Expression of BRAK/CXCL14 is associated with antitumor efficacy of gefitinib in head and neck squamous cell carcinoma

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Objectives: The clinical efficacy of gefitinib (ZD1839, Iressa), which is an inhibitor specific for the epidermal growth factor (EGF) receptor tyrosine kinase, has been demonstrated in non-small cell lung carcinoma patients with EGF receptor mutations, and so these mutations are a useful marker(s) to find responders to this drug. However recent studies showed that the EGF receptor gene mutation is rare in squamous cell carcinomas of the esophagus and head and neck regions. In the present study we investigated the relationship between BRAK expression and gefitinib efficacy for tumor suppression.

Methods: HNSCC lines were cultured Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum. Nearly confluent cells were cultured overnight in serum-free DMEM. After starvation, they were incubated with or without EGF (10 ng/ml) and/or gefitinib (1 mM). HSC-3 cells were subcutaneously injected into athymic nude mice. HSC-3-xenografted mice were daily administered gefitinib (50 mg/kg) orally.

Results: Gefitinib attenuated the effect of EGF, or even stimulated BRAK mRNA expression of HNSCC cell lines in vitro. Oral administration of gefitinib reduced the size of the tumors formed by HSC-3 cells in the nude mice concomitantly increased BRAK mRNA expression in vivo.

Conclusions: Our results indicate that oral administration of gefitinib reduced tumor size, at least in part, through elevation of BRAK expression. Thus, the use of gefitinib for treatment of patients with HNSCC in whom there is an inducing effect of the drug on the BRAK expression of their cancer cells may be advantageous. Furthermore, BRAK may be a promising molecule for gene therapy of HNSCC.

Functional analysis of promoter region of human BRAK/CXCL14, a tumor progression suppressor

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Objectives: CXCL14/BRAK, a non-ELR motif chemokine, is highly expressed in all normal cells, but is not expressed or expressed at a negligible level in most of head and neck squamous cell carcinomas examined. Earlier we reported that the BRAK expression level is inversely related to tumor size (Ozawa et al., Biochem. Biophys. Res. Commun. 348: 406-412, 2006). However, the mechanisms by which the gene is regulated are still unclear. Thus, to elucidate the mechanisms regulating BRAK gene expression, we determined the transcriptional start site and promoter motifs of the gene.

Methods: For determination of the transcriptional start-site, the 5' Rapid Amplification of cDNA End (5'-RACE) method was employed by use of a 5'-RACE CORE SET (TAKARA). For determination of the promoter region of the gene, we constructed vectors containing presumptive promoter regions for the BRAK gene connected to the luciferase reporter gene and introduced them into HSC-3 cells. Promoter activities were determined by use of the Dual-GloTM Luciferase Assay System (Promega). Cells were cultured in the presence or absence of okadaic acid (20 nM).

Results: The transcriptional start site was found to be in the previously reported exon 1 region (+284) of the gene. Determination of luciferase activities by use of deletion and/or mutation constructs clarified that a TATA-like sequence, TATTAA was essential for the transcription of the gene. Also an AP-1 binding sequence was necessary for stimulating the expression of the gene. Okadaic acid up regulated the expression level of BRAK. When HSC-3 cells were transfected with the control and mutated luciferase constructs and treated with okadaic acid, only the cells transfected with the mutated AP-1 binding sequence or deletion construct lost sensitivity to okadaic acid.

Conclusions: Our data indicate that the TATA-like sequence forms an essential part of the promoter of the BRAK/CXCL14 gene and that an AP-1 binding sequence is responsible for the stimulation of BRAK transcription by okadaic acid.