltransferase-knock-out CHO cell line derived from dhfr- CHO-DG44 cells. The cells were engineered for the production of therapeutic IgG1 antibodies lacking fucose residues alpha 1,6-linked to the core of the oligosaccharides attached at the conserved Asn 297 glycosylation site of IgGs. Such glycoengineered antibodies show large increases in biological activity (immune effector cell-mediated killing of antibody-targeted cells). The basis for the increased biological activity was also characterized and presented in this symposium by Kyowa Hakko researchers, who showed a large increase in binding affinity of the engineered antibodies to antibody Fc-receptors found on immune system cells such as NK cells and macrophages.

Shinobu Kuwae from Mitsubishi Pharma presented a high-yielding fed-batch process for the production of recombinant human antithrombin (AT). Use of recombinant cell line generated with Lonza's CHO-GS expression system led to product titres above 1g/l in serum-free medium, allowing the researchers to conclude that this process can now compete economically with AT production from human plasma. Yet another example of the usefulness of the GS selection system for rapidly yielding very productive cell lines was presented by Amid Varma of MedImmune, who has developed a serum-free fed-batch process using an NSO-GS-cell line for the production of a recombinant human therapeutic antibody. The process reaches antibody titres of 5g/l. The researchers suggest though taking a close look at the intellectual property landscape governing commercial use of the NSO system for recombinant antibody production.