Expression of Sorsby’s Fundus Dystrophy Mutations in Human Retinal Pigment Epithelial Cells Reduces Matrix Metalloproteinase Inhibition and May Promote Angiogenesis*

Received for publication, November 13, 2001, and in revised form, January 24, 2002
Published, JBC Papers in Press, January 30, 2002, DOI 10.1074/jbc.M110870200

Jian Hua Qi, Quteba Ebrahem, Karen Yeow, Dylan R. Edwards, Paul L. Fox†, and Bela Anand-Apte‡‡

From the ‡Department of Ophthalmic Research, Cole Eye Institute and the ‡Department of Cell Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195 and the §§chool of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom

Sorsby’s fundus dystrophy (SFD) is an autosomal dominant degenerative disease of the macula caused by mutations in the tissue inhibitor of metalloproteinase-3 (TIMP-3) gene. Choroidal neovascularization is a hallmark of this disease, which closely resembles the exudative form of age-related macular degeneration. However, the mechanism by which TIMP-3 mutations induce the disease phenotype in SFD remains unknown. To address this question we established human retinal pigment epithelial cell lines expressing wild type or S156C (Ser156 changed to cysteine) mutant TIMP-3. S156C TIMP-3 had reduced matrix metalloproteinase (MMP) inhibitory activity in retinal pigment epithelial cells and resulted in increased secretion and activation of gelatinase A and B. The conditioned medium from these cells induced angiogenesis in “in vivo” chick chorioallantoic membrane assays that could be reversed with recombinant wild type TIMP-3. Our data indicate that the choroidal neovascularization in SFD may be a result of increased MMP activity, which could lead to the stimulation of angiogenesis. These results also suggest the potential therapeutic use of TIMP-3 or synthetic MMP inhibitors in this disease.

* This work was supported by National Institutes of Health Grant 1 R29 EY12105-01, a Foundation Fighting Blindness module grant, and a Cleveland Clinic Foundation seed grant (to B. A.-A.). 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 1754 solely to indicate this fact.
† To whom correspondence should be addressed: Dept. of Ophthalmic Research, Cole Eye Inst./I-131, Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195. E-mail: anandab@ccf.org.
‡‡ The abbreviations used are: SFD, Sorsby’s fundus dystrophy; TIMP, tissue inhibitor of metalloproteinase; MMP, matrix metalloproteinase; CAM, chorioallantoic membrane; RPE, retinal pigment epithelium; ECM, extracellular matrix; PBS, phosphate-buffered saline; ConA, concanavalin A; MT, membrane-type; CNV, choroidal neovascularization; CM, conditioned medium.

† This paper is available on line at http://www.jbc.org

© 2002 by The American Society for Biochemistry and Molecular Biology, Inc.

Inhibition and May Promote Angiogenesis*

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 277, No. 16, Issue of April 19, pp. 13394–13400, 2002

Sorsby’s fundus dystrophy is an autosomal dominant degenerative disease of the macula caused by mutations in the tissue inhibitor of metalloproteinase-3 (TIMP-3) gene. Choroidal neovascularization is a hallmark of this disease, which closely resembles the exudative form of age-related macular degeneration. However, the mechanism by which TIMP-3 mutations induce the disease phenotype in SFD remains unknown. To address this question we established human retinal pigment epithelial cell lines expressing wild type or S156C (Ser156 changed to cysteine) mutant TIMP-3. S156C TIMP-3 had reduced matrix metalloproteinase (MMP) inhibitory activity in retinal pigment epithelial cells and resulted in increased secretion and activation of gelatinase A and B. The conditioned medium from these cells induced angiogenesis in “in vivo” chick chorioallantoic membrane assays that could be reversed with recombinant wild type TIMP-3. Our data indicate that the choroidal neovascularization in SFD may be a result of increased MMP activity, which could lead to the stimulation of angiogenesis. These results also suggest the potential therapeutic use of TIMP-3 or synthetic MMP inhibitors in this disease.
**EXPERIMENTAL PROCEDURES**

**Cell Culture—**ARPE-19 cells (American Type Culture Collection) were maintained in T75 flasks in filter medium consisting of Dulbecco’s modified Eagle’s medium/Ham’s F-12 plus 10% fetal bovine serum (Hyclone). All cultures of ARPE-19 grown on tissue culture plastic were fed weekly. Cultures were routinely passaged by dissociation with 0.05% (w/v) trypsin and 0.02% (w/v) EDTA, tetrasodium salt, followed by resuspension at a cell ratio ranging from 1:1 to 1:6.

**Generation and Analysis of Timp-3 Transfectant RPE Cell Lines—**Wild type (WT)-Timp-3 and S156C-Timp-3 inserts from human cDNA clones were fused in-frame with FLAG epitope DYKDDDK at their C-terminal end and cloned into expression vector pcDNA3.1 (CLONTECH, Palo Alto, CA). ARPE-19 cell lines were transfected by lipofection (LipofectAMINE reagent, Invitrogen) and in medium containing neomycin (0.5 mg/ml). As a control, cells were transfected with the vector alone. Conditioned medium, lysate, and ECM samples were prepared as described previously (16). Expression of transfected genes was confirmed by Western blot analysis with a polyclonal antibody to Timp-3 (a kind gift of Suneel Apte, Cleveland, OH) or monoclonal anti-FLAG (M2) antibody (Sigma).

**Preparation of ECM—**Cells were plated onto six-well culture plates (Costar). When confluent, the cells were removed from the culture dishes following a 15-min incubation in Ca2+-Mg2+-free phosphate-buffered saline (PBS) containing 5 mM EGTA and 1 mM phenylmethylsulfonyl fluoride. After several rinses in PBS and water the ECM was scraped in a small volume of electrophoresis sample buffer without reducing agent. For protein estimations, ECM was scraped in PBS containing 1 mM phenylmethylsulfonyl fluoride. Protein concentrations were determined using the Bio-Rad protein assay reagent.

**Zymography and Reverse Zymography—**Equal amounts of protein were loaded on a 7.5% polyacrylamide gel with 1 mg/ml gelatin for zymography or a 12% gel with 1 mg/ml gelatin plus baby hamster kidney cell-conditioned medium as a source of MMPs for reverse zymography. Following electrophoresis, gels were processed as described previously (10). Briefly, gels were incubated in a solution of 25 mg/ml Triton X-100, 0.1 M Tris-HCl (pH 7.5), containing 5 mM CaCl2 and 0.2 mg/ml sodium azide at 37 °C. Gels were stained with 5% Coomassie Blue R-250 in acetic acid/methanol/water (1:3:6) for 2 h and destained with acetic acid/methanol/water (1:3:6). Gelatinase activity was also determined using a gelatinase activity assay kit (Chemicon International, Temecula, CA) according to the manufacturer’s instructions.

**Cell Adhesion Assays—**Human laminin (Collaborative Biomedical Inc., Bedford, MA) was immobilized on 96-well nontissue culture-treated plates. Wells were washed and incubated with 3% bovine serum albumin in PBS for 30 min. Nonadherent cells were incubated for 16 h in 50 mM Tris-HCl (pH 7.5) containing 5 mM CaCl2 and 0.2 mg/ml sodium azide at 37 °C. Gels were stained with 5 mg/ml Coomassie Blue R-250 in acetic acid/methanol/water (1:3:6) for 2 h and destained with acetic acid/methanol/water (1:3:6). Gelatinase activity was also determined using a gelatinase activity assay kit (Chemicon International, Temecula, CA) according to the manufacturer’s instructions.

**Cell Migration Assay—**A modified Boyden chamber assay was carried out as described previously (17). 8.0-μm pore polivinylpyrrolidone-free polycarbonate membranes were precoated with collagen type I (100 μg/ml). For migration of RPE cell transfecants, laminin (5 μg/ml) or 10% fetal bovine serum was placed in the lower wells, and RPE cells (1.0 × 105) were placed in the upper wells. For endothelial cell chemotaxis, human dermal microvascular endothelial cells (Clonetics, San Diego, CA) (3.0 × 104) were placed in the upper wells and allowed to migrate toward conditioned medium placed in the lower wells. Chambers were incubated for 4 h at 37 °C in a 5% CO2 humidified incubator. Cells remaining on the top of the filter were removed. The bottom surface of the filter was fixed, stained, and mounted. The number of cells migrating per well was counted microscopically, and mean ± S.E. was calculated from quadruplicate samples.

**Matrigel Collagen Gel Invasion Assay—**This assay was carried out as described previously (18) with some modifications. Boyden chambers were used, and 8.0-μm polivinylpyrrolidone-free filters were coated with matrigel or collagen type I gel (Collaborative Biomedical Inc.). The assay was carried out at 37 °C for 18–24 h, and filters were fixed, stained, and mounted. Invaded cells were counted under a microscope, and mean ± S.E. was calculated from quadruplicate samples.

**Endothelial Cell Proliferation Assay—**This assay was carried out as described previously (17). Human dermal microvascular endothelial cells were incubated for 72 h in the presence of conditioned medium from RPE cell transfecants. Cells were trypsinized and counted using a Coulter particle counter.

**Chick Chorioallantoic Membrane (CAM) Assays—**The CAM assay was performed as described previously (19) with slight modifications. Fertilized 3-day-old White Leghorn eggs (Sunnyside Inc., Beaver Dam, WI) were cracked, and embryos with the yolk intact were placed in 100–200-mm glass-bottom Petri dishes. After incubation for 3 days at 37 °C in 3% CO2, CAMs were implanted with 5-mm diameter sterilized gelatin sponges (Gelfoam, Upjohn Co., Kalamazoo, MI) loaded with equal protein amounts of conditioned medium from RPE cell transfecants and photographed on day 3. CAMs implanted with sponges loaded with serum-free medium alone or with basic fibroblast growth factor or vascular endothelial growth factor were used as negative and positive controls, respectively. Recombinant Timp-3 (a kind gift of Gillian Murphy, University of East Anglia, United Kingdom) was added to some pellets. Samples were always compared on the same CAM to avoid egg to egg variability. Responses were graded as >, =, or < by independent readers.

**RESULTS**

**SFD Mutant (S156C)-Timp-3 Protein Expressed in Human RPE Cells Is an Inefficient Timp—**To mimic the RPE cells in SFD, we generated stable human RPE cell lines expressing WT or mutant (S156C) Timp-3. The stable expression of protein was confirmed by Western blot analysis using polyclonal antibodies to Timp-3 (Fig. 1a) and was quantified by scanning densitometry (Fig. 1b). Like the endogenous Timp-3 in RPE cells, both wild type and mutant forms of Timp-3 were expressed predominantly in the ECM (Fig. 1, a and b) with a small fraction being secreted into the conditioned medium (Fig. 1, c and d). Wild type and mutant Timp-3 proteins were expressed at levels 3–5-fold over that of endogenous Timp-3 present in ARPE-19 cells. Antibodies to the FLAG epitope also confirmed the expression of these proteins in the ECM (Fig. 1e). MMP inhibitory activity in the ECM of these cell lines was analyzed by reverse zymography (Fig. 1f) and quantified by scanning densitometry (Fig. 1, g and h). Mock-transfected RPE cells (pcDNA3.1 alone) expressed endogenous functional Timp-3 inhibitor in the ECM with an approximate molecular mass of 24 kDa corresponding to the protein seen on Western blots (Fig. 1a) as well as the 27 kDa glycosylated form (Fig. 1f). This inhibitory activity was increased in cells that were transfected with the wild type Timp-3 construct. The glycosylated as well as the nonglycosylated forms of mutant (S156C)-Timp-3 in the ECM demonstrated reduced MMP inhibitory activity when compared with WT-Timp-3 (Fig. 1, g and h).

We hypothesized that a reduction in Timp activity in the ECM of RPE cells expressing mutant Timp-3 may affect the protease activity secreted by these cells. Gelatin zymograms were used to test the gelatinase activity in the conditioned medium, lysates, and ECM of the transfected RPE cells. Endogenous 72-kDa gelatinase (pro-MMP-2) activity was observed in the conditioned medium of mock-transfected RPE cells (Fig. 2a). Densitometric quantitation (data not shown) determined a marked increase in the expression of this activity in the conditioned medium (60.4%) (Fig. 2a), cell lysates (57.2%) (Fig. 2c), and ECM (72%) (Fig. 2e) of cells expressing S156C mutant Timp-3. Like other MMPs, gelatinase is secreted as an inactive proenzyme, and its cell-mediated activation requires binding of the C-terminal domain of the proenzyme to a metalloproteinase complex of the membrane-type matrix metalloproteinase MT1-MMP and Timp-2. Subsequent sequential proteolysis of the propeptide by MT1-MMP and gelatinase A is believed to generate the active form of gelatinase A, and this process can be regulated by Timp-2 or Timp-3 (20). Concanavalin A (ConA) can induce clustering of MT1-MMP, which plays a role in the acti-
vation of MMP-2 in other systems (21). To study the potential role of mutant TIMP-3 in this process, RPE cells were treated with ConA, and the gelatinase activity in the conditioned medium (Fig. 2b) and cell lysate/ECM (Fig. 2d) fractions were examined by gelatin zymograms. RPE cells expressing mutant TIMP-3 showed an induction of the active forms in the conditioned medium (62 and 43 kDa) and lysate/ECM (62 kDa) (Fig. 2, b and d, respectively). MMP-9 activity was observed only in the conditioned medium of cells expressing mutant TIMP-3 (Fig. 2, a and b). Active MMP-2 in the conditioned medium of these cells was also quantitated with a gelatinase activity assay utilizing a biotinylated gelatinase substrate. This assay (Table I) confirmed an increase in the gelatinase activity in the conditioned medium of RPE cells expressing S156C TIMP-3 in the absence or presence of ConA.

Thus, in terms of MMP activity, expression of S156C TIMP-3 in RPE cells induced an increase in both pro-MMP-2 and active MMP-2 in the conditioned medium, lysate, and ECM of the cells possibly as a result of attenuated MMP inhibitory activity.
of the expressed mutant TIMP-3 protein. Since there is no decrease in the expression of MMP-2 in cells overexpressing WT-TIMP-3, the possibility that the increase in MMP-2 activity in the cells expressing mutant TIMP-3 may be a direct induction or activation of gelatinase A by the mutant TIMP-3 protein cannot be ruled out.

Expression of SFD Mutant (S156C) TIMP-3 Protein in RPE Cells Results in Altered Cell Adhesion, Migration, and Invasion—Quiescent RPE cells rest on the inner aspect of Bruch’s membrane, which contains collagen type IV and laminin. MMPs play a key role in the regulation of a variety of cellular functions. The adhesion of RPE cells to ECM and the migratory and invasive properties of the cell are critical for the maintenance of their physiological phenotype. RPE cell attachment was measured on laminin (Fig. 3a). Overexpression of WT-TIMP-3 had no effect on the adhesion of RPE cells to laminin relative to the mock-transfected cells. However, expression of S156C TIMP-3 resulted in a small but statistically significant decrease in adhesion to laminin (Fig. 3a). Migration of S156C TIMP-3-expressing RPE cells toward laminin (Fig. 3b) as well as 10% fetal bovine serum (Fig. 3c) was significantly increased. Invasive capability of RPE cells through matrigel and collagen

| RPE cells | Untreated | ConA |
|-----------|-----------|------|
| Vector    | 18.5 ± 1.3 | 40.1 ± 2.9<sup>a</sup> |
| WT-TIMP-3 | 17.7 ± 0.5  | 39.9 ± 3.0<sup>a</sup> |
| S156C TIMP-3 | 22.3 ± 0.5<sup>a</sup> | 53.4 ± 3.6<sup>a,b</sup> |

<sup>a</sup> p < 0.01 versus untreated.
<sup>b</sup> p < 0.01 versus wild type.
<sup>c</sup> p < 0.05 versus mock.

Fig. 3. Adhesion, migration, and invasion of WT-Timp-3- (WT-1 and WT-5), S156C-Timp-3- (156-1 and 156-3), and vector alone (C1 and C3)-transfected ARPE-19 cells. a, adhesion of transfected cells on laminin; b and c, migration of RPE transfectants toward laminin (b) and 10% fetal bovine serum (c). d and e, invasion of RPE transfectants through matrigel (d) and collagen type I (e). Data are expressed as the mean of triplicate samples ± S.D. and are representative of two independent experiments.
type I matrix revealed an increase in the invasive potential of RPE cells expressing S156C TIMP-3 (Fig. 3, d and e, respectively). Overexpression of WT-TIMP-3 resulted in a slight decrease in invasion through both matrigel and the collagen matrix. Thus, expression of S156C TIMP-3 in the RPE resulted in attenuated adhesion to ECM with a concomitant increase in the migratory and invasive potential.

**S156C TIMP-3 Mutation Induces Angiogenic Potential in RPE Cells**—Choroidal neovascularization (CNV) is a major complication of Sorsby’s fundus dystrophy. RPE cells are believed to play a key role in the pathogenesis of CNV (22–24). Therefore, we tested the angiogenic potential of RPE cells expressing the S156C TIMP-3 mutation. We first examined the ability of the conditioned medium of RPE cells to induce migration and proliferation of endothelial cells. Conditioned medium from RPE cells expressing S156C TIMP-3 increased the migration of microvascular endothelial cells when compared with mock-transfected as well as WT-TIMP-3-expressing RPE cells (Fig. 4a) but had no effect on their proliferation (Fig. 4b).

We also evaluated the effects of the conditioned medium on angiogenesis using the CAM assay. Interestingly conditioned medium from cells expressing S156C TIMP-3 showed an increase in the neovascularization response compared with that induced by WT-TIMP-3 (in 89% of the CAMs tested, n = 18) and control transfected cells (in 65% of the CAMs tested, n = 17) (Fig. 4, c, d, and e). Addition of recombinant human TIMP-3 to the S156C TIMP-3-conditioned medium inhibited the angiogenic response (Fig. 4f). These results are consistent with the data obtained from the migration experiments and suggest that the SFD mutant (S156C TIMP-3) induces a proangiogenic phenotype in RPE cells.

**DISCUSSION**

The RPE is believed to play a modulating role in the maintenance of a vascularized and highly permeable fenestrated choriocapillaris on its outer basal side. *In vivo* studies as well as clinical observations have determined that the absence of RPE can lead to secondary atrophy of the choriocapillaris (25–27). Polarized secretion of vascular endothelial growth factor by the RPE toward the basal surface where its receptor KDR (vascular endothelial growth factor receptor-2) is localized on the inner choriocapillaris suggests a possible mechanism for trophic paracrine signaling between RPE and choriocapillaris (28). CNV is an important pathological feature of eye diseases such as Sorsby’s fundus dystrophy and the more common age-related macular degeneration. During the course of CNV, encapsulation of growing vessels by RPE coincides with the stasis of further vascular growth (29). This suggests that the RPE might play a critical role in controlling the initiation and progression of CNV.

A precise spatial and temporal regulation of extracellular proteolysis is required for neovascularization. Recent studies have indicated that members of the MMP family play an important role in angiogenesis (30–33). Mice deficient in MMP-2 and MMP-9 exhibit reduced angiogenesis *in vivo* (34, 35). MMP-2 is the most widely distributed MMP and is localized on
Expression of S156C TIMP-3 in human RPE cells results in a reduction of MMP inhibitory (TIMP) activity in the ECM. Recombinant S156C TIMP-3 generated in NSO myeloma cells has association rate constants for a range of MMPs that are 2–3-fold reduced compared with wild type TIMP-3 (40). However, the decrease in TIMP activity in RPE cells is in contrast to that reported previously with expression in COS-7 and baby hamster kidney cells (10, 40). In both of these cell types, high levels of expression of mutant TIMP-3 protein gives rise to dimers, which are not present in our study with RPE cells. We expressed protein at relatively low levels in cells that expressed endogenous wild type protein to mimic the heterozygous autosomal dominant phenotype. That expression from the choriocapillaris by inhibiting MMP activity, results in an intact matrix, and/or binding directly to the surface of angiogenic blood vessels (36). TIMP-3, a regulator of MMPs, is deposited by RPE cells into Bruch’s membrane where it is a component of ECM (8, 37). We have previously demonstrated that TIMP-3 is a potent inhibitor of angiogenesis (17). Since CNV is a prominent feature of SFD, with accumulation of TIMP-3 deposits in Bruch’s membrane (7), we propose to examine the mechanisms by which mutations in the TIMP-3 gene lead to the characteristic ocular phenotype (38, 39).

In this study we show that the CNV phenotype seen in SFD may be a result of reduced MMP inhibitory activity with a concomitant increase in MMP-2 activity. This appears to provide the cells with the ability to induce angiogenesis as shown by both in vitro and in vivo assays. These results might explain the pathophysiological mechanism of CNV in SFD and suggest the potential use of MMP inhibitors locally in the subretinal space as a means of therapy.

Acknowledgments—We thank Jim Lang for photography and Suneel Apte for critical reading of the manuscript. We extend a sincere apology to colleagues whose work was not cited because of space limitations.

REFERENCES
1. Sorbey, A., and Joll Mason, M. E. (1949) Br. J. Ophthalmol. 62–77
2. Holz, F. G., Haimovici, R., Wagner, D. G., and Bird, A. C. (1994) Retina 14, 599–623
3. Jacobson, S. G., Cideciyan, A. V., Regunath, G., Rodriguez, F. J., Varnum, R. J., Feldman, V. E., and Stone, E. M. (1995) Nat. Genet. 11, 81–87
4. Kalms, H., and Seedhold, D. (1976) J. Med. Genet. 13, 271–276
5. Polkinghorne, P. J., Bird, A. C., Timp-3, in J. Clin. Ophthalmol. 32, 143–152
6. Bird, A. C. (1989) Arch. Ophthalmol. 113, 512–520
7. Blauweijers, H. G., Holtkamp, G. M., Putten, H., Witmer, A. N., Koolwijk, P., Partanen, T. A., Alitalo, K., Kroon, M. E., Kijlstra, A., van Hinsbergh, V. V., and Schlingemann, R. O. (1999) Am. J. Pathol. 155, 421–428
8. Miller, H., Miller, B., and Ryan, S. J. (1985) J. Clin. Investig. 76, 875–877
9. Ryan, S. J., and Schlingemann, R. O. (1998) Curr. Eye Res. 17, 1271–1277
10. Sakamoto, T., Sakamoto, H., Hinton, D. R., Spee, C., Ishibashi, T., and Ryan, S. J. (1995)Curr. Eye Res. 14, 621–627
11. Sakamoto, T., Sakamoto, H., Hinton, D. R., Spee, C., Ishibashi, T., Hinton, D. R., and Ryan, S. J. (1995) Arch. Ophthalmol. 113, 512–520
12. Blauweijers, H. G., Holtkamp, G. M., Putten, H., Witmer, A. N., Koolwijk, P., Partanen, T. A., Alitalo, K., Kroon, M. E., Kijlstra, A., van Hinsbergh, V. V., and Schlingemann, R. O. (1999) Am. J. Pathol. 155, 421–428
13. Miller, H., Miller, B., and Ryan, S. J. (1985) Invest. Ophthalmol. Vis. Sci. 27, 1644–1652
14. Hiraoka, N., Allen, E., Apel, I. J., Gyetko, M. R., and Weiss, S. J. (1998) Acta Anat. (Basel) 165, 365–377
15. Struve, L., Weingeist, A. C., and Zetter, B. (1997) Am. J. Pathol. 150, 155, 1048–1051
16. Leco, K. J., Waterhouse, P., Sanchez, O. H., Gowing, K. L., Poole, A. R., Wakeham, A., Mak, T. W., and Khokha, R. (2001) J. Clin. Investig. 108, 817–829
17. Yeow, K. M., Ishibashi, T., and Ryan, S. J. (1995)Curr. Eye Res. 14, 621–627
18. Sakamoto, T., Sakamoto, H., Hinton, D. R., Spee, C., Ishibashi, T., and Ryan, S. J. (1995)Curr. Eye Res. 14, 621–627
Angiogenesis in Sorsby's Fundus Dystrophy

41. Baker, A. H., George, S. J., Zaltsman, A. B., Murphy, G., and Newby, A. C. (1999) Br. J. Cancer 79, 1347–1355
42. Baker, A. H., Zaltsman, A. B., George, S. J., and Newby, A. C. (1998) J. Clin. Investig. 101, 1478–1487
43. Bond, M., Murphy, G., Bennett, M. R., Amour, A., Knauper, V., Newby, A. C., and Baker, A. H. (2000) J. Biol. Chem. 275, 41358–41363
44. Ahonen, M., Baker, A. H., and Kahari, V. M. (1998) Adv. Exp. Med. Biol. 451, 69–72
45. Ahonen, M., Baker, A. H., and Kahari, V. M. (1998) Cancer Res. 58, 2310–2315
46. George, S. J., Lloyd, C. T., Angelini, G. D., Newby, A. C., and Baker, A. H. (2000) Circulation 101, 296–304
Expression of Sorsby's Fundus Dystrophy Mutations in Human Retinal Pigment Epithelial Cells Reduces Matrix Metalloproteinase Inhibition and May Promote Angiogenesis

Jian Hua Qi, Quteba Ebrahem, Karen Yeow, Dylan R. Edwards, Paul L. Fox and Bela Anand-Apte

J. Biol. Chem. 2002, 277:13394-13400.
doi: 10.1074/jbc.M110870200 originally published online January 30, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M110870200

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 45 references, 17 of which can be accessed free at http://www.jbc.org/content/277/16/13394.full.html#ref-list-1