[EP289] NOREPINEPHRINE REGULATES MESENCHYMAL STEM CELLS SURVIVAL IN VITRO

Cheng Biao[1], Guangdong Province, China , Kong Yanan[1], Guangzhou, China , Xuan Min[1], Guangzhou, China , Pan Liangli[2], Guangzhou, China

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Thursday, May 14, 2015

E-poster Session: Basic Science 2

Background: High glucose can induce apoptosis in a variety of cells. In vivo high glucose is often complicated by the sympathetic deactivation. Sympathetic nervous system can regulate various bone marrow-derived cells behavior. The hypothesis for the study was that, high glucose induced mouse bone marrow mesenchymal stem cells (MSC) apoptosis.

Abstract: Sympathetic neurotransmitter norepinephrine (NE) regulates the MSC apoptosis and/or other behaviors.

Methods: To isolate and culture C57BL / 6 mouse bone marrow mesenchymal stem cells. MSCs were cultured in low glucose medium(4.5 mM) with or without NE(5μM). Cell proliferation was monitored by CCK8 and MSC migration was assessed in a scrape wound and transwell. MSC was incubated with high glucose (25 mM) or high glucose and NE for 5days. Cell apoptosis was detected by TUNEL and ELISA.

Results: Norepinephrine promotes MSC proliferation ($P<0.05$). NE inhibited MSC migration in a wound-healing assay ($P<0.05$) that was associated with a change in F-actin organization. MSC undergoing apoptosis induced by high glucose ($P<0.05$). NE increased MSC survival ($P<0.05$).

Conclusions: NE is functional in MSC and may play an important role in the progression of diabetic, by regulating MSC loss and influencing the activation state and recruitment of MSC.

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[1355] THE BIOLOGIC ROLE OF SYMPATHETIC NERVE ON ANGIOGENESIS

Cheng Biao[1], Guangdong Province, China , Pan Liangli[2], Guangzhou, China

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Thursday, May 14, 2015

E-poster Session: Basic Science 2

Angiogenesis is an essential part of wound healing and angiogenesis impair may result in the occurrence of chronic ulcer. Sympathetic nervous system plays an important role on angiogenesis. It plays its biologic role primarily through the interaction between neurotransmitters released from nerve terminals and receptors in target organs. Among this, the activation or inhibition of adrenergic receptors (primarily β-adrenergic receptor) affects angiogenesis most. In addition, sympathetic nerves locate near pericytes of microvascular, go along the capillaries and there are adrenergic receptors on the surface of endothelial cells and pericytes. As a result, sympathetic nerve may participate in angiogenesis by influencing endothelial cells and pericytes of new capillaries.

[EP291] ISOLATION OF LUNG MULTIPOTENT STEM CELLS USING A NOVEL MICROFLUIDIC MAGNETIC ACTIVATED CELL SORTING SYSTEM

Jiang Jian-Xin[1], Daping, China , Lin Qiu[2], Chongqing, China , Xue-Tao Yang[3], Daping, China , Juan Du[3], Daping, China , Hai-Yan Wang[3], Daping, China , Jian-Hui Sun[3], Daping, China , Ling Zeng[3], Daping, China , Ce Yang[3], Daping, China

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Thursday, May 14, 2015

E-poster Session: Basic Science 2

Recent studies have shown that the lung has a remarkable reparative capacity, many progenitor cell populations that can be induced to proliferate in response to injury as well as differentiate into one or more cell types. Mouse lung multipotent stem cells (MLSCs) could regenerate many different lung components including bronchioles, alveoli, and pulmonary vessels. But the research of MLSCs were limited due to its rarity. In this study, we introduced a novel microfluidic magnetic activated cell sorting system in the isolation of MLSCs. The sorted MLSCs had better viability and purity. They were identified by colony formation efficiency and differentiation ability, they have possed self-renewal and differentiation capacities, highlighting their stem cell properties.

[EP292] THE MILIEU OF ACUTE LUNG INJURY STIMULATES RAT ALVEOLAR TYPE Ⅰ CELLS PROLIFERATE AND EXHIBIT PHENOTYPIC PLASTICITY IN VITRO

Jiang Jian-Xin[1], Daping, China , Yong Li[2], Daping, China , Ling Zeng[2], Daping, China , Xue-Tao Yang[2], Daping, China , Juan Du[2], Daping, China , Hai-Yan Wang[2], Daping, China , Jian-Hui Sun[2], Daping, China , Ce Yang[2], Daping, China

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Thursday, May 14, 2015

E-poster Session: Basic Science 2

As one of the most important organs of our body, lungs are susceptible to various injuries due to its unique anatomic structure and the character of being directly connected with the outside world. Trauma patients, particularly severe trauma patients, are often accompanied with acute lung injury (ALI). ALI and its severe form: Acute respiratory distress syndrome (ARDS), are major causes of death in trauma patients. Lungs will start endogenous repair after ALI. It has been found that a relatively small content of various types of cells located in special microenvironment play a key role in the process of repair after injury, including endogenous and exogenous stem / progenitor cells. In addition to the possibility of the role of stem / progenitor cells in repairing, more and more studies also showed that the terminally differentiated cells may also play a vital role in tissue repair after injury. In recent years, many studies have demonstrated that various kinds of terminally differentiated cells can dedifferentiate into stem / progenitor cells in multiple organs under certain conditions. However, it has not been reported whether type I alveolar epithelial cell (ATI) can dedifferentiate to mediate the repair of ALI.

The main findings are as follows: We successfully isolated ATIs of high purity from rat lungs by using a three-step method, including neutral protease digestion, IgG adherent and magnetic sorting. This method proved highly efficient and stable. The cultured cells expressed ATI cell-specific markers, including T1α, AQP5 and Cav-1,barely expressed ATII cell-specific marker pro-SPC, which indicated that these cultured cells were ATI's. We used the ALI milieu (BALF and LTH) to stimulate primary cultured ATIs in vitro and observed that day-3 BALF, day-2,day-3 and day-5 LTH from rat ALI model could significantly promote the proliferation of ATIs. Particularly, day-3 LTH had the strongest ability to promote ATIs proliferation. For further analysis, we used ALI-D3- LTH to stimulate ATIs and then detected the expression levels of phenotype related genes. We found that SPC and Nkx2.1 mRNA expression levels were significantly increased. Then we confirmed the protein expression of pro-SPC by immunofluorescence method and Western Blot. In addition, we found the mRNA expression level of Klf2 was significantly reduced in ALI-D3-LTH group, which was critical for the maturation of ATIs during development. These results revealed that the milieu of ALI could induce dedifferentiation of ATIs in vitro.

[EP293] DEVELOPMENT OF THE PARAMETERS OF TOPICAL OXYGEN THERAPY AND ITS EFFECT ON ISCHEMIA WOUND HEALING

Liu Hong-Wei[1], Guangzhou, China , Li-Ling Xiao[1], Guangzhou, China , Cong-Qiang Rao[1], Guangzhou, China , Sheng-Hong Li[1], Guangzhou, China , Fu Xiao-Bing[2], Bei-Jing, China

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Background: Topical oxygen therapy (TOT) has been used in human medicine to promote healing in chronic wounds. However, the effect and treatment parameters are still controversial.

Objective: The present study was conducted to investigate the effects of TOT on healing in ischemia wound, and explore the effective treatment parameters of TOT for chronic wound.

Methods: Wound surface oxygen concentration was measured under different treatment condition such as oxygen flow rate, oxygen-supplying methods, and dressing thickness of wound surface. Ischemia wounds were created on the legs of Wistar rats. Animals were treated with or without TOT at the developed parameters twice a day after wounding. Wound healing rate was observed by wound photography.

Results: TOT markedly promoted ischemia wound healing, accompanied by the increase in wound collagen fiber and new blood vessels at 7, 10 days after wounding, as well as the decrease in number of inflammatory cells in the wound at 7, 10 and 14 days after wounding, when compared with control group.

Conclusion: Our data indicated that TOT could be carried out in ischemia wound covered with a thin layer of dressing, and oxygen flow rate at 4L/min was a minimum effective dose for TOT. Moreover, TOT could promote collagen synthesis and angiogenesis, and reduce the infiltration of inflammation cells in ischemic wounds.

[EP294] EFFECT OF 650 NM LOW-LEVEL LIGHT IRRADIATION ON THE THERAPEUTIC POTENTIAL OF ADIPOSE-DERIVED STEM CELLS IN REGENERATIVE MEDICINE

Liu Hong-Wei[1], Guangzhou, China , Xuan Liao[1], Guangzhou, China , Cheng Biao[2], Guangdong Province, China , Sheng-Hong Li[1], Guangzhou, China , Yuan Xu[1], Guangzhou, China , Li-Ling Xiao[1], Guangzhou, China , Fu Xiao-Bing[3], Bei-Jing, China

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Background: Low-level light therapy has been shown to enhance proliferation and cytokine secretion of a number of cells. Human adipose-derived stem cells (ADSCs) are an attractive cell source for repairing injured tissue. It has been drowned intensive attention on how to enhance its regenerative capacity. A 650 nm GaAlAs laser is reported to have photobiological effects and is widely used for accelerating tissue repair and regeneration, however its effect on ADSCs has not yet been understood.

Objective: The present study was conducted to investigate the effects of GaAlAs laser irradiation on the proliferation, cytokine secretion and differentiation of cultured ADSCs.

Methods: The cultured ADSCs from human adipose tissue were treated by using 650 nm GaAlAs laser irradiation at 2J/m^2, 4J/m^2, 8J/m^2 respectively. Cell Proliferation was quantified by MTT assay, cytokine secretion was determined with ELISA assay, and adipogenic differentiation was examined with oil red staining respectively. In addition, the expression profiles of putative ADSC surface markers were determined by real-time PCR.

Results: Treatment of cells with GaAlAs laser at a radiant exposure of 4 J/cm^2 resulted in the enhancement of ADSC proliferation and adipogenic differentiation, as well as the increase of cytokine secretion. However, GaAlAs laser irradiation at the indicated dosages did not influence the expression profiles of putative ADSC surface markers.

Conclusion: These data suggest that low-level laser irradiation is an effective biostimulator of ADSCs, which might enhance the regenerative potential of ADSCs.

[EP295] INHIBITION OF ANGIOTENSIN-CONVERTING ENZYME IMPAIRED THE BIOLOGICAL FUNCTION OF EPIDERMAL STEM CELLS

Liu Hong-Wei[1], Guangzhou, China , Xuan Liao[1], Guangzhou, China , Jing Xiao[1], Guangzhou, China , Cheng Biao[2], Guangdong Province, China , Sheng-Hong Li[1], Guangzhou, China , Yuan Xu[1], Guangzhou, China , Li-Ling Xiao[1], Guangzhou, China , Fu Xiao-Bing[3], Bei-Jing, China

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E-poster Session: Basic Science 2

Background: The present study was undertaken to investigate the effect angiotensin-converting enzyme (ACE) inhibitor, captoprol on the epidermal regeneration of wound, and explored the underlining mechanisms.

Methods and Results: Full-thickness skin wounds created on the dorsum of Wistar rats increased the expression of ACE and the ratio of bromodeoxyuridine (BrdU) label-retention cells in the keratinocytes of wounded edge. Treatment of Wistar rats with ACE inhibitor, captopril at a dose of 10mg/kg/d attenuated wound closure rate, epidermal regeneration and decreased the ratio of BrdU label-retention cells of wounded edge. Additionally, 74.2% of the cultured human epidermal stem cells (ESCs) were positive cells for ACE expression detected by flow cytometric analysis. Addition of captopril at a dose of 10^{-6}mol/L significantly inhibited the proliferation, migration and adhesion of cultured ESCs, but did not influence the apoptosis and keratin 10 (K10) expression of cultured ESCs. Moreover, treatment of cultured ESCs with captopril decreased the production of Angiotensin II (Ang II), but did not affect the production of Angiotensin (1-7). Furthermore, Addition of Ang II markedly suppressed the proliferation, migration and adhesion as well as phosphorylation of extracellular signal-regulated kinase (ERK), signal transducer and activator of transcription (STAT)1 and STAT3 of cultured ESCs, which was attenuated by Ang II type 1 (AT_1) receptor blocker valsartan, and enhanced by Ang II type (AT_1) receptor antagonist PD123319.

Conclusions: Our data demonstrated that captopril via inhibiting Ang II-mediated signaling could impair epidermal regeneration during wound healing, suggesting a critical role of ACE in sustaining biological function of ESCs.

[EP296] LOW MICROMOLARCOPPER (II) IONS ENHANCE THE PROLIFERATION OF HUMAN KERATINOCYTES VIA APPROPRIATE GENERATION OF REACTIVE OXYGEN SPECIES

Daizhi Peng[1], Chongqing, China , Xia-Dong Duan[1], Chongqing, China , Yilan Zhang[1], Chongqing, China , Zhiyong Chen[1], Chongqing, China , Yalan Huang[1], Chongqing, China

[1]*Institute of Burn Research, Southwest Hospital, Third Military Medical University, State Key Laboratory of Trauma, Burns and Combined Injury*

Thursday, May 14, 2015

E-poster Session: Basic Science 2

Objective: To investigate the effects of copper (ii) ion (Cu^{2+}) concentrations in a wide range from nanomolar to milimolar levels on human keratinocyte proliferation as well as reactive oxygen species (ROS) generation, and verify the role of ROS in human keratinocyte proliferation.

Methods: Human skin keratinocyte line HaCaT cells were treated with 10^{-8} M～10^{-3} M of Cu^{2+}or(and) 5 mMN-Acetyl-L-cysteine(NAC), the proliferation rate and ROS production of these cells were measured by cell counting kit-8 (CCK-8) and fluorescent probe DCFH-DA, respectively. Both intracellular ROS levels and mitochondria localization were labeled by coculture with DCFH-DA and MitoTracker probes, and visualized under laser confocal microscopy.

Results: After cultured with 10^{-6} M and 10^{-5} M Cu^{2+} for 48h, the cell relative proliferation rates were significantly higher than that in negative control group (P<0.05） . Meanwhile, after exposure to 10^{-6} M and 10^{-5} M Cu^{2+}, intracellular ROS levels increased remarkably at both 30 min and 60 min P<0.01 for 10^{-6} M group, P<0.05 for 10^{-5} M group. In addition, 5 mM NAC can significantly antagonize the cell proliferation and ROS generation induced by Cu^{2+}. Although intracellular ROS generation also increased only after 60 min exposure under 10^{-8} M and 10^{-7} M Cu^{2+} （P< 0.05), but no obvious change was observed in keratinocyte proliferation.

Conclusion: Low micromolar concentrations of copper (ii) ions can promote the proliferation of human skin keratinocytes through up-regulated intracellular ROS generation at moderate level.

[EP297] THE IMPROVED DBCH BIOLOGICAL PROPERTIES AND THE POSSIBILITY OF ITS USE IN WOUND HEALING THERAPIES

Karolina Skołucka-Szary[1], Zakroczym, Poland , Wanda Piaskowska[1], Zakroczym, Poland , Ewa Bieniek[1], Zakroczym, Poland , Aleksandra Ramięga[1], Zakroczym, Poland , Ewelina Stoczyńska-Fidelus [1], Zakroczym, Poland , Piotr Rieske [1], Zakroczym, Poland , Sylwester Piaskowski [1], Zakroczym, Poland

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E-poster Session: Basic Science 2

Main functions of active dressing materials include protection against infections, absorption of exudates, thermoregulation and creation of appropriate moist environment for wound healing. Nowadays, the available materials range from traditional cotton gauze to the modern multifunctional systems made from natural and synthetic polymers. Current trend in wound healing therapies is switching toward biodegradable dressings made of innovative biopolymers.

It is desirable for the polymer to resorb during the healing process so that the dressing removal does not affect the healing tissue. Moreover, the biodegraded dressing material may become a scaffold for migrating cells, accelerating the granulation process.

Chitin is proposed as candidate material for biomedical application, especially in wound therapies due to its biological properties, including good biocompatibility, biodegradability and wound healing effects [1-2]. The problem of chitin insolubility in common organic solvents and any further difficulties in its processing may be solved by using chitin derivatives. Dibutyryl chitin (DBCH) is a chitin ester derivative esterified with butyryl groups at positions 3 and 6 of the N-acetylglucosamine units. The *in vitro* and *in vivo* investigations of DBCH biological properties confirm its biocompatibility according to the EN ISO 10993 requirements [3-6]. Synthesis conditions are crucial for maintaining DBCH biological properties affecting fibroblasts colonization of DBCH surface. Due to its ability to biodegrade on the wound surface and its high sorption capacity, DBCH is one of the materials giving the most encouraging results and one of the most promising polymers to be used in the treatment of chronic wounds.

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[1362] DEVELOPMENT OF THE PARAMETERS OF TOPICAL OXYGEN THERAPY AND ITS EFFECT ON ISCHEMIA WOUND HEALING

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Conclusions: Our data demonstrated that captopril via inhibiting Ang II-mediated signaling could impair epidermal regeneration during wound healing, suggesting a critical role of ACE in sustaining biological function of ESCs.

[1365] LOW MICROMOLARCOPPER (II) IONS ENHANCE THE PROLIFERATION OF HUMAN KERATINOCYTES VIA APPROPRIATE GENERATION OF REACTIVE OXYGEN SPECIES

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Objective: To investigate the effects of copper (ii) ion (Cu^{2+}) concentrations in a wide range from nanomolar to milimolar levels on human keratinocyte proliferation as well as reactive oxygen species (ROS) generation, and verify the role of ROS in human keratinocyte proliferation.

Methods: Human skin keratinocyte line HaCaT cells were treated with 10^{-8} M～10^{-3} M of Cu^{2+}or(and) 5 mMN-Acetyl-L-cysteine(NAC), the proliferation rate and ROS production of these cells were measured by cell counting kit-8 (CCK-8) and fluorescent probe DCFH-DA, respectively. Both intracellular ROS levels and mitochondria localization were labeled by coculture with DCFH-DA and MitoTracker probes, and visualized under laser confocal microscopy.

Results: After cultured with 10^{-6} M and 10^{-5} M Cu^{2+} for 48h, the cell relative proliferation rates were significantly higher than that in negative control group (P<0.05）. Meanwhile, after exposure to 10^{-6} M and 10^{-5} M Cu^{2+}, intracellular ROS levels increased remarkably at both 30 min and 60 min P<0.01 for 10^{-6} M group, P<0.05 for 10^{-5} M group. In addition, 5 mM NAC can significantly antagonize the cell proliferation and ROS generation induced by Cu^{2+}. Although intracellular ROS generation also increased only after 60 min exposure under 10^{-8} M and 10^{-7} M Cu^{2+} （P< 0.05), but no obvious change was observed in keratinocyte proliferation.

Conclusion: Low micromolar concentrations of copper (ii) ions can promote the proliferation of human skin keratinocytes through up-regulated intracellular ROS generation at moderate level.

[1385] THE IMPROVED DBCH BIOLOGICAL PROPERTIES AND THE POSSIBILITY OF ITS USE IN WOUND HEALING THERAPIES

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Main functions of active dressing materials include protection against infections, absorption of exudates, thermoregulation and creation of appropriate moist environment for wound healing. Nowadays, the available materials range from traditional cotton gauze to the modern multifunctional systems made from natural and synthetic polymers. Current trend in wound healing therapies is switching toward biodegradable dressings made of innovative biopolymers.

It is desirable for the polymer to resorb during the healing process so that the dressing removal does not affect the healing tissue. Moreover, the biodegraded dressing material may become a scaffold for migrating cells, accelerating the granulation process. Chitin is proposed as candidate material for biomedical application, especially in wound therapies due to its biological properties, including good biocompatibility, biodegradability and wound healing effects [1-2]. The problem of chitin insolubility in common organic solvents and any further difficulties in its processing may be solved by using chitin derivatives. Dibutyryl chitin (DBCH) is a chitin ester derivative esterified with butyryl groups at positions 3 and 6 of the N-acetylglucosamine units. The *in vitro* and *in vivo* investigations of DBCH biological properties confirm its biocompatibility according to the EN ISO 10993 requirements [3-6]. Synthesis conditions are crucial for maintaining DBCH biological properties affecting fibroblasts colonization of DBCH surface. Due to its ability to biodegrade on the wound surface and its high sorption capacity, DBCH is one of the materials giving the most encouraging results and one of the most promising polymers to be used in the treatment of chronic wounds.

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