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Loss of malt1 expression enhances the progression of oral squamous cell carcinomas

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Background: A signaling pathway mediated through IkB kinases (IKKs) activates NFkB and plays a central role in apoptotic pathway of TNF-a-stimulated immune cells. Recent findings indicated that numbers of molecules connect the signaling sequence from cell surface receptor to NFkB, including BCL10 and MALT1, and phosphorylate IKKs. Although impediments of NFkB pathway disturb the epithelial development, the role and regulatory elements in normal and neoplastic epithelial cells remains unknown.
Materials and Methods: Total RNA isolated from normal gingiva and oral carcinomas under informed consent to patients were subjected to RT-PCR for MALT1 and BCL10. Genomic DNA of carcinoma cells and normal counterparts was isolated from paraffin-embedded oral carcinoma specimens (n = 36) by microdissection, and investigated microsatellite instability (MSI) of the MALT1 locus by STS-PCR. Tissue localization of MALT1 protein was analyzed on 85 carcinoma tissues. To examine biological roles of MALT1, we constructed fkbpΔMALT1, which exerts active MALT1 protein in the presence of AP20187, and generated a constitutively expressing oral carcinoma cell line (fkbpΔMALT1HSC2). fkbpΔMALT1HSC2 was applied for biological assays. HSC2 cells expressing full-length MALT1 or dominant-negative form MALT1 (ΔMALT1) were subctaneously transplanted into athymic mice.
Results: MALT1 and BCL10 mRNA were coordinately expressed in normal gingiva and keratinocytes, but frequently absent in carcinoma tissues and cell lines. MSI of the MALT1 locus was identified in 18 cases (50.0%), and closely associated with the decrease of immunoreactivities. Patients with little or no MALT staining showed lower disease-specific survival rate. Proliferation and invasion into basement membrane matrix of fkbpΔMALT1HSC2 cells were extensively increased in the absence of AP20187, while decreased when MALT1 signaling was activated by AP20187. Using a mouse model, we examined effects of MALT1 on *in vivo* tumor growth. Full-length MALT1-expressing HSC2 dramatically decreased tumor growth and BrdU-labeling index when compared to parental cells transfected vector alone. In contrast, ΔMALT1-expressing cells grew rapidly and increased BrdU labeling.
Conclusions: These data demonstrated that MALT1 is downregulated in carcinoma cells in parallel with genomic instability and the loss of expression associates with carcinoma progression, and suggest that MALT1 plays a role in suppression of oral carcinoma progression.

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Parathyroid Hormone-related Protein (PTHrP) expression in human bone and liver metastases

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Background: PTHrP has been implicated in the pathogenesis of bone metastases (BM), particularly in breast carcinoma. However, PTHrp expression in liver metastasis (LM) is unknown.
Methods: Bone pathologic fractures or BM biopsies from 64 patients, median age 63 (36–85), 36 female, were compared with LM from 28 patients, median age 57 (33–79), 18 female. In the BM patients 32 had breast carcinoma, 10-gastrointestinal, 6-lung, 6-prostate, and 10 patients had other types of cancer. In the LM patients 12 had breast carcinoma, 11-gastrointesinal, and 5 patients had other types of cancer. Formalin fixed, decalcified (BM), paraffin embedded sections of metastatic lesions were stained by an immunohistochemical (IHC) method using a mouse monoclonal antibody (Chugai Pharmaceutical Co., Ltd.) to human PTHrP. The expression of PTHrP in cancer cells was graded according to the percentage of stained cells (0; 1: <25%; 2: 25–75%; 3: >75%) and the intensity of staining (1, 2 or 3). A final IHC score (0–9) was obtained for each patient; Pathologic fractures (PF) were observed in 19/64 patients of the BM group after the diagnosis of BM. The Mann-Whitney test was employed to analyse the correlation among clinical factors and the IHC score.
Results: 56/64 (87.5%) of BM patients and 25/28 (89.2%) of LM patients showed PTHrP expression in the cancer cells. There was no statistically significant difference in PTHrP expression regarding the primary tumour site (breast versus non-breast) in the BM group (mean score: 4.8/3.9; p = 0.22) and in the LM group (mean score: 6.3/4.7; p = 0.12). There was a statistically significant difference in the PTHrp expression in patients who had PF (mean score = 6.3) compared to patients without PF (mean score = 3.4), p = 0.0007.
Conclusions: PTHrP expression in bone metastases and liver metastases was high and independent of the primary tumour origin. PTHrP expression was significantly higher in patients who had pathologic fractures. This study suggests that PTHrP may have a role in the pathogenesis of both BM and LM.

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HSV-TK gene therapy of human lung adenocarcinoma xenografts using a hypoxia/radiation dual-sensitive promoter

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Background: The aim of present study is to investigate whether the hypoxia responsive element(HRE) can be used to enhance the suicide gene(HSV-tk) expression and tumoricidal activity in radiation controlled gene therapy of Human Lung Adenocarcinoma Xenografts.
Materials and methods: A chimeric promoter, HRE-Egr, was generated by directly linking a 0.3 kb-fragment of HRE to a 0.6 kb-human Egr-1 promoter. Retroviral vectors containing luciferase or HSV-tk gene driven by Egr-1 or HRE-Egr were constructed. Human adenocarcinoma cell line (A549) was stably transfected by above vectors using the Lipofectamine method. The Sensitivity of transfected cells to prodrug ganciclovir (GCV) and cell survival rates were analyzed after exposure to the dose of 2 Gy radiation and/or hypoxia(1%). *In vivo*, tumor xenografts in BALB/c were transfected by the constructed retroviruses and irradiated to a total dose of 6 Gy, followed by GCV(20 mg/Kg *14 days) treatment.
Results: When the HSV-TK gene controlled by HRE-Egr promoter was introduced into A549 cells by a retroviral vector, the exposure to 1% O_2 and 2 Gy radiation induced significant enhancement of GCV cytotoxicity to the cells. Moreover, in nude mice bearing solid tumor xenografts, only the tumors infected with the hybrid promoter-containing virus gradually disappeared by GCV administration and radiation. Conclusion These results indicate that HRE can enhance transgene expression and tumoricidal activity in HSV-TK gene therapy controlled by ionizing radiation in hypoxic Human Lung Adenocarcinoma.

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TGF-beta-induced Stat3 phosphorylation mediated growth regulation in lung cancer

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Background: Transforming growth factor-beta (TGFβ) is a well-known potent growth inhibitor for many tumor cells of epithelial origin. Loss of response to TGFβ-mediated growth inhibition is frequently associated with malignant progression in a variety of human cancers. However, during anticancer chemotherapy, tumor cells often develop a coordinate resistant to that drug and cytotoxic cytokines. Recent study has revealed the cross-resistance of adriamycin and TGFβ in tumor cells. However, no distinct mechanisms have been proposed. Thus, this study tried to dissect the relationship between acquisition of drug resistant of tumor cells and subsequent acquisition of resistance to TGFβ.
Materials and methods: NCI-H23 parental (H23P) and NCI-H23 adriamycin resistant (H23ADR) lung adenocarcinoma cell lines were used in this study. The variations of biological functions induced by TGFβ in these two cell lines were studied by cell proliferation assays, flow cytometric analyses, transcription reporter assays, and Western blotting.
Results: We demonstrated that, after TGFβ1 treatment, H23ADR cells are much more refractory to TGFβ-mediated growth suppression than their parental (H23P) cells. The action is not either attributed to differential expression of TGFβ receptors, or involved in TGFβ1-induced cell cycle arrest and cell death between these two cell lines. By comparison studies on differential activations of TGFβ-induced signaling molecules, we first demonstrate that TGFβ1 can augment endogenous Stat3 activity in H23P cells with slow kinetics, but barely affects on H23ADR cells. Transfection of constitutively active Stat3 into H23ADR cells led to growth suppression in the absence of TGFβ1; additionally, expression of dominant-negative Stat3 significantly rescued H23P cells from TGFβ1-mediated growth suppression.
Conclusion: Our data suggest that modulation of Stat3 activity play a critical role in the TGFβ1-regulated cell growth during acquired adriamycin resistance, and provide the first evidence that Stat3 activity is involved in the cell growth suppression elicited by TGFβ1 in cancer cells.