Genes Genom (2013) 35:411–413
DOI 10.1007/s13258-013-0109-1

EDITORIALS

Meeting report: genetics and genome engineering

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Received: 15 April 2013 / Accepted: 15 April 2013 / Published online: 7 May 2013

The first of the Genetics Society of Korea's mini symposium series was held on April 5th at Kangwon National University in Chuncheon, Korea. The mini symposium aims to encourage discussion and interaction among Korean research communities and bolster science communication. This year four symposia are scheduled to be held at different locations nationwide of Korea and are open to all local and international researchers. The opening symposium "Genetics and Genome Engineering" was co-hosted by members of the Kangwon National University: Institute of Bioscience and Biotechnology, Institute of Antibody Research and the College of Biomedical Science.

Dr. Jin Soo Kim of the National Creative Research Initiatives Center for Genome Engineering and the Department of Chemistry at Seoul National University opened the meeting with his talk "Precision genome editing in cells and organisms TALENs and RNA-Guided Endonucleases (RGENs)". Gene knockout in animal models is essential in addressing in vivo function. The development of genome editing technologies has allowed reverse genetics to be routinely applied to the mouse, and to invertebrates such as *Caenorhabditis elegans* and *Drosophila melanogaster*, making these species the most popular choice among model organisms used to understand the molecular function and relevance of selected genes during development and disease.

Engineered enzymes are powerful and versatile tools of genome editing: zinc finger nucleases (ZFNs) are constructed by fusing custom-engineered DNA-binding zinc finger arrays to the *Fok*I nuclease domain (Urnov et al. 2010) and via transcription activator-like effector nucleases (TALENs) are made by fusing TALE arrays to the *Fok*I nuclease domain (Miller et al. 2011; Zhang et al. 2011). ZFNs and TALENs act as artificial restriction enzymes; they can be designed to target specific DNA sequences and thus can be used to precisely excise, replace, or mutate particular regions of the genome. Dr. Kim's research team has optimized the TALEN architecture to improve efficiency and specificity, and has developed a high-throughput Golden-Gate cloning system to assemble TALEN plasmids at a genomic scale (Kim et al. 2013). The researchers used these TALENs to establish single- and double-gene knockout cells in which NF-κB signaling pathways were disrupted. Compared to siRNA-treated cells, these cell lines showed unambiguous suppression of signal transduction. They also applied both ZFNs and TALENs to induce site-specific mutations in mice (Sung et al. 2013), as well as in other animals.

Dr. Kim also reported on a novel genome-editing technology based on RNA-guided endonucleases (RGENs) that was developed recently in his lab (Cho et al. 2013) and two other groups (Cong et al. 2013; Mali et al. 2013). *Cas9* is a sequence-specific endonuclease in type II CRISPR/*cas* systems, which confer prokaryotes with adaptive immunity

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against invading phages and plasmids. *Cas9* recognizes and cleaves target DNA sequences complementary to small synthetic guide RNAs embedded in the protein, generating site-specific DNA double-strand breaks in vitro and in human cells, where spontaneous repair mechanisms induce targeted genome modifications at high frequencies (Cho et al. 2013). Unlike ZFNs and TALENs, RGENs are customizable without any cloning step, making them a broadly useful, scalable, and expeditious platform for genome editing in cells and organisms.

The second speaker, Dr. Cheol-Hee Kim from the Department of Biology at Chungnam National University, Daejun, Korea, gave a presentation entitled 'Functional study of human genes in zebrafish'. The zebrafish is an excellent animal model system for understanding the mechanism of vertebrate development and for modeling human diseases. However, effective lab-based tools for reverse genetics have been unavailable until the recent development of TALEN mediated genome editing.

Zebrafish mutants have been isolated traditionally by ENU-based chemical mutagenesis followed by positional cloning to identify genes. To complement chemical mutagenesis, Dr. Kim developed a transgene reporter system for insertional mutagenesis and identified a mutant "ondal", which he characterized and mapped to a gene involved in axonal myelination and human schizophrenia. Other interesting mutants identified by the group include "samdori" and "hayan-4".

Using a different approach, his lab also screened human UniGene collections of Korean UniGene Information (KUGI) by in silico analysis, from which more than 15,000 human full-length cDNAs are currently available. More than 2,700 human full-length cDNAs of interest were microinjected and overexpressed in zebrafish embryos, resulting in the identification of 50 functionally active genes with variable phenotypes from developmental defects to neuronal cell death in injected embryos. Many of these genes may be implicated not only in developmental processes but also in diseases such as cancer.

From this study, his group identified a human clone named 'ottogi' that may be a novel developmental regulator of vertebrate head formation acting via Wnt signal inhibition; another interesting clone named ZNF312b is specifically overexpressed in gastric cancer cells and may play a role in tumor progression and metastasis via transcriptional activation of the K-ras oncogene (Song et al. 2009).

Finally Dr. Kim also talked about the "ZEN" (Zebrafish & Engineered Nucleases) consortium that he has organized in the Asia-Oceania region. Recent developments in genome editing techniques have heralded a new era in zebrafish genetics. TALEN and RGEN/CRISPR methods allow genomic engineering and gene knockdown in zebrafish to be performed in almost any laboratory; in contrast ZFN-based techniques require resources available to only large research labs or companies. ZEN is part of the effort to efficiently share the techniques and resources generated from this expansion of zebrafish genetic technologies. More than 60 genes of interest are currently represented from a number of different laboratories in collaboration with ToolGen, Inc (Seoul, Korea). At present, 32 stable gene knockouts have been successfully generated, with additional genes currently under screening.

The third speaker, Dr. Hae Chul Park from the Graduate School of Medicine in Korea University, Ansan, Korea, gave a presentation under the tile, 'Zebrafish as a vertebrate model for oligodendrocyte development and neuropeptide study'. During vertebrate neural development, proliferative neuroepithelial precursors give rise first to neurons and later to glial cells. A particularly good example of this process is seen in a specific subset of ventral spinal cord precursor cells known as pMN precursors, which produce motor neurons during neurogenesis. The production of motor neurons is followed by the generation of oligodendrocyte progenitor cells (OPCs), which subsequently form oligodendrocytes, the myelinating cell type of the central nervous system. Sonic Hedgehog signaling is required for the generation of motor neuron and OPCs from the pMN precursors, however, it is not clear exactly how Hedgehog (Hh) signaling directs the complex sequential specification of motor neurons and OPC during development. Dr. Park showed that Indian Hedgehog b (Ihhb, previously known as Echidna Hedgehog), another member of the Hh signaling pathway, is expressed in the floor plate cells of the ventral spinal cord at the time of OPC specification in zebrafish embryos, and that neural precursors failed to specify OPCs in the absence of Ihhb function (Chung et al. 2013). This indicates that Ihhb function is required for the specification of OPCs from pMN precursors by negatively regulating cell proliferation. In addition, Dr. Park's lab has an interest in neuropeptide research. Neuropeptides are small protein-like molecules that are expressed by neurons, released, and mediate neuronal communication by acting on cell surface receptors. Neuropeptides influence the activity of the brain in specific ways and are thus involved in particular brain functions, like analgesia, reward, food intake, sleep, learning and memory. For these reasons, identification of novel neuropeptides may be important in the discovery of potential drug target molecules. Dr. Park's lab recently isolated zebrafish orthologs of human genes encoding novel neuropeptides and large-scale functional analysis of these neuropeptides in zebrafish is under way.

The last speaker, Dr. Ji-Yun Lee of the Department of Pathology, College of Medicine, Korea University, Seoul, Korea, talked about 'Application of whole genomic technology from benchside to bedside in genetic disorders'. Whole genomic technology, including both array and

sequencing based methods, are revolutionary tools that are rapidly becoming an integral part of diagnostic procedures in clinical laboratories. These technologies were first developed as research tools for the investigation of genomic imbalances and changes that are associated with various diseases (e.g., genomic disorders, diabetes, congenital heart defects, cancer etc.). They allow for high-resolution and high-throughput assessment of genetic alterations ranging from single base pair substitutions in single genes to millions of bases of contiguous DNA, depending on their design and platform. She introduced how these technologies, especially array CGH (Comparative Genomic Hybridization) and exome sequencing, can be applied in the clinic for disease diagnosis and to identify disease-causing mutations in patients with genetic disorders; they also have advantages over conventional technologies such as cytogenetic analysis or Sanger sequencing. These technologies will deliver great benefits in the field of personalized medicine and have applications in diagnosis, prognosis and treatment, although challenges remain, such as how to accurately interpret which genomic changes are pathogenic in nature and which are benign.

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