IA-2, a Transmembrane Protein Tyrosine Phosphatase, Is Expressed in Human Lung Cancer Cell Lines with Neuroendocrine Phenotype

Hong Xie, Abner L. Notkins, and Michael S. Lan
Laboratory of Oral Medicine, National Institute of Dental Research, NIH, Bethesda, Maryland 20892-4322

Abstract
IA-2 is a transmembrane protein tyrosine phosphatase isolated recently from a human insulinoma subtraction library. Its expression in normal human tissues is restricted primarily to the pancreatic islets and brain. In this report, we describe the expression of IA-2 mRNA in a panel consisting of 20 lung tumor cell lines with neuroendocrine and non-neuroendocrine phenotype and 17 non-lung tumor cell lines. IA-2 mRNA was detected in 8 of 11 neuroendocrine small cell lung carcinomas, 4 of 4 non-small cell lung carcinomas with neuroendocrine phenotype, and 11 of 12 non-lung neuroendocrine tumor cell lines. In contrast, IA-2 mRNA was not detected in five non-neuroendocrine lung carcinomas, nor in a panel of other non-neuroendocrine tumor cell lines. The expression pattern of IA-2 mRNA suggests that IA-2 may represent a new marker for neuroendocrine differentiation in human lung cancer cells and perhaps other neuroendocrine tumors.

Introduction
Recently, we isolated two novel cDNAs, IA-1 and IA-2, from a human insulinoma subtraction library (1, 2). IA-1 cDNA encodes a protein with a prohormone domain and five zinc-finger DNA binding motifs (1). Clinical studies on a panel of 64 human lung cancer cell lines demonstrated that IA-1 mRNA was detected in 97% (30/31) of SCLC cell lines and 13% (4/30) of NSCLC cell lines with neuroendocrine phenotype (3). IA-2 cDNA encodes a 979-amino acid protein consisting of an extracellular domain, a transmembrane region, and a single intracellular PTP domain (2). PTPs are enzymes that catalyze the dephosphorylation of phosphorylated tyrosine residues and play an important role in signal transduction, which in turn governs cell growth, differentiation, and transformation (4). Clinical studies showed that up to 70% of sera from newly onset IDDM patients had autoantibodies to IA-2, and that IA-2 is a major autoantigen in IDDM (5, 6). Pancreatic islets of Langerhans are endocrine cells that secrete hormones that regulate glucose metabolism. Although islet cells are derived from the endoderm layer, it is known that islet cells share certain biochemical properties with neuronal cells and express markers such as presence of dense core granules by electron microscopy, tyrosine hydroxylase, gastrin-releasing peptide, IA-1, and synaptophysin (3, 16). SCLCs, in contrast to NSCLCs, display increased sensitivity to chemotherapy and radiotherapy. Identification of new neuroendocrine markers may not only help distinguish between neuroendocrine and non-neuroendocrine lung tumors, but also provide clues as to the mechanism involved in responsiveness to chemotherapy. In this report, we show that IA-2 is a neuroendocrine marker that differentiates neuroendocrine from non-neuroendocrine human lung cancers.

Materials and Methods
Cell Lines. Cell lines obtained from the American Type Culture Collection (Rockville, MD) included the following: (human cell lines) SCLC (NCI-69, NCI-82, NCI-128, NCI-146, NCI-187, NCI-209, NCI-345, NCI-446, NCI-510, NCI-889, and NCI-1688), NSCLC (NCI-292, NCI-441, NCI-460, NCI-520, NCI-596, NCI-727, NCI-810, NCI-1155, and SK-MES), retinoblastoma (Y-79 and WERI-Rb1), medulloblastoma (D341Med and D283Med), and glioblastoma (U-118-MG); (mouse cell lines) embryonal carcinoma (F-9), adrenal cortex tumor (Y-1), and pituitary tumor (AtT-20); (rat cell lines) pituitary tumor (GH-3), pheochromocytoma (PC-12), medullary thyroid carcinoma (6–23), testicular tumor (LC-540), and insulinoma (RIN); and (hamster cell line) insulinoma (HIT). Mouse α-TC-1 and β-TC-1 cell lines were provided kindly by Dr. E. H. Leiter (Jackson Laboratory, Bar Harbor, ME). Tumor cell lines were cultured in modified Eagle’s medium supplemented with 10% FCS or according to supplier’s instructions.

Northern Analysis. Total RNA was isolated from cell lines by the acid guanidinium thiocyanate/phenol/chloroform extraction method (17). RNA (20 μg) was fractionated by 1% agarose/formaldehyde gel electrophoresis and then transferred to Nytran via capillary blotting. Hybridization was performed at 52.5°C overnight with 40% formamide, 5X saline sodium phosphate EDTA (10 mm phosphate-buffered saline, 1 mm EDTA; pH 7.4) saline-sodium phosphate-EDTA, 10 μg/ml sheared salmon sperm DNA, 6X Denhardt’s solution, and 106 cpm/ml of labeled probe. Full-length IA-2 cDNA probe (2) was labeled by random priming with [32P]dCTP (Amersham). Expression of IA-1 gene in human lung tumor cell lines was determined previously (3).

Results and Discussion
A panel of 20 human lung cancer cell lines was examined for the expression of IA-2 message. Fig. 1A and B, shows that IA-2 mRNA (3.8 kb) was expressed in 8 of 11 (73%) neuroendocrine SCLC cell lines and 4 of 4 (100%) NSCLC cell lines with neuroendocrine phenotype. In contrast, IA-2 mRNA was not detected in five non-neuroendocrine NSCLC cell lines. Comparing the expression pattern of IA-2 with that of IA-1, a previously identified neuroendocrine marker in human lung cancer (3), we find that IA-2 is comparable to IA-1 as a neuroendocrine marker for human lung cancer (Table 1).
Because IA-2 message is highly conserved in different species (18-20), we further examined the expression of IA-2 message in other tumor cell lines derived from human, mouse, rat, and hamster. Fig. 1C shows that IA-2 message was expressed strongly in two retinoblastomas, a thyroid medullary carcinoma, two pituitary tumors, three insulinomas, a glucagonoma, and a pheochromocytoma. IA-2 message was expressed weakly in one of the two medulloblastomas (D283Med) examined. It was not detected in adrenal cortex carcinoma (Y-1), embryonal carcinoma (F-9), glioblastoma (U-118-MG), and testicular carcinoma (LC-540). Taken together with our earlier work (2), it is concluded that the expression of IA-2 is associated closely with tumors of neuroendocrine origin.

IA-2 was isolated originally from a human insulinoma subtraction library and found to be expressed primarily in islets and brain (2). Subsequent studies showed that it is a major autoantigen in IDDM (5, 6). In this connection, it is interesting to note that other neuroendocrine markers such as glutamic acid decarboxylase(65 and aromatic L-amino acid decarboxylase are neuroendocrine markers as well as islet cell autoantigens is consistent with previous reports that pancreatic islet cells have neuron-like properties, express markers found in the nervous system (23), and in vitro can display neurite-like processes (24).

The neuroendocrine-specific tissue distribution and the fact that IA-2 is a transmembrane PTP make IA-2 a unique marker for neuroendocrine differentiation in human lung cancer. Although the precise biological function of IA-2 is still unclear, it is very likely that it plays a role in the signal transduction of neuroendocrine cells. Expression of IA-2 message was found not only in 8 of 11 SCLCs, but also in 4 of 4 NSCLCs with neuroendocrine phenotype. Because many neuroendocrine lung tumors respond to chemotherapy, it is of considerable clinical interest to determine whether the expression of IA-2 correlates with response to chemotherapy. If the expression of IA-2 can predict the sensitivity to chemotherapy, then further determination of its biological function may reveal some clues as to the mechanism of chemotherapeutic responsiveness in these neuroendocrine tumors.

Acknowledgments

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References

2. Lan, M. S., Russell, E. K., Lu, J., Johnson, B. E., and Notkins, A. L. IA-1 and IA-2 gene expression were examined by Northern analysis. a Number positive/number tested.
3. Cell lines that express chromogranin A and/or l-dopa decarboxylase. NE, neuroendocrine.

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