

## Meeting Report

# Disease and animal research: a meeting review

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Animal models have been playing an important role in disease research. They have advanced our knowledge about the genetics, development, environmental effects, and in turn, the mechanism of diseases. A recent review has pointed out that one-third of the high-impact animal research published in seven leading journals has been through clinical trial, and one-tenth of these studies have succeeded and been applied to disease treatment (Hackam and Redelmeier, 2006; van der Worp et al., 2010). With the completion of the whole genome sequencing of various organisms, including human and model organisms such as yeast, fly, worm, and mouse, current focus is on the functional characterization of these genomes. Researchers hope to reveal the mechanism of more diseases, especially the multi-factorial diseases, in a wide scale and with high efficiency so as to establish effective preventions and interventions. Efforts have been invested in two important aspects: large-scale mutagenesis and large-scale phenotypic studies. This meeting ‘Disease and Animal Research’ was held in Shanghai from June 30th to July 2nd in 2010. Hosted by the Institute of Developmental Biology and Molecular Medicine (IDM), Fudan University, China, this meeting aimed at providing a chance for international scholars to exchange recent progress in the field of disease and animal research, form productive collaborations, and promote future development. This review describes up-to-date advances in the areas that this meeting focused on, including both phenotypic studies and mutagenesis screens: (i) large-scale phenotyping in the European Mouse Disease Clinic (The EUMODIC Programme); (ii) application of a mammalian transposon

Sleeping Beauty (SB) in cancer; (iii) recent developments in various disease mechanism studies, such as neurodegenerative diseases, hypoxia, and developmental defects; (iv) current usage of invertebrate genomics; (v) rodent application in drug discovery and development; (vi) evolutionarily conserved natural PB insertion in the human genome and Cockayne syndrome; (vii) PB technology workshop by IDM: development and application of the PB technology in mutagenesis screening; and (viii) better communication with other researchers through publication: how to get published. *The EUMODIC Programme*. The International Knockout Mouse Consortium (IKMC), including the European Conditional Mouse Mutagenesis Program (EUComm, <http://www.eucomm.org/>), the Knock Out Mouse Project (KOMP, <http://www.komp.org/>), and the North American Conditional Mouse Mutagenesis Project (NORCOMM, <http://www.norcomm.org/index.htm>), planned to systematically generate conditional mutants for every mouse gene. So far, 447 mutant mice are available through EUComm.

Meanwhile, the European Commission started the European Mouse Disease Clinic Programme (EUMODIC, <http://www.eumodic.org/>) to carry out broad-spectrum phenotyping studies on up to 500 mutants as a first step toward the establishment of a complete mouse phenotyping network (<http://www.eumorphia.org/publications.html>). Dr Brown highlighted the current progress for this project. Four hundred and fifty-one mutants have been put through the phenotypic screens for 406 phenotype parameters and 155 metabolic parameters included in 20 phenotyping platforms inside the four mouse clinics

belonging to EUMODIC. More than 150 standard operating procedures have been developed through the European Mouse Phenotyping Resource for Standardised Screens (EMPRESS). One hundred and forty-seven lines have data published in the EuroPhenome database. More than half of the lines analyzed display convincing phenotypes and a majority of them have shown that the genes disrupted are involved in more than one phenotype category. Not only homozygote (70%), but also heterozygote mutants (53%) exhibit significant abnormalities.

*The SB transposon system and cancer*. The SB transposon, a member of the Tc1/mariner family harnessing a ‘cut-and-paste’ mechanism, is active in mammalian genomes (Ivics et al., 1997). Drs Copeland and Jenkins have successfully applied this system somatically to screen for genes associated with various cancers, including mouse hepatocellular carcinoma, colorectal cancer and T-cell lymphoma (Keng et al., 2009; Starr et al., 2009). They discovered that ubiquitous expression of the SB transposase led to hematopoietic cancer, while change either in the SB transposon structure or the expression of the transposase to be tissue and/or developmental stage-specific would result in different types of cancers with dramatic difference in genetic mutation profiles. In addition, the cancer subtypes appear to dependent on the type of cells they arise from. Recent progress includes the development of new SB systems with different combinations and the application of these new systems to cancer modeling, such as pancreatic, prostate, and liver cancer in hepatitis B surface antigen sensitized mice.

**Neurodegenerative diseases.** Neurodegenerative diseases are becoming more severe with high occurrence in old people: 5%–10% in the population over 60 years old, up to 50% in that over 80 years old. Misfolded characteristic proteins are commonly seen in most neurodegenerative diseases, including both Lou Gehrig's disease and CJD/Kuru disease.

Dr Horwich showed that misfolded SOD1 enzyme is associated with the Lou Gehrig's disease in two model organisms—worm and mouse (Wang et al., 2009a,b). Accumulation of the misfolded protein leads to the dominantly inherited dysfunction and loss of motor neurons. Detailed mechanistic studies are underway.

Dr Collinge brought the good news to the meeting that the development of effective treatment for this prion caused deadly disease, CJD and kuru in humans, and BSE and scrapie in animals, now seems feasible. Dr Collinge described the discovery, phenotypic description, molecular characterization, and mechanism mining of this disease, as reviewed in his paper published recently (Collinge, 2001, 2010). The misfolded protein transmits via recruiting the normal and harmless prion protein into the assembly without any help from DNA or RNA. Prion protein species may play crucial role in the mutant species transmission. Deletion of the normal gene appears have no effect on the health status of the animal, while the brain-specific deletion rescued the disease phenotype in mice. These pre-clinical data indicated that this gene is a potential drug target.

**Hypoxia.** Hypoxia presents a huge challenge to aerobic organisms whether during physiological status or pathological status. Dr Haddad has cultured a lethal level hypoxia tolerant fly strain after selection over 200 generations. Deep re-sequencing and bioinformatics tools have revealed that a number of DNA regions, with a majority on chromosome X, are associated with the tolerance, including several signaling transduction pathway and metabolic genes (Zhou et al., 2008). Here, involvement of notch signaling was presented. Over-expression and gain-of-function alleles all lead to considerably higher hypoxia tolerance in flies while inhibitor application, gene deletion

and RNAi all confer significant opposite effects.

**Developmental defects.** The typical way of understanding the normal developmental process is through the analysis of the effects of mutated genes. More knowledge about the mechanism of developmental defects always accompanies deep insights into the functions of genes in development. Five talks in this meeting have been devoted to this area.

The distinction between undifferentiated cells and differentiated cells lies in their attitude towards programming, with the former one facilitating it and the latter one resisting it. Dr Spradling showed that the differentiation is closely linked to the type of cell cycles. Differentiation resulted from the unfaithful passage of epigenetic information from the pluripotent cells to their progenitors.

The actin-nucleation factors are involved in basic composition and various life processes at the cellular level. Focusing on deciphering the role of two actin-nucleation factors, the Arp1/3 complex and the formin-family protein diaphanous (Dia) (Massarwa et al., 2007; Gildor et al., 2009), Dr Shilo discovered that Arp2/3 is involved in the fusion of muscle cells in flies and can also be detected in mice. Dia is in the polarized apical secretion in epithelial tubes.

A group of transmembrane proteins, the ERM proteins (ezrin, radixin, and moesin), are essential to the formation of the well-organized membrane domains, which was reviewed by Dr Fehon recently in both publication (Fehon et al., 2010) and this meeting. Though the ERM proteins have striking structure similarity, slight differences contribute to their functional diversity. Deficiency in any single ERM protein is associated with various different developmental and/or cellular defects, respectively.

As the first members of the intramembrane protease family and the major activators of the EGF signaling pathway in fruit fly, as characterized by Dr Freeman (Freeman, 2009), the rhomboids are evolutionarily conserved and are implicated in cancer, mitochondrial diseases and parasitic infection. Further biological and functional dissection of these genes with both mammalian and invertebrate models is in progress.

Hedgehog signaling directs a vast number of animal developmental processes, ranging from embryogenesis to adult homeostasis (Jiang and Hui, 2008). Malfunction of this signaling results in numerous disorders such as birth defects and cancer. Dr Scott found that Patched1 regulates this pathway in the primary cilium (Rohatgi et al., 2007). Dr Scott also examines the mechanism of this hedgehog signaling in cerebellum development and cancer (Lee et al., 2010).

**Invertebrate genomics.** Invertebrate model organisms have contributed much to our knowledge of development and disease mechanisms. Whole genome sequences and the fast development of genomics study and bioinformatics study toolboxes enable more comprehensive and systematic studies.

Dr Sternberg, one of the principal investigators of the Wormbase (<http://www.wormbase.org/>), is applying whole genome sequencing and comparative genomics to the study of the unique biology of insect-killing nematodes, which can jump up to 10 times of their body length. He is also studying the function and biology of the worm male linker cell (Kato and Sternberg, 2009), which is important for worm gonadogenesis. Ongoing work consists of analyzing the transcriptome of this cell to define the developmental program and mechanism of cell migration.

Dr Perrimon has successfully developed whole genome RNAi screens in the *Drosophila* tissue culture system (Friedman and Perrimon, 2004). He is using functional genomics to unearth the secrets of physiological homeostasis and the tissue/regenerative homeostasis of animals in response to injury.

**Rodent application in drug discovery and development.** The ultimate aim for disease and animal research is to discover effective methods for disease prevention and treatment. A sustainable cost-effective model is the key to the development and ultimate successful application of new medicines. Dr Vogt presented the recent application of rodent models to this area by pharmaceutical companies in the post-genomics era: (i) establishment of an integrated infrastructure covering all aspects needed for drug target discovery: from rodent model production to comprehensive

phenotyping. A database (MouseTrap) for archiving, retrieving, and analyzing data are also essential; (ii) application of new and effective technologies such as RNAi and using zinc-finger domains in genetic manipulation of the rodent genome to support drug distribution studies.

**Natural PB insertion in human genome and Cockayne syndrome.** The inheritable Cockayne syndrome (CS) leads to severe progeria. The majority of this disease results from mutations in the Cockayne Syndrome Group B (CSB, also known as ERCC6) gene encoding a SWI/SNF-like DNA-dependent ATPase (Christiansen et al., 2003). Dr Weiner has recently discovered that a piggyBac transposable element known as PGBD3 may play a role in this disease (Newman et al., 2008). CS patients normally lose the normal CSB protein while continuing to express the evolutionarily well-conserved CSB-PGBD3 fusion protein resulting from PGBD3 insertion into the CSB gene. This fusion protein appears to be involved not only in immune responses but also in the regulation of the genes near MER85s elements, short PGBD3-derived elements widespread in the human genome.

**PB technology workshop by IDM.** IDM recently developed a mammalian high-efficiency transposition system, the piggyBac (PB) system (Ding et al., 2005). With this method, IDM has produced a large collection of mutant alleles: 5191 mutant lines carrying PB insertion in genes with each insertion site mapped, providing a gold mine for further large-scale phenotypic and pre-clinical drug screens. Information about all of these mutant lines is available at the PBmice (piggyBac Mutagenesis Information Center: <http://idm.fudan.edu.cn/PBmice/> or <http://www.idmshanghai.cn/PBmice/>) (Sun et al., 2008). A flow-control production mode and a lab information management system (LIMS) of MP-PBmice greatly enhanced the speed of production (Yang et al., 2009). Cryopreservation of embryos and/or sperms has been established to archive the mutant lines.

Initial phenotyping studies are underway, including lethality, metabolic abnormality, developmental defect, autoimmune characterization, and cancer modifier screens. The

phenotypes caused by PB insertions are similar to the reported knock out alleles. The future usage of this mutant collection was envisioned to: (i) serve as disease models for known disease genes; (ii) enable phenotypic studies for mutants carrying disease related insertion sites predicted by large-scale genomics and bioinformatics studies, such as Genome Wide Association Studies; (iii) examine and determine the candidate disease related genes; (iv) phenotype screen to discover new disease genes; (v) functional inhibition screen for drug targets.

PB insertion mutagenesis has also proven of high efficiency in rats, a good animal model for various clinically related areas of studies, including physiology, neurobiology, pharmacology, toxicology, and pre-clinical trials. IDM has generated a number of mutant rats, including a significant number with abnormal phenotypic characters. PB is a universal mutagenesis tool across the animal kingdom.

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