thus manifesting both tip cell and stalk cell traits. We provide experimental evidence that such intermediate cell states do exist by staining of endothelial cell cultures with CD34, a known tip cell marker, thus substantiating our enhanced model.

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Flashing Brownian Ratchet Mechanism of Collective State Transition in Mouse Embryonic Stem Cell Revealed by using Single Cell Analysis

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Embryonic stem cell (ESC) is pluripotent cell, which is capable of self-renewal and can differentiate to all types of cells. The ESCs collectively differentiate to the right cell in the right place in a group during tissue development. Because the mouse ESC (mESC) can be considered as a simple spontaneously fluctuating individual that does not have any oscillators or circadian clock, it is thought that the collective differentiation in stem cell conceals the mechanism to generate orderliness from assemble of randomness. In order to find the basic role to generate the collective differentiation, we here investigate relationships of heterogeneity of cells and of colonies during the early differentiation stage in mESC by using single cell microscopy. We collected and analyzed total of 100 images of mESC colony containing approximately 30 cells every day for 8 days after differentiation induction. The cellular state could be defined by the fluorescent intensities of the reporters, Venus and mKate2, respectively reporting expressions of Nanog and Oct4, which are core transcription factors for pluripotency maintenance. The heterogeneity in cellular state could be exhibited not only in individual cell but also in colony. We found that the cell tended to resemble its state to the neighboring cells with some kinds of cooperativity, causing the colonial heterogeneity with restricting the cellular fluctuation in a colony. The quantitative analysis of the experimental data lead us the hypothesis that the imbalance of spontaneous fluctuation in a cell and cooperativity between cells realizes a flashing Brownian ratchet, causing the avalanche-like state transition of differentiation state. The present hypothesis was further reinforced by the numerical toy-model with simple rules. The fluctuation and cooperativity play a role for preserving the fractal heterogeneity in multilayer, and the flashing Brownian ratchet is possibly an intrinsic common feature in collective decision making in fluctuating individuals.

1372-Pos Board B440

Protective Effects of Extremely Low Frequency Magnetic Field (50 Hz, 1 mT) and Pterostilbene Treatment on Renal ischemic Injury

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Ischemia-reperfusion injury is one of the most important pathological outcomes observed after organ trasnplantation. Although the application of magnetic field has been proposed as a new treatment strategy for ischemic injury, there have been contraversial results reporting magnetic field treatment as being effective or uneffective on ischemic injury. This study aims to determine the therapeutic role of extremely low frequency electromagnetic field (ELF-EMF) application and the administration of pterostilbene, an antioxidant which is a derivative of resveratrol, on renal ischemic injury.

In the study, rats were divided into control, ischemia-reperfusion, ELF-EMF (50 Hz, 1 mT) treatment and 10 mg/kg dose of pterostilbene injection together with magnetic field treatment groups. After the establishment of renal ischemia-reperfusion model by clamping the arteries for 20 minutes and a 24 hour reperfusion, treatments were applied for 5 consecutive days. Then the kidneys were dissected, homogenised and analyzed by Fourier transform infrared (FTIR) spectroscopy. Protein secondary structures were predicted using second derivative spectra in amide I region.

Ischemia-reperfusion injury caused a decrease in lipid and protein amount, lipid/protein ratio; a decrease in the level of unsaturated lipids and unsaturated/saturated lipid ratio; an increase in membrane fluidity and lipid peroxidation in components of rat kidneys. Moreover, ischemia-reperfusion injury led to an alteration in the structure of proteins and lipids and a higher content of long chained lipids were observed in kidneys of ischemia-reperfusion group. ELF-EMF application (50 Hz, 1 mT) was not sufficient to repair all ischemia-induced alterations; however pterostilbene injection together with magnetic field treatment successfully restored ischemia-induced changes in all of the investigated parameters.

The results revealed that pterostilbene treatment together with magnetic field application can be a promising prevention for renal ischemic injury.

1373-Pos Board B441

Possible Therapeutic Effects of Gamma-Tocotrienol Conjugation Therapy with Doxorubicin on Hepatocellular Carcinoma Cell Line HEPG2

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Owing to its latent progression characteristics, poor diagnosis ratio, sub-10% 5-year survival rate and high mortality rate, Hepatocellular Cancer (HCC) is deemed as one of the deadliest cancers. Classified as the primary cancer of the liver tissue, possible causes of HCC can be listed as not only genetic factors influencing its onset and progression, but also excessive use of alcohol and exposure to aflatoxins, advanced iron accumulation, cyrosis, obesity and diabetes. Clinical success of most commonly used chemotherapeutical agent, doxorubicin, is hampered by the acquired resistance of HCC cells and unwanted side effects. It is widely accepted that the acquired resistance of HCC cells to doxorubicin might be due to signals perceived from the microhabitat of the tumour cells as well as activation of certain signalling pathways inside the cell, such as ABC transporter, ALDH, BCL2 pathways. It has been previously shown that gamma-tocotrienol has a pro-apoptotic impact on HCC cells. It also somehow aids the HCC cells to regain control on the otherwise uncontrollable cell division. On the other hand, the molecular mechanism of this action of gamma-tocotrienol is poorly documented. Therefore, we set to investigate the molecular profile changes that occur in the HCC cells upon treatment of the cells with gamma-tocotrienol either alone or alongside doxorubicin, as we would suggest to be done in a conjugation therapy against HCC. For this purpose, we investigated the impacts of this conjugation therapy on cell survival, mobility, lipid metabolism and molecular profiles using techniques of XTT, Wound Healing Assay, TBARS Assay and ATR-FTIR spectroscopy combined with multivariate analysis. Our initial findings show that addition of gamma-tocotrienol to doxorubicin treatment advances the effects of the treatment and significantly impacts the molecular profiles of the HCC cells.

1374-Pos Board B442

Development of Aged Erythrocytes Separation Device using Lorentz Force

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Under the use of mechanical circulatory support devices, serious hemolysis occurs immediately after transfusion because of mechanical shear stress generated by heart-lung machines. This hemolysis has been found to cause by rupturing of aged and weak red blood cells (RBCs) called old RBCs. To reduce this problem, it is considered that only the young RBCs should be transfuse separately. The membrane of young RBCs has negative charge and gradually diminish this electricity along with the aging. In this study, the RBC separation device, based on a principle of the Lorentz force and negative charge characteristics of young RBCs, was designed, prototyped, and evaluated. To assess the feasibility of proposed device, Mean Corpuscular Volume (MCV) of treated blood was calculated using Complete Blood Counter (CBC) as an index of youngness of RBCs. (It is known that the volume of young RBCs are larger than that of old RBCs.) As a result of experiment, treated blood had 1.011 ± 0.008 and 1.007 ± 0.007 times larger MCV than control blood (separation speed of 0.1 L/min and 0.3 L/min respectively). There was no correlation between efficiency of RBC separation and flow rate of separation device. In conclusion, it was suggested that transfusion-associated hemolysis during extracorporeal circulation can be prevented by using proposed method.

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Modulation of EGFR Signaling by Antibody Conjugated DNA Nano-Forceps

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The spatial distribution of EGFR (Epithelial Growth Factor Receptor) is tightly regulated for the maintenance of various cellular metabolisms as proliferation, differentiation, polarity and migration in mammalian cell. However, in case of cancer cells, the spatial distribution of EGFR is dysregulated by the abnormal expression of EGFR and its phosphorylation still generates regardless of the binding of EGF (Epithelial Growth Factor).

Therefore, in order to modulate EGFR signaling regardless of EGF in cancer cells, we developed EGFR antibody conjugated DNA Nano-Forceps with multiple arms and measured the phosphorylation level of EGFR by the treatment of this DNA Nano-Forceps. EGFR conjugated DNA Nano-Forceps inhibited the phosphorylation by EGFR dimerization in dose dependent manner even if we treated EGF in A549 as an EGFR hyper-activated lung cancer cell line. Also, EGFR downstream signaling pathways as the phosphorylation of ERK2