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Molecular cloning of the human kidney differentiation antigen gp160: Human aminopeptidase A

(cDNA cloning/renal cancer/ectopeptidases/angiotensins)

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ABSTRACT **gp160 is a cell surface differentiation-related glycoprotein of 160 kDa expressed by epithelial cells of the glomerulus and proximal tubule cells of the human nephron but only by a subset of renal cell carcinomas (RCCs). We have reported that gp160 expression correlates with the resistance of cultured RCCs to the antiproliferative effects of α interferon, while lack of expression correlates with sensitivity to α interferon. In this study, we have purified gp160 protein, obtained partial sequences of random peptides, and isolated a full-length cDNA. The gp160 cDNA possesses 78% homology to the murine BP-1/6C3 antigen, a B-lymphocyte differentiation protein that exhibits aminopeptidase A (APA; EC 3.4.11.7) activity. Enzymatic assays on human RCC cell lines indicated a 100% concordance between APA activity and gp160 expression. APA activity of gp160-expressing RCC cells was increased or decreased by a panel of APA activators or inhibitors, respectively. Furthermore, anti-gp160 monoclonal antibodies immunoprecipitate APA activity from RCC cell lysates and selectively deplete APA activity from RCC cell extracts. These data indicate that the gp160 human kidney/RCC glycoprotein is human APA.**

The gp160 antigen is a differentiation-related kidney glycoprotein of 160 kDa that is expressed on the surface of epithelial cells of the glomerulus and proximal tubule cells of the human nephron (1). However, despite the fact that virtually all renal cell carcinomas (RCCs) derive from proximal tubule cells (2, 3), ≈20% of RCCs lack gp160 expression (2, 4, 5). This observation indicates an altered regulation or other defect of this glycoprotein upon malignant transformation of the proximal tubule cell. We have also shown that expression of gp160 by cultured RCCs correlated with the resistance of RCC cells to the antiproliferative effects of α interferon (α-IFN), while lack of expression correlated with sensitivity to α-IFN (6). One implication of these data is that gp160 may modulate the activity of α-IFN and impair its efficacy as an antitumor agent in the treatment of advanced RCC. Therefore, to explore the functional role of gp160 in malignant and normal renal cells, we have purified gp160, obtained partial sequences of random peptides, and isolated a cDNA encoding gp160.†† Sequence and functional analyses indicate that gp160 is human aminopeptidase A [APA; L-α-aspartyl (L-α-glutamyl)-peptide hydrolase, EC 3.4.11.7].

MATERIALS AND METHODS

Protein Purification and Amino Acid Sequencing. Fifty grams of human kidney cortex was lysed in 50 mM Tris, pH 7.4/1% Nonidet P-40 containing protease inhibitors (aprotinin at 10 μg/ml, pepstatin at 1 μg/ml, and leupeptin at 1 μg/ml), centrifuged at 10,000 × *g*, and incubated with wheat germ agglutinin (Pharmacia LKB). Bound glycoprotein was eluted with 10% *N*-acetyl-D-glucosamine (Sigma) (7) and applied to an Affi-Prep hydrazide column (Bio-Rad) coupled with monoclonal antibody (mAb) S4. The column was eluted with 200 mM glycine, pH 2.5/150 mM NaCl/0.1% Nonidet P-40, and a portion of each fraction was separated on an SDS/7.5% PAGE gel and analyzed by silver staining. Fractions containing a 160-kDa protein species were pooled, concentrated by a Bio-Gel resin (Bio-Rad), separated by SDS/PAGE, electroeluted onto nitrocellulose membranes (Schleicher & Schuell) (8), and digested *in situ* with trypsin as described (9). Proteolyzed fragments were separated by HPLC and subjected to liquid-phase amino acid sequencing (10).

Screening of a cDNA Library and DNA Sequencing. The amino acid sequences of gp160 peptides were used to construct two 20-bp oligonucleotides, which were used as sense and antisense primers for PCR amplification of first-strand cDNA (11) derived from the SK-RC-28 cell line. PCR amplification was performed as described (12), using either *Taq* polymerase (Perkin–Elmer/Cetus) or *Pfu* polymerase (Stratagene). PCR-amplified DNA fragments were purified and sequenced with a double-stranded DNA cycle sequencing kit (GIBCO/BRL) as described (13, 14). By using this sequence data, two additional 20-bp oligonucleotides were synthesized and used in conjunction with λ phage specific oligonucleotide primers to amplify a gp160 cDNA from a λgt11 cDNA library constructed from normal human kidney (Clontech) (15, 16).

Northern Blot Analysis. Total RNA was isolated from cultured RCC cells, and 20 μg was electrophoresed in 1% agarose/formaldehyde gels, transferred to nitrocellulose membranes, and hybridized with PCR-amplified gp160-specific DNA fragments end-labeled with ^{32}P (Stratagene) (17).

Cells and Antibodies. Normal kidney and RCC cell lines used have been described (5). Mouse mAbs used included S4 (IgG2a), M1 (IgG1), and F33 (IgG1), each of which recognizes a different epitope on gp160 (6, 18); S27 (IgG1), which recognizes the adenosine deaminase binding protein (gp120) (19); and T43 (IgG1), which recognizes a glycoprotein of 85 kDa (3).

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Abbreviations: APA, aminopeptidase A; APB, aminopeptidase B; APN, aminopeptidase N; α-IFN, α-interferon; RCC, renal cell carcinoma; mAb, monoclonal antibody.

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††The sequence reported in this paper has been deposited in the GenBank data base (accession no. L14721).

Immunoprecipitation and Immunodepletion. Immunoprecipitation studies were performed as described (20). In immunodepletion experiments, Sepharose-protein A beads (Repligen, Cambridge, MA) were incubated at room temperature for 1 hr with mAb, washed with phosphate-buffered saline, and incubated for 2 hr with RCC cell lysates suspended in 1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate/150 mM NaCl/50 mM Tris, pH 7.4, containing aprotinin at 10 μg/ml. After centrifugation to remove the beads, aliquots of supernatant were tested for residual enzymatic activity. The beads were washed with lysis buffer and tested for enzymatic activity.

Enzyme Assays. Enzymatic activities were measured spectrophotometrically at 405 nm with aminoacyl *p*-nitroanilide derivatives as substrates (21, 22). Hydrolysis of α-L-glutamyl *p*-nitroanilide (Bachem) was assayed with 1.5 mM substrate in 0.1 M Tris (pH 7.0) at 37°C. Other aminoacyl *p*-nitroanilides were assayed in 50 mM phosphate buffer (pH 7.0). Enzyme inhibitors included EDTA, 1,10-phenanthroline, 2,2′-dipyridyl, amastatin, bestatin, angiotensin II, angiotensin III, and [βAsp^1]angiotensin II. $CaCl_2$, $BaCl_2$, and $SrCl_2$ (Sigma) were used as enzyme activators.

RESULTS

Purification and Amino Acid Sequencing of gp160. gp160 was isolated from normal human kidney, which expresses high levels of this protein (1, 2), by first enriching for glycoproteins with lectin chromatography followed by immunoaffinity purification using anti-gp160 mAb S4. A comparison of the amino acid sequences of three gp160 tryptic peptides to those in the translated GenBank data base (23) revealed incomplete homology to the BP-1/6C3 differentiation antigen (24), a murine early B-lymphocyte cell surface glycoprotein.

Isolation of gp160 cDNA. cDNA prepared from gp160-expressing SK-RC-28 cells was amplified by PCR using primers derived from the sequence of the gp160-specific tryptic peptides, yielding a 400-bp DNA fragment. Additional oligonucleotides based on the sequence of this fragment were used to amplify gp160-encoding cDNAs from a human kidney cDNA library. Nucleotide sequencing of the resulting gp160-specific cDNAs showed overlap of the original 400-bp DNA fragment and included the remainder of the gp160 coding sequence. To confirm that the nucleotide sequence did not contain errors as a result of polymerase infidelity, both the sense and antisense strands of at least four independent PCR clones were sequenced (Fig. 1).

The gp160 Antigen Contains a Zinc-Binding Domain and a Tyrosine-Phosphorylation Motif. The complete cDNA nucleotide sequence of gp160 revealed a single open reading frame of 957 aa starting with an initiator methionine codon at nt 84 and ending with a termination codon at nt 2955 (Fig. 1). The existence of two in-frame stop codons 5′ to the first methionine at nt 84 confirmed this as the initiating codon. All the residues of the three gp160 peptides determined by protein sequencing were included in the reading frame, confirming that the cDNA sequence represented gp160 (Fig. 2). The gp160 cDNA predicts a protein with a polypeptide core of 109 kDa, which is consistent with an observed molecular mass in SDS/PAGE of 110–120 kDa for the gp160 protein following neuraminidase treatment and removal of all N-linked oligosaccharides (C.L.F., unpublished data). There are 13 potential N-linked glycosylation sites in the gp160 sequence, as well as two motifs near the N terminus that have a putative consensus sequence believed to be required for the attachment of glycosaminoglycan chains (Fig. 2) (25). In addition, there is a potential tyrosine phosphorylation site at Tyr-115 (26) and a zinc-binding region at aa 390–400, identifying gp160 as a member of the zinc-dependent metallopeptidase family (27).

A comparison of the complete amino acid sequence of gp160 to those in the translated GenBank data base (version 72) (23) revealed 33% amino acid homology to human aminopeptidase N (APN; EC 3.4.11.2) (28, 29) and 78% amino acid homology to the murine B-lymphocyte differentiation glycoprotein BP-1/6C3 (Fig. 2) (24). Hydrophobicity analysis (30) indicated that the 5′ ends of gp160 and BP-1/6C3 are similar. Both proteins lack an amino-terminal signal peptide but contain a 22-aa hydrophobic segment suggestive of a membrane-spanning region (24, 30). The first 15 aa form a typical polypeptide domain found on the cytoplasmic side of transmembrane proteins. Within this domain, Ser-9 and Tyr-12 are potential sites for cytoplasmic phosphorylation. The human gp160 also contains two potential sites for the addition of glycosaminoglycans, at residues 46–49 and 181–184, which are not found in the BP-1/6C3 amino acid sequence. The human and mouse genes are, however, highly homologous in the region surrounding a conserved zinc-binding domain. The combined structural data suggest that,

```
   1 tccaattcaa aaagaaagtc tctgacgtta gttagtttaa tttaacatct ttttatgtgt aacacttgac tttggaagca aaaATGAACT TTGCGGAGAG
 101 AGAGGGCTCT AAGAGATACT GCATTCAAAC GAAACATGTG GCCATTCTCT GTGCGGTGGT GGTGGGTGTA GGATTAATAG TGGGACTTGC CGTGGGCTTG
 201 ACCAGATCGT GTGACTCCAG CGGGGACGGC GGGCCGGGCA CTGCGCCAGC TCCTTCCCAC CTGCCTTCTT CCACGGCCAG CCCCTCAGGT CCTCCTGCCC
 301 AGGACCAGGA CATCTGCCCG GCCAGTGAGG ATGAGAGCGG ACAGTGGAAA AACTTTCGAC TGCCGGACTT CGTCAACCCA GTCCACTACG ACCTGCACGT
 401 GAAGCCCCTG TTGGAGGAGG ACACCTACAC GGGCACCGTG AGCATCTCCA TCAACCTGAG CGCTCCCACC CGGTACCTGT GGCTGCACCT CCGGGAGACC
 501 AGGATCACCC GGCTCCCGGA GCTGAAGAGG CCCTCTGGGG ACCAGGTGCA AGTCCGGAGG TGTTTCGAGT ACAAAAAGCA GGAGTACGTG GTGGTCGAGG
 601 CGGAGGAAGA GCTTACCCCC AGCAGTGGAG ATGGCCTGTA TCTCCTGACC ATGGAGTTCG CCGGCTGGCT GAACGGCTCC CTCGTGGGAT TTTATAGAAC
 701 CACCTACACG GAGAACGGAC GAGTCAAGAG CATAGCGGCC ACCGATCATG AACCAACAGA TGCCAGGAAA TCTTTTCCTT GTTTTGATGA GCCCAACAAA
 801 AAGGCAACTT ATACAATATC TATCACCCAT CCCAAAGAAT ACGGAGCACT TTCAAATATG CCAGTGGCGA AAGAAGAGTC AGTGGATGAT AAATGGACTC
 901 GAACAACTTT TGAGAAGTCT GTCCCCATGA GCACGTACCT GGTGTGCTTT GCTGTACATC AATTTGACTC TGTAAAGAGA ATATCAAATA GTGGAAAACC
1001 TCTTACAATT TATGTCCAGC CAGAGCAAAA GCACACAGCC GAATATGCTG CAAACATAAC TAAAAGTGTG TTTGATTATT TTGAAGAATA CTTTGCTATG
1101 AATTATTCTC TTCCTAAATT AGATAAAATC GCTATTCCAG ATTTTGGCAC TGGTGCCATG GAGAACTGGG GACTCATCAC GTACAGAGAA ACGAACCTGC
1201 TTTATGACCC TAAGGAATCA GCCTCATCAA ACCAACAGAG GGTGGCCACT GTGGTTGCCC ATGAACTTGT GCATCAGTGG TTTGGAAATA TTGTGACCAT
1301 GGACTGGTGG GAAGACTTGT GGCTAAATGA AGGATTTGCT TCTTTCTTTG AGTTTCTGGG AGTAAACCAT GCAGAAACAG ACTGGCAAAT GCGTGACCAA
1401 ATGTTACTTG AAGATGTATT ACCTGTTCAA GAGGATGATT CTTTGATGTC TTCGCATCCA ATTATTGTGA CTGTGACAAC CCCTGATGAA ATAACATCTG
1501 TTTTTGATGG AATATCCTAT AGCAAGGGAT CTTCTATTTT GAGAATGCTT GAAGACTGGA TAAAACCAGA GAATTTTCAA AAAGGATGTC AGATGTACTT
1601 GGAAAAATAC CAATTCAAGA ATGCAAAAAC TTCTGATTTT TGGGCAGCAC TGGAAGAGGC AAGTAGGCTA CCAGTGAAAG AAGTAATGGA CACCTGGACC
1701 AGACAGATGG GTTATCCTGT GCTTAACGTG AACGGTGTCA AGAACATCAC ACAGAAACGC TTTTTGTTGG ACCCAAGAGC TAACCCTTCT CAGCCCCCTT
1801 CAGATCTTGG TTATACATGG AATATCCCAG TTAAATGGAC TGAAGATAAT ATAACAAGCA GTGTGTTATT TAATAGGTCA GAAAAAGAAG GAATCACTTT
1901 GAACTCCTCT AATCCTAGTG GAAATGCTTT TCTCAAAATA AACCCAGATC ATATTGGGTT TTATCGTGTA AATTATGAAG TAGCAACTTG GGACTCGATA
2001 GCTACAGCGC TCTCCTTGAA CCACAAGACA TTTTCTTCAG CAGATCGTGC AAGTCTTATT GATGATGCTT TTGCCTTGGC AAGAGCTCAA CTTCTAGATT
2101 ATAAGGTGGC TTTGAACTTG ACCAAGTATC TCAAAAGGGA AGAGAATTTT TTACCATGGC AGAGAGTAAT TTCAGCTGTA ACCTACATCA TTAGCATGTT
2201 TGAAGATGAT AAAGAGCTAT ATCCTATGAT TGAGGAATAC TTCCAAGGTC AAGTGAAGCC TATTGCAGAT TCTCTGGGAT GGAATGATGC TGGAGACCAT
2301 GTCACAAAGT TACTCCGTTC CTCCGTGTTA GGGTTTGCGT GCAAGATGGG AGACAGAGAA GCCTTGAACA ATGCTTCCTC GTTATTTGAG CAGTGGCTAA
2401 ATGGGACTGT AAGCCTTCCC GTAAATCTCA GGCTTCTGGT GTATCGGTAT GGGATGCAGA ACTCTGGCAA TGAGATTTCA TGGAACTACA CTCTTGAGCA
2501 ATACCAGAAA ACTTCATTAG CTCAAGAAAA AGAAAAACTG CTGTATGGAT TAGCATCAGT GAAGAACGTT ACTCTTTTGT CAAGGTATTT GGATTTGCTC
2601 AAGGACACGA ACCTTATTAA AACTCAGGAT GTGTTTACAG TCATTCGATA TATCTCATAT AACAGCTATG GGAAGAACAT GGCCTGGAAT TGGATACAAC
2701 TCAACTGGGA CTATCTAGTC AACAGATATA CACTCAATAA CAGAAACCTT GGCCGAATTG TCACAATAGC AGAGCCATTC AACACTGAAC TGCAACTGTG
2801 GCAGATGGAG AGCTTTTTTG CAAAATATCC ACAAGCTGGA GCAGGAGAAA AACCTAGGGA ACAAGTGCTG GAAACAGTGA AAAACAATAT AGAGTGGCTA
2901 AAACAACATA GAAACACCAT CAGAGAATGG TTTTTTAATT TACTTGAGAG TGGTTAAtgt attcaaatgt tagagtttaa ttttgtgaat ctattgtttc
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FIG. 1. Nucleotide sequence of the gp160 cDNA. The sequence shown is a composite of PCR-derived clones. The coding region is shown in uppercase letters, whereas the 5′ and 3′ noncoding regions are in lowercase letters. The initiation codon (ATG) and the stop codon (TAA) are underlined.

```
1                                                          60
MNFAEREGSKRYCIQTKHVAILCAVVVGVGLIVGLAVGLTRSCDSSGDGGPGTAPAPSHL
.....E.P..K...KG.....I.G...A.......S.......       EQD.T....Q

61                                                        120
PSSTASPSGPPAQDQDICPASEDESGQWKNFRLPDFVNPVHYDLHVKPLLEEDTYTGTVS
.PPE..TAL.. ...NV..D......E.........I.......E..A.M...R...I.T

121                                                       180
ISINLSAPTRYLWLHLRETRITRLPELKRPSGDQVQVRRCFEYKKQEYVVVEAEEELTPS
..V...K...D....I...K..K....R....E..PI.............IQ.A.D.AAT

181                                                       240
SGDGLYLLTMEFAGWLNGSLVGFYRTTYTENGRVKSIAATDHEPTDARKSFPCFDEPNKK
...SV.R.....K...........K...M.D.QIR.........................

241                                                       300
ATYTISITHPKEYGALSNMPVAKEESVDDKWTRTTFEKSVPMSTYLVCFAVHQFDSVKRI
S..S...I.....S......EE.S.M...N.KK...V...............R.TAIE.K

301                                                       360
SNSGKPLTIYVQPEQKHTAEYAANITKSVFDYFEEYFAMNYSLPKLDKIAIPDFGTGAME
.R.....KV....N..E.........QA......D....E.A..................

361                                                       420
NWGLITYRETNLLYDPKESASSNQQRVATVVAHELVHQWFGNIVTMDWWEDLWLNEGFAS
....V...........LL..........S..............T......D.........

421                                                       480
FFEFLGVNHAETDWQMRDQMLLEDVLPVQEDDSLMSSHPIIVTVTTPDEITSVFDGISYS
...........K....LS.V.....F.............VV...S..A............

481                                                       540
KGSSILRMLEDWIKPENFQKGCQMYLEKYQFKNAKTSDFWAALEEASRLPVKEVMDTWTR
..A......Q...T..K......I..K.F..A........DS.Q...N...........S

541                                                       600
QMGYPVLNVNGVKNITQKRFLLDPRANPSQPPSDLGYTWNIPVKWTEDNITSSVLFNRSE
......VT.S.RQ..........SK.D......E.........R.ADNDNSRITVY..LD

601                                                       660
KEGITLNSSNPSGNAFLKINPDHIGFYRVNYEVATWDSIATALSLNHKTFSSADRASLID
.G..... A.L..D.....................GG...W..E...S..TR..A...S.F..

661                                                       720
DAFALARAQLLDYKVALNLTKYLKREENFLPWQRVISAVTYIISMFEDDKELYPMIEEYF
...........N..I.....M...S..D....E...S.S.........R.......T..

721                                                       780
QGQVKPIADSLGWNDAGDHVTKLLRSSVLGFACKMGDREALNNASSLFEQWLNGTVSLPV
......V..L...Q.T.S.I.....A.I.............G...Q..DS..K.SA.I..

781                                                       840
NLRLLVYRYGMQNSGNEISWNYTLEQYQKTSLAQEKEKLLYGLASVKNVTLLSRYLDLLK
.................AA...........................D.K..A...EM..

841                                                       900
DTNLIKTQDVFTVIRYISYNSYGKNMAWNWIQLNWDYLVNRYTLNNRNLGRIVTIAEPFN
.P.I...................T..............S.F.I.D.Y............

901                                                    957
TELQLWQMESFFAKYPQAGAGEKPREQVLETVKNNIEWLKQHRNTIREWFFNLLESG
........Q.......N...A.................NVN.QS.....AS.P
```

FIG. 2. Alignment of the predicted gp160 protein (upper sequence) with the mouse BP-1/6C3 amino acid sequence (lower sequence). The translated gp160 cDNA sequence predicts a 957-aa protein with 13 potential N-linked glycosylation sites (three amino acids are underlined to indicate each site). The three gp160 trypsin proteolytic polypeptides identified by protein microsequencing are boldfaced and underlined. The single 22-aa hydrophobic segment is boxed. The potential zinc-binding motif is double underlined, and the potential glycosaminoglycan attachment sites are boldfaced. The sequences were aligned with the sequence of mouse BP-1/C63 and are identical except where another amino acid has been inserted. The gaps were left as spaces.

like BP-1/6C3, gp160 is a type II integral membrane protein with an inverted membrane orientation (i.e., extracellular carboxyl terminus) (24, 31).

RNA Expression. A Northern blot analysis was performed with RNA extracted from several gp160-positive and -negative RCC cell lines and a radiolabeled 469-bp DNA fragment corresponding to positions 399–868 of the gp160 cDNA. A 4.1-kb mRNA transcript was detected in all gp160-positive, but not in any gp160-negative, RCC cell lines (Fig. 3).

APA Activity and gp160 Expression by RCC Cells. The murine homologue of gp160 possesses APA activity (22). We examined several human RCC cell lines for APA activity by assessing the hydrolysis of α-L-glutamyl *p*-nitroanilide. Equal amounts of lysate derived from cultured normal human kidney cells and RCC cells that expressed gp160 (SK-RC-7, SK-RC-28, and SK-RC-45) exhibited approximately a 5-fold increase in enzymatic activity as compared to lysates derived from RCC cells that lacked gp160 expression (SK-RC-18, SK-RC-29, SK-RC-39, SK-RC-42, and Caki I) (Fig. 4) (5).

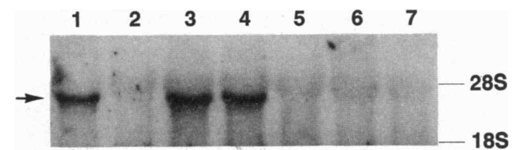

FIG. 3. Northern blot analysis of gp160 expression. Each lane contains 20 μg of total RNA. Lane 1, cultured human proximal tubule cells; lane 2, SK-RC-49; lane 3, SK-RC-7; lane 4, SK-RC-38; lane 5, SK-RC-42; lane 6, SK-RC-2; lane 7, LNCAP prostate cancer cells. The membrane was hybridized with a ^{32}P-labeled 469-bp probe.

Depletion of APA Activity from Renal Cancer Cells by S4 mAb. To determine if APA activity directly associates with gp160, we immunodepleted RCC cell protein extracts with anti-gp160 mAbs and assessed the extracts for residual APA activity, as well as for aminopeptidase B (APB; EC 3.4.11.6) and APN, which are structurally and functionally related to APA (32). mAb S4 could selectively deplete APA but not APN or APB enzymatic activity from a gp160-positive RCC cell line (Table 1). Similar results were obtained with mAbs M1 and F33, which detect epitopes of gp160 different from mAb S4 (data not shown). As shown in Fig. 4, a small amount of APA enzyme activity was detected in SK-RC-39 cells, a cell line that lacks both gp160 protein expression and gp160 RNA expression. APA activity could not be immunodepleted from gp160-negative SK-RC-39 cells with any of the gp160-specific mAbs.

APA Activity of gp160 Immunoprecipitates. To prove that APA activity correlated with the presence of gp160 molecules, we determined that APA activity was demonstrable only in immunoprecipitates formed using anti-gp160 mAbs (S4, M1, or F33) from gp160-positive RCC cells but not with a control antibody (Fig. 5). Since functional APA was detected in gp160 immunoprecipitates, these data also indicate that the S4, M1, and F33 mAbs do not sterically interfere with the catalytic site of APA. In similar experiments with gp160-negative cell lines, APA activity could not be detected in immunoprecipitates.

Activators and Inhibitors of APA Activity on RCC Cells. The effects of divalent cations on APA activity are presented in Table 2. Alkaline earth metals such as Ca^{2+}, Ba^{2+}, and Sr^{2+} enhanced the APA activity of gp160-positive cells. Activity

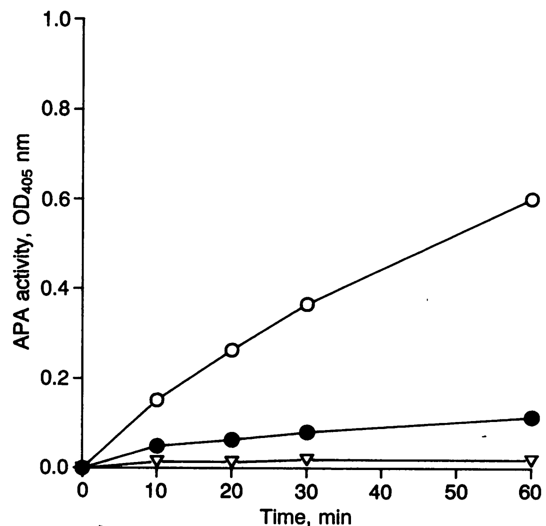

FIG. 4. APA activity of RCC cell lines. Representative data assessing APA activity of a gp160-positive (SK-RC-45; ○) and gp160-negative (SK-RC-39; ●) RCC cell lines. Bovine serum albumin (▽) was used as control.

Table 1. Selective removal of APA activity from SK-RC-28 cells by mAb S4

Enzyme	Substrate	Enzymatic activity, units/ml		% inhibition
		Control antibody	S4 mAb	
APA	α-L-Glutamyl *p*-nitroanilide	175	37	79
APB	L-Lysl *p*-nitroanilide	155	153	1
APN	L-Leucyl *p*-nitroanilide	233	242	0

One enzymatic unit is defined as the amount of enzyme required to cause a change in relative fluorescence at 405 nm of 0.01 OD unit per min under the assay conditions described in *Materials and Methods*. The results were confirmed in two separate experiments.

was lower in the presence of 5 mM EDTA and markedly inhibited by the chelating agents 2,2′-dipyridyl and 1,10-phenanthroline. Enzyme activity of gp160-positive RCC cells was also affected by the APA competitive inhibitors amastatin, bestatin, angiotensin II, and angiotensin III. As expected, the analogue of angiotensin II with β-aspartic acid as its amino-terminal amino acid was noninhibitory at 0.5 mM. The small amount of APA activity detected in gp160-negative SK-RC-39 cells (Fig. 4) was not inhibited by 1,10-phenanthroline or angiotensin II (data not shown).

DISCUSSION

The data presented here indicate that gp160, a normal human kidney differentiation antigen that is differentially regulated in RCCs, is human APA, an aminopeptidase that catalyzes the removal of amino-terminal acidic residues from unblocked oligopeptides. This conclusion is based on the following evidence: (*i*) the amino acid sequence of gp160 is highly homologous to the murine BP-1/6C3 glycoprotein (24), a B-lymphocyte differentiation antigen that exhibits APA activity (22); (*ii*) APA activity correlates precisely with the expression of gp160 protein and mRNA in a panel of RCC cell lines; (*iii*) gp160-specific mAbs immunoprecipitate APA activity from gp160-expressing RCCs but not from gp160-nonexpressing RCCs; (*iv*) gp160-specific mAbs selectively immunodeplete APA activity from gp160-expressing RCCs but not other related aminopeptidases; and (*v*) APA activity from gp160-positive RCC cells exhibited characteristic biochemical responses upon treatment with a spectrum of activators and inhibitors of APA. Finally, we have previously shown that gp160 protein in noncultured human kidney is localized to the glomerulus and proximal tubule cells, which correlates precisely with the known localization of APA activity within the nephron (1, 33).

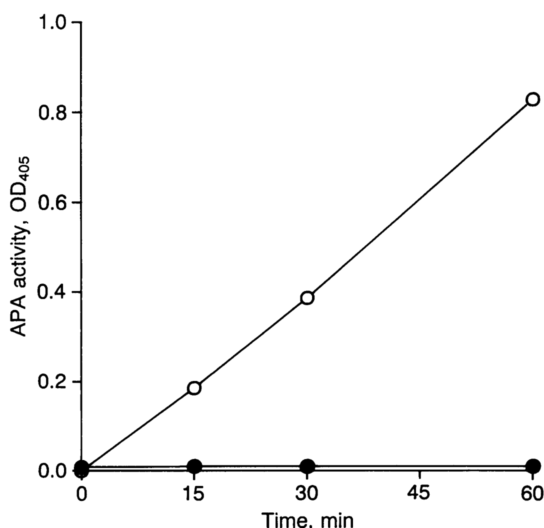

FIG. 5. APA activity of immunoabsorbed gp160 antigen. Molecules immunoprecipitated by mAb S4 (○) or control antibody mAb T43 (●) from equal amounts of a cell lysate preparation of SK-RC-28 cells were assayed for APA activity.

The predicted amino acid sequence of gp160 is 78% homologous to the murine BP-1/6C3 antigen. BP-1/6C3 is a homodimeric, phosphorylated cell surface glycoprotein of 130–140 kDa with a 110-kDa protein backbone (34). The molecular mass of APA solubilized from pig kidney epithelial membranes ranges from 270 to 400 kDa (35, 36), while that from human placenta is 280–500 kDa (37, 38). These additional molecular forms may be due to different degrees of glycosylation, dimeric structures, or, alternatively, to more than one APA gene.

The amino acid sequences of gp160 and BP-1/6C3 predict identical zinc-binding domains, which define a family of zinc-dependent metallopeptidases (27, 39). However, the enzymatic activity of APA is reportedly zinc-independent, requiring instead Ca^{2+} for full activity (32, 40). Therefore, it has been suggested that the zinc-binding consensus sequence may not solely define zinc-dependent metalloproteases but may reflect a conserved evolutionary relationship with metalloproteases that do require zinc for activity (22). Alternatively, the zinc-binding domain may be involved in modulating APA activity in an unknown way (22) or may be critical to another function of the protein that is independent of aminopeptidase activity.

While the full range of biological functions of APA remains to be elucidated, APA appears to have a role in the catabolic pathway of the renin–angiotensin system (41). The amino-terminal amino acid aspartyl residue of angiotensin II can be removed by APA to yield the less active angiotensin III. Thus, aminopeptidase A is also defined as an angiotensinase and is implicated in the regulation of blood pressure by affecting circulating angiotensins. Furthermore, APA may modulate angiotensin-mediated local cerebral blood flow, in part by preventing circulating angiotensins from crossing the blood–brain barrier (42). Cell membrane-bound and soluble APA in serum have been identified (42), as has soluble gp160

Table 2. Effect of various effectors on APA activity

Effector	Concentration, mM	% residual activity
EDTA	5.0	42
$CaCl_2$	1.0	217
$BaCl_2$	1.0	124
$SrCl_2$	1.0	176
1,10-Phenanthroline	1.0	12
2,2′-Dipyridyl	5.0	7
Amastatin	0.25	14
Bestatin	2.0	29
Angiotensin II	0.5	13
	0.05	53
Angiotensin III	0.5	70
[β Asp^1]Angiotensin II	0.5	86
	0.05	88

Equal amounts of a membrane preparation from SK-RC-7 and SK-RC-45 cells were each preincubated with effectors for 5 min and then incubated at 37°C for 30 min with the substrate α-L-glutamyl *p*-nitroanilide at a final concentration of 1.5 mM. Values are the average of results obtained from the two cell lines.

in the serum of RCC patients (N.H.B., unpublished data). The soluble form of APA secreted by placental cells may be an indicator of placental function and is believed to play a role in controlling blood pressure during pregnancy (37, 38).

By analogy to other ectopeptidases, APA may also regulate autocrine and paracrine signals by proteolytic activation or inactivation of specific regulatory peptides (43). For example, interleukin 7 (IL-7) can simultaneously upregulate proliferation and APA (BP-1/6C3) expression in pre-B lymphocytes (44). This proliferative effect can be blocked with the BP-1 antibody, suggesting that APA is involved in regulating IL-7 signal transduction (22). However, IL-7 has no similar effect on the APA activity of RCC cells (D.M.N. and D.E., unpublished data). In addition, anti-gp160 mAbs do not depress the proliferation of RCC cells *in vitro* or abolish α-IFN resistance in gp160-positive RCC cell lines (D.M.N., and G.A.G., unpublished data).

In a previous study, we have shown that high expression of gp160/APA correlates with the resistance of cultured RCCs to the antiproliferative effects of α-IFN, whereas lack of expression correlates with sensitivity to α-IFN (6). These data suggest that APA affects α-IFN action on renal cells in an as yet unknown manner. Direct proteolysis of α-IFN by APA is unlikely since conditioned medium from gp160-expressing RCC cells treated with radiolabeled α-IFN showed no evidence of degraded IFN (L. M. Pfeffer, personal communication). Another possibility is that APA activates or inactivates a factor(s) essential for the antiproliferative effects of α-IFN on RCC cells.

Two previous studies have reported lack of APA activity in RCCs and have suggested that this peptidase may be a marker of transformation (45, 46). In contrast, we have detected gp160 expression in most cultured and noncultured RCCs (2, 5, 6), suggesting that APA is a kidney differentiation antigen and not related to transformation. Similarly, murine APA (BP-1/C63) in pre-B lymphocytes is believed to define a differentiation stage unrelated to malignant potential (47). However, the loss of gp160 expression and concomitant APA activity in a subset of RCCs suggests that gp160 may play a role in the biology of RCCs in an unknown way. Future studies should define the biological functions of gp160/APA in both normal and malignant human kidney cells.

Note Added in Proof. Max Cooper and colleagues (48) have simultaneously cloned human APA.

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