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Up-regulation of heat shock Protein 27 in irinotecan resistant human colon cancer cells

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In this study investigated the relationship between irinotecan resistance and expression of stress proteins in colon cancer cells. Several human colon cancer cell lines (KM20, KM12C, COLO320HSR and KM1214) were analyzed for their susceptibility to irinotecan by MTT assay and confocal image using TUNEL staining.
We selected two cell lines: one, susceptible and the other resistant to irinotecan. There was no significant difference in the expression level of all kinds of stress proteins between two cells under normal condition except a high expression of Hsp27 in only pretreated Colo320HSR colon cell line by RT-PCR and real time PCR. Among colon cancer cells used for this study, Colo320HSR was resistant but KM1214 was susceptible to irinotecan. The results of MTT assay and TUNEL staining showed that irinotecan induce apoptosis in KM1214 cells but not in Colo320HSR.. Also the expression levels of hsp27 was significantly up regulated in Colo320HSR after irinotecan treatment by RT-PCR and real time PCR.
In order to investigate the role of Hsp27 in irinotecan-induced apoptosis of colon cancer cells, we investigated the relationship between irinotecan and oxaliplatin resistance and expression of stress proteins in naive pretreated and treated colon cancer cells and colorectal cancer's specimen clinically. Also we discovered that naive pretreated cancer cells naturally have a original resistance to chemotherapeutic drug caused by heat shock proteins 27. In order to investigate the role of Hsp27 in irinotecan-induced apoptosis of colon cancer cells, we suppressed the expression of Hsp27 in irinotecan-resistant Colo320 cells by using antisense oligodeoxynucleotides (A-ODN) specifically directed against Hsp27. Also we induced the overexpression of Hsp27 in irinotecan-resistant Colo320 cells by using sense ORF specifically directed against Hsp27.
These results suggest that Hsp27 plays important role in the induction of resistance against irinotecan. A-ODN(antisense oligodeoxynucleotides) treatment increased the irinotecan-induced apoptosis in Colo320 cells by 3–4 fold. These results suggest the possibility that hsp27 plays some role in irinotecan resistance in colon cancer cells and has a independent potential capacity of resistance to chemotherapeutic drugs.

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Loss-of-heterozygosity of chromosome 12 in malignant lymphomas from mice exposed to continuous low-dose rate gamma-ray irradiation

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Our previous report (Tanaka, S. et al., Radiat. Res. 160, 376–379, 2003) suggested that the shortened lifespan induced by continuous gamma-ray irradiation of B6C3F1 mice (C57BL/6J/Nrs × C3H/He/Nrs) at a low-dose rate (21 mGy/day, total dose = 8000 mGy) may be caused by early death due to the onset of malignant lymphomas.
To investigate the biological mechanisms of early lymphomagenesis induced by chronic exposure to low dose rate radiation, specimens of malignant lymphomas collected from 74 irradiated and 69 unirradiated mice were analyzed for loss of heterozygosities (LOHs) in their chromosomes 4, 11 and 12. Analyses were carried out using simple sequence length polymorphism-based PCR assay.
Frequent LOH were found at the region of D12Mitl33 on the telomeric site of chromosome 12 in 19.5% of lymphomas from irradiated mice and in 36.1% of lymphomas from unirradiated mice. LOH on chromosomes 4 and 11 were infrequent. These results differ from previous reports wherein radiation-induced thymic lymphomas have frequent LOH on these chromosomes.
The results suggest that chronic gamma-ray irradiation at low-dose rates may reduce the frequency at D12Mit133 LOH in lymphomagenesis. Mapping of possible candidate genes in the LOH region of D12Mit133 on the telomeric site of chromosome 12 that may be closely related to the early onset of lymphomas are now in progress. This work was carried out with the financial support of Aomori Prefecture, Japan.

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Tumor growth and angiogenesis induced by C6 glioma cells are regulated by heparin affin regulatory peptide *in vivo* and *in vitro*

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Background: Glioblastoma multiforme is the most aggressive malignant glioma and among the highly angiogenesis-dependent tumors. Heparin affin regulatory peptide (HARP) seems to be involved in the progression of brain tumors, such as the glioblastoma multiforme, with mechanisms yet to be elucidated. In this study, we tried to determine the role of HARP in glioblastoma multiforme.
Materials and Methods: An antisense strategy for inhibition of HARP expression in rat C6 glioma cells was used to study the role of HARP on cancer cell growth and angiogenic potential *in vivo* and *in vitro*.
Results: Decrease of the expression of endogenous HARP protein in C6 cells (C6-AS cells) significantly increased proliferation rate and anchorage-independent growth. Implantation of C6-AS cells onto chicken embryo chorioallantoic membranes resulted in a significant increase of tumor-induced angiogenesis, compared with angiogenesis induced by non transfected or C6 cells transfected with the plasmid alone (C6-PC cells). In the same line, conditioned medium from C6-AS cells significantly increased endothelial cell proliferation, migration and tube formation *in vitro* compared with the effect of C6 or C6-PC cells. Finally, there was a significant increase in vascular endothelial growth factor mRNA and protein levels in cultures of C6-AS cells compared with C6 or C6-PC cell cultures.
Conclusions: HARP seems to play a significant role in the regulation of tumor growth and angiogenesis induced by C6 glioma cells.
We thank European Social Fund (ESF), Operational Program for Educational and Vocational Training II (EPEAEK II), and particularly the Program IRAKLEITOS, for funding the above work.

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The potential role of oval cell presence in experimental liver fibrosis

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Background: Oval cells (OC) are liver stem cells involved in the progress of liver disease (when liver regeneration is suppressed) and hepatocellular carcinoma development, in experimental models. This study investigates whether OC develop and proliferate in a model of experimental liver fibrosis without suppression of liver regeneration.
Material and Methods: The study comprised 36 male Wistar rats divided in 2 groups: A (n = 4): controls and B (n = 32): CCl_4 injection (intraperitoneally 2 ml/Kg BW, 1:1 vol in corn oil twice weekly). Rats were sacrificed at 4, 8 and 12 weeks. SGPT values were measured in blood samples. Liver tissues were evaluated for:

i. degree of fibrosis (Masson's trichrome stain)
ii. AFPmRNA expression (RT-PCR and in-situ hybridization)
iii. cell-proliferation (Ki67 antigen stain)
iv. expression of antibodies for cytokeratins 19 (CK19) and 7 (CK7), alpha-fetoprotein (AFP), leukocyte common antigen (LCA) and CD34 antigen.

In addition, double stain (ISH+IHC) was applied to detect cells that co-expressed CK7/AFPmRNA, CK19/AFPmRNA, AFP/AFPmRNA. Cells with morphologic features of OC that were AFPmRNA or AFP+/CK19+/CK7+ and LCA(−)/CD34(−) were scored.
Results: Oval cells were present in all 36 specimens but in the control group (A) their percentage was significantly lower compared to group B ($p < 0.001$). They were located in periportal areas and were also recognized more often in association with fibrous bands and accompanied inflammatory infiltrates. Morphometric analysis revealed that the number of OC increased towards evolution of liver scarring In addition, OC show significant proliferation as the liver fibrosis progresses. Double stain revealed that OC co-expressed CK19/AFPmRNA, CK7/AFPmRNA, AFP/AFPmRNA. The percentage of CK19+, CK7+, and AFP(protein or mRNA)+ cells were directly correlated with SGPT values ($p < 0.05$).
Conclusions: This study demonstrates that oval cells develop and proliferate in a model of experimental liver fibrosis without suppression of liver regeneration. This may provide additional prognostic and therapeutic implications in the management of progressive liver fibrosis associated with liver failure.