



FONDAZIONE
INTERNAZIONALE
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Native and Induced Pluripotent Stem Cell Standardization

March 19–21, 2012, Florence, Italy

Organized by:



PROGRAM

Chiostro del Maglio
Via Venezia, 5 – 50121 Florence (Italy)

March 19, 2012

Dear Speakers, dear Participants,

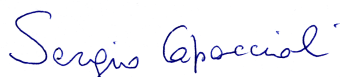
On behalf of the Executive Committee of the Workshop “Embryonic Stem Cell Standardization” to be held in **Florence**, Italy, on **March 19-21, 2012**, it is our great pleasure to invite you to participate in the Event. This Workshop has been organized in view of the potential exploitation of the embryonic stem cell and, above all, iPS technology by industry and clinical medicine.

The failure so far experienced in applying stem cell technology to repair parenchymal organs can be ascribed to the lack of sufficient knowledge on basic mechanisms, but also to the absence of standardized criteria and protocols. Very often, each laboratory follows its own “recipe”, using arbitrary nomenclature and non comparable, if not confusing, experimental protocols. All this makes it difficult to learn from the others and, ultimately, hampers the progression of knowledge on stem cell behavior and applicability.

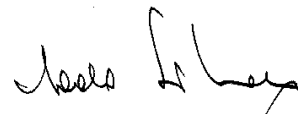
The ambitious goal of this meeting is to gather the most innovative and scientifically robust knowledge and technologies on stem cells and involve investigators from academic, public and private Research Institutions and Industry in formulating recommendations to standardize the isolation and manipulation of stem cells using solid and well-documented knowledge rather than fragmentary and often unrepeatable experimental reports.

This is the Third meeting of a series of events named “Disputationes”. This is a latin word for “Discussions, Disputes”. In fact, we invite the attendees to discuss and argue on very hot topics and mark the progress in the knowledge and technology of a specific field. “Disputationes” has also an historical significance: centuries ago, like now, when the scientific method was at its beginning, scholars used to discuss their opinions in public events, in duel-like debates.

Our kindest regards,



Sergio Capaccioli, PhD
President AIT ONLUS



Paolo Di Nardo, MD
Head LCMC

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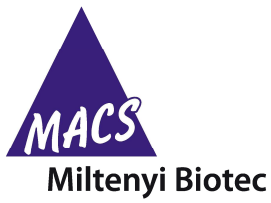
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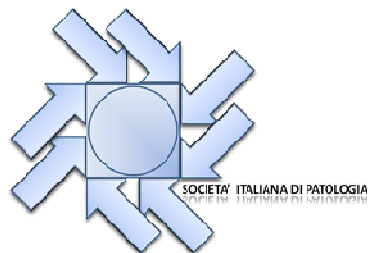
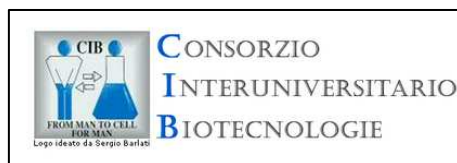
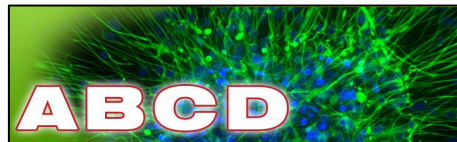
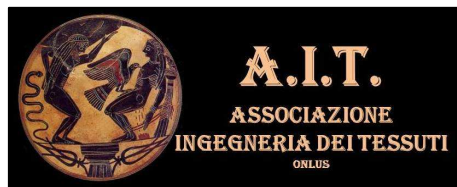


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ACKNOWLEDGEMENTS



ENDORISING INSTITUTES



GENERAL INFORMATION

CONGRESS VENUE

Chiostro del Maglio

Via Venezia, 5 – 50121 Florence (Italy)

Tel./Fax 0039 055/496803

A brief description of the congress venue:

This historic site is an ancient convent and the word “Maglio” indicates the ancient game practiced in late Renaissance, which consisted in striking an iron ball with a long hammer towards a target. The church, which nowadays is used as “Aula Magna” (main room), was built at the end of the XIII century and is decorated with frescoes by Giotto’s School. Its structure is strangely modern: thin columns support the whole building, and were appreciated by Vasari.

The cloister was built more recently than the convent, and it was classified as “not concluded”. Actually, the south east side of the cloister is not present and since 1924 it is replaced by an artistic wrought iron gate, which symbolizes both the glory and the pain of medical duties in war. In 1865 this building was used as a barracks for Italian Bersaglieri, then in 1882 it became the Italian Army Medical School. Since 1998, Medical headquarters are located in this building. In the garden you can find the important bronze memorial by Arrigo Minerbi dedicated to Medical Doctors victim of wars.

ORGANISING SECRETARIAT



O.I.C. Srl

Organizzazione Internazionale Congressi

Viale G. Matteotti, 7 – 50121 Florence (Italy)

Tel. 0039/055/50351 – Fax 0039/055/5001912

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The organising secretariat desk is located at Chiostro del Maglio near to the meeting room (Aula Magna) and is open according to the following schedule:

Monday, March 19	10.00-18.30
Tuesday, March 20	8.30-19.00
Wednesday, March 21	8.30-13.30

REGISTRATION FEES

Quotations in EUR €	By 15/02/2012	After 15/02/2012
VAT included		and on site

Participant	€ 250,00	€ 300,00
Student *	€ 100,00	€ 125,00
Welcome Dinner	€ 50,00	€ 50,00

**Copy of course certificate or chief letter is necessary.*

The registration fees include:

- access to the scientific sessions;
- congress kit;
- certificate of participation;
- personalised name badge;
- programme and abstract book;
- coffee breaks
- working lunch on March 20.

CONGRESS NAME BADGE

A personalised name badge is included in the congress kit for all registered participants to be collected at the secretariat desk upon arrival according to the official opening times before the congress start. Participants are required to wear the badge during the scientific sessions and at all times for security reasons as this is an official document and proof of registration.

WELCOME DINNER – HARRY'S BAR FIRENZE on Tuesday, March 20 at 20.30

Dinner reservations are accepted first-come-first-served basis due to space limits, participants are required to check availability at the Secretariat Desk in the congress venue by March 20 at 12.00 at the latest.

Short description of the restaurant: since 1953, Harry's Bar has been one of the most famous restaurants in the world. Today, the style that made it famous continues to both fascinate and delight. The impeccable service, the newly interpreted dishes of the excellent cuisine, a wine cellar offering the best selections and the American Bar make the story of Harry's Bar legendary. The elegant ambience, enhanced by the light of the exclusive Murano art glass lamps provides a perfect meeting place to enjoy the special Bellini cocktail or a delicious cup of coffee. The magnificent terrace, overlooking the Arno river with the Ponte Vecchio in the background, is a wonderful spot to admire a beautiful Florence sunset.

The most suggestive way to reach Harry's Bar - A short stroll along the Arno river...

Reaching the Harry's Bar is very easy and fascinating. Coming from the Uffizi Gallery and Ponte Vecchio keep walking along Lungarno Acciaiuoli, Lungarno Corsini and once you have crossed Piazza Goldoni you get to the Harry's Bar (address Lungarno Amerigo Vespucci, 22 Firenze)

SLIDE CENTRE

A computerised slide centre for the management of all scientific presentations is located in the meeting room (Aula Magna) and available for speakers to rehearse and upload their presentations according to the following schedule:

Monday, March 19	13.00-18.30
Tuesday, March 20	8.30-19.00
Wednesday, March 21	8.30-13.30

Speakers are invited to strictly follow the allotted times for a smooth flow of the scientific sessions. **Computer projection only is available**, no slide projector is at disposal in the meeting room and it is not possible to use personal laptops at the podium. The management of the scientific projections is guaranteed by this single computerised system that automatically forwards each presentation to the podium and on the main screen. Speakers are kindly requested to use PowerPoint for windows (XP version). Those speakers with PowerPoint presentations on CD Rom or USB pens must go to the slide centre at least one hour before presentation, meanwhile those with presentations on their own laptop and/or using Macintosh/Apple at least two hours before presentation in order to convert the files into windows format and/or download them on the slide system.

COFFEE BREAKS AND WORKING LUNCH

During the congress coffee breaks and a working lunch are scheduled according to the official programme. The catering area is located near to the meeting room (Aula Magna) and to the secretariat desk.

EXHIBITION AREA

During the congress an exhibition display of the latest instruments and technologies in the field is held near to the catering area, where participants have the opportunity to meet the representatives of the following companies: **CARL ZEISS SpA, M-MEDICAL Srl, MILTENYI BIOTEC Srl.**

POSTER AREA

The poster area is located near to the catering area and to the secretariat desk, where tape is available for fixing the posters to the boards. Posters must be 70cm wide and 100cm high, the title and names of the authors must be included in the top side of the posters.

On March 19 from 8.30 to 12.00 presenters must fix the posters on the assigned boards.

On March 21 posters must be removed from 11.00 to 13.00; the posters left on the boards after 13.00 will be disposed of.

On March 20 during the working lunch (13.30-14.30) presenters are required to wear the name badge and to stay close to the assigned poster boards to reply to the questions by the participants.

No poster discussion is scheduled in the meeting room.

The registration is absolutely necessary to have access to the congress venue and to present the poster.

CLOAKROOM

During the congress coats and luggage can be left in an unattended room near to the secretariat desk. The organising secretariat can not be held liable for this area.

TRANSPORTATION TO THE CONGRESS VENUE

The congress venue is located near to Florence historical city centre, walking distance from the main railway station of Santa Maria Novella and most hotels reserved by the organising secretariat.

By plane

From Florence airport the congress venue is about 15-20 minutes by taxi or by bus (Ataf-Sita bus service “Vola-in-bus”, main stop at the railway station of Santa Maria Novella with departures every 30 minutes).

For bus information www.ataf.net

For taxi reservation dial 055/4242 or 055/4798.

For taxi information www.radiotaxifirenze.it

By train

The most comfortable railway station is Santa Maria Novella, located downtown at about 15 minutes walking distance from the congress venue.

For train information www.trenitalia.com

By car

From North: exit Firenze Nord, follow directions for the city centre “Centro”.

From South: exit Firenze Sud, follow directions for the city centre “Centro”.

For maps and routes www.viamichelin.it

Near to the congress venue different fee parking lots are available for delegates to park their cars.

The organisers can not be held liable for accidents or mishaps that may occur to participants or to their accompanying persons during the congress or as a result of it, therefore a personal insurance is recommended for all participants according to the usual National procedures in each country of origin.

According to the strict Italian law smoking is not permitted in any area of the congress venue, except for the external cloister under the arches.

Participants are kindly requested to switch off the mobile phones in the main room during the scientific sessions.

PROGRAM

Monday, March 19

13.30 – 15.00 Registration

15.00 – 15.30 OPEN CEREMONY

15.30 – 18.10 SESSION 1: EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS (I)

CHAIRPERSONS: Hans-Werner Denker (Universität Duisburg-Essen, Germany) -
Mariusz Z. Ratajczak (University of Louisville, USA)

15.30 – 16.00 **Fiorella Altruda** (University of Torino, Italy) - Germline cell-derived pluripotent stem cells: factors controlling transdifferentiation and potential applications in animal models of disease

16.00 – 16.30 **Jose Cibelli** (Michigan State University, USA and LARCel, Spain) - Reprogrammed transcriptome in rhesus-bovine interspecies somatic cell nuclear transfer embryos

16.30 – 16.50 COFFEE BREAK

16.50 – 17.20 **Massimo De Felici** (University of Rome “Tor Vergata”, Italy) - Generation of oocytes from stem cells: a biological view

17.20 – 17.50 **Graham C. Parker** (Wayne State University, USA) - How best to advance neurodegenerative research using pluripotent stem cells

17.50 – 18.10 DISPUTATIO

Tuesday, March 20

9.30 – 11.20 SESSION 2: EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS (II)

CHAIRPERSONS: Anne L. Plant (National Institute of Standards and Technology, USA) - Massimo De Felici (University of Rome “Tor Vergata”, Italy)

9.30 – 10.00 **Marco Onorati** (University of Milano, Italy) - Developmentally coordinated extrinsic signals drive human pluripotent stem cell differentiation towards fully functional DARPP-32+ medium-sized spiny neurons

10.00 – 10.30 **Neil J. Harrison** (The University of Sheffield, UK) - Culture adaptation of human ES cells – evolution *in vitro*

10.30 – 11.00 **Paola Rebuzzini** (University of Pavia, Italy) - Genomic stability of mouse embryonic stem cells after γ -rays treatment

11.00 – 11.20 DISPUTATIO

11.20 – 11.40 COFFEE BREAK

11.40 – 13.30 SESSION 3 - PHYSICAL FACTORS AND STEM CELLS

CHAIRPERSONS: Paolo Di Nardo (University of Rome “Tor Vergata”, Italy) - Neil J. Harrison (The University of Sheffield, UK)

11.40 – 12.10 **Masahiro Kino-Oka** (Osaka University, Japan) - Manufacturing of cells and tissues based on flexible Modular Platform

12.10 – 12.40 **Monica Monici** (Laboratorio Congiunto ASAcampus, and University of Florence, Italy) - The role of physical factors in stem cell differentiation and tissue engineering

12.40 – 13.10 **Murli Dhar Tiwari** (University of Delhi, India) - Adhesion or anti adhesion? What is the significance of stem cell antigen CD34 in hematopoietic biology?

13.10 – 13.30 DISPUTATIO

13.30 – 14.30 LUNCH AND POSTER SESSION

14.30 – 16.20 SESSION 4 - ETHICAL AND LEGAL ASPECTS IN STEM CELL RESEARCH

CHAIRPERSONS: Jose Cibelli (Michigan State University, USA and LARcel, Spain) - Graham C. Parker (Wayne State University, USA)

14.30 – 15.00 **Hans-Werner Denker** (University of Duisburg-Essen, Germany) - A quest for redefining stem cell induction strategies: How to deal with ethical objections and patenting problems

15.00 – 15.30 **Maria Cristina Galli** (Istituto Superiore di Sanità, Rome, Italy) - Regulatory issues in developing Advanced Therapy Medicinal Products with stem cells in EU

15.30 – 16.00 **Anne L. Plant** (National Institute of Standards and Technology, USA) - NIST Activities in Metrology for Stem Cells

16.00 – 16.20 DISPUTATIO

16.20 – 16.40 COFFEE BREAK

16.40 – 18.45 SESSION 5 - STEM CELLS AND CANCER

CHAIRPERSONS: Lucio Luzzatto (Istituto Toscano Tumori, Florence, Italy) - Sergio Capaccioli (University of Florence, Italy)

16.40 – 17.10 **Persio Dello Sbarba** (University of Florence, Italy) - Cancer and Leukaemia Stem Cells and their environment in cancer resistance to treatment

17.10 – 17.40 **Enrico Mini** (University of Florence, Italy) - The impact of the concept of Tumor Stem Cells on cancer therapy

17.40 – 18.10 **Roberta Santini** (Istituto Toscano Tumori, Florence, Italy) - Hedgehog signaling in Melanoma Stem Cells

18.10 – 18.25 **Lucio Luzzatto** (Istituto Toscano Tumori, Florence, Italy) - Concluding remarks

18.25 – 18.45 DISPUTATIO

Wednesday, March 21

9.30 – 11.20 SESSION 6 - STEM CELL MODELS AND THERAPEUTIC APPLICATIONS

CHAIRPERSONS: Marco Onorati (University of Milano, Italy) - Robert Passier (Leiden University Medical Center, The Netherlands)

9.30 – 10.00 **Alberto d’Onofrio** (IFOM-IEO, Milano, Italy) - Mathematical Modelling of Stem Cell Kinetics

10.00 – 10.30 **Maurilio Sampaolesi** (University of Pavia, Italy and KU Leuven, Belgium) - Epigenetic memory of iPS cells to enhance muscle differentiation

10.30 – 11.00 **Mariusz Z. Ratajczak** (University of Louisville , USA) - Very Small Embryonic Like Stem Cells (VSELs) – our key to regeneration, longevity and passkey to cancer

11.00 – 11.20 DISPUTATIO

11.20 – 11.40 COFFEE BREAK

11.40 – 13.30 SESSION 7: PLURIPOTENT VS ADULT STEM CELLS IN HEART REPAIR

CHAIRPERSONS: Fiorella Altruda (University of Torino, Italy) - Maurilio Sampaolesi (University of Pavia, Italy and KU Leuven, Belgium)

11.40 – 12.10 **Robert Passier** (Leiden University Medical Center, The Netherlands) - Human pluripotent stem cells as models for cardiac development, disease and drug screening.

12.10 – 12.40 **Dinender K. Singla** (University of Central Florida, USA)- Cardiac Regeneration in the Diabetic Heart

12.40 – 13.10 **Paolo Di Nardo** (University of Rome “Tor Vergata”, Italy) - Engineered human cardiac progenitor cells in hearth repair

13.10 – 13.30 DISPUTATIO

ABSTRACTS

Germline cell-derived pluripotent stem cells: factors controlling transdifferentiation and potential applications in animal models of disease

Sharmila Fagoonee, Letizia De Chiara and Fiorella Altruda

Molecular Biotechnology Center, University of Torino

Stem cells hold the key to replacing cells lost in many genetic and degenerative diseases. Stem cells can be differentiated into specialised cell types and used as a platform for gene delivery to correct single gene defects, especially those which can be corrected by only a small amount of functional protein.

The adult testis contains a pool of stem cells known as spermatogonial stem cells, which continuously generate differentiating daughter cells for subsequent production of haploid spermatozoa through meiosis. Cultured spermatogonial stem cells (SSCs) derived from testis of adult mice and human undergo a spontaneous conversion to ESC-like pluripotent stem cells. We have demonstrated that these germ line-cell derived pluripotent stem cells or GPSCs can be differentiated into functional hepatocytes in vitro. GPSCs are thus a promising tool for the treatment of hereditary metabolic diseases whilst avoiding ethical concerns over embryo use for the production of pluripotent human stem cells.

Reprogrammed transcriptome in rhesus-bovine interspecies somatic cell nuclear transfer embryos

Jose Cibelli DVM, PhD

Michigan State University, East Lansing, Michigan, USA and LARCel, Sevilla, Andalusia, Spain.

In the post induced pluripotent stem cells (iPSCs) era, new knowledge about these cells has emerged showing unsettling facts such as aberrant reprogramming, gene deletions, epigenetic memory and stochastic differentiation potential. Against this backdrop, there is rekindled interest in the reprogramming potential of the oocyte. Our work focuses on interspecies somatic cell nuclear transfer (iSCNT), defined as the fusion of an enucleated oocyte from one species with a cell from another, as a way of providing new insights into the molecular processes responsible for reprogramming somatic cells.

This concept is not new. iSCNT, has been proposed as tool to rescue endangered or already extinct species. The evidence that the procedure may work producing full-term pregnancies is however scant. Out of 21 different species cloned using SCNT, four were produced by iSCNT. When examined more closely, two of these are parent-child species and the evolutionary distance between the other two species is 1 million and 10.1 million years ago for *Felis catus* - *Felis Sylvester* and *Equus caballus* – *Equus asinus* respectively. Clearly, the further one stretches that distance, the lower the likelihood of obtaining a viable fetus becomes.

A more tangible goal is to attempt to produce embryonic stem cells using iSCNT. In our laboratory we set up a series of experiments trying to determine whether the fusion between bovine oocytes and non-human primate somatic cells, such as rhesus macaque and chimpanzee, would be capable of reinitiating embryonic development. We examined the extent of preimplantation development these embryos are capable of achieving, the epigenetic changes that their nucleus is subject to, and the reactivation of genes that are typical of that developmental stage as well as the silencing of fibroblastspecific genes. We concluded that regardless of the incredibly large divergence distance between bovine and non-human primate - 94.4 million years – molecules present in the cow oocyte are capable of reactivating monkey embryonic genes. Our data provides encouraging evidence in support of the iSCTN model as a research tool and potentially leading to a more powerful and reliable method of producing isogenic pluripotent cells for human therapy.

Generation of oocytes from stem cells: a biological view

Massimo De Felici

Dipartimento di Sanità Pubblica e Biologia Cellulare, Facoltà di Medicina e Chirurgia, Università di Roma Tor Vergata, Via G. Montpellier 1, 00133 Roma; e-mail: defelici@uniroma2.it

Stem cells (SCs) from various sources exposed to appropriate and specific conditions differentiate into cell types of all three germ layers (ectoderm, mesoderm and endoderm) and also into germ cells. The latter had raised speculation that SCs may have a potential role in reproductive medicine. So that in vitro development of germ cells to obtain mature male and female gametes having the capacity to participate in normal gametogenesis has been actively attempted for the last few years. We believe that methods to produce oocytes from SCs should utilize well-designed protocols that will establish a controlled, rather than spontaneous, oocyte development and differentiation. These can be based on protocols developed to obtain functional oocytes from in vivo-derived primordial germ cell (PGCs) and tissues of embryonic ovaries. I will outline the researches carried out mainly in my laboratory designed to achieve these objectives and their relevance toward improving oocyte development from SCs.

Cancer and Leukaemia Stem Cells and their environment in cancer resistance to treatment

Persio Dello Sbarba

Dipartimento di Patologia e Oncologia Sperimentali, Università degli Studi di Firenze.

Cancer Stem Cells (CSC) and Leukaemia Stem Cells (LSC) in particular represent a small minority of virtually all neoplastic cell populations which retains most features of normal stem cells sustaining physiological tissue regeneration. CSC/LSC have been shown to be the ultimate responsible for tumor initiation and expansion, as well as for the long-term maintenance of Minimal Residual Disease (MRD) and the consequent risk of late relapse of disease even in patients who had brilliantly responded to induction therapy with complete clinical as well cytogenetic (when applicable) remission.

Evidences obtained in our laboratory and confirmed later by others indicated that severe hypoxia (0.1-1% oxygen) is a critical environmental condition for the maintenance of the haematopoietic stem cell (HSC) potential. In hypoxia, HSC are maintained better than in normoxia, whereas non-stem clonogenic progenitors are suppressed. On this basis, we introduced the concept of “hypoxic stem cell niches” where HSC would be capable to reside due to their adaptation to hypoxia, that, in turn, would contribute to the maintenance of their potential. HSC were found, indeed, capable to cycle in hypoxia and hypoxia to allow a limited HSC cycling finalized to support self-renewal.

We addressed the regulation of LSC maintenance within the above context. It is worth pointing out that, in the case of CSC, hypoxia-adaptation would be crucial not only for their long-term persistence in tissues, but also for cancer progression to more aggressive phenotypes, being hypoxia itself a strong inducer of genomic instability. Stabilized and cloned leukaemia cell lines were found highly heterogeneous, to comprise cells with a hypoxia-adapted “stem” phenotype as well as others with a hypoxia-sensitive “progenitor”, suggesting these phenotypes to be not genetically “frozen”, but flexibly, and reversibly, expressed. Furthermore, in hypoxia, while surviving leukaemia cells are generally growth-arrested, most LSC are cycling, indicating that cycling in hypoxia is a specific property of LSC. Such a property is the main candidate feature to sustain MRD of leukaemia within the hypoxic stem cell niches.

On the basis of all above, leukaemia cell lines emerged as suitable models to assess independently the sensitivity to chemotherapy of the “stem” or the “progenitor” leukaemia cell phenotypes, provided an experimental system capable to select the one from the other (such as incubation in hypoxia for different times) is established. The idea was exploited to test the drug-sensitivity of chronic myeloid leukaemia (CML) cells. The expression and phosphorylation of the oncogenetic BCR/Abl fusion protein of CML, but not BCR/abl transcription, were found suppressed in hypoxia, indicating that hypoxia-selected LSC, while remaining genetically neoplastic, are independent of BCR/Abl signalling. Hypoxia-selected LSC, indeed, exhibited complete refractoriness (or “primary” resistance) to Imatinib-mesylate (IM), the first-choice drug for CML therapy. This resistance is the characteristic property of CML cells responsible for MRD. On the other hand, the clonal expansion of LSC was paralleled by the rescue of BCR/Abl protein expression, necessary for maximal neoplastic growth, so that LSC progeny reverted to IM sensitivity.

The above-summarized results represent the first experimental evidence supporting the association of an environmentally-enforced metabolic profile -hypoxia-adaptation- with a specific phenotype -BCR/Abl suppression- affecting CML cell resistance to treatment. This view is in keeping with the recent proposal put forward by others to take a “metabolic profiling” of cancer cells as the basis for a “context-specific” therapeutic strategy.

A quest for redefining stem cell induction strategies: How to deal with ethical objections and patenting problems

Hans-Werner Denker

Lehrstuhl für Anatomie und Entwicklungsbiologie, Universität Duisburg-Essen (Germany)

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A recent ruling of the European Court of Justice in Luxembourg (EU-CJ, 18 October 2011) on ES cell patenting has renewed the interest in attacking so-far unsolved ethical problems of stem cell research. In this contribution I will outline ethical and patenting problems that appear to arise in the modern field of induced pluripotent stem cell (iPS cell) technology. The focus will be on stem cell potentiality, and I will argue that potentiality rather than the act of sacrificing embryos will have to be a central point of concern in stem cell ethics and patenting in the future. Possible solutions will be discussed.

When somatic cells are reprogrammed to gain “full” pluripotency, they acquire (so to say as a by-product) the capability to form viable embryos if tetraploid complementation (TC) is performed (addressed as “gold standard” by some authors). I argue that human cells possessing this capability cannot be patented. In analogy to the arguments used by the EU-CJ, this must apply not only to patenting cell lines themselves but also to patenting technologies using these cells. The fact that the problem is being created by the process of iPS cell induction itself asks for alternative strategies of stem cell derivation as well as for stringent criteria how to define and to test pluripotency vs. lower levels of potentiality. It will have to be discussed which genes should be seen here to be crucial (e.g. genes involved in early embryonic pattern formation processes). For ethical reasons it cannot be defended to use TC as a test for “full” pluripotency with human cells. It is thus necessary to discuss alternative test criteria.

Recent reports suggest that it may indeed be possible to directly induce multipotency while bypassing a pluripotent state, thus avoiding the addressed problems. It thus appears timely and prudent to redefine goals and strategies for stem cell derivation, in addition to stem cell quality testing criteria, in order to find an escape from the ethical and patenting dilemma.

Mathematical Modelling of Stem Cell Kinetics

Alberto d'Onofrio, PhD

Department of Experimental Oncology, European Institute of Oncology (IFOM-IEO), Milano, Italy

The current knowledge on the differentiation and proliferation of pluripotent Stem Cells and of multipotent progenitors has received important inputs from computational and systems biology (Roeder and Lorenz, 2006; MacArthur et al, 2008; Michor et al 2008). Indeed, the itself concept of asymmetric vs symmetric stem cell division is expressed in mathematical language. In this talk, after a review of some basic concept, we shall focus on two classical stem-cells based systems: colon crypts and hematopoiesis.

References

d'Onofrio A and Tomlinson IPM, A nonlinear mathematical model of cell turnover, differentiation and tumorigenesis in the intestinal crypt, *Journal of Theoretical Biology*, 244:367-374, 2007

MacArthur BD, Ma'ayan A, Lemischka IR. Toward stem cell systems biology: from molecules to networks and landscapes. *Cold Spring Harb Symp Quant Biol.* 2008;73:211-5

Michor F Mathematical models of cancer stem cells. *J Clin Oncol.* 2008 Jun 10;26(17):2854-61

Roeder I, Lorenz R. Asymmetry of stem cell fate and the potential impact of the niche: observations, simulations, and interpretations. *Stem Cell Rev.* 2006;2(3):171-80

Regulatory issues in developing Advanced Therapy Medicinal Products with stem cells in EU

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The views expressed in this article are author's personal views, and may not be understood or quoted as being made on behalf of the CAT/EMA or reflecting the position of the CAT/EMA.

Advanced therapy medicinal products (ATMP), including stem cell therapy and regenerative medicine, represent a significant emerging field in which new treatment opportunities are offered to patients. The main EU regulatory framework for ATMP includes Regulation 1394/2007 and Directive 2001/20/EC. Medicinal products for stem cell therapy and regenerative medicine are considered as cell therapy (CPMP) or tissue engineered medicinal products (TEP), if genetically modified they are considered gene therapy products (GTMP).

With the Regulation 1394/2007, ATMP are authorized for the EU-wide market by the European Medicine Agency (EMA) through the centralized procedure, in which market authorisation application is evaluated by the Committee for Advanced Therapy (CAT) and final opinion to the European Commission is given by CHMP. CAT working parties CPWP and GTWP has issued a number of guidelines describing quality, preclinical and clinical requirements for CPMP/TEP as well as for GTMP, to help applicants in developing their products.

According to Directive 2001/20/EC, clinical trial approval is the responsibility of each EU Member State. Therefore clinical development takes place at national level, with each EU Member State performing a separate evaluation and authorization procedure even for a multinational trial. Procedures and initiatives have been put in place by EMA and national Competent Authorities to facilitate an efficient translation of research discoveries into effective ATMP.

The challenges faced during stem cells-based ATMP development in EU will be discussed.

Culture adaptation of human ES cells – evolution *in vitro*

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Human pluripotent stem cells are not immune to mutation, nor are they maintained in optimal conditions. Consequently, these cells have both the means and motive to adapt to culture, and will evolve by the same Darwinian principles as all other living organisms. This culture adaptation has been documented in both human ES and iPS cells, with epi/genetic changes observed which increase the growth capacity of these cells. For a stem cell this increased growth capacity must result from an increased propensity for self-renewal over differentiation and/or death, and as such culture adaptation will likely influence how these cells can be grown and differentiated. Further, that the most common genetic changes in culture adapted pluripotent cells are also frequently observed in embryonal carcinoma cells, their malignant equivalent, is clearly a safety concern.

The minimisation of genetic change in stem cell cultures requires an understanding of the factors that impact on both mutation and selection of these variants, and with this in mind the genotype and phenotype of culture adapted cells will be discussed. The large scale effort of the International Stem Cell Initiative (ISCI) has better defined the amplicons associated with culture adaptation, and also the impact of growth conditions on the appearance of genetic changes, and these data will also be mentioned. Whilst the maintenance of 'normal' stem cell lines remains a challenge, culture adaptation also presents an opportunity to gain insight into those mechanisms which control cell fate, and may ultimately be utilised to increase our understanding of human pluripotent cells.

Manufacturing of cells and tissues based on flexible Modular Platform

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The cell sheet engineering is one of the most critical techniques to make rapid construction of three-dimensional (3-D) structure as cultured tissues. The monolayer cell sheet can be harvested using the temperature-responsive surface to maintain intact vital cell-cell junctions and extracellular matrix (ECM) which plays a role of glue for sheet assembling as well as the host after transplantation.

Recently autologous transplantation of multilayer myoblast sheet is emerging as a new technique for curing myocardial infarction, which is associated with the dysfunction of cardiomyocytes and irreversible cell loss. This method can overcome the disadvantages such as less take ratio of transplanted cells through the direct injection of myoblast suspension. Skeletal myoblasts are easy to be harvested from patients, have ability to become active, self-renew and differentiate, permitting muscle regeneration upon muscle injury. The sheet of myoblasts also has ability to source the cytokines which improves heart function due to paracrine system including the facilitation of angiogenesis and the attraction of progenitors on affected part. Many researches have been tackled concerning cell source exploring, cell culture, sheet assembling, and in vivo animal tests.

In manufacturing, strict management against contamination and human error are compelled due to direct use of un-sterilizable products and the laboriousness of culture operations, respectively. Therefore, the system developments for ensuring a stable process and quality of therapeutic products are the critical steps, leading to the active commercialization using the cell sheets.

The comparison of management between cell processing facility (CPF) and cell aseptic processing system (CAPS) based on the isolator system revealed that CAPS leads to reductions of the running cost as well as operational laboriousness in the small production. Then, we, recently, conducted the design of manufacturing facility to develop the automation system for sheet manufacturing with flexible modular platform (fMP). Our developed isolator system for the sheet assembly can make automated formation of multilayered sheets and their incubation, and the machinery operations were successfully performed. This system can realize some procedures by having flexible connections with various modules required for the culture operations under sterile conditions, suggesting the broad versatility for the production in other types of multilayered sheets.

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The impact of the concept of Tumor Stem Cells o cancer therapy

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Cancer stem cells (CSCs) comprise a unique subset of cells capable of self-renewal and pluripotency that can drive tumor initiation, maintainance, mutation accumulation and metastasis as revealed in functional assays.

First identified in hematological malignancies, CSCs have been recognized to play an important role in several solid tumors. Work in a variety of cancers has suggested that CSCs are an important source of resistance to chemotherapy. This might explain the current limitations of anticancer drugs in curing human malignancies.

Several resistance mechanisms have been proposed including high expression of specific ABC drug transporters. Pathways important for the growth and tumorigenesis of CSCs include the DNA damage checkpoint pathway, the Wnt, Notch, Hedgehog and the PI3K/AKT/mTOR signalling pathways. Activation of these cellular pathways has been identified as a likely mechanism causing the insensitivity of CSCs to chemotherapy.

Although much work is still needed to identify and characterize CSCs, efforts are now being directed towards the design of therapeutic strategies capable to target the molecular mechanisms sustaining cancer stemness and treatment resistance, with the goal of improving the efficacy of current cancer therapies.

The role of physical factors in stem cell differentiation and tissue engineering

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Introduction and Aims. It is known that physical factors play an important role in regulating biological processes. As well as the biochemical factors, also physical factors may induce the cells to reprogram their functions to adapt dynamically to the environmental conditions. Therefore they can be applied in biomedicine and biotechnology in order to drive and modulate cell behavior. Here, we report the results of our research on the application of physical stimuli (gravitational and mechanical stresses, laser radiation) for modulating cell commitment, differentiation and function with the final aim to understand when and how physical stimuli can be useful for promoting tissue repair and formation of functional tissue constructs.

Methods. The experiments that have allowed us to obtain the results here described have been performed on human Mesenchymal Stem Cells (hMSCs) by using a Random Positioning Machine (angular velocity of rotation 60°/s, exposure 72 h) to model microgravity, a hyperfuge to expose the samples to hypergravity conditions (5 periods of 10 min at 10 x g spaced with 10 min recovery periods at 1 x g) and a high power, pulsed Nd:YAG laser to irradiate the cells with IR pulses (λ 1064 nm, 200 μ s pulse duration, 10 Hz repetition rate, 458.65 mJ/cm² energy fluence, 73 sec exposure). RT-PCR, immunofluorescence microscopy and image analysis have been applied in order to analyze the effects induced by the treatments.

Results. Our findings show that the exposure to microgravity conditions inhibits the differentiation of hMSCs towards mature elements belonging to tissues with antigravitational function (osteoblasts, chondrocytes, etc), favouring adipogenesis. On the contrary, hypergravity promotes osteoblastogenesis/chondrogenesis and inhibits adipogenesis. Interestingly, a similar effect can be obtained by irradiating hMSCs with IR pulses emitted by a Nd:YAG laser.

Conclusions. Physical stimuli can be used to switch hMSCs from a differentiation pathway to an alternative one. Now we know that differentiation processes and cell functions are directed through the concerted effects of many different stimuli and it is becoming increasingly clear that the only way to approach functional tissue regeneration and repair is to supply combined humoral and physical stimuli in a dose- and time-dependent manner. In perspective, the possibility of modulating hSMCs behavior by applying suitable physical stimuli could be of consequence in cell transplantation therapy, tissue repair, regeneration and engineering.

Developmentally coordinated extrinsic signals drive human pluripotent stem cell differentiation towards fully functional DARPP-32+ medium-sized spiny neurons

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Medium-sized spiny neurons (MSNs) are the only neostriatum-projection neurons and their degeneration is at the core of the extrapyramidal dysfunctions in Huntington's disease. We show that MSNs can be efficiently generated from human pluripotent - both naïve or induced - stem cells, exposed to key neurodevelopmental molecules. In a feeder-free adherent culture, ventral-telencephalic neuroectodermal specification is induced by BMP/TGF- β inhibition and subsequent SHH treatment. The emerging FOXG1+ telencephalic progenitors are terminally differentiated, resulting in the systematic line-independent generation of CTIP2+/DARPP-32+ MSNs. Similarly to mature MSNs, the in vitro generated neurons show single and repeated action potentials, spontaneous activity and dopamine neuromodulation of GABA currents. In sum, human pluripotent stem cells can be efficiently driven to acquire a functional striatal fate using a stepwise method representing a platform for in vitro developmental neurobiology studies, drug screening approaches and regenerative medicine.

How best to advance neurodegenerative research using pluripotent stem cells

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Introduction and Aims. Stem cell research offers the ability both to study development and the nascent potential to develop therapeutics beyond bone marrow and cord blood hematopoietic reconstitution. By their very intractability, few areas of potential stem cell therapy have engendered more excitement and investment as neurological disorders. This presentation will review recent progress in clinical translation, attempt to explain current trends in stem cell research, and summarize recent findings that relate to spinal muscular atrophy, an autosomal recessive motor neuron disease caused by a genetic defect carried by as many as one in 75 people

Human pluripotent stem cells as models for cardiac development, disease and drug screening

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Pluripotent human embryonic stem cells (hESC) have the potential to differentiate to any cell type of the human body. This characteristic has sparked researchers to study the use of hESC for regenerative medicine, drug screenings and embryonic development. We have recently optimized differentiation of hESC to cardiomyocytes, including growth factor directed differentiations as monolayers or as three-dimensional aggregates (embryoid bodies or EBs). Previously, we have demonstrated that hESC-derived cardiomyocytes (hESC-CM) faithfully recapitulate the early molecular events during embryonic development. Recently, we have generated a cardiac reporter line by introducing Green Fluorescent Protein (GFP), in the genomic locus of the early cardiac transcription factor NKX2-5, which enables us to visualize the derivation of NKX2-5+ cardiomyocytes during in vitro differentiation and purify these cells by Fluorescent Activated Cell Sorting (FACS). The combination of different transcription factor-coupled fluorescent reporters in this so-called “rainbow” hESC cell line, covering sequential stages of the cardiac lineage, will allow us to identify and characterize pathways for specific subtypes of the cardiac lineage at early and later stages during differentiation. Furthermore, a better understanding of these developmentally related processes will be further important for progress in fields of tissue engineering, disease modelling, drug toxicity and discovery, which most likely will lead to improved tailor-made therapies and better and safer medicines on the market.

NIST Activities in Metrology for Stem Cells

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The manufacture and deployment of stem cells for medical therapies pose special challenges compared to other kinds of pharmaceutical products. While regulation of these products requires quantitative assessment of their potency, it is difficult to determine which in vitro assays provide relevant measures of potency of the product. Assessing biological activity is challenging in part because cell-based assays are complex, and there are many sources of measurement uncertainty. NIST is pursuing several strategies to improve metrology and standards that will enable discovery and regulatory approval of stem cell and regenerative medicine technologies.

One project involves developing algorithms that can classify stem cell colonies according to their appearance to determine their state of pluripotency. An unbiased, mathematical description of phase contrast microscopic images of pluripotent colonies would enable reliable selection of colonies for expansion, and possibly enable the automation of pluripotent cell scale up. Another activity is to work with the US FDA to describe assays for cell-based therapy products in terms of classical metrology concepts. In fields such as analytical chemistry, the activities required for achieving accuracy, precision and robustness are relatively straightforward. For assays meant to assess the biological activity of cells, such concepts are often difficult to describe and challenging to achieve. We focus on experimental approaches for reducing uncertainty such as benchmarking of instrument response.

These and other efforts are aimed at encouraging unbiased and rigorous quantitation of complex biological phenomena. Carefully acquired data will, in themselves, be benchmarks against which other data can be compared. Furthermore, reducing uncertainty in in vitro measurements will make it easier to identify which assays best predict the in vivo response.

Very Small Embryonic Like Stem Cells (VSELs) – our key to regeneration, longevity and passkey to cancer

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Pluripotent very small embryonic/epiblast derived stem cells (VSELs) as we hypothesize are deposited at begin of gastrulation in developing tissues and play an important role as backup population of pluripotent stem cells (PSCs) for tissue committed stem cells (TCSCs). Molecular analysis of adult bone marrow (BM)-derived purified VSELs revealed that they i) express pluripotent stem cells markers e.g., Oct4, Nanog, Klf-4, SSEA-1 ii) share several markers characteristic for epiblast as well as migratory primordial germ cells (PGCs), and iii) possess a unique pattern of genomic imprinting (e.g., erasure of differently methylated regions at *Igf2-H19* and *Rasgrf1* loci and hypermethylation at *KCNQ1* and *Igf2R* loci). We hypothesize that these pluripotent stem cells play an important role in tissue/organ rejuvenation, and their proliferation and potentially premature depletion is negatively controlled by epigenetic changes of imprinted genes that regulate insulin factor signaling (*Igf2-H19* locus, *Igf2R* and *RasGRF1*). Since the attenuation of insulin/insulin growth factor (Ins/Igf) signaling positively correlates with longevity, we propose, based on our experimental data in animal models, that gradual decrease in the number of VSELs deposited in adult tissues, which occurs throughout life in an Ins/Igf signaling-dependent manner is an important mechanism of aging. In contrast, a decrease in Ins/Igf stimulation of VSELs as seen for example during calorie restriction extends the half life of these cells in adult organs and has a beneficial effect on life span. On other hand the same epigenetic changes in the epigenetic signature of imprinted genes keep these cells quiescent in adult tissues and prevent them from teratoma formation. In contrast epigenetic changes/mutations that lead to activation of imprinted genes could potentially lead to tumor formation by these cells. Mounting evidence accumulates that perturbation of expression of imprinted genes is a common phenomenon observed in developing tumors. We envision that mutated VSELs in addition to teratomas are origin of several malignancies including germinal tumors and pediatric sarcomas.

Genomic stability of mouse embryonic stem cells after γ -rays treatment

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Embryonic stem cells (ESCs) are pluripotent, self-renewing and undifferentiated cells derived from the inner cell mass (ICM) of the blastocyst. Because of their origin, they have a strict control on their genome integrity to ensure the correct transmission of undamaged DNA.

We characterise, during a period of up to 96 h of culture, the cell cycle phase distribution and apoptosis of R1 mouse ESC (mESC) line treated with a single dose of 2 or 5 Gy γ -rays. After 2 Gy treatment and 24 h of culture, a significant majority of cells was blocked at the G2/M phase and a massive apoptosis was observed, in comparison to control population. Between 48-72 h after irradiation, the parameters used to describe the cell cycle and apoptosis returned similar to those of control. When mESCs were irradiated with 5 Gy, a small fraction of cells still presented clear evidences of a G2/M block and apoptosis, even after 96 h of culture. At 96 h of culture, for a characterisation of treated populations, the chromosome complement, the differentiation capacity and the transcriptome expression were evaluated in comparison to the control cells. The survived mESCs maintained their undifferentiated status and capacity to differentiate into the three germ layers. A structural stability of the aberrations was observed among populations and the microarrays data showed few differences in gene expression profile of 5 Gy-treated cells compared to control.

Overall, our results indicate a commitment of mESCs to maintain pluripotency and genome stability.

Epigenetic memory of iPS cells to enhance muscle differentiation

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In the growing field of regenerative medicine, stem cell therapies hold promising potential for innovative treatments of several degenerative diseases, including muscular dystrophies. Muscular dystrophies (MDs) are a heterogeneous group of genetically inherited diseases, mainly characterized by a severe and chronic wastage of skeletal and cardiac muscle. MD outcome is still fatal, due to respiratory or cardiac failure, and clinical strategies to counteract the biological causes are still missing. Pluripotent stem cells offer a precious source of self-renovating and multi-lineage differentiating cells. Induced pluripotent stem cells (iPSCs) are generated from somatic cells via transient overexpression of pluripotency factors, such as Oct4, Sox2, Klf4 and Nanog. Depending on diverse protocols and epigenetic signatures, iPSCs differentiate into multinucleated skeletal fibers and into beating cardiomyocyte foci in vitro and can participate in the myogenic regeneration in dystrophic animal models. Stem cell-based approaches aim at providing the affected muscles with new, functional myofibers and restore, at least partially, cardiac and skeletal muscle architecture and motility. Although heart and skeletal muscles share the expression of several proteins, including all the contractile machinery, their differentiation programs are strictly specific and finely controlled. We have recently reported that specific miRNAs can regulate muscle lineage switch. Thus, targeting those miRNAs could be used to drive specific iPSC differentiation or considered as an adjuvant strategy for regenerative process.

Nonetheless, novel strategies are still required to optimize the differentiation efficiency and reduce the teratogenic risk of iPSCs. In this view, the epigenetic memory of the donor cell type could be deeper investigated and exploited as a fine tool to increase the myogenic commitment of the iPSC-derived differentiating cell pool.

Hedgehog signaling in Melanoma Stem Cells

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Melanoma represents the most malignant and lethal among skin tumors due to its high infiltration capacity and invasion, and for its resistance to chemotherapy and radiotherapy treatments. Despite new therapeutic approaches performed in the last decade, prognosis for metastatic melanoma patients remains still poor (1). Recent observations have led to the hypothesis that tumorigenesis is a plagiarism of embryonic development in which stem cells assume a central role. Indeed, solid tumors have been demonstrated to contain a subset of cells that are endowed with tumorigenic potential and stem cell properties, that is the capacity for symmetric and asymmetric cell division, allowing them to self-renew and differentiate. Accumulating evidence suggests that cancer-stem like cells might exist in melanoma, although their identity and frequency is controversial, due to the limitation of xenografts models (2,3). We have previously demonstrated that growth, recurrence and metastasis of melanoma in mice require Hedgehog-GLI signaling (4), a key communication pathway involved in many aspects of development and cancer. Using a combination of functional in vitro and in vivo assays we have isolated putative human melanoma stem-like cells. In addition, we are identifying signaling pathways that may have promise in disrupting melanoma stem cell self-renewal and survival. We will discuss how these findings might hold important implications for the development of novel therapeutic approaches for melanoma.

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Cardiac Regeneration in the Diabetic Heart

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Recently, we reported that induced pluripotent stem (iPS) cells generated from H9c2 cells following transplantation into infarcted heart can inhibit apoptosis and differentiate into cardiac myocytes. iPS cells can have wide application in various other diseases such as diabetes. Therefore, we determined the potential of iPS cells or factors released from iPS cells in the regeneration as well as repair of the injured myocardium in the different models of diabetes. Our streptozotocin (STZ) induced diabetic rat hearts shows a significant decrease in cardiac myocyte apoptosis and fibrosis. Furthermore, we also examined that an increased apoptosis is mediated by oxidative stress. However, transplanted iPS cells in STZ-induced diabetes significantly reduced apoptosis, fibrosis and increased oxidative stress. Furthermore, our echocardiography data shows significantly improved cardiac function. Next, we generated myocardial infarction in db/db mice to understand the effects of transplanted iPS cells on neovascularization, apoptosis, and fibrosis. Furthermore, we also explored the role of factors released from iPS cell on the activation of endogenous c-kit positive cardiac progenitor cells. Our data suggest that there were a significantly increase in neovascularization. Moreover this increase in neovascularization was also contributed by the activation of endogenous c-kit positive cells. Additionally, increased neovascularization demonstrated a significantly improved cardiac function. Overall, our data from these models suggest that transplanted iPS cells in the injured diabetic heart provide beneficial effects via inhibiting apoptosis, fibrosis and increasing neovascularization.

Adhesion or anti adhesion? What is the significance of stem cell antigen CD34 in hematopoietic biology?

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Introduction. CD34 have been famous for more than two decades among clinicians and researchers for its unique capability to identify cells with supreme engraftment quality in a majority of blood borne disorders. There have been infamous corporate battle due to various CD34 based reagents in past. However, its significance has reduced in clinics for identification/ purification of Hematopoietic Progenitor Cells/Hematopoietic Stem Cells (HPCs/HSCs) with the advents of newer stem cell identification methodology and with the concept of induced pluripotent stem cells / mesenchymal stem cells (IPSC/MSCs). Here we are focused more on its functional significance in HSCs adhesion/ migration which largely remain obscure. Preliminary work indicated its key participation in cellular trafficking to the site of hematopoiesis or injury in some cases at least.

Objectives. Modulation of hematopoietic stem progenitors/stem cells' adhesion/migration through ectopic expression of stem cell specific genes.

Methods. Gene cloning, stable transfection, induced expression and adhesion assay

Results. A 1.5 KB cDNA was cloned in a eukaryotic gene expression vector (pTet-On) by recombinant gene cloning methods and sequence ORFs was confirmed through gene sequencing. The vector containing human CD34 cDNA was transfected in Human T lymphoblastic cell line Jurkat E6.1 by using Lipofectamine 2000 and surface expression of wild type Human CD34 (Hu-CD34WT) was analyzed by flowcytometric methods. The effect of induced expression of ectopic gene demonstrated no significant effect on the surface expression level of surface adhesion molecules viz. ICAM-1, Integrin- alpha L, Integrin-beta-2/ beta-1, Integrin- alpha 5 as evaluated by flowcytometric methods. Similarly, cellular proliferation remains unaffected on ectopic expression as indicated by cellular proliferation assays.

The adhesion of the Hu-CD34WTcells was evaluated by calculating their binding to the fibronectin-coated (Fn-coated) plastic surfaces. The fraction of the adhered cells was calculated by measuring optical density in an MTT assay. The CD34WT expressing cells showed O.D. values (that indicates adherent cellular fractions) $0.4692 \pm SD 0.057168$ in comparison to control (untransfected) $0.3154 \pm SD 0.025696$, CD34WT (uninduced) $0.2698 \pm SD 0.082577$ indicating 1.744 fold enhancement of Jurkat E6.1 cells expressing CD34WT (induced) in comparison to the CD34WT (uninduced) cells to the Fn-coated surface (p-value 0.004014). These findings indicate ability of CD34WT over-expression to potentially enhance cellular adhesion of Jurkat E6.1 cells to the extracellular matrix proteins (like Fn) However, potential phosphorylation site Ser306 might be playing crucial in the underlying mechanism, which remains to be defined at molecular level.

The effect of CD34 over-expression on adhesion of Jurkat E6.1 cells to the stromal cell layer was assessed by Calcein-AM method. For determination of cellular adhesion, the cells were stained with Calcein-AM dye and laid over precultured stromal cell layer. The adhesion was facilitated for 2 hrs at 37°C with 5% CO₂. Both adherent and non adherent cellular fractions were calculated by measuring the fluorescence intensity and % adherence was calculated. We assessed the adhesion effect of Hu-CD34WTcells to stromal cell layer that was irradiated at different doses. The growth of stromal cells has been demonstrated not to be affected by radiation doses as high as 100 Gy during in vitro conditions. However, the protein synthesis was demonstrated to be inhibited at more than 10Gy. We irradiated stromal cells at 5Gy and 10 Gy at 24 hrs and 48 hrs prior to the adhesion assay and measured the % adhesion of all three types of cells.

The cellular adhesion was enhanced at both radiation doses irrespective of expression of CD34 genes. There was a meager upmodulation in adhesion of the all groups of cells including control (untransfected) and Hu-CD34WT (transfected) cells to the stromal layer in comparison to the unirradiated cells.

Conclusion: cells expressing human CD34 gene shown a moderate increase in their adhesion to the surface coated with extracellular matrix proteins and stromal cells. The studies presented here clearly indicate a positive role for CD34 surface molecule in human T-Lymphoblasts at least. Though the exact mode of signaling and their regulation is still under defined.

POSTER ABSTRACTS

P1

Differentiative potential of fibroblast-like cells from human peripheral blood

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Introduction and aims. Cells with clonogenic proliferation have been identified in human and animal peripheral blood. When ex vivo cultured, they adhere to plastic dishes, form typical fibroblastoid colonies and respond to differentiative stimuli acquiring osteogenic, adipogenic and chondrogenic properties. As circulating multipotent stem cells could represent an important autologous cell source for muscle tissue engineering, in the present work adherent fibroblastic cells from human peripheral blood (hPBCs) have been phenotypically characterized and tested in vitro for myogenic differentiation.

Material and methods. After Ficoll density gradient separation, mononucleated cells from peripheral blood samples were collected and then cultured in α -MEM, 16,5% FBS, 1% antibiotic solution. Fibroblastoid colony forming unit populations (CFU-F) were harvested and characterized by cytometry using FacsCanto II (BD), doubling population study, SEM, differentiation tests and gene expression analysis (RT-PCR).

Results. hPBCs were morphologically stable during subculturing as demonstrated by optical and SEM microscopy. When characterized by cytometrical assay, hPBCs revealed a mesenchymal stem cell profile being CD73+/CD90+/CD105+/CD166+/CD133med/ntegrin β 1med/CD34low and showed a doubling population time of 48 hours over 31 passages. The responsivity to osteogenic and adypogenic stimuli was defined by cytochemistry. In particular, after 14 days from induction with myogenic factors, hPBCs organized into syncitium-like structures as shown by optical and SEM microscopy. Furthermore, RT-PCR and cytometry revealed the expression of myogenic markers.

Conclusions. In this work, the procedure to isolate hPBCs with multidifferentiative potential was defined. Taken together, our results have demonstrated that these cells possess stem plasticity which bodes well for their future application in tissue engineering.

P2

Effects of diode laser 635 nm stimulation on K⁺ and Ca²⁺ channel currents in mesenchymal stem/stromal cells isolated from mouse bone marrow

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Introduction and Aims. Undifferentiated mesenchymal stem cells (MSCs) from bone marrow express multiple functional K⁺ channel currents and in a less extent Na⁺ and L-type Ca²⁺ current. These two latter currents increase in expression with MSCs proliferation and differentiation. Low laser intensity (LLL) therapy is described in the literature as causing increases in the amount of DNA synthesis, osteoclasts proliferation in bone defects tibia and osteoporosis. The aim of this study was to characterize the effects of LLL stimulation on functional K⁺ and Ca²⁺ channel currents in cultured MSCs.

Material and methods. Mouse bone marrow mesenchymal stromal cells (MSCs) were isolated from femura and tibiae of male C2F1 mice . MSCs were cultured in growth medium (DMEM containing 1% Glutamine, 1% P/S and 20 % FBS), in the presence or absence of 0.5 mM Ba²⁺, for 24 and 48 h. MSCs were stimulated for 10-26 s with a diode laser operating at a wavelength a 632 nm in continuous irradiation mode (power 89 mW; energy density dose of 0.26 J/cm²; laser output was coupled to a 0.6-mm-diameter optical fiber). Cell proliferation was assayed by cell counting, EdU incorporation assay, and Notch-1 activation. Ionic currents were recorded with the whole-cell patch clamp technique. Experiments were made before (time 0) and 24 or 48 hours after laser treatment.

Results. Laser treatment increased significantly MSC proliferation and up-regulated Notch-1 expression. These effects were paralleled by a reduced value of the cell membrane capacitance, index of a reduced surface membrane area, and an increase of the specific resting membrane conductance. Laser stimulated cells showed significant increase of K(Ca), Kir and T- and L-type Ca²⁺ currents. Notably, the above changes were reduced in cells pretreated with Ba²⁺.

Conclusions. Taken together, these results, suggest a role for diode laser in the stimulation of MSC and underscore novel intriguing mechanisms responsible for laser biostimulation.

P3

Autologous progenitor cells in a hydrogel form a supernumerary and functional skeletal muscle *in vivo*

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Introduction and Aims. Extensive loss of skeletal muscle tissue results in incurable mutilations and severe loss of function. *In vitro* generated artificial muscles undergo necrosis when transplanted *in vivo* before host angiogenesis may provide the amount of O₂ required for muscle fibre survival. Skeletal muscle tissue engineering has met with limited success, due to the complex tissue architecture and the presence of a dense microvascular network without which muscle fibers do not survive once implanted *in vivo*. Here we report a novel strategy exploiting the good survival and differentiation of mouse mesoangioblasts in a recently discovered biomaterial, PEG-Fibrinogen, and their ability, once engineered to express Placenta derived Growth Factor and embedded in this material, to attract host vessels and nerves while myotubes begin to form. Mesoangioblasts, embedded into PEG-Fibrinogen hydrogel, generate an additional muscle on the surface of the Tibialis Anterior.

Material and methods. We report a novel strategy exploiting the good survival and differentiation of mouse mesoangioblasts in a recently discovered biomaterial, PEG-Fibrinogen, and their ability, once engineered to express Placenta derived Growth Factor and embedded in this material, to attract host vessels and nerves while myotubes begin to form.

Results. When PF embedded Mabs are implanted underneath the skin on the surface of the tibialis anterior (TA), the normal contractile activity of this muscle induces fiber orientation and maturation, resulting in an extra muscle that is morphologically and functionally very similar to the underlying TA

Conclusions. This strategy opens the possibility of *in vivo* autologous muscle creation for a large number of pathological conditions.

P4

Reprogramming bovine fibroblast to create induced pluripotent stem cells

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Introduction and Aims. Stem cell technology has recently been of great interest in veterinary field, with potential applications in improved production and health through the establishment of genetically modified animals, the in vitro study of host-pathogen interaction as a model for human and veterinary medicine and the screening of putative pharmaceutically active molecules. Cattle are economically important worldwide and are an attractive target for biotechnology research. Recent studies have shown that induced pluripotent stem (iPS) cells are comparable with embryonic stem cells (ES). However most attempts to create ES for cattle have failed. The purpose of the present study was to establish an effective protocol to generate bovine iPS cells using Yamanaka's four transcription factors (Oct4, Sox2, Klf4, c-Myc – OSKM).

Material and methods. Skin fibroblasts were transduced with retrovirus carrying the mouse OSKM and two days later were replated onto mitomycin C treated feeder cells. Three media were compared: A with leukemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF), B with LIF; and C with serum replacement, LIF and bFGF. Virus integration and exogenous and endogenous gene expression were analyzed, and an in vitro differentiation assay was performed.

Results. iPS-like colonies were identified 7-9 days post transduction in media A and B. Two colonies that showed good growth rate and morphology were expanded and characterized. Genomic PCR and RT-PCR for the mOSKM showed that the genes were integrated into the genomes of the fibroblasts and transcribed. Endogenous pluripotent genes were also activated. Embryoid bodies were observed in suspension culture without growth factors.

Conclusions. Preliminary results suggest that bovine iPS cells can be derived by the addition of OSKM however, if it is possible to maintain the cells in an undifferentiated state has still to be demonstrated.

Characterization of human adipose-derived stromal cells harvested through different techniques of liposuction

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Introduction and Aims. This study investigates the yield and function of adipose-derived stromal cells (ASC) which are present in the aspirates obtained by wet liposuction (WL) and dry liposuction (DL).

Material and methods. The aspirates were processed to investigate the yield of the stromal-vascular fraction (SVF) cells (trypan blue staining) and the yield (CFU-F assay), growth rate and apoptosis (fluorimetric assays) and differentiation potential (western blotting, immunofluorescence) of ASC.

Results. Proliferation, viability and adipogenic, osteogenic and endothelial cell commitment of ASC were similar irrespective to the surgical techniques. The amount of freshly isolated WL-SVF cells was significantly lower than that obtained from DL as well as the number of CFU-F was lower in WL- than DL-aspirates.

Conclusions. Although the final yield of ASC harvested from a WL aspirate was about half of a same volume of DL aspirate, the main biological features of WL-derived ASC, i.e. growth rate, viability and differentiation potential were satisfactory. Therefore, WL can be favourably considered not only to remove the adipose mass from plastic surgery patients but also as a procedure to be followed for tissue engineering applications.

P6

Laser biostimulation of mouse bone marrow mesenchymal stromal cells: effects on cell proliferation and Notch-1 expression

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Introduction and Aims. Mesenchymal stromal cells (MSCs) are a promising cell candidate in regenerative medicine. Their proliferative potential can be increased by low-level laser irradiation (LLLI), but the mechanisms involved remain to be clarified. Here we investigated the effects of 635 nm diode laser on mouse MSC proliferation paying particular attention to the ability of the laser light to stimulate Notch-1 signaling pathway, a key determinant of MSC self-renewal. We also searched for an involvement of KCa1.1 ion channels, previously shown to be a target of the laser action, in the laser-mediated effects on Notch-1 regulation.

Material and methods. Irradiated and control mouse bone marrow MSCs were assayed for cell viability and proliferation by MTS assay, time lapse videomicroscopy EdU incorporation. Expression of Notch-1 and its target gene Hes-1 were evaluated in MSCs cultured in the absence or presence of 0.5 mM Ba²⁺, to inhibit KCa1.1 channels, using RT-PCR, Western blotting and confocal immunofluorescence.

Results. Compared with non-irradiated MSCs, those irradiated with diode laser showed increased proliferation; this phenomenon was associated with the up-regulation and activation of Notch-1, as also demonstrated by increased nuclear expression of Hes1 by the irradiated cells. Of interest, KCa1.1 channel inhibition was able to prevent the stimulatory effects induced by laser irradiation on MSC growth and Notch-1 expression, providing clues to advance our knowledge on the mechanisms leading to stimulation of cell proliferation by laser light. Of note, diode laser did not affect MSC viability or caused morphological alterations.

Conclusions. Our findings demonstrate that the mitogenic effects observed in the diode laser-irradiated MSCs involve stimulation of Notch-1 signaling through KCa1.1 channel activation and suggest that LLLI may be a valid and safe approach for preconditioning of MSCs prior their transplantation for regenerative medicine purposes.

P7

Canine Mesenchymal Stem Cells from visceral and subcutaneous adipose tissue for cell-based therapy

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Objectives. This study compared some characteristics of canine Adipose tissue-Derived Mesenchymal Stem Cells (cAD-MSCs) from subcutaneous and visceral fat. These findings were directed to obtain high quantity and quality cAD-MSCs for clinical cell-based therapy.

Material and methods. Subcutaneous and visceral fat samples were collected from 2 different groups of 10 healthy donor dogs. The cAD-MSCs were isolated from each sample and were cultured in Dulbecco's modified Eagle's medium-Low Glucose with 20% Fetal Bovine Serum. Cell yield (number of cells/g of fat) was evaluated. cAD-MSCs were sub-cultured up to passage (P) 6 and their proliferation potential was evaluated. The identity of cAD-MSCs of each passage was verified by their ability to attach to flasks surface, to produce colony-forming units (CFU) and to differentiate in chondrocytes, adipocytes and osteocytes. The presence of transcription factors indicating self-renewal and undifferentiation (Oct4, Nanog and Sox2) were also investigated by RT-PCR analysis.

Results and conclusion. The isolated cAD-MSCs adopted a fibroblast-like shape. Statistical analysis demonstrated that subcutaneous and visceral fat yielded the same number of cells/g of fat. We also demonstrated that in a non-inductive medium the cAD-MSCs reached the highest proliferative capacity, especially when derived from subcutaneous fat. Data shown that the obtained CFU number grew up to P2 and decreased in subsequent passages. Cells derived from subcutaneous fat gave higher mean values of CFU than those of cells deriving from visceral fat in all passages. cAD-MSCs differentiated in the three lineages above mentioned up to P4. RT-PCR analysis revealed that cells expressed pluripotency-associated transcription factors Oct4, Nanog and Sox2 up to P6, but the mRNA expression level was higher at P2. Finally, the results of our study showed that the cAD-MSC from subcutaneous fat, grown up to P2 in our culture condition were most suitable to use in regenerative therapy.

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Morphofunctional interactions between mouse mesenchymal stromal/stem cells and myoblasts in co-culture: clues for skeletal muscle regeneration

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Introduction and Aims. Mesenchymal stromal cells (MSCs) have been successfully used to improve skeletal muscle repair/regeneration; however, the mechanisms responsible for their beneficial effects remain to be clarified. On these basis, in the present study, we evaluated in a co-culture system the ability of bone-marrow MSCs to influence C2C12 myoblast behavior and analyzed the cross-talk between the two cell types at the cellular and molecular level.

Material and methods. C2C12 cells were cultured alone or in co-culture with mouse MSCs and their proliferative attitude were assayed by Time lapse videomicroscopy, cyclin A expression and EdU incorporation. Notch-1 expression and activation were also evaluated by RT-PCR, Western Blot and confocal immunofluorescence in the myoblasts cultured alone or immunomagnetically separated from MSCs after co-culture. Paracrine interaction between the two cell types was evaluated by analyzing the involvement of MSC-derived vascular endothelial growth factor (VEGF) in the myoblastic response.

Results. Myoblast proliferation was greatly increased in the co-culture; this phenomenon was accompanied by the up-regulation of Notch-1 signalling, a key determinant of myoblast activation and proliferation. The myoblastic response was mainly depended on the paracrine release of VEGF by MSCs. Indeed, the addition of MSC-derived conditioned medium (CM) to C2C12 cells yielded similar results as those observed in the co-culture and increased the phosphorylation and expression levels of VEGFR. Treatment with the selective pharmacological VEGFR inhibitor, KRN633, attenuated receptor activation and inhibited the effects of MSC-CM on C2C12 cell growth and Notch-1 signalling.

Conclusions. This study provides novel evidence for a role of MSCs in stimulating myoblast cell proliferation and suggests that the functional interaction between the two cell types may be exploited for the development of new cell-based skeletal muscle repair strategies.

P9

Electric field stimulation induces the expression of cardiomyogenic markers in murine and human stem/progenitor cells isolated from different sources

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Introduction and Aims. Recent studies have shown that an electric field (EF) induces stem cell commitment towards a cardiac phenotype. However, the effects of pulsed EF involved in myogenic commitment are still poorly understood. The aim of this work was to investigate the effects of mono and biphasic electrical stimulation on a mouse stem cell line (m17.ASC) and on adult stem cells isolated from human adipose tissue and heart.

Material and methods. Device: the bioreactor system is composed of a chassis carrying an electrical wiring system and is equipped with housings for multiple culture PDMS chambers, in which stainless steel electrodes are embedded. Electrical stimulation is driven by graphical-interface software able to set and control the stimulation patterns. Biological experiments: m17.ASC cell line and human adult stem cells were seeded on glass slides and 24 hours later the EFs (square mono-phasic 2ms, 1Hz, 8V amplitude or bi-phasic 2ms, 1 Hz, ± 4 V amplitude) were applied for different time points (3, 8, 24, 72 hours). Cell proliferation was evaluated by crystal violet staining and on the basis of the expression of the early cardiac markers Connexin 43 (Cx-43) and Gata-4 by IF.

Results. Mouse m17.ASCs upon both electrical stimulation protocols showed proliferation rates similar to those of not-stimulated cells. After 3 days of biphasic stimulation m17.ASCs up-regulated the expression of Cx-43. similar results were observed for both human cell types. In the case of human cells bi-phasic stimulation induced also the up-regulation of GATA-4 transcription factor.

Conclusions. These results suggest that the bi-phasic EF can be an appropriate signal to induce myogenic commitment in mesenchymal stem cells. Experiments which combine this stimulus with stiffness and topography of the culture substrate are in progress.

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