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.
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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.

Conclusions: HLDF6 peptide exhibited antitumor activity in mice with experimental hemoblastosis, which was characterized by the inhibition of the primary tumor growth and the potentiation a cytostatic effect of CP. Besides, it has been shown that HLDF6 had a direct antiproferative effect on tumor cells.

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Antitumor activity of fragments of the HL-60 cell differentiation factor in mice with Lewis lung carcinoma

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Background: In the course of studies of the HL-60 cell differentiation factor two peptide fragments, hexamer HLDF6 and octamer HLDF8, with antiproliferative, differentiating and apoptogenic properties were identified (Kostanyan et al, 1995; 1999). It has been demonstrated that HLDF6 was an effective antitumor agent in mice with NSO myeloma (Kostanyan et al, 2000). The peptide had steroidogenic properties (Rzhevsky D.I., 2003), which made us think that it could influence the growth of hormone-dependent tumors. In this connection the aim of the present paper was to study antitumor activity of HLDF fragments in mice with transplanted Lewis lung carcinoma (LLC).
Materials and Methods: HLDF6 and HLDF8 peptides were i.p. administered to tumor-bearing female C57Bl/6 mice in a dose of 25 mg/kg five times at an interval of 24 h, with the first injection 24 h or 12 days after LLC transplantation. Cyclophosphamide (CP, 50 mg/kg) was i.p. administered on day 11 after transplantation. The mouse groups that were given saline, CP or the peptides in equivalent doses were used for comparison. Antitumor activity of the peptides was evaluated by the dynamics of the tumor growth node and the parameters of metastatic spreading of the tumor in lungs.
Results: It has been shown that administration of HLDF6 and HLDF8 peptides at different times after tumor transplantation did not have an effect on the primary tumor growth and did not enhance a cytostatic effect of CP. At the same time the injections of HLDF6 and HLDF8 at the early stage of the tumor development markedly decreased the number of lung metastases (by 40 and 48%, respectively, as compared to controls). Administration of HLDF6 in combination with CP caused a decrease in the parameters of metastatic spreading as compared to the animals injected with CP alone. When the peptide was administered prior to CP at the early stage of tumor process, a 20% decrease in the number of metastases was observed. Five-fold administration of HLDF6 at the late stage of the tumor development following the injection of CP decreased the number of lung metastases by 2.6 times and increased the animal life expectancy by six days.
Conclusions: HLDF6 and HLDF6 peptides did not have a marked effect on the Lewis lung carcinoma growth when they were used alone or in combination with cyclophosphamide. However, these peptides attenuated the process of metastatic spreading of the tumor and enhanced cytostatic effect.

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Different regulation of growth signal according to HER2 amplification status in primary breast cancer

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Background: HER2 amplification affects the cell proliferation through the modulation of multiple G1 cell cycle regulators in breast tumor cells.
Materials and Methods: We analyzed expression profiles of retinoblastoma protein (pRB) and p27Kip1 according to the HER2 amplification status on tissue microarray (TMA) from 153 breast cancers.
Results: HER2 amplification was observed in 39 (25.5%) of 153 breast cancers. The frequency of HER2 amplification was significantly increased in high grade tumors ($p = 0.023$). pRB was expressed in 54 (39.5%) of 114 tumors without HER2 amplification whereas it was expressed in 27 (69.2%) of 39 tumors with HER2 amplification. pRB expression was significantly associated with HER2 amplification. p27Kip1 expression was preserved in 75 (49%) of 153 tumors. p27Kip1 expression was preserved in 57 (50%) of 114 tumors without HER2 amplification whereas its expression was preserved in 18 (46.2%) of 39 HER2-amplified tumors. There was no significant relationship between HER2 amplification and p27 expression. We analyzed the change of proliferative index (PI) according to the HER2 amplification status. In 39 HER2-amplified tumors, PI was increased in 66.7% of pRB negative tumors. In contrast, PI was increased in 22.3% of 27 pRB expressing tumors. Degree and frequency of PI increase was significantly decreased in pRB expressing tumors ($p = 0.036$). The change of PI was also analyzed according to the p27Kip1 expression, but the association between PI and p27Kip1 was not observed in HER2 amplified tumors. In 114 HER2 non-amplified tumors, PI was increased in 21 tumors (36.8%) out of p27Kip1 repressed tumors. In contrast, PI was increased in 12 tumors (24.5%) out of 57 tumors with p27Kip1 preservation. The PI was significantly decreased in the tumors with p27kip1 expression ($p = 0.001$). The association between pRB expression and PI was not observed in these tumors.
The PI of the breast cancers was associated with pRB expression in HER2 amplified tumors whereas it was associated with p27Kip1 expression in HER2 non-amplified tumors. The results of the current study indicate that the cell proliferative activity of the breast cancer is under different regulation of growth signals according to HER2 amplification status.

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Influence of the long-term action of the flavonoid: curcumin or quercetin on the DNA damage induced by etoposide in the LT12 cell line

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Background: Plant flavonoids like curcumin or quercetin are famous for their numerous beneficial properties including antioxidant, anti-inflammatory and anticarcenogenic effects. Recently great hope has been lying in the introduction of flavonoids to chemotherapy as an adjuvant therapy. The aim of such a therapy is to minimize the side-effects of chemotherapy without decreasing the antitumor/antineoplastic effects of cytostatics. The aim of study was to ascertain the influence of curcumin or quercetin on the DNA damage induced by etoposide in the LT12 cell line derived from rat BNML leukemic model.
Materials and Methods: LT12 cells were treated for 24, 48 and 72 hours in the presence of curcumin or quercetin in the concentration range 0−20 μM. After those times (without discarding flavonoids), the cells were simultaneously treated with etoposide at a concentration of 1.5 μM for 1 hour. Then the amount of DNA damage was estimated by a single cell electrophoresis in agarose gel using Comet assay 2.6 software.
Results: When used in low doses (1−10 μM), neither curcumin nor quercetin caused DNA damage, even when they were present in the culture medium for 72 hours. High doses of quercetin (20 μM) caused a statistically significant DNA damage. After its 24-hour presence in the culture medium, curcumin induced DNA damage; that damage was very severe after 48 and 72 hours (above 95%; not measurable by the program). On the other hand, curcumin in the concentration range 1−10 μM and quercetin in the contrentation range 1−5 μM, present for 24 hours in the culture medium, protected against the DNA damage induced by etoposide. Such an effect did not appear during a longer action (48 or 72 h) of the flavonoids; on the contrary, an increase in the amount of DNA damage was then observed. After 48 and 72 hours and already at a concentration of 10 μM, curcumin increased the amount of DNA damage induced by etoposide, while quercetin only at a concentration of 20 μM enhanced the DNA-damaging action of etoposide.
Conclusions: The effect of the flavonoids tested is dose- and time-dependent. A shorter time (24 h) of their action may attenuate the effect of cytostatics by causing lesser damage to tumor cells. A longer action (48 and 72 h) of the flavonoids may enhance the effect of etoposide by causing a larger amount of DNA damage. Curcumin exerts a stronger effect than does quercetin via an increase in the amount of DNA damage induced by etoposide in rat leukemic LT12 cells.
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