with prolonged disease-free survival after curative surgery. The biological function of CLDN-10 in carcinogenesis is lacking. The aim of the current study is to evaluate the biological function of CLDN-10 in HCC by functional assays.
Material and Methods: CLDN-10 was overexpressed in Hep3B, an HCC cell line with low invasive ability and siRNA-mediated knockdown of CLDN-10 was performed in a highly invasive HCC cell line, HLE. The effect on invasion, migration, proliferation and survival was then investigated by in vitro function assays. MMP levels were evaluated by gelatin zymography. Expressions of MT1-MMP and claudin family members were examined by semi-quantitative RT-PCR and Western blotting.
Results: Functional studies demonstrated that increased expression of CLDN-10 enhanced the metastatic potential of HCC by promoting cancer cell survival, motility and invasiveness. More importantly, in the CLDN-10 transfectants, there was increase in mRNA transcription and protein expression of MT1-MMP, a protease shown to promote intrahepatic metastasis in HCC in our earlier study. In addition, CLDN-1, -2 and -4 was up-regulated in CLDN-10 overexpression transfectants, indicating that the expression of claudin family members in cancer cells might affect each other. On the contrary, CLDN-10 siRNA strongly inhibited invasion, MMP2 and MT1-MMP expression. These findings highlighted that CLDN-10 promotes metastatic potential in HCC by enhancing invasion through up-regulation of MT1-MMP and MMP2 expression.
Conclusion: CLDN-10 is important for MMP activation, HCC invasion and migration. It also modifies the claudin family expression profile. These findings underline the contributions of CLDN-10 in HCC progression.

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The difference in two types of HSV thymidine kinase's antiviral activity depends on cellular localization – in vitro study

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Background: The administration of Herpes simplex virus thymidine kinase gancyclovir-dependent (HSV-TK-GCV) viral constructions is among the first clinical protocols in cancer gene therapy. In spite the advantages of non-toxicity and safety the clinically certified effectiveness is relatively low compared to methods of standard cancer therapy. The aim of our work was to study the possible attitudes of TK activity enhancement comparing two types of viral thymidine kinases transfected into mammalian cells.
Methods: The cDNA of standard therapeutic constructions HSV-TK1 was amplified from pUT649 plasmid, wild type HSV-TK2 was amplified from Herpes simplex virus type 2 genome with primers: for. aa aga tct ATG GCT TCT CAC GCC GGC CA, rev. aa aag ctt CTA AAC YYC CCC CAY CTC GCG GGC AA. Both HSV-TK genes were ligated into pDsRed2-C1 frame downstream of the red fluorescent reporter gene and into p2FP-RNAi replaced turboGFP gene without any tags. pDsRed2-C1-TK1, pDsRed2-C1-TK2, p2FP-RNAi-TK1 and p2FP-RNAi-TK2 were transfected into Cos-7 and CHO-K1 cell cultures. Protein expression and intracellular distribution were analyzed with scanning confocal microscopy. TK expression was determined with Western blot using antibodies produced in laboratory. The GCV-mediated cell suicide activity was assessed with MTT cytotoxicity assay.
Results: Transfection efficiency for all vectors with TK was 50% and higher. The expression of TK1 and TK2 in CHO-K1 cells was higher than in Cos cell line. Confocal examination after different periods of transfectant's selection showed TK1 tagged with DsRed2 enters the nucleus unlike DsRed2-TK2 that was distributed more evenly throughout the cytoplasm. It occurred to be easy to obtain stably transfected cell lines with TK2 and no one TK1 transfectants survived 6 weeks of G418 selection. We observed interference in p2FP-RNAi transfectants appeared in increasing of TK expression after several days since transfection was made. Comparison of two TK types in short-time MTT assays proved TK1 GCV-mediated cytotoxic activity was 2 times higher than for TK2. However after several days of expression and G418 pre-selection TK2 in both pDsRed2 and p2FP-RNAi vectors provided 2−3-folded higher cytotoxic activity starting with lower doses of GCV versus TK1.
Conclusion: We suppose intracellular localization of the TK1 and TK2 proteins due to sequence alterations and able to affect apoptotic activity of the ferment. TK2 cytoplasmic distribution might be important factor for new gene therapeutic vectors development.

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A probiotic strain of enterecoccus faecium CRL183 reduces DMH-induced large intestinal tumors in male Wistar rats

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Background: Lactic acid bacteria have been reported to have a positive effect on colon cancer. We investigated the influence of the Enterococcus faecium CRL 183 on 1,2-dimethylhydrazine (DMH)-induced colon cancer, aberrant crypt foci (ACF) and modulation the immune reponse in male Wistar rats.
Material and Methods: 8-week old rat were given subcutaneous DMH injections at 20 mg/kg once a week during three months. Four groups were used: (1) non-treatment control; (2) DMH control; (3) Enterococcus faecium CRL 183-DMH; (4) Enterococcus faecium CRL 183 control. The all groups were compared histologically and TNF-α, IFN-γ and IL-4 cytokines.
Results: The non-treatment control and Enterococcus faecium CRL 183 control not develop tumor. The E. faecium CRL 183-DMH group showed a 50% inhibition in incidence in average number of tumors compared to DMH-control. ACF formation decreased in Enterococcus faecium CRL 183-DMH group and the results were statistically significant in the DMH control. TNF-α, IFN-γ and IL-4 cytokines increasing in this group.
Conclusions: These results show that Enterococcus faecium CRL 183 reduced tumor progression by modulation the immune response

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Involvement of AP-1 in cannabinoid antiproliferative action

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We have previously observed that δ^9-tetrahydrocannabinol (THC), the most important cannabinoid in terms of potency and abundance, reduces human breast cancer cell proliferation by blocking the progression of the cell cycle and by inducing apoptosis. In order to study in further detail the mechanism of cannabinoid action, we performed a DNA microarray-based study of human breast cancer cell response to THC. After normalization and filtering we obtained a total of 28 genes modulated by THC that might be involved in its action. Amongst these genes, we focused our attention on JunD, a member of the activator protein-1 (AP-1) transcription factor family, which is up-regulated by cannabinoid challenge. We validated the involvement of JunD in THC action by two different approaches:

i. By means of siRNA, we observed that breast cancer cells lacking JunD were more resistant to THC than the corresponding controls.
ii. We compared the effect of THC on wt vs. junD−/− immortalized fibroblasts. As for siRNA junD-knocked down breast cancer cells, JunD-deficient fibroblasts were more resistant to THC than their corresponding wt partners.

Taken together, these data support that the AP-1 family, especially JunD, is involved in the antiproliferative effect of cannabinoids in breast cancer cells. These results expand our knowledge on the mechanism of cannabinoid action and might set the bases for a cannabinoid-based therapy for the management of breast cancer.

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Spleen tyrosine kinase as a novel candidate metastasis suppressor for human oral squamous cell carcinoma

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Background: Spleen tyrosine kinase (Syk) is a non-receptor type of protein-tyrosine kinase that is widely expressed in several epithelial cells. Firstly, aberrant expression of Syk has been reported in breast cancer. Furthermore, recent finding suggests that loss of Syk is linked to poor prognosis and metastasis. However, expression level of Syk in oral cavity remains unclear. In the current study, we investigated the expression levels of Syk mRNA and protein expression in oral squamous cell carcinoma (OSCC)-derived cell lines and human primary OSCCs to elucidate the potential involvement of Syk in OSCC.

Materials and Methods: The 7 human OSCCs-derived cell lines used in this study were Ca9−22, Ho-1-N-1, HSC-2, Ho-1-u-1, HSC-4, KON and KOSC-2. Tumors with patient-matched normal oral tissues (when available) were obtained at the time of surgical resection at Tokyo dental college Chiba Hospital after informed consent had been obtained from the patients according to a protocol that was approved by the institutional review board of Tokyo Dental College.
Results: Using quantitative real-time reverse transcription polymerase chain reaction and immunofluorescence analyses on 7 OSCC-derived cell lines and normal oral keratinocytes (NOKs), Syk mRNA and protein expression were commonly down-regulated in all cell lines compared with the NOKs. Although no sequence variation in the coding region of the Syk gene was identified in these cell lines, we found a frequent hypermethylation in the CpG island region. In clinical samples, high frequencies of Syk down-regulation were detected by immunohistochemistry [19 of 30 (63%)]. Furthermore, the Syk expression status was significantly correlated with lymph node metastasis.
Conclusions: These results suggest that the Syk gene is frequently inactivated during oral carcinogenesis and that an epigenetic mechanism may regulate loss of expression, which may lead to metastasis.

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Antiangiogenic effect of newly synthesized chalcones

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Backround: Angiogenesis, the process by which new blood vessels are formed, is an important event in physiologic or pathological conditions. Several clinical studies showed a positive correlation between the number of vessels in the tumor and the metastases formation and the prognosis of the disease. Therefore antiangiogenesis is an important area of therapeutic development for treatment of cancer, since tumor growth and metastasis depends on angiogenesis.
Chalcones are precursors of flavonoids in their biosynthetic pathway. Variety of biological activities have been demonstrated for these compunds such as antiinflammatory, analgesic, antiviral, antibacterial, gastroprotective, antioxidant as well as cytotoxic properties. However, there is only a limited amount of literature concerned with antiangiogenic effects of chalcones.
Materials and Methods: In the present work, we tested four newly synthesized chalcones: 4-Hydroxychalcone (**1**), E-2-(X-benzylidene)-1-tetralones (**2a**, **2b**) and E-2-(4'-methoxybenzylidene)-1-benzosuberone (**3**) for their antiangiogenic effect using human umbilical vein endothelial cells (HUVEC). Effects of these compounds were tested by employing MTT cytotoxicity assay, capillary tube formation (CTF), endothelial cell migration (ECM), gelatinase zymography or vascular endothelial growth factor (VEGF) detection.
Results: From chalcones tested only compound **3** possess significant cytotoxic effect on HUVECs. It also completely inhibited CTF by HUVECs in concentrations 10^{-7}-10^{-8} mol/L. Moreover, this chalcone in the same concentrations effectively block also ECM. In biochemical analysis, chalcone **3** treatment of HUVEC for 24 h resulted in a concentration-dependent decrease in the secretion of matrix metalloproteinase (MMP-9). Furthermore, exposure of HeLa cells (cervix cancer) to chalcone **3** resulted in a dose-dependent decrease in the secreted VEGF level in conditioned media.
Other chalcone tested possess similar effects only in the highest concentration used (10^{-4} mol/L).
Conclusions: The present study demonstrate antiangiogenic properties of chalcone **3**. Further studies are necessary to elucidate its mechanism of action, nevertheless, this compound might have a potential to enter preclinical trials as a new angiostatic drug.
This study was supported by grants: VEGA 1/4236/07 and VEGA 1/3365/06.

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Expression of Gb3/CD77 and effect of verotoxin-1 treatment of cisplatin-resistant mesothelioma and NSCLC cells

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The aim of the study was to quantify the expression of globotriasosylceramide (Gb3/CD77) and the treatment effects of verotoxin-1 on cisplatin-sensitive and -resistant mesothelioma and NSCLC cell lines. Gb3 is a neutral glycosphingolipid which also acts as the receptor for verotoxin-1. The targeting of the toxin to a specific intracellular transport pathway is determined by the Gb3 isoform expressed on the cell surface and by the presence or absence of Gb3 in the lipid raft microdomains of the cell membrane. Gb3 is expressed on many tumour cells and tumour cells that express Gb3 will often become drug-resistant through induction of MDR1, which pumps anti-cancer drugs out of cells.
We studied the expression of Gb3 in the cisplatin-sensitive and -resistant pulmonary mesothelioma (P31) and NSCLC H1299) cell lines. The cisplatin-resistant sub-lines both expressed much higher amounts of Gb3 than the cisplatin-sensitive sub-lines. The cisplatin-resistant sub-lines were much more sensitive to verotoxin-1 than the cisplatin-sensitive sub-lines as noted by viability assays and TUNEL staining. Two umol/L of the Gb3-inhibitor PPMP (1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol) totally abolished GB3 expression of the cisplatin-resistant cell sub-lines and also abolished VT-1-induced cytotoxicity and apoptosis to the cells.
Our results suggest that increased Gb3 expression of cisplatin-resistant mesothelioma and NSCLC tumour cells makes them sensitive to verotoxin-1 cytotoxicity and apoptosis induction. Gb3 expression of cisplatin-resistant tumour cells may provide the basis to a new treatment approach using verotoxin-1 to enhance cancer therapy in inherited or acquired cisplatin resistance of tumours.

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An inhibitory effect of the hexamer fragment of HLDF differentiation factor on the development of experimental hemoblastosis

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Background: Since pathogenesis of many malignant tumors is related to aberrations in normal cell differentiation (Lyne J.C. et al., 1997), the attention of many researchers is now focused on preparations that induce differentiation process (Kanai M. et al., 2003; Filleur S. et al., 2005). One of the promising preparations of this type is HLDF6, hexamer fragment of HLDF, differentiation factor of human promyelocytic cell line HL-60 (Kostanyan I.A. et al., 1995, 2000). The aim of this investigation was to study antitumor activity of HLDF6 on experimental hemoblastosis models and in the tumor cell culture.
Materials and Methods: The studies were carried out in male DBA/2 mice with transplanted P-388 lympholeukosis and female CBA mice with LS lymphosarcoma. HLDF6 was i.p. administered in doses of 25 and 50 mg/kg five times a day or three times at an interval of 24 h at different times after tumor transplantation. The preparation was administered alone or in combination with cyclophosphamide (CP, 10−50 mg/kg, i.p.). The effectiveness of therapy was evaluated by the inhibition of the tumor growth and variations in the animal lifetime. To evaluate a direct antiproliferative effect of the HLDF6 peptide on the tumor cells proliferative activity and the survival of cells were measured by the method of T. Mosmann (1983).
Results: Five-fold administration of HLDF6 in a dose of 25 mg/kg to mice with P-388 lympholeukosis at early stage of tumor process led to a 34% increase in the animal life expectancy, but did not enhance the effect of CP. The dynamics of the LS lymphosarcoma growth was not influenced by the injections of HLDF6 at different periods of the tumor development. At the same time administration of HLDF6 in a dose of 50 mg/kg preceding or following the injection of CP contributed to the more marked inhibition of the tumor growth as compared to administration of CP alone. In the case of three-fold administration of the peptide following the injection of the cytostatic a 5-fold increase of tumor growth inhibition was observed. HLDF6 diminished the tumor cell survival level during prolonged cultivation in vitro, but did not enhance the effect of CP.