Amyloid Precursor-like Protein 2 Promotes Cell Migration toward Fibronectin and Collagen IV*

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Previous studies have established that in response to wounding, the expression of amyloid precursor-like protein 2 (APLP2) in the basal cells of migrating corneal epithelium is greatly up-regulated. To further our understanding of the functional significance of APLP2 in wound healing, we have measured the migratory response of transfected Chinese hamster ovary (CHO) cells expressing APLP2 isoforms to a variety of extracellular matrix components including laminin, collagen types I, IV, and VII, fibronectin, and heparan sulfate proteoglycans (HSPGs). CHO cells overexpressing either of two APLP2 variants, differing in chondroitin sulfate (CS) attachment, exhibited a marked increase in chemotaxis toward type IV collagen and fibronectin but not to laminin, collagen types I and VII, and HSPGs. Cells overexpressing APLP2-751 (CS-modified) exhibited a greater migratory response to fibronectin and type IV collagen than their non-CS-attached counterparts (APLP2-763), suggesting that CS modification enhanced APLP2 effects on cell migration. Moreover, in the presence of chondroitin sulfate, transfectedants overexpressing APLP2-751 failed to exhibit this enhanced migration toward fibronectin. The APLP2-ECM interactions were also explored by solid phase adhesion assays. While overexpression of APLP2 isoforms moderately enhanced CHO adhesion to laminin, collagen types I and VII, and HSPGs lines, especially those overexpressing APLP2-751, exhibited greatly increased adhesion to type IV collagen and fibronectin. These observations suggest that APLP2 contributes to re-epithelialization during wound healing by supporting epithelial cell adhesion to fibronectin and collagen IV, thus influencing their capacity to migrate over the wound bed. Furthermore, APLP2 interactions with fibronectin and collagen IV appear to be potentiated by the addition of a CS chain to the core proteins.

Amyloid precursor protein (APP) is the precursor of 39–43 amino acid polypeptides–Aβ, the major component of cerebrovascular and neuritic plaque amyloid deposits found in the brains of Alzheimer’s patients. APP is a member of a protein family including amyloid precursor-like proteins (APLP)-1 and -2 (1–5). Members of the APP/APLP family are type I integral membrane proteins that contain a single membrane-spanning domain with a large extracellular N-terminal domain and a short C-terminal cytoplasmic domain (1, 2). Both APP and APLP2 are ubiquitously expressed in mammalian tissues and cells and their in vivo roles largely remain to be determined (6, 7). APP and APLP2 are encoded by alternatively spliced mRNAs (2, 4, 5). One of the spliced exons has structural/functional homology to the Kunitz-type serine protease inhibitors (8). The other spliced exon encodes a 15- (APP) or 12- (APLP2) amino acid insert that disrupts a consensus sequence required for the addition of a chondroitin sulfate (CS) chain (9, 10). Hence, the isoforms of APP and APLP2 lacking these small polypeptide inserts are subject to CS modification. We previously showed that the majority of APLP2 molecules in rat corneal epithelium and in olfactory sensory axons are modified by the addition of a CS glycosaminoglycan (CSPG) chain (11, 12). Following wounding, the levels of APLP2 mRNA and protein are increased markedly in the basal epithelial cells that are actively migrating (11), implicating a role(s) for APLP2 in mediating epithelial migration during re-epithelialization.

Cell migration plays a central role in many biological processes, including embryonic development, wound healing, immunoresponses, and tumor metastasis. Cell migration requires a dynamic interaction between the cell, its substrate, and the cytoskeleton-associated motile apparatus (13, 14). Cell surface adhesion receptors serve to connect the substratum with the cytoskeleton, and thus they are central to the migratory process (14). The best characterized cell surface receptors for matrix components are the integrin family of proteins (15). Integrins play a key role in cell migration, both as receptors connecting the ECM to intracellular cytoskeletal proteins and as receptors transducing information from ECM to affect cell behavior (15, 16). Another group of cell surface molecules capable of binding ECM components are cell surface proteoglycans including the syndecans, CD44 and NG2. Syndecans, via their covalently attached heparan sulfate chains, bind fibronectin (FN), interstitial collagens, thrombospondin, and tenasin (17–19). Syndecans are thought to play important roles in cell-matrix and cell-cell adhesion, migration, and proliferation (20). CD44 binds several ECM components including hyaluronan (21), FN (22), and collagen type IV (23), and it is linked to the cytoskeleton by ezrin (24) and ankyrin (25). Another cell surface CSPG is NG2 (26, 27). NG2-expressing B28 glioma cells exhibited a greater migratory response toward type VI collagen.

trix; HSPG, heparan sulfate proteoglycan; FN, fibronectin; PBS, phosphate-buffered saline; BSA, bovine serum albumin.
than do non-NG2 expressing cells (28). These cell surface proteins, by serving as adhesion molecules, are thought to promote cell migration during normal development, in vitro tumor invasion, and wound repair. The APP family of proteins is known to interact with selected ECM proteins such as heparin sulfate proteoglycan (29), laminin (30), FN (31), and collagen (32). APP has also been shown to promote adhesion of a number of cell types in culture (33–35). Thus, the APP family of proteins might be components of a multidimensional mechanism for the regulation of spatial and temporal cell-matrix interactions during tissue morphogenesis and wound healing.

To investigate the role of APLP2 in cell adhesion and cell migration, we analyzed Chinese hamster ovary (CHO) cell lines overexpressing APLP2 isoforms and report in this article that APLP2 overexpression caused a significant increase in migratory response of CHO cells to FN and type IV collagen. The ability of APLP2 transfectants to migrate toward FN and type IV collagen is closely related to the increases in cell adhesion to these ECM proteins. These findings suggest that APLP2-ECM interaction may play a functional role in cell behavior. They also provide support for APLP2 to function in epithelial wound healing. Because the APP family of proteins is highly conserved and similarly processed (2), information about the biology of APLP2 should add to our knowledge regarding the functional role of these proteins.

EXPERIMENTAL PROCEDURES

Cell Lines—CHO cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Inc.) supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) (complete medium). The stable CHO lines expressing APLP2-751 and APLP2-763 were generated by co-transfecting APLP2 expression vector pSVAPLP2-751 (10) and pSVAPLP2-763 (9) with a neovector, respectively, and were maintained in complete DMEM containing 200 μg/ml Geneticin. Three APLP2-751-transfected cell lines, B2, C1, and D1 (10) and three APLP2-763-transfected cell lines D + 127, 8, and 16 were used. For control, untransfected parental and mock-transfected CHO cells were used.

Assays of APLP2 Overexpression and Identification of APLP2 at the Cell Surface—Cells were allowed to grow to confluence in 25-cm² flasks. The conditioned medium from each cell line was collected and cells were then washed twice with PBS. To determine relative levels of APLP2 in transfectants, CHO cells were lysed with a buffer containing 50 mM Tris, pH 8.0, 150 mM NaCl, 5 mM EDTA, 0.5% Triton X-100, and protease inhibitors (50 μg/ml pepstatin, 50 μg/ml leupeptin, 10 μg/ml aprotinin, and 0.25 mM phenylmethylsulfonyl fluoride). The homoge-

![Fig. 1. Immunoblot detection of cell surface APLP2.](image)

**Fig. 1.** Immunoblot detection of cell surface APLP2. To detect cell surface APLP2, CHO cells without transfection (lane 1), transfected with APLP2-763 (line D + 1216, lane 2) or APLP2-751 (line B2, lane 3) from one confluent 100-mm tissue culture dish were surface biotinylated. Biotinylated proteins were precipitated from subcellular fractions (cytosol, 100,000 x g supernatant; membrane, 100,000 x g pellet) with avidin-Sepharose and separated by SDS-polyacrylamide gel electrophoresis. APLP2 was detected by immunoblotting with rabbit polyclonal APLP2 antiserum D2II (1:1000 dilution) using enhanced chemiluminescence. The positions of molecular markers (myosin, 202 kDa; β-galactosidase, 109 kDa) are indicated at the right.

![Fig. 2. Migration of CHO cells toward ECM proteins as a function of APLP2 overexpression.](image)

**Fig. 2.** Migration of CHO cells toward ECM proteins as a function of APLP2 overexpression. Migration of cells overexpressing mouse APLP2-751 (■, line B2) or 763 (■, line D + 1216) and mock-transfected (□) or untransfected CHO cells (□) were analyzed using a modified Boyden chamber. Cells in serum-free DMEM were added to the upper chamber and allowed to migrate for 4 h through 8-mm porous membranes toward the lower chamber to which 20 μg/ml ECM proteins, as indicated, had been added. Motility was quantified by counting the number of cells that migrated to the undersides of the membrane. The results are averages of 12 random fields; error bars show standard errors. *, p < 0.0001, ANOVA followed by Scheffe’s multiple comparison procedure.
The results are averages of at least 12 random fields; error bars show standard errors.

FIG. 3. CHO cell lines expressing various levels of APLP2 isoforms and their migratory response toward fibronectin. A, three APLP2 transfectant cell lines for each APLP2 isoform were selected. To determine relative level of APLP2 (A), detergent lysates (5 μg/lane for APLP2 transfectants, lanes 2–7; or 20 μg/lane, control, lane 1) prepared from CHO cells grown in DMEM medium with G418 till confluence were incubated at 37 °C in the presence of chondroitinase ABC. After digestion, the samples were fractionated on SDS gels and immunoblotted using APLP2 antibody, D2II (A). Lanes 2–4 cell lines (designated D + 127, 8 and 16, respectively (10)) transfected with APLP2-763; and lanes 5–7 cell lines (designated B2, C1, and D1, respectively (10)) transfected with APLP2-751. B, cells in serum-free DMEM were added to the upper well of a Boyden chamber and allowed to migrate for 4 h through 8-mm porous membranes toward the lower chamber to which 10 μg/ml proteins had been added. Motility was quantified by counting the number of cells that migrated to the undersides of the membrane. The results are averages of 12 random fields; bars show standard errors.

FIG. 4. Migration of CHO cells that overexpress APLP2 isoforms toward fibronectin. Cells were allowed to migrate for 6 h toward lower chambers with fibronectin added at different concentrations, as indicated. Motility was quantified by counting the number of cells that migrated to the undersides of the membrane. ○, cells overexpressing APLP2-751 (line B2); ◯, cells overexpressing APLP2-763 (line D + 1216); △, control, CHO cells. The results are averages of at least 12 random fields; error bars show standard errors.

FIG. 5. Time course of migration toward type IV collagen of CHO cells expressing APLP2 isoforms. Cell migration assays were performed for control (A) and two cell lines expressing APLP2 isoforms (○, APLP2–751 line B2; ◯, APLP2-763 line D + 1216) using 10 μg/ml type IV collagen. After various times as indicated, cells that migrated to the underside of the membrane were quantified. The results are averages of at least 12 random fields; error bars show standard errors.

FIG. 6. Inhibition of migration of CHO cells that express APLP2-751 by chondroitin sulfate. CHO cells overexpressing APLP2-763 (line D + 1216), APLP2–751 (line B2), or control (untransfected CHO cells) were resuspended in DMEM containing 0.1% BSA and preincubated with medium alone (filled bars) or medium containing CS (open bars) for 20 min. Cells were then allowed to migrate for 4 h toward the lower chamber to which 10 μg/ml fibronectin had been added. Motility was quantified by counting the number of cells that migrated to the undersides of the membrane. The results are averages of at least 12 random fields; bars show standard errors. (*, p < 0.005, as determined by Student’s t test)
proteins were released from avidin-agarose by boiling for 5 min in Laemmli sample buffer and fractionated by SDS-polyacrylamide gel electrophoresis. Cell surface APLP2 was detected by Western blotting as described above. To determine the ratio of modified/non-modified APLP2, the image of autoradiograph was captured by the BDS Image System and analyzed by NIH Image 1.55-Gel Plotting Micros. Each desired band was marked; the image was acquired and plotted. The area beneath each plotted curve (APLP2 band intensity) was automatically calculated.

**Boyden Chamber Migration Assay**—Human plasma FN, laminin, and type I collagen were purchased from Collaborative Biomedical (Becton Dickinson, Franklin Lakes, NJ); types IV and VII collagen and heparan sulfate proteoglycan (HSPG) were purchased from Sigma. Migration assays were carried out in 12- or 48-well Neuroprobe chambers (Cabin John, MD). CHO cells were harvested by trypsinization, washed once with PBS + 10% FCS, and then washed twice with DMEM. Cells were resuspended in DMEM + 0.1% BSA and then added to the upper chamber (1.8 × 105 cells for 12-well chambers and at 7.8 × 103 cells for 48-well chambers). For blocking assay, chondroitin sulfate was preincubated with the cells before they were added to the upper compartment of the Boyden chamber. The lower compartment was filled with either DMEM containing 0.1% BSA as control, or with various extracellular matrix proteins at 20 μg/ml (Fig. 2). For subsequent fibronectin and collagen IV studies, 10 μg/ml protein or as otherwise indicated (see figure legends) were used. The two compartments of the Boyden chamber were separated by a polycarbonate filter (8 μm pore size, Poretics, Livermore, CA). Cells were allowed to migrate for 2–10 h at 37 °C in a humidified atmosphere containing 5% CO₂. The membranes were briefly fixed with methanol and stained with Diff Quick Stain (Dade Diagnostics, Aguada, Puerto Rico). Cells on the upper side of the filter were removed mechanically. The filters were mounted on glass slides and the number of cells that had migrated to the lower surface were counted or photographed and then counted on the micrograph. A random microscope field (×200 magnification) was counted per well. Each assay was carried out in 12 wells and repeated at least once.

Conditioned medium from CHO B2 cells was obtained from a confluent CHO culture (100-mm dish) that was maintained in 6 ml of serum-free medium (Opti-DMEM, Life Sciences) for 24 h. Under this condition, the maximum amount of secreted APLP2-751 molecules was accumulated in the media. One milliliter of collected medium was dialyzed twice against 200 ml of DMEM + 0.1% BSA. The dialyzed medium was used to resuspend CHO cells and added to the lower chamber to determine the effects of secreted APLP2 molecules on cell migration.

**Cell Adhesion Assay**—Purified matrix proteins (10 μg/ml, Fig. 7) tested included collagen I, collagen IV, collagen VII, FN, laminin, and HSPG. To test concentration dependence, 0.5–10 μg/ml fibronectin or collagen IV was used with 1% BSA as control. Matrix proteins were diluted in PBS and 100-μl aliquots were added to the wells of Nunc Immuno plates. Proteins were allowed to absorb overnight at 4 °C. The wells were then washed three times with PBS and nonspecific binding sites were blocked for 1 h with 1% BSA in PBS at 37 °C. Cells were detached by treatment with 0.25% trypsin-EDTA, washed once with DMEM, 104 cells for 48-well chambers) or APLP2–751 (line B2) were resuspended in regular DMEM or medium containing secreted APLP2, then allowed to migrate toward the lower chamber containing FN or collagen IV with and without secreted APLP2, respectively. Motility was quantified by counting the number of cells that migrated to the underside of the membrane. The data were presented as averages of 8 random fields with standard errors. *, p < 0.005; **, p < 0.01, as determined by paired Student’s t test.

**RESULTS**

**Expression and Post-translational Modification of APLP2 in CHO Cells Transfectants**—Naive CHO cells express detectable levels of APLP2 molecules. We previously reported that permanent CHO cell lines transfected with cDNAs encoding mouse APLP2-751 and APLP2-763 have markedly increased levels of APLP2 (see Refs. 9 and 10, and also, Fig. 3). APLP2-751 in transfected cells were modified by addition of CS chains of various lengths (10, 11). Furthermore, the cell lines overexpressing APLP2 also release ectodomain fragments derived from corresponding APLP2 precursor isoforms into the conditioned media (10). Cell surface localization of APLP2 in CHO transfectants was determined in experiments in which cell surface proteins were biotinylated briefly (5 min at room temperature) and precipitated with avidin-agarose from high speed pellet or cytosol fractions (Fig. 1). While no APLP2 immunoreactivity was detected in cytosol, APLP2 was detected for three cell lines at the surface of live CHO cells (Fig. 1, membranes, lanes 1, 2, and 3). The levels of APLP2 in the fraction of cell surface proteins from APLP2 transfectants (Fig. 1, cell line D + 1216, lanes 2 and line B2, lane 3) were markedly higher than that from untransfected cells (lane 1). Band intensity analysis with original x-ray film revealed in lane 3 (membrane) ~60% of APLP2 staining was in ~120–200 kDa (CS modified) forms. Similar results were obtained when biotinylation was performed at 4 °C for 30 min (data not shown).

**Migration Studies**—Our in vivo wound healing study suggested that APLP2 isoforms might be involved in mediating epithelial sheet migration (11). To determine whether APLP2 affects the ability CHO cells to migrate in response to ECM proteins, we used Boyden chamber assays to compare the rel-
ative ability of parental or mock-transfected cells, cells overexpressing APLP2-763 (non-CS-modified) and cells overexpressing APLP2-751 (CS-modified) to migrate toward a variety of ECM components (Fig. 2). For these assays, ECM proteins were placed in the lower chamber of the Boyden apparatus to serve as a soluble chemoattractant. As shown in Fig. 2, ECM proteins collagen type I and VII, laminin, and HSPG had low stimulative effects on CHO cell migration. Overexpression of APLP2 did not alter the migratory response of CHO cells toward these proteins. On the other hand, while the control cells exhibited low migratory responses to type IV collagen, APLP2-transfected cell lines exhibited significantly increased migration toward this basement membrane-specific collagen ($p < 0.0001$). We observed approximately 1.7 times more cells crossing the membrane for APLP2-763 cells and 3.6 times more for cells expressing APLP2-751, compared with control CHO cells. Among the ECM proteins tested, FN had the highest stimulatory effects on CHO migration. CHO cells overexpressing APLP2 displayed markedly enhanced migration toward FN ($p < 0.0001$), being 1.8 times greater for APLP2-763 and 2.5 times greater for APLP2-751 cells when compared with parental or mock-transfected cells (Fig. 2). Furthermore, while overexpression of both isoforms of APLP2 promoted CHO cell migration toward collagen IV and FN, cells overexpressing APLP2-751 exhibited a significantly greater (~2.1 times toward collagen IV and ~1.4 times toward FN, $p < 0.0001$) migratory response to these ECM proteins than those overexpressing APLP2-763.

Several lines overexpressing each isoform were generated. Although these cell lines expressed different levels of APLP2 as determined by chondroitinase digestion and Western blotting (Fig. 3A), all cell lines expressing the same isoform (lines D 127, 8 and 16 expressing APLP2-763; lines B2, C1, and D1 expressing APLP2-751) exhibited similar levels of enhanced cell migration toward FN (Fig. 3B), indicating that the migratory response of these cell lines correlates with the isoform expressed but not with the levels of APLP2 overexpression.

Fig. 4 shows migration of CHO cells toward different concentrations of FN. All three cell lines exhibited increasing migration with increasing FN concentration. The pattern of APLP2 isoforms promoting CHO migration, APLP2-751 > APLP2-763 > control cells, was observed at all concentrations tested. Fig. 5 shows that the increased migration of APLP2-transfected CHO cell lines, when compared with control cells, toward type IV collagen can be observed within the first 2 h in the modified Boyden chamber assays, but was more obvious after longer incubations (6–10 h). This effect was much more pronounced in cells overexpressing CS-modified APLP2 molecules.

To test whether CS modification contributes to the effects of APLP2 molecules on promoting CHO cell migration, we preincubated cell suspensions with chondroitin sulfate (20 µg/ml) before adding cells to the upper chambers in the migration assays (Fig. 6). Clearly, chondroitin sulfate interfered with FN-mediated migration of CHO cells expressing CS-APLP2 (2.84 times reduction, $p < 0.005$), but had no effect on parental or cells expressing APLP2-763. These results suggest that modification of APLP2 by addition of CS chains potentiates the effects of APLP2 core protein on CHO cell migration.

Transfected CHO cells release abundant APLP2 over time by ectodomain shedding and these secreted molecules may play a role in enhancing CHO cell migration. To determine the effects of secreted APLP2 on migration, we added dialyzed conditioned medium from 24-h cultures of APLP2-751-transfected cells to

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**Fig. 8. Adhesion of CHO cells to ECM proteins as a function of overexpression of APLP2.** CHO cells overexpressing APLP2-763 (Ⅲ, line D + 1216), APLP2-751 (Ⅱ, line B2), or control (Ⅳ, untransfected CHO cells) were seeded onto microtiter plates coated with ECM proteins (10 µg/µl). After allowing cells to attach for 30 min at 37 °C, nonadherent cells were removed by washing. Adherent cells were fixed and stained with crystal violet and adhesion was quantified by reading the optical density at 570 nm. Mean data from at least eight independent experiments are shown. Error bars represent standard deviations. C, collagen; LN, laminin; ✞, $p < 0.0001$, ANOVA; *, $p < 0.0001$; **, $p < 0.001$; ***, $p < 0.01$, as determined by Scheffe’s test for pairwise comparison.
the migration assay (Fig. 7). This medium contains abundant
CS-modified secreted APLP2 molecules. If secreted APLP2 enhances CHO cell migration, one would expect addition of soluble APLP2 in upper well to increase parental CHO cell migration. Instead, migratory response of CHO cells and APLP2 transfected cells toward collagen IV and FN was significantly decreased (p < 0.005, for APLP2-763 toward collagen IV, p < 0.01) when soluble APLP2 was added both wells of a Boyden chamber, suggesting that soluble APLP2 alone is not sufficient to enhance CHO cell migration and that CS-APLP2 may have inhibitory effects on CHO migration over these ECM proteins.

Adhesion Studies—To determine if the increased migration of APLP2 transfecteds was due to altered cell adhesion to collagen IV and FN, APLP2 expressing CHO cells were evaluated in standard cell adhesion assays (Fig. 8). We first determined to which ECM proteins CHO cells expressing APLP2 adhere. CHO cells, both parental and mock-transfected, exhibited limited adhesion to 10 μg/ml type I and VII collagens, laminin, and HSPG and overexpression of APLP2, especially CS-modified molecules, resulted in an increase in adhesion of CHO cells to these ECM proteins (p > 0.01 for group comparison with ANOVA). Cells expressing APLP2 had markedly enhanced adhesion to collagen IV (p < 0.0001, 2 times more for cells expressing APLP2-763 and 4 times more for cells expressing APLP2-751 when compared with parental cells). CHO cells had the highest capacity to adhere to FN and cells overexpressing APLP2 exhibited significantly increased CHO cell adhesion (p < 0.0001 overall comparison, ~1.85 times more for cells expressing APLP2-763, p < 0.001, and ~2.3 times more for cells expressing APLP2-751, compared with control CHO cells, p < 0.0001). Here, the effects of CS modification on APLP2 enhancing CHO cell adhesion (1.24 times greater, p < 0.01) was similar, but not exactly proportional to its effects on enhancing cell migration (1.39 times greater, Fig. 2). Taken together, the ability of APLP2 to promote CHO cell migration is consistent with its capacity to enhance CHO cell adhesion.

We also examined the concentration-dependent adhesion of CHO cells overexpressing APLP2 to type IV collagen and FN (Fig. 9). CHO cells exhibited detectable adhesion to 2 μg/ml type IV collagen. However, much lower concentrations (0.5 μg/ml) of type IV collagen supported adhesion by the CHO cells overexpressing APLP2, especially CS-APLP2. At all concentrations tested, adhesion by APLP2-expressing cells was greater than that seen with native CHO cells and APLP2-751 expressing cells exhibited increased adhesion relative to cells expressing APLP2-763 (OD570 0.178 for APLP2-751 versus OD570 0.127 for APLP2-763, 10 μg/ml collagen IV). Similarly, both cell lines exhibited adhesion to FN at concentrations as low as 0.5 μg/ml, markedly above the level seen for untransfected cells and the difference is more obvious when FN concentration was 1 μg/ml or higher. Adhesion to FN by CHO cells overexpressing APLP2-751 (OD570 0.334, 10 μg/ml FN) was greater than that of cells overexpressing APLP2-763 (OD570 0.272, 10 μg/ml FN).

**DISCUSSION**

We previously described that APLP2, an APP-related protein, was post-translationally modified by the addition of CS chains. APLP2 accumulated in the migratory corneal epithelia and in the olfactory sense neurons which are in a state of continual turnover (11, 12). Here, we present evidence that APLP2 promotes cell migration toward selected substrates. Using CHO cells overexpressing APLP2 we report that APLP2 enhances CHO cell migration toward FN and collagen IV. Furthermore, by overexpressing CS-modified or non-CS containing isoforms of APLP2 by transfecting CHO cells with alternatively spliced cDNAs of APLP2, our system allowed for comparison of the effects of CS modification on cell migration toward ECM proteins. Cell expressing APLP2-751 that were modified by addition of a CS GAG chain exhibited a greater increase in enhanced migration toward FN and collagen IV when compared with those expressing non-modified APLP2, suggesting that both the core protein and the GAG chain are important for cell migration in these transfected cells. Similarly, cells overexpressing APLP2 isoforms also exhibited a marked increase in adhesion on FN and type IV collagen. Thus, we propose that APLP2 supports epithelial cell adhesion to the ECM proteins FN and collagen IV and may influence cell migratory property in vivo.

The biological significance of APLP2 is underscored by the observations that FN and collagen IV stimulate corneal epithelial cell migration in vitro and in vivo (36, 37). FN is a key component of the provisional matrix during wound repair (38). It is a multifunctional cell adhesion protein and its suggested functions include mediating cellular adhesion, promoting cell migration, and helping to regulate cell growth and gene expression (16). In the cornea, FN normally is not present in the basement membrane; however, as a result of injury, it accumulates in the wound region (39). Collagen IV is a unique collagen providing scaffold for basement membrane (40, 41). In addition to serving a structural role, type IV collagen interactions with cells have been implicated in affecting processes such as cell adhesion, migration, and differentiation (41). Increased levels of type IV collagen are found to occur along certain pathways during development (42, 43), tissue remodeling after injury (44, 45), and tumor invasion (46, 47). In normal adult

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2 F-S. X. Yu and X-F. Li, unpublished results.
cornea, α3/α4(IV) chains of collagen IV are found in the epithelial basement membrane (48, 49). However, wounding induces a switch from α3/α4(IV) to α1/α2(IV) (50). Interestingly, a heparin-binding peptide derived from the α1 chain of collagen IV promotes corneal epithelial cell adhesion and migration (37, 51). Hence, newly expressed type IV collagen such as α1/α2(IV) isoforms may effectively promote epithelial cell migration using a mechanism involving cell surface adhesion receptors such as APLP2. Significantly, the changes in the spatial and temporal expression of APLP2 take place concomitantly with changes in the ECM composition (39, 50), suggesting a close interplay of these two groups of molecules during wound healing. Thus, together with the present study showing that APLP2 molecules promote CHO cell migration toward FN and collagen IV, we suggest that during re-epithelialization, the wound-induced basal cell-specific APLP2 would interact with provisional ECM protein such as FN or α1/α2 type IV collagen accumulated in the wound bed and mediate cell migration to cover the defects. Since APLP2 was also found to be abundant in olfactory sensory axons, the sensory neurons that are in a state of continual turnover (12), it may be part of a mechanism regulating synaptogenesis during neuronal development.

What is the mechanism by which APLP2 facilitates cell migration? We showed that ECM proteins type IV collagen and FN, but not others, stimulate migration and support adhesion of APLP2 transfectants. Studies with cultured cells have shown a close correlation between cell-substratum adhesion and migration (13). The close correlation between cell adhesion and cell migration supported by APLP2 is in accordance with the notion that APLP2 may function as a cell surface adhesion receptor, promoting cell migration on FN and type IV collagen. Cell migration requires a dynamic interaction between the cell and its substrate. It is known that the well characterized integrins play a major role in cell substrate adhesion (15). Our study did not address the relationship between APLP2 and integrins in mediating cell adhesion and migration; it is, however, interesting to note that APP at the cell surface has been suggested to collaborate with integrins to enhance cell adhesion (52). We have also shown in CHO transfectants that mature APLP2 is found at the cell surface and undergoes proteolytic processing to release the large soluble ectodomain. Furthermore, secreted APLP2 alone apparently does not enhance CHO cell migration. Thus, in the complex multistep process of cell migration, APLP2 might function in initial epithelial cell-substratum interaction by binding to ECM components fibronectin and/or collagen type IV (52). It remains to be determined whether matrix interacting APLP2 is proteolytically cleaved (ectodomain shedding) as cells migrate. Furthermore, the observation that secreted CS-APLP2 in conditioned medium inhibits CHO cell migration over FN and collagen IV is intriguing. Further investigation into the role of secreted APLP2 isoforms with purifying protein in cell migration and/or adhesion will be important.

Our data also showed that cells overexpressing APLP2-751 exhibited a migratory response to FN and type IV collagen significantly larger than those expressing APLP2-763. Cellular responses to FN and type IV collagen are likely to have a complex molecular basis involving the interactions between multiple functional domains of these proteins and specific cell surface molecules. One such molecular interaction is between cell surface CSPG and the extracellular matrix of mammalian cells (53–55). Both FN and type IV collagen contain heparin-binding domains that are capable of interacting with cell surface proteoglycans including CSPGs (51, 56). Peptides corresponding to heparin-binding sequences of FN and type IV collagen promote chemotactic- and haptotactic-directed cell migration of a number of cell types, including corneal epithelial cells (36, 51). Thus, transmembrane APLP2 with CS-GAG attached might interact with the heparin-binding regions of FN and type IV collagen. This interaction may potentiate the effects of APLP2 in the CHO transfectants by further enhancing their migratory response to FN and type IV collagen. This may provide an explanation for the distinct response of cells overexpressing APLP2-751 to FN and type IV collagen. Hence, cells might use post-translational modification of APLP2 as a means to influence and modify their behavior. This would allow cells fine tuning of their response to changes in microenvironments. However, it is not clear why the addition of CS chains reduced APLP2-751 migration response to a level that is less than APLP2-763 and similar to the control. One possible explanation is while CS-GAG may facilitate APLP2 core-ECM interaction, unbound CS-GAG of APLP2-751 in the presence of exogenous CS chain may hinder APLP2 core protein from binding to collagen IV or fibronectin. As such, in the future it might be possible in certain clinical situations to use CS to manipulate cell-matrix interaction and cell migration in vivo.

In summary, our studies, for the first time, provide evidence that APLP2 serves as an adhesion molecule in cell-substrate interactions and promotes cell migration. APLP2 molecules are abundantly expressed in developing and migratory epithelia and in olfactory sensory axons. We suggest that APLP2 proteins play an important role in physiological processes such as morphogenesis and wound healing. A full description of the behavior of these intriguing molecules including APP under normal and pathological conditions awaits further study.

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