1P457**Radiation inhibits protein downregulation by RNA interference and antisense oligodeoxynucleotides in human breast cancer cells.**

○Tetsuo Nakajima, Mitsuru Neno

Research Center for Radiation Safety, Natl. Inst. Radiol. Sci.

A lot of efforts have focused on the RNA interference(RNAi) using small interfering RNAs(siRNAs) as a tool to knock down the expression of a target protein, although all of the processes of RNAi has not been clarified yet. It is a potential therapeutic tool. In radiation therapy field, particularly, in fractionated radiation therapy and cures of patients in accidental radiation exposure, treatment with siRNA after irradiation of tissues and cells is expected to be performed in its application. However its efficacy remains unknown. We evaluated effects of radiation on siRNA function. In human breast cancer cells (MCF7), siRNA against PKC delta downregulated PKC delta expression 24 h after treatment. Irradiation with 5Gy inhibited the down-regulation. In the case of the siRNA against PKC alpha as well as PKC delta, radiation also inhibited the down-regulation by the siRNA. Though effects of radiation on transfection efficiency by lipofection was also assessed in the process of siRNA functioning, radiation had no effect on the transfection efficiency. These results suggest that radiation inhibits regulations of siRNA function for downregulation of targeted proteins after the internalization. To understand the mechanism of radiation-induced inhibition of siRNA function, comparative experiments using antisense oligodeoxynucleotides(AS-ODN) were performed. Radiation also inhibited downregulation by AS-ODN against PKC delta. Currently change in internalized RNA degradation after irradiation is under investigation.

1P459**A regional gradient of intrinsic rhythmicity depicted in embryonic cultured multiple-hearts**○Tetsuro Sakai², Kohtaro Kamino¹¹Dept. of Physiology, Tokyo Medical and Dental Univ. Graduate Sch. and Faculty of Medicine, ²Dept. of Physiology, Univ. of the Ryukyus Sch. of Medicine

We have used optical methods to examine the spatial gradient of intrinsic rhythmicity in the early stage multiple-heart chick embryos which were induced experimentally in whole embryo culture. The embryos were cut microsurgically through the tissue of the anterior intestinal portal at the 5- to early 7-somite developmental stage. Spontaneous electrical activity in 4 to 6 segmented hearts, during the 7- to 10-somite stages of development, were monitored simultaneously by means of multiple-site optical recordings of membrane potential activity, using a voltage-sensitive merocyanine-rhodanine dye (NK2761). Each segment of the heart exhibited its own inherent rhythmicity. In quadruple-hearts, the rhythmicity was often in the order of [left-caudal segment], [right-caudal segment], [left-cephalic segment], and [right-cephalic segment]; the heart rate in the left-caudal segment was often faster than that in the other segments. An atypical pattern of "bursting" rhythm was observed in the cephalic segments, suggesting that, in these segments, the development of the rhythmicity is relatively poor. These findings strongly emphasize the concept that, in the early phases of cardiogenesis, the formation of a regional gradient of pacemaker activity (i.e. a spatial gradient of intrinsic rhythmicity) results in the functional self-organization of the pacemaking area.

1P458**Cu²⁺ release and inactivation of tyrosinase induced by 1MHz ultrasound**○Tsukasa Takahashi¹, Takashi Kondo², Wakako Hiraoka¹¹Dept. of Physics, Meiji Univ., ²Dept. of Radiological, Toyama Univ.

We have studied the mechanism of inactivation of mushroom tyrosinase induced by 1 MHz ultrasound. Recent works have reported the extensive application of sonotherapy to dermatological disorder and maintenance of skin homeostasis. Tyrosinase is one of target enzymes of sonotherapy for melanin deposition. In this report, we tried to inactivate tyrosinase in vitro by ultrasound. As an enzymatic assay of tyrosinase, continuous spectrophotometric rate determination was employed. Reactive oxygen species (ROS) were detected by the method combining ESR and spin-trapping. Released Cu²⁺ was quantified with kinetic-catalytic spectrophotometric method. Sonication was performed with 1 MHz Ultrax-UX601 (0 - 1.4 W/cm²). Acoustic intensity was calibrated with HNR-1000 hydrophone (ONDA, Japan). As a result, tyrosinase activity was reduced as increasing power of 1 MHz ultrasound. When the reaction mixture containing spin trap was irradiated, OH-adduct was detected by ESR. However, there was no effect of mannitol (OH scavenger) on the reduction of tyrosinase activity after irradiation. When excess Cu²⁺ was added to the solution, tyrosinase activity was not reduced after irradiation. The analysis of free Cu²⁺ showed that ultrasound mediated Cu²⁺ release from tyrosinase. These results indicate that ultrasound-induced inactivation of tyrosinase is caused by not ROS generated from the cavitation but the Cu²⁺ release from activation center of the enzyme.

1P460**Cell division of primary stromal-vascular cells during adipose differentiation**○Masafumi Nagayama¹, Tsutomu Uchida¹, Toshio Taira², Kyoko Shimizu², Masato Sakai², Kazutoshi Gohara¹¹Division of Applied Physics, Graduate School of Engineering, Hokkaido University, ²Primary Cell Co., Ltd.

Adipocyte proliferation and differentiation have been investigated in various disciplines because hypertrophy and hyperplasia of adipocytes in adipose tissue are associated with obesity and lifestyle-related diseases. According to previous reports, preadipocytes proliferate until they undergo growth arrest at confluence and also before growth-arrested cells start to accumulate lipid droplets (LDs) in their cytoplasm. However, it has never been clarified whether adipocytes proliferate in the course of adipocyte differentiation after they have started to accumulate LDs, although they have been considered to lack proliferative activity.

To clarify how individual cells proliferate and differentiate into adipocytes, we captured time-lapse images of primary stromal-vascular cells (SVCs) derived from rat mesenteric adipose tissue. The results revealed that adipocytes were still able to proliferate through cell division even though they were in the process of differentiation: The adipocyte that had accumulated many LDs (a few μm in diameter) suddenly became rounded and then the rounded adipocyte divided into two daughter cells within the following 30 min. The total number and size of the LDs in the two daughter cells corresponded roughly with those in the parent adipocyte. The result suggests that LDs in a parent adipocyte are evenly distributed between two daughter cells without fusing or dividing. We propose that the SVCs can proliferate and accumulate LDs simultaneously rather than in sequence.