Abstract. Background & Materials and Methods: Recently, it has been proved that PGP9.5 is an oncogene candidate for squamous cell carcinomas. To examine the PGP9.5-related oncogenic pathway, we tested for global patterns of gene expression in cancer cells following PGP9.5 gene introduction using an oligonucleotide microarray approach. Results: Plasminogen activator inhibitor-1 (PAI-1) was identified as an overexpressed gene in a PGP9.5-expressed esophageal squamous cancer cell line. To confirm the data obtained, we performed Northern analysis using a PGP9.5 or a PAI-1 cDNA probe and found that PAI-1 mRNA was induced by PGP9.5 expression in NUEC1 cells. We further examined endogenous PGP9.5 and PAI-1 expression in 6 esophageal cancer cell lines. One cell line (NUEC2) with PGP9.5 expression exhibited PAI-1 expression, suggesting the possibility that PGP9.5 might induce PAI-1 directly or indirectly. Conclusion: These results suggested that PAI-1 might be a novel downstream mediator of PGP9.5 in esophageal squamous cell carcinomas.

Recent advances in molecular biology have revealed various genetic changes in dominant oncogenes, such as cyclin D1 and AIS, together with the inactivation of tumor suppressor genes, such as p53 and p16, to be involved in the carcinogenesis of esophageal cancer (1-3). It is still important to search for novel genetic changes to estimate the malignancy of esophageal cancer.

PGP9.5 is a ubiquitin hydrolase, widely expressed in neuronal tissue at all stages of neuronal differentiation, and has been used as a neuroendocrine marker (4,5). The ubiquitin-proteasome pathway that degrades cytosolic and nuclear proteins via an ATP- and ubiquitin-dependent mechanism involves cell-cycle regulation genes (6-8). Using a serial analysis of gene expression method (SAGE), it was proved that the PGP9.5 transcript is highly expressed in lung cancer (9). Subsequent study showed that PGP9.5 expression was independent of neuroendocrine differentiation and closely associated with advanced stages of non-small cell lung cancer (NSCLC) (10). Moreover, PGP9.5 expression was frequently observed in squamous cell carcinoma of lung cancer and significantly associated with the pathological stages of cancer, suggesting that its expression in NSCLC makes it a potential marker for the malignancy.

These results prompted us to examine the PGP9.5 expression in esophageal cancer, which is one of the most common squamous cell cancers. To better characterize its role in this cancer, we examined PGP9.5 expression retrospectively in resected esophageal cancers using immunohistochemistry. Of 40 esophageal cancer specimens, 19 (48%) exhibited positive staining with PGP9.5 in most tumor cells (11). This result supported the idea that PGP9.5 might be a potential marker for squamous cell carcinoma. However, it remains unclear how PGP9.5 would advance the tumorigenic pathway in these cancers.

In an effort to gain further insight into this pathway, we examined global patterns of gene expression in cancer cells following PGP9.5 gene introduction using the oligonucleotide microarray approach. Here, we identified the plasminogen activator inhibitor-1 (PAI-1) as a novel downstream mediator of PGP9.5.

Abbreviations: PAI-1, plasminogen activator inhibitor-1.

Correspondence to: Kenji Hibi, Gastroenterological Surgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8560, Japan. Tel: +81-52-744-2245, Fax: +81-52-744-2255, e-mail: khibi@med.nagoya-u.ac.jp

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Materials and Methods

PGP9.5 adenovirus. A full-length PGP9.5 EST clone was purchased from Genome Systems (Huntsville, AL, USA) and PGP9.5 adenovirus was constructed as previously described (12). Briefly, PGP9.5 cDNA was subcloned into the shuttle vector, pAdTrack-CMV. The resultant plasmid was linearized by digesting with the restriction endonuclease PmeI, and subsequently cotransformed into Escherichia coli BJ5183 cells with an adenoviral backbone plasmid, pAdEasy-1. Recombinants were selected for kanamycin resistance, and recombination was confirmed by restriction digest analysis. The linearized recombinant plasmid was then transfected into the adenovirus packaging cell line, HEK-293.

Oligonucleotide microarray. A human esophageal squamous cancer cell line, NUEC1, was infected by 50 multiplicities of infection (MOI) of a PGP9.5 adenovirus or an empty adenovirus (control). NUEC1 cell line does not express PGP9.5, as shown later. Therefore, changes in gene expression could be evaluated using an oligonucleotide microarray. Affymetrix software was used to calculate the fold changes of gene expression induced by the infection of PGP9.5 adenovirus, by comparing the fluorescent intensity of each array with respect to the control. Seven genes were chosen as candidates for downstream mediator of PGP9.5, according to several criteria set in advance and validated by the following analyses.

Northern analysis. Four cell lines were established in our laboratory (NUEC1, 2, 3, 4). The other cell lines were purchased from the American Type Culture Collection (TE1, 2). Cultured cell lines were lysed in guanidine buffer and total RNA was isolated using the CsCl gradient method. Northern blot hybridization using the cDNA probe was performed as described previously (13). cDNA included the 3' part of the PGP9.5 or PAI-1 gene. The human β-actin gene was used as an internal control to standardize the relative amount of RNA in each lane.

Results

To evaluate the transcriptional effects of PGP9.5, we studied a human esophageal squamous cancer cell line (NUEC1) infected by a PGP9.5 adenovirus or an empty adenovirus (control). The NUEC1 cell line does not express PGP9.5, as shown later. Therefore, changes in gene expression could be evaluated using an oligonucleotide microarray. Affymetrix software was used to calculate the fold changes of gene expression induced by the infection of PGP9.5 adenovirus, by comparing the fluorescent intensity of each array with respect to the control. This analysis revealed that 3 genes were overexpressed and 4 genes were repressed in PGP9.5-
We expressed NUEC1 cells (Table 1). We performed semi-quantitative RT-PCR for these 7 genes in PGP9.5-expressed or non-expressed NUEC1 cells to examine the result from the oligonucleotide microarray. Only plasminogen activator inhibitor-1 (PAI-1) was overexpressed in PGP9.5-expressed NUEC1 cells (data not shown).

To further confirm the data obtained from the oligonucleotide microarray, we performed Northern analysis using a PGP9.5 or a PAI-1 cDNA probe (Figure 1). PAI-1 mRNA was induced by PGP9.5 expression in NUEC1 cells. To assess the endogenous expression pattern of PGP9.5 and PAI-1, we then examined the expression of these genes in esophageal cancer cell lines by Northern analysis. Out of 6 esophageal cancer cell lines, 1 (NUEC2) and 2 cell lines (NUEC2 and 4) exhibited the expression of PGP9.5 and PAI-1 genes, respectively (Figure 2). One cell line (NUEC2) with PGP9.5 expression exhibited PAI-1 expression, suggesting the possibility that PGP9.5 might induce PAI-1 directly or indirectly. This result also implied that other factors would be involved in regulating PAI-1 expression, because 1 cell line (NUEC4) without PGP9.5 expression exhibited PAI-1 expression.

Discussion

Esophageal squamous cell carcinoma is one of the most aggressive cancers. Its management involves surgical operation followed by chemotherapy and radiotherapy. In addition, gene therapy has recently been developed to attack cancer cells directly based on their molecular defects (14). Therefore, it is important to investigate the concurrent genetic alterations to better characterize the tumorigenic pathway of this cancer.

To date, little is known about the role of PGP9.5 in cancer. PGP9.5 belongs to the ubiquitin carboxyl-terminal hydrolase (UCH) family. Current accumulating data suggest that these enzymes play an important role in the cellular proteolytic pathway that regulates many cellular processes, including cell cycle and death (4,8). UCHs are 25 kDa enzymes involved in the translational processing of pro-ubiquitin gene products as well as in the release of ubiquitin from tagged proteins, i.e., de-ubiquitination, which may decrease the degradation of oncogenes, possibly contributing to their overexpression in tumors. However, which gene is controlled by PGP9.5 remains unclear.

In this study, we examined global patterns of gene expression in cancer cells following PGP9.5 gene introduction, using the oligonucleotide microarray approach. Although our data implied that other factors would be involved in regulating PAI-1 expression, we identified PAI-1 as a novel downstream mediator of PGP9.5. PAI-1 is known to be a dual function protein, that inhibits the activation of plasminogen activators and interferes with cell binding to vitronectin (15). In the last decade, evidence of PAI-1 involvement in cancer growth and metastasis has steadily increased. Several studies of different human cancers have consistently shown that high levels of PAI-1 in tumors correlate with poor patient outcome, including our recent report (16-19). The reason for this phenomenon is presently unknown, but experiments in PAI-1-deficient mice have suggested that PAI-1 may be necessary for local invasion and angiogenesis (20). In this regard, PAI-1 would work as an oncoprotein in these cells and its expression may be partially controlled by PGP9.5.

This study provides solid evidence for further studies on the molecular mechanism of the PGP9.5-related oncogenic pathway in esophageal squamous cell carcinoma, and also confirms that PAI-1 may be a novel mediator of this pathway. Work in progress on the interaction of proteins derived from these genes will further elucidate a part of the genetic regulation of esophageal squamous cell carcinoma.

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References


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