Cell Biology
21. Expression, Subcellular Localization and Chromosome Localization of Hamster Ku70 and Ku80

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Keywords: Ku70, Ku80, hamster, mapping, GFP

Ku, a heterodimer of Ku70 and Ku80, plays a key role in multiple nuclear processes, e.g. DNA repair, chromosome maintenance, and transcription regulation. To understand the fundamental characteristics of Ku proteins, we examined the electrophoretic mobility, subcellular localization and expression of hamster Ku70 and Ku80 and determined the chromosome locations of their genes. When transfected into hamster cells, exogenous Ku70 and Ku80 tagged with green fluorescent protein (GFP) accumulated in the nucleus, indicating that both Ku proteins can localize in the nucleus of hamster cells. The electrophoretic mobility of hamster Ku proteins is different from that of human Ku proteins. No significant changes in the quantity of Ku proteins were observed in CHO-K1 cells treated with 10 Gy of ionizing radiation, suggesting that both proteins are expressed constitutively in amounts adequate to repair DNA DSBs. The chromosome locations of the Ku genes were determined by direct R-banding fluorescence in situ hybridization. The Ku70 gene was localized to Syrian hamster chromosome 4qa4.1 → qa4.2 and Chinese hamster chromosome 2p3.1, and the Ku80 gene was localized to Syrian hamster chromosome 4qb5 → qb6.1 and Chinese hamster chromosome 2p3.5 → p3.6. These results provide clues to the biological functions of Ku, as well as useful information for constructing comparative chromosome maps between hamsters and other mammalian species, including human, mouse, and rat.

Publications:

22. Endothelins Control the Proliferation and Differentiation of Murine Melanocytes from UVB-induced Pigmented Spots

Tomohisa Hirobe

Keywords: melanocyte, keratinocyte, UVB, proliferation, differentiation

Long-term exposure to ultraviolet radiation B (UVB) is known to induce pigmented spots in the dorsal skin of hairless mice. By using a serum-free culture medium supplemented with dibutyryl adenosine 3:5-cyclic monophosphate and basic fibroblast growth factor, it has been shown that the proliferation of epidermal melanoblasts and melanocytes from UVB-induced pigmented spots is greatly stimulated, and the stimulation is regulated by keratinocytes rather than melanocytes. In this study, we tried to understand what factors derived from keratinocytes were involved in regulating the proliferation and differentiation of epidermal melanoblasts and melanocytes from UVB-induced pigmented spots. For the purpose, antibodies towards growth factors and cytokines were added to the serum-free culture medium in the presence of keratinocytes. Results showed that antibodies towards endothelin (ET)-1, ET-2 and ET-3 inhibited the proliferation and differentiation of cultured epidermal melanoblasts/melanocytes from UVB-induced pigmented spots of hairless mice, but not from non-irradiated mice. In contrast, antibodies towards hepatocyte growth factor and leukemia growth factor failed to affect the proliferation and differentiation of cultured epidermal melanoblasts/melanocytes from both control and irradiated mice. These results suggest that ETs are keratinocyte-derived factors which are involved in regulating the proliferation and differentiation of epidermal melanoblasts/melanocytes from both control and irradiated mice. These results provide clues to the biological functions of Ku, as well as useful information for constructing comparative chromosome maps between hamsters and other mammalian species, including human, mouse, and rat.

Publication:

23. Effect of Nitric Oxide on Sertoli Cell Tight Junction-Associated Proteins

Makoto Onoda, Takanori Katsube and Hiroshi Inano

Keywords: Sertoli cell, nitric oxide, tight junction, occludin, ZO-1, cortactin, NOC18

Nitric oxide (NO) is an important biological molecule with a wide variety of functions. NO appears to be present in all parts of the male reproductive system and to play diverse roles in testicular, epididymal and vas deferent functions. We, therefore, undertook clarification of the effects of NO on the Sertoli cells (SC) tight junction (TJ)-associated proteins by using a culture
Fig. 17. Immunocytochemistry of tight junction-associated proteins in cultured rat Sertoli cells. Sertoli cells (1.5×10^6 cells/well) were isolated and cultured on Matrigel coated-cover glass in serum free defined medium with (A' and B') or without (A and B) NOC18 (400 μM). Sertoli cells were subjected to immunocytochemistry with specific antibodies. A and A': Cultures stained with anti-occludin antiserum. B and B': Cultures stained with anti-ZO-1 antiserum.

SC were isolated from 16-day-old immature rat testes by a three-step sequential enzyme digestion procedure, and cultured on 15-mm round glass cover slides coated with reconstituted extracellular matrix (Matrigel) in the well of a 24-multiwell plate. The cultures were maintained in serum free defined medium throughout the experimental period. 2,2’-(Hydroxynitrosohydrazone) bis-ethanamine (NOC 18), a NO donor, was added to the cultures, and the distribution and expression of the TJ-associated proteins, such as occludin, ZO-1 and actin cytoskeleton, were determined by immunocytochemistry and Western blot analysis, respectively, with specific antibodies. Cortactin (p80/85), a prominent substrate for Src protein tyrosine kinase, was also examined.

The amount of NO produced by SC in culture was 3.4 ±1.4 nmol/ml and 7.3 ± 0.9 nmol/ml for 24 hr- and 48 hr-incubation periods, respectively. This suggests that SC produce NO spontaneously under the culture condition, although the amount is relatively minute. On the other hand, the exogenously released NO from an NO donor into the medium was 147.8 ± 2.7 nmol/ml and 333.9 ±10.1 nmol/ml in the cultures with 200 μM and 400 μM of NOC 18, respectively, in a 24 hr-culture period. These values are in agreement with the theoretical values based on the half-life time (21 hr) of NOC 18. Immunostaining for occludin, ZO-1 and cortactin in untreated-SC showed continuous labeling around the cell periphery in the region of the cell-cell junctional complex, and these colocalized with TJ-associated F-actin filaments. However, incubation with NOC 18 (200 μM and 400 μM) caused complete disorganization of occludin staining and partial disruption of ZO-1 staining within 48 hr (Fig. 17), while alteration in the immunolabeling pattern for cortactin and F-actin was inconclusive in NOC 18 treated SC. Furthermore, Western blotting of occludin, ZO-1 and cortactin exhibited descending expressions of these TJ-associated proteins in a dose-dependent manner of NOC 18 in the NO donor treated SC (Fig. 18). These results suggest that excessive production of NO within testis during acute or chronic pathophysiological conditions disrupts TJ-associated proteins of SC and that NO may perturb blood-testis barrier formation and then, in turn, the regulation of normal spermatogenesis.

24. Chemically Induced Premature Chromosome Condensation in Human Fibroblast Cell Lines: Fundamental Study for Applications to the Biodosimetry of Local Exposure

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Keywords: cell cycle, human fibroblasts, premature
chromosome condensation (PCC), phosphatase inhibitors, X-rays

The premature chromosome condensation (PCC) of human peripheral lymphocytes treated with inhibitors of protein phosphatase has been demonstrated to be an excellent tool for the estimation of high dose whole-body exposure. To develop a new biodosimetry for local exposure, the cytogenetical reaction of human fibroblast lines to PCC inducers was examined and compared with that of lymphocytes.

The efficiency of the induction by calyculin A was greater than that by okadaic acid in both lymphocytes and fibroblasts. Calyculin A induced PCC in 5 Gy-irradiated and unirradiated samples at almost the same frequency in the lymphocytes, whereas the efficacy was considerably lower in irradiated fibroblasts than unirradiated ones. Calcium ionophore enhanced the induction of PCC in irradiated fibroblasts, although PCC frequencies were still much lower than those in the lymphocytes.

Fig. 19(a) shows the time-course of the induction of PCC when fibroblasts were irradiated, cultured and treated with calcium ionophore and calyculin A. The proliferative cycle of 2 Gy-irradiated fibroblasts seemed to be synchronous so that a wave of PCC reactions was evident. However, no synchrony was observed in 5 and 10 Gy-irradiated samples, presumably due to a difference in the fractions of the cells in cycling states after exposure. The frequency of ring chromosomes observed in 2 and 5 Gy-irradiated fibroblasts was too low to be used as a marker for cytogenetic dosimetry, but that of excess fragments, scored as the observed chromosome number minus 46, might be substituted.

The frequency of excess fragments for 2, 5 and 10 Gy-irradiated fibroblasts was less than 0.75, about 1 and a few per cell, respectively, although these values changed with the culture period (Fig. 19(b)). As for cultured fibroblasts, the frequencies of PCC and excess fragments for the period of Day 2 to Day 5 could give a very rough estimation of exposed doses in vitro.

To apply PCC techniques to fibroblasts for cytogenetic dosimetry, there are two major problems: First the in vitro cell cycle of fibroblasts is more difficult to control than that of lymphocytes. This may be solved by the analysis of PCC in a non-cycling phase but preliminary attempts have not been successful. Second, human fibroblasts are refractory to PCC induction by calyculin A compared with tumor cell lines. On the other hand, our PCC method has an advantage for cytogenetic analysis of fibroblasts: in the process of PCC induction, okadaic acid and calyculin A detached cells from culture plates as well, and the trypsin treatment was skipped. The trypsin treatment is necessary for conventional cytogenetic analysis of adhesive cells, although it affects cell membranes so to make chromosome preparation difficult.

Publication:

Fig. 19: Time-course of the PCC induction in hTERT-BJ1 human fibroblasts. After fibroblasts in the confluent phase were irradiated at 0 (▲), 2 (△), 5 (□) and 10 Gy (○) and held at 37°C for 3 hours, they were trypsinized, plated and cultured for 1-9 days. At 5 hours and 30 minutes before harvesting, 1 M A23187 and 250 nM calyculin A were added to the cultures, respectively. (a) The percentage of cells having prematurely condensed chromosomes (PCC cells). (b) The frequency of excess fragments per cell, scored as the observed chromosome number minus 46.
25. Targeted Disruption of Np95 Gene Renders Murine ES Cells Hypersensitive to DNA Damaging Agents and DNA Replication Blocks

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Keywords: X-rays, UV, MNNG, SCE, cell cycle, hydroxyurea, replication fork

Np95, which contains a ubiquitin-like domain, a cyclin A/E-Cdk2 phosphorylation site, Rb binding motif and ring finger domain, has been shown to be colocalized as foci with PCNA in early and mid-S phase nuclei. We established Np95 null murine ES cells by replacing the exons 2-7 of the Np95 gene with a neo cassette and by selecting out a spontaneously occurring homologous chromosome crossing-over with a higher concentration of neomycin. Np95-null cells were more sensitive to X-rays, ultraviolet light (UV), N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and hydroxyurea (HU) than ES wild type (Np95+/+) or heterozygously inactivated (Np95+/-) cells. Expression of transfected Np95 cDNA in Np95-null cells restored the resistance to X-rays, UV, MNNG or HU concurrently to a level similar to that of Np95+/+ cells, though slightly below that of wild type (Np95+/+) cells. These findings suggested that NP95 played a role in the repair process for DNA damages incurred by these agents. The frequency of spontaneous sister chromatid exchange was significantly higher for Np95-null cells than for Np95+/+ cells or Np95+/- cells (P < 0.001). We inferred that NP95 functioned as a common component in the multiple response pathways against DNA damages and replication arrest and thereby contributed to the genomic stability.

Publications:

26. Significance of Fractionated Irradiation for the Biological Therapeutic Gain of Carbon Ions

Koichi Ando, Sachiko Koike, Akiko Uzawa, Nobuhiko Takai, Takeshi Fukawa, Yoshiya Furusawa, Mizuho Aoki, Chisa Oohira, Manami Monobe, Ryonfa Lee, Masao Suzuki and Kumie Nojima

Keywords: relative biological effectiveness, mouse, tumor, skin

It is well established that the RBE (relative biological effectiveness) for cell killing depends on LET (linear energy transfer), and that a maximum RBE is observed at ~150 keV/µm. However, the therapeutic gain depends on the ratio of the RBEs for the effects on the cancer cell population and the effects on normal tissues. The RBE of a given radiation quality depends on LET but also on dose, biological system and effect, and irradiation conditions. There are no data available to answer the question: which LET is suitable to improve the biological therapeutic gain of carbon ions? Here, we selected 3 different LET values of 290 MeV/µm carbon ions, and compared the relative biological effectiveness between tumor growth retardation and skin damage using a murine transplantable tumor. Larger RBE values for tumors than the skin type were obtained when carbon ions of intermediate LET were delivered daily for 2 through 5 fractions (Fig. 20). The biological therapeutic gain would be high for the carbon ion SOBP when the number of fractions has been correctly selected in clinical trials.

Publication:
Number of Fractions

Fig. 20. RBE values of carbon ions for tumors and skin. The RBE value is calculated by comparing the isoeffect dose of carbon ions with that of X rays for the corresponding number of fractions. The open circles and closed triangles represent tumors and skin, respectively.

27. Time-Course of Reoxygenation in Experimental Murine Tumors after Carbon-Beam and X-Ray Irradiation

Sachiko Koike, Kumie Nojima, Yoshiya Furusawa, Koichi Ando, Natsuo Oya¹, Keisuke Sasai¹, Toru Shibata¹, Takehisa Takagi¹, Keiko Shibuya¹ and Masahiro Hiraoka¹ (Kyoto Univ.)

Keywords: high-LET radiation, reoxygenation, SCCVII, EMT6

We compared the tumor reoxygenation patterns in three different murine tumor cell lines after X-ray irradiation with those after carbon beam irradiation using the heavy ion medical accelerator (HIMAC) system. The tumors of the cell lines SCCVII, SCCVII-variant-1 and EMT6 on the hind legs of mice received local priming irradiation with a carbon beam (8 Gy, 73 keV/μm in LET, 290 MeV/u, 6 cm SOBP) or X-rays (13 Gy, 250 kVp). After various intervals, the mice were given whole-body test irradiation (16 Gy, 250 kVp X-rays) either in air or after they were killed. The hypoxic fractions were estimated as the proportions of the surviving fractions of the tumors in killed mice to those in air-breathing mice. In the SCCVII tumors, the hypoxic fractions at 0.5 h were 50% and 21% (p < 0.05) after the priming X-ray irradiation and carbon beam irradiation, respectively. In the SCCVII-variant-1 tumors, the hypoxic fractions were 85% and 82% at 0.5 h, 84% and 20% at 12 h (p < 0.01), and 21% and 31% at 24 h after X-ray irradiation and after carbon beam irradiation, respectively. In the EMT6 tumors, the reoxygenation patterns after X-irradiation and carbon beam irradiation were similar. We concluded that the reoxygenation pattern differed among the three tumor cell lines, and that reoxygenation tended to occur more rapidly after carbon beam irradiation than after X-ray irradiation for SCCVII and SCCVII-variant-1 tumors.

Publication:

28. Chromatid Breaks Induced in Normal Human Cells by Heavy Ions at Initial, Metaphase and Interphase States

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Keywords: chromosome aberrations, high-LET radiations, initial breaks, cell-cycle, PCC-FISH
To investigate initial chromatid breaks and how cell-cycle delays in human cells affect the expression of complex chromosome damage in metaphase following high- and low-LET radiations, we irradiated whole blood or exponentially growing human fibroblast cells AG1522 in vitro with a low and a high dose of iron, neon, carbon, and silicon particles or γ-rays.

Lymphocytes were cultured and meta-phase cells were collected at different time points after 48-84 h in culture. Interphase chromosomes were prematurely condensed using calyculin-A, either 48 or 72 h after exposure to iron particles or γ-rays. Cells in the first division were analyzed using a combination of FISH whole-chromosome painting and DAPI/Hoechst 33258 harlequin staining. Chromosomes of AG1522 cells were prematurely condensed using calyculin-A. Initial chromatid-type and isochromatid breaks in G2 cells were scored.

The dose-response for total chromatid breaks was linear regardless of radiation type. The relative biological effectiveness (RBE) increased with LET, and showed a maximum at 55-80 keV/µm, then decreased at higher LET. Induction of isochromatid-type breaks was linear to dose for high-LET radiations, but linear-quadratic for γ-rays or 13 keV/µm carbon beams. The RBE for the induction of isochromatid breaks obtained from linear components increased rapidly between 13 keV/µm and 80 keV/µm carbon beams and decreased gradually for higher LET beams. High-LET radiations were more effective in induction of isochromatid breaks, while low-LET radiations were more effective in induction of chromatid-type breaks. The densely ionizing track structures of high-LET radiation and the geometry of sister chromatids in G2 cells resulted in an increase in isochromatid breaks.

The delay of chromosome damage expression in metaphase was LET- and dose-dependant. This delay was mostly related to the late emergence of complex-type damage into metaphase. Yields of damage in PCC collected 48 h after irradiation with iron particles were similar to that obtained from cells undergoing mitosis after prolonged incubation. The yield of high-LET radiation-induced complex chromosome damage could be underestimated when analyzing metaphase cells collected at one time point after irradiation. Chemically induced PCC method might be a more accurate technique, because the problems with complicated cell-cycle delays would be avoided.

Publications:

29. Hsp25 Regulates the Expression of p21 through Multiple Mechanisms

Park Sang-Hee, Toshiyasu Hirama, Naoyuki Anzai, Yoshiko Kawase, Misao Hachiya, Hisayoshi Kondo, Saori Kawanura, Yasunari Takada, Daisaku Takai and Makoto Akashi

Keywords: Hsp25, p21, translational and post-translational regulation

Exposure of cells to external stresses leads to the induction or activation of certain proteins. Expression of heat shock proteins (Hsp) is induced in response to these stresses. Hsps are known to have molecular chaperone activities; but recent studies have shown that Hsps have a variety of functions such as triggering proliferation, differentiation, and apoptosis of cells. Previously, we found that overexpression of a 25 kDa Hsp (Hsp25) induced the expression of the cell cycle inhibitory protein p21 (WAF1/Cip1/Sdi1) in murine fibroblastoid L929 cells. However, the mechanisms for the induction of p21 by Hsp25 are unknown. In the present study, we have investigated the mechanisms of the regulation of p21 expression by Hsp25 in these cells. The introduction of Hsp25 cDNA stimulated the accumulation of p21 transcripts through transcriptional but not posttranscriptional regulation in these cells. We also found that overexpression of Hsp25 markedly increased the translational rate of p21 and stabilized the protein. Studies using proteasome inhibitors and Western blot analysis for ubiquitination of p21 have demonstrated that the stabilization of p21 is regulated through a ubiquitin-independent pathway. However, no direct association of Hsp25 with p21 was observed. These findings suggest that Hsp25 induces p21 expression through multiple mechanisms and that transcriptional, translational, and post-translational regulations are important in the regulation of p21.