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Corin-Mediated Processing of Pro-Atrial Natriuretic Peptide in Human Small Cell Lung Cancer Cells

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ABSTRACT

Corin is a recently discovered pro-atrial natriuretic peptide (ANP) convertase that is abundantly expressed in the heart. ANP is a cardiac hormone but can be secreted ectopically by certain cancers including small cell lung cancer (SCLC). In this study, we examined the role of corin in ANP production by SCLC cells. Reverse transcription-PCR detected corin mRNA expression in all nine SCLC cell lines examined and ANP mRNA expression in seven of the nine cell lines. In contrast, arginine vasopressin mRNA was detected in only five of the nine SCLC cell lines studied. Corin-expressing SCLC cells were capable of converting recombinant human pro-ANP to biologically active ANP, as determined by Western analysis and a cyclic GMP assay. Transfection of small interfering RNA duplexes directed against the *corin* gene completely blocked the processing of pro-ANP in the SCLC cells. Our results show that corin functions as a pro-ANP convertase in SCLC cells. We also suggest that the expression of corin may contribute to the pathogenesis of the syndrome of inappropriate secretion of antidiuretic hormone associated with certain cancers.

INTRODUCTION

ANP[1] is a hormone important in maintaining body fluid and sodium homeostasis (1, 2). The peptide is synthesized primarily in atrial cardiomyocytes. Under pathological situations, such as congestive heart failure, ANP expression is highly up-regulated in the cardiomyocytes of the ventricle, which could then become a major source of ANP production (3). In addition to normal tissues, ANP expression has been reported in a variety of cancer tissues. For example, ANP antigen or mRNA was detected in cardiac rhabdomyomas (4), hepatic carcinomas (5), and SCLCs (6, 7). Studies have suggested that the overexpression of ANP may contribute to the development of hyponatremia in patients with SCLC (8, 9).

In cardiomyocytes, human ANP is synthesized as a 151-amino acid prepropeptide. After the signal peptide is removed by the signal peptidase, pro-ANP is stored in the dense granules of the cell (10, 11). On stimulation, pro-ANP is released from the dense granules and activated on the cell surface by proteolytic cleavage at residue arginine 98, generating an NH_2-terminal propeptide and a 26-amino acid COOH-terminal peptide that is biologically active (12, 13). Recently, we identified a cardiac serine protease, corin (14), which is a member of the type II transmembrane serine protease family (15–17). We and others have shown that corin mRNA and protein are highly expressed in cardiomyocytes (14, 18) and that recombinant corin converts pro-ANP to biologically active ANP in a highly sequence-specific manner (19). In cultured cardiomyocytes, overexpression of an active site mutant corin or transfection of siRNA duplexes against the *corin* gene prevented the processing of pro-ANP, indicating that corin is the pro-ANP convertase in the heart (20).

In addition to the heart, corin mRNA expression was detected in other tissues such as developing kidneys and bones and pregnant uterus (14). Corin mRNA was also detected in cancer cells derived from osteosarcoma, leiomyosarcoma, and endometrial carcinoma (14). In this study, we examined corin and ANP expression in SCLC cells and the role of corin in pro-ANP processing in these cells. Our results showed that both corin and ANP mRNAs were expressed in the majority of SCLC cell lines. Corin-expressing SCLC cells were capable of converting pro-ANP to biologically active ANP. Moreover, transfection of siRNA duplexes directed against the human *corin* gene blocked the processing of pro-ANP in these cells. These data indicate that corin plays a role in ANP production in cancer cells and may contribute to hyponatremia commonly observed in patients with SCLC.

MATERIALS AND METHODS

Materials. Cell culture media, L-glutamine, FBS, BSA, and HEPES were from Invitrogen (Rockville, MD). Endothelial basal medium, smooth muscle growth mediators (SMGM2), and human cardiomyocytes were from Cambrex Bioscience, Inc. (Walkersville, MD). HEK 293 cells, BHK cells, and human SCLC cell lines [NCI-H69, NCI-H82, NCI-H345, NCI-H510A, NCI-H1284, NCI-H1341, NCI-H1618, NCI-H1688, and DMS 114 (6, 7, 21–28)] were from the American Type Culture Collection. Rabbit antihuman ANP polyclonal antibody was from Bachem Laboratories (San Carlos, CA).

Cell Culture. 293 cells were cultured in α-MEM supplemented with 10% FBS and 1% L-glutamine. Human cardiomyocytes were cultured in endothelial basal medium supplemented with SMGM2 and 10% FBS. SCLC cells were cultured under the conditions recommended by American Type Culture Collection. All cells were cultured at 37°C in humidified cell culture incubators with 5% CO_2 and 95% air.

RT-PCR. mRNA samples were isolated from cultured cells using the Micro-Fast Track 2.0 mRNA isolation kit (Invitrogen, Grand Island, NY) according to the manufacturer's instructions. RT-PCR reactions were performed using the Titanium One-Step RT-PCR Advantage kit (Clontech, Palo Alto, CA). The reactions for reverse transcription were performed at 50°C for 60 min. PCR reactions for human corin mRNA were performed using oligonucleotide primers [sense, 5′-CAT-GGA-CTC-CTG-CCC-TGT-AGG-3′; antisense, 5′-GGG-CGT-CGC-TAC-GAG-ACA-3′ (14)] with 30 cycles of amplification (30-s annealing at 65°C, 1-min extension at 68°C, and 30-s denaturation at 94°C). PCR reactions for human pro-ANP mRNA were performed using oligonucleotide primers [sense, 5′-AGA-GAC-AGA-GCA-GCA-AGT-G-3′; antisense, 5′-CAT-GGC-CTT-CGA-CAA-TGT-CGG-G-3′ (29)], with 30 cycles of amplification (30-s annealing at 56°C, 1-min extension at 68°C, and 30-min denaturation at 94°C). PCR reactions for human arginine vasopression mRNA were performed using oligonucleotide primers [sense, 5′-AAT-AGG-CAG-CCA-GCA-GAG-GCA-3′; antisense, 5′-TCT-CGT-CGT-TGC-AGC-AAA-CG-3′ (30)], with 30 cycles of amplification (30-s annealing at 55°C, 1-min extension at 68°C, and 30-s denaturation at 94°C).

Transfection and Western Analysis. To study pro-ANP processing in SCLC cells, transfection experiments were performed using plasmids encoding human pro-ANP (pcDNAproANP) and corin (pcDNACorin), as described previously (19). Recombinant pro-ANP and corin encoded by these vectors contain a viral V5 tag at their COOH termini, which facilitates the detection of the proteins. HEK 293 cells or SCLC cells were transiently transfected with the expression vectors using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA). To examine pro-ANP processing by SCLC cells, conditioned medium containing

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[1] The abbreviations used are: ANP, atrial natriuretic peptide; cGMP, cyclic GMP; FBS, fetal bovine serum; RT-PCR, reverse transcription-PCR; SIADH, syndrome of inappropriate secretion of antidiuretic hormone; siRNA, small interfering RNA; SCLC, small cell lung cancer; HEK, human embryonic kidney; BHK, baby hamster kidney.

recombinant pro-ANP from transfected 293 cells was incubated with NCI-H1284, NCI-H1688, and NCI-H1341 cells at 37°C for 4 h. Pro-ANP and its derivatives in the conditioned medium were immunoprecipitated by an anti-V5 antibody (Invitrogen). Proteins were separated by SDS-PAGE and analyzed by Western blotting using a peroxidase-conjugated anti-V5 antibody.

cGMP Assay. To examine the activity of recombinant ANP, a cGMP assay was performed using an enzyme immunoassay kit (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom), as described previously (20). In these experiments, synthetic human ANP (Peninsula Laboratories, San Carlos, CA) was used as a standard. Each experimental condition was assayed in triplicate.

Effects of RNA Interference. Oligonucleotide siRNAs, 5′-AATTTGCT-TCTCACCTCAGCA-3′, were designed based on sequences specific for human corin cDNA (14). Sense and antisense siRNAs with dTdT 3′-overhangs were synthesized by Dharmacon Research, Inc. (Lafayette, CO). A control scrambled siRNA, 5′-AACTTACGTACGCTACTCTCT-3′, was also synthesized. The sequence of the scrambled siRNA does not match any registered human genes as indicated by searching genomic databases. Human SCLC NCI-H1688 cells were selected for the RNA interference experiments because these cells are adherent and fast-growing in culture and easy to transfect. The cells were grown in 24-well culture plates and transfected with the siRNA duplexes against the human *corin* gene or the control scrambled siRNA using Oligofectamine 2000. Twenty-four h after the transfection, cells were washed twice with serum-free medium and incubated with the conditioned medium containing recombinant pro-ANP at 37°C for 4 h. Pro-ANP and its derivatives in the conditioned medium were analyzed by immunoprecipitation and Western blotting using an anti-V5 antibody.

RESULTS

Detection of Corin, Pro-ANP, and Arginine Vasopressin mRNA in SCLC Cell Lines. To examine the expression of corin and pro-ANP mRNA in human SCLC cells, we screened a panel of SCLC cells and selected eight cell lines (NCI-H69, NCI-H82, NCI-H345, NCI-H510A, NCI-H1341, NCI-H1618, NCI-H1688, and DMS 114) that are adherent and grow at a reasonable rate in culture. We also included a nonadherent cell line, NCI-H1284, which has been characterized extensively for the expression of ANP and arginine vasopressin (6, 25–27). By RT-PCR, human corin mRNA, represented by a 417-bp PCR product, was detected in all nine SCLC cell lines (Fig. 1). The intensity of the PCR product band was, however, relatively weak in two cell lines, NCI-H1618 and NCI-H1341. Pro-ANP mRNA, represented by a 447-bp PCR product, was detected in seven cell lines (NCI-H510A, NCI-H82, NCI-H345, NCI-H1284, NCI-H1618, NCI-H1341, and NCI-H1688) but not in the other two cell lines (NCI-H69 and DMS 114; Fig. 1). We also examined the expression of arginine vasopressin that has been reported in SCLC cells (6, 7). Arginine vasopressin mRNA, represented by a 493-bp PCR product, was identified in five cell lines (NCI-H510A, NCI-H69, NCI-H82, NCI-H345, and NCI-H1618) but not in the other four cell lines (NCI-H1284, NCI-H1341, NCI-H1688, and DMS 114; Fig. 1). In controls, no PCR products were found if mRNA samples were omitted in the RT-PCR reactions containing oligonucleotide primers for corin, pro-ANP, or arginine vasopressin. In addition, the PCR products from corin and pro-ANP, but not arginine vasopressin, gene transcripts were amplified in samples from cultured human cardiomyocytes (Fig. 1). The results indicate that corin and pro-ANP mRNAs are expressed in most SCLC cells examined in this study.

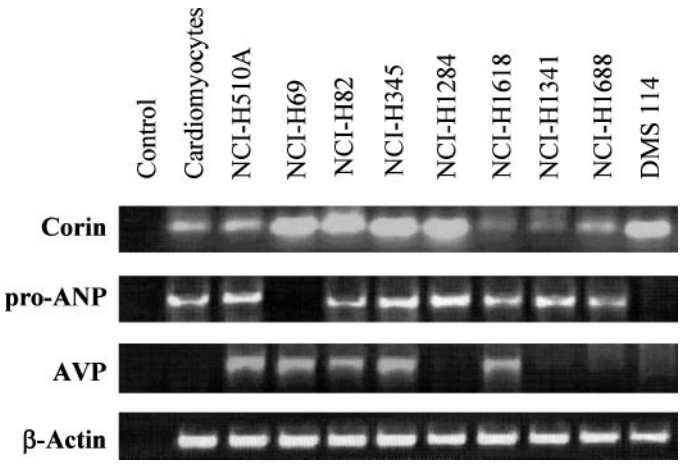

Fig. 1. Analysis of human corin, pro-ANP, and arginine vasopressin mRNA expression in SCLC cells by RT-PCR. RNA samples were isolated from human primary cardiomyocytes and SCLC cell lines. RT-PCR experiments were performed using oligonucleotide primers derived from human corin, pro-ANP, and arginine vasopressin cDNA sequences. PCR products for corin (417 bp), pro-ANP (447 bp), and arginine vasopressin (*AVP*; 493 bp) were separated on agarose gels and visualized by ethidium bromide staining. As a positive control, PCR products for β-actin (500 bp) were detected in samples from cardiomyocytes and all SCLC cell lines (*bottom panel*). As a negative control, no PCR products were detected when RT-PCR reactions were performed in the absence of mRNA samples (control lane in all panels).

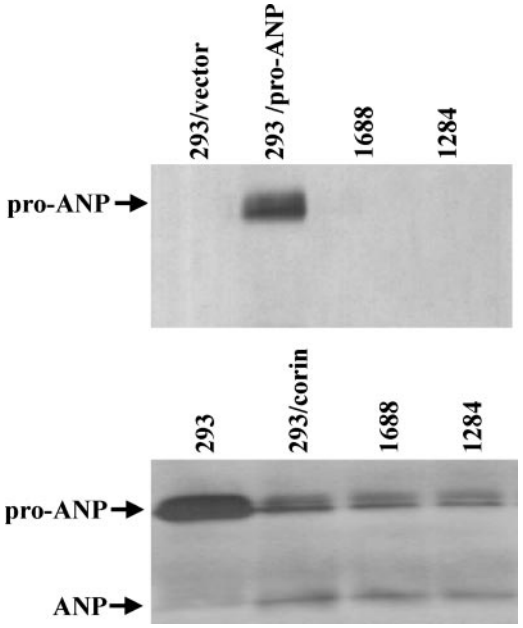

Fig. 2. Processing of recombinant pro-ANP in SCLC and transfected 293 cells. Human pro-ANP was expressed in 293 cells using plasmid pcDNAproANP (*293/proANP*), as shown by Western analysis of the conditioned medium using an anti-V5 antibody (*top panel*). As controls, no specific bands were detected in the conditioned media from 293 cells transfected with a control vector (*293/vector*) or from untreated NCI-H1284 (*1284*) and NCI-H1688 (*1688*) cells (*top panel*). The conditioned medium containing recombinant pro-ANP was then incubated with SCLC cell lines NCI-H1284 and NCI-H1688, parental 293 cells (*293*) that did not express corin, or a stable 293 cell line expressing human corin (*293/corin*). Recombinant pro-ANP and its derivatives in the conditioned medium were analyzed by Western blotting (*bottom panel*). At high resolution, two bands of pro-ANP were detected on the Western blot, possibly caused by differences in glycosylation.

Processing of Pro-ANP by SCLC Cells. Because antihuman corin antibodies are not available, we were unable to directly determine corin protein expression in the SCLC cell lines. Instead, we examined the activity of corin in these cells in a pro-ANP processing assay. NCI-H1688, an adherent and fast-growing cell line, and NCI-H1284, a nonadherent but well-characterized cell line, were selected for these studies. Human recombinant pro-ANP was first expressed in 293 cells by transfection of an expression vector, pcDNAproANP. The conditioned medium containing recombinant pro-ANP was collected and analyzed by Western blotting. As controls, conditioned media from 293 cells transfected with a control vector and from untreated NCI-H1284 and NCI-H1688 cells were also included. As shown in the *top panel* of Fig. 2, recombinant pro-ANP was detected only in the

conditioned medium from 293 cells transfected with the pro-ANP-expressing vector. The conditioned medium containing recombinant pro-ANP was then incubated with NCI-H1284, NCI-H1688, parental 293 cells (which do not express corin), or a 293 cell line that stably expresses human corin. Processing of pro-ANP was analyzed by immunoprecipitation and Western blotting. As shown in the *bottom panel* of Fig. 2, conversion of recombinant pro-ANP to ANP was observed in 293 cells expressing recombinant human corin and in NCI-H1284 and NCI-H1688 cells, but not in control parental 293 cells. In a separate experiment, we also observed similar pro-ANP processing by another SCLC cell line, NCI-H1341 (data not shown). The results indicate that corin protein is present and functional on the surface of these SCLC cells.

The Activity of SCLC Cell-Processed ANP. The biological function of ANP is mediated by binding to its receptor and stimulating its guanylyl cyclase activity, leading to generation of intracellular cGMP (1, 2). To determine whether the SCLC cell-processed recombinant ANP is biologically active, the conditioned medium incubated with NCI-H1284 and NCI-H1688 cells was tested for cGMP-stimulating activity in a BHK cell-based assay. As shown in Fig. 3, cGMP-stimulating activities were significantly higher in the conditioned medium treated with NCI-H1284, NCI-H1688, and corin-expressing 293 cells as compared with that in control 293 cell-treated medium. The results are consistent with the observed pro-ANP processing in NCI-H1284 and NCI-H1688 cells and indicate that recombinant ANP processed by these SCLC cells was biologically active.

We also performed transfection experiments in adherent 293 and NCI-H1688 cells using plasmids expressing corin and pro-ANP and examined the cGMP-stimulating activity in the conditioned media. As shown in Fig. 4, low levels of cGMP-stimulating activity were detected in the conditioned medium from untransfected NCI-H1688 cells. The activity is most likely derived from endogenous ANP processed by endogenous corin because both corin and pro-ANP mRNAs were detected by RT-PCR in these cells (Fig. 1). Transfection of a corin-expressing plasmid did not significantly increase the cGMP-stimulating activity in the NCI-H1688 cell-derived medium (Fig. 4), suggesting that endogenous corin is not a rate-limiting factor in processing endogenous pro-ANP. In contrast, the cGMP-stimulating activity was significantly increased when NCI-H1688 cells were transfected with a pro-ANP-expressing plasmid alone or together with a corin-expressing plasmid (Fig. 4). In control 293 cells, little cGMP-stimulating activity was found unless the cells were transfected with both pro-ANP- and corin-expressing plasmids (Fig. 4). These results were consistent with the observation that corin is expressed in NCI-H1688 cells and is capable of processing pro-ANP to biologically active ANP (Figs. 2 and 3).

To further demonstrate that the observed cGMP-stimulating activity was mediated by ANP, we tested the effect of a polyclonal antihuman ANP antibody on the conditioned medium derived from NCI-H1688 cells transfected with plasmids expressing corin and pro-ANP. As shown in Fig. 5, the cGMP-stumulating activity was inhibited dose dependently when increasing concentrations of the antibody were added to the conditioned medium. These results indicate that the observed cGMP-stimulating activity is indeed mediated by human ANP.

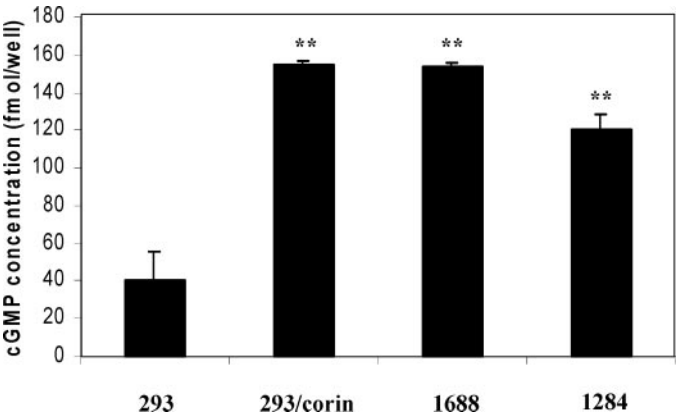

Fig. 3. cGMP-stimulating activity in SCLC cell-treated culture medium. BHK cells were cultured in 96-well plates. The conditioned medium containing recombinant pro-ANP was treated with parental 293 cells (*293*), corin-expressing 293 cells (*293/corin*), NCI-H1688 cells (*1688*), or NCI-H1284 cells (*1284*) and then added to each well and incubated at 37°C for 10 min. The cells were lysed in a lysis buffer, and the intracellular concentrations of cGMP were measured with the Biotrak kit, as described under "Materials and Methods." Each experimental condition was assayed in triplicate. The data are presented as mean ± SD from three independent experiments. **, $P < 0.01$ *versus* samples from parental 293 cells by Student's *t* test.

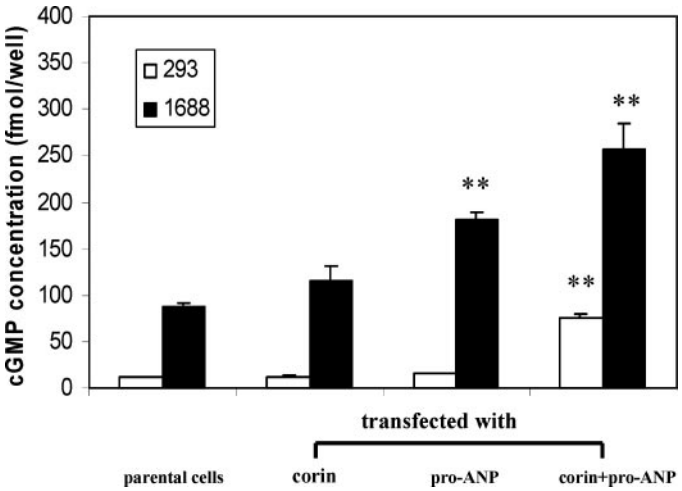

Fig. 4. cGMP-stimulating activity in the conditioned media from transfected cells. NCI-H1688 (■) and 293 (□) cells were transfected with plasmids expressing human corin and pro-ANP, either individually or together. The conditioned media were collected and tested for the cGMP-stimulating activity in a BHK cell-based assay, as described under "Materials and Methods." Each experimental condition was assayed in triplicate. The data are presented as mean ± SD from three independent experiments. **, $P < 0.01$ *versus* samples from respective parental cells by Student's *t* test.

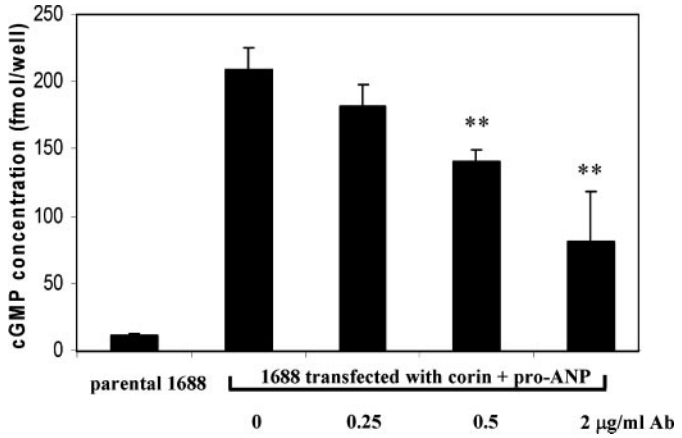

Fig. 5. Effects of an anti-ANP antibody on the cGMP-stimulating activity. NCI-H1688 cells (*1688*) were transfected with plasmids expressing human corin and pro-ANP. The conditioned medium was collected after 24 h and incubated with increasing concentrations of an antihuman ANP antibody at 25°C for 30 min. As a negative control, the conditioned medium from parental NCI-H1688 cells was included. The conditioned media were tested for the cGMP-stimulating activity in a BHK cell-based assay. The data are presented as mean ± SD from three independent experiments. **, $P < 0.01$ *versus* samples from transfected NCI-H1688 cells without antibody treatment by Student's *t* test.

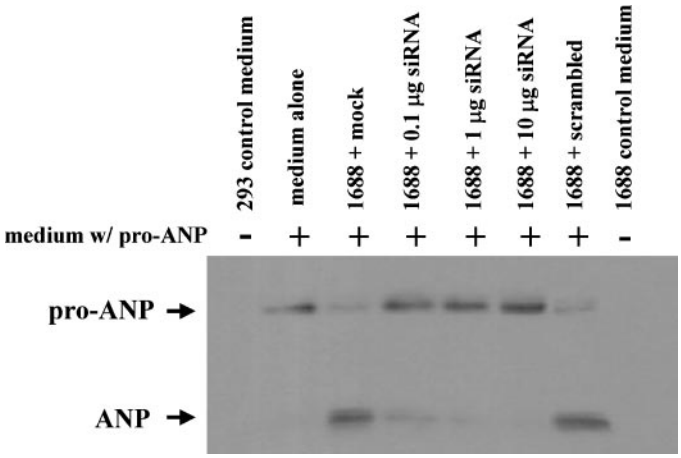

Fig. 6. Effects of siRNA duplexes against the human *corin* gene. Recombinant pro-ANP was expressed in 293 cells. The conditioned medium containing recombinant pro-ANP was collected and incubated at 37°C for 4 h with NCI-H1688 cells transfected with increasing concentrations (0.1, 1, and 10 μg) of siRNA duplexes against the human *corin* gene. As controls, mock transfected NCI-H1688 cells (*1688 + mock*) and NCI-H1688 cells transfected with a scrambled siRNA (10 μg; *1688 + scrambled*) were included in the experiments. In addition, the conditioned media from parental 293 and NCI-H1688 cells were also included. The processing of pro-ANP in the conditioned medium was analyzed by Western analysis using an anti-V5 antibody.

Effect of siRNA on NCI-H1688 Cell-Mediated Pro-ANP Processing. To confirm that the processing of pro-ANP observed in NCI-H1688 cells was mediated by corin, we tested the effect of siRNA duplexes against the human *corin* gene in NCI-H1688 cells. In our previous studies, the siRNA-based gene silencing technique (31) was used to block corin-mediated pro-ANP processing in cultured cardiomyocytes (20). Recombinant pro-ANP was first expressed in transfected 293 cells and then incubated with either mock-transfected NCI-H1688 cells or NCI-H1688 cells transfected with a scrambled siRNA or increasing concentrations of siRNA directed against the human *corin* gene. Processing of pro-ANP was analyzed by immunoprecipitation and Western blotting. As shown in Fig. 6, the processing of pro-ANP was not affected in mock-transfected or scrambled siRNA-transfected NCI-H1688 cells but was inhibited in the cells transfected with increasing concentrations of siRNA against the human *corin* gene. In other controls, no recombinant pro-ANP or ANP was detected in the conditioned media derived from parental 293 or untreated NCI-H1688 cells. These results support the conclusion that the processing of pro-ANP in NCI-H1688 cells is mediated by corin.

DISCUSSION

In this study, we examined the expression of corin, pro-ANP, and arginine vasopressin mRNA in a panel of human SCLC cells. Corin and pro-ANP mRNAs were detected in most of the cell lines tested: corin mRNA was detected in all nine cell lines examined; and pro-ANP mRNA was detected in seven of the cell lines. In contrast, arginine vasopressin mRNA was detected in only five cell lines. Although we did not directly measure corin and pro-ANP proteins, functional studies indicated that both proteins were present and functional in these cells. We showed that the SCLC cells are capable of processing recombinant human pro-ANP, indicating the presence of functional endogenous corin. We demonstrated that the SCLC cell-processed ANP is biologically active in a cGMP assay. In addition, we showed that transfection of siRNA directed against the human *corin* gene prevents the pro-ANP processing in the cancer cells. Together, these results provide direct evidence that corin is expressed in SCLC cells and capable of processing pro-ANP to functional ANP.

We and others have shown that corin mRNA and protein are abundant in the heart (14, 18). In contrast, corin expression was not detected in the lung by Northern, *in situ* hybridization, and RT-PCR analyses (14, 20). Like corin, ANP is normally expressed in the heart but not detectable in the lung. It is possible that corin and ANP are expressed in normal lungs by a neuroendocrine cell population that is too small to be detected by conventional methods using whole lung preparations. These neuroendocrine cells could later become the precursor cell type for SCLC. Therefore, corin and ANP expression might be consistent with the phylogeny of SCLC. Alternatively, the *corin* and *ANP* genes are turned on ectopically in SCLC cells by an unknown mechanism. In a recent study, we have characterized the promoters of the human and mouse *corin* genes that contain conserved binding elements for TBX5, GATA, NKX2.5, and Krüppel-like transcription factors (32). These conserved binding elements are also present in the promoters of the *ANP* gene from various species (33, 34). We showed that mutations in a conserved GATA element in the corin promoter impaired GATA-4 binding and its promoter activity in cultured cardiomyocytes, indicating that GATA-4 plays an important role in the cardiac expression of the *corin* gene (32). Previous studies have also demonstrated that GATA-4 is critical for the cardiac expression of the *ANP* gene (33, 35). It would be interesting to determine whether GATA-4 is also involved in the expression of *corin* and *ANP* genes in SCLC cells.

Our findings of corin expression and processing of biologically active ANP in SCLC cells provide a new insight into molecular mechanisms for the hyponatremia that frequently occurs in patients with SCLC (6, 36, 37). For many years, overproduction of arginine vasopressin by cancer cells has been considered to be the primary cause of cancer-associated hyponatremia, and therefore, the disease is also called SIADH (38). High levels of plasma arginine vasopression have indeed been found in SCLC patients with hyponatremia (39). Ectopic expression of arginine vasopressin has been confirmed in cultured SCLC cells (6, 39). The mode of action of arginine vasopressin to promote water retention can explain the observed dilutional hyponatremia. In many cases, however, high concentrations of plasma arginine vasopressin are not detected in SCLC patients who exhibit hyponatremia, suggesting that other mechanisms may be responsible for SIADH (7, 27, 40). Consistent with these observations, we found that arginine vasopressin mRNA was detected in only about half of the SCLC cell lines examined in this study. More importantly, in NCI-H1284 cells, which were derived from a patient with documented hyponatremia (6), arginine vasopressin mRNA expression was not detected in our RT-PCR analysis (Fig. 1), confirming a previous finding by Gross *et al.* (6).

High levels of ANP have been found in plasma samples (7, 8, 41, 42) and tumor cells (6, 7, 26, 27) derived from SCLC patients who exhibit SIADH, indicating that secretion of natriuretic peptides by cancer cells is another mechanism for the hyponatremic phenotype. Because ANP is synthesized as a prepropeptide, proteolytic cleavage is required to activate the precursor. Our recent studies have shown that corin is the pro-ANP convertase in cardiomyocytes (19, 20). It was not known, however, if the processing of pro-ANP in SCLC cells is also mediated by corin. In an early study, Johnson *et al.* (26) detected a pro-ANP processing activity in NCI-H1284 cells, but the identity of the proteolytic enzyme remained unknown. In the study presented here, we showed that corin mRNA was present in all SCLC cell lines examined, including NCI-H1284, and that corin-expressing cancer cells were capable of converting pro-ANP into biologically active ANP. The siRNA-based studies demonstrate that corin is critical for the processing of pro-ANP in NCI-H1688 cells. Because NCI-H1284 cells are nonadherent and difficult to transfect as compared with adherent NCI-H1688 cells, we were unable to perform the siRNA-based experiments in these cells. Taken together, however, our

data strongly support that corin is responsible for the processing of pro-ANP in SCLC cells, which may contribute to the pathogenesis of cancer-associated SIADH. At this time, our data are derived mainly from cultured SCLC cell lines. It will be important to further confirm corin expression in primary SCLC tumors.

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