Spanish National Cancer Research Centre

CNIO FRONTIERS Meetings 2011

Recapturing Pluripotency: links between cellular reprogramming and cancer

7-9 NOVEMBER 2011

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CNIO FRONTIERS Meetings 2011

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Recapturing Pluripotency: links between cellular reprogramming and cancer

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Meetings 2011

Detailed programme

MONDAY, November 7th

09:30 **Welcome Address**
Maria A. Blasco & Konrad Hochedlinger

Session I: PLURIPOTENCY

Chair: Rudolf Jaenisch

09:45 **Rudolf Jaenisch,** Whitehead Institute for Biomedical Research, Cambridge, USA
Stem cells, pluripotency and nuclear reprogramming

10:20 **Pablo Menéndez,** GENyO (*Pfizer-Universidad de Granada-Junta de Andalucía* Centre for Genomics and Oncological Research), Granada, Spain
Short talk: *Residual expression of ectopic reprogramming factors prevents differentiation of iPSCs generated from human neonatal fibroblasts and cord blood-derived CD34+ progenitors*

10:35 **Alexander Meissner,** Harvard Medical School, Cambridge, USA
Epigenetic dynamics in stem cells and development

11:10 ***Coffee break and poster session***

11:40 **Azim Surani,** The Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, UK
Resetting the epigenome beyond pluripotency in the germ line

12:15 **Pentao Liu,** Wellcome Trust Sanger Institute, Cambridge, UK
Short talk: *Co-expressing Rarg and Lrh1 enables fast and efficient reprogramming to ground state pluripotency*

12:30 **Hans R. Schöler,** Max Planck Institute for Molecular Biomedicine, Münster, Germany
Induction of pluripotency by Oct4

13:05 ***Lunch and poster session***

Session II: REPROGRAMMING

Chair: Konrad Hochedlinger

15:05 **Konrad Hochedlinger,** Harvard University and HHMI, Boston, USA
Dissecting the mechanisms of cellular reprogramming

15:40 **Ángel G. Martín,** *Fundación Inbiomed*, San Sebastián, Spain
Short talk: *Sox2 expression in breast tumours and activation in breast cancer stem cells*

15:55 **Frank D. McKeon,** Genome Institute of Singapore; Harvard Medical School, Boston, USA
Origins of Barrett's esophagus: targeting stem cells in precursors of cancers

16:30 ***Coffee break and poster session***

17:00 **Maria A. Blasco,** CNIO, Madrid, Spain
The TRF1 telomere protein is essential for the generation of iPS cells and marks both pluripotent and adult stem cells

17:35 **Purificación Muñoz,** IDIBELL, Barcelona, Spain
Short talk: *Expansion and mobilization of hair follicle stem cells promote squamous cell carcinoma development in mouse skin*

17:50 **Juan Carlos Izpisua Belmonte,** Salk Institute for Biological Studies, San Diego, USA
Generation and correction of laminopathy-associated LMNA mutations in patient specific iPSCs

18:25 ***END of session***

TUESDAY, November 8th

Session III: CANCER – part 1

Chair: John E. Dick

09:30 **John E. Dick,** Princess Margaret Hospital, University Health Network and University of Toronto, Toronto, Canada
Towards unification of the cancer stem cell and clonal evolution models of cancer

10:05 **Tomomi Tsubouchi,** University of Sussex, Falmer, UK
Short talk: *Contribution of cell cycle stages to successful reprogramming towards pluripotency*

10:20 **Emmanuelle Passegué,** University of California, San Francisco, USA
Stress-response mechanisms and genome maintenance in normal and cancer stem cells

10:55 ***Group picture. Coffee break and poster session***

11:25 **Meinrad Busslinger,** Research Institute of Molecular Pathology, Vienna, Austria
Lineage commitment and plasticity in the lymphoid system

12:00 **Catriona Jamieson,** University of California, San Diego, USA
Malignant reprogramming in leukaemia

12:35 **María L. Toribio,** *Centro de Biología Molecular "Severo Ochoa"* (CBMSO-CSIC), Madrid, Spain
Short talk: *Notch1 signalling reprograms T-cell progenitors resident in the human thymus into bone-marrow grafting precursors: implications in leukaemia development*

12:50 **Scott Armstrong,** Children's Hospital, DFCI, Harvard Medical School, Boston, USA
Epigenetic programmes and cancer stem cell development

13:25 ***Lunch and poster session***

Session IV: CANCER – part 2

Chair: *Michael F. Clarke*

15:25 **Michael F. Clarke,** Stanford Institute for Stem Cell and Regenerative Medicine, Stanford University, Stanford, USA
Molecular analysis of normal and malignant mammary gland stem cells

16:00 **Pilar Sánchez,** *Instituto de Salud Carlos III* (ISCIII), Madrid, Spain
Short talk: *DYRK1A modulates the self-renewal capacity of neural stem cells and glioblastoma initiating cells through regulation of EGFR stability*

16:15 **Charlotte Kuperwasser,** Molecular Oncology Research Institute, Tufts University School of Medicine, Boston, USA
Cellular reprogramming during the formation of breast cancer

16:50 ***Coffee break and poster session***

17:20 **Fiona Watt,** Cancer Research UK Cambridge Research Institute, Cambridge, UK
Contribution of stem cells and differentiated cells to development of tumours of multilayered epithelia

17:55 **Mirna Pérez-Moreno,** CNIO, Madrid, Spain
Short talk: *Connections between macrophages and epithelial progenitor cells during hair follicle regeneration*

18:10 **Elaine Fuchs,** Howard Hughes Medical Institute and The Rockefeller University, New York, USA
Stem cells in skin homeostasis and cancer

18:45 ***END of session***

WEDNESDAY, November 9th

Session V: CANCER – part 3

Chair: *Anton Berns*

09:30 **Anton Berns,** The Netherlands Cancer Institute, Amsterdam, The Netherlands
Cell-of-origin studies in mouse models of small cell and non-small cell lung cancer

10:05 **Josema Torres,** *Universidad de Valencia*, Spain
Short talk: *p21Waf1/Cip1 preserves neural stem cell self-renewal by regulating Sox2 gene expression*

10:20 **Manuel Serrano,** CNIO, Madrid, Spain
Tumour suppressors and reprogramming

10:55 ***Coffee break and poster session***

11:25 **Maria Pia Cosma,** Centre for Genomic Regulation, Barcelona, Spain
Wnt signalling and the reprogramming of cell fate to pluripotency

12:00 **Viviane Tabar,** Memorial Sloan-Kettering Cancer Center, New York, USA
Cancer stem cells and tumour angiogenesis

12:35 **Steven Pollard,** UCL Cancer Institute, London, UK
Short talk: *Highly aneuploid human glioblastoma cells can be epigenetically reprogrammed using two transcription factors*

12:50 ***END of session***

Note: **Talks:** 30 minutes / **Short talks:** 10 minutes
Discussion: 5 minutes after each **talk** / 5 minutes after each **short talk**

notes

Recapturing Pluripotency: links between cellular reprogramming and cancer

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Session 1

PLURIPOTENCY

Stem cells, pluripotency and nuclear reprogramming

Rudolf Jaenisch

Whitehead Institute for Biomedical Research, Cambridge, USA

The recent demonstration of *in vitro* reprogramming using transduction of four transcription factors by Yamanaka and colleagues represents a major advance in the field. However, major questions regarding the mechanism of *in vitro* reprogramming as well as the definition of pluripotent cell states need to be understood and will be one focus of the talk.

Human and mouse embryonic stem cells (ESCs) are derived from blastocyst stage embryos but have very different biological properties, and molecular analyses suggest that the pluripotent state of human ESCs isolated so far corresponds to that of mouse derived epiblast stem cells (EpiSCs). We have rewired the identity of conventional human ESCs into a more immature state that extensively shares defining features with pluripotent mouse ESCs. This was achieved by exogenous induction of Oct4, Klf4 and Klf2 factors combined with LIF and inhibitors of glycogen synthase kinase 3 (GSK3) and mitogen-activated protein kinase (ERK) pathway. In contrast to conventional human ESCs, these epigenetically converted cells have growth properties, X chromosome activation state (aXa), a gene expression profile, and signaling pathway dependence that are highly similar to that of mouse ESCs. The generation of "naïve" human ESCs will allow the molecular dissection of a previously undefined pluripotent state in humans, and may open up new opportunities for patient-specific, disease-relevant research.

A major impediment in realising the potential of ES and iPS cells to study human diseases is the inefficiency of gene targeting. Using Zn finger or TALEN mediated genome editing we have established efficient protocols to target expressed and silent genes in human ES and iPS cells. Finally, our progress in using iPS cells for therapy and for the study of complex human diseases will be summarised.

Residual expression of ectopic reprogramming factors prevents differentiation of iPSCs generated from human neonatal fibroblasts and cord blood-derived CD34+ progenitors

Verónica Ramos-Mejía, Rosa Montes, Clara Bueno, Verónica Ayllón, Pedro J. Real, René Rodríguez and **Pablo Menéndez**

GENyO (*Pfizer-Universidad de Granada-Junta de Andalucía* Centre for Genomics and Oncological Research), Granada, Spain

Human induced pluripotent stem cells (hiPSCs) have been generated from different tissues, with the age of the donor, tissue source and specific cell type influencing the reprogramming process. Reprogramming haematopoietic progenitors to hiPSCs may provide a very useful cellular system for modelling blood diseases. We report the generation and complete characterisation of hiPSC lines from human neonatal fibroblasts and cord blood (CB)-derived CD34+ haematopoietic progenitors using a single polycistronic lentiviral vector containing an excisable cassette encoding the four reprogramming factors Oct4, Klf4, Sox2 and c-myc (OKSM). The ectopic expression of OKSM was fully silenced upon reprogramming in some hiPSC lines and was not reactivated upon differentiation whereas other hiPSC lines failed to silence the transgene expression. The inability of some hiPSCs to silence the reprogramming factors was not associated to the cell type/tissue origin. All hiPSCs were induced to differentiate towards haematopoietic and neural lineages and it was found that those hiPSC lines which had silenced OKSM ectopic expression displayed fair haematopoietic and neural differentiation potential. In contrast, those hiPSC lines which failed to switch off OKSM expression were unable to differentiate towards either lineage, suggesting that the residual expression of the reprogramming factors functions as a developmental brake impairing hiPSC differentiation. Successful adenovirus-based Cre-mediated excision of the provirus cassette in CB-derived CD34+ hiPSCs with residual transgene expression resulted in transgene-free hiPSC clones with significantly improved differentiation capacity, confirming that residual expression of the reprogramming factors impairs hiPSC differentiation.

Epigenetic dynamics in stem cells and development

Alexander Meissner

Harvard Medical School, Cambridge, USA

The developmental potential of human pluripotent stem cells suggests that they can produce diseaserelevant cell types for biomedical research. However, substantial variation has been reported among pluripotent cell lines, which could affect their utility and clinical safety. Such cell-line-specific differences must be better understood before one can confidently use embryonic stem (ES) or induced pluripotent stem (iPS) cells in translational research. Toward this goal we have established genome-wide reference maps of DNA methylation and gene expression for 20 previously derived human ES lines and 12 human iPS cell lines, and we have measured the *in vitro* differentiation propensity of these cell lines. This resource enabled us to assess the epigenetic and transcriptional similarity of ES and iPS cells and to predict the differentiation efficiency of individual cell lines. The combination of assays yields a scorecard for quick and comprehensive characterization of pluripotent cell lines.

Resetting the epigenome beyond pluripotency in the germ line

Azim Surani

The Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, UK

Germ cells undergo comprehensive epigenetic reprogramming towards acquiring totipotency, which is a prerequisite for pluripotency *in vivo*. Notably, the full extent of epigenetic reprogramming in the germ line erases most if not all the pre-existing epigenetic information in the genome. Any defects present in experimentally generated pluripotent cells are apparently corrected upon differentiation into the germ cell lineage and following germ line transmission. The underlying mechanisms are complex but events *in vivo* may be explored in cell-based assays to gain deeper insights on underlying mechanisms. Unraveling the mechanisms responsible for germ cell-specific epigenetic reprogramming will likely have important implications for both basic and clinical stem cell research.

Co-expressing Rarg and Lrh1 enables fast and efficient reprogramming to ground state pluripotency

Wei Wang, Jian Yang and **Pentao Liu**

Wellcome Trust Sanger Institute, Cambridge, UK

Somatic cells can be reprogrammed to Induced Pluripotent Stem Cells (iPSCs) by expressing four transcription factors, Oct4, Sox2, Klf4 and c-Myc. We recently found that enhancing retinoic acid (RA) signalling profoundly promoted reprogramming, whereas inhibiting it completely blocked it. Co-expressing Rarg (retinoic acid receptor gamma) and Lrh-1 (liver receptor homolog 1, Nr5a2) with the four factors greatly accelerated reprogramming. Reprogramming of mouse embryonic fibroblast cells (MEFs) to ground state iPSCs requires 3-4 days' induction of these six factors. We are also able to readily reprogrammed primary human neonatal and adult fibroblast cells to exogenousfactor-independent iPSCs, which resembled ground state mouse ES cells in growth properties, gene expression and signalling dependency. The new human iPSCs should facilitate functional analysis of the human genome.

Induction of pluripotency by Oct4

Daniel Esch[1], Juha Vahokovski[2], Guangming Wu[1], Hannes Drexler[1], Matthew R. Grooves[2], Vivian Pogenberg[2], Marcos Arauzo-Bravo[1], Matthias Wilmanns[2] and **Hans R. Schöler[1]**

[1]Max Planck Institute for Molecular Biomedicine, Münster, Germany; [2]European Molecular Biology Laboratory (EMBL), Hamburg, Germany

The reprogramming of mouse and human somatic cells into pluripotent stem cells, termed induced pluripotent stem (iPS) cells, using fibroblasts (somatic cells) and initially requiring the virally-expressed transcription factor quartet of Oct4, Sox2, c-Myc, and Klf4, was first described in 2006. Later, we reported that Oct4 alone is sufficient to directly reprogram adult mouse and human fetal neural stem cells (NSCs) into iPS cells, indicating that Oct4 plays a crucial role in the reprogramming process. We recently showed that induced epiblast stem cells (iEpiSCs) can be obtained by directly reprogramming somatic cells with the quartet under EpiSC culture conditions. In contrast to somatic cells, primordial germ cells (PGCs) were first induced to pluripotency 20 years ago by the mere modulation of the culture conditions. We converted adult germline stem cells (GSCs) into germline-derived pluripotent stem (gPS) cells. GSCs are unipotent testicular cells capable of not only self-renewing, but also giving rise to sperm. Like embryonic stem (ES) cells, GSCs exhibit significant levels of Oct4 and Klf4, but low levels Sox2 and c-Myc.

In my presentation, I will discuss new insights into the molecular mechanism underlying the induction of pluripotency. This will also concern how Oct4 attracts the reprogramming machinery onto DNA.

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Session 2

REPROGRAMMING

Dissecting the mechanisms of cellular reprogramming

Konrad Hochedlinger

Harvard University and HHMI, Boston, USA

My lab is studying the mechanisms of cellular reprogramming using transcription factor-mediated conversion of somatic cells into induced pluripotent stem cells (iPSCs). We are mapping the transcriptional and epigenetic changes cells undergo during iPSC formation with the goal to identify regulators that determine cellular identity. For example, we have shown that the Tgf-beta and p53 pathways act as barriers during iPSC derivation, and their manipulation enhances reprogramming efficiency. Similarly, the differentiation state of the starting cells can influence reprogramming, with less differentiated cells being more amenable to reprogramming than terminally differentiated cells. We have generated the first integration-free iPSC lines, thus eliminating a major roadblock for the therapeutic application of reprogramming technology. More recently, we identified a gene expression signature that distinguishes iPSCs from embryonic stem cells, allowing us to isolate highest quality iPSCs.

Sox2 expression in breast tumours and activation in breast cancer stem cells

O. Leis, A. Eguiara, E. López-Arribillaga, M.J. Alberdi, S. Hernández-García, K. Elorriaga, A. Pandiella, R. Rezola and **Ángel G. Martín**

Fundación Inbiomed, San Sebastián, Spain; Onkologikoa, San Sebastián, Spain; CIC-Salamanca, Spain

The cancer stem cell (CSC) model does not imply that tumours are generated from transformed tissue stem cells. The target of transformation could be a tissue stem cell, a progenitor cell, or a differentiated cell that acquires self- renewal ability. The observation that induced pluripotency reprogramming and cancer are related has lead to the speculation that CSCs may arise through a reprogramming-like mechanism. Expression of pluripotency genes (Oct4, Nanog and Sox2) was tested in breast tumours by immunohistochemistry and it was found that Sox2 is expressed in early stage breast tumours. However, expression of Oct4 or Nanog was not found. Mammosphere formation in culture was used to reveal stem cell properties, where expression of Sox2, but not Oct4 or Nanog, was induced. Over-expression of Sox2 increased mammosphere formation, effect dependent on continuous Sox2 expression; furthermore, Sox2 knockdown prevented mammosphere formation and delayed tumour formation in xenograft tumour initiation models. Induction of Sox2 expression was achieved through activation of the distal enhancer of Sox2 promoter upon sphere formation, the same element that controls Sox2 transcription in pluripotent stem cells. These findings suggest that reactivation of Sox2 represents an early step in breast tumour initiation, explaining tumour heterogeneity by placing the tumourinitiating event in any cell along the axis of mammary differentiation.

Origins of Barrett's esophagus: targeting stem cells in precursors of cancers

Frank D. McKeon

Genome Instute of Singapore; Harvard Medical School, Boston, USA

Barrett's esophagus is an intestinal metaplasia in patients with chronic acid reflux and the dominant risk factor for esophageal adenocarcinoma. This metaplasia is irreversible and proving to be resistant to physical methods of elimination such as radiofrequency ablation. While generally assumed to arise from the transcommitment of esophageal squamous stem cells, our mouse models that lose esophageal stem cells show a robust formation of Barrett's in a matter of days. We traced the origins of this Barrett's metaplasia to a discrete group of residual embryonic cells that reside at the squamocolumnar junction in all individuals that expands in an opportunistic fashion during damage to the esophageal cells. Given the apparent high rates of recurrence of Barrett's following RFA, presumably due to the failure to eradicate every stem cell in Barrett's glands, we set out to develop strategies focused on targeted elimination of these stem cells. Early results indicated that we have cloned Barrett's as well as esophageal and gastric stem cells, and that their comparative expression profiles reveal cell surface antigens and discrete pathways for selective therapeutic intervention.

The TRF1 telomere protein is essential for the generation of iPS cells and marks both pluripotent and adult stem cells

Maria A. Blasco

CNIO, Madrid, Spain

Telomeres are bound by a protein complex named shelterin, which includes TRF1, responsible for preventing telomere fragility and fusions. In the context of the organism, TRF1 is essential at the blastocyst stage of embryo development as well as to maintain adult hair follicle stem cells, suggesting a role for TRF1 in stem cell biology. Here, we describe mice carrying a *knock-in* (KI) allele in which TRF1 is fused to the reporter protein eGFP. In agreement with the notion that adult stem cells have longer telomeres, we find that eGFP-TRF1 expression is maximal in known stem cell niches in the mouse, including hair follicle stem cells and the Lgr5-positive (Lgr5+) cells at the intestinal crypts. Interestingly, expression of eGFP-TRF1 decreases more abruptly than telomere length when going from the more primitive to the more differentiated compartments. In line with this, when we analysed the expression of eGFP-TRF1 in induced pluripotent stem (iPS) cells we observed extraordinarily high levels, which are uncoupled from telomere elongation associated to reprogramming, and that are heterogeneous and coincident with the in-built heterogeneity of Nanog expression in iPS cell colonies. In fact, selection of high eGFP-TRF1 iPS cells correlated with higher pluripotency as indicated by their ability to form teratomas and chimeras. Finally, TRF1 is dramatically upregulated during reprogramming to generate iPS cells and the absence of TRF1 completely prevents reprogramming even in the absence of p53 and despite normal proliferation rates. These results indicate that TRF1 plays a critical role in the maintenance of stemness and that pluripotent cells are extremely sensitive to telomere instability.

Expansion and mobilisation of hair follicle stem cells promote squamous cell carcinoma development in mouse skin

Victoria da Silva-Diz[1], Sònia Solé-Sánchez[1], Maria Urpí[1], Antonio Valdés-Gutiérrez[1], Rosa M. Penin[2], Gloria Pascual[3], Eva González-Suárez[1], Oriol Casanovas[4], Francesc Viñals[4], Eduard Batlle[5] and **Purificación Muñoz[1]**

[1]Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain; [2]Bellvitge University Hospital/IDIBELL, Barcelona, Spain; [3]Centre for Genomic Research (CRG), Biomedical Research Park (PRBB), Barcelona, Spain; [4]Catalan Institute of Oncology (ICO/IDIBELL), Barcelona, Spain; [5]Institute for Research in Biomedicine (IRB), Barcelona, Spain

Epidermal and hair follicle stem cells have been associated to the origin of oncogene-induced squamous cell carcinomas (SCCs). However, it is still unclear how stem cells localised in the lower region of hair follicles, contribute to SCC generation in the epidermis. Here, we determine alterations in the homeostasis and dynamics of bulge and hair germ stem cells that are involved in SCC development. We used a skin cancer mouse model that expresses, in stem cells and basal keratinocytes, the oncoproteins E6 and E7 from human papillomavirus, which are related to human SCC development. We show that during early steps of tumourigenesis, E6 and E7 induce an aberrant expansion of keratin 15 (K15)-expressing cells that exhibit hair germ markers (HG-like cells). Lineage tracing assays demonstrate that the leucine-rich G protein-coupled receptor 5 (Lgr5)-expressing bulge stem cells and their progeny are the origin of the HG-like cells. These expanded stem cell population is produced by activation of bulge stem cell proliferation and attenuation of the hair follicle differentiation programme of their descendents. A large subset of the HG-like cells is mobilised to the infundibulum and to the interfollicular epidermis, accumulates at pre-neoplastic lesions and is in the origin of E6/E7-driven tumours. These findings indicate that aberrant accumulation of altered hair germ stem cells in hair follicles and their subsequent mobilisation to the interfollicular epidermis promote skin tumour initiation.

Generation and correction of laminopathy-associated *LMNA* mutations in patient specific iPSCs

Juan Carlos Izpisua Belmonte

Salk Institute for Biological Studies, San Diego, USA

Combination of stem cell-based approaches with gene-editing technologies represents an attractive strategy for studying human disease and developing therapies. However, gene-editing methodologies described to date for human cells suffer from technical limitations including limited target gene size, low targeting efficiency at transcriptionally inactive loci, and off-target genetic effects that could hamper broad clinical application. To address these limitations, and as a proof of principle, we focused on homologous recombination-based gene correction of multiple mutations on lamin A (LMNA), which are associated with various degenerative diseases. We show that helper-dependent adenoviral vectors (HDAdVs) provide a highly efficient and safe method for correcting mutations in large genomic regions in human induced pluripotent stem cells and can also be effective in adult human mesenchymal stem cells. This type of approach could be used to generate genotype matched cell lines for disease modeling and drug discovery and potentially also in therapeutics.

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Session 3

CANCER – PART 1

Towards unification of the cancer stem cell and clonal evolution models of cancer

John E. Dick

Princess Margaret Hospital, University Health Network and University of Toronto, Toronto, Canada

The cellular and molecular basis for intra-tumoural heterogeneity is poorly understood. Tumour cells can be genetically diverse due to mutations and clonal evolution resulting in intra-tumoural functional heterogeneity. Often proposed as mutually exclusive, cancer stem cell (CSC) models postulate that tumours are cellular hierarchies sustained by CSC heterogeneity due to epigenetic differences (i.e. long term tumour propagation only derives from CSC). The clinical relevance of CSC has been challenged by recent reports that some tumours may actually not adhere to a CSC model when the xenograft system is enhanced. Two lines of evidence support the CSC model in AML and B-ALL. We have recently developed gene signatures specific to either AML LSC or normal HSC and found they share a set of genes that define a common stemness programme. Only these stem cell related gene signatures were found to be highly significant independent predictors of patient survival when large clinical databases were introgated. Thus, determinants of stemness influence clinical outcome of AML establishing that LSC are clinically relevant and not artifacts of xenotransplantation. Second, we have carried out a series of combined genetic and functional studies of Ph+ B-ALL leukaemic initiating cells (L-IC) that point to commonalities between clonal evolution and CSC models of cancer. L-IC from diagnostic patient samples were genetically diverse and reconstruction of their genetic ancestry showed that multiple L-IC subclones were related through a complex evolutionary process that involved both linear or branching leukaemic progression. The discovery that specific genetic events influence L-IC frequency and that genetically distinct L-IC evolve through a complex evolutionary process indicates that a close connection must exist between genetic and functional heterogeneity. Finally, our study points to the need to develop effective therapies to eradicate all genetic subclones in order to prevent further evolution and recurrence.

Contribution of cell cycle stages to successful reprogramming towards pluripotency

Tomomi Tsubouchi*, Jorge Soza-Ried, Karen Brown, Francesco Piccolo, C. Filipe Pereira, Matthias Merkenschlager and Amanda G. Fisher

MRC Clinical Sciences Centre, Imperial College, London, UK; *Present address: MRC Genome Damage and Stability Centre, University of Sussex, Falmer, UK

Nuclear reprogramming is functional conversion of the genome contained within a differentiated somatic cell to a state of developmental pluripotency, through factors that reside in the pluripotent stem cells. We take advantage of the cell fusion system to study nuclear reprogramming of human B (hB) cells induced by mouse ES (mES) cells. Curiously, only ~15% of hB nuclei forming heterokaryons reprogram.

We presume heterogeneity among mES- and/or hB- cell populations that affect reprogramming efficiencies and focused on cell cycle stages of mES cells. To gain insight into the contribution of cell cycle stage for successful reprogramming, we have optimized counterflow centrifugal elutriation method to enrich mES populations at progressive stages of cell cycle. We show that cell populations enriched for G2/M stage of cell cycle have enhanced reprogramming capabilities compared to those enriched for other stages of cell cycle. Our preliminary observation suggest that this enhanced reprogramming capacity of G2/M population is not due to the fact that nuclear proteins in mES cells are more readily available to hB through nuclear membrane breakdown of M-phase cells. Some of the known reprogramming factors are more abundant in G2/M fractions, which may contribute to the enhanced reprogramming capacities. We are currently characterising cellular events within heterokaryons obtained from fusion between hB and either G1-enriched or G2/M-enriched mES populations to further gain insight into how enhanced reprogramming is accomplished using G2/M population.

Together we aim to advance our understandings in the reprogramming process, which we believe would also advance our understandings in ES cell identity and the nature of differentiation.

Stress-response mechanisms and genome maintenance in normal and cancer stem cells

Emmanuelle Passegué

University of California, San Francisco, USA

Haematopoietic stem cells (HSC) are the only cells within the haematopoietic system that self-renew for life, whereas all other haematopoietic cells are short-lived and committed to the transient production of mature blood cells. Recently, we have shown that HSCs are intrinsically vulnerable to mutagenesis following extrinsic genotoxic stress. We found that their unique quiescent cell cycle status restricts them to the use of the error-prone non-homologous end joining (NHEJ) repair mechanism, hence rendering HSCs prone to misrepaired DNA double strand breaks (DSB) and chromosomal instability. This finding provide the beginning of an explanation why HSCs, despite being protected at the cellular level, are more likely than other haematopoietic cells to initiate blood disorders. We reasoned that HSCs have other unique protective features, which allow them to contend with a variety of cellular insults and damaged cellular components while maintaining their life-long functionality and genomic integrity. We will present some of our recent findings on the fundamental mechanisms of stress-response that preserve HSC fitness during periods of metabolic stress. It is now clearly established that oncogenic insults in diseases such as myeloproliferative neoplasms (MPN) can transform HSCs and dramatically alter their biological functions leading to the emergence of leukaemia-initiating stem cells (LSC). We will discuss how LSCs may take advantage of some deregulated features of these stress-response mechanisms that normally protect HSCs to escape therapeutic killing and mediate cancer development.

Lineage commitment and plasticity in the lymphoid system

Roger Revilla-i-Domingo, Ivan Bilic, Bojan Vilagos, Hiromi Tagoh, Anja Ebert, César Cobaleda and **Meinrad Busslinger**

Research Institute of Molecular Pathology, Vienna, Austria

Haematopoietic stem cells undergo self-renewal and regenerate all blood cell types throughout life. They do so by first differentiating to lymphoid and erythro-myeloid progenitors. The common lymphoid progenitors (CLPs) generate B and T cells, which provide acquired immunity to foreign pathogens. The entry of CLPs into the B cell pathway is regulated by several transcription factors including the B cell commitment factor Pax5. Pax5 restricts the developmental potential to the B cell lineage by repressing genes controlling signalling pathways of other haematopoietic lineages and by simultaneously activating B-cell-specific genes contributing to B cell signalling and differentiation. Importantly, Pax5 maintains the identify of B cells throughout B lymphopoiesis, as the loss of Pax5 allows mature B cells from peripheral lymphoid organs to develop into functional T cells in the thymus via dedifferentiation to uncommitted progenitors in the bone marrow.

By using genome-wide approaches, we have recently demonstrated that Pax5 binds to 40% of the cis-regulatory genome defining 8,000 target genes in pro-B and mature B cells. However, Pax5 regulates only 4% of its target genes in both cell types by inducing enhancers at activated genes and eliminating DNase I hypersensitive sites at repressed genes. Pax5-regulated genes in pro-B cells account for a large part of the expression changes between CLPs and pro-B cells, thus identifying Pax5 as a global regulator of this developmental transition. Surprisingly however, the regulated Pax5 target genes overlap only minimally in pro-B and mature B-cells. Hence, Pax5 controls distinct transcriptional programmes in these cell types despite the fact that Pax5 controls the identity of B lymphocytes in both early and late B cell development.

Malignant reprogramming in leukaemia

Catriona Jamieson

University of California, San Diego, USA

Leukaemia stem cells evolve as a result of sequential genetic and epigenetic alterations that enhance self-renewal, survival and dormancy in supportive microenvironments. Full transcriptome RNA sequencing and nanoproteomics have highlighted splice isoform switching in committed progenitors which enable them to co-opt primitive stem cell properties and to evade therapies that target proliferating cells.

Development of highly active anti-leukaemia stem cell therapy together with splice isoform biomarkers of response may obviate therapeutic resistance and provide an essential paradigm for cancer stem cell detection and eradication in other refractory malignancies.

Notch1 signalling reprogrammes T-cell progenitors resident in the human thymus into bone-marrow grafting precursors: implications in leukaemia development

Marina García-Peydró and **María L. Toribio**

Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Universidad Autónoma de Madrid, Madrid, Spain

T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive haematological malignancy that arises from the transformation of thymic T-cell precursors, which then infiltrate the bone marrow (BM). Deregulation of NOTCH1 signalling is a common cause of T-ALL, as activating NOTCH1 mutations arte found in over 60% of T-ALLs. However, our knowledge of the early oncogenic events induced by NOTCH1 is very scarce, as functional biology of human T-ALL has relied so far on *in vitro*-expanded patient-derived cell lines. Here, we have assessed the biological impact and leukaemogenic potential of oncogenic NOTCH1 mutations in humans using a xenotransplantation *in vivo* model. We show that primary human HSCs overexpressing constitutively active NOTCH1 (ICN1) engrafted efficiently into the BM of immunodeficient transplanted mice, as did their wild-type counterparts, but selectively displayed a 25-fold increased expansion which paralleled the ectopic generation of aberrant T cells in the BM. Strikingly, ICN1 overexpression in progenitors that have already migrated from the BM into the thymus also resulted in BM grafting and aberrant T-cell generation, while control thymic precursors were devoid of both functions. Analysis of the molecular mechanisms underlying NOTCH1-induced reprogramming of BM grafting showed that the adhesion molecule CD44 is a critical NOTCH1 target required for BM grafting and expansion of human progenitors expressing mutant NOTCH1. More importantly, CD44 function supports the leukaemogenic potential of already established primary human T-ALL cells *in vivo*. Although aberrant T cells induced by oncogenic NOTCH1 were unexpectedly polyclonal and had a maximal life span of 10-wks, suggesting that leukaemia progression requires the cooperation of additional mutations, our results may be clinically relevant, as initiating lesions that participate in the establishment of the transformed state in cancer cells are envisioned as the most promising therapeutic targets.

Epigenetic programmes and cancer stem cell development

Scott Armstrong

Children's Hospital, DFCI, Harvard Medical School, Boston, USA

Acute myelogenous leukaemia stem cells (LSC) are the subset of leukaemia cells capable of extensive self-renewal and maintenance of the leukaemia. We are interested in the mechanisms of leukaemia development as they relate to activation and maintenance of gene expression programmes by epigenetic pathways responsible for deregulated self-renewal in leukaemia. We have demonstrated that oncogenic fusion proteins can activate a self-renewal associated gene expression programme when expressed in myeloid progenitor cells that normally have limited self-renewal capabilities. Detailed characterisation of LSC from multiple different mouse leukaemias has shown that LSC are most similar to differentiating myeloid cells that express a limited haematopoietic stem cell (HSC) associated signature. Detailed analysis of the gene expression data from normal HSC and progenitors as compared to LSC identified the Wnt/β-catenin pathway as potentially important for acute myeloid leukaemia LSC, and we have subsequently validated this pathway as critical using conditional mouse models. We are continuing to assess the gene expression programmes subverted during the transition from normal HSC and progenitor cells to LSC and are comparing these data to genome wide epigenomic analyses to determine which specific epigenetic programmes are required for leukaemia stem cell maintenance. Identification of epigenetic pathways required to maintain leukaemia self-renewal should provide new targets for novel therapeutic approaches.

notes

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Session 4

CANCER – PART 2

Molecular analysis of normal and malignant mammary gland stem cells

Michael F. Clarke and Neethan Lobo

Stanford Institute for Stem Cell and Regenerative Medicine, Stanford University, USA

Primary human tissue contains a complex mixture of cell types which can only be partially purified by cell sorting based on surface markers. To gain further insight into mammary gland and tumour biology, we performed highly parallel single cell multiplexed gene expression measurements on individual normal and malignant mammary cells. This approach enabled discovery of novel normal mammary stem cell markers and gave insights into normal and malignant tissue architecture. We identified two distinct normal mammary stem cell populations. Both populations had similar engraftment ability *in vivo*, demonstrating that there are likely two physiological stem cell states. Single cell analysis of estrogen receptor positive (ER+) breast tumours revealed similar cellular populations in cancer cells that resemble the normal mammary duct architecture. Remarkably, the expression of therapeutic targets such as growth factor receptors and the estrogen receptor differed between the different cell compartments. Our results show that single cell analyses might be useful for developing new strategies for treating cancer by identifying the expression of therapeutic targets by each of the cancer cell populations that make up a particular tumour.

We regret to inform that due to family reasons, Dr. Michael Clarke has cancelled his participation. In his place, we are glad to announce the participation of Dr. Christopher Heeschen.

Identifying the Achilles heal of metastatic cancer stem cells

Christopher Heeschen

CNIO, Madrid, Spain

New opportunities for novel therapeutic strategies against carcinomas are emerging with the accumulating evidence for the existence of cancer stem cells. These cells represent a subpopulation distinguishable from the bulk of the tumour by their exclusive ability to drive in vivo tumourigenesis based on their extensive self-renewal capacity and their ability to repopulated the entire tumour. Most importantly, they play a crucial role in disease relapse due to their inherent resistance to current therapies such as chemo- or radiotherapy. Therefore, the elucidation of the regulatory machinery of cancer stem cells including their interplay with the tumour microenvironment is of crucial relevance for the development of clinically more efficient therapies. Our most recent studies now provide evidence for the re-activation of a developmental pathway reminiscent of those found in embryonic stem cells. We were able to identify Nodal and Activin as two critical components determining the two-way communication between cancer stem cells and an embryonic-like microenvironment. Pancreatic cancer stem cells reactivate the potent embryonic morphogens Nodal and Activin, which strongly increases their plasticity and aggressiveness. This pathway is essential for their self-renewal capacity as well as invasiveness and therefore represents a novel therapeutic target for this deadly disease. Intriguingly, the Nodal/Activin is not only expressed in pancreatic cancer stem cells, but also in pancreatic stellate cells, which are abundantly present in the stroma surrounding pancreatic cancer cells, and serve as a supportive niche for cancer stem cells. Our preclinical studies showed that the cancer stem cell compartment can be severely altered by inhibition of this pathway resulting in chemo-sensitisation of the cancer stem cells rendering them susceptible to elimination by standard chemotherapy resulting in disease stabilization. However, as the hallmarks of this disease are poor vascularisation and the newly described protective cancer stem cell niche, achieving therapeutically relevant drug concentrations in the tumour capable of disrupting this bilateral signalling remained challenging. Intriguingly, simultaneous targeting of the sonic hedgehog pathway as a crucial signalling component of pancreatic stellate cells and other stroma cells was able to eventually conquer this biological barrier. The resulting triple therapy containing a Nodal/Activin inhibitor, a hedgehog pathway inhibitor, and a chemotherapeutic agent resulted in long-term survival of all mice.

Christopher Heeschen

CNIO, Madrid, Spain

Christopher Heeschen studied Medicine in Budapest, Munich and Berlin. After three years of clinical training in Internal Medicine and participating in several international multicenter studies for novel anti-platelet therapies in patients with acute coronary syndromes, he joined the Falk Cardiovascular Research Center at Stanford University (USA) in 1999 where he worked on basic mechanisms of angiogenesis and vasculogenic stem cells. He obtained his PhD in 2001 and subsequently joined Johann-Wolfgang-Goethe University in Frankfurt/Main as Junior Group Leader in 2003 to work on the clinical translation of stem cell-based therapies for cardiovascular regeneration. He became an independent investigator in 2004 as Professor of Experimental Oncology and Transplantation and Head of the Department Experimental Medicine at Ludwig-Maximilian-University in Munich (Germany). Shortly after entering into the newly emerging field of cancer stem cells in solid tumors, his group identified a distinct population of exclusively metastatic cancer stem cells. In 2009, he joined the CNIO as Senior Group Leader in the newly founded Clinical Research Programme to further study the role and characteristics of cancer stem cells and their microenvironment for developing novel multimodal therapies.

DYRK1A modulates the self-renewal capacity of neural stem cells and glioblastoma initiating cells through regulation of EGFR stability

N. Pozo, C. Zahonero, P. Fernández, A. Pérez, A. Hernández-Laín, J.R. Ricoy, J.M. Sepúlveda, P. González and **Pilar Sánchez**

Instituto de Salud Carlos III, Madrid, Spain; *Universidad CEU-San Pablo*, Madrid, Spain; Hospital *Universitario 12 de Octubre*, Madrid, Spain

Glioblastomas (GBMs) are very aggressive primary brain tumours, being resistant to chemo and radio-therapy. Several groups have demonstrated that there are important differences in the differentiation status inside a given GBM, with cells resembling normal neural stem cells (NSCs) on top of the cellular hierarchy. This subpopulation of cells has greater potential of tumour initiation and shares with the NSCs their self-renewal, differentiation and sustained-proliferation capacities. These so-called tumour initiating cells (TICs) can be enriched *in vitro* with the protocols developed initially for the culture of NSCs in the absence of serum. Apart from common *in vitro* protocols, TICs and NSCs share also many regulatory mechanisms. We have recently demonstrated that DYRK1A (Dualspecificity tyrosine(Y)-phosphorilation-Regulated Kinase 1A) controls the response of adult NSCs to EGF in the adult brain. Levels of DYRK1A activity determine EGFR turnover rates, affecting stem cells expansion and maintenance in adult neurogenic niches so that in heterozygous mice there is a premature loss of NSCs.

Interestingly EGFR is one of the most important therapeutic targets as it is altered in more than 50% of GBMs. However, most of the tyrosin-kinase inhibitors assayed in GBMs have shown poor beneficial effects. Our results indicate that inhibition of DYRK1A in GBM TIC-enriched cell lines promotes EGFR degradation affecting proliferation and especially the survival of the TICs. Interestingly, there is a strong correlation between DYRK1A and EGFR expression in a panel of gliomas. Moreover, DYRK1A kinase inhibition or shRNA strategies clearly impair tumour growth *in vivo*. In resume our data suggest that recapitulation of EGF receptor stability is an essential oncogenic event in a big percentage of GBMs, underlying the special nature of this type of tumours and the dependence of TICs (like NSCs) self-renewal on high levels of EGFR in the membrane.

Cellular reprogramming during the formation of breast cancer

Patricia J. Keller, Adam Skibinski, Stephen P. Naber, Jonathan A. Garlick and
Charlotte Kuperwasser

Molecular Oncology Research Institute, Tufts University School of Medicine, Boston, USA

Invasive human breast cancer is a multifaceted disease consisting of tumours that exhibit a wide spectrum of histological and molecular features. Ductal carcinomas are the most common type of breast cancer accounting for ~80% of all invasive tumours. They are broadly cathegorised based on whether they express the estrogen receptor and can be further subdivided molecularly and histologically into distinct subclasses with different prognostic outcomes and therapeutic sensitivities. There also are rare types of breast cancers such as metaplastic carcinomas where tumour cells exhibit features of alternate cell types that no longer resemble breast epithelium and exhibit features of skin, apocrine, bone, and even cartilaginous differentiation . Until now, it has been difficult to identify the cell types in the human breast that give rise to these various forms of breast cancer. We will present our findings using human reduction mammoplasty tissues that common forms of human ductal carcinomas are derived from luminal epithelial cells while rare metaplastic tumours are derived from basal/myoepithelial (ME) cells. We will also discuss that the development of metaplastic tumours from basal/ME-lineage cells is due to their intrinsic ability to spontaneously lose mammary commitment and to reprogram back into ectodermal/epidermal stem cells that can form adult human skin, cutaneous appendages, and other epidermal tissues. We have identified various epigenetic chromatin modifying enzymes that facilitate mammary cell reprogramming back into ectodermal/epidermal cells. Collectively, these findings identify the normal cellular precursors to human breast cancer and link early epigenetic events ascribed to premalignant changes to cellular reprogramming, increased pluripotency, and the acquisition of embryonic differentiation states even prior to neoplastic transformation.

Contribution of stem cells and differentiated cells to development of tumours of multilayered epithelia

Fiona Watt

Cancer Research UK Cambridge Research Institute, Cambridge, UK

Multilayered epithelia such as the epidermis and oral mucosa are maintained throughout adult life by self-renewal of stem cells and differentiation of their progeny. It is widely believed that stem cells are the tumour initiating cells in squamous cell carcinomas because the more differentiated cells are resident in the tissue for a relatively short time. Nevertheless, there is strong evidence that differentiating cells can contribute to tumour development. I will describe an experimental model in which the underlying signalling pathways, which involve communication of differentiated cells with stem cells and cells of the bone marrow, are being elucidated.

Connections between macrophages and epithelial progenitor cells during hair follicle regeneration

Donatello Castellana and **Mirna Pérez-Moreno**

CNIO, Madrid, Spain

Skin stem cells reside in the hair follicles in a special niche termed the bulge. These cells are responsible for the cyclic replenishment of hair follicles during the lifetime of mammals. The reactivation of a new hair cycle requires a crosstalk between epithelial progenitor cells and a cluster of specialised mesenchymal cells, known as dermal papilla. Additionally, it has been recently demonstrated that other cells in the niche such as melanocytes, adipocytes and nerve cells may also provide cues to hair progenitor cells and regulate their activation. However, until now is not fully understood if inflammatory cells participate in this event. To this regard, we have analysed the presence of different inflammatory cells during the hair cycle and observed that macrophage numbers significantly decrease at the onset of stem cells activation and hair follicle growth. Interestingly, chemical depletion of macrophages during early telogen, the resting phase of the hair cycle, was sufficient to induce hair growth with fully differentiated characteristics. Gene expression analysis of macrophages, at different phases of hair cycle, revealed that the levels of several Wnts were increased before the onset of hair follicle growth. Overall, our results suggest that macrophages have a crucial role in the regulation of hair follicle stem cells activation. Further studies will provide more insights about the role of the crosstalk between macrophages and skin stem cells in skin physiology, and their possible implications in skin diseases, such inflammatory diseases, and cancer.

Stem cells in skin homeostasis and cancer

Elaine Fuchs

Howard Hughes Medical Institute and The Rockefeller University, New York, USA

Even though many adult tissues undergo relatively infrequent turnover, they still require stem cells which are then used sparingly to replenish cells during normal homeostasis and in response to injury. How stem cells balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair. Moreover, increasing evidence suggests that the regulatory circuitry governing this balancing act is at the root of some types of tumours both in mice and in humans. The skin is an excellent model system to understand how stem cells function in normal tissue generation and how this process goes awry in cancer. Using skin as our paradigm, we've been dissecting how extrinsic signalling to stem sets off a cascade of changes in transcription that governs the activation of stem cells during tissue development, homeostasis and hair regeneration. Our findings have provided us with new insights into our understanding of the process of stem cell activation, and in so doing have revealed mechanisms which are also deregulated in a variety of different human cancers. As importantly in understanding how stem cells are activated is learning about the signals that instruct stem cells to stop making tissue. Our recent findings on this topic have led us to the realm of identifying cancer stem cells of squamous cell carcinomas. We've demonstrated that when isolated by FACS and introduced directly into the skin of a host recipient, a single cancer stem cell can generate an SCC that is similar in properties to the parent SCC. These findings have major implications for our understanding of cancer and how its stem cells generate and respond to their own microenvironment.

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Session 5

CANCER – PART 3

Cell-of-origin studies in mouse models of small cell and non-small cell lung cancer

Kate Sutherland, Joaquim Calbo, Min-chul Kwon, Natalie Proost and **Anton Berns**

The Netherlands Cancer Institute, Amsterdam, The Netherlands

Small cell lung cancer (SCLC) is one of the most lethal human malignancies, due to its high metastatic potential and chemo-resistance upon relapse. Using the Rbf/f;p53f/f mouse model for SCLC, we found that the tumours are often composed of phenotypically different cells, characterised by mesenchymal and neuroendocrine markers. These cells had a common origin as they shared specific genomic aberrations. Crosstalk between mesenchymal and neuroendocrine cells can endow the neuroendocrine cells with metastatic capacity, illustrating the potential relevance of tumour cell heterogeneity in dictating functional tumour properties. Interestingly, these neuroendocrine cells can convert into the mesenchymal component by Ras pathway activation, suggesting that these cell types might interconvert. This raises the question of the cell-of-origin of this tumour.

To investigate this, we inactivated Trp53 and Rb1 in distinct subsets of cells in the adult lung by targeting Cre-recombinase expression to Clara (CC10 positive), neuroendocrine (CGRP positive), and alveolar type 2 (SPC positive) cells using adenoviral vectors. Using these cell-type-restricted Adeno-Cre viruses we could show that inactivation of Trp53 and Rb1 can efficiently transform CGRP and SPC-positive cells leading to SCLC, albeit SPC positive cells at a lesser efficiency. In contrast CC10-expressing clara cells were largely resistant to transformation. The results indicate that NE cells serve as the predominant cell of origin of SCLC. A different cell type restriction was found when a Kras driven non-small-cell lung cancer (NSCLC) model was used to reveal the cell- of-origin of NSCLC emphasising the notion that both cell type specific features and the nature of the oncogenic lesion(s) are critical factors for the tumour initiating capacity of a cell.

p21Waf1/Cip1 preserves neural stem cell self-renewal by regulating Sox2 gene expression

M. Angeles Marqués-Torrejón[1], Ana Banito[2], Eva Porlan[1], Jesús Gil[2], **Josema Torres**[1*] and Isabel Fariñas[1*]

[1] *Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Universidad de Valencia*, Spain; [2]MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College, Hammersmith Campus, London, UK

Tissue renewal and homeostasis during the lifetime relies on the ability of adult stem cells to balance proliferation with the maintenance of their developmental potential. In the adult brain, continual neurogenesis to the olfactory bulb is sustained by the existence of self-renewing neural stem cells (NSCs) in the subependymal neurogenic niche1. The cell cycle inhibitor p21Waf1/Cip1 (p21) is an important regulator of NSC self-renewal and its elimination *in vivo* leads to premature exhaustion of the subependymal NSC pool2. The p21-null phenotype suggests a relationship between cell cycle control and long-term self-renewal, but the molecular mechanisms underlying the control of adult NSC maintenance by p21 remain unexplored. Here we identify a novel function of p21 in NSC self-renewal through direct regulation of the expression of Sox2, a key factor in the specification and maintenance of NSCs and neural progenitors3. We observe, both *in vitro* and *in vivo*, that p21 negatively regulates the levels of the endogenous Sox2 protein in NSCs. Augmented levels of Sox2 in p21-null cells leads to DNA damage-independent accumulation of the tumour suppressor protein p53 and cell growth arrest. Higher levels of Sox2 also decrease the differentiation potential of NSCs. Reduction of the endogenous levels of either Sox2 or p53 eliminates the cell growth block and allows NSC expansion. Our results demonstrate a novel regulation of the NSC pool driven by a p21/Sox2/p53 axis.

Tumour suppressors and reprogramming

Manuel Serrano

CNIO, Madrid, Spain

Our laboratory is interested in the interplay between tumour suppressors and the process of reprogramming because we hypothesize that this can illuminate novel aspects of the tumourigenic process. In recent years, we have identified the tumour suppressors p53 and Ink4a/Arf as main barriers for reprogramming. We have extended this approach to other tumour suppressors and we will present a summary of our current projects. Similarly, we are also exploring the implication of core stem factors in the process of tumourigenesis. We have focused on the stem factor Nanog and we will present an update of our findings on the role of Nanog in cancer.

Wnt signalling and the reprogramming of cell fate to pluripotency

Maria Pia Cosma

Centre for Genomic Regulation, Barcelona, Spain

Spontaneous cell fusion between two cells of different lineages can originate new hybrid cells that have different features from the original parent cells. If one of the fusing parent cells is highly plastic, such as a stem cell, and the other is a somatic cell, their fusion can be followed by reprogramming events that generate new hybrid pluripotent cells. However, whether cell-fusion-mediated reprogramming can occur *in vivo* in higher vertebrates, and what are the molecular mechanisms and genes that drive the reprogramming, remain to be defined. We have shown that activation of the Wnt/β-catenin signalling pathway enhances reprogramming of somatic cells after their fusion with embryonic stem cells. We are currently dissecting out the gene networks and studying the mechanisms of *in vivo* somatic-cell reprogramming, to determine whether reprogrammed hybrids have the potential to differentiate and regenerate neural tissues.

Cancer stem cells and tumour angiogenesis

Viviane Tabar

Memorial Sloan-Kettering Cancer Center, New York, USA

Recent data from our lab (Wang et al. Nature 2010:829-33) demonstrate that glioblastomas comprise a cancer stem-cell like subpopulation that is capable of differentiation into endothelial cells. In fact we demonstrate that a proportion of endothelial cells within tumour vessels harbor somatic genomic aberrations identical to those seen within the tumour cells, regardless of the tumour genotype or transcriptomal class. In addition, we show that a defined subpopulation of GBM cells can transition to an endothelial lineage, *in vitro* and under single cell clonal conditions, as well as *in vivo*. Interestingly VEGF inhibition via pharmacological or genetic means can control maturation of the tumour-derived vessels, but does not interfere with tumour cell differentiation into endothelial progenitors. However, the suppression of the Notch pathway via gamma-secretase inhibitors or shRNA for Notch1, resulted in significant but incomplete inhibition of GBM cancer stem cell differentiation into endothelial progenitors. The functional significance of these findings and their impact on the design of anti-angiogenic treatments will be determined in future studies.

Highly aneuploid human glioblastoma cells can be epigenetically reprogrammed using two transcription factors

Stefan H. Stricker[2], Andrew Feber[1], Kathreena M. Kurian[3], Pär G. Engström[4], Yasuhiro Takashima[5], Colin Watts[7], Peter Dirks[8], Paul Bertone[4,5], Stephan Beck[1], Austin Smith[5] and **Steven M. Pollard[1,2]***

[1]UCL Cancer Institute, University College London, UK; [2]Samantha Dickson Brain Cancer Unit, UCL, UK; [3]Frenchay Hospital, Frenchay Park Road, Bristol, UK; [4]EMBL, European Bioinformatics Institute, Wellcome Trust Genome Campus, Cambridge, UK; [5]Genome Biology and Developmental Biology Units, EMBL, Heidelberg, Germany; [6]Wellcome Trust Centre for Stem Cell Research, University of Cambridge, UK

Cancer cells are driven by both genetic and epigenetic changes, but their relative contribution in driving the malignant phenotype remains unclear. We have used induced pluripotent stem (iPS) methodology to demonstrate that highly malignant and aneuploid human glioblastoma cells can be epigenetically reprogrammed. Glioblastoma-iPS cells (GiPSCs) activate expression of early embryonic markers such as NANOG, and display widespread reconfiguration of DNA methylation patterns including reactivation of aberrantly silenced tumour suppressor genes. Removal of epigenetic restrictions enables these GiPSCs to enter alternative differentiation programmes *in vitro* and *in vivo*. GiPSCs now provide a tractable model system to explore whether transcriptional resetting and epigenetic reprogramming in human cancer can restore normal cellular behaviour to malignant cells.

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Speakers' biographies

Rudolf Jaenisch

Whitehead Institute for Biomedical Research, Cambridge, USA

Rudolf Jaenisch, M.D., is a Founding Member of Whitehead Institute for Biomedical Research and Professor of Biology at MIT. Jaenisch studies the epigenetic regulation of gene expression with the goal of efficiently changing one differentiated cell type into another. This has lead to groundbreaking work with mammalian embryonic stem cells and adult cells that have been reprogrammed to an embryonic stem cell like-state, called induced pluripotent stem (iPS) cells. Jaenisch continues to push iPS cell methodology forward and has demonstrated iPS cells' therapeutic potential in models of Sickle Cell anemia and Parkinson's disease. For his work, Jaenisch has been honored with the first Peter Gruber Foundation Award in Genetics, the Brupracher Foundation Cancer Award, Cozzarelli Prize from the Proceeding of the National Academy of Sciences (PNAS), Robert Koch Prize for Excellence in Scientific Achievement, Meira and Shaul G. Massry Prize, Ernst Schering Prize, Vilcek Prize, and the Wolf Prize in Medicine. Jaenisch is a Member of the National Academy of Sciences, a Member of the Institute of Medicine, and a Fellow of American Academy of Arts and Sciences.

Alexander Meissner

Harvard Medical School, Cambridge, USA

Alex Meissner trained in Rudolf Jaenisch's laboratory at the Whitehead Institute, before he joined Harvard University as assistant professor in the department of stem cell and regenerative biology in 2008. He is also a member of the Harvard Stem Cell Institute and a senior associate member of the Broad Institute.

Working with his colleagues at the Broad Institute, Alex is developing and applying high-throughput bisulfite sequencing technologies for DNA methylation analysis. This should ultimately lead to creating reference epigenomes – maps of what happens outside the DNA sequence -- for many cell types, with the goal of better understanding normal and diseased cellular states. He is co-directing the NIH Reference Epigenome Mapping Center at the Broad Institute. Complementing his work on epigenomics, his lab has a major interest in cell states and how they can be altered through ectopic transcription factors. He has led several of the early studies in the induced pluripotent stem (iPS) field while in the Jaenisch lab. His lab has since then made notable contributions to the understanding of the process and he was recently named a Pew Scholar in the Biomedical Sciences.

Azim Surani

The Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, UK

Azim Surani obtained his PhD in Mammalian Development at the University of Cambridge under Professor R G Edwards. He was appointed as the Marshall-Walton Professor at the Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge in 1992. His past work includes the discovery of the phenomenon of Genomic Imprinting in mice. His recent work has focussed on the specification and properties of the mouse germ cell lineage, including epigenetic reprogramming of the genome. He was elected a Fellow of the Royal Society in 1990. He is an Associate Fellow of the Third World Academy of Sciences (1992), Fellow of the Academy of Medical Sciences (2001), a Member of EMBO (1993). The Royal Society awarded him the Gabor Medal in 2001, and the Royal Medal in 2010.

Hans R. Schöler

Max Planck Institute for Molecular Biomedicine, Münster, Germany

Hans Schöler received his PhD at the Center for Molecular Biology in Heidelberg. 1991 he started his own laboratory at the European Molecular Biology Laboratory. 1999 he became Director of the Center for Animal Transgenesis and Germ Cell Research and Full Professor at the University of Pennsylvania. 2004 he returned to Germany to become Director of the Max Planck Institute for Molecular Biomedicine in Münster. More than 20 years ago he identified the transcription factor Oct4. Since then his research focuses on the molecular biology of the mammalian germline (i.e. pluripotent cells and germ cells).

Konrad Hochedlinger

Harvard University and HHMI, Boston, USA

Dr. Hochedlinger is an Associate Professor of Stem Cell and Regenerative Biology at Harvard University and an Early Career Scientist at the Howard Hughes Medical Institute. Dr. Hochedlinger is a native of Austria where he studied biology before moving to MIT to perform graduate and postdoc training. He head his own lab since 2006 at the Massachusetts General Hospital Cancer Center and Center for Regenerative Medicine.

Dr. Hochedlinger's research centers around pluripotency and cellular reprogramming with a particular interest in induced pluripotency and its similarities to cancer. For example, his lab demonstrated that iPS cells can be generated without integrating viruses, which eliminated a major roadblock for translating this technology into a clinical setting. In addition, his lab discovered that pathways involved in cancer, such as p53 and Tgf-beta, also play a role in cellular reprogramming. His lab continues to dissect the mechanisms of cellular reprogramming by employing genome-wide RNAi screens, deep sequencing and by developing novel transgenic tools in mouse and human cells.

Frank D. McKeon

Genome Instute of Singapore; Harvard Medical School, Boston, USA

Frank McKeon and Wa Xian jointly oversee a highly interactive group of researchers devoted to the cloning of stem cells of regenerative tissues and cancer precursors to understand the genetic basis of self-renewal and to forward novel therapies in regenerative medicine and cancer preemption. McKeon received his graduate and postdoctoral training in Biochemistry and Biophysics at UCSF, while Xian received her graduate and postdoc training at MD Anderson in Molecular Genetics and the Baylor College of Medicine. Both work in the Department of Cell Biology at the Harvard Medical School, the Institute of Medical Biology, and the Genome Institute of Singapore.

Maria A. Blasco

CNIO, Madrid, Spain

After obtaining her PhD in 1993 Maria A. Blasco joined the Cold Spring Harbor Laboratory in 1993 New York (USA) to carry out her postdoctoral training under the leadership of C. W. Greider.

In 1997 she started her own research Group at the *Centro Nacional de Biotecnología* in Madrid. She joined the CNIO in 2003 and became its Director in 2011.

Her major research achievements include:

- Isolation of the core components of mouse telomerase and generation of the first knockout mouse for telomerase.
- Generation of the first mouse with increased telomerase expression in adult tissues. The finding that mammalian telomeres and subtelomeres have marks characteristic of constitutive heterochromatin.
- Discovery of telomeric RNAs, which are potent telomerase-inhibitors whose expression is altered in cancer.
- Demonstration that telomerase activity and telomere length determine the self-renewal capacity of adult stem cells.
- Identification of the longest telomeres as a universal feature of adult stem cell niches.
- The finding that telomerase over-expression in the context of cancer resistant-mice improves organismal fitness, produces a systemic delay in ageing and an extension in median life-span.
- Discovery that telomeres rejuvenate after nuclear reprogramming.
- Identification of the molecular mechanisms by which short telomeres/DNA damage limit nuclear reprogramming of defective cells.
- Discovery that telomeric protein TRF1 can act as both a tumour suppressor and as a factor in ageing prevention.

Blasco has received numerous awards, is an elected EMBO member and has served on its Council since 2008.

Juan Carlos Izpisua Belmonte

Salk Institute for Biological Studies, San Diego, USA

Juan Carlos Izpisua Belmonte graduated from the University of Valencia, Spain and received his Ph.D. from the University of Bologna, Italy and the University of Valencia, Spain in 1987. After postdoctoral stages at the EMBL in Heidelberg, Germany, and UCLA, Los Angeles, USA, in 1993 he moved to the Salk Institute for Biological Studies in La Jolla, California where he is currently a professor in the Gene Expression Laboratories. Since 2005 he is also the Director of the Center for Regenerative Medicine in Barcelona. He has published over 250 articles in internationally peer reviewed journals and book chapters. He has also organized and spoken at numerous meetings and seminars. His key scientific interests include the establishment of organ left-right asymmetry, limb and heart development, and stem cell biology and regeneration. He has received several notable honors and awards, including the William Clinton Presidential Award, the Pew Scholar Award, the National Science Foundation Creativity Award, the American Heart Association Established Investigator Award, and the Roger Guillemin Endowed Nobel Chair for his endeavors in these fields. Through the years, Dr. Izpisua Belmonte has been at the forefront of developmental biology research. He has produced novel, groundbreaking results, such as uncovering the role of some homeobox genes in limb patterning and specification, as well as the identification of the molecular mechanisms that determine how the different cell type precursors of internal organs are organized spatially along the embryonic left right axis. Furthermore, his work has started to give us a glimpse into the molecular basis implicated during organ regeneration in higher vertebrates, the differentiation of human stem cells into various tissues, and the molecular basis underlying somatic cell reprogramming.

John E. Dick

Princess Margaret Hospital, University Health Network and
University of Toronto, Toronto, Canada

John Dick is a Senior Scientist at Princess Margaret Hospital, a Professor, Molecular Genetics, University of Toronto, and the Director, Cancer Stem Cell Programme, Ontario Institute for Cancer Research. He is internationally known for developing normal and leukaemia human stem cell assays in immune-deficient mice. His group identified leukaemia and colon-cancer initiating cells, providing support for the hierarchical organization of some human cancers. He has been recognized for pioneering the normal and cancer stem fields with numerous awards, including the Dameshek and Thomas Prizes (ASH), Clowes Memorial Award (AACR), and shared the Diamond Jubilee Award (NCIC) with Drs. Till and McCulloch.

Emmanuelle Passegué

University of California, San Francisco, USA

Dr. Passegué earned her Ph.D. degree from the University Paris XI, France. She first trained as a mouse geneticist at the Institute for Molecular pathology (IMP) in Vienna, Austria, where she worked with Dr. Erwin Wagner, and then as a stem cell biologist at Stanford University, where she worked with Dr. Irving Weissman. She joined the UCSF faculty in 2006 and is currently an Associate Professor in the Department of Medicine, Division of Hematology Oncology with the Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research. Her research focuses on understanding the mechanisms controlling haematopoietic stem cell (HSC) and early progenitor cell functions during normal and leukeamic haematopoiesis.

Meinrad Busslinger

Research Institute of Molecular Pathology, Vienna, Austria

Meinrad Busslinger characterized sea urchin histone genes with Prof. M. Birnstiel (Zürich) and studied the regulation of globin genes with Prof. R. Flavell (London). In 1983, he became a group leader at the Institute of Molecular Biology (Zürich) and, in 1988, a senior scientist at the Institute of Molecular Pathology (Vienna). The research of Meinrad Busslinger is focused on the molecular mechanisms that control the commitment of lymphoid progenitors to the B and T cell pathways. He made the important discovery that Pax5 is the critical B cell lineage commitment factor, for which he was awarded the Wittgenstein prize.

Catriona Jamieson

University of California, San Diego, USA

Dr. Jamieson earned her MD and PhD from the University of British Columbia (UBC). She did her Internal Medicine Residency and BMT fellowship at Vancouver General Hospital/St. Paul's Hospital, a part of the UBC Health Sciences Center. Then, she completed a BMT fellowship, a Hematology Fellowship and a Postdoctoral Research Fellowship at Stanford University. Dr. Jamieson specialized in myeloproliferative disorders and leukaemia, and she currently studies the mutant stem cells and progenitor cells in myeloproliferative neoplasms that can give rise to cancer stem cells. Her goal is to find more selective, less toxic therapies.

Scott Armstrong

Children's Hospital, DFCI, Harvard Medical School, Boston, USA

Scott Armstrong received an MD and PhD from the University of Texas Southwestern Medical School. He completed an internship and residency at Children's Hospital Boston and a fellowship at Children's Hospital Boston/Dana-Farber Cancer Institute. He is currently Co-director of the Harvard Stem Cell Institute Cancer Programme and Co-director of the Leukaemia programme in the Dana-Farber Harvard Cancer Center. He received the Wilson S. Stone Memorial Award from the M.D. Anderson Cancer Center in 2006, which recognises a young researcher who has made outstanding contributions to biomedical sciences in the United States, and received the McCulloch and Till Award from the International Society of Experimental Hematology in 2009, which recognises emerging international leaders in stem cell biology. He was elected to the American Society for Clinical Investigation in 2010.

Michael F. Clarke

Stanford Institute for Stem Cell and Regenerative Medicine,
Stanford University, Stanford, USA

Dr. Michael F. Clarke is the Associate Director of the Stanford Institute for Stem Cell and Regenerative Medicine. In addition to his clinical duties in the division of Oncology, Dr. Clarke maintains a laboratory focused on two areas of research: i) the control of self-renewal of normal stem cells and their malignant counterparts; and ii) the identification and characterisation of cancer stem cells. A central issue in stem cell biology is to understand the mechanisms that regulate self-renewal of haematopoietic stem cells, which are required for haematopoiesis to persist for the lifetime of the animal. Until recently, the molecular mechanisms that regulate adult stem cell self-renewal were not known. His laboratory recently found that the proto-oncogene Bmi-1 regulates stem cell self-renewal via an epigenetic mechanism. By investigating the pathways upstream and downstream of Bmi1, the laboratory is actively investigating the molecular pathways that regulate self-renewal.

Charlotte Kuperwasser

Molecular Oncology Research Institute, Tufts University School of Medicine, Boston, USA

Dr. Charlotte Kuperwasser is a tenured Associate Professor in the Department of Anatomy and Cellular Biology at Tufts University School of Medicine and an investigator at the Molecular Oncology Research Institute (MORI) at Tufts Medical Centre. She has been working in breast cancer research since her graduate training at the University of Massachusetts, Amherst, where she completed her PhD in 2000.

As a Jane Coffin Child's Postdoctoral Fellow in the laboratory of Robert Weinberg, she developed novel humanised models to study normal and malignant human breast development as well as breast cancer metastasis to human bone. Since that time, Dr. Kuperwasser has been focusing on understanding the relationship between normal human breast development and cancer formation. Her laboratory has been studying the mechanisms that regulate normal and cancer stem cell biology and the role of the tissue microenvironment during development and cancer progression. Dr. Kuperwasser has received several awards including the COG/Aventis Young Investigator Award, the Raymond & Beverly Sackler Award, and the Natalie V. Zucker Award. She is supported by grants from the NIH/NCI, the DOD, the Breast Cancer Research Foundation, and the Silvian Foundation.

Fiona Watt

Cancer Research UK Cambridge Research Institute, Cambridge, UK

Fiona Watt obtained her D.Phil from Oxford University and was a postdoc at M.I.T. She initially established a laboratory at the Kennedy Institute in London and then moved to the Cancer Research UK (CR-UK) London Research Institute (formerly known as the Imperial Cancer Research Fund). She is currently the Herchel Smith Professor of Molecular Genetics at the University of Cambridge. She is Deputy Director of the Wellcome Trust Centre for Stem Cell Research and Deputy Director of the CR-UK Cambridge Research Institute. In 2012 Fiona Watt will move to King's College London as the inaugural director of the Centre for Stem Cells and Regenerative Medicine. She is a member of EMBO, a fellow of the Academy of Medical Sciences and the Royal Society and an Honorary Foreign Member of the American Academy of Arts and Sciences.

Elaine Fuchs

Howard Hughes Medical Institute and The Rockefeller University, New York, USA

Elaine Fuchs is the Rebecca C. Lancefield Professor in Mammalian Cell Biology and Development at The Rockefeller University. She is also an Investigator, Howard Hughes Medical Institute. Fuchs has published >260 papers and is internationally known for her research in skin biology and associated human genetic disorders, which include skin cancers. Fuchs' current research focuses on the molecular mechanisms that underlie how multipotent stem cells respond to external cues, change their programme of gene expression, exit their niche and adopt specific fates to make the epidermis, sebaceous glands and hair follicles of the skin in normal homeostasis and wound repair. She is also interested in how these pathways in normal stem cell biology go awry in squamous cell carcinomas.

Fuchs received her Ph.D. in Biochemistry from Princeton University, and after her postdoctoral research at the Massachusetts Institute of Technology, she joined the faculty at the University of Chicago. She stayed there until 2002 when she relocated to The Rockefeller University. Fuchs' awards and honors include the Presidential Young Investigator Award, the Richard Lounsbery Award from the National Academy of Sciences, the Novartis-Drew Award for Biomedical Research, the Dickson Prize in Medicine, the FASEB Award for Scientific Excellence, the Beering Award, the National Medal of Science, the L'Oreal-UNESCO Award and Charlotte Friend Memorial Award from the American Association for Cancer Research. This year, she received the Madison Medal, the Passano Award and the Albany Prize. Fuchs is a member of the National Academy of Sciences, the Institute of Medicine of the National Academy of Sciences, the American Academy of Arts and Sciences and the American Philosophical Society, and she holds honorary doctorates from Mt. Sinai/New York University School of Medicine and from the University of Illinois, Champaign-Urbana. Fuchs is also a past President of the American Society of Cell Biology and is immediate past-President of the International Society for Stem Cell Research.

Anton Berns

The Netherlands Cancer Institute, Amsterdam, The Netherlands

My laboratory focuses on mouse models of cancer. Through the years we have made contributions to the development of these models by advancing techniques to manipulate the mouse genome. Insertional mutagenesis has been one of the employed strategies that have yielded a range of new cancer-inducing genes some of which were studied in more detail. Our current research focuses on mesothelioma and lung cancers with a specific interest in the genetic heterogeneity of these tumours and their cell of origin as we believe this will likely influence their response to therapy.

Manuel Serrano

CNIO, Madrid, Spain

Manuel Serrano obtained his PhD in 1991 at the University of Madrid. From 1992 to 1996, Serrano worked as postdoctoral fellow in the laboratory of David Beach, at Cold Spring Harbor Laboratory (NY, USA). During this time, Serrano made his most important discovery with the cloning and characterization of p16, which defined a new class of cell cycle regulators and was soon recognised as a key tumour suppressor. Serrano returned to Spain in 1997 as an independent investigator and this year, in collaboration with Scott Lowe, they described the phenomenon of oncogene-induced senescence, which is now widely accepted as a main anti-tumourigenic barrier. Serrano joined the Spanish National Cancer Research Center in 2003. The main contributions of Serrano during these years have been related to the concept of oncogene-induced senescence as a tumour suppression mechanism, the regulation and function of Ink4a/Arf, and the generation of mouse models with increased cancer resistance and delayed aging. More recently, Serrano´s lab reported the anti-reprogramming activity of Ink4aArf and p53. Serrano´s laboratory is interested in the role of tumours suppressors in cancer and beyond (metabolism, stemness, aging).

Maria Pia Cosma

Centre for Genomic Regulation, Barcelona, Spain

Maria Pia Cosma was a Marie Curie Postdoc at IMP, Vienna, from 1997-2000. In 2003, she became a Young Group Leader at TIGEM, Naples, and an EMBO Young Investigator (YIP). From 2004 to 2010, she was a Lecturer at the European School of Molecular Medicine, Naples. In April 2010, she moved to the CRG, Barcelona, Spain, as a Senior Scientist and ICREA Research Professor. She is an awardee of: Marie Curie Excellence Award (2005); ERC Starting Grant (2009); HFSP Grant (2010). She received the Order of Merit of the Italian Republic in 2007, and became an EMBO Member in 2010.

Viviane Tabar

Memorial Sloan-Kettering Cancer Center, New York, USA

Viviane Tabar is a board certified Neurosurgeon with expertise in brain tumour surgery at Memorial Sloan Kettering Cancer Center in New York. In parallel, Dr. Tabar runs her own laboratory with a focus on the biology of stem cells in brain repair and cancer. The lab has also pioneered the use of mouse nuclear transfer embryonic stem cells for neural repair and was first to demonstrate integration of human ES derived neural precursors in neurogenic niches of the adult rat brain. Over the last few years the Tabar lab has increasingly focused on human glioma biology. Tabar is a member of the Sloan-Kettering Center for Stem Cell Biology, an associate professor of Neurosurgery at Sloan-Kettering and the Weill Cornell School of Medicine and the founding director of the Sloan-Kettering Pituitary Tumour Centre.

notes

Recapturing Pluripotency: links between cellular reprogramming and cancer

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Poster session

Active demethylation in non-CG context during differentiation of mouse Embryonic Stem cells

María Abad, Osvaldo Graña, Orlando Domínguez, Almudena R. Ramiro, David G. Pisano and Manuel Serrano

CNIO, Madrid, Spain

DNA methylation is one of the best-characterized epigenetic marks, and it is involved in a variety of important biological processes such as transcriptional repression, transposable element silencing, genomic imprinting and X chromosome inactivation. Although it has been viewed as a stable modification, experiments in the last decade have shown that DNA methylation is dynamic. In particular, active DNA demethylation has been observed during embryonic development and in somatic cells responding to specific signals. However, the mechanisms through which these processes take place remain unclear, and growing evidences suggest the existence of different pathways acting in a context dependent manner.

We have profiled the DNA methylation status in mouse embryonic stem cells (ES cells) and differentiated cells through Reduced Representation Next Generation Sequencing. ES cells are enriched in non-CG methylation compared with differentiated cells, and this non-CG methylation is lost during cell differentiation. Interestingly, demethylated cytosines are enriched in WRC sequence contexts, which are the consensus context for AID (Activation-Induced (cytidine) Deaminase), an enzyme that has been implied in active DNA demethylation. Finally, upon differentiation, AID deficiency impairs the expression of certain genes regulated by DNA methylation.

Together, our results demonstrate active non-CG demethylation during differentiation, that is mediated, at least in part, by AID.

Chronic TGF-β treatment of hepatoma cells changes their tumourigenic potential showing a cancer stem cell phenotype

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Chronic treatment of FaO rat hepatoma cells with TGF-β selected cells that have undergone epithelial–mesenchymal transition (EMT). We have previously established a cell line (TβT-FaO, from TGF-β-treated FaO) that shows a mesenchymal, de-differentiated and migratory phenotype when maintained in culture in the presence of TGF-β (Bertran et al., Cell Signal 2009). How this change in phenotype would affect to the *in vivo* tumourigenic potential of these cells was not explored yet.

Here we have analysed the molecular and phenotypic characteristics of tumours after inoculation of FaO and TβT-FaO cells into immunosuppressed mice. Subcutaneous injection of TβT-FaO cells produced tumours with similar doubling time and latency than the parental FaO cells, although histology reflected differences in cell structure and size. Intrasplenic injection of TβT-FaO cells showed less agresivity, respect to their parental FaO cells, but severe anemia. Liver tumour formation derived from intrasplenic injection of FaO cells induced a multifocal hepatocarcinoma in all mice, whereas parallel inoculation of TβT-FaO cells promoted less foci, but heterogeneous hepatic lesions. Most of the lesions were more irrigated and less differentiated, reminding hepatoblastoma-like tumours. However, white lesions also appeared, reminding hepatocholangiocarcinoma-like tumours. Primary cultures from both TβT-FaO-induced lesions showed positive staining for CK19 and Albumin. The cholangiocarcinoma-like lineage showed an increased expression of E-cadherin and c-kit respect to the hepatoblastoma–like lineage which displayed positive staining for CK7, CK18, Vimentin and SPARC. Conclusions: Chronic *in vitro* TGF-β treatment of FaO cells changed their tumourigenic potential. Tumour growth was similar, but phenotype of lesions reflected a stem-like phenotype which provokes the appearance of less differentiated tumours (hepatoblastomas) or transdifferentiation to a different liver tumour lineage (cholangiocarcinoma).

Placenta-derived mesenchymal stem cells as a stem cell-based therapy for experimental breast tumours

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Recent evidence suggests that mesenchymal stem cells (MSCs) selectively home to tumours, where they contribute to the formation of tumour-associated stroma providing a proof of principle for using MSCs as vehicles for antitumour agents for targeted delivery and local production of biologic agents in tumours. Bone marrow represents the main source of MSCs. However, MSCs are a rare population of cells and the number significatively decreases with age. Therefore, the search for alternatively sources of MSC is imperative. The placenta and amniotic fluid are alternative sources of stem cells with several advantages over other adult stem cells.

Recently, we described a population MSCs from the human placenta (DMSCs). Carcinogenesis induced in rats by N-Nitrosomethylurea (NMU) is an important model used for the study of breast cancer. Recent reports from our group indicated that two doses of NMU and metoclopramide induced breast cancer tumours in the 85% of the rats.

The aim of this study was to use placental mesenchymal stem cells as cellular vehicles to home and deliver anticarcinogenic agents specifically into tumour breast cancer sites.

The results show that DMSCs migrate *in vitro* and *in vivo* to rat's mammary tumours indicating a future application as cellular vehicles to deliver anticarcinogenic agents to tumour sites. Besides, transplantation of DMSCs has an important effect in the progression and evolution of the tumour. This effect is related with an important effect in the survival rate of the rats and is related to a paracrine action of the DMSCs in the tumour site.

Human lung adenocarcinoma cells in floating spheroid colonies display stem-like phenotypic hallmarks

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The specific cellular subset of a tumour responsible for initiating and maintaining the disease is accepted as coincident with a niche of so-called cancer stem cells (CSCc), or tumour-initiating cells (TICs), endowed with properties such as aberrant differentiation, self-renewal and drug resistance. We showed that the bronchoalveolar duct junctions of normal lung harbour epithelial-specific stem cells (Tesei A., et al. Cell Proliferation 2009; 42: 298-398). Here we aimed to isolate and characterise CSCc/TICs from 1) a human adenocarcinoma cell line (RAL) that we originally established at *the Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumouri (I.R.S.T.) at Meldola* (Gasperi-Campani A., et al., Cancer Genetics and Cytogenetics 1998; 107:11–20) and from 2) surgical human tissue samples. Cellular suspensions derived from the RAL cell line were cultured in ultralow attachment plates in a serum-free medium. Neoplastic lung tissue samples, derived from patients affected by adenocarcinoma at different stages, were mechanically and enzymatically dissociated before expansion in culture. Expression of stem-like phenotype-related genes in cell clusters was evaluated with RT-PCR and Real Time RT-PCR. Floating spherical colonies developed within about ten days in culture. The spheroids obtained by our originally established lung adenocarcinoma cell line RAL, resulted enriched in CD133 and OCT-3/4 transcripts. RT-PCR analysis of spheres obtained from surgical tissue samples showed a higher expression of BCRP-1, CD133, BMI-1, OCT-3/4, Lef-1, CD44 and Slug, when compared to original tissue. Floating spherical RAL cell colonies and the original adherent cell culture counterpart was also evaluated for their level of CpG methylation in OCT-3/4 and CD133 gene promoters, by means of bisulfite sequencing. They showed the same profile of hypermetilation suggesting that this level of transcriptional control is not involved in modulating their expression in our *in vitro* model.

Sphere forming assays to reveal stem like features in breast cancer cell lines: more than meets the eye

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The origin of cancer stem cells is still controversial. Their origin could be a tissue stem cell, a progenitor cell or a differentiated cell acquiring self-renewal ability, even their origin could be different depending on the type of cancer. Recently our group found that the expression of the reprogramming factor Sox2 is increased in early breast cancer lessions and during mammosphere formation in MCF7 and T47D cell lines. These observations suggest that mammosphere cultures can be used to study the reactivation of pluripotency and stemness programs during tumour progression.

Sphere forming assays are broadly used to assay stem cell activity in tissues, tumours and cell lines and several authors reported that breast cancer cell lines contain a small population of cells with stem cell properties being able to grow as mammospheres in suspension culture and behaving as tumourigenic in nude mice. We tested the ability to grow as mammospheres of 6 breast cancer cell lines representing the major breast cancer subtypes. Only MCF7, BT474 and T47D were successfully propagated as long term mammosphere cultures, measured as increase in the number of viable cells upon serial non-adherent passages. Interestingly these cell lines show typical luminal epithelial features, such as ER and E-cadherin expression. Other cell lines tested (MDA-MB-231, MDA-MB-468 and SK-Br3) formed cell clumps that can be disaggregated mechanically, without need for trypsinization, but the cell viability drops dramatically on serial passages. These cell lines show typical mesenchymal like features, shuch as spindle like morphology and SNAIL upregulation. These cell lines behave as highly aggressive and metastasic in xenotransplantation assays, demonstrating their tumour initiating ability, despite the lack of mammosphere formation. Therefore the mammosphere assay may be suitable to reveal stem like features in breast cancer cell lines, showing luminal epithelial markers, while for mesenchymal-like cell lines caution my be exerted.

Sox2 expression in breast tumours and activation in breast cancer stem cells

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The cancer stem cell (CSC) model does not imply that tumours are generated from transformed tissue stem cells. The target of transformation could be a tissue stem cell, a progenitor cell, or a differentiated cell that acquires self- renewal ability. The observation that induced pluripotency reprogramming and cancer are related has lead to the speculation that CSCs may arise through a reprogramming- like mechanism. Expression of pluripotency genes (Oct4, Nanog and Sox2) was tested in breast tumours by immunohistochemistry and it was found that Sox2 is expressed in early stage breast tumours. However, expression of Oct4 or Nanog was not found. Mammo- sphere formation in culture was used to reveal stem cell properties, where expression of Sox2, but not Oct4 or Nanog, was induced. Over-expression of Sox2 increased mammosphere formation, effect dependent on continuous Sox2 expression; furthermore, Sox2 knockdown prevented mammosphere formation and delayed tumour formation in xenograft tumour initiation models. Induction of Sox2 expression was achieved through activation of the distal enhancer of Sox2 promoter upon sphere formation, the same element that controls Sox2 transcription in pluripotent stem cells. These findings suggest that reactivation of Sox2 represents an early step in breast tumour initiation, explaining tumour heterogeneity by placing the tumour-initiating event in any cell along the axis of mammary differentiation.

p27Kip1 regulates Sox2 function in stem cells, development and differentiation through direct transcriptional repression

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Mice lacking the cell cycle inhibitor p27 exhibit an increased body size, organ hyperplasia, pituitary tumours, and retinal dysplasia 1-3 but these phenotypes are not caused by excessive activity of cell cycle kinases 4. Notably, Sox2 haploinsufficiency results in reduced body size and pituitary hypoplasia in mice 5, and a variety of human genetic syndromes including anophthalmia and hypopituitarism are due to SOX2 mutations 5-8. Here, we show genetically that Sox2 haploinsufficiency rescues all the examined phenotypes associated with p27 deficiency in mice, indicating a functional interplay between these two genes. Interestingly, p27-null tissues, including brain, pituitary and retina, show upregulation of Sox2 expression. Moreover, pluripotent stem cells lacking p27 fail to fully repress Sox2 upon differentiation. At a mechanistic level, we found that upon differentiation p27 directly binds to the critical Sox2-SRR2 enhancer together with a p130-E2F4-Sin3a repressive complex. In further support of these findings, p27-null embryonic fibroblasts present increased basal levels of Sox2 expression, which allows reprogramming to induced pluripotent stem cells (iPSCs) in the absence of ectopic Sox2. Collectively, these results establish an unprecedented connection between a cell cycle regulator and a stem cell factor.

Nodal/Activin signalling drives self-renewal and tumourigenicity of pancreatic cancer stem cells and provides a target for combined drug therapy

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Pancreatic adenocarcinoma is currently the fourth leading cause for cancer-related mortality. Despite developments in detection and management of pancreatic cancer, 5-year survival has remained miserable with about 4%. Cancer stem cells (CSCs) have been implicated in tumour growth and metastasis. Primary human pancreatic cancer tissue contains CSCs that are exclusively tumourigenic, highly metastatic, and resistant to standard chemotherapy. However, the regulatory machinery defining their self-renewal capacity and *in vivo* tumourigenicity has not been defined yet. We used primary human pancreatic CSCs derived from a representative set of patients to study CSCs *in vitro* and *in vivo*. CSCs displayed a plastic and multipotent phenotype reminiscent of embryonic stem cells consistent with emerging evidence that regulators of cell fate during embryonic development may also play a role in tumourigenesis. We therefore investigated whether the embryonic morphogens Nodal/Activin are involved in this process. Nodal/Activin were hardly detectable in more differentiated pancreatic cancer cells, while CSCs and stroma-derived pancreatic stellate cells representing a putative niche for CSCs markedly overexpressed Nodal/Activin, but not TGF-β. Knockdown or pharmacological inhibition of the Nodal/Activin receptor Alk4/7 in CSCs virtually abrogated their self-renewal capacity, *in vivo* tumourigenicity, and reversed the resistance of orthotopically-engrafted CSC to treatment with gemcitabine. However, engrafted primary human pancreatic cancer tissue containing substantial stroma showed no response due to limited drug delivery. The addition of a stroma-targeting hedgehog pathway inhibitor enhanced delivery of the Nodal/Activin inhibitor and translated into long-term progression-free survival. Therefore, the Nodal/Activin pathway is crucially involved in the self-renewal capacity of CSC. Inhibition of this pathway, if combined with hedgehog pathway inhibition and gemcitabine, provides a novel therapeutic strategy for targeting cancer stem cells.

Cancer stem cells characteristics of U-118 glioblastoma cell line

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Glioblastomas (GBM) are the most malignant primary brain tumours. The therapy success has been limited by the genetic and cellular heterogeneity of GBM, by the presence of cancer stem cells and resistance to the chemotherapeutic agents. Previous studies reported that the cancer stem cells were characterised by the expression of CD133, but the validity of CD133 as a cancer stem cell marker is still in debate. Recent data showed that CD133 (+) and CD133 (-) cells share similar stemness and tumourigenic properties. In order to determine the contribution of CD133 expression to the tumourigenic potential of glioma cells we used U-118 glioma cell line and we evaluated whether these cells have ability to organise in gliomaspheres and their resistance to chemotherapeutic agents. For that, U-118 cells were incubated with temozolomide, rapamycin, wortmannin and U-0126 and the cell cycle and apoptotic cells were analysed by flow cytometry. Our results demonstrated that the U118 cells didn't express CD133 but have the ability to divide and form spheres. The U-118 cells also show resistance to the chemotherapeutic drugs, as referred above. When chemotherapeutics were used alone, the higher percentage of apoptosis, about 33.3%, was achieved in the presence of U-0126 (15mM). However, when the chemotherapeutics agents were combined the percentage of apoptotic cells increased to 71.0%. Altogether, our results indicated that in spite of the absence of CD133 expression, this glioma cell line has characteristics of stem cells such as the ability to organise in spheres and to exhibit resistance to the chemotherapeutic agents.

Regulation of human melanoma cells by the post-implanted mouse embryo microenvironment

Elixabete López Sánchez-Sarachaga, A. Díez-Torre, R. Andrade and J. Aréchaga

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Embryonic and cancer cells share several biological characteristics, such as the undifferentiated phenotype and plasticity. An increasing number of studies support the idea that these features are regulated, at least partially, by common mechanisms. The current cancer stem cell theory postulates that neoplasias originate from a small population of stem-like, tumour-initiating cells. Supporting this theory, cells that show stem cell properties have been found in many tumours: brain tumours, including medulloblastoma and glioblastoma, head and neck squamous cell-, colon-, prostate-, lung-, pancreas-, ovarian-, breast-, hepatic carcinoma, multiple myeloma and melanoma. Over the passed few years, the reversion of the metastatic phenotype of human melanoma cells has been shown using zebrafish (Lee et al., 2005) and chick (Kasemeier-Kulesa et al., 2008) embryos which are useful for these kinds of studies, since all developmental stages can be followed *in vitro*. However, the use of a mammalian model, such as the mouse embryo, could provide conditions and cues closer to those found in the human microenvironment. The aim of our work was to test the regulation of human melanoma cells by post-implanted mouse embryos. We have used for this purpose an embryo culture system, which allows the maintenance of post-implanted mouse embryos for a few days *in vitro*, using New's method (Aréchaga, 1997). Human melanoma cells of the A375 cell line, transfected with the Nucleoplasmin-green fluorescent protein (Nucleoplasmin-GFP) hybrid gene, were adhered to the visceral endoderm of 7.5 days mouse embryos in rotary culture and co-cultured *in vitro* for three days. The location and quantification of human GFP-expressing melanoma cells inside the embryo body were evaluated at different times by confocal microscopy. Our results show that melanoma cells were internalised and migrated inside the embryo body in a way reminiscent of neural crest cells, which normally give rise to melanoblasts. The absence of localised tumoural growth, after 72 hours of *in vitro* embryo culture, suggests that malignant phenotype inhibiting factors are active at the gastrulating stage, as was shown previously during later developmental stages (Gerschenson et al, 1986). Although further research is needed to elucidate the signalling pathways involved in cancer inhibitory mechanisms by the embryo, the present results confirm the regulation of melanoma growth by developmental microenvironments and thus represents a good starting point for the future progresses in biological anticancer therapies in human melanoma.

Study of a novel RNA Pol II-associated protein in pluripotency and differentiation

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Fundamental principles underlying the pluripotency/differentiation decision may be conserved across all metazoans. For example, the regulation of RNA Polymerase II-mediated transcriptional elongation is emerging as a common developmental checkpoint in both plants and animals. Moreover, transcriptional elongation is rate-limiting in ~40% of all ES cell gene expression, where it is thought to permit the coordinated launch of complex transcriptional programmes of differentiation genes in parallel.

RNA Polymerase II-associated protein-1 (RPAP1) is a recently described and highly conserved subunit of the mammalian RNA pol II complex, which has been linked to the regulation of transcriptional elongation in plants. Here we have studied RPAP1 expression and function in stem and differentiated cells during differentiation/reprogramming.

Suv4-20h abrogation enhances telomere elongation during reprogramming and confers a higher tumourigenic potential to iPS cells

Rosa María Marión[1], Gunnar Schotta[2], Sagrario Ortega[1], and Maria A. Blasco[1]

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Reprogramming of adult differentiated cells to induced pluripotent stem cells (iPS) cells has been achieved by over-expression of specific transcription factors.

Nuclear reprogramming induces a series of profound changes at the telomeres of the parental differentiated cells, including a telomerase-dependent telomere elongation and the remodeling of telomeric chromatin. In particular, iPS cells show a decreased density of H4K20me3 heterochromatic mark at telomeres compared to the parental cells. Suv4-20h1 and Suv4-20h2 histone methytransferases (HMTases) are responsible for the trimethylation of H4K20 at telomeres, as cells deficient for both HMTases show decreased levels of H4K20me3 at telomeric chromatin. Here, we set to address the role of the Suv4-20h enzymes in telomere reprogramming by generating bona-fide iPS cells from mouse embryonic fibroblasts (MEFs) double null for both HMTases (Suv4-20dn MEFs). We found that Suv4-20h deficiency enhances telomere elongation during reprogramming without altering their ability to protect the chromosome ends or the efficiency of reprogramming. Moreover, teratomas generated from Suv4-20dn iPS cells also have elongated telomeres and an increased growth rate when compared to wild-type controls. These results indicate that abrogation of Suv4-20h enzymes and loss of heterochromatic mark H4K20me3 at telomeric heterochromatin facilitates telomere reprogramming and provides an increased tumourigenic potential to the resulting iPS cells.

Claspin loss of function may constitute an important step in cellular reprogramming leading to breast carcinogenesis

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Hereditary breast cancer is associated with germline mutations in BRCA1/2. These have incomplete penetrance, suggesting involvement of other factors. It is likely that alterations in genes that encode BRCA1/2 interacting DNA damage repair proteins may modify the risk of breast cancer development in BRCA1/2 mutation carriers or in familial cases with no identifiable BRCA1/2 mutations. Claspin has central role in checkpoint control, namely through monitoring of DNA replication and bridging checkpoint responses to the DNA repair machinery, by interaction with different proteins, including BRCA1. However, little is known about its role in cancer. Claspin inactivation seems to be important during human carcinogenesis. We have investigated whether alterations in CLSPN were associated with increased breast cancer risk as well as gliomagenesis. DNA from familial and sporadic breast cancer cases, and healthy controls, and from glioma cells, was screened for mutations in CLSPN using PCR and DNA sequencing. We have detected several CLSPN mutations and polymorphisms in both types of cancer. Two of the mutations were only found in breast cancer patients. Gly6Asp variant was over-represented in sporadic breast cancer patients, suggesting its association with an increased risk for breast cancer development. Co-segregation of three polymorphisms was associated with loss of expression of Claspin in breast tumour cells, expression being retained in normal cells. These data suggest a role for Claspin as a tumour suppressor, which may be related to its function in the control of DNA replication and triggering of cell cycle checkpoint responses. Our results, together with the currently available data on the role of Claspin in cell homeostasis/cancer, suggest that loss of Claspin function may be an important step during cancer cellular reprogramming.

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Mathematical modelling of Wnt/β-catenin dependent somatic cell reprogramming

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Somatic cell reprogramming, i.e. de-differentiation of somatic cells in pluripotent stem-like cells, is possible *in vitro* by fusing embryonic stem cells (ESCs) with somatic cells. We showed that the activation of Wnt/β-catenin signalling pathway enhances reprogramming of somatic cells *in vitro* after their fusion with mouse ES cells; in particular, ESCs can reprogramme somatic cells only after periodic and limited accumulation of β-catenin in ESC nuclei. We are now dissecting out such molecular mechanism. The first step is the derivation and identification of a differential equations based mathematical model that will allow capturing in a quantitative fashion the threshold and timing effects of nuclear β-catenin. This will allow elucidating the dynamics of the system in dependence on the presence of negative and positive feedback loops controlling the pathway, and defining the periodicity and level of nuclear β-catenin accumulation that triggers reprogramming.

Once this step will be assessed, the mathematical model will guide the engineering of ESCs that accumulate nuclear β-catenin with the desired period and at a specific threshold. These cells will be independent of Wnt activity and tested for their reprogramming of somatic cells after fusion. At present, we are collecting data, both via time-course and time-lapse experiments, to be used for the derivation and identification of the mathematical model. Preliminary data confirmed the presence of β-catenin dependent genes oscillations.

Role of GRK2 in tumourigenesis of stratified epithelium

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G-protein-coupled receptor kinase 2 (GRK2) causes desensitization of GPCRs, such as chemokine receptors, turning down their signalling. It has been described that reduced GRK2 levels potentiate leukocyte chemotaxis, and emerging evidence suggest that GRK2 expression modulates epithelial cell migration in a cell-type and stimulus-dependent manner. Our main focus has been to study the functional role of GRK2 expression in tumours of stratified epithelia. Interestingly, we detect GRK2 expression in differentiated areas of these tumours but not in the invasive front areas. Moreover, there is a significant negative correlation between GRK2 expression and tumour malignancy and grade, that could be useful in tumour prognosis. In this regard, overexpression of GRK2 in spindle-like cells induces an epithelial phenotype, and preliminary results indicated that reduced GRK2 levels increase migration of human keratynocytes. Moreover, the skin of GRK2 hemizygous mice displays an altered pattern of expression of genes involved in cell cycle and cell proliferation. We also find that in the skin of these animals there is a down-regulation of miR-145, a tumour supresor miRNA that directly targets several proteins involved in cancer and inhibits proliferation. In conclusion, our results support a putative inhibitory role of GRK2 in tumoural progression in stratified epithelium.

Exploring NANOG variants in human cancer

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The homeobox transcription factor NANOG is one of the key molecules implicated in the maintenance of embryonic stem (ES) cell identity. Despite its very well characterised role in ES cells, very little is known about the function of this gene in adult tissues. Very recent studies have reported the expression of NANOG in some specific human tumours, suggesting that this stemness transcription factor could also play a role in cancer. Interestingly, several copies of NANOG have been found throughout the human genome: the original NANOG (also called NANOG1), a tandem duplicated gene (NANOG2) and nine retrotrasposed variants (NANOGP2-P10) sharing different degrees of homology with NANOG1. Among them only three generate NANOG proteins: NANOG1, NANOG2 and NANOGP8.

In order to deepen our knowledge of the role of NANOG family genes in adult tissues and cancer, we decided to perform a detailed expression analysis of NANOG variants transcripts in a large panel of human cells, including cancer cell lines of different origins. Our results indicate that every cell line expresses a specific combination of NANOG genes.

We are currently analysing the expression of NANOG proteins in cell lines and in tissue microarrays (TMAs), containing normal and cancer tissues. Furthermore, we are performing functional assays in order to discriminate if these NANOG variants share the same function of NANOG1 protein or show novel non-redundant functions.

Our final aim is to elucidate the contribution of NANOG variants during carcinogenesis and to characterise their individual roles in pathological and non-pathological conditions.

Dyrk1A regulates egfr levels in glioblastoma tumour initiating cells

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Malignant astrocytic gliomas such as Glioblastoma (GBM) are the most common and lethal intracranial tumours. Treatment outcome after multimodal therapies, including surgical resection, radiotherapy, and chemotherapy, remain poor. Glioblastoma is the first solid cancer in which tumour growth (*in vitro* and *in vivo*) was described to be driven by a sub-population of cancerous cells. These so-called tumour initiating cells (TICs) has been hypothesized to be involved in resistance to treatment, and tumour relapse. The behaviour of TICs of aggressive gliomas resembles that of normal Neural Stem Cells (NSCs) and they respond to the same signalling pathways. Our previous work shows that Dyrk1A-kinase is implicated in the regulation of EGFR protein levels in adult NSCs. Dyrk1A antagonizes EGFR degradation, favouring recycling of the receptor back to the membrane. Our results demonstrated that this regulation of EGFR turnover controls maintenance and mobilisation of NSCs. Given that the most frequent genetic alterations on GBMs consist of amplification and/or over-expression of EGFR, we hypothesized that DYRK1A function could be relevant for glioma growth. Here we show that DYRK1A gene expression is elevated in glioblastoma, especially on those with high levels of EGFR mRNA. Reduction of DYRK1A in the U87 glioblastoma cell line using RNA interference resulted in diminished membrane and total levels of EGFR measured by western blot and flow-cytometry, and in a decreased in the number of neurosphere formed in clonal analysis. We performed intracranial trasplantation of U87 cells into nude mice and we found a 80% reduction in tumour volume in U87 shRNADYRK1A compared to cells infected with shRNA-SCRAMBLE. The inhibition of DYRK1A provoked also the appearance of distinct histological features on the grafts. We have been able to generate several primary GBM cell lines grown as neurospheres. In those TIC-enriched cultures DYRK1A protein levels are heterogeneous and it correlates with EGFR expression. We have shown that, even slight reductions in DYRK1A levels or kinase activity, inhibit EGFR and provoke differences in proliferation and neurosphere forming capacity. All these results suggest that DYRK1A controls the behaviour of TICs and is a potential target for GBM therapeutic strategies, especially those designed against EGFR.

Role of the extracellular protease ADAMTS1 for the acquisition of an endothelial-like phenotype in plastic tumour cells

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GENyO (*Pfizer-Universidad de Granada-Junta de Andalucía* Centre for Genomics and Oncological Research), Granada, Spain

Cancer stem cells have been hypothesised to explain tumour plasticity, including the capability to adopt distinct differentiation commitments. Among the mechanisms of tumour neo-vascularisation, the ability of some malignant cells to mimic an endothelial phenotype has been recognised by a capacity to form matrix-enriched pseudovascular structures. In addition to the expression of genes associated with an endothelial nature, the molecular dynamism of specific microenvironments may also be critical. We report the identification of the extracellular protease ADAMTS1 as a critical molecule for tumour cells to acquire endothelial-like properties. By analysing different tumour models, first we observed that ADAMTS1 appeared to increased tumour growth rate in an angiogenesis-independent manner, influencing the tumour cells to display an exclusive endothelial-like gene signature. Also, we documented the relevant expression of ADAMTS1 in aggressive and highly plastic melanoma and Ewing sarcoma cells, already reported as tumour cells with endothelial-like properties. Notably, inhibiting ADAMTS1 action compromised the endothelial mimetic attributes observed in this setting. Still, it is essential an active search of specific substrates to understand the contribution of these proteases either in normal or pathological neo-vascularisation, including phenomena as endothelial transdifferentiation from tumour cells, either in a primary tumour or in a prometastatic niche. Using alternative proteomic approaches, we have been able to identify target molecules for ADAMTS1 during neo-vascularisation, including important basement membrane (BM) components. Our findings provide insights into how the tumour microenvironment can elicit endothelial mimicry by tumour cells.

Function of 4.1R protein in cell migration

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Centro de Biología Molecular 'Severo Ochoa', Madrid, Spain

4.1R protein, originally identified in human red blood cells, is the founding member of the protein 4.1 superfamily which is characterised by the presence of a conserved N-terminal 4.1/ezrin/radixin/moesin (FERM) domain. Some members of the family establish linkages between membrane proteins and the cortical cytoskeleton. There is now strong evidence for their involvement in other cellular functions, including regulation of cell cycle progression, cell adhesion, and establishment of cell polarity.

In erythrocytes, 4.1R protein stabilises the spectrin-actin network and anchors it to the plasma membrane. However, the existence of multiple isoforms of 4.1R in nucleated cells and its distribution at different intracellular sites, suggest potencial roles of 4.1R in different cellular processes.

Among them, our group has shown that 4.1R is associated with tubulin and regulates microtubule organisation during interphase and mitosis. Our interests are contributed to the characterisation of functional role of 4.1R in non-erytroid cells, analysing the participation of protein 4.1R in cell migration. Regarding the studies on the functional roles of nonerythroid 4.1R isoforms, by using overexpression and gene silencing experiments, we observe important morphological changes in the cells. In response to 4.1R knockdown, cells acquire a non-polarysed phenotype, their directional and random migration is impaired and microtubule architecture distribution altered. These results suggest a relevance of protein 4.1R in cell motion and polarity.

Glioblastoma tumour initiating cells enriched cultures establishment and biological characterisation

Cristina Zahonero[1], P. Fernández[2], N. Pozo[1], Y. Noriega[1], R. Pérez[1], A. Pérez[3], A. Hernández[3], J. R. Ricoy[3], J. M. Sepúlveda[3], P. González[3], A. Ayuso[4] and P. Sánchez[1]

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Glioblastoma multiforme (GBM) is the most common and lethal primary human brain tumour. Highly mitotic activity, invasive behavior, genetic instability and inter and intra-tumoural heterogeneity, could explain the resistance of this kind of cancer to current therapies. In the last years, a new protocol developed initially for the culture of neural stem cells, have allowed the establishment of tumour initiating cells (TICs) enriched cultures, facilitating the study of glioma's biology. These cultures maintain the molecular features and genetic alterations of the original tumour. When implanted into immunocompromised mice these neurospheres mimic the invasive behavior of tumour cells in the patient. Although some groups have defended that the heterogeneity of GBMs is lost *in vitro*, our results show that TIC enriched cell lines can be classified in at least two different subtypes with distinct gene expression profile. In fact, they resemble the proneural and mesenchymal subtypes defined by array studies with human tissue. They also have different morphology and migratory capacity. Interestingly, the proneural cell lines are difficult to derivate but they can be amplified subcutaneously in nude mice xenografts preserving their characteristics over multiple passages. All these results suggest that improving *in vitro* techniques can increase the spectrum of GBM models for therapeutic approaches.

Recapturing Pluripotency: links between cellular reprogramming and cancer

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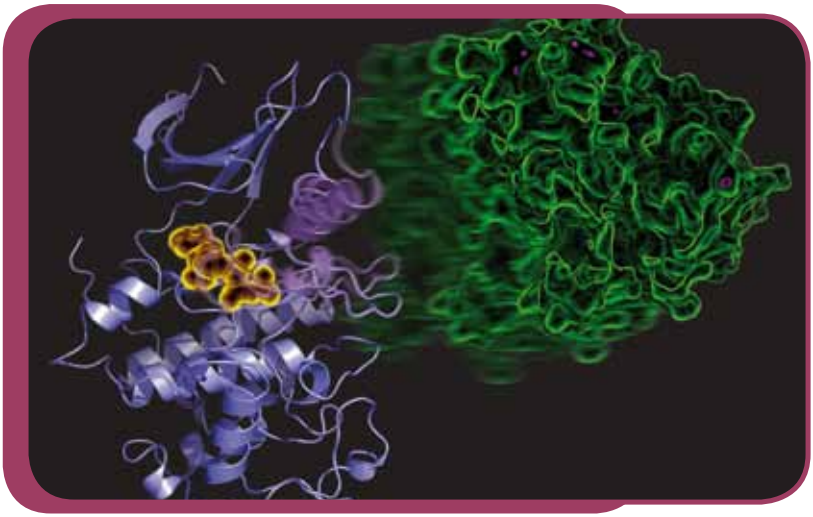

Allosteric Regulation of Cell Signalling

Madrid, 17-19 September 2012

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Ermanno Gherardi, MRC Laboratory of Molecular Biology, Cambridge, UK

Daniel Lietha, CNIO, Madrid, Spain

Giulio Superti-Furga, CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

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Previous CNIO Frontiers Meetings and CNIO Cancer Conferences

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2011

Canceromatics II: Multilevel Interpretation of Cancer Genome
28/03/2011 – 30/03/2011
Organisers: Søren Brunak, Stephen Chanock, Núria Malats, Chris Sander, Alfonso Valencia

Breast Cancer
07/02/2011 - 09/02/2011
Organisers: Joaquín Arribas, José Baselga, Miguel Ángel Piris, Lajos Pusztai and Jorge Reis-Filho

2010

Cancer pharmacogenetics: Personalizing medicine
22/11/2010 - 24/11/2010
Organisers: Javier Benítez, William E. Evans, Miguel Martín and Magnus Ingelman-Sundberg

Nature-CNIO Cancer Symposium on "Frontiers in Tumour Progression"
24/10/2010 - 27/10/2010
Organisers: Douglas Hanahan, Zena Werb and Erwin Wagner

4th ESO-CNIO Familial Cancer Conference
07/06/2010 - 08/06/2010
Organisers: Javier Benítez, Rosalind Eeles and Hans Vasen

Molecular cancer therapeutics
08/03/2010 - 10/03/2010
Organisers: Gail Eckhardt, Roy S. Herbst and Manuel Hidalgo

2009

The energy of cancer
02/11/2009 - 04/11/2009
Organisers: Toren Finkel, David M. Sabatini, Manuel Serrano and David A. Sinclair

Cancer-om-atics: Multilevelinterpretation of cancer genome data
06/07/2009 - 08/07/2009
Organisers: Søren Brunak, Núria Malats, Chris Sander and Alfonso Valencia

Stem cells and cancer
23/02/2009 - 25/02/2009
Organisers: Elaine Fuchs, Maria A. Blasco, Eduard Batlle and Mirna Pérez-Moreno

2008

Signalling upstream of mTOR
03/11/2008 - 05/11/2008
Organisers: Dario R. Alessi, Tomi P. Mäkelä and Montserrat Sánchez-Céspedes

Structure and mechanisms of essential complexes for cell survival
23/06/2008 - 25/06/2008
Organisers: Niko Grigorieff, Eva Nogales and Jose María Valpuesta

Development and cancer
04/02/2008 - 06/02/2008
Organisers: Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

2007

Links between cancer, replication stress and genomic integrity
05/11/2007 - 07/11/2007
Organisers: Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez and André Nussenzweig

Myc and the transcriptional control of proliferation and oncogenesis
11/06/2007 - 13/06/2007
Organisers: Robert N. Eisenman, Martin Eilers and Javier León

Molecular mechanisms in lymphoid neoplasm
19/02/2007 - 21/02/2007
Organisers: Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe and Miguel Angel Piris

2006

Telomeres and telomerase - CNIO / Joséf Steiner Cancer Conference
13/11/2006 - 15/11/2006
Organisers: Maria A. Blasco and Jerry Shay

Medicinal chemistry in oncology
02/10/2006 - 04/10/2006
Organisers: Fernando Albericio, James R. Bischoff, Carlos García-Echeverria and Andrew Mortlock

Inflammation and cancer
22/05/2006 - 24/05/2006
Organisers: Curtis Harris, Raymond DuBois, Jorge Moscat and Manuel Serrano

PTEN and the AKT route
08/05/2006 - 10/05/2006
Organisers: Ana Carrera, Pier Paolo Pandolfi and Peter Vogt

2005

Cancer and aging
07/11/2005 - 09/11/2005
Organisers: Maria A. Blasco, Kathy Collins, Jan Hoeijmakers and Manuel Serrano

MAP kinases and cancer
30/05/2005 - 01/06/2005
Organisers: Philip Cohen, Roger Davis, Worcester, Chris Marshall and Ángel Nebreda

Animal tumour models and functional genomics
07/03/2005 - 09/03/2005
Organisers: Allan Balmain, Mariano Barbacid, Anton Berns and Tyler Jacks

2004

Cadherins, catenins and cancer
29/11/2004 - 01/12/2004
Organisers: Amparo Cano, Hans Clevers, José Palacios and Franz Van Roy

Structural biology of cancer targets
27/09/2004 - 29/09/2004
Organisers: Ernest Laue, Guillermo Montoya and Alfred Wittinghofer

2003

Apoptosis and cancer
01/12/2003 - 03/12/2003
Organisers: Gabriel Nuñez, Marisol Soengas and Scott Lowe

Small GTPases in human carcinogenesis
16/06/2003 - 18/06/2003
Organisers: Juan Carlos Lacal, Channing Der and Shuh Narumiya

Targeted search for anticancer drugs
17/03/2003 - 19/03/2003
Organisers: Amancio Carnero and David H. Beach

2002

Mechanisms of invasion and metastasis
18/11/2002 - 20/11/2002
Organisers: Joan Massagué and Richard Hynes

The cell cycle and cancer
30/09/2002 - 02/10/2002
Organisers: Marcos Malumbres, Charles Sherr and Jiri Bartek

Cancer epigenetics: DNA methylation and chromatin
29/05/2002 - 31/05/2002
Organisers: Manel Esteller and Stephen B. Baylin

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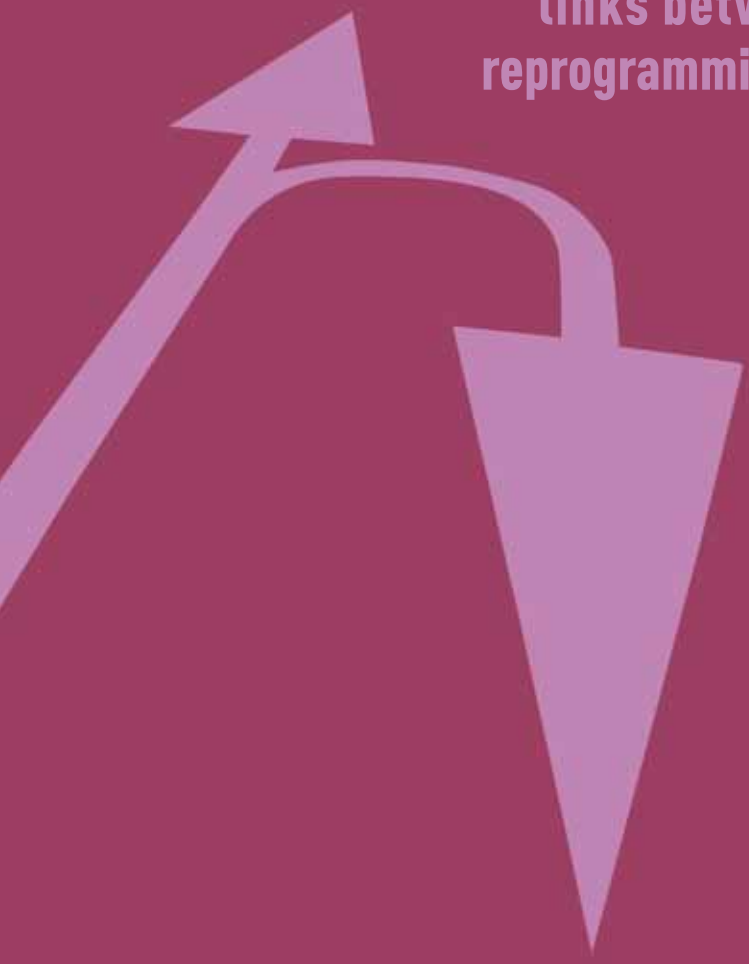

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