Background

Extensive molecular genetic studies have shown that lung cancer cells have acquired multiple genetic and epigenetic changes as a result of increasing genomic instability. One of the important features of solid tumors is hypoxia, and the transcription factor hypoxia inducible factor-1 (HIF-1) plays an essential role in adaptive response to reduced oxygen levels through controlling gene expression levels. HIF-1 was also reported to regulate DNA repair system, affecting on genomic stability in hypoxia. These data support concept that defective of DNA repair pathways causes genomic instability within the tumor microenvironment.

Variant alleles of 2 HIF1A polymorphisms, C1772T (P582S) and G1790A (A588T) were previously reported to have a significantly higher transcriptional activity than wild-type in vitro. However, the association between HIF1A polymorphisms and susceptibility to cancers is still a controversy and their functions remain unclear. Based on these facts, the genotype frequency of HIF1A polymorphisms at exon 12 and the potential role of these polymorphisms in relation to the genomic instability in lung cancer were analyzed.

Materials and Methods

A total of 83 patients with lung cancer (42 adenocarcinomas: AD, 30 squamous cell carcinomas: SCC, 7 small cell lung carcinomas: SCLC, and 4 adenosquamous cell carcinomas: ADSCC), whose lung cancer tissues have been analyzed for 1p34 deletion mapping, loss of heterozygosity (LOH) of TP53 and RB1, p16 deletion and promoter methylation, and EGFR mutation and amplification, and whose non-cancerous DNA was available for this study were selected.

The specific primer set was used to amplify a 178-bp fragment in HIF1A exon 12 by PCR, and direct-sequencing using Big Dye Terminator Cycle Sequencing Kit™ and automated sequencing system ABI PRISM 310 Genetic Analyzer™ was carried out.

Luciferase reporter assays were performed with using a lung adenocarcinoma cell line (A549), which expresses wild-type p53. Transcriptional activity of vector (FLAG), wild-type (WT), P582S or A588T HIF-1α was analyzed in a co-transfection assay using reporter plasmid HRE-Luc without or with mutant-type of p53 (HA-p53 R248W). Luciferase activities were measured after incubation under normoxic or hypoxic conditions for 36 hours, and relative transcriptional activities were calculated as ratio to those of pRL-SV40 in each well.

StatView Version 5.0 (SAS Institute Inc., Cary, NC, USA) was used to perform statistical analyses, and Chi-square test, Fisher’s exact test, or student’s t-test was
employed to compare other variables.

Results

The frequencies of the HIF1A C1772T and G1790A genotypes in the lung cancer patients obtained in the present study were compared with those of controls reported in our previous study. No significant differences were found in genotype frequencies or haplotype frequencies between them. Also there was no significant difference among the histology. All genotype distributions were within the Hardy-Weinberg equilibrium. The HIF1A polymorphisms were not associated with the pathological stage of lung cancer or with the overall survival of the patients.

Genotype frequency of HIF1A polymorphisms in lung cancer patients were compared with the genetic/epigenetic aberrations found in their lung cancer tissues in our previous studies, i.e., LOH at TP53, RB1, or 1p34, deletion and methylation of p16, and deletion/mutation and amplification of EGFR. The frequency of HIF1A C/T genotype was significantly higher in patients with TP53 LOH in cancer tissues among overall lung cancer patients ($p = 0.015$) as well as among AD patients ($p = 0.047$). The frequency of HIF1A G/A genotype was significantly higher in patients with 1p34 LOH in AD tissues ($p = 0.009$). Thus, lung cancers with TP53 and/or 1p34 LOH were associated with the genotypes with enhanced transcriptional activity ($p = 0.089$), especially in AD ($p = 0.008$). No significant relationship was observed between p16/Rb aberrations or EGFR mutation/amplification and HIF1A genotypes.

Then, the transcriptional activity of the HIF-1 wild-type, P582S, and A588T were compared without or with mutant-type p53 (R248W) cotransfection in A459 lung cancer cell line that has wild-type p53. Under hypoxic condition, the P582S or A588T variant showed 1.4-1.5 fold times higher transactivation capacity than the wild-type ($p < 0.005$). Such transcriptional activities were dramatically enhanced in cells with mutant-type p53, and both HIF-1α variants showed significantly enhanced transactivation capacity compared with the wild-type HIF-1α in hypoxic condition ($p < 0.005$ and $p < 0.01$, respectively).

Discussion

HIF-1 has been shown as one of the key factors in repressing DNA repair system, resulting in increasing genomic instability. Genetic alterations in TP53, RB1, p16, and 1p34 region are frequently observed in lung cancer. Although we did not carried out a multivariate analysis due to strong linkage among TP53, RB1, p16, and 1p34 alterations, the present study found that patients with HIF1A 1772T variant allele had significantly
more frequently acquired $TP53$ LOH in their lung cancer tissues ($p = 0.015$). In patients with adenocarcinoma, $TP53$ and 1p34 LOH were significantly more frequently observed in individuals with the $1772T$ or $1790A$ variant allele, respectively, and in combination of them. Inactivation of p53 is well known to be associated with genomic instability, and loss of 1p34 was reported to be significantly associated with aneuploidy, i.e., chromosome instability. Moreover, loss of 1p34 was found in some hemangioblastomas without $VHL$ allelic loss, indicating the possible existence of a tumor suppressor gene in this region related to hypoxia. These data indicate that functional activation of HIF-1α due to these polymorphisms may have induced mutations in some tumor suppressor genes involved in lung cancer development, possibly through its repressive function on DNA repair systems.

Both HIF-1α variants demonstrated higher transcriptional activities than the wild-type under hypoxia in vitro, regardless of the p53 status. Since HIF-1α showed dramatically enhanced transcriptional activities with p53 inactivation, combination of $HIF1A$ variation with p53 complete inactivation, i.e., $TP53$ LOH representing complete inactivation possibly with precedent mutation in the remaining allele, may have enhanced the HIF-1α activity maximally in hypoxic condition in vivo.

These findings suggest that the HIF-1α polymorphisms may have an important impact on lung carcinogenesis, especially in adenocarcinoma, possibly through increasing genomic instability.