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.