Original Article

Down-Regulation of the Human PRL-3 Gene Is Associated with the Metastasis of Primary Non-Small Cell Lung Cancer

Shin-ichi Yamashita, MD,[1,2] Yoshiko Masuda, MD,[1] Katsutaka Matsumoto, MD,[1] Yasuhiro Okumura, MD,[1] Hosei Matsuzaki, MD,[1] Takashi Kurizaki, MD,[1] Yoshio Haga, MD,[1] Shigeru Katafuchi, MD,[1] Toshihiko Murayama, MD,[3] Satoshi Ikei, MD,[1] and Katsunobu Kawahara, MD[2]

Background: Phosphatase of the regenerating liver (PRL)-3 protein tyrosine phosphatase gene is expressed in colon cancer metastasis. To investigate the role of this gene in metastatic lung cancer, we compared PRL-3 gene expression between primary cancers, metastatic lung cancer, and normal lung tissue.

Materials and Methods: Five metastatic tumor and normal samples from non-small cell lung cancer patients were obtained at the National Hospital Organization Kumamoto Medical Center (Kumamoto, Japan). For a quantitative evaluation of RNA expression by PCR, we used Taqman PCR methods.

Results: Although PRL-3 gene expression levels in the primary lesions were slightly decreased compared with those in the normal tissues, those in the metastatic lesions were extremely down-regulated in the synchronous metastatic case. In 2 of these 3 cases, the metastatic tumors showed down-regulated PRL-3 gene expression at 10 times less than that of the normal tissue, and the other tumor showed a slightly weaker expression.

Conclusion: These data suggest that a down-regulation of the PRL-3 gene is important in lung cancer metastasis and provide a new hypothesis of lung cancer metastases. (Ann Thorac Cardiovasc Surg 2007; 13: 236–239)

Key words: phosphatase of the regenerating liver, metastasis, lung cancer

Introduction

Metastatic cancer cells use a pathway that includes cytoskeleton change, loss of adhesion, enhanced mobility, and degradation of the basement membrane.[1)]

Recently, it was reported that human phosphatase of the regenerating liver (PRL)-3 protein tyrosine phosphatase gene was expressed at high levels in cancer metastases, but at lower levels in nonmetastatic tumors and normal colorectal epithelium.[2)] PRL-3 is a 22-kDa tyrosine phosphatase that is expressed in adult human muscle and heart.[3)] In a previous report, an overexpression of this gene induced the enhanced growth of human embryonic fibroblasts. PRL-3 gene has an important role in cancer metastases and has been suggested as a powerful tool for cancer-targeting therapy.[2)] It is interesting to evaluate the expression of the PRL-3 gene in metastases of other types of cancer. We hypothesized that primary lung cancer metastasizes to other lung fields in the same fashion. In this study, we investigated the expression of metastatic lesions from non-small cell lung cancer and from normal lung tissue.

From Departments of [1]Surgery and [3]Pathology, National Hospital Organization Kumamoto Medical Center, Kumamoto, and [2]Department of Surgery II, Faculty of Medicine, Oita University, Yufu, Japan

Received August 28, 2006; accepted for publication November 6, 2006

Address reprint requests to Shin-ichi Yamashita, MD: Department of Surgery II, Faculty of Medicine, Oita University, 1–1 Idaigaoka, Yufu, Oita 879–5593, Japan.

Materials and Methods

Patients and samples

Five metastatic lung cancers from non-small cell lung cancers were obtained at the National Hospital Organization Kumamoto Medical Center (Kumamoto, Japan). The samples were examined histologically for the metastatic or second primary cancer. All were diagnosed as metastatic tumors according to Martini's criteria.[4] None of the 5 cases had received radiation therapy or chemotherapy before surgery. Adjacent normal lung tissue was also taken from all cases. The tissue specimens were frozen immediately and stocked at –80°C until RNA extraction.

Quantitative PCR analysis

For a quantitative evaluation of RNA expression by PCR, we used Taqman PCR methods, as previously reported.[5] The PRL-3 gene was amplified by a set of the following primers: reverse, ggggacttctcaggtcgtgt; forward, tggac ttcttcatgccccga.

The PRL-3 gene internal probe was ttgcggtgcgagtcg tggaagtaa.

The PCR amplification conditions were one cycle of 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. The measured value was calculated by comparative Ct methods,[5] and glyceraldehyde-3 phosphate dehydrogenase (GAPDH) gene amplification was used as a control.

Results

PRL-3 gene expression in human metastatic lung carcinoma from non-small cell lung carcinoma was evaluated by real-time PCR. Three out of the 5 patients had metachronous metastases to other lung fields, and 2 patients had synchronous metastases. The median follow-up period was 4.1 years (from 1.2 to 6.5 years); 2 patients died from carcinomatosis after a resection of metastases, but the other patients are still alive with no evidence of recurrence. The tumor specimens were diagnosed by the same pathologist, and metastasis was confirmed according to histological grade and subtype.

The metastatic tumors of cases 1 and 2 were resected 1 week after the first primary lung cancer resection. Because the metastatic tumors in the other cases were obtained by operations for metastases after 1, 3, and 5 years, i.e., metachronous metastases, the primary tumors could not be obtained as fresh samples.

Because the purpose of this study was to compare the expression level of the PRL-3 gene between normal lung tissue or primary tumors and metastatic tumors, we set the expression level of the PRL-3 gene in normal tissue as control in all cases. Figure 1 shows the expression levels of this gene in each case. All cases were informative. Intriguingly, although the PRL-3 gene expression levels in the primary lesions were slightly decreased compared with those in the normal tissues, those in the metastatic lesions were extremely down-regulated in the synchronous metastatic cases (1 and 2). On the basis of these results, we evaluated the other 3 metastatic lesions. In 2 of these cases, the metastatic tumors showed down-regulated PRL-3 gene expression at 10 times less than that of the normal tissue, and the other tumor showed a slightly weaker expression.

Discussion

The molecular mechanism of tumor metastasis is still unclear. A recent study reported that PRL-3 is a candidate to be a metastasis-promoting gene, and PRL-3 is overexpressed in metastatic tumors derived from colon cancer.[2] From these results, we speculated that metastatic lung cancer from primary non-small cell lung cancer has the same potential of PRL-3 to invade and metastasize.

In this study, we examined only 7 tumors derived from 5 patients because patients who have metastatic tumors or recurrence in the lung are not candidates for resection, and it can be difficult to obtain tumor specimens. As mentioned earlier, we could not evaluate a small number of the tumors tested. However, the results were strikingly contrary, and the PRL-3 gene was down-regulated. These results lead us to speculate on a different mechanism in the metastasis of primary lung cancer into other lung fields.

Most endogenous PRL-3 is associated with the plasma membrane and endosomal structures in a prenylation-dependent manner.[6] In a previous report, the transfection of the PRL-3 gene into Chinese hamster ovary (CHO) cells promoted tumor migration, invasion in vitro, and metastasis in vivo.[7] PRL-3 phosphatase activity may be required for tumor cell migration and invasion. It has been suggested that overexpressed PRL-3 in metastatic liver tumors from colorectal cancers has the potential to metastasize. In other reports, metastatic tumor cells outside the liver, for example, lung, brain, and ovary tumor cells, from colorectal cancer expressed PRL-3.[8] However, no liver or lung metastatic tumor from esophageal, stomach,

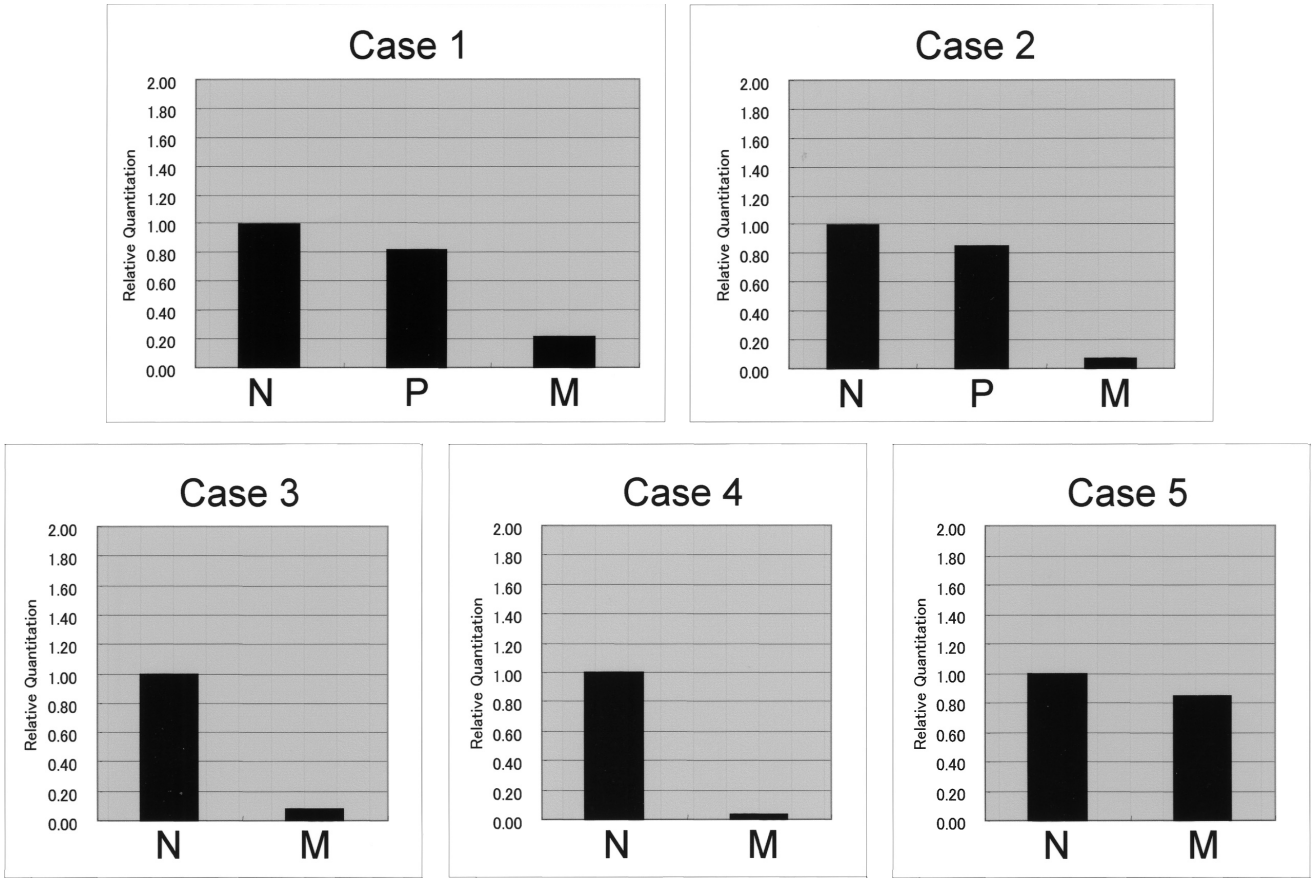

Fig. 1. Relative quantitation of PRL-3 gene.
Gene expression levels in primary lesions and metastatic lesions were compared with those in normal tissues (1.00). N, normal lung tissue; P, primary lesion; M, metastatic lesion.

or pancreatic cancers expressed the PRL-3 gene at detectable levels.[8] Nevertheless, our data showed that a PRL-3 gene expression of metastatic lung cancer from primary lung cancer was down-regulated. These results may demonstrate that the process of metastasis is associated with cell-specific gene expression and microenvironment-specific gene induction. PRL-3 is located at the plasma membrane, but not in the nucleus in normal intestinal epithelium.[8] On the other hand, because PRL-3 was down-regulated in the lung metastasis, this might be evidence of a tumor suppressor-like function. The location of PRL-3 in normal pulmonary epithelium is unclear, and this gene could function to inhibit tumor metastasis if it is in the nucleus.

PRL-3 shares some homology with PTEN/MMAC1, a lipid phosphatase, and it has been reported that PTEN is a tumor suppressor gene.[9] The loss of heterozygosity of PTEN was frequently detected in metastases derived from the lung, and inactivation mutations were found in squamous cell carcinoma of the lung.[10] Because the homologous sites between PRL-3 and PTEN are the phosphatase-active sites, it is suggested that both genes have the same function and act to suppress tumor progression.

As previously reported, phosphotyrosine levels are increased in melanoma,[11] which is apparently consistent with reports of elevated protein tyrosine kinase activity. Some protein tyrosine kinases are encoded by oncogenes and have been implicated in carcinogenesis. Decreased protein tyrosine phosphatase activity may also increase phosphotyrosine. Protein tyrosine phosphatase genes are candidate tumor suppressors, and loss of expression may contribute to carcinogenesis. The loss of their protein tyrosine phosphatase expression may contribute to the abnormal tyrosine phosphorylation seen in metastatic lung carcinoma; this gene is a candidate tumor suppressor.[12]

In conclusion, although the sample size is small and

our result may be a preliminary study, the data suggest that PRL-3 gene inactivation may play a key role in the progression of tumors. Further experiments will be required to determine the role of PRL-3 gene expression in large numbers of patients with lung cancer metastasis and other types of cancer.

Acknowledgment

This study was supported by a grant for National Hospital Clinical Research from the Ministry of Health, Labor and Welfare of Japan.

References

1. Weiss L. Metastasis of cancer: a conceptual history from antiquity to the 1990s. *Cancer Metastasis Rev* 2000; **19**: 193–383.
2. Saha S, Bardelli A, Buckhaults P, et al. A phosphatase associated with metastasis of colorectal cancer. *Science* 2001; **294**: 1343–6.
3. Matter WF, Estridge T, Zhang C, et al. Role of PRL-3, a human muscle-specific tyrosine phosphatase, in angiotensin-II signaling. *Biochem Biophys Res Commun* 2001; **283**: 1061–8.
4. Martini N, Melamed MR. Multiple primary lung cancers. *J Thorac Cardiovasc Surg* 1975; **70**: 606–12.
5. Aarskog NK, Vedeler CA. Real-time quantitative polymerase chain reaction. A new method that detects both the peripheral myelin protein 22 duplication in Charcot-Marie-Tooth type 1A disease and the peripheral myelin protein 22 deletion in hereditary neuropathy with liability to pressure palsies. *Hum Genet* 2000; **107**: 494–8.
6. Zeng Q, Si X, Horstmann H, et al. Prenylation-dependent association of protein-tyrosine phosphatases PRL-1, -2, and -3 with the plasma membrane and the early endosome. *J Biol Chem* 2000; **275**: 21444–52.
7. Zeng Q, Dong, JM, Guo K, et al. PRL-3 and PRL-1 promote cell migration, inva and metastasis. *Cancer Res* 2003; 63: 2716–22.
8. Bardelli A, Saha S, Sager JA, et al. PRL-3 expression in metastatic cancers. *Clin Cancer Res* 2003; **9**: 5607–15.
9. Diamond RH, Cressman DE, Laz TM, et al. PRL-1, a unique nuclear protein tyrosine phosphatase, affects cell growth. *Mol Cell Biol* 1994; **14**: 3752–62.
10. Hahn M, Wieland I, Koufaki ON, et al. Genetic alterations of the tumor suppressor gene PTEN/MMAC1 in human brain metastases. *Clin Cancer Res* 1999; **5**: 2431–7.
11. McArdle L, Rafferty M, Maelandsmo GM, et al. Protein tyrosine phosphatase genes downregulated in melanoma. *J Invest Dermatol* 2001; **117**: 1255–60.
12. Simpson L, Parsons R. PTEN: life as a tumor suppressor. *Exp Cell Res* 2001; **264**: 29–41.