Johji Inazawa

1 .Topic in Research Achievements in the Year 2006

In order to identify novel disease-associated genes, we performed genomic and epigenomic analyses in hvariou types of cancers including oral ell carcinoma (OSCC) and soft tissue tumors as well as congenital genomic disorders. In the course of a program to screen 39 OSCC cell lines for genomic copy-number aberrations in a genome-wide manner using our in-house array-based comparative genomic hybridization (array-CGH), we identified some of candidate genes located in homozygous deletions or gene amplifications, and performed the detailed analysis of these cancer-related genes now (**unpublished data**). On the other hands, we identified a homozygous loss of *protocadherin 20* (*PCDH20*, 13q21.2) in the study of non–small-cell lung cancer (NSCLC) cell lines for genomic copy number aberrations using in-house array-CGH analysis (1/20, 5%). *PCDH20* mRNA was not expressed in the majority of NSCLC cell lines without a homozygous deletion of this gene (10/19, 52.6%). In primary NSCLC cases, the methylated *PCDH20* promoter was frequently observed (32/59, 54.2%), and seemed to be associated with a shorter overall survival (P = 0.0140 and 0.0211 in all and stage I tumors, respectively). In addition, restoration of *PCDH20* expression in NSCLC cells reduced cell numbers in colony formation and anchorage-independent assays. These results suggest that epigenetic silencing by hypermethylation of the CpG-rich promoter region of *PCDH20* leads to loss of PCDH20 function, which may be a factor in the carcinogenesis of NSCLC (***Cancer Res.***, 66, 4617-4626, 2006). Previously, we have reported frequent silencing of the expression of *low-density lipoprotein receptor-related protein 1B* (*LRP1B*) by genetic and epigenetic mechanisms in esophageal squamous cell carcinoma (***Cancer Res.***, 64: 3741-3747, 2004). We examined intragenic homozygous deletions, expression levels, and methylation status in the CpG island of *LRP1B* in OSCC, and demonstrated that frequent inactivation of *LRP1B* mainly occurs via epigenetic mechanisms in cell lines and primary cases of OSCC (***Cancer Sci.***, 97: 1070-1074, 2006). Moreover, we analyzed 14 OSCC cell lines as well as 108 primary OSCC tumors with regard to the frequency of mutations within hotspot regions and the changes in the genomic copy-number of *PIK3CA*. *PIK3CA* missense mutations in exons 9 and 20 were identified in 21.4% (3/14) of OSCC cell lines and 7.4% (8/108) of OSCC cases by genomic DNA sequencing. A significant correlation between somatic mutations of *PIK3CA* and disease stage was observed: the frequency of mutations was higher in stage IV (16.1%, 5/31) than in a subset of early stages (stage I-III) (3.9%, 3/77; P = 0.042). In contrast, the amplification of *PIK3CA* was observed at a similar frequency among all stages. AKT was highly phosphorylated in OSCC cell lines with *PIK3CA* mutations compared with those without mutations, despite the amplification. The results suggest that somatic mutations of the *PIK3CA* gene are likely to occur late in the development of OSCC, and play a crucial role through the PI3K-AKT signaling pathway in cancer progression (***Cancer Sci.***, 97: 1351-1358, 2006).

2 .Publications in the year 2006

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3 . Abstracts in the year 2006

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