INSTITUT DE FRANCE
Académie des sciences

Cellules souches : nouveaux développements et perspectives / *Stem cells: new developments and prospects*

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COLLOQUE DE L'ACADÉMIE DES SCIENCES

1ER JUIN 2010

à l'Institut de France

Grande salle des séances
23 quai de Conti - 75006 Paris

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avec
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Programme

8h30 – 9h00 ***Accueil***

9h00 – 9h20 **Ouverture du Colloque** / *Welcome Address*
Alain Carpentier, Vice-Président de l'Académie des sciences
Jean-François Bach, Secrétaire perpétuel de l'Académie des sciences
Introduction :
Nicole Le Douarin, Secrétaire perpétuelle honoraire de l'Académie des sciences

SESSION I
Présidente : Nicole Le Douarin

9h20-10h10 **Keynote lecture.**
Défis et choix pour les thérapies avec cellules souches / *Challenges and Choices for Stem Cell Therapies*
Helen Blau, Baxter Laboratory for Stem Cell Biology, Stanford University (États-Unis)

10h10-10h50 **Les potentialités des cellules épithéliales thymiques** / *Capturing potency of thymic epithelial cells*
Yann Barrandon, Laboratoire « Dynamique des cellules souches » (LDCS), École Polytechnique fédérale de Lausanne (Suisse)

10h50-11h10 ***Pause***

11h10-11h50 **Signalisation Wnt, cellules souches Lgr5 et le cancer** / *Wnt signaling, Lgr5 stem cells and cancer*
Hans Clevers, , Hubrecht Institute, Utrecht (Pays-Bas)

11h50-12h30 **Thérapie génique de maladies héréditaires du système hématopoïétique** /*Gene therapy for inherited diseases of the hematopoietic system*
Alain Fischer, Membre de l'Académie des sciences, UMR 768 « Développement normal et pathologique du système immunitaire » (CNRS IFR 94 et Inserm), Groupe hospitalier Necker - Enfants Malades, Paris

SESSION II
Président : Henri Korn

14h30-15h10 **Principes généraux de la pluripotence** / *Design principles of pluripotency*
Austin Smith, Department of Biochemistry, Wellcome Trust Centre for Stem Cell Research, University of Cambridge (Grande-Bretagne)

15h10-15h50 **Les cellules souches pluripotentes comme modèles en physiopathologie : intérêt en thérapie cellulaire** / *Pluripotent stem cells as cellular models for physiopathology ; relevance to cell therapy*
Daniel Aberdam, U898 Inserm « Physiopathologie et biothérapie : cellules souches, développement et cancer », Faculté de médecine, Nice

15h50-16h10 ***Pause***

16h10-16h50 **Thérapie cellulaire en pathologie cardiovasculaire** / *Cell therapy in cardiovascular diseases*
Philippe Menasché, U633 Inserm, Groupe Hospitalier Hôpital européen Georges Pompidou – Broussais, Paris

16h50-17h30 **Développement d'une niche pour les cellules souches chez les plantes** / *Stem cell niche development in plants*
Thomas Laux, Developmental Biology and Biotechnology of Plants, Institut für Biologie III, Universität Freiburg (Allemagne)

17h30 **Conclusions**
Jean-François Bach

17h40 ***Cocktail***

Organisation : Service des colloques de l'Académie des sciences
Inscription obligatoire à colloques@academie-sciences.fr

Résumés

« Challenges and Choices for Stem Cell Therapies »

Helen M. Blau

Baxter Laboratory for Stem Cell Biology,
Stanford University School of Medicine
Stanford, CA 94305, USA

Stem cells have remarkable potential to impact human health in the next decade. Here I describe three diverse sources of cells for use in regenerative medicine.
(1) *Induced pluripotent stem cells (iPS)* have embryonic stem cell (ES) properties, they overcome the ethical and immunological problems of ES, and enable modeling of human diseases in culture. However, the mechanisms leading to iPS generation are not understood and difficult to elucidate, because reprogramming to pluripotency is a slow, stochastic and inefficient process. To address this need, we developed a synchronous high efficiency and rapid reprogramming approach: cell fusion to produce non-dividing heterokaryons, that enable early mechanistic studies. Our recent heterokaryon studies provide evidence of a novel role for the lymphocyte enzyme AID (Activation Induced Cytosine Deaminase) in two developmental genetic “black boxes”: the initiation of mammalian DNA demethylation and reprogramming toward pluripotency.
(2) *Tissue-specific stem cells* with potent regenerative properties are present in many adult tissues including blood and muscle, but their “stemness” is rapidly lost upon culture in traditional plastic dishes. We hypothesized that a bioengineered substrate which recapitulated key biophysical and biochemical niche features could overcome this limitation. Using a novel hydrogel culture substrate in conjunction with timelapse microscopy and a highly automated data analysis algorithm, we tracked the behavior of clones derived from single muscle stem cells (MuSC) in culture, and then subjected them to a stringent assay of function: transplantation into mouse muscles followed by a quantitative assessment of regeneration by non invasive bioluminescence imaging. We found that MuSCs cultured on a substrate with the elastic modulus of muscle tissue and tethered with a niche extracellular matrix protein, proliferated without loss of regenerative capacity illustrating the power of biomaterials to direct stem cell fate and overcome roadblocks to stem cell therapeutic utility.
(3) *Dedifferentiation to generate progenitors*: tissue regeneration in mammals is extremely limited, while urodele amphibians and teleost fish regenerate major structures, including the heart and entire limbs, largely by cell cycle reentry. Using genetic, biochemical, and single-cell analyses (time lapse microscopy and live cell laser capture micro dissection and catapulting), we showed that a mammalian tumor suppressor that is absent in regenerative vertebrates, the *Ink4a* product, ARF (Alternative Reading Frame), is a regeneration suppressor. Transient suppression of *ARF* together with *Rb* in differentiated muscle cells yielded primary colonies of myoblasts that retained the ability to differentiate and fuse into myofibers upon transplantation *in vivo*. These findings suggest that tumor suppression was acquired at the expense of regeneration in evolution. We propose that *transient* induction of dedifferentiation and generation of progenitors could serve as an adjunct to many types of stem cells.

Capturing Potency of Thymic Epithelial Cells

Yann Barrandon

Laboratory of Stem Cell Dynamics
School of Life Sciences
École Polytechnique Fédérale Lausanne
1015 Lausanne
and
Experimental Surgery
Lausanne University Hospital
1011 Lausanne, Switzerland

Maintenance of organ function relies on the same basic mechanisms involved during morphogenesis; in those tissues and organs undergoing extensive remodeling, there are cells termed stem cells that are responsible for long-term renewal, tissue regeneration and repair. Stem cells have two fundamental properties, the capacity to self-renew and to generate a differentiated progeny for an extended period of time (theoretically for a lifetime). The thymus is of endodermal origin, has a unique 3D epithelial structure that does not resemble that of a simple or stratified epithelium, and it supposedly only contains progenitor epithelial cells with limited growth capacities. We have demonstrated that the thymus of the rat contains a population of clonogenic epithelial cells with astonishing capabilities. These clonogenic cells can be extensively cultured and cloned while maintaining a proper thymic identity *in vitro*. Moreover, they can express MHC class II and Aire when transplanted into a reconstituted thymus. Surprisingly, they can adopt the fate of *bona fide* multipotent stem cells of the hair follicle when exposed to skin morphogenetic signals, a property maintained in serial transplantation. Gene profiling experiments have demonstrated that several transcription factors important for thymic identity were either down regulated or silenced in thymic epithelial cells recovered from skin. This clearly represents a crossing of lineage boundaries, an increase in potency and the demonstration that adult stem/progenitor cells can be robustly reprogrammed by microenvironmental cues.

Wnt signaling, Lgr5 stem cells and cancer

Hans Clevers

Hubrecht Institute and University Medical Center Utrecht,
Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

Stem cells hold great promise for regenerative medicine, but have remained elusive in many tissues because of a lack of adequate definitive markers. Progress in mouse genetics has provided the tools for characterization and validation of stem cell markers by functional and/or lineage tracing assays. The Wnt target gene Lgr5 has been recently identified as a novel stem cell marker of the intestinal epithelium and the hair follicle. In the intestine, Lgr5 is exclusively expressed in cycling crypt base columnar cells. Genetic lineage-tracing experiments revealed that crypt base columnar cells are capable of self-renewal and multipotency, thus representing genuine intestinal stem cells. In the stem cell niche of the murine hair follicle, Lgr5 is expressed in actively cycling cells. Transplantation and lineage tracing experiments have demonstrated that these Lgr5(+ve) cells maintain all cell lineages of the hair follicle throughout long periods of time and can build entire new hair follicles. Expression of Lgr5 in multiple other organs indicates that it may represent a global marker of adult stem cells. This review attempts to provide a comprehensive overview of the stem cell compartments in the intestine and skin with a focus on the cycling, yet long-lived and multipotent, Lgr5(+ve) stem cell populations.

Haegebarth A, Clevers H : Wnt signaling, lgr5, and stem cells in the intestine and skin
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Thérapie génique : le modèle des déficits immunitaires combinés sévères

Alain Fischer

Membre de l'Académie des sciences
UMR 768 « Développement normal et pathologique du système immunitaire »
CNRS IFR 94 et Inserm
Groupe hospitalier Necker – Enfants Malades, Paris

En 1968, l'allogreffe de cellules souches hématopoïétiques est devenue une thérapeutique des maladies graves du système sanguin. Les déficits immunitaires combinés sévères (DICS) furent les premières pathologies à en bénéficier. Environ 30 ans plus tard, les DICS sont à nouveau les premières maladies à bénéficier de la thérapie génique. Entre temps, les gènes responsables de ces pathologies ont été identifiés, alors que le concept et la technologie de transfert de gène ont émergé. Le transfert de gène *ex vivo* dans les cellules progénitrices de l'hématopoïèse à l'aide de vecteurs rétroviraux permet le développement de lymphocytes T et la correction durable du DICS avec un recul aujourd'hui supérieur à 10 ans. Deux formes de DICS (DICS liés à l'X et déficit en adénosine désaminase) ont ainsi été traitées avec un bénéfice pour 38 patients (5 essais cliniques au total). Ces résultats ont apporté une preuve de principe de l'efficacité d'une thérapie génique d'une maladie héréditaire. Ils ont néanmoins été marqués par la survenue d'un effet adverse grave (à type de leucémie) chez 5/19 malades atteints de DICS lié à l'X, complication inhérente aux propriétés du vecteur utilisé. La compréhension du mécanisme laisse aujourd'hui concevoir une modification de l'approche qui permette de concilier l'efficacité obtenue avec une prévention de ce risque. Par ailleurs l'utilisation de vecteurs lentiviraux, plus efficaces pour transduire des cellules souches ouvre la perspective d'élargissement de l'utilisation de la thérapie génique à d'autres maladies génétiques de l'hématopoïèse. Les premiers résultats obtenus dans le traitement de l'adrénoleucodystrophie par cette approche sont de fait encourageants.

Design principles of pluripotency

Austin Smith

Department of Biochemistry
Wellcome Trust Centre for Stem Cell
University of Cambridge (UK)

Pluripotency is the capacity of an individual cell to initiate formation of all lineages of the mature organism plus the germline directed by extrinsic cues from the embryo. A pluripotent cell has no predetermined programme. In mice and rats this naïve state at the foundation of mammalian development can be captured in culture in the form of self-renewing embryonic stem (ES) cells. Activation of the extracellular signal regulated kinase (Erk) cascade triggers differentiation of pluripotent cells. ES cells can be most efficiently derived and maintained by inhibiting this pathway and in parallel reducing activity of glycogen synthase kinase-3 (Gsk3). The cytokine LIF also supports derivation and propagation of ES cells through activation of Stat3 without reducing Erk activity. We are investigating how Erk antagonism, Gsk3 inhibition and Stat3 activation contribute to creating, maintaining and recreating authentic pluripotency.

Pluripotent stem cells as cellular models for physiopathology : relevance to cell therapy

Daniel Aberdam
INSERM U898, Nice, France
and
Rappaport Institute of the TECHNION, Haifa, Israel

Pluripotent stem cells are able to differentiate into many cell types in vitro, thus providing a potential unlimited supply of cells for cell-based therapy. As they recapitulate the main steps of embryogenesis, they represent as well a powerful cellular model for cognitive in vitro studies on normal development and congenital diseases. We reported their efficient ability to recapitulate the reciprocal instructive ectodermal-mesodermal commitments, for the formation of an embryonic skin and that the transcription factor p63, a member of the *p53* family, is mandatory for epidermal commitment. The production of pluripotent cell (iPS) lines derived from patient affected by ectodermal dysplasia (ED) fibroblasts further allowed us to decipherate the congenital p63-linked pathways defective in ED skin formation.
p63 gene encodes two main isoforms, TAp63 and Np63, with opposing functions. Recently, we report an unexpected role of p63 in heart development. TAp63 deficiency prevents expression of pivotal cardiac genes and in turn cardiogenesis, resulting in the absence of beating cardiomyocytes. Our observations indicate that TAp63, expressed by sox-17 endodermal cells, acts in a non-cell-autonomous manner by modulating expression of cardiogenic factors. Remarkably, we found that p63-null mouse embryos exhibit severe defects in embryonic cardiac development, including pronounced dilation of both ventricles, a defect in trabeculation and abnormal septation. This was accompanied by myofibrillar disarray, mitochondrial disorganization and reduction in spontaneous calcium spikes. This unexpected discovery was made on knock-out mice that have been produced a decade ago and thus confirms the powerful of pluripotent stem cells for cognitive studies linked to physiopathology.
In summary, our findings uncover a critical role for p63 in both epidermal and cardiovascular fate and suggest that p63 could be a candidate gene for orphan congenital heart diseases. Therapeutic potential of these findings will be discussed.

Cell Therapy in Cardiovascular Diseases

Philippe Menasché

Unité de Chirurgie Cardiaque, Hôpital Européen Georges Pompidou
Université Paris Descartes
Inserm U 633

Cardiac cell therapy has different objectives and modalities depending on the target clinical indication. In acute myocardial infarction, the objective is, in conjunction with early revascularization of the culprit vessel, to prevent late left ventricular remodeling which is associated with a poor prognosis. In refractory angina, the objective is to stimulate angiogenesis to alleviate symptoms. In both settings, autologous bone marrow mononuclear cells or, less commonly, autologous or allogeneic mesenchymal stem cells have been used clinically, either by direct intracoronary infusion or by left ventricular endoventricular delivery (in the case of angina). Meta-analyses of controlled trials show a functional benefit which remains modest in the infarct indication; it is more convincing in angina patients but the number of studies is smaller. In all situations, however, the benefit, when present, is mainly attributed to the paracrine effects of the cells and the subsequent activation of signaling pathways leading to increased tissue protection (stimulation of angiogenesis, reduction in apoptosis, extracellular matrix remodeling leading to a more compliant scar), and not to their differentiation in cardiomyocytes. The case of chronic heart failure is more complex. The challenging objective is to restore some function in akinetic scarred areas. So far, clinical studies have entailed the use of skeletal myoblasts or bone marrow-derived cells which have been delivered either by transepicardial injections during coronary artery bypass operations or by stand-alone endoventricular catheterization. Overall, outcomes have been rather disappointing but three major lessons have been drawn which should be helpful for framing future studies. The first is that “regeneration” of the chronically failing myocardium likely requires the provision of cells endowed with a true cardiomyogenic differentiation potential. Uncertainties surrounding the existence and potential therapeutic use of cardiac stem cells account for the interest paid to human embryonic stem cells which can be driven *in vitro* towards a cardiac phenotype. Induced pluripotent cells reprogrammed from adult somatic cells are also generating a great deal of interest. However, the mechanisms and consequences of this reprogramming process still remain elusive so that it is likely that the first applications of these cells will pertain to drug screening or disease modeling rather than to cell therapy. The second lesson is that the current methods of cell transfer are poorly efficient and require to be optimized for increasing the number of cells actually delivered to the heart. The third lesson is that the high rate of cell death mandates the search for complementary strategies designed to enhance cell viability by tackling the three main causes of cell loss, i.e., ischemia, apoptosis specifically due to the loss of cell-to-cell cell-to-matrix attachments and rejection if allogeneic cells are to be used. The paradigm has thus shifted from the simple injection of isolated cells to the more elaborate delivery of a composite product providing cells along with their vascular and matrix support, which is a prerequisite for cells to survive and thus for the therapy to be efficacious.

Stem cell niche development in plants

Thomas Laux

Institute of Biology III, Schaenzlestr.a, 79104 Freiburg

The stem cell populations of the shoot and root meristem are reliably maintained by signals from surrounding cells, the stem cell niche, although cells continuously leave the meristem and are replaced by new ones. The stem cells in the shoot meristem are controlled by signalling from an underlying organizing center, expressing the *WUSCHEL* gene (Mayer et at., 1998), and the size of the stem cell population is dynamically regulated by a feedback loop between stem cells and organizing center (Schoof et al.2000). This signalling circuitry has the potential to act as a self-regulatory system that is integrated into a larger regulatory network to control cell fate in the shoot apex (Tucker and Laux 2008). Here we will discuss recent findings with a focus on development of the stem cell niche during embryogenesis.

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