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ABSTRACTS

Remodelling of paternal chromatin in mammalian zygotes

Helena Fulka[1,2], Sugako Ogushi[3] and Josef Fulka, Jr.[2]

[1]*Institute of Experimental Medicine, Czech Academy of Sciences, Videnska 1083, CS-142 20 Prague 4, Czech Republic*

[2]*Institute of Animal Production, POB 1, CS-104 01 Prague 10, Czech Republic*

[3]*Graduate School of Science and Technology, Kobe University, Nada-Ku, Kobe 657-8501, Japan*

Recent results indicate that some abnormalities are more frequent in children conceived by ICSI (intracytoplasmatic sperm injection). The ICSI is widely used in human assisted reproduction. Where the abnormalities originate from is not known but it has been speculated that they are caused by dysregulation of parental genomes and probably are epigenetic in nature. During ICSI procedure many steps, such as sperm-oocyte binding, or gamete fusion, occurring during normal fertilization are bypassed. It has been speculated that these steps might be important for later processes taking place after fertilization. For example, data from Rhesus monkeys indicate that after ICSI different abnormalities in male pronucleus formation can be observed. Therefore it is desirable to study the techniques used in human assisted reproduction in a step-by-step manner.

For these reasons we have evaluated zygotes from natural mating, IVF (in vitro fertilization) and ICSI in the mouse for the presence of certain epigenetics marks (H3/K9 dimethylation and DNA methylation) which are known to localize to parental pronuclei asymmetrically and the formation of nuclear envelope (indicated by labelling for the nuclear pore complex – NPC). Results from these experiments show that there is no difference between individual groups. Therefore, if the embryo genome is dysregulated, this problem either arises later in development or is limited to certain sequences only.

There are also some indications that both the maternal (oocyte) and the paternal component (sperm) play an important role in the epigenetic remodelling of paternal genome after fertilization. To study these questions in humans is a very difficult task. The main reason is the lack of material and also ethical issues. Therefore, we have devised a system to study the remodelling of the paternal genome after interspecific ICSI. We have injected pig and human sperm into mouse oocytes. We have evaluated several epigenetic parameters on interspecific ICSI produced zygotes – localization of dimethylated H3/K9, labelling for methylated DNA, trimethylated H3/K4, HP1β and NPC. Our results indicate that both pig and human sperm injected into mouse oocytes can undergo normal remodelling showing the expected pattern of labelling for all the above mentioned antibodies. This also shows that mouse oocytes have a very strong remodelling capacity. The remodelled paternal pronuclei (especially in humans) can be further used for example in cytogenetic analysis.

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Allogeneic in utero bone marrow transplantation rescues the mutant phenotype in a murine model of osteopetrosis

A. Frattini[1], M. G. Sacco[1], F. Cerisoli[1], M. C. Magli[1], A. Pangrazio[1], H. C. Blair[2], A. Villa[1], **P. Vezzoni**[1]

[1]*Istituto Tecnologie Biomediche, Consiglio Nazionale delle Ricerche, Segrate,* [2]*Department of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, United States*

Infantile autosomal recessive osteopetrosis (ARO) is a severe bone disease, due to osteoclast malfunction, that causes many severe abnormalities as macrocephaly, deafness, blindness, hepatosplenomegaly and severe anemia, beginning in early infancy or in fetal life. Deafness and blindness are thought to be the effects of bone pressure on nerves. In more than 50% of cases, the defect is in the TCIRG1 gene, coding for the a3 subunit of the acidifying proton pump of the osteoclast. The only available treatment is bone marrow transplantation (BMT), which needs a HLA-matched donor and a conditioning regimen in order to avoid graft versus host disease (GVHD). However, post-natal BMT does not usually cure all the stigmata of the disease and even when engraftment is achieved, neither the growth impairment nor the cranial nerve defects are rescued. These considerations make ARO the best candidate to verify whether the in utero BMT approach could benefit patients who need to be treated early during fetal life. We exploited a spontaneous mutant mouse strain (*oc/oc*) whose underlying genetic defect and phenotype are identical to TCIRG1-dependent human ARO patients. Affected mice die early in life at 3 weeks of age with severe anemia, high bone density and complete absence of tooth eruption. We used this mouse model as recipient of in utero bone marrow transplantation (IUT). BM cells from a GPF positive outbred mouse strain (CD-1) were used as cell donors, simulating what occurs in patients who do not have HLA-matched donors. From thirty-eight females treated by in utero injection at 14.5 p.c ($5x10^6$ BM cells/per fetus), 14 *oc/oc* mice were born, five of these were alive after 4 weeks of life, showing a drastic improvement of the phenotype. Dramatic rescue of the phenotype is obtained by both permanent and transient engraftment, suggesting that differentiation of donor hematological progenitors along the osteoclast lineage in the critical perinatal period is sufficient to prevent most of the skeletal changes wich are the basis of the severity of ARO. Moreover, all the mice examined in our study did not show any sign of GVHD, which represents the major complication in postnatal BMT. The results described here suggest that in utero BMT in ARO patients could greatly improve and even normalize their clinical picture and could also of benefit in other diseases in which severe symptoms develop in fetal life.

Does fusion play a role in normal liver?

Francesca Faggioli°, Maria Grazia Sacco°, Juan Pablo Casado*, Paolo Vezzoni°

°Istituto di Tecnologie Biomediche, CNR, via Cervi 93, Segrate, Milano

**European Commission – DG JRC Institute of Health and Consumer Protection Joint Research Center, Ispra (VA)*

Cell fusion is part of normal development and tissue homeostasis. It has been shown to occur during the formation of myotubes, osteoclasts, trophoblast cells and macrophage-derived giant cells. We tried to investigate whether cell fusion also plays a physiological role in liver formation. Liver cells are known to have a DNA content greater than 2n and this feature is usually attributed to endoduplication. However, recent experiments performed in mice transplanted with exogenous cells showed that liver cells are particularly prone to fusion with injected cells. This raises the possibility that cell fusion could also occur as a physiological event. Nevertheless, these experimental models are based on an artificial system in which animals are heavily irradiated and the liver itself is acutely or chronically damaged.

To explore this issue, we created a "more" physiological system in which chimeric mice are produced by aggregation of two morulae, the first positive for the GFP, the second for the LacZ marker. These two genes have been extensively used as reporter genes. The presence in the liver of cells bearing both markers should definitively prove that cell fusion occurs in liver in physiological circumstances. The results obtained so far will be discussed.

Isolation, expansion & *in vitro* differentiation of sheep bone marrow mesenchymal cells

Vackova, J. Fulka Jr., CG. Cereser*, K. Matzukawa *, G. Ptak, P. Loi,
**Research Institute of Animal Production, Prague, Uhrineves, Czech Republic,*
** Department of Comparative Biomedical Sciences, Teramo, Italy*

Background. Adult, as well as embryonic stem cells could be the source of cells for human tissue replacement and stem cell therapy. It is necessary to verify this procedure first on an animal model. In the first part of our work we tried to isolate, propagate and differentiate adult stem cells - bone marrow (BM) derived - from sheep, a suitable animal model.

Materials and Methods. Bone marrow was collected from the iliac crest of female sheep and separated on Ficoll-Hypaque. Isolated mononuclear cells were cultured in αMEM with 10 % foetal bovine serum, 2 mM L-glutamine, 40 µl/ml gentamicin at 38.5°C in a humified atmosphere of 5 % CO2 in air. *Hepatic differentiation:* 2 weeks in initiation medium (αMEM, 2% FBS, 0.5µM dexamethasone, 50 mg/ml ITS+ premix, 50ng HGF), 2 weeks in maturation medium (αMEM, 2% FBS, 0.5µM dexamethasone, 50 mg/ml ITS+ premix, 50ng/ml oncostatin).
Neural differentiation: αMEM with 2% FBS + 200ng/ml NGF for 2 weeks.
Electroporation: BM cells were electtroporated on the passage 5 with Nucleofector II device (Amaxa). On passage 5 we checked the karyotype of the cells by standard procedures.

Results. The cells start to grow 2-3 days after seeding in clumps with fibroblast like morphology. They were able of clonal expression from a single cell. After culturing in hanging drops or on a glass surface, they were able to produce „marrow bodies". After culturing in basic conditions, these „bodies" start to grow up in fibroblast like shape. These newly attached cells proliferated as confirmed by BrdU incorporation.
Hepatic differentiation was detected both by immuno-histochemical detection of cytokeratin 18, (a marker of hepatic differentiation, ~ 50%) after one month of induction, and by the observation of the phenotype. In fact, the morphology of BM cells changed from fibroblast-like to oval cells. After 14 days culture with NGF we were able to see some neuron-like cells (~15%).
The karyotype on the passage 5 was normal (54 XX). The electroporation on the passage 5 resulted in more than 70% of GFP positive cells.

Conclusions. We were able to isolate, in vitro culture, propagate and successfully freeze sheep bone marrow mesenchimal cells after culture with appropriate growth factors these cells differentiated into hepatocyte-like and neuron-like cells. The cells had normal 2N karyotype and were successfully transfected with GFP protein.

Preliminary data on pig-bovine interspecies nuclear transfer embryo development

Irina Lagutina, Dario Brunetti, Giovanna Lazzari and Cesare Galli.
Laboratorio di Tecnologie della Riproduzione, ISILS, CIZ, Cremona, Italy

We constructed with zona-free method NT embryos using bovine (Bo) or porcine (Po) oocytes matured in vitro and bovine fetal fibroblasts (BFF), pig adult fibroblasts (PAF) and fetal (PFF) GFP-positive fibroblasts. Constructs were fused by double pulse of 30 µsec DC 1.2 kV/cm. Three-four h post fusion embryos with Bo were activated by 5 µM ionomycin for 4 min and incubated in 2 mM 6-DMAP in SOFaa for 4 h while embryos with Po were activated by double pulse of 30 µsec DC 1.2 kV/cm in the fusion medium with 1 mM Ca and incubated in 5 µg/ml cytochalasin B in SOFaa for 4 h. Embryos were cultured in SOFaa in 5% CO_2, 5% O_2 at 38.5°C. The NT embryo development and GFP expression (D7) were monitored.

Donor cell	Oocytes	N	Cleavage (%)	≥8 cells (%)	BL D6 (%)	GFP, %
BFF	bovine	39	38 (97.4)	29 (74.4)	29 (74.4)	NA
	pig	34	25 (73.5)	18 (52.9) *	0	NA
PAF 1	pig	51	46 (90.2)	25 (49)	10 (19.6)	NA
	bovine	41	40 (97.6)	25 (61)	0	NA
PAF 2	pig	79	69 (87.3)	33 (41.8)	30 (38)	NA
	bovine	99	99 (100)	61 (61.6)	0	NA
PFF	pig	83	70 (84.3)	37 (44.6)	23 (27.7)	100 (BLD6)
GFP+	bovine	234	212 (90.6)	119 (50.9)	0	84.4 (all)

*All embryos arrested at 4 cell stage

'Pig fibroblasts into Bo' embryos arrested at the 8-21-cell stage while 'BFF into Po' embryos – at the 4-cell stage. Overall significantly more 'Pig fibroblast into Bo' embryos were able to progress through 4-cell stage pig developmental block than normal pig NT embryos (57.8±3.5% vs 47.1±1.3%, t-test P=0.02). This study shows that early embryo development is driven by recipient cytoplasm up to the stage when genome activation should occur. The arrest of interspecies NT embryos at the stage of embryonic genome activation suggests that this developmental step is impaired. Grant ISS CS 11 and Eurostells programme from ESF.

The involvement of histone methylation in placenta-specific imprinting

Alexandre Wagschal, Heidi Sutherland*, Katia Delaval, Wendy Bickmore* and Robert Feil.

Institute of Molecular Genetics, CNRS, UMR-5535, Montpellier, France

**MRC, Western General Hospital, Edinburgh, UK*

Genomic imprinting is an essential mechanism whereby the expression of genes depends on whether the allele is inherited from the mother or the father. To date, some eighty imprinted genes have been identified in humans and mice, and imprinting is conserved in ruminant species as well. Many imprinted genes are essential for cellular proliferation and growth during development, others influence behaviour. The majority of these rather unusual genes are clustered in large chromosomal domains1.

In the mouse, several imprinted domains comprise genes that are imprinted in the trophoblast lineage only. One of these is the *Kcnq1* domain on distal chromosome 7, corresponding to the Beckwith-Wiedemann Syndrome region on human chromosome 11p15. The paternal repression along this >800-kb domain is controlled by an intronic 'imprinting control region' (ICR), the KvDMR1. This ICR is marked by DNA methylation on the maternally inherited allele. On its paternally inherited allele, it produces a non-coding RNA that could potentially be involved in the paternal silencing along the domain. We reported recently, that the paternal silencing along the *Kcnq1* domain arises early in development, and involves histone deacetylation and acquisition of lysine-27 trimethylation and lysine-9 dimethylation (and deacetylation) on histone H3 (Figure 3B). The Polycomb repressor complex PRC2 was found to be recruited to the paternal chromosome and could potentially be involved in the K27 trimethylation along the silenced chromosome.

In our current studies, we explore which histone methyltransferase (HMT) are involved in the paternal H3 lysine-9 methylation, and the silencing along the domain. Specifically, we have studied embryos and placentae obtained from a gene-trap mouse line deficient in the HMT G9a. G9a is involved in lysine 9 dimethylation on histone H3, in particular at euchromatic regions of the genome. Similar to published studies, we found G9a-/- conceptuses to be embryonic lethal, confirming that G9a is essential in development. At the *Kcnq1* domain, the genes that are imprinted in the trophoblast only, showed frequent loss of imprinting in the placenta. Interestingly, this paternal de-repression occurred to a different extent in the different G9a-/- placentae analysed. These findings suggest that G9a is one of the factors involved in the maintenance of imprinting in the placenta, specifically at genes whose allelic repression is independent of DNA methylation. An additional question that is being addressed is whether G9a is also important for imprinting maintenance at genes that have differential DNA methylation, and to which extent it plays a role in maintaining their differential DNA methylation.

Nuclear lamin A/C expression in bovine parthenotes and nuclear transfer embryos

R.D.W. Kelly [A] , R. Alberio [A] and K.H.S. Campbell [A]

School of Biosciences, The University of Nottingham, Loughborough, Leics, LE12 5RD, UK

Despite the apparent successes of nuclear transfer (NT) technology, numerous recent reports have indicated de-regulation of key gene expression patterns in NT embryos as compared to their in vivo and IVF counterparts. Aberrant expression of lamin A/C has been reported in mouse (Moreira *et al.,* 2003 *J. Cell Sci.* **116**, 3713-3720) and bovine (Sullivan *et al.,* 2004 *Biol. Rep.* **70**; 146-153) SCNT embryos, leading to the hypothesis that the presence of lamin A/C might affect subsequent. Lamin A/C expression is a potential marker for reprogramming due to the induced expression and remodelling during differentiation. Previously using immunofluorescence in bovine IVF embryos we have demonstrated the persistence of lamin A/C until the 2-cell stage (Kelly *et al.,* 2005 *Reprod. Fert. Dev.* **17**, 205-206). This study was initiated to further characterize lamin A/C expression in bovine parthenogenetic and NT embryos using a monoclonal antibody specific to lamin A/C. Bovine oocytes were matured in vitro as previously described (Fouladi-Nashta *et al*., 1998 *Biol. Rep.* **59**; 255-262). SCNT embryos were constructed using lamin A/C positive, primary bovine fetal fibroblasts (BFF1) and in vitro matured, enucleated MII bovine oocytes. Oocyte cell couplets were fused at 24 hours post onset of maturation 1hour prior to activation. Oocyte activation was achieved with 7% ethanol for 7min followed by a 6hr incubation in mSOF containing 10µg/ml cycloheximide and cytochalasin B for the production of both NT and parthenogenetic embryos. Embryos were cultured in mSOFacci supplemented with 10% FCS and collected at various stages for immunofluorescence staining. Prior to fixation embryos were incubated in 2mg/ml protease to remove the zona pellucida. Samples were fixed in 100% methanol at −20°C for 20 min and then blocked for 1 h (4% goat serum in PBS) at RT. Embryos were then incubated overnight at 4°C with mouse anti-lamin A/C antibody (IgM; Santa Cruz, California) or with blocking solution as a control. Following the primary incubation, embryos were washed extensively in 1% BSA in PBS and then incubated with TRITC goat anti-mouse IgM (1:400) (Chemicon, USA) for 1 h at RT. Unbound secondary antibody was removed by washing with 1% BSA in PBS, and embryos were mounted in VectaSheild containing 4',6-diamidino-2-phenylindole (2 µg/mL). Images were viewed using epifluorescence (Leica DMR, Germany) and confocal microscopy (Leica TCS). Immunofluorescence labelling for of lamin A/C was indistinguishable for both parthenogenetic and NT preimplantation embryos. The pro-nuclei of parthenogenetic and NT zygotes were positively labelled for lamin A/C. Nuclear labelling was also observed in 2-cell embryos for each embryo type. All parthenogenetic and NT embryos examined from the 4-cell stage through to hatched blastocysts were devoid of any immunfluorescence labelling. Theses data suggest that remodelling of the nuclear lamina occurs correctly in bovine NT embryos.

An unexpected function for IL-3 in the embryonic development of hematopoietic stem cells

Catherine Robin[1], Katrin Ottersbach[1], Charles Durand[1], Marian Peeters[1], Lesley Vanes[2], Victor Tybulewicz[2] and Elaine Dzierzak[1]

[1]*Dept. of Cell Biology and Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands*

[2]*Division of Immune Cell Biology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.*

Cytokines play an important role in adult hematopoiesis, yet the function of many of them has not been examined in the embryonic hematopoietic system. During development, adult repopulating hematopoietic stem cells (HSCs) arise first in the E10.5 aorta-gonad-mesonephros (AGM) region. For the normal generation of AGM HSCs, the Runx1 transcription factor is required. Since *IL-3* is a known Runx1 target, we examined whether IL-3 or other cytokines can act on AGM HSCs. Using the Runx1 haploinsufficient mouse model, we show that IL-3 uniquely amplifies E11 AGM HSCs and is expressed *in vivo* in the AGM. Blocking experiments and transplantation studies with *IL-3* mutant AGMs implicate IL-3 in the expansion of HSCs in the embryo. Moreover, IL-3 promotes the growth of AGM HSCs prior to the stage E10.5, indicating that adult repopulating HSCs can be elicited at earlier stages. Thus, our studies support a novel and unexpected function for IL-3 during development.

GDNF signalling in kidney morphogenesis and spermatogenesis

Hannu Sariola[1]

[1]*Professor in Developmental Biology, Developmental Biology, Institute of Biomedicine, University of Helsinki, Finland*

Glial-cell-line-derived neurotrophic factor (GDNF) was originally identified as a survival factor for midbrain dopaminergic neurons. GDNF and related ligands, neurturin (NRTN), artemin (ARTN) and persephin (PSPN), maintain several neuronal populations in the central nervous systems, including midbrain dopamine neurons and motoneurons. In addition, GDNF, NRTN and ARTN support the survival and regulate the differentiation of many peripheral neurons, including sympathetic, parasympathetic, sensory and enteric neurons. GDNF has further critical roles outside the nervous system in the regulation of kidney morphogenesis and spermatogenesis. GDNF family ligands bind to specific GDNF family receptor alpha (GFRalpha) proteins, all of which form receptor complexes and signal through the RET receptor tyrosine kinase. The biology of GDNF signalling is much more complex than originally assumed. The neurotrophic effect of GDNF, except in motoneurons, requires the presence of transforming growth factor beta, which activates the transport of GFRalpha1 to the cell membrane. GDNF can also signal RET independently through GFR1alpha. Upon ligand binding, GDNF in complex with GFRalpha1 may interact with heparan sulphate glycosaminoglycans to activate the Met receptor tyrosine kinase through cytoplasmic Src-family kinases. GDNF family ligands also signal through the neural cell adhesion molecule NCAM. In cells lacking RET, GDNF binds with high affinity to the NCAM and GFRalpha1 complex, which activates Fyn and FAK.

Transplantation of expanded ventral mesencephalic progenitor cells leads to functional recovery in a rat model of Parkinson's disease

Pia Jensen[1], Emil G. Pedersen[1], Jens Zimmer[1], and Morten Meyer[1]

[1]*Anatomy and Neurobiology, Institute of Medical Biology, University of Southern Denmark, Winslwparken 21, Odense C, Denmark*

Among the dopaminergic neurons in substantia nigra pars compacta and in the ventral tegmental area, subpopulations express the calcium-binding proteins calbindin (CB) and calretinin (CR), and the CB-containing neurons are supposed to be less prone to degeneration in Parkinson's disease. Glial cell line-derived neurotrophic factor (GDNF) is a potent survival factor for nigrostriatal dopaminergic neurons. Using free-floating roller-tube (FFRT) cultures derived from fetal rat (E14) ventral mesencephalon we found that GDNF (10 ng/ml) significantly increased the number of surviving tyrosine hydroxylase (TH)-immunoreactive neurons. The possible effects of GDNF treatment on CB-immunoreactive (CB-ir) and CR-ir neurons in such cultures were examined in the present study. The neuronal cell densities were measured by quantifying the numbers of CB-ir and CR-ir neurons in areas of sections through the most extensive parts of the spherical cultures. In 4-day-old and 8-day-old cultures GDNF treatment increased the density of CB-ir neurons by 50% and 59%, respectively. Partial co-existence of TH and CB was shown using the method of double immunolabeling. The density of CR-containing neurons was unaffected by GDNF treatment as confirmed by Western blotting for CR. Parallel effects of GDNF treatment were obtained for cultures of human fetal ventral mesencephalon (8 weeks postconception). In conclusion, our findings identify GDNF as a potent factor for fetal rat and human nigral CB-ir neurons able to promote their survival in culture. Referring to a suggested neuroprotective role of CB, the results may be of relevance in the context of neuronal transplantation of patients suffering from severe Parkinson's disease.

Redundant functions of Fgfr1, Fgfr2 and Fgfr3 in the development of the mid- and hindbrain

Juha Partanen[1]

[1]*Institute of Biotechnology, Biocenter 1, P.O. Box 56, 00014 University of Helsinki, Finland*

Fibroblast growth factor 8 (FGF8) mediates the function of the midbrain-hindbrain organizer (MHO). FGF signals are transmitted by means of four known FGF receptors (FGFRs). Studies of Fgfr expression in early vertebrate development have shown that Fgfr1 is expressed along the entire neural tube, whereas Fgfr2 and Fgfr3 expression has been shown to spare the tissue adjacent to the MHO. The FGF8 signal from the MHO, therefore, was believed to be transmitted by FGFR1 exclusively. However, incongruent results from conditional mutants of Fgf8 and Fgfr1 in the midbrain-hindbrain (MHB) region contradict this hypothesis. Therefore, we reexamined the expression of the Fgfrs in this region. Fgfr1 is expressed all over the neural tube. Strikingly, Fgfr2 is expressed throughout the floor plate of the MHB region. In the basal plate, Fgfr2 directly abuts the Fgf8 expression domain at the MHO, anteriorly and posteriorly. Fgfr3 expression is in contact with the Fgf8 expression domain only in the rostroventral hindbrain. Based on these findings, we postulate a role for FGFR2 and FGFR3 in FGF signaling in the ventral midbrain and hindbrain.

In vivo and *in vitro* fate of cortical progenitor cells

Ondrej Machon[1], Mattias Backman[1], Line Mygland[1], Zbynek Kozmik[2], Stefan Krauss[1]

[1]*Norwegian Center for Stem Cell Research (NCS), Department of Cellular and Genetic Therapy, Institute of Microbiology, the National Hospital, University of Oslo, Oslo, Norway*

[2]*Department of Transcriptional Regulation, Institute of Molecular Genetics, Videnska 1083, 142 00 Prague 4, Czech Republic*

Neural progenitors of the mouse forebrain can be propagated in vitro as neurospheres in the presence of bFGF and EGF. However, less is understood whether regional characteristics or developmental stage properties of these cells are maintained in neurosphere cultures. Here we show that the original cell fate is lost in neurosphere cultures. We isolated neural progenitors from the dorsal telencephalon of D6-GFP mice and cultured them in vitro. The expression profile was specifically changed in cultured cells in just three passages. Markers of the dorsal forebrain were downregulated and several ventrally-expressed genes were induced. The altered gene expression led to a profound phenotypic change of cultured cells. D6-GFP positive cortical progenitors produce excitatory neurons in the cortex and few astrocytes in vivo but after culture in vitro, these cells differentiate into many astrocytes and also oligodendrocytes and inhibitory neurons. Wnt signaling in cultured neurospheres was downregulated in the same manner as other dorsal markers but dominant active Wnt signaling slowed down the loss of the dorsal identity in neurospheres.

Endothelial cells in breast morphogenesis

Valgardur Sigurdsson [1,2,4] Geir Tryggvason[2] Magnus Karl Magnusson[1,3] Thorarinn Gudjonsson[1,2,4]

[1]*Molecular and Cell Biology Research Laboratory, Icelandic Cancer Society*
[2]*Department of Laboratory Hematology, Landspitali University Hospital*
[3]*Genetics & Molecular Medicine, Landspitali University Hospital*
[4]*Faculty of Medicine, University of Iceland*

Recent genome-wide expression analysis of breast cancer has brought new life to the classical idea of tumors as caricatures of the process of tissue renewal as envisioned by Pierce and Speers (Cancer Res 1988;48:1996-2004) more than a decade ago. The search for a cancer founder cell or different cancer founder cells is only possible if a hierarchy of differentiation has been established for the particular tissue in question. In the human breast, the luminal epithelial and myoepithelial lineages have been characterized extensively in situ by increasingly elaborate panel of markers, and methods to isolate, culture, and clone different subpopulations have improved dramatically. Comparisons have been made with the mouse mammary gland in physiological three-dimensional culture assays of morphogenesis, and the plasticity of breast epithelial cells has been challenged by immortalization and transformation. As a result of these efforts, several candidate progenitor cells have been proposed independently of each other, and some of their features have been compared. This research has all been done to better understand breast tissue homeostasis, cell-type diversification in general and breast cancer evolution in particular. The present review discusses the current approaches to address these issues and the measures taken to unravel and maintain cell type diversification for further investigation.

Regulation of spermatogonial stem cell self renewal and differentiation

Dirk G. de Rooij[1], A.M.M. van Pelt[1]

[1]*Endocrinology, Department of Biology, Faculty of Science, Utrecht University, The Netherlands*

The continuation of the spermatogenic process throughout life relies on a proper regulation of self-renewal and differentiation of the spermatogonial stem cells. These are single cells situated on the basal membrane of the seminiferous epithelium. Only 0.03% of all germ cells are spermatogonial stem cells. They are the only cell type that can repopulate and restore fertility to congenitally infertile recipient mice following transplantation. Although numerous expression markers have been helpful in isolating and enriching spermatogonial stem cells, such as expression of THY-1 and GFRalpha-1 and absence of c-kit, no specific marker for this cell type has yet been identified. Much effort has been put into developing a protocol for the maintenance of spermatogonial cells in vitro. Recently, coculture systems of testicular cells on various feeder cells have made it possible to culture spermatogonial stem cells for a long period of time, as was demonstrated by the transplantation assay. Even expansion of testicular cells, including the spermatogonial stem cells, has been achieved. In these culture systems, hormones and growth factors are investigated for their role in the process of proliferation of spermatogonial stem cells. At the moment the best culture system known still consists of a mixture of testicular cells with about 1.33% spermatogonial stem cells. Recently pure SV40 large T immortalized spermatogonial stem cell lines have been established. These c-kit-negative cell lines did not show any differentiation in vitro or in vivo. A telomerase immortalized c-kit-positive spermatogonial cell line has been established that was able to differentiate in vitro. Spermatocytes and even spermatids were formed. However, spermatogonial stem cell activity by means of the transplantation assay was not tested for this cell line. Both the primary long-term cultures and immortalized cell lines have represented a major step forward in investigating the regulation of spermatogonial self-renewal and differentiation, and will be useful for identifying specific molecular markers.

Role of Rac and Myc in epidermal homeostasis

Paul Newman[1], Salvador Aznar Benitah, Michaela Frye and Fiona Watt
[1]*Keratinocyte Lab, Cancer Research UK, 44 Lincolns Inn Fields, London, UK*

Mammalian epidermis is maintained by self-renewal of stem cells, but the underlying mechanisms are unknown. Deletion of Rac1, a Rho guanosine triphosphatase, in adult mouse epidermis stimulated stem cells to divide and undergo terminal differentiation, leading to failure to maintain the interfollicular epidermis, hair follicles, and sebaceous glands. Rac1 exerts its effects in the epidermis by negatively regulating c-Myc through p21-activated kinase 2(PAK2) phosphorylation. We conclude that a pleiotropic regulator of cell adhesion and the cytoskeleton plays a critical role in controlling exit from the stem cell niche and propose that Rac and Myc represent a global stem cell regulatory axis.

Stem cells in tooth development

Szabolcs Felszeghy[1], Marika Suomalainen[1] and Irma Thesleff[1]
[1]*Institute of Biotechnology, University of Helsinki, Finland*

Calvarial bone and suture development is under complex regulation where bone morphogenetic protein (Bmp) and fibroblast growth factor (Fgf) signalling interact with Msx2/Twist and Noggin and regulate frontal bone primordia proliferation and suture fusion, respectively. We have shown previously that the winged helix transcription factor Foxc1, which is necessary for calvarial bone development, is required for the Bmp regulation of Msx2. We now show that FGF2 regulates the expression of Foxc1, indicating that Foxc1 integrates Bmp and Fgf signalling pathways. We also show that Foxc1 is not needed for the acquisition of osteogenic potential or for the differentiation of osteoblasts. The expression of Fgf receptors and Twist were normal in Foxc1-deficient calvarial mesenchyme, and ectopic FGF2 was able to induce the expression Osteopontin. Furthermore, we demonstrate that Foxc1 does not participate in the regulation of Noggin expression. Our findings indicate that Foxc1 integrates the Bmp and Fgf signalling pathways independently of Twist or Noggin. This signalling network is essential for the correct patterning and growth of calvarial bones.

Developing human embryonic stem cell-based therapy for Parkinsons disease

Sofia Correia[1], Anke Brederlau[2], Sergey Anisimov[1], Cesine Paul[1], Laurent Roybon[1], Peter Eriksson[2], Patrik Brundin[1], and Jia-Yi Li[1]

[1] *Neuronal Survival Unit, Wallenberg Neuroscience Center, Lund University, Sweden*

[2] *Cellartis AB, Götheborg, Sweden*

Motor dysfunctions in Parkinson's disease are considered to be primarily due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta. Pharmacological therapies based on the principle of dopamine replacement are extremely valuable, but suffer from two main drawbacks: troubling side effects (e.g. dyskinesia) and loss of efficacy with disease progression. Transplantation of embryonic dopaminergic neurons has emerged as a therapeutic alternative. Enthusiasm following the success of the initial open-label trials has been dampened by the negative outcome of double-blind placebo controlled trials. Additionally, the emergence of graft-related dyskinesia indicates that the experimental grafting procedure requires further refinement before it can be developed into a therapy. Shortage of embryonic donor tissue limits large-scale clinical transplantation trials. We review three of the most attractive tissue sources of dopaminergic neurons for cell replacement therapy: human embryonic ventral mesencephalic tissue, embryonic and adult multipotent region-specific stem cells and embryonic stem cells. Recent developments in embryonic stem cell research and on their implications for a future transplantation therapy in Parkinson's disease are described. Finally, we discuss how human embryonic stem cells can be differentiated into dopaminergic neurons, and issues such as the numbers of dopaminergic neurons required for success and the risk for teratoma formation after implantation.