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.