# PROGRAM

Date: November 29, 2006
Venue: Lecture Hall, Dental Hospital TMDU

## Opening Remarks

09:55-10:00 **Masaaki NODA** [Director, Medical Research Institute (MRI), Tokyo Medical and Dental University (TMDU)]

## Session I Cancer Genomics and Epigenomics I

Chairperson: **Kristian HELIN** [University of Copenhagen, Denmark],
**Akinori KIMURA** [Dept. of Molecular Pathogenesis, MRI, TMDU]

10:00-10:30 Association study of major psychiatric disorders
**Draga TONCHEVA** [Medical University Sofia, Bulgaria]

10:30-10:55 Prediction of the therapeutic response to Paclitaxel and Docetaxel by gene expression profiling in primary chemotherapy for breast cancer
**Yoshio Miki** [Dept. Molecular Genetics, MRI, TMDU]

10:55-11:40 Genome-Epigenome Interactions in Tumors with Excessive Proliferation
**Joseph F. COSTELLO** [UCSF, USA]

11:40-12:05 High-throughput genomic and epigenomic analyses in cancer and genomic disorders on array platforms
**Johji INAZAWA** [Dept. Molecular Cytogenetics, MRI, TMDU]

## Session II Chromatin Regulation

Chairperson: **Wolf REIK** [The Babraham Institute, UK],
**Fumitoshi ISHINO** [Dept. of Epigenetics, MRI, TMDU]

13:30-14:15 Chromatin modifiers and cancer
**Kristian HELIN** [University of Copenhagen, Denmark]

14:15-14:40 Molecular features of histone methlytransferase Ash1 and its role in hematopoiesis
**Yujio Tanaka, Shigetaka KITAJIMA** [Dept. Biochemical Genetics, MRI, TMDU]

14:40-15:00 Coffee break

## Session III Epgenetics in Development

Chairperson: **Masatoshi HAGIWARA** [Dept. Functional Genomics, MRI, TMDU], **Joseph F. COSTELLO** [UCSF, USA]

15:00-15:45 Molecular control of the oocyte to embryo transition
**Davor SOLTER** [Max Plank Institute, Germany]

15:45-16:10 Retrotransposon-derived imprinted genes essential for development
**Fumitoshi ISHINO** [Dept. Epigenetics, MRI, TMDU]

16:10-16:55 Regulation of imprinting and epigenetic reprogramming in mammalian development
**Wolf REIK** [The Babraham Institute, UK]

16:55-17:40 Epigenetic programming of the genome in the embryo and germ cells
**Azim SURANI** [University of Cambridge, UK]

## Closing Remarks

17:40-17:50 **Takeshi TSUBATA** [Director, School of Biomedical Science, Tokyo Medical and Dental University]

Date: November 30, 2006
Venue: Lecture Hall, TMDU

## Joint Special Lecture at

## International Genomic Imprinting Workshop 2006

13:20-14:00 DISCOVERY OF IMPRINTED X-INACTIVATION IN EUTHERIAN MAMMALS
**Nobuo TAKAGI** [Hokusei Gakuen University, Sapporo]

14:00-14:40 EVENTS LEADING TO THE DISCOVERY OF IMPRINTING
**Bruce CATTANACH** [MRC, Harwall, UK]

# Association study of major psychiatric disorders

**Draga Toncheva**

Department of Medical Genetics, Medical University of Sofia, Bulgaria

Bipolar affective disorder (BP) and schizophrenia (SZ) have a strong genetic component, but the pathogenetic mechanism is still unknown. Current treatments are empirical, of limited effectiveness and do not address the basic defects underlying the development of psychiatric disorders. Association studies are powerful tool for detecting genetic causes of these severe complex disorders.

We have collected probably the largest collection of parent-offspring DNA samples from patients with major psychiatric disorders in the world: 622 families with schizophrenic proband, 278 families with bipolar proband, 109 families with schizoaffective proband. Additionally we have collected case-control DNA samples from 300 schizophrenic patients and 800 healthy controls.

Our aim was to identify the genetic polymorphisms that increase the susceptibility to bipolar affective disorder and schizophrenia and to test their influence on the clinical trait and the disease treatment.

So far we have investigated SNPs in genes with neurobiological function, gene from phosphatidylinositol signalling system, genes encoding components of neural synapses, genes located in linkage regions for major psychiatric disorders.

We obtained several positive results of SNPs association with schizophrenia and bipolar disorder and we found some new genetic variants in linked regions.

# Prediction of the therapeutic response to Paclitaxel and Docetaxel by gene expression profiling in primary chemotherapy for breast cancer

**Yoshio Miki**

Medical Research Institute, Tokyo Medical and Dental University

Suboptimal efficacy of most drugs for cancer therapy may be viewed as a result of our failure to account for individual differences in cancer etiology and drug response. Full understanding of the factors underlying these individual characteristics should allow the development of more specific diagnoses and therapeutics. Paclitaxel and docetaxel are taxoid drugs, and are now the most active agents for breast cancer. They both work by interfering with mitosis, but they each do it a little differently and the sensitivity is heterogeneous. To avoid unnecessary treatment, identification of a predictive marker is desired to distinguish between patients who are likely to respond and those who are not. We report the discovery of a gene expression profile that predicts response to paclitaxel or docetaxel in breast cancer patients. We took core needle samples from patients with primary breast cancer before treatment and then assessed tumor response to neoadjuvant under IC. Patents were divided into five groups according to pathological responses (Grade 0, extremely resistant; Grade 1a, resistant; Grade 1b, moderate responder; Grade 2, responder; Grade 3, high responder). RNA extracted from biopsy samples using microdissection method were profiled on oligomicroarrays of 21,000 human transcripts. Differentially expressed approximately 50 genes between responder (Grades 2 and 3) and extremely resistant (Grade 0) groups were selected by Mann-Whittney U-test. Secondly, correlation between RNA expression measured by the arrays and semiquantitative RT-PCR was ascertained on the selected genes. Using the semiquantitative RT-PCR data of selected genes, we performed machine-learning method (AdaBoost) to determine the greatest estimated accuracy between responders (Grades 2 and 3) and non-responders (Grades 0, 1a and 1b), and high-scored predictive sets were selected. Thus, we will demonstrate the application of large-scale genetic studies in breast cancer patients to molecular prediction of drug response to specific drug treatments.

# Genome-Epigenome Interactions in Tumors with Excessive Proliferation

**Joseph F. Costello**

Dept. of Neurological Surgery
University of California, San Francisco

Genetic and epigenetic mechanisms both contribute to, and interact during tumorigenesis. In genetic mouse models of tumors, disruption of DNA methylation modifies dramatically the incidence of tumor formation and the spectrum of tumor types. Methylation imbalance alone is also sufficient to induce tumors in mice. These studies illustrate a functional role of epigenetic imbalance in tumorigenesis, and also emphasize the interaction of genetic and epigenetic mechanisms in determining tumor incidence and tumor type. In human tumors, single gene analyses also show that these mechanisms can cooperate directly. Epigenetic mechanisms can also cause genetic alterations, and *vice versa*. These initial studies raise important questions about the degree to which genetic and epigenetic pathways cooperate in human tumorigenesis, the identity of the specific cooperating genes and how they interact functionally to determine the differing biological and clinical course of tumors.

To address this gap in our knowledge we developed tools for integrating large scale genomic and epigenomic analysis of human tumors. We applied our large scale analysis to glioblastomas (GBM) which are the most common and most malignant of human brain tumors. We describe a new state of severe hypomethylation that occurs in human GBM, affecting up to an estimated 10 million CpG dinucleotides per haploid tumor genome. Demethylation involves pericentromeric, subtelomeric and interspersed repetitive elements, and appears to be one factor predisposing to specific genetic alterations commonly occurring in GBM. In individual tumors we find evidence for all three proposed consequences of hypomethylation, including specific sites of chromosomal instability, reactivation of normally silent genes and imprinting defects. The cases of severe hypomethylation are also hyperproliferative. These data will be discussed in the context of an integrated genetic and epigenetic model that connects inadequate methyl-donor production and genome-wide hypomethylation to genomic instability and cellular hyperproliferation.

| High-throughput genomic and epigenomic analyses in cancer and genomic disorders on array platforms | |
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**Johji Inazawa**

Department of Molecular Cytogenetics, Medical Research Institute,
Tokyo Medical and Dental University, Japan

Comparative genomic hybridization (CGH) has provided huge information about copy number aberrations in cancer. We performed CGH analysis in a total of more than 1000 tumors in various cancers, and identified many cancer-related genes as the target within novel amplifications we detected. Among those, we identified GASC1 (Gene Amplified in Squamous Cell Carcinoma 1) as an amplification target within 9p23-24 amplification detected in esophageal squamous cell carcinoma (Yang et al., Cancer Res. 2000). Interestingly, Dr. Helin's group showed that GASC1 can demethylate H3K9me3/me2 in vitro (Cloos PA et al., Nature 2006). However, conventional CGH to metaphase chromosomes can provide only limited resolution at 5-10 Mb level. To circumvent this limitation, array-based CGH has been devised. We have constructed different types of BAC-based CGH-arrays. The first consisted of ~4500 BACs for genome-wide analysis (named MCG Whole Genome Array-4500); this array provided a resolution of ~0.7Mb. The second consisted of ~800 BACs harboring 800 known cancer-related genes, intended for diagnosis of cancer-specific copy-number aberrations (MCG Cancer Array-800). The third of our arrays contains 212 contiguous BACs spanning a ~20-Mb region at 1p36 (MCG 1p36 Contig Array). In addition, X-tiling array harboring 1008 BACs throughout the X-chromosome except pseudoautosomal region and a custom-made array for the diagnosis of genomic disorders have been constructed to explore disease-related cryptic chromosome aberrations in mental retardation (MR) and/or multiple congenital anomalies (MCA). Further, to accomplish high-throughput screening for methylated CpG sites in the whole genome, we combined array-CGH with the methylated CpG island amplification (MCA) method. This "BAC array-based MCA (BAMCA)" can discriminate BAC clones that harbor methylated CpGs on our BAC-arrays. On array platforms, we have succeeded in identifying novel cancer-related genes and candidate genes responsible for unknown genomic disorders.

# Chromatin modifiers and cancer

**Kristian Helin**

BRIC, University of Copenhagen, Denmark

Eukaryotic DNA is packaged into highly ordered chromatin and a crucial control mechanism for the interaction of transcription factors with specific genes is the post-translational modifications of the histones around which DNA is wrapped into nucleosomes. This control mechanism is important for the cell's memory, and since it is not inherited from one generation to the next, it is an *epi*genetic control mechanism.

The research in my laboratory has in the last decade focused on the identification and characterization of genes involved in the regulation of normal proliferation that also contribute to the development of human cancer. Consistent with an essential role of epigenetics in controlling cell-fate decisions, we have identified several novel oncogenes that function as chromatin modifiers.

In my presentation I will present a couple of stories illustrating this:

*BRAD1* (Bromodomain and Atpase Domain 1) is a novel E2F target gene, whose expression is required for the entry into the S phase of the cell cycle. We have shown that BRAD1 is associated with chromatin remodeling activity, binds the MYC oncoprotein and stimulates its transcriptional activity. *BRAD1* maps to a region that is frequently found amplified in cancer. Consistent with this, BRAD1 expression is high in several human tumors and correlates with clinical outcome in breast cancer patients. Based on these results we suggest that BRAD1 links the E2F and MYC pathways and contributes to the development of aggressive cancer through the enhancement of MYC-dependent transcription.

*GASC1* (Gene Amplified in Squamous Carcinoma 1) is a putative oncogene, which belongs to the JMJD2 subfamily of the Jumonji family, and is also known as JMJD2C. We have shown that three members of this subfamily of proteins demethylate tri- and di-methylated H3K9 *in vitro.* Furthermore, ectopic expression of GASC1 or other JMJD2 members dramatically decreases tri-and dimethylated H3K9, increases monomethylated H3K9, delocalizes HP1 and reduces heterochromatin *in vivo*. Previously *GASC1* was found to be amplified in several cell lines derived from esophageal squamous carcinomas, and in agreement with a contribution of GASC1 to tumor development, inhibition of GASC1

expression decreases cell proliferation. Thus in addition to identifying GASC1 as a histone trimethyl demethylase we suggest a model for how this enzyme could be involved in cancer development, and propose it as a target for anticancer therapy.

# Molecular features of histone methlytransferase Ash1 and its role in hematopoiesis

**Yujiro Tanaka and Shigetaka Kitajima**

Gene Structure and Regulation, School of Biomedical Science, and
Biochemical Genetics, Medial Research Institute

Chromatin structure plays important roles in regulating gene expression and genome stability. Over the past decade, various enzymes have been shown to covalently modify core histones that in turn regulate structure and function of nucleosomes. However, molecular mechanisms that orchestrate a large number of chromatin regulatory protein complexes in the nucleus remain elusive. Here we show that two members of mammalian trithorax-group, i.e. ASH1 and MLL1, constitute a part of chromatin regulatory network in the nucleus. Mammalian ASH1 is essentially required for expression of select Hox genes and functionally co-operates with MLL1 to activate transcription in both Hox promoter-specific and non-specific manners. We show that mammalian ASH1 specifically methylates histone H3 lysine 36 (K36), in contrast to previous reports suggesting that *Drosophila* ASH1 methylates K4. Methylation of K36 by ASH1 leads to gene suppression, and therefore ASH1 has both positive and negative functions in gene regulation. Importantly, ASH1 partially collaborates with an oncoprotein MLL-AF9 suggesting its role in leukaemogenesis. We also show that reduction of ASH1 drives a multipotent human leukaemia cell line K562 towards the erythroid lineage and away from the megakaryocytic lineage suggesting that ASH1 is involved in cell fate determination during normal haematopoiesis.

# Molecular control of the oocyte to embryo transition

**Davor Solter, A. V. Evsikov, A. E. Peaston, K. W. Hutchison B. B. Knowles**

Max-Planck Institute of Immunobiology, Freiburg, Germany, The Jackson Laboratory, Bar Harbor, USA and Department of Biochemistry, Microbiology and Molecular Biology, The University of Maine, Orono, USA

The full-grown mammalian oocyte, arrested in prophase of the first meiotic division, contains all of the molecules that will be utilized to bridge the period of transcriptional silence that begins upon completion of oocyte growth and lasts till the activation of the embryonic genome. Nuclei from differentiated somatic cells can be reprogrammed to totipotency in the oocyte milieu during the oocyte to embryo transition. During this period, approximately two days in the mouse, stores of maternal messages are selectively utilized resulting in the synthesis of known and novel proteins. The Gene Ontology vocabulary was used to annotate the molecular functions of the full-grown oocyte and 2 cell embryo transcriptomes and compare it with a composite transcriptome of all other cells and organs in the Mouse Genome Database. The 2 cell embryo is enriched in transcripts encoding translation regulators and RNA binding proteins and is depauperate in those encoding ligands and receptors. Gene expression during the oocyte to embryo transition is controlled by timely translation and homologues of factors described in non-mammalian cells, which bind to specific cis-sequences in the 3'UTR of mRNAs are also found in the mouse oocyte and early embryo. Expression of specific retroviral elements varies in a stage-specific fashion and may change expression of adjacent genes. These mobile elements affect gene evolution and my play a role in epigenetic restructuring of the embryonic genome.

# Retrotransposon-derived imprinted genes essential for development

**Fumitoshi Ishino[1], Ryuichi Ono[1], Yoichi Sekita[1], Shunsuke Suzuki[1], Mie Naruse[1,2], Takashi Kohda[1] and Tomoko Kaneko-Ishino[3]**

[1] Medical Research Institute, Tokyo Medical and Dental University, [2] JSPS research fellow, [3] School of Health Sciences, Tokai University

The completion of three mammalian genome projects, those of the human, mouse, and rat, clearly showed that 30-40% of mammalian genomes are derived from retrotransposons; however, most have lost their jumping activity and have long been considered junk. Recently, we and other groups have reported that there are some actively transcribed Ty3/gypsy retrotransposon-derived genes that are highly conserved in mammalian genomes. Among them, two were identified as imprinted genes *Peg10* and *Peg11/Rtl1*, located on mouse proximal chromosome 6 and distal chromosome 12, respectively. To elucidate functions of such evolutionarily conserved retrotransposon-derived genes, we produced *Peg10* and *Peg11/Rtl1* knockout mice. Surprisingly, both genes were demonstrated to be essential for mammalian development: *Peg10* KO mice showed early embryonic lethality and *Peg11/Rtl1* KO mice showed late embryonic/neonatal lethality associated with retarded growth. These phenotypes are corresponding well to those in mice with maternal duplication of the proximal chromosome 6 and of the distal chromosome 12, respectively. Importantly, severe placental problems were observed and related to these phenotypes in both cases.

Our work demonstrates that *Peg10* and *Peg11/Rtl1* are, at least, one of the responsible genes in these imprinted regions and suggests the significant role of some retrotransposon-derived for acquisition of functional placentas during mammalian evolution.

# Regulation of imprinting and epigenetic reprogramming in mammalian development

**Wolf Reik, Chun-Fung Chan, Lisa Redrup, Diana Lucifero, Claire Dawson, Fatima Santos, Myriam Hemberger, and Wendy Dean**

Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Cambridge CB22 3AT, UK

We are interested in the epigenetic mechanisms that regulate genomic imprinting and epigenetic reprogramming in mammals. In genomic imprinting our attention is currently focussed on the role of the non-coding RNA Kcnq1ot1 whose paternal expression is crucial for the paternal inactivation of a cluster of imprinted genes. The Kcnq1ot1 promoter region is methylated in oocytes but not sperm and Kcnq1ot1 is paternally expressed from the 2 cell embryo stage. Adjacent genes in the imprinting cluster are either ubiquitously imprinted and acquire gene silencing and repressive histone modifications by the blastocyst stage, or are only imprinted in the placenta and acquire silencing and repressive histone marks during differentiation of extraembryonic lineages. The properties of the Kcnq1ot1 non-coding RNA and how it may induce gene silencing in cis are being studied.

While non-coding RNAs and histone marks are involved in postzygotic gene silencing in imprinting clusters, DNA methylation is the primary mark in the parental germlines. This means that methylation imprints need to be erased or reprogrammed in primordial germ cells. Substantial epigenetic reprogramming including demethylation of DNA also occurs during early development in the zygote and preimplantation embryo. We are interested in the biological purposes and the molecular mechanisms of reprogramming. Reprogramming is likely to be important for the erasure of imprints, genetic conflict, and pluripotency of embryo and stem cells. Demethylation of DNA is of special significance for reprogramming. We show that cytidine deaminases when targeted to a methylated imprinted region in vivo can cause efficient demethylation. These results together with recent insights from plants suggest that DNA deaminases and glycosylases together with DNA repair are involved in demethylation.

# Epigenetic programming of the genome in the embryo and germ cells

**Azim Surani**

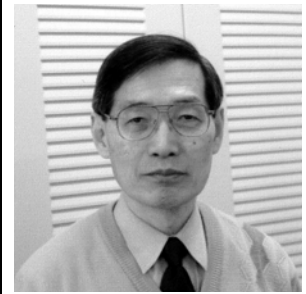
Wellcome Trust cancer Research UK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK

Extensive and dynamic epigenetic programming events occur during early mammalian development, and in the germ cell lineage. In the zygote, parental genomes exhibit asymmetrical histone and DNA methylation changes. Nuclear transplantation of somatic nuclei to the oocyte reveals that the oocyte and the epiblast have distinct properties for stepwise reprogramming of the genome. This is clearly evident when comparing reprogramming of Xi and Xa in female somatic nuclei transplanted into the mouse oocyte. These epigenetic events are also unusual compared to the epigenetic changes associated with normal development, suggesting that reprogramming events are subject to the existing epigenetic modifications in the donor nuclei.

The pluripotent epiblast that is established in the blastocyst is the source of both the somatic and germ cell lineage. The epiblast cells immediately after blastocyst implantation are destined to form somatic cells. This also evident because a key pluripotency-specific marker, *nanog*, is rapidly down regulated in the epiblast at the start of postimplantation development. However, in a few cells that acquire germ cell fate, there is active repression of the somatic programme by Blimp1, a known transcriptional repressor with a SET/PR domain. Recent evidence shows that Blimp1 forms a novel complex with PRMT5, an arginine methylase for H2A and H4R3me2s.

Following specification of PGCs, there are further extensive epigenetic changes, which may represent erasure of the somatic programme, together with the establishment of the germ cell-specific chromatin signature. This includes up regulation of H3trimeK27 (together with Ezh2) and loss of H3dimeK9. These changes are accompanied by re-expression of nanog immediately following specification of PGCs. Further dynamic changes in the chromatin and histone modifications occur following the entry of PGCs into the genital ridges, which include DNA demethylation and re-activation of the X chromosome and erasure of imprints.

# DISCOVERY OF IMPRINTED X-INACTIVATION IN EUTHERIAN MAMMALS



**Nobuo Takagi**

Hokusei Gakuen University

The novel method of chromosome preparation from mouse embryos during early organogenesis (Wroblewska and Dyban, 1969) made it possible to delineate metaphase chromosomes even from such small embryos at E6.5 and E7.5 with minimal cell loss. Later, Dutrillaux et al. (1973) showed that the inactivated X chromosome and autosomes are readily identified by acridine orange staining after incorporation of BrdU in the latter half of the S phase. The generous gift of the mouse stock carrying the Cattanach translocation (Cattanach, 1961) from Dr. Mary Lyon was the last push to the detection of the imprinted paternal X inactivation in extraembryonic tissues. Furthermore, the cell lineage relationships established by Richard Gardner and his associates (1975) contributed greatly to the elucidation of the data obtained. Thus, a road had apparently been paved for the detection of imprinted inactivation, but completely unexpected was that we still do not know whether it is the rule or the exception in placental mammals.

# EVENTS LEADING TO THE DISCOVERY OF IMPRINTING

**Bruce Cattanach**

MRC Mammalian Genetics Unit, Harwell, Oxford UK.

The recognition of imprinting, the phenomenon whereby genomes, chromosomes, chromosome regions and ultimately a small subset of genes are differentially expressed according to parental origin, could be traced back to at least four different lines of work. There was the early insect work, X-inactivation studies, the embryological experiments, and finally work with chromosome translocations.

It would probably be fair to say that the concept that maternally and paternally derived genomes could be marked or imprinted during parental gametogenesis was first recognized by Crouse (1960). She was working upon the selective elimination of the paternal X chromosome during male meiosis in the scale insect, Sciara. Other researchers applied the term to the heterochromatinisation of the whole paternal set that occurs in mealy bugs.

In mammals the first clear example was the preferential heterochromatic behaviour of the paternal X chromosome in the extraembryonic membranes of mice as recognized by Takagi & Sasaki (1975). The complete non-random paternal X-inactivation that occurred in marsupials provided a further example (Sharman, 1971), as also a consistent skew in the randomness of X inactivation in the somatic tissues of mice; the paternal X was observed by Cattanach & Perez (1970) to be more likely to become the active chromosome.

A bigger breakthrough occurred in embryological studies. Initially there had been evidence that parthenogenotes (Markert,1982) and androgenotes (Kagi & Ohama,1977) do not survive. However, prompted by the controversial Illmansee (1977) reports that survival could be induced, Surani et al (1984) and McGrath & Solter (1984) showed that both the male and female genomes were essential to embryonic development. The term imprinting was applied to the process by which the two genomes were marked in the parental gametes such that functioned differently in the zygote. Indications that the effect might involve chromosomes or even

genes was prompted by the non-random X inactivation work and by a parent of origin effect detected by Johnston involving a chromosome 17 effect.

The chromosome translocation work of Cattanach & Kirk (1985) conducted about the same time brought the phenomenon more into focus. Clues were again present many years earlier. Parental non-equivalence of a chromosome 7 region was indicated in Snell's (1946) translocation studies but the objectives of the work primarily concerned the mapping of chromosomes. Searle & Beechey (1975), in continuing this approach, found what they called non-complementation lethality, this arising with parental imbalance for certain chromosome regions. But the discovery of phenotypic abnormalities at birth, including growth and behavioural effects, with chromosome 11 disomies and chromosome 2 partial disomies clearly illustrated that it is not the whole genome that is involved; only certain chromosomes, certain chromosome regions, implying certain genes, may function differently according to parental origin. Since then further such work has identified some 10 regions of the genome that are imprinted.

By 1993 four genes had been found that were subject to imprinting. Currently, the number is over 80 and the majority of these lie within the recognized imprinting regions.