Type XXIII Collagen, a New Transmembrane Collagen Identified in Metastatic Tumor Cells*

Received for publication, October 17, 2002, and in revised form, March 14, 2003 Published, JBC Papers in Press, March 18, 2003 DOI 10.1074/jbc.M210616200

Jacqueline Banyard, Lere Bao‡, and Bruce R. Zetter§

From the Program in Vascular Biology and Department of Surgery, Children's Hospital/Harvard Medical School, Boston, Massachusetts 02115

We have identified a transmembrane collagen, collagen XXIII, in rat prostate carcinoma cells. Differential display of mRNA expression in prostate carcinoma sublines with varying metastatic potential revealed overexpression of this transcript in the metastatic AT6.1 subline. cDNA cloning identified a 2733-bp transcript from AT6.1 RNA, encoding a protein of 532 amino acids, together with a 3067-bp human homologue, resulting in a 540-amino acid protein. Collagen XXIII is predicted to be a type II membrane protein consisting of an aminoterminal cytoplasmic domain, a transmembrane region, and three collagenous domains flanked by short noncollagenous domains. Collagen XXIII is a new member of the transmembrane collagen family, showing structural homology with the transmembrane collagens XIII and XXV. We present evidence that collagen XXIII is expressed as a 75-kDa protein at the cell surface and that it can be cleaved by furin protease activity. Cleavage results in a 60-kDa soluble protein that forms a multimeric complex and exhibits a low affinity interaction with heparin.

The extracellular environment consists of a complex mix of matrix macromolecules together with sequestered growth factors. Each of these components can interact with cells to influence their function and behavior. Proteolytic cleavage of these molecules can release soluble growth factors and matrix fragments that also possess biological activity. The collagen superfamily represents the most abundant group of extracellular matrix macromolecules, and it is becoming clear that this family encompasses a structurally and functionally diverse group of proteins (1). Collagens can be divided into two major groups: fibrillar and nonfibrillar, with a key characteristic of both being the presence of a repeating Gly-Xaa-Yaa, where Xaa and Yaa are frequently proline and hydroxyproline, respectively. A subfamily of the nonfibrillar collagens is the transmembrane collagens. This group currently consists of three members, collagens XIII and XVII and the recently identified collagen XXV, each containing a single-pass hydrophobic transmembrane domain. Both collagens XIII and XVII show widespread distribution in epithelia (2, 3), whereas collagen XXV is specifically overexpressed in neurons (4). Functionally both collagen XIII and XVII have been co-localized in cell adhesions, collagen XVII has been co-localized in hemidesmosomes (3), collagen XIII has been co-localized in focal contacts (2), and their extracellular domains have been shown to support cell adhesion (6–8). We report here the cloning of a new transmembrane collagen overexpressed in rat prostate adenocarcinoma cells and characterize its cellular localization, its cleavage, and the properties of its soluble ectodomain.

EXPERIMENTAL PROCEDURES

Cell Culture—The Dunning rat R-3327 prostate carcinoma sublines, AT2.1, AT3.1, and AT6.1, provided by J. Isaacs (Johns Hopkins University) were maintained as previously described (9). K562 and LoVo cells were purchased from ATCC and grown in recommended culture media. Transfection was performed in AT2.1 cells, unless otherwise stated, using LipofectAMINE 2000 (Invitrogen). The furin inhibitor decanoyl-Arg-Val-Lys-Arg-chloromethylketone (CMK), was purchased from Bachem.

Differential Display—mRNA differential display PCR was performed on AT2.1, AT3.1, and AT6.1 cells as described previously (10).

cDNA Cloning and Construct Preparation—An oligo(dT) primed cDNA library was constructed in the agt10 vector (Amersham Biosciences), using poly(A) RNA obtained from AT6.1 cells. The library was screened using a 32P-labeled cDNA fragment, identified from differential display, as a probe. Inserts from positive clones were subcloned into pBluescript II SK(+) and subjected to DNA sequencing. Partial rat and human collagen XXIII cDNAs were extended using a Marathon cDNA amplification kit (Clontech) with AT6.1 mRNA and Marathon-ready human brain (cerebellum) cDNA (Clontech), respectively. Further extension of the 5′ sequence was performed using a SMART RACE cDNA amplification kit (Clontech), according to the manufacturer’s instructions, using human heart poly(A)+ RNA and brain (cerebellum) poly(A)+ RNA (Clontech). Inverse RACE on AT6.1 mRNA was performed as described (11), using ThermoScript (Invitrogen), 10% GC-Melt (Clontech), and 5% MeSO2, reverse transcribing with primer R1 (5′-GGCGGGCCAGGGCAACAGTTCG-3′) and forward primer F1 (5′-GGCAACTGTGGGGCGCTGCMG-3′). Circularized DNA was amplified by PCR using nested reverse primer R2 (5′-CACGGCCGCGCATCCGCAAG-3′) and forward primer F1 (5′-GGCAACTGTGGGGCGCTGCMG-3′). The genomic sequence was amplified from adapter-ligated, restriction enzyme-digested rat genomic DNA from the Rat Genome Walker kit (Clontech) with Advantage-PC Genomic PCR reaction mix (Clontech) with adapter and gene-specific PCR primers. Amplified PCR products were inserted into pcCR2.1-TOPO (Invitrogen) and plasmid DNA-sequenced.

Amino-terminal truncated rat collagen XXIII(del 1–48)Myc corresponding to amino acids Ala49–Lys102, was obtained by PCR using LA Taq (Panvera, WI) with forward primer 5′-GGCTTCTGCGGCGCGCTGCGGCGG-3′ and reverse primer 5′-GCTCTGACTAGCATACTTTCCTTTCTCAAGAATATTTTTTGTTCCCTGATCGACCAATACGAGCAGG-CA-3′ and inserted into pDNA4/HisMax(C) (Invitrogen) in frame with an amino-terminal His tag. Full-length (wild type) rat collagen XXIII-Myc was constructed by overlap extension PCR. Exon 1 sequence was amplified by PCR from rat genomic DNA, as determined by genome

* This work was supported by Grant R01 CA 37393 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Present address: Pintex Pharmaceuticals, 313 Pleasant St., Watertown, MA 02472.
‡ To whom correspondence should be addressed: Dept. of Surgery, Enders 1074, Children's Hospital, 300 Longwood Ave., Boston, MA 02115. E-mail: bruce.zetter@tch.harvard.edu.
§ To whom correspondence should be addressed: Dept. of Surgery, Enders 1074, Children's Hospital, 300 Longwood Ave., Boston, MA 02115. E-mail: bruce.zetter@tch.harvard.edu.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY158985 and AY158896.

1 The abbreviations used are: CMK, chloromethylketone; RACE, rapid amplification of cDNA ends; COL, collagenous; NC, noncollagenous; RT, reverse transcription; PBS, phosphate-buffered saline.
walking, to overlap cloned cDNA sequence by a primer length. Fragments were annealed, extended using Advantage-2 GC Genomic polymerase, and amplified by PCR. The soluble cleaved form of human collagen XXIII, corresponding to Glu$^{111}$-Lys$^{440}$, was obtained using forward (5'-GGGGTACCGAAGCTTACCCATGAAATTTGC-3') and reverse (5'-CCGCTCGAGGCTTATGCCAGCAGCCAGGCACAG-3') primers and inserted into pSecTag2HygroB (Invitrogen) with an amino-terminal secretion signal and carboxyl-terminal His and Myc tags. DNA was sequenced using the Sequenase 2.0 DNA sequencing kit (U.S. Biochemical Corp.) or submitted to the DNA core facility (Boston, MA) for DNA sequencing. Sequence data were compiled using GeneToolLite Multi-Align (BioTools, Inc., Alberta, Canada) and peptides aligned using GCG PileUp, PEP comparison matrix. Amino acid and nucleotide distances were compared using the Jotun Hein method.

RT-PCR Analysis—Total RNA was isolated from tissue culture cells using a RNeasy miniprep kit (Qiagen), treated with 25 units of RNase-free DNase (Promega), phenol-chloroform extracted, and ethanol-purified. 2 µg of total RNA was reverse transcribed using 200 units of Superscript II (Invitrogen) and 500 ng of oligo(dT)$_{12}$-18 at 45 °C. Serial dilutions of cDNA were amplified by PCR using 18 cycles with the rat glyceroldehyde-3-phosphate dehydrogenase primers (forward primer, 5'-TGAAGATGGGTCGGTGAAACGTTGTCG-3'; reverse primer, 5'-CATCAGGCACTAGGTCACCCAC-3'). PCR bands were analyzed by National Institutes of Health Scion Image (Scion Corporation), and cDNA concentration was equalized accordingly such that housekeeping gene amplification was equivalent (data not shown). Equalized cDNA was amplified by PCR using LA Taq using primers designed to span multiple exons (rat collagen XXIII forward primer, 5'-GACTCGAGCGTTCCCTGGACCCA-3'; reverse primer, 5'-GATACAGCTTGTGAGTTTACGAGGCC-3'). PCR was performed using a PTC-200 Peltier Thermal Cycler (MJ Research) as follows: 94 °C for 3 min; 38 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min; followed by a 72 °C 5-min elongation step. Housekeeping genes were amplified using the same PCR conditions for 21 cycles. RNA analysis of collagen XXIII expression in normal tissues was performed on a rat multiple tissue cDNA panel (Clontech) using the PCR conditions listed above and primers (forward primer, 5'-GGCCCTCAAAGGTGCTGC-3'; reverse primer, 5'-TTGGGGCTTACGCCAGGTCACC-3').

Preparation of Collagen XXIII Antisera—Rat AT6.1 cDNA encoding the carboxyl-terminal 108 amino acids of collagen XXIII was cloned into pGEX3X vector (Amersham Biosciences). Protein was expressed in Escherichia coli DH5α (Invitrogen) and purified over a glutathione-Sepharose column (Amersham Biosciences). New Zealand White rabbit antisera were immunized with 100 µg of collagen XXIII gluthathione S-transferase fusion protein emulsified with complete Freund's adjuvant. The rabbits were boosted by a single injection of the glutathione S-transferase fusion protein. Positive antisera from rabbit 22225B, hereafter known as 6.1B, was used for subsequent analysis.

Protein Preparation—The cells were collected in RIPA or M-PER extraction reagent (Pierce) supplemented with Complete EDTA-free protease inhibitors (Roche Applied Science). For serum-free conditioned medium analysis, the cells and debris were removed by centrifugation at 250 × g for 5 min, followed by 0.2-µm filtration. For conditioned medium analysis following transfection of wild type collagen XXIII-Myc, protein was concentrated using PAGEprepp slurry (Pierce) according to the manufacturer's instructions and eluted in 100 mM sodium citrate, 50 mM Tris, pH 7.5, and 1% SDS. For hirapin and hirapin binding studies, conditioned medium from collagen XXIII/Glu$^{111}$-Lys$^{440}$ transfected cells was analyzed without concentration. Heparin- and H11032 (5 µg/ml) binding studies were performed with 22225B serum, followed by horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences) and detected by an enhanced choliniumnescence detection system (PerkinElmer Life Sciences).

Identification and Cloning of a Novel Collagen Expressed in Metastatic Prostate Adenocarcinoma Cells—Sublines of the Dunning rat R-3327 prostate adenocarcinoma (9) were analyzed for differences in mRNA expression by differential display PCR. An enriched mRNA transcript was identified that was highly overexpressed in the metastatic subline AT6.1 (Fig. 1A). The cloned cDNA fragment was used to screen an AT6.1 cDNA library, isolating a 1412-bp partial cDNA. Sequence analysis revealed a novel mRNA containing an 108-amino acid open reading frame containing the repeating amino acid motif [Gly-Xaa-Yaa], where Xaa and Yaa were frequently proline and hydroxyproline, characteristic of the collagen superfamily of proteins (1). The partial mRNA sequence was extended by both the 5’ and 3’ RACE methods (12). Sequence obtained via multiple 5’ RACE reactions suggested an incomplete transcript, because of an additional collagenous coding sequence upstream of potential start sites. Consequently PCR genomic walking (13) was used to amplify additional 5’ sequence, revealing a potential translation start site upstream of the known exon sequence. Inverse RACE (11) confirmed this sequence in rat AT6.1 mRNA and further determined a potential transcriptional start site 128 bp upstream of the translation start site (Fig. 1B). Thus we identified a 2733-bp transcript from AT6.1 RNA. BLAST (14) comparison of the new collagen nucleotide sequence against available data base information revealed an expressed sequence tag fragment from human retina with homology. Northern blot and RT-PCR was used to identify human tissues and cell lines expressing this new collagen, revealing expression in human heart and brain and in the human leukemia cell line, K562 (data not shown). RNA from these sources was used to amplify the human homologue using RACE and PCR, identifying a 3067-bp transcript. BLAST search revealed identity with the carboxyl-terminal end of an incomplete sequence.
human cDNA from testis, DKPZp434K0621 (accession number AL137461), and most recently to the complete *Mus musculus* collagen α1 type XXIII (accession number AP410792). Thus we have identified and cloned both the human and rat type XXIII collagen α1-chains. At the nucleotide level, human and rat collagen α1(XXIII) show 76% identity. Rat and mouse collagen α1(XXIII) predictably show even greater cDNA identity, at 91%. Considering the high identity between human, rat, and mouse cDNAs, and the longer 5′ sequences identified in human and mouse, we note the possibility of additional transcriptional start sites in the rat collagen XXIII gene, in addition to the site we have identified by Inverse RACE. Certainly multiple transcriptional start sites have been identified in the structurally similar collagen XIII (15). BLAST searching the National Institutes of Health genome data base located the α1(XXIII) gene on *Homo sapiens* chromosome 5q5 (NT_023132.9) and *M. musculus* chromosome 11B1 + 2 (NW_000039.1), hereafter referred to simply as collagen XXIII, in the absence of known alternate α-chains. Across the open reading frames human and rat collagen XXIII show 65% nucleotide identity, translated into 532- and 540-amino acid proteins, respectively, with 91% identity (Fig. 2). Structurally, collagen XXIII consists of a long amino-terminal noncollagenous (NC) domain, NC-1, containing a short cytoplasmic region and a putative membrane spanning domain, followed by three collagenous (COL1–COL3) domains in the extracellular region of the protein that are interrupted by short noncollagenous domains (NC2–NC4), as shown in the schematic (Fig. 3). The structural organization of human and rat collagen XXIII is similar to the published transmembrane collagens XIII (16) and collagen XXV (4). At the amino acid level collagen XIII and XXV show 54 and 56% identity with collagen XXIII, respectively, although this homology exists primarily across the collagenous domains. However, in all three structurally similar transmembrane collagens, types XXIII, XIII, and XXV, there is high identity across the 20-amino acid carboxy-terminal noncollagenous domain (Fig. 4). In addition, the COL1 domain of collagen XXIII contains a conserved consensus binding motif for the α5β1 integrin, RGD, and multiple copies of a RGD motif reportedly used for integrin-mediated cell adhesion by collagen type XVII (8).

**Collagen XXIII Is a Type II Transmembrane Protein—**Sequence analysis revealed the presence of a group of hydrophobic amino acids close to the amino terminus of the collagen XXIII protein. Multiple sequence analysis algorithms, including TMHMM2.0 (17) and TMPred (18), predict this region to be a transmembrane helix, in accord with the structural homology with transmembrane collagens XIII and XVII. To investigate the cellular localization of collagen XXIII, a truncation mutant, rat collagen XXIII(del1–48)Myc, was constructed by deletion of the putative cytoplasmic and transmembrane domains. The length of the putative transmembrane helix is variable between prediction programs. Because the ectodomain sequence is important for collagen XIII chain association (19), a minimal deletion was made. AT2.1 prostate carcinoma cells were transfected with either wild type rat collagen XXIII-Myc or the amino-terminal truncation mutant, rat collagen XXIII(del1–48). Cellular localization was determined by immunofluorescence staining using antibody 6.1B, which recognizes the carboxy terminus of rat collagen XXIII. Fig. 5 demonstrates that wild type collagen XXIII could be detected either with or without cell permeabilization. In contrast, collagen XXIII(del1–48) was detected only following permeabilization of the cells, indicating an intracellular localization for the amino-terminal truncation mutant. This demonstrates that the carboxy terminus of wild type collagen XXIII is present on the cell surface and that the amino-terminal cytoplasmic and...
transmembrane domains are required for cell surface targeting.

**Collagen XXIII Expression Is Up-regulated in Metastatic Tumor Cells**—We first identified collagen XXIII in the highly metastatic AT6.1 subline from the Dunning Rat R-3327 prostate adenocarcinoma model. To investigate its expression pattern, RT-PCR analysis was performed on additional sublines in this rat prostate cancer series. Fig. 6A shows that nonmetastatic NbE cells and poorly metastatic AT2.1 cells did not express collagen XXIII, whereas highly metastatic AT6.1 cells show high levels of expression, and further revealed that an additional metastatic subline, MatLyLu, expressed low levels of collagen XXIII. We have examined collagen XXIII expression in normal rat tissues by RT-PCR analysis and found expression in brain and lung (data not shown).

To investigate expression of collagen XXIII at the protein level, a rabbit polyclonal antibody, antibody 6.1B, was raised to a glutathione S-transferase fusion protein encoding the carboxyl-terminal 108 amino acids of rat collagen XXIII. Fig. 7A demonstrates antigenic recognition of transfected rat collagen XXIII(del 1–48)Myc by 6.1B antibody. Specificity was compared with immunoblot using anti-Myc 9E10 monoclonal antibody, as shown in Fig. 7B. Endogenous collagen XXIII was detected in rat AT6.1 and MatLyLu metastatic prostate carcinoma cells by immunoblot using 6.1B antibody, as a protein with a molecular mass of ∼70,000–75,000 Da. No collagen XXIII protein was detectable in the nonmetastatic NbE cells or in the poorly metastatic AT2.1 subline (Fig. 7C), confirming the expression pattern at the RNA level. These results suggest that collagen XXIII expression is up-regulated in certain metastatic prostate cancer cells relative to nonmetastatic or poorly metastatic prostate cancer cells.

**Collagen XXIII Is Cleaved from the Cell Surface by a Furin Protease**—Because most collagens are secreted proteins, we explored whether collagen XXIII was retained on the cell surface by analyzing cell lysates and cell-conditioned media of cells transfected with Myc-tagged wild type rat collagen XXIII. Western blot analysis revealed the presence of a 70,000–75,000-Da form on the cell surface along with a smaller, ∼60-kDa soluble form of the protein in the cell media (Fig. 8, A and B). Amino acid analysis of the extracellular domain of collagen XXIII revealed potential sites for cleavage by furin, a protease that requires the minimal basic amino acid motif Arg-Xaa-Xaa-Arg (20). Collagen XXIII contains a suitable basic motif, 19KLRTVR in rat collagen XXIII and 105KIRTAR in human collagen XXIII. Interestingly, these motifs show homology with the furin cleavage sites reported in the transmembrane collagens XIII (19), XVII (3), and XXV (4). To investigate the
role of furin proteases in collagen XXIII cleavage, wild type rat collagen XXIII-Myc overexpressing cells were treated with a synthetic furin inhibitor, decanoyl-RVKR-CMK. Fig. 8 (A and B) demonstrates that cleavage of collagen XXIII was reduced by CMK treatment. Equal loading of lysate protein is indicated by β-actin immunoblot (Fig. 8C). In addition, when wild type collagen XXIII-Myc was overexpressed in LoVo cells, a human colon carcinoma cell line that lacks endogenous furin activity (21), full-length protein was seen in the cell lysate, but no soluble cleavage product was detectable in the medium fraction (Fig. 8D). Taken together, these data indicate a role for furin protease cleavage in the processing of collagen XXIII.

**Multimer Formation and Heparin Binding of Soluble Collagen XXIII**—Collagen XXIII is a new member of an increasingly structurally diverse collagen superfamily, many members of which associate with homotypic or heterotypic collagen polypeptide chains to form multimers. To investigate the association of collagen XXIII α-chains, the truncation mutant collagen XXIII(del1–48)Myc was used, such that protein would be retained in the cell lysate and not cleaved into the cell conditioned medium. The cells were transfected with rat collagen XXIII(del1–48)Myc, and cell lysate was analyzed by immunoblot under nonreducing and reducing conditions. Fig. 9A shows the formation of multimeric complexes, indicative of a dimer and trimer, which were reduced to a monomer upon addition of the reducing agent β-mercaptoethanol. Because we have shown that the wild type protein can be cleaved from the cell surface, we next determined whether the cleaved collagen remains in this multimeric form. Conditioned medium from cells overexpressing wild type collagen XXIII-Myc was collected and analyzed by immunoblot under nonreducing and reducing conditions (Fig. 9B). These data indicate that furin-cleaved soluble collagen XXIII can exist as an α3(XXIII) homotrimer and also suggest that the amino acids required for α-chain association are beyond the site of furin cleavage. To further investigate the function of soluble collagen XXIII, an expression plasmid was constructed encoding the cleaved form of human collagen XXIII, amplified from K562 human leukemia cell cDNA, in frame with a secretion signal sequence. Serum-free conditioned medium from collagen XXIII(Glu111-Lys546)-transfected cells was immunoblotted under nonreducing conditions, resulting in equivalent multimer formation, validating α-chain association of this secreted protein (data not shown).

Because a soluble collagen may be available for cell surface and/or extracellular matrix binding, we next investiga-

![Fig. 9. Multimer formation of collagen XXIII α-chains.](image)

![Fig. 10. Heparin binding of collagen XXIII.](image)
be dependent on collagen chain association and folding, because the peptide motifs RGD and KGD lie within collageneous domains and could be hidden within a collagen triple helix. Such cryptic motifs are exposed upon proteolytic cleavage or unfolding via denaturation, as shown for collagen types I, V, VI, and XVII (8, 23–25). Conversely, other peptide motifs may depend on triple helix conformation for ligand presentation to integrin receptors, as observed for the collagen recognition motif GFOGER (26). Clearly the state of collagen folding could influence cellular interaction and function. We have shown that recombinantly expressed forms of collagen XXIII formed multimers suggestive of a dimer and trimer under nonreducing conditions. Although the trimeric complex might be predicted to contain nucleated triple helical domains characteristic of collagens, it has been observed that expression of other recombinant transmembrane collagens also results in dimeric structures. Expression of recombinant collagen types XIII (19, 27), XVII (3), and XXV (4) generates both dimers and trimers. However, rotary shadowing electron microscopy of the structurally similar collagen XIII has revealed the formation of rodlike structures (27), consistent with triple helix folding. This might imply that these collagen dimers represent a processing intermediate, although dimeric collagen XXV/CLAC is a major form detected in brain tissue from patients with Alzheimer’s disease (4). We have not yet been able to determine whether endogenous collagen XXIII shows the same distribution of monomer and multimers because of expression levels and/or detection sensitivity. However, collagen XXIII chain association will be an important consideration in the functional analysis of this protein.

Collagens can also interact with other components of the extracellular matrix, as demonstrated by collagen XXV binding to fibrillized β-amyloid peptide, a key component of the characteristic senile plaques deposited in Alzheimer’s disease (4), and collagen XIII interaction with fibronectin, nidogen-2, and heparin (27). We report here low affinity heparin binding of collagen XXIII. Indeed, collagen XXIII contains clusters of basic amino acid residues that might mediate heparin interaction. Heparan sulfate glycosaminoglycans exist both at the cell surface and as a matrix component; thus any interaction may be relevant in the context of either cell-matrix or cell-cell contact (28), and we are currently further defining this binding activity.

Our results demonstrate that cleavage of type XXIII collagen is a furin convertase/ paired basic amino acid-cleaving enzyme-mediated event. Many transmembrane proteins are cleaved by this group of proteases, including growth factors such as transforming growth factor-β1 and other proteases, such as stromelysin 3 (matrix metalloprotease-11) and membrane type matrix metalloprotease-1 (reviewed in Ref. 20). Furin cleavage of many proteins is a required activation event, resulting in the release of a soluble mature molecule from an inactive precursor. This raises the possibility that soluble collagen XXIII represents the mature form of collagen XXIII. Alternatively, membrane proteins can possess activities independent of the cleaved protein, as demonstrated with soluble sHB-EGF and proHB-EGF (29). Because furin protease cleavage may control the rate of release of soluble collagen XXIII, it is interesting to note that increased furin expression has been reported in lung (30) and head and neck tumors (31). Moreover, reduced tumorigenicity resulted from inhibition of furin activity (32). Because collagen XXIII expression was up-regulated in more aggressive cell lines of the Dunning rat prostate carcinoma model, concurrent furin activity level may be an important factor regulating transmembrane collagen cleavage in the tumor environment. A useful consequence of increased cleavage of a membrane protein up-regulated in tumor cells is its potential use as a diagnostic or prognostic marker. We have preliminary evidence correlating increased collagen XXIII immunohistochemical staining with increasing tumor stages in human prostate cancer (data not shown) and are further characterizing expression to explore this possibility.

Acknowledgments—The Dunning rat prostate carcinoma variants were generously provided by J. T. Isaacs (Johns Hopkins University). The NBE rat prostate cell line was provided by M. R. Freeman (Children’s Hospital, Boston, MA).

REFERENCES
1. Myllyharju, J., and Kivirikko, K. I. (2001) Ann. Med. 33, 7–21
2. Peltonen, S., Hentula, M., Hagg, P., Yla-Outinen, H., Tuukkanen, J., Lakkaroki, J., Rehn, M., Pihlajaniemi, T., and Peltonen, J. (1999) J. Invest. Dermatol. 113, 635–642
3. Schacke, H., Schumann, H., Hammami-Haussli, N., Raghunath, M., and Bruckner-Tuderman, L. (1998) J. Biol. Chem. 273, 25937–25942
4. Hashimoto, T., Wakahayashi, T., Watanabe, A., Kowa, H., Hosoda, R., Nakamura, A., Kanazawa, I., Arai, T., Takio, K., Mann, D. M., and Iwatsubo, T. (2002) EMBO J. 21, 1524–1534
5. Hookfin, S. N., Findlay, K., deHart, G. W., and Jones, J. C. (1998) J. Invest. Dermatol. 111, 1015–1022
6. Hagg, P., Vaisanen, T., Tuomisto, A., Rehn, M., Tu, H., Huhtala, P., Eskelinen, S., and Pihlajaniemi, T. (2001) Matrix Biol. 19, 727–742
7. Nykvist, P., Tu, H., Ivaska, J., Kajpy, J., Pihlajaniemi, T., and Heino, J. (2000) J. Biol. Chem. 275, 8255–8261
8. Nykvist, P., Tasanen, K., Viitasalo, T., Kajpy, J., Jokinen, J., Bruckner-Tuderman, L., and Heino, J. (2001) J. Biol. Chem. 276, 38673–38679
9. Isaacs, J. T., Isaacs, W. B., Feitz, W. F., and Scheres, J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9094–9099
10. Siebert, P. D., Chenchik, A., Kellogg, D. E., Lukyanov, K. A., and Lukyanov, S. A. (1995) Nucleic Acids Res. 23, 1087–1088
11. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
12. Krist, A. P., Latvanlehto, A., Sund, M., Horelli-Kuitunen, N., Rehn, M., Palotie, A., Beier, D., and Pihlajaniemi, T. (1999) Matrix Biol. 18, 261–274
13. Davis, G. E. (1992) Proc. Natl. Acad. Sci. U. S. A. 91, 75–80
14. Hagg, P., Rehn, M., Huhtala, P., Vaisanen, T., Tamminen, M., and Pihlajaniemi, T. (1998) J. Biol. Chem. 273, 15396–15397
15. Sonnhammer, E. L., von Heijne, G., and Krogh, A. (1998) Proc. Int. Conf. Inff. Syst. Mol. Biol. 6, 175–182
16. Hofmann, K., and Stoffel, W. (1993) Biol. Chem. Hoppe-Seyler 374, 166
17. Snellman, A., Tu, H., Vaisanen, T., Kvist, A. P., Huhtala, P., and Pihlajaniemi, T. (2000) EMBO J. 19, 5051–5059
18. Nakayama, K. (1997) Biochim. Biophys. Acta 1328, 1067–1071
19. Nakayama, K., and Klagsbrun, M. (2000) J. Biol. Chem. 275, 5051–5059
20. Takahashi, S., Kashi, K., Hatazawa, K., Takahashi, S., Soker, S., and Klagesbrun, M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 2573–2577
21. Davis, G. E. (1992) Biochem. Biophys. Res. Commun. 182, 1025–1031
22. Ruggiero, F., Champliaud, M. F., Gareeze, M., and Aumailley, M. (1994) Exp. Cell Res. 210, 215–223
23. Pfaff, M., Aumailley, M., Specks, U., Knole, J., Zerres, H. G., and Timpl, R. (1993) Exp. Cell Res. 206, 167–176
24. Knight, C. G., Morton, L. F., Peachey, A. R., Tuckwell, D. S., Farndale, R. W., and Barnes, M. J. (2000) J. Biol. Chem. 275, 35–40
25. Tu, H., Sanai, T., Snellman, A., Gehring, W., Pirila, T., Rimpi, R., and Pihlajaniemi, T. (2002) J. Biol. Chem. 277, 23092–23099
26. Park, P. W., Reizes, O., and Bernfield, M. (2000) J. Biol. Chem. 275, 29923–29926
27. Miyoshi, E., Higashiyama, S., Nakagawa, T., Hayashi, N., and Taniguchi, N. (1997) J. Biol. Chem. 272, 14349–14355
28. Miyakawa, S., Siros, F., Yao, J., Sedita, N. G., and Chretien, M. (1997) Br. J. Cancer 75, 1509–1514
29. Bassi, D. E., Mahloghi, H., Al-Saleem, L., Lopez De Cicco, R., Ridge, J. A., and Klein-Sanzo, A. J. (2001) Mol Carcinog. 31, 224–232
30. Bassi, D. E., Lopez De Cicco, R., Mahloghi, H., Zucker, S., Thomas, G., and Klein-Sanzo, A. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 10326–10331
Type XXIII Collagen, a New Transmembrane Collagen Identified in Metastatic Tumor Cells
Jacqueline Banyard, Lere Bao and Bruce R. Zetter

J. Biol. Chem. 2003, 278:20989-20994.
doi: 10.1074/jbc.M210616200 originally published online March 18, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M210616200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 32 references, 14 of which can be accessed free at http://www.jbc.org/content/278/23/20989.full.html#ref-list-1