ASTORIA PARK HOTEL
Riva del Garda

EMBO/SEMM Workshop on

HOMEODOMAIN PROTEINS, HEMATOPOIETIC DEVELOPMENT and LEUKEMIAS

23RD/25TH MARCH 2006

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SCIENTIFIC & ORGANIZING COMMITTEE

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SCIENTIFIC PROGRAM

22rd/25th March, 2006

Arrival and departure details

March 22, 2006 — *Afternoon*

h. 16.00/20.30	Registration and poster positioning
h. 20.30	Welcome Dinner

March 26, 2006 — *Morning*

In the morning departure of all participants

MARCH 23, 2006
Homeodomain Proteins in Development

Morning session: Other Species

h. 09.00/09.40	**RICHARD S. MANN**, New York, USA *"Hox, Hth and Exd Functions in Fly Development"*
h. 09.40/10.10	**DALE FRANK**, Israel *"Xenopus Meis3 Protein in Vertebrate Nervous System Development"*
h. 10.10/10.40	**CHARLES SAGERSTROM**, Worcester, USA *"Hox/TALE Regulated Pathways in Hindbrain Development"*
h. 10.40/11.10	coffee break
h. 11.10/11.40	**FRANCESCO ARGENTON**, Padua, Italy *"Functional Subfunctionalization of Pknox Genes in Zebrafish"*
h. 11.40/12.10	**BERNARD PEERS**, Liege, Belgium *"Role of Zebrafish Pbx and Meis Genes in the Patterning of Endoderm and in Pancreas Development"*
h. 12.30/14.00	Lunch

MARCH 23, 2006
Homeodomain Proteins in Development

Afternoon session: Mouse

h. 14.00/14.30	**LICIA SELLERI**, New York, USA *"Pbx1/Pbx2 are Required for Vertebrate Distal Limb Pattering"*
h. 14.30/15.10	**STEPHEN TAPSCOTT**, Seattle, USA *"The Role of Pbx in Skeletal Myogenesis"*
h. 15.10/15.40	**HORST SIMON**, Heidelberg, Germany *"The Engrailed Transcription Factors and the Survival of Mesencephalic Dopaminergic Neurons"*
h. 15.40/16.10	**FILIPPO RIJLI**, Strasbourg, France *"Hox Genes and the Development of Somatosensory Circuits in Mice"*
h. 16.10/16.40	coffee break
h. 16.40/17.10	**ALAIN TREMBLEAU**, Paris, France *"Homeodomain Proteins as Regulators of Local Translation in Developing Sensory Systems"*
h. 17.10/17.50	**MICHAEL CLEARY**, Stanford, USA *"Pbx Mutant Mice Provide a Multi-Genic Model for Congenital Heart Disease"*
h. 17.50/18.30	**MOISES MALLO**, Lisbon, Portugal *"Hox Genes and Signalling Pathways"*
h. 18.30/18.50	**DIRK GEERTS**, Amsterdam, Netherlands *"The MEIS Signal Transduction Pathways in Neuroblastoma"*
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h. 21.30/22.30	**Poster session I**

MARCH 24, 2006
Homeodomain Proteins

Morning session: Molecular Mechanisms

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h. 09.40/10.20	**MIGUEL TORRES**, Madrid, Spain *"Homeodomain Proteins in Embryonic Hematopoiesis"*
h. 10.20/10.40	**ANDREA BRENDOLAN**, New York, USA *"A Pbx1-Dependent Genetic and Transcriptional Network Regulates Spleen Ontogeny"*
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h.11.10/11.30	**LISA MAVES**, Washington, USA *"Pbx Provides Competence to Activate Myogenesis in Response to Myod"*
h. 11.30/11.50	**ELISABETTA FERRETTI**, New York, USA *"Analysis of Skeletal Development in Pbx 1/2/3 Compound Mice"*
h. 11.50/12.20	**VINCENZO ZAPPAVIGNA**, Modena, Italy *"Pbx1 and Emx2 Interact to Form the Scapular and Pelvic Regions of the Limb"*
h. 12.30/14.00	Lunch

MARCH 24, 2006
Homeodomain Proteins

Afternoon session: Hematopoiesis

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h. 15.00/15.30	**ANDREW WASKIEWICZ**, Edmonton, Canada *"Zebrafish PBX Genes Are Required for Primitive Hematopoiesis"*
h. 15.30/16.00	**DMITRI PENKOV**, Moscow, Russia *"Involvement of Prep1 in the T-Lumphocytic Potential of Hematopoietic Precursors"*
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h. 16.30/17.00	**FRANCESCA FICARA**, Stanford, USA *"The Pbx1 Proto-Oncogene and Homeodomain Transcription Factor Regulates Adult Hematopoiesis"*
h. 17.00/17.40	**MARIA CRISTINA MAGLI**, Pisa, Italy *"Otx Genes in Hematopoiesis"*
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MARCH 25, 2006
Homeodomain Proteins and Malignant Transformation

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h. 09.40/10.10	**ALESSANDRA CARÈ**, Rome, Italy *"HOXB7: a Master Gene of Neoplastic Transformation"*
h. 10.10/10.30	**BARBARA CAUWELIER**, Ghent, Belgium *"The TCRB-HOXA Rearrangement in T-ALL Leads to a Specific Increase of the Alternative HOXA10b Transcript"*
h. 10.30/11.00	coffee break
h. 11.00/11.20	**HANNES KLUMP**, Hannover, Germany *"HoxB4 Expression Levels Critically Determine the Competence of ES-CELL Derivates to Mediate Hematopoietic long-Term Repopulation, In Vivo"*
h. 11.20/12.00	**MARK P. KAMPS**, La Jolla, USA *"Differentiation Arrest vs. Leukemogenesis-Distinctive Roles for Hoxa9 and Meis1"*
h. 12.00/12.20	**STEFAN HEINRICHS**, Boston, USA *"HoxB9 is Highly Expressed in Blast Cells in a Subset of Acute Myeloid Leukemia Patients and Supports Proliferation of AML Cell Lines"*
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MARCH 25, 2006
Homeodomain Proteins and Malignant Transformation

Afternoon session: Part II

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h. 15.50/16.10	**OLAF HEIDENREICH**, Tubingen, Germany *"RNA Interference, siRNA, Gene Expression Profiling, Acute Myeloid Leukaemia, AML1/MTG8"*
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h. 16.40/17.00	**ASEEM Z. ANSARI**, Madison, USA *"Designing Chemical Mimics of Hox Proteins"*
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h. 17.40/18.40	**General Discussion**
h. 20.00	Goodbye Dinner

LECTURES

Richard S. Mann
New York, USA

Dale Frank
Haifa, Israel

Charles Sagerstrom
Worcester, USA

Francesco Argenton
Padua, Italy

Bernard Peers
Liege, Belgium

Licia Selleri
New York, USA

Stephen Tapscott
Seattle, USA

Horst Simon
Heidelberg, Germany

Filippo Rijli
Strasbourg, France

Alain Trembleau
Paris, France

Michael Cleary
Stanford, USA

Moises Mallo
Lisbon, Portugal

Dirk Geerts
Amsterdam, The Netherlands

Claus Nerlov
Monterotondo, Italy

HOMEODOMAIN PROTEINS IN DEVELOPMENT

Morning session *Other Species*

Afternoon session *Mouse*

DIVISION OF LABOR AT THE HOMOTHORAX LOCUS OF DROSOPHILA

RICHARD S. MANN

Dept. Biochemistry and Molecular Biophysics, Columbia University

The homothorax (hth) gene of D. melanogaster is required for executing Hox functions, for head development, and for forming the proximodistal (PD) axis of the appendages. We have found that alternative splicing of hth generates two types of protein isoforms, one that contains a DNA binding homeodomain (HthFL) and one that does not contain a homeodomain (HDless). Both types of Hth isoforms contain the evolutionarily conserved HM domain that mediates a direct interaction with Extradenticle (Exd), another homeodomain protein. I will provide evidence that although both HthFL and HDless isoforms of Hth can induce the nuclear localization of Exd, they carry out remarkably distinct sets of functions during development. Surprisingly, we find that the majority of hth's functions, including PD patterning and most Hox-related activities, can be executed by the HDless isoforms. In contrast, antennal development shows an absolute dependency on the HthFL isoform. Thus, alternative splicing of hth results in the generation of multiple transcription factor complexes that execute unique functions in vivo. Interestingly, Meis1 and Prep2 also encode HDless isoforms, suggesting that homeodomain-less variants of this gene family are evolutionarily ancient.

XENOPUS MEIS3 PROTEIN IN VERTEBRATE NERVOUS SYSTEM DEVELOPMENT

DALE FRANK

Department of Biochemistry, The Rappaport Family Institute for Research in the Medical Sciences, Faculty of Medicine, Technion - Israel Institute of Technology, Haifa 31096, Israel

The developing amphibian nervous system arises in the ectoderm in response to signals secreted from the dorsal mesoderm (Spemann's Organizer). The initial induced state of the neural ectoderm is anterior, with additional factors being required to generate more posterior tissues. The proper formation of the antero-posterior pattern is crucial for correct nervous system development. We are investigating the function of the XMeis3 TALE-homeobox protein in posterior neural cell fate specification during early Xenopus laevis development. In XMeis3 knock down embryos, hindbrain, neural crest and primary neuron cells are eliminated. In these XMeis3 morphant embryos, expression of genes regulating neurogenesis, the Sox and Zic family proteins is unaltered. Injection of the Pax3 MO or ectopic levels of the Zic5 dominant-negative protein extinguish embryonic expression of the XMeis3 gene, thus triggering the subsequent loss of hindbrain, neural crest and primary neuron cell fates. Ectopic expression of HoxD1 protein, which is an XMeis3 direct-target gene, rescues these cell fate losses in XMeis3 and Zic5 knock down embryos. In the genetic cascade controlling early embryonic neural cell fates, XMeis3 protein lies below neuralizing, but upstream of regional-specific cell-fate determining genes. Thus, XMeis3 protein is positioned at a crucial focal point simultaneously regulating multiple cell fates during early vertebrate nervous system development.

HOX/TALE REGULATED PATHWAYS IN HINDBRAIN DEVELOPMENT

CHARLES SAGERSTROM

Dept. of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, USA

TALE homeodomain proteins form complexes with other transcription factors to control axial patterning of the developing embryo. We have begun to delineate the pathways acting downstream of TALE proteins during patterning of the zebrafish nervous system and gut tube. We have demonstrated that paralog group (PG) 1 Hox proteins act together with Meis and Pbx proteins to induce a number of genes required for formation of rhombomere (r) 4 and 5 of the zebrafish hindbrain. Our recent work has focused on r5 and indicates that the PG1 Hox-regulated pathway branches in r5 such that a vhnf1-dependent branch is required for repression of r4-genes and a val-dependent branch is required for krox20 and hoxa3 expression. Our microarray analyses have identified a number of novel hoxb1b/meis3/pbx4-regulated genes in the hindbrain and these are being integrated into existing pathways. Lastly, we find that meis3 is expressed in the endoderm immediately anterior to the forming pancreas. In this location, Meis3 appears to act in a complex with Pbx4 to control expression of foxa2 and shh, which are in turn required to repress pancreas gene expression. meis3 therefore appears to also control positioning of the pancreas primordium and patterning of the gut tube.

FUNCTIONAL SUBFUNCTIONALIZATION OF PKNOX GENES IN ZEBRAFISH

Enrico Vaccari, Gianluca Deflorian, Elisa Bernardi, Marino Bortolussi, FRANCESCO ARGENTON

Dipartimento di Biologia, Universita' degli Studi di Padova

Meinox proteins form heterotrimeric complexes with Hox and Pbx members, increasing the DNA binding specificity of Hox proteins in vitro and in vivo. The abundance of Meinox genes in zebrafish, six meis and two pknox genes, is likely to be the outcome of the genome duplication event. Hence, this abundance, provides the opportunity to study Meinox embryological functions in a model which is less affected by pleiotropy of phenotypes as the partial genetic redundancy of zebrafish genome has led to subfunctionalization (Force et al., 1999). In our laboratory, we have dissected the specific contributions of pknox1.1 and pknox1.2.
pknox1.1 is expressed maternally and ubiquitously up to 24 hours post-fertilization (hpf), and restricted to the head from 48 hpf onwards. Hindbrain segmentation and patterning is affected severely, as revealed by either loss or defective expression of several hindbrain markers including anteriorly expressed Hox genes. Furthermore, the heads of pknox1.1 morphants lacks all pharyngeal cartilages. This is caused by the inability of neural crest cells to differentiate into chondroblasts. Our results indicate that prep1.1 has a unique genetic function in craniofacial chondrogenesis and, acting as a member of Meinox-Pbc-Hox trimers, it plays an essential role in hindbrain development.
On the other side, pknox1.2 loss of function do not affect hindbrain segmentation and patterning; rather, injection of pknox1.2 morpholinos causes an altered identity of the cartilage of the hyoid arch, and the absence of cartilages in branchial arches 4-7. Embryological manipulations and promoter analysis shows that pknox1.2 mRNA expression is up-regulated by Retinoic Acid (RA). Our findings show that pknox1.2 plays an important role in the differentiation of cranial neural crest cells and in pharyngeal endoderm segmentation, while pknox1.1 is more important in hindbrain patterning and neural crest chondrogenesis, providing evidence of the functional specialization of pknox genes in zebrafish craniofacial development as well as head segmentation and morphogenesis.

ROLE OF ZEBRAFISH PBX AND MEIS GENES IN THE PATTERNING OF ENDODERM AND IN PANCREAS DEVELOPMENT

BERNARD PEERS

Université de Liège, BELGIUM

Studies in zebrafish have revealed an important function of the Meis/prep and Pbx genes in hindbrain patterning while their role in the endoderm and mesoderm derived organs is much less known. By studying transcription factors involved in the pancreatic expression of the zebrafish Pax6b gene, we identified Pbx/Meis/Prep complexes binding to 2 conserved regulatory motifs of the Pax6b promoter. This prompted us to search for TALE homeodomain factors expressed at significant levels in the developing pancreas. The expression pattern of 5 Meis/Prep genes and 5 Pbx genes was investigated in zebrafish embryos by in situ hybridisation. Pbx3, Pbx4 and Meis3 were detected closed to the pancreatic anlage but not within the endocrine islet as revealed by double in situ hybridisation experiments. Strong expression of these 3 TALE homeobox genes were located in mesodermal cells juxtaposed to the ventral pancreatic bud which generates the exocrine tissue. Loss of function studies, by injection of anti-sense Pbx4 or Meis3 morpholinos, mainly affected the formation of the pancreatic exocrine tissue while the other endodermal markers were much less affected. Interestingly, we observed that the locations of these endodermal markers were shifted anteriorly in the Pbx4 morphant embryos.

PBX1/PBX2 REQUIREMENT FOR DISTAL LIMB PATTERNING IS MEDIATED BY THE HIERARCHICAL CONTROL OF HOX SPATIAL DISTRIBUTION AND SHH EXPRESSION

T. Capellini, G. Di Giacomo, V. Salsi, V. Zappavigna and L. SELLERI

Department of Cell and Developmental Biology, Cornell University Weill Medical School and Graduate Program of Cornell University/ Sloan Kettering Institute New York, NY 10021, USA

Vertebrate limb development occurs along three cardinal axes: proximal-distal (P-D), anterior-posterior (A-P) and dorsal-ventral (D-V) that are established via the organization of signaling centers, such as the zone of polarizing activity (ZPA). Distal limb development, in turn, requires a molecular feedback loop between the ZPA expression of Sonic hedgehog (Shh) and the apical ectodermal ridge (AER). As of yet, the molecular triggers that activate Shh expression are mostly unknown, although a few candidate genes have been proposed. Hox genes, which encode homeodomain-containing DNA binding proteins, have been more recently proposed as upstream regulators of Shh and determinants of A-P and P-D axis formation. Finally, in vertebrates, functional ablation of multiple 5' Hox genes leads to distal limb truncations partially mediated by the lack of Shh. While the early phase of Hox expression appears essential for Shh onset, the dynamic expression of Hox genes throughout limb bud development aids in patterning elements along the P-D axis. Indeed, patterning alterations occur in limb skeletal elements of specific developmental modules in mice where multiple paralogous Hox genes have been genetically ablated, suggesting an internal functional redundancy within paralogous groups. These findings highlight that Hox genes act as global regulators of both axial and limb patterning throughout development. The present understanding of Hox function is that they exert their roles partially through the aid of cofactors, such as Pbx TALE homeoproteins that increase Hox DNA binding specificity and selectivity. We elucidated roles of Pbx genes in organogenesis and skeletal development. Indeed, we found that Pbx1 is required for proximal limb patterning. Specifically, in Pbx1 homozygous mutant (Pbx1-/-) embryos, skeletal structures of girdles (i.e., scapula and pelvis) and proximal limb stylopod (i.e., humerus and femur) that normally express nuclear Pbx1 at early developmental stages, are malformed, while, in contrast, their distal elements and joints appear normal. These findings parallel the role of Exd (the Drosophila Pbx homolog) in governing proximal domains of the fly appendage, where its expression is restricted. Conversely, we found that Pbx2 or Pbx3 loss does not determine skeletal or limb phenotypes. Here, we report that Pbx1 and Pbx2 are co-expressed in the early vertebrate limb field and that later, while Pbx2 is expressed throughout the limb bud mesenchyme, Pbx1 is expressed only proximally. Next, by exploiting a Pbx1/Pbx2 loss-of-function mouse model, we determine that, despite the lack of skeletal and/or limb abnormalities in Pbx2-/- embryos, decreasing Pbx2 dosage in the absence of Pbx1 does indeed affect limb development more severely than the loss of Pbx1 alone. Indeed, we demonstrate that compound Pbx1/Pbx2 embryos, in addition to their proximal limb defects, exhibit the appearance of novel and severe distal limb abnormalities; Pbx1-/-; Pbx2+/- embryos display loss of distal hindlimb elements; whereas Pbx1-/-; Pbx2-/- embryos lack limbs altogether. Furthermore, we establish that, unlike in flies, where the leg develops independently of Hox and Exd is required for specification of proximal, but not distal limb segments, in vertebrates distal limb patterning is Pbx1/Pbx2-dependent. Indeed, we demonstrate that Pbx genetic requirement is mediated, at least in part, through their hierarchical control of Hox spatial distribution and Shh expression. Overall, we establish that, by controlling Hox gene spatial expression in the posterior limb and regulating ZPA function, Pbx1/Pbx2 exert a primary hierarchical function on Hox genes, rather than behaving merely as Hox ancillary factors.

MYOD AND THE REGULATION OF SKELETAL MUSCLE GENE TRANSCRIPTION: THE ROLE OF PBX/MEIS

CA Berkes, L Maves, BH. Penn, DA Bergstrom, and SJ. TAPSCOTT

Fred Hutchinson Cancer Research Center, Seattle, WA USA

The expression of MyoD is sufficient to convert a fibroblast to a skeletal muscle cell, and, as such, is a model system in developmental biology for studying how a single initiating event can orchestrate a highly complex and predictable response. A combination of expression analysis and chromatin immunoprecipitation studies show that MyoD directly activates genes expressed both early and late during the program of cell differentiation. Temporal patterning of gene expression is achieved, at least in part, through a feed-forward mechanism: MyoD is sufficient to activate early genes, however, the activation of late genes requires both MyoD and additional transcription factors induced by MyoD. Specifically, MyoD regulates the expression of Mef2 isoforms and the activity of the p38 MAPK pathway. Subsequently, Mef2 and p38 cooperate with MyoD to regulate RNA polymerase II recruitment and progression at a subset of genes. Similarly, MyoD initiates the expression of Myog, and Myog is necessary together with MyoD to activate the expression of a set of late genes. Thereby, MyoD directly activates genes expressed throughout the program of muscle differentiation and uses a feed-forward regulatory circuit to generate a multi-stage transcriptional program. In the absence of MyoD, the other participating transcription factors are not sufficient to efficiently initiate expression of the target genes. This is due, at least in part, because these factors cannot bind to the promoters in the absence of MyoD, either because they cannot locate the promoters in chromatin or because they cannot recruit necessary chromatin remodeling proteins, or both. At a subset of promoters, the homeobox protein Pbx is necessary for MyoD to localize a gene within chromatin and initiate transcription. Pbx appears to be resident at a subset of promoters in both muscle and non-muscle cells and interacts with MyoD through the MyoD H/C and Helix 3 domains. This demonstrates a specific mechanism of targeting MyoD to loci in inactive chromatin and reveals a critical role of homeodomain proteins in marking specific genes for activation in the muscle lineage. These studies are beginning to merge our understanding of how lineage-specific information is encoded in chromatin with how master regulatory factors drive programs of cell differentiation.

THE ENGRAILED TRANSCRIPTION FACTORS AND THE SURVIVAL OF MESENCEPHALIC DOPAMINERGIC NEURONS

HORST SIMON

Centre for Neuroscience (IZN), Department of Neuroanatomy, University of Heidelberg, Germany

The homeobox transcription factors Engrailed-1 and -2 are expressed by mesencephalic dopaminergic neurons from midgestation into the adult. The two genes are cell-autonomously and in a gene-dose dependent manner required for the survival of this neuronal population. In homologous recombinant mutant mice null for both genes, the neurons are generated in the ventral midbrain, start to express their neurotransmitter phenotype, however, die briefly thereafter by apoptosis leading to the total loss of the cells at birth. Correlated to the allelic composition, intermediate genotypes between wildtype (En1+/+;En2+/+) and the engrailed double mutants (En1-/-,En2-/-) are characterized by various degrees of loss of mesencephalic dopaminergic neurons. We show here that a viable and fertile genotype, heterozygous null for En1 and homozygous null for En2 (En1+/-;En2-/-), exhibits a Parkinson-like progressive degeneration of DA neurons in the lateral and ventral tiers of the substantia nigra during the first three postnatal months. This specific loss results in a reduced storage and release of dopamine in the caudate putamen, to motor deficits reminescent of akinsesia and bradykinesia during Parkinson's disease, and to a loss of weigth, caused by a reduction in food uptake. Furthermore, in vitro gain and loss of function experiments demonstrate that the level of Engrailed expression determines the sensitivity of mesencephalic dopaminergic neurons to mitochondrial insult and to compounds which induce the mitochondrial pathway of apoptosis. Taken together, our data suggest that engrailed mutant mice are a new genetic model system for Parkinson's disease, which could be employed to unravel the molecular mechanism underlying the degeneration of the nigrostriatal system in man.

HOX GENES AND THE DEVELOPMENT OF SOMATOSENSORY CIRCUITS IN MICE

Frank Oury, Yasunori Murakami, and FILIPPO M. RIJLI

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The examination of the receptive properties of neurons in the somatosensory cortex of the brain has revealed a physical representation of the body surface in our brain, the so–called sensory homunculus. However, the molecular and cellular mechanisms underlying the building of the homunculus and the establishment of somatosensory maps in the developing brain are still poorly understood. In the rodent, the trigeminal pathway relays sensory information from the face to the cerebral cortex. Sensory inputs from the face are topographically mapped in the somatosensory cortex, via relay stations in the thalamus and hindbrain. Little is known about the molecular mechanims involved in the establishment of somatotopic order in this system. Hox genes are best known for providing positional identity and patterning information at early stages of central nervous system (CNS) development. There is still little evidence, however, about their potential involvement in later stages of CNS maturation, including the formation of neural circuitry. By means of ad hoc genetic tools, we found a direct correlation between rhombomeric origin of hindbrain trigeminal nuclei and their somatotopic organisation. Moreover, by way of both conventional and conditional knockout alleles we present evidence implicating Hoxa2 in the development of topographic wiring in the trigeminal system.

HOMEODOMAIN PROTEINS AS REGULATORS OF LOCAL TRANSLATION IN DEVELOPING SENSORY SYSTEMS

ALAIN TREMBLEAU

CNRS UMR 8542, Ecole Normale Supérieure and University Pierre and Marie Curie Paris 6, Paris, France

Local synthesis of proteins in neuronal processes plays key functions in the development of the nervous system (axon guidance, synaptogenesis) and synaptic plasticity. The molecular mechanisms at work in this regulated process are poorly understood. Our recent work show that homeodomain protein transcription factors are involved in these processes taking place locally in axons, by interacting with the protein synthesis machinery. Evidence for these new roles of homeodomain proteins in neurons were obtained in two vertebrate sensory systems, the developing visual system and the olfactory system.
The Engrailed-2 (En-2) homeodomain transcription factor is expressed in a caudal to rostral gradient in the developing midbrain where it plays an instructive role in patterning the optic tectum, the target of topographic retinal input. Besides its well-known role in regulating gene expression through its DNA-binding domain, En-2 has other domains that are involved in nuclear export, secretion, and internalisation, suggesting that it may also play a role in cell-cell communication. Consistent with this possibility, we demonstrated that an external gradient of En-2 protein strongly repels growth cones of Xenopus axons originating from the temporal retina and, conversely, attracts nasal axons. En-2 accumulates inside growth cones within minutes of exposure, and a mutant form of the protein that cannot enter cells fails to elicit turning. Once internalised, En-2 stimulates the rapid phosphorylation of proteins involved in translation initiation and triggers the local synthesis of new proteins. Furthermore, the turning responses of both nasal and temporal growth cones to En-2 are blocked by inhibitors of protein synthesis. The differential guidance of nasal and temporal axons reported here suggests that En-2 may participate directly in topographic map formation in the vertebrate visual system.
The Emx2 homeodomain protein is highly expressed by developing and adult olfactory sensory neurons (OSN) of the mouse. We showed that this protein is transported in the axonal compartment of these neurons, in which it is present in high density synaptosomal subfractions containing the eIF4E translation initiation factor. Emx2 and eIF4E can be co-immunoprecipitated from olfactory mucosa and bulb extracts and interact directly, as demonstrated in pull-down experiments. Our data suggest that Emx2 may have translational regulatory function in OSN axons. We are currently investigating these functions using transgenic approaches and translation reporters for mRNAs known to be transported in OSN axons, that encode proteins playing a role in OSN axonal guidance.

References :
Isabelle Brunet, Christine Weinl, Michael Piper, Alain Trembleau, Michel Volovitch, William Harris, Alain Prochiantz et Christine Holt: The transcription factor Engrailed-2 guides retinal axons (2005) Nature, 438, 94-98.
Stéphane Nedelec, Isabelle Foucher, Isabelle Brunet, Colette Bouillot, Alain Prochiantz et Alain Trembleau: Emx2 homeodomain trancription factor interacts with the translation initiation factor eIF4E in the axons of olfactory sensory neurons (2004) Proc. Natl. Acad. Sci. USA , 101, 10815-10820.

PBX MUTANT MICE PROVIDE A MULTI-GENIC MODEL FOR CONGENITAL HEART DISEASE

Ching-Pin Chang, Lei Chen, Gerald R. Crabtree, Licia Selleri, and MICHAEL L. CLEARY

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Formation of cardiac outflow tracts is a complex morphogenetic process, whose failure results in a variety of congenital heart malformations with major clinical consequences. We have observed that the construction of cardiac outflow tracts requires cooperative interactions among the family members of Pbx homeodomain transcription factors. The contributions of each member to outflow tract formation were analyzed in mice with ten different allelic combinations of Pbx1, 2 or 3 null mutations. Combined Pbx mutations result in a spectrum of cardiac anomalies representative of defects at specific stages of conotruncal, branchial artery, and semilunar valve formation. A major role is served by Pbx1, whose absence leads to persistent truncus arteriosus with failure of conotruncal septation and defects in branchial artery patterning. The reduction or absence of Pbx2 or Pbx3 results in Pbx1 haplo-insufficiency and specific malformations that resemble tetralogy of Fallot, overriding aorta with ventricular septal defect, and bicuspid semilunar valves. The results support a cooperative role for Pbx proteins in the regulation of conotruncal septation, equality of septation, aortic alignment and valvulogenesis. Thus, Pbx deficiencies define specific stages of cardiac outflow tract development and provide a multi-genetic model for congenital heart disease involving the cardiac outflow tract.

HOX GENES AND SIGNALING PATHWAYS

MOISÉS MALLO

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To understand the molecular mediators of Hoxa2 activity during mouse development we performed an Affymetrix GeneChip screen on the second branchial arches of wild type and Hoxa2 mutant embryos. We identified several genes that were up regulated in the absence of the Hoxa2 gene. As expression of these genes seems down regulated by Hoxa2 activity, we reasoned that there must be a mechanism for the activation of these target genes, which would be modulated by the Hoxa2 activity. A variety of analyses performed on some of these targets revealed that Hoxa2 interferes with FGF signaling during patterning of the second branchial arch. We also evaluated if Hox genes could also act in other biological contexts by modulating signaling processes. Our recent findings about the role of Hoxb4 on the expansion of hematopoietic progenitors will be discussed.

THE MEIS SIGNAL TRANSDUCTION PATHWAYS IN NEUROBLASTOMA

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Neuroblastoma is the most common extra-cranial solid tumor in children. It is a tumor of the peripheral sympathetic nervous system that probably already originates before birth. Neuroblastoma has an extremely diverse clinical outcome: some patients show spontaneous regression, but most patients die from rapidly metastasizing tumors despite aggressive treatment. So far, no genes have been identified that can fully explain the mechanism of neuroblastoma pathogenesis. The embryonic nature of neuroblastoma suggests the involvement of one or more key developmental genes.
We discovered genomic amplification of the MEIS1 homeobox gene in neuroblastoma cell line IMR32, and demonstrated high expression of MEIS1-3 genes in 23 other neuroblastoma cell lines using Northern blot. We then performed Affymetrix gene expression profiling of a series of 125 neuroblastoma tumors. MEIS gene expression showed significant difference between benign, well-differentiated ganglioneuroma tumors (n=13), where MEIS1 and MEIS3 expression is low, and aggressive, non-differentiated, neuroblastoma tumors (n=90), where they are highly expressed. Mixed-type ganglioneuroblastoma tumors (n = 22) showed an intermediate MEIS1 and MEIS3 expression. MEIS1 and MEIS3 expression correlated well with other important neuroblastoma oncogenes (e.g. PHOX2B and cyclin D1). Also, MEIS1 and MEIS3 showed significant correlation with cellular pathways like growth factor signaling, cell cycle, and cell death. Together, these results suggest an important role for MEIS genes in neuroblastoma differentiation.
We therefore analyzed the downstream pathways of MEIS genes by manipulating MEIS1 expression in neuroblastoma cell lines. Neuroblastoma cell lines were transfected with tetracycline-inducible expression vectors containing either MEIS1E dominant-negative cDNA, or MEIS1-specific siRNA. Induction of MEIS1 siRNA expression resulted in severely reduced cellular proliferation and increased cell death. Sustained MEIS1 expression appears vital for efficient neuroblastoma cell growth. To identify all MEIS1 downstream target genes, RNA of time course experiments of these transfectants was analyzed using Affymetrix DNA-micro arrays. Genes regulated by MEIS1 siRNA expression include genes involved in cell cycle, differentiation, and cell death. We now investigate the mechanisms by which MEIS genes regulate these cellular processes and interconnect with other important neuroblastoma oncogenes.

C/EBPALPHA IN DEVELOPMENT AND LEUKEMIA

CLAUS NERLOV

Mouse Biology, European Molecular Biology Laboratory, Monterotondo, Italy

The C/EBPalpha transcription factor is the founding member of the C/EBP family of basic region-leucine zipper proteins. C/EBPs are widely expressed during development and in the adult mammalian organism, and play a variety of roles in cellular differentiation, metabolism and immune function.
Of these proteins C/EBPalpha has emerged as a crucial regulator of the switch from a proliferative progenitor state to the non-proliferative terminally differentiated state. This is accomplished by incorporating two molecular functions into the C/EBPalpha protein: the ability to directly activate promoters associated with the differentiated phenotype of adipocytes, granulocytes and eosinophils, and the ability to block cell cycle progression through interference with the activity of E2F family transcription factors. Consistent with this copupling being important for suppressing neoplasia we have found that mice in which the E2F repressing function of C/EBPalpha has been disrupted through point mutation undergo transformation of the granulocyte lineage. Mutation of the CEBPA gene is observed in 7-10% of patients with acute myeloid leukemia, and the most frequent mutation involves selective ablation of translation of the 42kDa C/EBPalpha isoform (p42), while preserving translation of the shorter 30kDa isoform (p30). Such mutations also prevent C/EBPalpha-mediated E2F repression, and lead to increased progenitor proliferation and development of AML in a mouse model lacking specifically lacking p42. Interestingly, p30 still mediates the generation of granolocyte-macrophage progenitors, which are absent when the entire Cebpa gene is deleted, suggesting that the selective disruption of p42 allows the formation of, but not adequate proliferation control in, committed myeloid progenitors. Recent results indicate that this may lead to direct transformation of committed progenitors, in the absence of HSC-like leukemic stem cells.

Poster session I

A1

HOXB GENES FUNCTION IN THE MATURATION OF THE DORSAL HORN NEURON

Dinko Blazevic (MDC-Berlin), Carmen Birchmeier (MDC-Berlin)

The dorsal horn of the spinal cord receives and integrates sensory information from the body and transmits it to higher brain centers. Neurons of the dorsal horn are born in the second wave of neurogenesis in the spinal cord (E11.5- E13.5 in mice). As they leave the progenitor zone, they exit the cell cycle and undergo final differentiation. We have found that several of the HoxB genes, unlike their paralogs from the other clusters, are expressed in the maturing neurons of the dorsal horn along the whole length of the spinal cord. To address their function in this process we first raised the antibodies against HoxB3, B6, B7, B8, B9 to examine their expression on a cellular level, and analyzed the spinal cord phenotype of the HoxB cluster deletion mutant. Although all cell types characteristic for the dorsal spinal cord seem to be generated in normal numbers, the dorsal horn shows severe disorganization as early as E14.5. To identify the downstream targets of HoxB genes in the dorsal horn we performed a differential gene expression analysis using Affymetrix chips and identified a list of about 150 differentially expressed genes.

A2

A PREP-1 NULL MUTATION IS EMBRYONIC LETHAL AT E7.5

Luis C Fernandez-Diaz[1], Maria Teresa Fiorenza[2], Franco Mangia[2], Nancy A Jenkins[3], Neal G Copeland[3] and Francesco Blasi[1]

[1]Universita' Vita Salute San Raffaele and IFOM (FIRC Institute of Molecular Oncology), Milan, Italy. [2]Universita' di Roma La Sapienza, Rome, Italy. [3]NCI, National Institutes of Health, Frederick, MD, USA.

The Prep-1 Meinox protein is able to interact with all Pbx proteins, to prevent their export from the nucleus and to cooperate in DNA binding site selection. We have generated a Prep-1 null mouse by deleting its homeodomain by homologous recombination. Importantly, we have analysed in detail the mutation to exclude the production of a mutant protein (expressing the amino terminal region of the protein) acting as a dominant negative.The phenotype of the Prep1-/- mutation is embryonic lethality at E7.5. The result of an initial analysis of gastrulation markers, like brachyury, suggests a defect in early gastrulation, with no formation of the primitive streak. We have studied the expression of Prep1, Pbx1, Pbx2 and Pbx3 genes in the very early stages (from one cell to blastocyst) and found that Prep1, Pbx1 and Pbx3 were expressed already in the unfertilized oocyte and in the 2-cell stage. Prep1 continues to be expressed up to the blastocyst stage, while Pbx1 and Pbx3 are not observed at the 4-cells and morula stages but reappear in the blastocyst. Pbx2, on the other hand, was never expressed at none of the stages. Further expression studies at later developmental times will be important to understand the function of Prep1 in gastrulation.

A3

HDAC INHIBITOR AFFECTS HOXB2 EXPRESSION AND PREP1-PBX1-HOXB1 DNA-BINDING ACTIVITY IN THE NT2-D1 CELL LINE

Elena Longobardi and Francesco Blasi

Universita' Vita Salute San Raffaele and IFOM

Hox genes control cell fates and specify regional identities in vertebrate development. Despite intensive work many aspect of Hox expression regulation remain obscure. Furthermore a growing number of data suggest that dysregulation of homeobox-containing genes is involved in many hematologic malignancies as well as in development of solid tumors. We have analyzed the effect of inhibitors of histone-deacetylases on the expression of Hoxb genes in a retinoic-acid inducible human cell line, NT2-D1. The present work provide new insight on the mechanisms underlaying Hoxb2 regulation. We show that TSA (an HDACs inhibitor) blocks retinoic acid-induced Hoxb2 expression in the NT2-D1 cell line. Prep1, Pbx1 and Hoxb1 bind to PM/PH sites of the Hoxb2 enhancer as a ternary complex and the binding is required for Hoxb2 expression in rhombomere r4. Here we show that TSA abolishes the RA induced Hoxb2 expression in NTD21 cells by preventing the formation of the ternary complex. Western blot and RT-PCR data show that the addition of TSA does not alter either the level or the localization of Pbx1, Prep1 and Hoxb1 but nevertheless the DNA-binding activity of these proteins is strongly reduced (EMSA and Chromatin Immunoprecipitation).

A4

ROLE OF THE TRANSCRIPTION FACTOR PREP1 IN APOPTOSIS

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Laboratory of Molecular Genetics, Di.Bi.T. - S. Raffaele Scientific Insititute, Milano, Italy

Prep1 is an homeodomain protein, belonging to the TALE superfamily, that heterodimerizes with Pbx proteins, in a DNA-independent manner. The Prep/Pbx complex is translocated to the cell nucleus, where it acts as a transcriptional regulator. In this study, we have investigated the role of Prep1 in apoptosis in mammalian cells, using Mouse Embryo Fibroblasts (MEFs) obtained from WT and Prep1 hypomorphic mice (Prep1i/i). Prep1i/i MEFs were more sensitive to apoptosis after UV induction than the wt couterpart. We also observed a strong increase of active Caspases 9 and 3 and an altered expression level of p53 and other pro and antiapoptotic proteins. Interestingly, we observed that also the overexpression of Prep1, in different cell lines, induces cells to be more sensitive to apoptosis after UV irradiation. The results strongly indicate a role for Prep1 in programmed cell death and suggest a important role for cellular homeostasis.

A5

PBX AND MEIS FAMILY MEMBERS IN ZEBRAFISH FOREBRAIN DEVELOPMENT

F Pezzimenti[1], AJ Waskiewicz[2], M Ekker[3], M Mione[1]

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[3]Center for Advanced Research in Environmental Genomics Department of Biology, University of Ottawa, Canada

Forebrain patterning is orchestrated by the combined expression of transcription factors, which decode inductive interactions generated by signalling centers. Homeobox genes, including dlx family members, play important roles in maintaining ventral forebrain identity; moreover these genes are involved in the differentiation of diverse ventral domains along the antero-posterior axis. In this study we have explored the hypothesis that AP patterning of forebrain ventral domains may be coordinated by interactions of Dlx and Meis proteins. We focused on two meis genes, meis1 and meis2.2, which in zebrafish are expressed in discrete domains of the forebrain. We studied the expression of the two meis genes in the zebrafish forebrain throughout development and observed that meis2.2 is expressed at the pallial/subpallial boundary, in the preoptic area and in the caudal hypothalamus and is largely co-expressed with dlx2 and dlx4. Meis1 is more widely expressed throughout the telencephalon, thalamic region and hypothalamus. Next we explored the effects of meis2.2 morpholino knockdown on the differentiation of the ventral forebrain nuclei using specific markers for subregions of the ventral forebrain. Knockdown of meis2.2 alone or in combination with meis1 morpholino generates specific ablations of distinct nuclei, thus indicating that meis genes are differentially required for the development of forebrain nuclei. We are also exploring the interactions of meis and dlx homeoproteins with a pbx gene using the same panel of markers in morpholino co-injected zebrafish embryos.We hope that these studies will help to decipher the roles of meis proteins in the differentiation of specific ventral forebrain domains.

A6

PREP1 CONNECTS NUCLEAR ACTIN TO HOX GENE EXPRESSION

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Hox genes encode transcription factors that control patterning development. They are organised in group of clusters disposed in tandem. The colinearity of the HoxB cluster expression has been demonstrated also in mammalian cell lines. Retinoic acid induces the HoxB cluster expression in NT2 human cells over the time. Prep1 is an important component in the transcriptional regulation of Hox gene expression. A Prep1-HoxB1-Pbx trimer is required to activate the Hoxb1 and HoxB2 transcription and Prep1 down regulation in vivo (zebrafish and mouse) leads to decreased expression of several Hox genes.We have employed a TAP (Tandem Affinity Purification) strategy to identify and isolate Prep1-interacting proteins. We found that Prep1 interacts with non muscle myosins (NMMIIa and NMM1b), as well as beta-actin and gamma-actin. Little is known about the role of these proteins in the nucleus, and nothing on their possible role on the expression of the HoxB cluster. In NT2 cells, ChiP analyses shows that Pbx1b and inactive RNApolII are associated to the HoxB2 enhancer in control cells. Prep1 and beta-actin are found associated to the Hoxb2 enhancer after retinoic acid treatment, together with Pbx1b and the activated form of RNApolII (phosphorylated in serine 2). Using confocal fluorescence microscopy, we find that G-actin concentrates in active transcriptional zones in the nucleus of NT2 cells and co-localizes with hHpr1/p84/Thoc1, an mRNA processing factor associated with 2Pser-RNApolII. Latrunculin A, a G-actin sequestrator, enhances Hoxb2 but not HoxB1 expression, i.e. Hoxb2 is transcribed also in the absence of retinoic acid. Swiholide A, an actin dimer sequestrator, does not exert any effect over HoxB2 expression, reinforcing the hypothesis that monomeric actin could play a role in HoxB2 expression. We are presently studying the importance of NMMHC in Hox gene expression and the mechanisms connecting Prep1 to actin/myosin and to the expression of the Hox genes.

A7

XENOPUS MEIS3 PROTEIN LIES AT A KEY FOCAL POINT REGULATING MULTIPLE CELL-FATES DURING EARLY NERVOUS SYSTEM DEVELOPMENT

Rachel Ofir and Dale Frank

In Xenopus embryos, XMeis3 protein activity is required for normal hindbrain formation. Knock down of the XMeis3 protein also causes a loss of primary neuron and neural crest cell lineages. Expression of more upstream genes regulating neurogenesis of the Sox and Zic family proteins was unaltered by the knockdown of XMeis3 protein. Injection of the Pax3 MO or ectopic levels of the Zic5 dominant-negative protein extinguished embryonic expression of the XMeis3 gene, thus triggering a subsequent loss of hindbrain, neural crest and primary neuron cell fates. Ectopic expression of HoxD1 protein which is an XMeis3 direct-target gene rescues the loss of these cell fates in XMeis3 and Zic5 knock down embryos. In the genetic cascade controlling early embryonic neural cell fates, XMeis3 lies below neuralizing, but upstream of regional-specific cell-fate determining genes. Thus, XMeis3 protein is positioned at a crucial focal point simultaneously regulating multiple neural cell fates during early vertebrate nervous system development.

A8

ANALYSIS OF PREP1.2 ROLE DURING EMBRYOGENESIS PROVIDES THE EVIDENCE OF THE PREP GENES FUNCTIONAL SPECIALIZATION IN ZEBRAFISH HEAD SEGMENTATION AND MORPHOGENESIS

Gianluca Deflorian, Enrico Vaccari, Stefan Pauls, Marino Bortolussi and Francesco Argenton.

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In this study, we focused our attention on a second Meinox gene: prep1.2, the nearest prep1.1 paralog. In-situ hybridization experiments showed that prep1.2 is maternally and ubiquitously expressed up to the tail bud stage, and is more concentrated in the cranial neural crest cells at 24 hpf. prep1.2 loss of function did not affect hindbrain segmentation and patterning, as revealed by expression of several hindbrain markers including Hox genes. However, injection of prep1.2 morpholinos caused an altered identity of the cartilage of the hyoid arch, and the absence of cartilages in branchial arches 4-7. This latter effect was due to the absence of posterior migratory cranial neural crest cells, as shown by the lack of expression of, dlx2 and dlx3, as well as hoxa2, hoxb2 and valentino. Furthermore, we found that in prep1.2 morphants the posterior pharyngeal pouches fail to form. Our functional experiments also showed that prep1.2 mRNA expression is up-regulated by Retinoic Acid (RA). This data was confirmed by the analysis of the 5í-UTR of prep1.2 genomic sequence, in which we found a 3íRARE (Retinoic Acid Responsive Element). Using the luciferase reporter gene, we have demonstrated that prep1.2 3íRARE sequence responds positively in vivo to RA.

A9

NEW POTENTIAL ROLE FOR TALE PROTEINS IN THE CYTOSOL: TRANSCRIPTION FACTORS THAT BIND RNA

J. Carlos Villaescusa* and Francesco Blasi*^

**IFOM -FIRC Institute of Molecular Oncology, via Adamello 16, 20139 Milan, Italy.^DIBIT, Università Vita Salute San Raffaele, via Olgettina 58, 20132 Milan, Italy.*

Hox specificity is generally thought to be enhanced by the interaction with members of the TALE superclass of homeodomain proteins Meis/Prep and Pbx. However there is increasing evidence that Meis/Prep and Pbx proteins also function as partners for other non-Hox transcription factors. A novel cap-dependent translational control mechanism has recently been described that is not mediated by the canonical eIF4E protein. In Drosophila, this mechanism involves a highly conserved protein called eIF4E Homolog Protein (4EHP) and the homeodomain protein Bicoid, and the dimeric complex 4EHP-Bicoid is able to block translation of certain mRNAs. Potential 4EHP interacting proteins contain the 4EHP-binding motif. Prep and Meis members of the TALE superclass contain the 4EHP-binding motif, which is conserved throughout different species. Here, we show that Prep1 and 4EHP can be co-immunoprecipitated from ovary cytosolic fractions, and they both colocalize in the cytosol of mouse oocytes. Furthermore, specific anti-Prep and Pbx antibodies are capable of immunoprecipitating certain Hox gene mRNAs from oocytes.A similar result has been obtained with the leukemic cell line L1210, in which Prep1 and 4EHP are co-expressed in the cytosol. Immunoprecipitation of cytosolic extracts from cross-linked L1210 cells shows that both Prep1 and Pbx2 immuno-precipitate certain Hox mRNAs, suggesting that some TALE proteins have a role in mRNA translation.

A10

HOX11 PARALOGOUS GENES INTERACT WITH PAX2 AND EYA1 TO ACTIVATE SIX2 AND GDNF DURING KIDNEY DEVELOPMENT

Alisha Yallowitz, Nancy Gong, Hanshi Sun, Deneen Wellik

University of Michigan Medical School

Analyses of targeted mutations in mice have demonstrated that Six2 and Gdnf are genetically downstream of the Hox11 paralogous genes, Pax2 and Eya1 in early metanephric development. Pax2 has been shown to be directly involved in the downstream activation of Gdnf. Pax2 is not sufficient, however, as Pax2 protein is expressed in Hox11 and Eya1 mutants, yet no Gdnf expression is seen in either of these mutant kidneys. Our data demonstrates that Hox11, Eya1 and Pax2 are capable of synergistically activating both Six2 and Gdnf reporter constructs. Deletion of a conserved, putative Pax2/Hox binding site abrogates this expression. We have also demonstrated phsyical interactions between Hox11, Pax2 and Eya1, and Pax2 binding to the proposed site in the Six2 upstream sequence. We are currently testing this conserved site in transient transgenic experiments. These data, along with previous genetic results suggest that Hox genes interacting with the Pax-Eya-Six network may be a conserved mechanism during mammalian development.

LECTURES

Mark Featherstone
Montreal, Canada

Miguel Torres
Madrid, Spain

Francesco Blasi
Milan, Italy

Maria Cristina Magli
Pisa, Italy

ORAL PRESENTATIONS

Andrea Brendolan
New York, USA

Lisa Maves
Washington, USA

Elisabetta Ferretti
New York, USA

Vincenzo Zappavigna
Modena, Italy

Pengbo Zhou
New York, USA

Andrew Waskiewicz
Edmonton, Canada

Dmitri Penkov
Moscow, Russia

Francesca Ficara
Stanford, USA

HOMEODOMAIN PROTEINS

Morning session *Molecular Mechanisms*

Afternoon session *Hematopoiesis*

PKA-INDUCIBLE TRANSACTIVATION BY THE MEIS1A C-TERMINUS

MARK FEATHERSTONE

McGill Cancer Centre, Montreal, Canada

The murine Meis1 gene was first discovered as a common site of retroviral integration leading to myeloid leukemia in BXH2 mice. Experiments in mouse models have since established the leukemogenic potential of MEIS1 protein, and human MEIS1 activation has been shown to be a hallmark of human acute myeloid leukemia (AML). Increased MEIS1 expression has been documented for other human cancers as well. We have shown that C-termini of the MEIS1A and 1B isoforms harbour protein kinase A (PKA) -inducible transactivation domains. This activity is dependent on both common and unique domains in the 1A and 1B C-termini, but not on homeodomain-proximal residues most highly conserved across species. By contrast, the C-terminus of the related PREP1 protein fails to mediate PKA-inducible transactivation, providing an insight into the differential functions of these regulators. More recently, our collaborator, Dr. Guy Sauvageau (U. Montreal), has shown that this same short MEIS1A C-terminus is absolutely required for the leukemogenic properties of MEIS1A in mouse bone marrow. Moreover, C-terminal oncogenic function can be replaced with the VP16 transcriptional activation domain, consistent with a transcriptional mechanism underlying the leukemogenic properties of the MEIS1A C-terminus. The talk will present recent results addressing the mechanism by which the MEIS1A C-terminus exerts its transcriptional and oncogenic functions.

HOMEODOMAIN PROTEINS IN EMBRYONIC HEMATOPOIESIS

MIGUEL TORRES, Catalina Ana Castillo-Roselló, Valeria Azcoitia

Departamento de Inmunología y Oncología, Centro Nacional de Biotecnología, CSIC, Madrid

Homeodomain proteins of the Meis subfamily are expressed dynamically in several organs during embryogenesis and exert potent regulatory activity through their interaction with Hox proteins and other transcription factors. Here we show that Meis1 is expressed in the hematopoietic stem cell (HSC) compartment in the fetal liver, and in the primary sites of definitive hematopoiesis, including the aorta-gonad-mesonephros (AGM) mesenchyme, the hemogenic embryonic arterial endothelium, and hematopoietic clusters within the aorta, vitelline and umbilical arteries. We inactivated the Meis1 gene in mice and found that Meis1 mutant mice die between embryonic days 11.5 and 14.5, showing internal hemorrhage, liver hypoplasia and anemia. In Meis1 mutant mouse fetal liver and AGM, HSC compartments are severely underdeveloped and colony-forming potential is profoundly impaired. AGM mesenchymal cells expressing Runx1, an essential factor for definitive HSC specification, are almost absent in mutant mice. In addition, hematopoietic clusters in the dorsal aorta, vitelline and umbilical arteries, are reduced in size and number. These results show a requirement for Meis1 in the establishment of definitive hematopoiesis in the mouse embryo. Meis1 mutant mice also displayed complete agenesis of the megakaryocyte lineage and localized defects in vascular patterning, which may cause the hemorrhagic phenotype.
We are currently analyzing the involvement of Hox, Pbx and Prep proteins in Meis1 functions during hematopoiesis and determining Meis1 functions in other developmental processes.

A PBX1-DEPENDENT GENETIC AND TRANSCRIPTIONAL NETWORK REGULATES SPLEEN ONTOGENY

ANDREA BRENDOLAN, Joseph Giacalone, Richard Harvey*, and Licia Selleri

*Weill Medical College of Cornell UniversityDepartment of Cell and Developmental BiologyNew York, 10021 NY*Victor Chang Cardiac Research Institute, St Vincent's Hospital, Darlinghurst, New South Wales 2010, Australia*

The genetic control of cell fate specification, morphogenesis and expansion of the spleen, a crucial lymphoid organ, is poorly understood. In this study, we establish a genetic network that regulates spleen ontogeny, by analyzing asplenic mice mutant for the transcription factors Pbx1, Hox11, Nkx3.2 and Pod1. We show that Hox11 and Nkx2.5, earliest markers for splenic progenitors, are absent in the splenic anlage of Pbx1 homozygous mutant embryos, implicating the TALE homeoprotein Pbx1 in cell specification. Pbx1 and Hox11 genetically interact in spleen formation and loss of either is associated with a similar reduction of progenitor cell proliferation and failed expansion of the splenic primordium. Chromatin immunoprecipitation assays show that Pbx1 binds to the Hox11 promoter in spleen mesenchymal cells, which co-express Pbx1 and Hox11. Furthermore, Hox11 binds its own promoter in vivo and acts synergistically with TALE proteins to activate transcription, supporting its role in an auto-regulatory circuit. These studies establish a Pbx1-Hox11-dependent genetic and transcriptional pathway in early spleen ontogeny. Finally, specific inactivation of Pbx1 in spleen mesenchyme circumvents embryonic lethality associated with ìglobalî Pbx1 loss. Our preliminary data show that Pbx1 spleen specific loss disrupts spleen morphogenesis, further confirming the central role of Pbx1 in spleen development and organ expansion.

PBX PROVIDES COMPETENCE TO ACTIVATE MYOGENESIS IN RESPONSE TO MYOD

LISA MAVES, Cecilia B. Moens and Stephen J. Tapscott

Fred Hutchinson Cancer Research CenterSeattle, Washington, USA

The basic helix-loop-helix transcription factor MyoD initiates skeletal muscle differentiation by directly regulating muscle-specific gene expression. We are investigating how MyoD regulates its target genes to activate myogenesis. Pbx homeodomain factors have been found in a complex with MyoD and have been proposed to mark promoters of certain target genes, including myogenin, for activation by MyoD. Using a combination of approaches in zebrafish, we have found that Pbx proteins are required for proper myogenin expression and muscle differentiation. myogenin expression is delayed and reduced in zebrafish embryos lacking Pbx function. Muscle patterning initially appears normal in Pbx-null embryos; however, muscle differentiation is delayed and incomplete. Slow muscle development appears normal in Pbx-null embryos, but fast muscle differentiation is inhibited. In wild-type zebrafish embryos, misexpression of myod causes widespread ectopic expression of myogenin and the muscle differentiation marker Myosin Heavy Chain. Loss of Pbx function blocks the ability of MyoD to activate ectopic myogenesis. Taken together, our results reveal a requirement for Pbx in vertebrate muscle development and show that Pbx is necessary to provide competence to respond to MyoD and properly activate a muscle differentiation program.

ANALYSIS OF SKELETAL DEVELOPMENT IN COMPOUND PBX1/2/3 MUTANT MICE

ELISABETTA FERRETTI*, TERENCE CAPPELINI*, Michael Depew, and Licia Selleri

Morphogenesis and patterning of the craniofacial and limb skeleton are built on complex, genetically controlled molecular networks. Genes encoding a number of regulatory proteins, such as secreted growth factors (and their receptors) and transcription factors, are crucial to initiate the establishment of patterns of gene expression that will result in developmental changes. Homeodomain-containing transcription factors are essential to these processes. One such homeodomain protein is Pbx1, a homolog of Drosophila Extradenticle (Exd), whose function in patterning the fly body plan has been demonstrated genetically. Pbx1 belongs to a family of four closely related mammalian homeodomain proteins, which collaboratively bind DNA with a large subset of Hox proteins to modulate their DNA binding specificities and to execute their developmental programs. To investigate the contributions of Pbx proteins to mammalian patterning and morphogenesis, we have generated knockout mice harboring null mutations for three family members: Pbx1 (Pbx1-/-), Pbx2 (Pbx2-/-) and Pbx3 (Pbx3-/-). Specifically, we have shown that Pbx1 deficiency results in embryonic lethality at gestational day 15/16, with widespread axial and appendicular skeleton patterning defects. Unlike Pbx1-/-, both Pbx2-/- and Pbx3-/- mice do not display abnormalities in skeletal development, indicating that Pbx1 plays a dominant role in these processes. Nonetheless, Pbx1 shares overlapping expression domains with both Pbx2 and Pbx3 in many tissues, including the craniofacial and limb skeleton, suggesting hidden, overlapping roles for these Pbx family members. Indeed, our recent results indicate that Pbx1;Pbx2 and Pbx1;Pbx3 double mutant embryos display an exacerbation of most Pbx1-/- skeletal defects with the appearance of novel, striking craniofacial and limb phenotypes. Results will be shown illustrating craniofacial and limb defects observed in compound Pbx mutants. The data obtained so far strongly suggest that critical, novel roles for Pbx2 and Pbx3 in skeletal development have yet to be uncovered.

**Equal contributions*

PBX1 AND EMX2 INTERACT TO FORM THE SCAPULAR AND PELVIC REGIONS OF THE LIMB

Giulia Vaccari∞, Massimo Pellegriniß, and VINCENZO ZAPPAVIGNA∞

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The master regulatory genes and the genetic pathways controlling the development of the most proximal limb elements, the limb girdles, are still largely unknown. Knockout mouse models for two genes expressed in the most proximal regions of the limbs, Emx2 and Pbx1, have provided new insight into the possible genetic pathways controlling limb girdle formation. Targeted disruption of Emx2 or Pbx1 is associated with severe developmental defects of the limb girdles. Structural features of the Emx2 protein suggest that it may heterodimerise with Pbx proteins. We therefore speculated that Emx2 and Pbx1 interact both genetically and functionally to pattern specific structures within the limb girdles. We have verified this possibility by testing for phenotypic interactions between Emx2 and Pbx1 in limb skeletal development in vivo. We generated Emx2 and Pbx1 double mutants and found that reducing the dosage of Emx2 exacerbates the defects caused by the loss of Pbx1. We are currently analysing the expression of other limb girdle patterning genes to understand the hierarchical relationships between these and Emx2/Pbx1 in girdle development. The formation of an Emx2/Pbx1 heterodimer and its molecular properties will be analysed as well.

PREP1 ROLE IN DEVELOPMENT IS EPISTATIC TO MEIS AND PBX. IDENTIFICATION OF KEY TARGET GENES AND OF NEW INTERACTORS

FRANCESCO BLASI

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A null Prep1 mutation causes embryos to die before gastrulation. Hypomorphic Prep1i/i embryos die mostly between E17.5-P0. These embryos are characterized by overall hypoplasy of all organs, deficient hematopoiesis with anemia, delayed erythrogenesis, decrease in Fetal Liver CFC activity and Long Term Repopulating-HSC .
Prep1i/i embryos also exhibit angiogenesis and eye defects, with decreased blood vessels in cultured allantois, decreased lens size and anomalies in retinal epithelium.
cMyb, Pax6 and Hoxa3genes, important in blood, eye and vasculogenesis , are strongly down-regulated in Prep1i/i embryos. Moreover, all Pbx and Meis proteins and/or mRNAs are decreased in Prep1i/i embryos.
Thus, Prep1 regulates the level of expression of its homologs and partners, Meis and Pbx, which results in a decrease of the expression of at least some Hox genes and of key developmental genes (like cMyb , Pax6 and Hoxa3).
Finally, we have identified a novel interactor , p160MBP, that competes with Pbx for Prep1 binding and that appears to be involved with Prep1 in regulating hematopoiesis.

UBIQUITIN-PROTEOLYTIC CONTROL OF HOX HOMEODOMAIN PROTEINS

Jianxuan Zhang[1], Jae-Hung Shieh[2], Yue Zhang[1], Gianni Morrone[2], Xiaoai Chen[1], Malcolm A. S. Moore[2], and PENGBO ZHOU[1]

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The HOX homeodomain proteins are key regulators of hematopoiesis and embryonic development. There are 39 HOX genes in vertebrates that are organized into four separate chromosomal clusters. HOX genes that are expressed in primitive hematopoietic cells, and their prompt downregulation is associated with hematopoietic differentiation and maturation. Although transcriptional inactivation of HOX genes during hematopoietic differentiation has been established, little is known about the biochemical mechanisms underlying the subsequent removal of HOX proteins. Here we report that the CUL-4A ubiquitination machinery controls the stability of HOXA9 and other HOX proteins by promoting their ubiquitination and proteasome-dependent degradation. The homeodomain of HOXA9 that mediates DNA-binding is also responsible for CUL-4A-dependent ubiquitination and degradation. Interfering CUL-4A biosynthesis resulted in alterations of the stability of HOXA9, mirrored by impairment of the 32D myeloid progenitor cells to undergo proper terminal differentiation into granulocytes. Furthermore, silencing of CUL-4A by RNA-mediated interference in human umbilical cord blood CD34+ cells significantly perturbed their self-renewal, expansion, and differentiation properties. These results revealed a novel regulatory mechanism of hematopoiesis by the ubiquitin-dependent proteolysis.

ZEBRAFISH PBX GENES ARE REQUIRED FOR PRIMITIVE HEMATOPOIESIS

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We are utilizing zebrafish Pbx-null embryos to investigate the role for Pbx proteins in the earliest stages of blood cell differentiation, primitive hematopoiesis in the lateral plate mesoderm. We demonstrate that Pbx is required for proper expression of both tal1 and fli1a (markers that label primitive precursors of both blood and vasculature). Furthermore, genes that specifically label hematopoietic cells (gata1 and runx1) are nearly eliminated in Pbx-null embryos. At later stages, Pbx null embryos lack detectable circulating blood cells, demonstrating that Pbx is required for primitive erythropoiesis. Given that Pbx proteins are cofactors of Hox proteins, but also are known to regulate hox gene expression in the hindbrain, we analyzed expression of caudal hox genes in Pbx null embryos. We find that expression of hoxb6b, hoxb7a, and hoxa9a are completely eliminated in Pbx null embryos. In a striking similarity to its function in the hindbrain, this clearly positions Pbx upstream of hox gene expression. We have identified a cryptic Pbx-binding hexapeptide in the para-hox protein Cdx4, and zebrafish Cdx4 is required for primitive hematopoiesis. As such, we are currently testing the hypothesis that Pbx cooperates with Cdx to regulate hox genes, and thereby control zebrafish primitive hematopoiesis.

INVOLVEMENT OF PREP1 IN THE T-LYMPHOCYTIC POTENTIAL OF HEMATOPOIETIC PRECURSORS

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Prep1 is an homeodomain transcription factor that acts by dimerizing with Pbx. The functions of Prep1 both in life and disease are not known. Since Prep1 null embryos die at gastrulation, we exploited Prep1i/i hypomorphic mice to study the post-natal role of Prep1. Reduced Prep1 expression caused an abnormal T cell development in the thymus, with increased DN thymocytes, decrease of SP thymocytes and increase in gamma delta T cells. Peripheral lymphoid organs of Prep1i/i mice contained fewer alpha beta T cells and more gamma delta T cells in comparison with wild-type littermates. Moreover, Prep1i/i DP thymocytes underwent more apoptosis and SP thymocytes proliferated less, than control littermates. Lethally irradiated mice transplanted with Prep1i/i fetal liver cells showed the same defects of the Prep1i/i mice. Among PBC family members, Pbx2 and very low levels of Pbx3 were observed in wild type thymus. In Prep1i/i thymocytes the level of Pbx2 protein was profoundly decreased, while the expression of Pbx3 was not changed at RNA level. We suggest that the deficient post-natal T-lymphocytic potential of the Prep1 hematopoietic progenitors depends on the combined, not compensated, absence of Prep1 and Pbx2. At the molecular level, an increase of expression of non-classical MHC class 1b genes (H2-T3, H2-T10/T22) was observed in Prep1i/i thymocytes. Since these genes are well-known ligands for gamma delta TCR, it is possible that their increase results in an enhancement of gamma delta T cell selection in Prep1i/i mice. We are currently investigating this possibility.

THE PBX1 PROTO-ONOCGENE AND HOMEODOMAIN TRANSCRIPTION FACTOR REGULATES ADULT HEMATOPOIESIS

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Pbx1 was originally discovered as the product of a proto-oncogene in pre-B cell leukemia. Pbx1 deficiency severely affects definitive hematopoiesis in the embryo by hampering hematopoietic stem cells (HSC) and/or progenitor proliferation. However, embryonic lethality prevents an assessment of its role in the adult HSC compartment and in lymphoid differentiation. To study Pbx1 in postnatal HSC a floxed Pbx1 gene was conditionally inactivated by Cre recombinase expressed under the control of the Tie2 promoter. In these animals, the size, cell number and microscopic structure of thymus and spleen were severely defective. We observed a three-fold decrease of B cells in the bone marrow (BM), mainly accounted for by a reduction of the pre-B cell subset. Most importantly, common lymphoid progenitors were reduced up to 10-fold, suggesting a possible target for Pbx1 function at the earliest stage of lymphoid development. Common myeloid progenitors and Lin-cKit+Sca1+ HSCs were also reduced, although to a lesser extent. BM cells from conditional ko mice were able to reconstitute congenic recipients in the absence of competition, but failed to engraft in competitive transplants, suggesting a possible defect of functional HSCs.In conclusion, Pbx1 has a crucial role in the development of the lympho-hematopoietic system, possibly controlling the proliferation and differentiation capacity of multiple stem and progenitor cell subsets.

OTX GENES IN HEMATOPOIESIS

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Homeobox-containing genes of different families are implicated in the control of hematopoiesis. Several lines of evidence, obtained in our laboratory, indicate that members of the Otx family play important roles in the regulation of hematopoietic stem and progenitor cells. Otx1, a homeobox gene of the paired class strictly required for brain morphogenesis, is expressed in murine fetal liver and bone marrow cells and loss of Otx1 function affects erythropoiesis and the differentiation of myelo-monocytic cells. Lack of Otx1 results in a cell-autonomous erythroid impairment and in altered ratios of granulocyte and macrophage progenitors. Conversely, Otx1 enforced expression in primary hematopoietic cells induces an increased number of erythroid cells and a concomitant decrease of myeloid cells. Furthermore, we have shown that Otx1 contributes to the control of erythropoiesis through a direct action on Scl/Tal1, a major hematopoietic regulator which, in contrast, is not relevant for Otx1 function in myelo-monocytic differentiation. Taken together, our data suggest that Otx1 is involved in the regulation of hematopoietic development and differentiation.

Poster session II:

Sandrine Sarrazin	B1
Catalina Ana Castillo Rosselló	B2
Elena Tenedini	B3
Karin Ackema	B4
Claudia Gemelli	B5
Jeroen Charite	B6
Monica Ballarino	B7
Takeaki Dohda	B8
Catherine Lavau	B9
Francesco Oriente	B10

B1

REDUCED PROLIFERATIVE CAPACITY OF HOX A3 AND HOX B3 DEFICIENT MYELOID PROGENITORS

Sandrine Sarrazin, Youssef Bakri, Athar Aziz, Noushine Mossadegh, Estelle Duprez and Michael H Sieweke

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We have analyzed the effect of Hox A3 and Hox B3 deficiency on hematopoietic progenitor development and proliferation. We found that Hox A3 and Hox B3 are expressed at several stages of myeloid differentiation and are required for normal proliferation potential of hematopoietic progenitors. Since Hox A3/Hox B3 double deficiency causes neonatal lethality we analyzed these populations in lethally irradiated mice reconstituted with Hox A3/Hox B3 deficient hematopoietic cells from fetal liver. We show that the number of both commune myeloid porgenitors (CMP) and bi-lineage committed progenitors (GMP, MEP) are significantly decreased which correlates with the reduced colony forming potential in semi-solid medium. This deficit in myeloid progenitors appears to involve a reduced proliferative capacity of these cells since we observed a reduced proliferation rate of Hox A3/Hox B3 double KO progenitors in response to SCM (Spleen Conditioned Medium) and specific myeloid cytokines (Il3, GM-CSF, G-CSF or M-CSF) which was not due to a trivial loss of expression of cytokine receptors, at least for M-CSF receptor, suggesting a more direct impact of Hox A3 and Hox B3 genes on proliferation control.So far studies of hematopoietic development in mice inactivated for Hox genes have only revealed weak abnormalities, probably due to strong compensatory effect particularly between genes from same paralog group. In this study using double knock-out mice, we define an important role of Hox A3 and Hox B3 in the control of myeloid progenitor proliferation.

B2

MEIS1 IN HEMATOPOIESIS

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Myeloid ecotropic viral insertion 1 (Meis1) gene is a member of the TALE family of transcription factors. In mouse models, Meis1, HoxA7, Hox A9 and Pbx1 (also member of the TALE family) are linked to induction myeloid & lymphoid leukemias. Meis1-deficient mice revealed that this gene affects the birth and establishment of the first definitive hematopoietic stem cells (HSC) in the mouse embryo.
The defects in Meis1 mutant mice may result from functional impairment of several Hox proteins with redundant functions in establishing the HSC population. On the other hand, Pbx1 represses HoxB4 HSC-promoting activity, suggesting that Pbx1 and Meis1 might antagonize each other in this process. Then, for the promotion of definitive hematopoiesis, Meis1 could thus function coupled to other Pbx proteins or independently of any Pbx activity. Alternatively, since both Meis and Pbx can also interact functionally with other non-Hox transcription factors, the role of Meis1 in establishing HSC could be unrelated to Hox protein activity. To answer these questions we are characterizing Hox genes expression in the aorta-gonad-mesonephros region of E10.5 mouse embryos and we are also working on the antagonism between Meis1 and Pbx using gof models in chick embryos.

B3

IDENTIFICATION OF A MOLECULAR SIGNATURE PREDICTIVE OF REFRACTORINESS IN ACUTE MYELOID LEUKEMIA

Elena Tenedini[1], Enrico Tagliafico[1], Rossella Manfredini[1], Francesco Ferrari[1], Enrica Roncaglia[1], Luca Fantoni[1], Alexis Grande[1], Sandra Parenti[1], Tommaso Zanocco-Marani[1], Claudia Gemelli[1], Tatiana Vignudelli[1], Monica Montanari[1], Roberta Zini[1], Simona Salati[1], Elisa Bianchi[1], Silvio Bicciato[2], Sergio Ferrari[1].

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Acute Myeloid Leukemia (AML) blast cells are immature committed myeloid cells unable to spontaneously undergo terminal maturation, characterized by heterogeneous sensitivity to natural differentiation inducers. No data are available so far by which infer the AMLís response to differentiating therapy. Thus, we have initially profiled by GeneChip arrays the gene expression of several AML cell lines: they derived by the original blast cell populations and are still characterized by the same immunophenotype, retain a different sensitivity or resistance to All-Trans Retinoic-Acid (ATRA) and Vitamin-D3 (VD) and never undergo spontaneously terminal maturation. Here we show that differences exist by which predict the cell line differentiation fate. Next we constructed a signature able to predict resistance or sensitivity to the differentiation induction and tested it, using a TaqMan platform, for its capability to predict the in-vitro response of 28 VD or ATRA treated AML blast cell populations. Finally, by a meta-analysis of public available microarray data we demonstrated that our signature of 11 genes, among them is particularly intriguing the presence of Meis1 and ID3, that was formerly designed to identify differentiation therapy resistant populations, turned out to be a good classifier for clusters of patients known to have poor prognostic significance.

B4

HOX GENES IN BONE MARROW MESENCHYMAL STEM CELLS

K.B. Ackema, Erasmus MCJ.Charite, Erasmus MC

Mesenchymal stem cells (MSC) constitute a rare cell population in the bone marrow of a wide range of mammalian species, capable of differentiating into several mesodermal lineages in vitro and in vivo, including osteoblasts, chondrocytes, adipocytes, and muscle cells. It is hypothesized that MSC play a role in regenerating the bone marrow stroma upon damage by various insults, and there is great interest in their clinical potential for tissue repair and gene therapy. To begin to understand the fundamentals of MSC biology, we are focusing on the Hox genes, which encode key regulators of mesoderm development. There is no cell surface marker profile that uniquely identifies MSC, and formalized in vivo transplantation assays analogous to those used for hematopoietic stem cells, have not been established to date. The closest approximation to the MSC are cells referred to as CFU-F, which are defined by their ability to give rise to plastic-adherent colonies of fibroblast-like cells in vitro, which can be expanded and differentiate into several mesenchymal lineages in vitro and in vivo. We have found that specific subsets of all four Hox clusters are expressed in murine CFU-F, and are currently investigating their regulation and their functions in these cells.

B5

ROLE OF HOXA10 HOMEOBOX PROTEIN IN HUMAN NORMAL MONOCYTOPOIESIS

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Several studies have proposed members of the HOX transcription factor family as key regulators of human hematopoiesis. In particular there is a great debate about the possible role played by HOXA10 in defining precise maturation lineages of hematopoietic stem cells. To dissect the biological function exerted by HOXA10 transcription factor in the process of human hematopoiesis, a cDNA coding for the investigated homeobox protein was retrovirally transduced in the U937 monoblastic cell line. The rationale of this experiment was supported by the observation that U937 cells efficiently undergo mono-macrophage differentiation upon exposure to the Vitamin D3 (VD) nuclear hormone and HOXA10 was demonstrated to behave as VD primary response gene in this cell context. The results obtained demonstrated that HOXA10 is able to induce the expression of the granulo-monocyte CD11b antigen with a remarkable inhibition of cell growth. To better characterize the role played by HOXA10 in a primary cell model, the same experiment was performed in human CD34+ stem / progenitor cells. Flow cytometry and gene expression analysis of transduced CD34+ cells revealed the acquisition of a clear monocyte phenotype that was achieved, at least partly, at the expense of other maturation lineages as the erythroid and granulocyte ones.

B6

TRANSCRIPTIONAL REGULATION OF HOX GENES IN THE HEMATOPOIETIC SYSTEM

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Hox transcription factors are among the most potent modulators of the self-renewal capacity of hematopoietic stem cells (HSC) currently known. Prerequisite to using this knowledge to devise strategies to enhance self-renewal in stem cell transplantation therapy in humans in a safe way, it is essential to know which of the Hox genes are endogenously involved in controlling self-renewal, and to understand how their expression is regulated.We use reverse genetics approaches to introduce viable reporter genes into endogenous Hox loci, to try to rigorously determine which Hox genes are actually expressed in HSC, and to follow their expression during HSC and hematopoietic progenitor development in vivo and in vitro.In addition, we are using mouse transgenesis to search for the cis-regulatory elements that drive expression of Hox genes in the adult hematopoietic system. Our data suggest that these elements are located at long distances from the genes they activate, and we are developing methods to speed up identification of such distant regulatory elements.

B7

IDENTIFICATION OF FACTORS REGULATING HAEMATOPOIETIC MICRORNAS TRANSCRIPTION

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MicroRNAs (miRNAs) are 21-23 nucleotides long RNA molecules that regulate the stability or the translational efficiency of target mRNAs. They are emerging as crucial players in several processes, particularly in cellular differentiation, proliferation and development. A number of evidences show that specific miRNA expression patterns are responsible for cell fate determination. Since miRNAs act in fine-tuning gene expression, our aim is to identify the upstream regulators of miRNA genes and to define their role in gene expression in concert with miRNAs themselves. Acute myeloid leukaemia represents an expansion of haematopoietic precursors blocked at different stages of differentiation. We previously identified a miRNA (miR-223) controlling the differentiation of human promyelocytic leukemia cells (NB4) into granulocytes (Fazi et al., 2005). We showed that miR-223 expression is controlled by a regulatory circuitry involving two transcriptional factors: NFIA and C/EBPalpha. To further investigate the mammalian transcription factors that regulate miRNAs during haematopoietic differentiation we intend to define the promoter elements required for their expression in different myeloid lineages.

B8

NOTCH1 GENE MUTATION LEADS DEREGULATION OF CELL CYCLE IN HUMAN T-ALL

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Notch signaling is essential for T-cell development, and Notch1 gain-of-function mutations are frequently found in T-cell acute lymphoblastic leukemia (T-ALL). We study how hyperactive Notch signaling leads to T-ALL, and whether this is mediated through the classical Notch downstream genes (Hes, Hey) and/or known T-cell oncogenes (e.g. Hox). We have confirmed previous observations that a number of cell lines from T-ALL patients exhibit elevated levels of the activated form of Notch1, with a new assay system that is fluorescence-based reporters to record Notch signaling in real time. Surprisingly, we observe only modest activation of Hes/Hey genes, while Notch activation is high. Based on these data, we hypothesize that the link between Notch signaling and cell cycle may involve components other than Hes/Hey. In support of this notion, we will show that p27 levels in T-ALL cell lines are altered in a Notch-dependent way. We are currently exploring how Hox genes are involved in this process.

B9

TRANSDUCTION OF MLL FUSION GENES IN FLT3 NULL BONE MARROW DOES NOT ALTER LEUKEMOGENESIS AND EX VIVO SENSITIVITY TO FLT3 INHIBITORS

Stèphanie Albouhair, Marta Libura, Ester Morgado and Catherine Lavau

CNRS UMR7151

Leukemias harboring chromosomal translocations involving MLL express high-levels of the receptor tyrosine kinase FLT3. To study its impact on leukemogenesis we used retroviral vectors to express MLL-ENL or MLL-CBP in Flt3+/+ and Flt3-/- bone marrow cells. Following transplantation, all mice succumbed to acute myeloid leukemia within 60 days (MLL-ENL) or 120 days (MLL-CBP) regardless of Flt3 status. Parameters reflecting cellular proliferation (leukocytosis, spleen weights) or maturation (morphology, immunophenotype) were unchanged by Flt3 genotype. Transplantability and clonality of the leukemias were also unaffected. The FLT3 protein was expressed in Flt3+/+ MLL-ENL leukemic blasts whose growth in vitro was inhibited by the FLT3 inhibitors PKC412 and AG1296. Surprisingly, Flt3-/- MLL-ENL leukemic cells were also responsive to these drugs. Furthermore, Flt3+/+ and Flt3-/- cells displayed the same sensitivity to the drugs with concentrations required to block MLL-ENL cell growth 10-fold higher than those sufficient to inhibit FLT3 signaling. This demonstrates that these compounds impair the growth of MLL-ENL leukemic cells through the inhibition of other kinases than FLT3. Altogether our data suggest that FLT3 is not a relevant therapeutic target to treat MLL-rearranged leukemias and stress the need for new insights into the mechanisms of these malignancies.

B10

THE HOMEODOMAIN TANSCRIPTION FACTOR PREP1: A NOVEL CANDIDATE FOR INSULIN RESISTANCE

Francesco Oriente, Pietro Formisano, Salvatore Iovino, Angela Vasaturo, Luis-Cesar Fernandez-Diaz*, Elisabetta Ferretti*, Claudia Miele, Angela Cassese, Chiara Romano, Giancarlo Troncone, Francesco Blasi* and Francesco Beguinot

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Prep1 is an homeodomain tanscription factor forming dimeric complexes with PBX and regulating the expression of developmental genes. Pbx1 null mice show pancreatic islet malformation and hypoinsulinemia with impaired glucose tolerance. We have studied the role Prep1 in regulating glucose homeostasis. Prep1i/+ mice displayed lower glucose levels than WT mice during insulin tolerance tests. Histology revealed smaller islets, consistent with a 35% decrease of glucagon and insulin secretion. The insulin/glucagon ratio was similar in Prep1i/+ and WT mice. However, streptozotocin-induced apoptosis of the islets caused diabetes only in WT, but not in Prep1i/+, mice. Pancreas morphology was abnormal in both types of animals and insulin secretion similarly reduced in WT and Prep1i/+ mice. Interestingly, insulin sensitivity was higher in Prep1i/+ mice. In fact, insulin receptor tyrosine phosphorylation and signalling were hyperactivated after insulin injection in muscle and in liver of the Prep1i/+mice. Experiments in L6 skeletal muscle and in HepG2 liver cell models overexpressing Prep1 showed decreased insulin signalling and reduced glucose transport and glycogen synthesis, suggesting that the Prep1 gene is a candidate for insulin resistance.

LECTURES

Takuro Nakamura
Tokyo, Japan

Alessandra Carè
Rome, Italy

Mark P. Kamps
La Jolla, USA

Francesco Lo Coco
Rome, Italy

Pier Giuseppe Pelicci
Milan, Italy

Margaret A. Goodell
Houston, USA

Myriam Alcalay
Milan, Italy

ORAL PRESENTATIONS

Barbara Cauwelier
Ghent, Belgium

Hannes Klump
Hannover, Germany

Stefan Heinrichs
Boston, USA

Olaf Heidenreich
Tubingen, Germany

Aseem Z. Ansari
Madison, USA

HOMEODOMAIN PROTEINS AND MALIGNAN TRANSFORMATION

Morning session *Part I*

Afternoon session *Part II*

IDENTIFICATION OF COOPERATIVE GENES FOR MEIS1 AND HOXA9 IN MYELOID LEUKEMOGENESIS

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Department of Carcinogenesis, The Cancer Institute, Japanese Foundation for Cancer Research, Tokyo.

Proper expression of AbdB-like Hox genes plays a key role in hematopoiesis and their deregulation perturbs myeloid differentiation, eventually resulting in leukemia with several cooperative genetic events. One of the important Hox genes in leukemogenesis is Hoxa9 that has been identified as a target for ecotropic retroviral integration in BXH2 murine AML as well as a fusion partner of NUP98 in human AML with t(7;11)(p15;p15). Importantly, it is observed that cooperative activation of Meis1 is essential for both Hoxa9- and NUP98-HOXA9-induced transformation of murine bone marrow cells. To gain further insights in Hoxa9/Meis1-induced leukemogenesis, retroviral insertional mutagenesis was carried out by using a Hoxa9 and Meis1 expressing retrovirus. Mouse bone marrow cells were transduced with the retrovirus and were transferred into sublethally irradiated mice. 100% of 28 mice died from AML in an average of 18 months and oligoclonal integrations of the retrovirus in leukemic cellswere detected. Total of 102 retroviral integration sites have been identified by inverse PCR and splinkerette PCR, and 6 were common integration sites (CISs). Three CISs at high incidence, Trib1, Mds1/Evi1 and Ahi1, were further analyzed, and these genes were found upregulated by retroviral integrations. These studies reveal Trib1, Mds1/Evi1 and Ahi1 as important genes to promote Hoxa9/Meis1-induced leukemia, and clarify the molecular pathway and genetic interaction in myeloid leukemogenesis.

HOXB7: A MASTER GENE OF NEOPLASTIC TRANSFORMATION

ALESSANDRA CARÈ

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We have shown a key role for HOXB7 in tumor proliferation and neoangiogenesis. In melanomas we demonstrated that basic fibroblast growth factor (bFGF), which represents the main autocrine growth factor, is a direct target of HOXB7. Accordingly, in the HOXB7-negative SkBr3 breast carcinoma, gene transduction of HOXB7 induced bFGF activation, independence from serum withdrawal, ability to form colonies in semisolid medium and increased growth rate. We also observed a strong up-modulation of several angiogenic and prometastatic factors, including VEGF, GRO□, IL-8, ANG-2 and MMP-9. We then studied the possible functional role of the HOX co-factors Pbx and Prep by co-expressing a deleted form of Pbx, acting as a dominant negative. The absence of functional Pbx/Prep molecules reduced the tumorigenic phenotype of the transduced cells, suggesting a role of these co-factors in HOXB7-induced tumor progression.
Parallel studies indicated HOXB7 involved in the modulation of the proliferative/differentiative program of hematopoietic stem/progenitor cells (HSCs/HPCs), where the HOXB7 proliferative stimulus may represent a pre-leukemic immortalization step, as suggested by a discrete population of blasts and immature precursors still present at late culture times.
Taken together these results indicate HOXB7 as a “master gene” involved in both normal and pathological remodeling and representing a good target to aim at to shut down many tumor-associated molecules through a single shot.

THE TCRB-HOXA REARRANGEMENT IN T-ALL LEADS TO A SPECIFIC INCREASE OF THE ALTERNATIVE HOXA10B TRANSCRIPT

BARBARA CAUWELIER, Frank Speleman

Recently, we described a new recurrent chromosomal aberration, inv(7)(p15q34) and t(7;7)(p15;q34) involving the TCRB (7q34) and the HOXA gene locus (7p15) in a subset of T-ALL patients (3.5%) leading to transcriptional activation of HOXA10 and HOXA11cluster genes. Screening of a cohort of 168 T-ALL cases revealed upregulated HOXA10 expression in 26% (44/168) of T-ALL cases whereas only 5 of 44 cases carried the TCRB-HOXA rearrangement. Since the HOXA10 gene shows two major transcripts (HOXA10a and HOXA10b), we wondered if the expression of the short transcript would be more specific for TCRB-HOXA rearranged cases. Therefore, 27 of 44 T-ALL patients and thymic subpopulations of increasing differentiation stages showing upregulated expression of HOXA10a transcript, were subsequently analysed for expression of the short HOXA10b transcript by real-time quantitive RT-PCR. This revealed an upregulated expression of HOXA10b in only 7 of the 27 samples analyzed. These 7 cases included all 5 TCRB-HOXA rearranged cases and only two additional patients without this rearrangement. Of further intrest, one MLL-AF9 and one CALM-AF10 positive patient with elevated expression of the HOXA10a transcript both lacked HOXA10b expression possibly suggesting a specific role for the HOXA10b short transcript in TCRB-HOXA mediated oncogenesis.

HOXB4 EXPRESSION LEVELS CRITICALLY DETERMINE THE COMPETENCE OFES-CELL DERIVATIVES TO MEDIATE HEMATOPOIETIC LONG-TERM REPOPULATION, IN VIVO

Sandra Pilat[1], Sebastian Carotta[1], Bernhard Schiedlmeier[2], Kenji Kamino[3], Manfred Schmidt[4], Christoph von Kalle[5], Peter Steinlein[6], Wolfram Ostertag[2], Christopher Baum[2,5], Hartmut Beug[6], and HANNES KLUMP[2]

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After in vitro differentiation of mouse embryonic stem (ES-) cells, efficient long term hematopoietic repopulation, in vivo, is especially efficient when the homeodomain transcription factor HOXB4 is ectopically expressed. We have recently shown that HOXB4-ES-cell derivatives behave similar to bone marrow cells also expressing this transcription factor ectopically, both in vitro and in vivo. Here we demonstrate that long term repopulation (>6 months) in Rag2(-/-)gammaC(-/-) recipient mice can also be achieved with ES-cell derived hematopoietic cells (ES-HCs) obtained from single ES-clones, but only when HOXB4 is expressed above a certain threshold level. Increased expression led to a high extent of chimerism in the bone marrow of transplanted mice (average 75%; range 45-95%, n=4) whereas ES-HC clones expressing lower levels only repopulated with very low efficiency (average 2.5% chimerism, range 1-4%, n=6 mice). These results suggest that HOXB4 can enforce the development of HSCs from ES-cells, but their long term repopulation capabilities are crucially dependent on the expression levels of HOXB4. Furthermore, mice reconstituted with ES-HC clones expressing high amounts of this protein recapitulated the morpho-histological phenotype observed in polyclonally reconstituted mice, including the bias towards myelopoiesis, ìbenignî myeloid proliferation in spleen and the incompatibility of HOXB4 expression with T-cell poiesis.

FUSION TO VP16 CONVERTS MEIS1 INTO AN ONCOPROTEIN THAT IMMORTALIZES PROGENITORS AND CAUSES AML IN THE ABSENCE OF COEXPRESSED HOX GENES: HOXA7 AND HOXA9 STRONGLY AUGMENT STEM CELL GENE TRANSCRIPTION IN VP16-MEIS1 PROGENITORS

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MEIS1 and Hoxa9 are homeobox transcription factors that promote self-renewal in hematopoietic progenitors. MEIS1 does not induce leukemia, but cooperates strongly with Hoxa9 to produce acute myeloid leukemia (AML). We have demonstrated that Hoxa7 or Hoxa9 block differentiation of myeloid progenitors that do not express MEIS1 and no not induce leukemic. Coexpression of MEIS1 induces transcription of genes that segregate with the leukemia-initiating subset of human AML blasts, such as CD34 and FLT3. We designate these genes as leukemic stem cell genes, or LSC genes. Meis1 promotes LCS gene transcription by a mechanism that requires interaction with PBX and DNA, and that also requires a short Meis1 C-terminal domain (CTD). To examine the transcriptional properties of Meis1 required for leukemic function, we use dominant transactivating or transrepressing forms of MEIS1 to determine whether the activation or repression function of Pbx:Meis1 complexes is sufficient to cause myeloid leukemia in combination with coexpressed Hoxa9. Fusion of MEIS1 to the Vp16 transactivation domain (but not the engrailed transrepression domain) produced an autonomous oncoprotein that immortalized progenitors, caused myeloid leukemias, and evoked weak expression of Meis1 LSC genes. Vp16-Meis1 required binding to Pbx and DNA; however, unlike Meis1, the CTD was not necessary in the context of Vp16-Meis1. This suggests that the CDT participates in target gene activation in AML blasts, a function replaced by Vp16 in its absence. Unlike the system reported recently by Mamo et al, VP16-Meis1 leukemias produced in our model did not express endogenous Hox genes. Retroviral expression of Hoxa9 or Hoxa7 within Vp16-Meis1 progenitors induced strong transcriptional up-regulation of LSC genes and elevated their leukemic potential to that of boni fied AML blasts. These data suggest that transactivation is the essential function of Pbx:Meis complexes in AML, and that Hox proteins cooperate with Meis:Pbx complexes to activate transcription of early progenitor genes whose expression is required for human AML. Consistent with this interpretation, chromatin IP experiments localize both Meis1 and Hoxa9 proximal to the FLT3 transcriptional initiation site.

HOXB9 IS HIGHLY EXPRESSED IN BLAST CELLS IN A SUBSET OF ACUTE MYELOID LEUKEMIA PATIENTS AND SUPPORTS PROLIFERATION OF AML CELL LINES

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HOXA9 has been identified as one of the most frequently deregulated genes in AML and MDS. However, other members of the HOX9 paralog group have not been implicated in leukemogenesis. Microarray gene expression analysis of 449 AML patients revealed that besides HOXA9 (61%), HOXB9 is upregulated in 15% of the cases, whereas the overexpression of HOXC9 and HOXD9 expression is a very rare event. The frequency of overexpression found in patient samples could be confirmed in AML cell lines. To identify downstream pathways driven by HOXB9 vector-based RNAi experiments were performed. In comparison to cells expressing a control shRNA, the cell lines transduced with HOXB9 specific shRNA vectors showed <5% of the control HOXB9 protein levels and a reduction in the cellular growth rate. Consequently, HOXB9 expression is required for the rapid growth of these AML cells. To gain insight into the molecular mechanisms underlying this phenotype global gene expression changes were analyzed. The HOXB9-dependent changes in the gene expression profile were determined by microarray experiments and analyzed by class neighbor identification and gene set enrichment analysis. Our results indicate that expression of HOXB9 maintains the cells of the leukemic clone in an undifferentiated and rapidly proliferative state.

ACUTE PROMYELOCYTIC LEUKEMIA AS A MODEL FOR TARGETED THERAPY OF HUMAN MALIGNANCIES

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Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia characterized by a block of differentiation at the promyelocytic stage. APL patients respond to pharmacological concentrations of all-trans retinoic acid (RA) and arsenic trioxide (ATO) and the basis of disease remission is terminal differentiation of leukemic blasts. The PML/RAR oncogenic transcription factor which recruits a histone deacetylase (HDAC) complex on target genes is responsible for both the pathogenesis of APL and for its sensitivity to RA and ATO. The molecular mechanisms of this disease have been studied with a belief that the results of these investigations could be applied to other acute myeloid leukemias (AMLs) and treatments that are based on such mechanisms would find a broad application in cancer therapeutics. Consistent with this view, several investigators described that HDACs may serve as common molecular targets in AML. Additionally, involvement of DNA methyl transferases (DMT) in the function of APL associated oncoproteins has been reported, thus pointing out that HDAC inhibitors and demethylating agents, may be a useful combination in anti-leukemic therapy for AML. Novel agents such as the callicheamicin anti-CD33 conjugate (Mylotarg) and are being used successfully in relapsed APL patients, but long-term results on both efficacy and toxicity of these drugs are are still awaited

P21 AS A TARGET OF LEUKEMIA-ASSOCIATED FUSION PROTEINS

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Stem/progenitor cells ensure for tissue and organism homeostasis and might represent a frequent target of transformation. Emerging concepts in tumor biology support the notion that tumors are hierarchically organized as abnormal tissues, which originate from, and are maintained by, transformed stem cells. Stem cells are relatively quiescent, while their more differentiated progeny have dramatic proliferative ability. The quiescence of stem cells is thought to be of critical biologic importance to prevent susceptibility to myelotoxic insults and consumption of the regenerative cell pool. In the absence of the cell cycle inhibitor p21, increased cell cycle leads to hematopoietic stem cell exhaustion and, under stress conditions (serial transplantation or myelotoxic treatments) haematopoietic cell depletion. We are investigating the existence, in leukemia animal models, of quiescent leukemic stem cells (LSC) and their role in the maintenance of the leukemic clone. We found that leukemic fusion proteins (AML1/ETO, PML/RAR, PLZF/RAR) induce up-regulation of p21 expression. Strikingly, the fusion protein AML1/ETO is unable to induce leukaemia in p21-/- mice and PML-RAR leukemias engineered into a p21-/- background fail to transplant into syngeneic animals. Ectopic expression of p21 into WT hematopoietic stem cells induces the expansion of the stem cell compartment, as evaluated by the long-term culture initiating-cell (LTC-IC) assay. Using a membrane stable fluorescent dye (PKH), we isolated a quiescent/slow-cycling leukemic cell population and showed that these cells have a stem cell phenotype and are able to transplant leukaemia. These data suggest that quiescient stem cells are pivotal in maintaining the leukaemia population.

NOVEL MASTER REGULATORS OF HEMATOPOIETIC DIFFERENTIATION REVEALED BY MULTI-LINEAGE GLOBAL EXPRESSION ANALYSIS

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Hematopoietic stem cells (HSC) gives rise to unique and divergent progeny to generate complete a hematologic and immune system. The mechanisms controlling this process are poorly defined, however several genes regulating aspects of differentiation have been identified. The genetic manipulation of these molecules results in aberrant differentiation, and several are implicated in the development of leukemias and lymphomas. In order to more comprehensively determine the factors governing hematopoietic differentiation, we have examined the transcriptome of highly purified mature hematopoietic cells (erythrocytes, granulocytes, monocytes, natural killer cells, activated and naïve T-cells, and B-cells), in addition to the long-term HSC, by using high-density oligonucleotide microarrays covering approximately two thirds of the coding mouse transcriptome. Gene expression cluster analysis indicates that HSC share many transcriptional similarities with lymphocytes, in particular, B-cells, NK cells, and activated T-cells, perhaps consistent with their similar lifestyle, or an evolutionary relationship. Furthermore, erythrocytes clustered separately from the myeloid branch lending support to the idea that erythrocytes rise from a progenitor other than the common myeloid progenitor. Determination of lineage-specific gene expression patterns revealed a unique transcriptional fingerprint of roughly 100 genes for each particular cell type, as well as nearly 9000 genes in common to all hematopoietic cells examined. Some of these are known markers or regulators for the cell type in which they were identified. Such genes include: 9 distinct NK cell lectin-like receptor family members, T-cell receptor on T-cells, Gata1 in erythrocytes, and macrophage scavenger receptor 1 on monocytes. Several of the fingerprint genes have been shown to be key regulators that are essential for generation of that lineage; for example, disruption of early B-cell factor 1 (Ebf1), a B-cell fingerprint gene, results in ablation of mature B-cells. The fingerprints also include many genes that have been scarcely studied, including a number of transcription factors, kinases, and G-protein-coupled receptors. We show that retroviral over-expression of some of these under-studied lineage-specific transcription factors results in significant skewing of hematopoietic differentiation toward the lineage in which they are found. Ongoing studies will determine whether other classes of fingerprint genes can similarly be used to increase, or decrease, the number of cells in a particular lineage, potentially leading to new therapeutic approaches to cancer and other hematopoietic diseases.

SIRNA-MEDIATED AML1/MTG8 DEPLETION AFFECTS DIFFERENTIATION AND PROLIFERATION-ASSOCIATED GENE EXPRESSION IN T(8;21)-POSITIVE CELL LINES AND PRIMARY AML BLASTS

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The chromosomal translocation t(8;21) is associated with 10 ñ 15% of al cases of acute myeloid leukaemia (AML). The resultant fusion protein AML1/MTG8 interferes with haematopoietic gene expression and is an important regulator of leukaemogenesis. We studied the effects of siRNA-mediated AML1/MTG8 depletion on global gene expression in t(8;21)-positive leukaemic cell lines and in primary AML blasts using cDNA arrays, oligonucleotide arrays and real-time RT-PCR. Suppression of AML1/MTG8 results in the increased expression of genes associated with myeloid differentiation such as AZU1, BPI, CTSG, LYZ and RNASE2 as well as of antiproliferative genes such as IGFBP7, MS4A3 and SLA both in blasts and in cell lines. Furthermore, expression levels of several genes affiliated with drug resistance or indicative of poor prognosis AML (BAALC, CD34, PRG2, TSPAN) are affected by AML1/MTG8 depletion. In conclusion, siRNA-mediated suppression of AML1/MTG8 cause very similar changes in gene expression pattern in t(8;21)-positive cell lines and in primary AML blasts. Currently, we identify direct AML1/MTG8 target genes. Furthermore, we analyze the contribution of selected target genes to the AM1L/MTG8-dependent leukaemic phenotype. The results obtained so far strongly suggest that the specific targeting of AML1/MTG8 function may be a promising approach for complementing existing treatment strategies.

DESIGNING CHEMICAL MIMICS OF HOX PROTEINS

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The Hox-family of transcription factors dictates the developmental program of various cells in an organism. In fact, several developmental defects are known to correlate with the disruption of HOX gene function.

Based on the crystal structures of Hox proteins we have created a class of small molecule analogs that mimic two key properties of Hox proteins in vitro (1). First, the compound targets DNA in a sequence-specific manner. Second, it interacts and recruits a key co-factor (Exd/Pbx1) that is thought to impart specificity and regulatory function on a subset of Hox proteins.

We intend to utilize synthetic Hox analogs to understand the functional properties of natural Hox proteins. The synthetic analogs have the potential to dissect transcriptional networks that govern developmental processes in cells and organisms. In addition, this class of 'artificial transcription factors' could, in the future, serve as precision-tailored therapeutic agents.

GENE EXPRESSION SIGNATURE OF LEUKEMIC STEM CELLS IN ACUTE MYELOID LEUKEMIA

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The precise nature of the hematopoietic cell targeted by the leukemogenic event is not known, although several investigations indicate that transformation occurs in very early progenitors. Leukemic stem cells (LSC), which are believed to represent a reservoir of leukemic cells with a low proliferative rate and ability to self-renew, may derive from transformed hematopoietic stem cells (HSC) or from more committed precursors that have reacquired stem cell characteristics.
The pathogenesis of acute myeloid leukemia (AML) is linked to oncogenic fusion proteins generated as a consequence of chromosomal translocations, found >50 % of AML cases. AML fusion proteins function as aberrant transcriptional regulators that interfere with myeloid differentiation and determine a stage-specific arrest of maturation. Approximately 35% of AMLs are instead associated with point mutations of the nucleophosmin (NPM) gene, which determine cytoplasmic localization of the NPM protein (NPMc+ AML). The mechanism of leukemogenesis triggered by NPM mutants is unknown, but is unlikely to depend on direct transcriptional regulation.
In order to gain insight into the molecular signature of LSCs, we have analyzed gene expression profiles in diverse types of AML models. Our results reveal that specific AML fusion proteins (AML1/ETO, PML/RAR*alfa*) are capable of directly activating genes involved in maintenance of the stem-cell phenotype, and repressing genes that regulate hematopoietic stem cell (HSC) commitment or differentiation, suggesting that fusion proteins may determine the activation/maintenance of stem cell circuits in LSC. NPMc+ leukemias display a specific gene expression profile dominated by a “stem cell” molecular signature, and in particular by the activation of numerous members of homeodomain-containing transcription factors, including HOX and TALE genes, some of which are implicated in hematopoietic development, and might reflect the molecular status of the LSC rather than represent a direct consequence of NPM mutations.

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