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SED1/MFG-E8: A Bi-Motif Protein That Orchestrates Diverse Cellular Interactions

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ABSTRACT

MFG-E8 was initially identified as a principle component of the Milk Fat Globule, a membrane-encased collection of proteins and triglycerides that bud from the apical surface of mammary epithelia during lactation. It has since been independently identified in many species and by many investigators and given a variety of names, including p47, lactadherin, rAGS, PAS6/7, and BA-46. The acronym SED1 was proposed to bring cohesion to this nomenclature based upon it being a *S*ecreted protein that contains two distinct functional domains: an N-terminal domain with two *E*GF-repeats, the second of which has an integrin-binding RGD motif, and a C-terminal domain with two *D*iscoidin/F5/8C domains that bind to anionic phospholipids and/or extracellular matrices. SED1/MFG-E8 is now known to participate in a wide variety of cellular interactions, including phagocytosis of apoptotic lymphocytes and other apoptotic cells, adhesion between sperm and the egg coat, repair of intestinal mucosa, mammary gland branching morphogenesis, angiogenesis, among others. This article will explore the various roles proposed for SED1/MFG-E8, as well as its provocative therapeutic potential. J. Cell. Biochem. 106: 957–966, 2009.

KEY WORDS: MFG-E8; SED1; CELL INTERACTIONS

In most species examined thus far, SED1/MFG-E8 occurs as a ~53 kDa glycoprotein that possess a cleavable signal peptide, followed by two N-terminal epidermal growth factor (EGF)-like repeats and two C-terminal Discoidin/F5/8C domains (referred to as F5/8C domains) (Fig. 1) [Stubbs et al., 1990; Larocca et al., 1991; Couto et al., 1996; Ogura et al., 1996; Andersen et al., 1997; Ensslin et al., 1998]. The second EGF domain also contains an arginine-glycine-aspartic acid (RGD) integrin-binding motif that engages $\alpha_v\beta_{3/5}$ integrin heterodimers to facilitate cell adhesion as well as induce integrin-mediated signal transduction [Andersen et al., 1997, 2000; Taylor et al., 1997; Ensslin and Shur, 2007; Raymond and Shur, in press]. Since the EGF domains are highly homologous to those that mediate binding between *Drosophila* Notch-1 and its ligand, Delta, it has been suggested that the EGF repeats may pair with one another to form SED1/MFG-E8 multimers, similar to the ability of EGF repeats to multimerize other types of cell adhesion molecules [Balzar et al., 2001].

The C-terminal F5/8C domains have sequence homology to the animal lectin discoidin and the C2 domain of blood coagulation Factor V and Factor VIII [Stubbs et al., 1990; Ogura et al., 1996]. Each F5/8C domain is composed of an eight-strand anti-parallel β-barrel, from which two or three hypervariable loops extend from the base [Shur et al., 2004; Lin et al., 2007; Shao et al., 2008]. The exposed amino acid residues that compose these hairpin loops dictate the protein's binding specificity. In some instances, such as in the discoidin protein from *Dictyostelium* and the chitobiase enzyme from *Arthrobacter*, these loops mediate binding to carbohydrate moieties on the surface of cells and in the extracellular matrix, whereas coagulation Factor V, Factor VIII, and the second C-terminal domain of SED1/MFG-E8 bind to anionic phospholipids of cellular membranes [Reitherman et al., 1975; Andersen et al., 1997, 2000; Macedo-Ribeiro et al., 1999; Pratt et al., 1999; Fuentes-Prior et al., 2002; Shi et al., 2004, 2008]. Crystallographic analysis suggests that hydrophobic residues of the F5/8C2 hairpin loops insert into the lipid bilayer, whereas basic residues at the base of the loop "dock" with the phospholipid headgroups [Macedo-Ribeiro et al., 1999; Pratt et al., 1999]. While both F5/8C domains of SED1/MFG-E8 maintain a similar overall structure to the C2 domains of the blood coagulation factors, the second F5/8C domain of SED1/MFG-E8 contains an amino acid substitution that results in considerably higher affinity for phosphatidylserine membranes than does the C2 domains of Factors V and VIII [Shao et al., 2008].

In mouse and rat, SED1/MFG-E8 also occurs as a ~66 kDa splice variant that includes an *O*-glycosylated, 37 amino acid (56 amino acid in rat) proline/threonine-rich sequence inserted between the second EGF domain and the first discoidin domain [Oshima et al., 1999; Burgess et al., 2006]. Expression of the two splice variants shows spatial and temporal specificity. For example, the 53 kDa variant is

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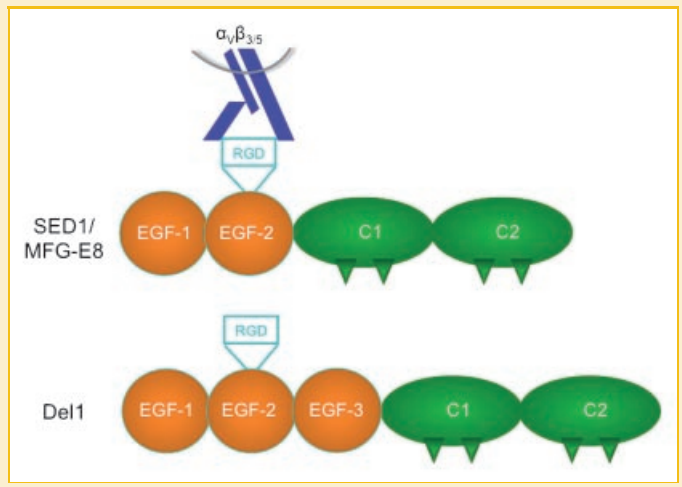

Fig. 1. Structural motifs of SED1/MFG-E8 and Del1. Both proteins contain Notch like-EGF repeats, the second of which possess an RGD integrin-binding motif, as well as two discoidin/F5/8 C domains that are able to bind phospholipid bilayers and/or extracellular glycosides via 2–3 hairpin loops projecting from the central barrel core. SED1/MFG-E8 and Del1 are believed to facilitate cell-matrix adhesion via RGD-dependent binding to cell surface $\alpha_v\beta_{3/5}$ integrin receptors, whereas the discoidin/F5/8C domain is also thought to bind to cell membranes via intercalation into the lipid bilayer. Alternatively, the discoidin/F5/8C domains may coordinate binding to extracellular glycoside substrates, similar to that seen in the sugar-binding discoidin domains. Del1 contains a third EGF repeat not found within SED1.

expressed in many tissues including the mammary glands of virgin female mice, whereas the 66 kDa variant shows limited tissue distribution and predominates during the late stages of pregnancy and during lactation [Oshima et al., 1999]. Nevertheless, the function of the proline/threonine rich insertion is unknown. It may increase the binding efficiency of SED1/MFG-E8 to phospholipids [Hanayama et al., 2002] and/or increase the efficiency of secretion [Oshima et al., 1999]. In this regard, it is interesting that when the two isoforms are expressed in epidermal keratinocytes, the long form is secreted into the culture supernatant, whereas the short form remains associated with the cells [Watanabe et al., 2005].

SED1/MFG-E8 CONTRIBUTES TO PHAGOCYTIC REMOVAL OF APOPTOTIC CELLS IN MANY TISSUES

MACROPHAGE REMOVAL OF APOPTOTIC LYMPHOCYTES

Given the known phospholipid binding specificity for the C-terminal F5/8C domains, it is not surprising that SED1/MFG-E8 has been identified as a key regulator of apoptotic cell removal in numerous systems [Hanayama et al., 2002; Leonardi-Essmann et al., 2005; Fens et al., 2008]. The first and best-studied example pertains to macrophage recognition of apoptotic lymphocytes in germinal centers. Upon activation, phagocytes secrete SED1/MFG-E8 as they encounter apoptotic cells. The C-terminal domains mediate attachment to phosphatidylserine and phosphatidylethanolamine residues exposed on the surface of the apoptotic lymphocyte, whereas the RGD motif binds to α_v integrins expressed on the advancing phagocyte [Hanayama et al., 2002; Leonardi-Essmann et al., 2005]. As expected, activated peritoneal macrophages harvested from SED1/MFG-E8-null animals have a reduced capacity to engulf apoptotic cells compared to wild-type [Hanayama et al., 2004]. Surprisingly though, these macrophages can still bind apoptotic cells suggesting that SED1/MFG-E8 is required for the process of engulfment rather than simply functioning as a molecular bridge between cellular debris and the phagocyte [Hanayama et al., 2004]. Mutation of the RGD motif to RGE has no effect on the protein's binding to apoptotic cells, which is mediated by the C-terminal domain; but rather blocks phagocyte engulfment, further implicating integrin binding in this processes [Asano et al., 2004]. Additionally, adult SED1/MFG-E8-null animals display characteristics of autoimmunity, including an enlarged spleen, a marked increase in serum antibodies against DNA and nuclear proteins, and glomerulonephritis, a condition caused by the deposition of circulating antibodies in the kidney [Hanayama et al., 2004]. These pathologies are phenocopied in wild-type mice injected with RGE-mutant SED1/MFG-E8, which serves as a dominant-negative protein inhibiting phagocytic engulfment of self-antigens [Asano et al., 2004].

Analogously, the human homolog of SED1/MFG-E8 (hMFG-E8) also binds phosphatidylserine and engages $\alpha_v\beta_3$ integrins [Yamaguchi et al., 2008]. As expected, low levels of hMFG-E8 enhance phagocytosis, however, at high concentrations, engulfment is inhibited in a dose-dependent fashion [Yamaguchi et al., 2008]. These results are of interest in light of the fact that some human patients suffering from the chronic autoimmune condition systemic lupus erythematosus have elevated serum levels of hMFG-E8 [Yamaguchi et al., 2008].

An interesting corollary has been suggested for microglial cells that assume a phagocytic role in the clearance of apoptotic neurons. Fractalkine is a chemokine expressed by non-hematopoietic cells, such as endothelia, neurons, and glial cells, that when released from the cell surface by the TACE protease, elicits a gradient-dependent chemoattraction of macrophages and other immune cells [Leonardi-Essmann et al., 2005]. Gene chip analysis of microglia transcripts following exposure to fractalkine revealed significant up-regulation of SED1/MFG-E8 [Leonardi-Essmann et al., 2005]. This raises the interesting hypothesis that fractalkine serves as both a chemoattractant leading microglia to damaged neurons, as well as a priming factor, inducing the production of SED1/MFG-E8 that

will subsequently mediate engulfment of the apoptotic cell [Leonardi-Essmann et al., 2005].

CLEARANCE OF MAMMARY EPITHELIAL CELLS DURING INVOLUTION

SED1/MFG-E8 performs a similar function during the clearance of apoptotic cells resulting from involution of the lactating mammary gland. During the first 48 h of gland involution, apoptotic cells are shed into the alveolar lumen where they are cleared by "nonprofessional" phagocytes, such as neighboring epithelial cells. During this time, SED1/MFG-E8-deficient mice show an increased level of apoptotic cells in the alveolar lumen, but the frequency of apoptotic cells in the alveolar epithelium is similar to control animals [Atabai et al., 2005]. This suggests that SED1/MFG-E8-null cells have impaired phagocytosis. Consistent with this, primary mammary epithelial cells from SED1/MFG-E8 mutants have significantly lower phagocytic potential when compared to controls. In later stages of involution, the residual milk fat globules and apoptotic cells, both of which have high phosphatidylserine content, are removed by macrophages that express high levels of SED1/MFG-E8. In the absence of SED1/MFG-E8, the apoptotic cells are not cleared efficiently and membranous material accumulates within the gland, leading to duct ectasia and mastitis [Hanayama and Nagata, 2005].

DIURNAL CLEARANCE OF PHOTORECEPTOR OUTER SEGMENTS

The phototransduction machinery of the retinal photoreceptors lies adjacent to the retinal pigmented epithelium (RPE), which expresses components that stabilize retinal adhesion as well as remove fragments of the photoreceptor outer segments (POS) that are shed daily. Previous studies indicated a requirement for $\alpha_v\beta_5$ integrin in both retinal adhesion and RPE-mediated phagocytosis of POS fragments [Nandrot et al., 2004, 2006]. Since SED1/MFG-E8 is a known ligand for $\alpha_v\beta_5$ integrins, and is localized to RPE, it raised the obvious possibility that SED1/MFG-E8 could be the "link" between the integrin receptor on RPE and shed POS fragments [Burgess et al., 2006].

The adhesion of the RPE to the neural retina is slightly (~20%), but significantly decreased in SED1/MFG-E8-deficient mice, coincident with the peak period of retinal adhesion [Nandrot et al., 2007]. Adhesion at non-peak times is unaffected in SED1/MFG-E8-deficient mice [Nandrot et al., 2007]. Since the loss of $\alpha_v\beta_5$ leads to a much greater loss of RPE-neural retina adhesion, the authors conclude that SED1/MFG-E8 does not likely play a major role in retinal adhesion, as does $\alpha_v\beta_5$ [Nandrot et al., 2007]. However, the mice used in these studies are not traditional SED1/MFG-E8 knockout mice, but rather, express SED1/MFG-E8 that is engineered to remain membrane-bound and not secreted, as is the wild-type protein [Atabai et al., 2005]. Consequently, the membrane-associated SED1/MFG-E8 may still be able to partially mediate intercellular adhesion via integrin binding [Nandrot et al., 2007]. Nevertheless, the lack of secreted SED1/MFG-E8 leads to a dramatic reduction in phagocytosis of outer segment markers [Nandrot et al., 2007]. Furthermore, RPE isolated from SED1/ MFG-E8-deficient mice show reduced (~50%) binding of POS, relative to RPE from control mice [Nandrot et al., 2007]. Finally, exogenous SED1/MFG-E8 can rescue the reduced binding of mutant RPE, as well as promote binding of POS to RPE cell lines [Nandrot et al., 2007]. These results support the notion that SED1/MFG-E8 is critical for the circadian removal of shed POS by the RPE [Nandrot et al., 2007]. It should be noted that other investigators were unable to confirm these studies using siRNA constructs to knockdown SED1/MFG-E8 mRNA in cultured RPE cells, as opposed to the use of genetically-engineered mice [Burgess et al., 2006].

CLEARANCE OF APOPTOTIC CELL DEBRIS IN ATHEROSCLEROTIC VESSELS

Recent data suggests that the opsonizing characteristics of SED1/MFG-E8 may also function in removal of pathogenic plaques. Atherosclerotic plaques accumulate cell debris, including apoptotic cells, which leads to plaque progression and disease. Phagocytic clearance of this apoptotic cell debris is critical for homeostatic maintenance and activation of anti-inflammatory pathways. To explore SED1/MFG-E8 function in the clearance of the atherosclerotic debris, *ladler*-/- mice, which are susceptible to atherosclerosis, were irradiated and reconstituted with either wild type or *Mfge8*-deficient bone marrow [Ait-Oufella et al., 2007]. After 8 weeks on an atherogenic diet, the mice reconstituted with wild-type bone marrow developed early lesions of atherosclerosis that were devoid of apoptotic cells [Ait-Oufella et al., 2007]. However, in mice reconstituted with *Mfge8*-null marrow, the frequency of apoptotic cells in lesions was markedly elevated, resulting in a 70% increase in lesion size [Ait-Oufella et al., 2007]. Interestingly, this phenotype is similar to that seen in $\alpha_v\beta_5$ deficiency, which participates in SED1/MFG-E8-mediated clearance of apoptotic cells [Weng et al., 2003]. Furthermore, the accumulated apoptotic debris is associated with decreased anti-inflammatory interleukins (IL-10) in the spleen, and increased IFNγ in both spleen and atherosclerotic arteries [Ait-Oufella et al., 2007]. These and other results suggest to the authors that SED1/MFG-E8 expression in bone marrow-derived cells is critical for maintenance of the normal systemic immune response [Ait-Oufella et al., 2007].

CLEARANCE OF Aβ AMYLOID PLAQUES IN ALZHEIMER'S DISEASE

A recent study reported that cultured astrocytes express SED1/MFG-E8, which can be found in their released exosomes [Boddaert et al., 2007]. Similarly, SED1/MFG-E8 expression is readily seen in astrocytes in cadaveric brains, but its expression is greatly decreased in the vicinity of Aβ plaques in brains from Alzheimer's disease (AD) patients. mRNA levels are also reduced by 35% in AD brains as compared to healthy controls [Boddaert et al., 2007]. Consistent with a possible casual relationship between SED1/MFG-E8 expression and the clearance of Aβ plaques, the authors report a direct interaction between recombinant MFG-E8 and the Aβ 1–42 peptide; substitution of the RGD motif with RGE inhibits interaction with the Aβ peptide [Boddaert et al., 2007]. More directly, FITC-conjugated Aβ peptide is taken up by blood-derived human macrophages, which are inhibited by incubation with anti-SED1/MFG-E8 antibodies [Boddaert et al., 2007]. Finally, peritoneal macrophages from SED1/MFG-E8-null mice have a severely reduced ability to phagocytose Aβ 1–42 peptide [Boddaert et al., 2007]. These data suggest that similar to resolution of atherosclerotic plaques, SED1/MFG-E8 serves a role in preventing the accumulation of Aβ amyloid.

SED1/MFG-E8 FACILITATES A NUMBER OF INTERCELLULAR INTERACTIONS

SED1/MFG-E8 MEDIATES SPERM-EGG BINDING

SED1/MFG-E8 was independently identified as a component of porcine sperm that has binding affinity for glycoproteins of the egg coat [Ensslin et al., 1998]. Sperm membranes where solubilized and applied to affinity columns containing immobilized egg coat glycoproteins [Ensslin et al., 1998]. Sequencing of the predominant eluted protein resulted in identification of porcine SED1/MFG-E8, originally named p47 [Ensslin et al., 1998]. The ability of SED1/MFG-E8 to function as a sperm receptor for the egg coat was confirmed by analysis of SED1/MFG-E8-null male mice, which are sub-fertile in vivo and whose sperm are unable to bind eggs in vitro [Ensslin and Shur, 2003].

Sperm acquire SED1/MFG-E8 during two phases of their development. The first evidence of SED1/MFG-E8 immunoreactivity occurs in the Golgi complex of spermatogenic cells, where it is presumably secreted onto the sperm surface [Ensslin and Shur, 2003]. However, the majority of sperm-associated SED1/MFG-E8 is derived from secretions of the initial segment of the epididymis, where it binds to the anterior dorsal aspect of the sperm plasma membrane, the area known to be responsible for mediating initial adhesion to the egg coat [Ensslin and Shur, 2003].

Studies with truncated proteins indicate that the gamete binding activity of SED1/MFG-E8 lies in the C-terminal F5/8C discoidin domains [Ensslin and Shur, 2003]. The function of the EGF domains in this circumstance remains uncertain, but it is thought that integrin binding does not participate in sperm-egg adhesion in mice [Ensslin and Shur, 2003]. One model for SED1/MFG-E8 function during sperm-egg adhesion suggests that the two different F5/8C domains have preferential binding affinities: the C2 domain binding to exposed phosphatidylserine residues on the sperm membrane, and the C1 domain binding to egg coat glycoproteins, analogous to the C1 domain of Del1, a SED1/MFG-E8 homolog that binds to the extracellular matrix (Fig. 2) [Ensslin and Shur, 2003; Shur et al., 2004; Hidai et al., 2007]. Since two EGF repeats are capable of mediating multimerization of other cell adhesion molecules, it is possible that SED1/MFG-E8 functions during sperm-egg binding as a dimer or multimer [Balzar et al., 2001; Ensslin and Shur, 2003].

More recent studies suggest that sulfation of SED1/MFG-E8 may also be important for its activity in sperm-egg binding. TPST-2 is a Golgi-localized sulfotransferase that mediates protein-tyrosine sulfation in the male reproductive tract [Hoffhines et al., 2008]. *Tpst-2* knockout mice are infertile, and their sperm exhibit reduced motility and fertilizing capability [Hoffhines et al., 2008]. SED1/MFG-E8 has been identified as substrate for TPST-2, and *Tpst-2* knockout mice produce non-sulfated SED1/MFG-E8, which suggests this post-translation modification is critical for its function in fertilization [Hoffhines et al., 2008].

MAINTENANCE OF THE EPIDIDYMAL EPITHELIUM

The loss of SED1/MFG-E8 from the epididymal epithelium, where it is normally secreted and associates with the sperm membrane, produces unexpected epididymal pathologies [Raymond and Shur, in press]. Most notable among these are detached epithelia and spermatic granulomas, which result from the exposure of sperm-associated antigens and a consequent immune response. This phenotype is suggestive of a tissue-intrinsic role in the epididymis, in addition to its role in sperm-egg adhesion. Using perfusion-based fixative procedures, SED1/MFG-E8 is found localized in basolateral domains of epididymal epithelial cells in vivo, and similarly, SED1/MFG-E8 is secreted both apically and basally from polarized

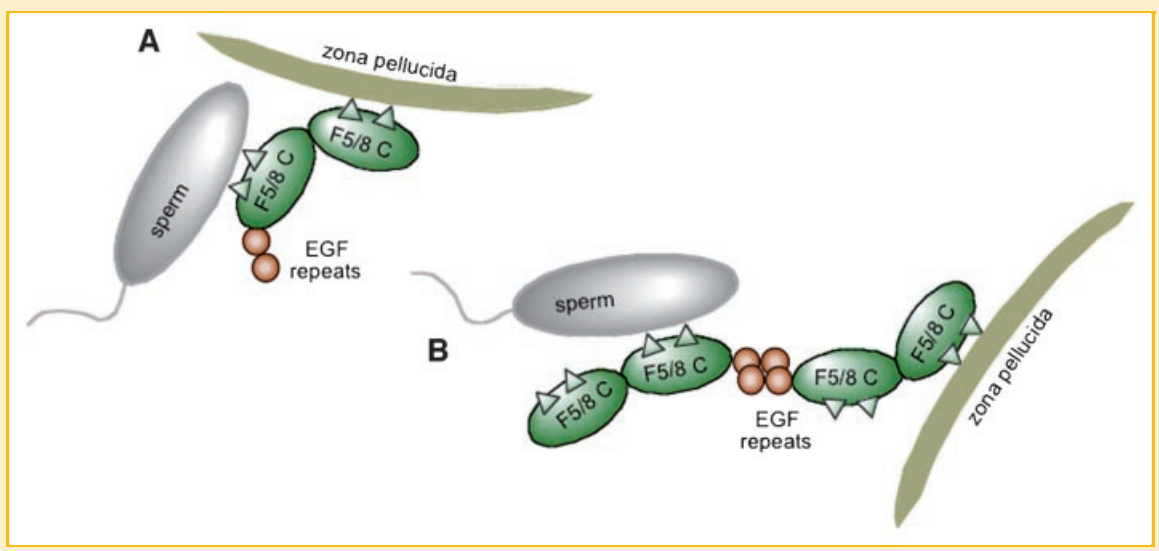

Fig. 2. Models for SED1/MFG-E8 function during sperm-egg binding. A: SED1/MFG-E8 binding to gamete surfaces appears to be mediated by the F5/8C domains, rather than the EGF repeats. Consequently, the simplest model suggests that SED1/MFG-E8 could function as a monomer, with one F5/8C domain binding to the sperm membrane and the other binding to the zona pellucida. B: Alternatively, SED1/MFG-E8 may facilitate sperm-egg binding as a dimmer, or oligomer, due to anti-parallel pairing of the EGF repeats, similar to that reported for the EGF domains of Ep-CAM [Balzar et al., 2001]. Reprinted from Ensslin and Shur [2003].

epididymal cells in vitro. The basolateral distribution of SED1/MFG-E8 suggested that it may play a role in epididymal cell adhesion. Quantitative in vitro assays demonstrate that SED1/MFG-E8 supports epididymal cell adhesion via RGD binding to α_V integrin receptors on epididymal epithelial cells. In support of these results, epididymal cells from SED1/MFG-E8-null males show reduced adhesion in vitro, a phenotype that can be rescued with exogenous SED1/MFG-E8. These results suggest that SED1/MFG-E8 may facilitate epididymal cell adhesion, and that its loss leads to breakdown of the epididymal epithelium and consequent development of spermatic granulomas [Raymond and Shur, in press].

MAINTENANCE AND REPAIR OF THE INTESTINAL EPITHELIUM

The epithelial lining of the gut undergoes continuous turnover, with stem cells located deep within the crypts being induced to differentiate into absorptive enterocytes and secretory cells as they migrate out of the crypt onto the villus surface. Not surprisingly, SED1/MFG-E8 is expressed in murine macrophages of the intestinal lamina propia, which led investigators to examine its potential role in repair and maintenance of the intestinal epithelium [Bu et al., 2007]. Unlike that reported in other systems, the addition of exogenous SED1/MFG-E8 accelerates the rate of migration of IEC-18 cells, an enterocyte cell line, in a PKC-dependent manner [Bu et al., 2007]. The exogenous SED1/MFG-E8 binds to the posterior region of the migrating cells, possibly to a patch of exposed phosphatidylserine. In vivo, SED1/MFG-E8 is found on crypt cells following injury, consistent with a potential role in epithelial repair. More directly, anti-SED1/MFG-E8 antibodies arrest the migration of BrdU-labeled cells out of intestine crypts, and a similar reduction in crypt cell migration is seen in SED1/MFG-E8-null mice [Bu et al., 2007]. As shown by others [Miksa et al., 2006, 2007], SED1/MFG-E8 expression is dramatically reduced following sepsis, which is also associated with reduced enterocyte migration from the crypts to the villus [Bu et al., 2007]. Remarkably, administration of SED1/MFG-E8 to septic mice restores crypt cell migration. How SED1/MFG-E8 promotes migration of intestinal epithelial cells is unclear, but may involve a relocalization of Arp2/3 and dissolution of actin-based stress fibers, along with the establishment of a new lamellipodia [Bu et al., 2007].

SED1/MFG-E8 FACILITATES MAMMARY GLAND BRANCHING MORPHOGENESIS

In addition to its role in the clearance of apoptotic cells during mammary gland involution, SED1/MFG-E8 also participates during development of the mammary gland. SED1/MFG-E8-null females show greatly diminished mammary glands, reflecting a severe reduction in the frequency of branching from both epithelial ducts and from terminal end buds, which are thin and poorly developed [Ensslin and Shur, 2007]. During normal development, the expanding epithelial tree develops from reciprocal inductive interactions between the two cell types that constitute the double-layered epithelial tube: luminal epithelial cells and myoepithelial cells. SED1/MFG-E8 is expressed by the epithelial cells where it binds to $\alpha_v\beta_{3/5}$ integrin receptors on myoepithelial cells, leading to MAPK activation and subsequent cell proliferation and duct outgrowth. The absence of SED1/MFG-E8 leads to a near total loss of MAPK activation in myoepithelial cells, with a concomitant reduction in cell proliferation and branching throughout the epithelial tree (Fig. 3) [Ensslin and Shur, 2007].

SED1/MFG-E8 AND ITS HOMOLOG DEL1 PROMOTE VASCULARIZATION

Development endothelial locus-1 (Del1) is a structural and functional homolog of SED1 that contains a signal sequence followed by three EGF-like, and two C-terminal F5/8C/discoidin-like domains. Identified via an enhancer-trap event in a transgenic mouse, Del1 is

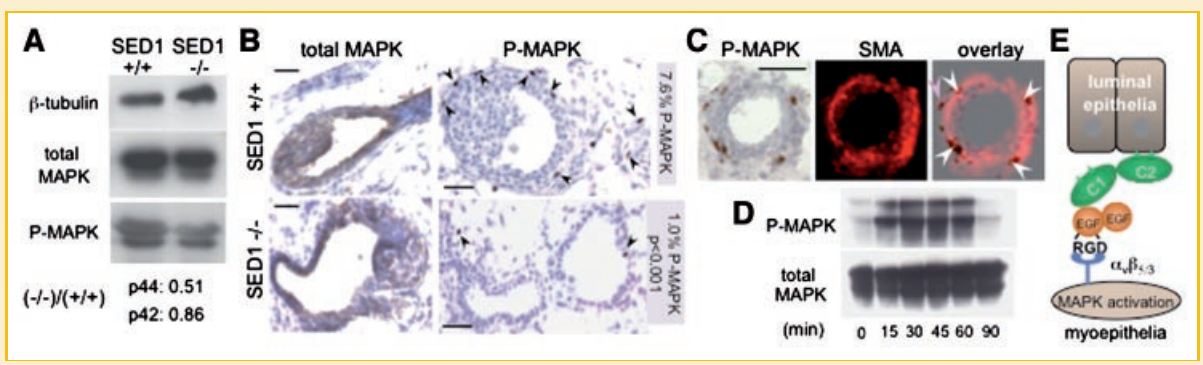

Fig. 3. The loss of SED1/MFG-E8 is associated with reduced MAPK activation in myoepithelial cells of the developing mammary gland. A: Although the level of total MAPK protein is similar between wild-type and SED1-null mammary gland organoids, as assessed by immunoblotting, the activated form of MAPK (P-MAPK) is greatly diminished in SED1-null organoids. Activation of the 44 kDa MAPK is reduced by nearly 50%, whereas activation of the 42 kDa isoform is closer to normal levels. B: MAPK (brown stain) is present in both the luminal and myoepithelial compartments, but activated MAPK (black arrows) is confined primarily to the myoepithelial compartment, consistent with the expression of α_v integrins on myoepithelial cells. MAPK activation is greatly reduced in the epithelial compartment of SED1-null organoids ($P < 0.001$). Bars = 0.05 mm. C: Most cells with activated MAPK colocalize with smooth muscle actin (SMA), indicative of myoepithelial cells (white arrows); whereas others lie adjacent or outside of the SMA reactivity (pink arrows). Bars = 0.05 mm. D: The addition of SED1/MFG-E8 to primary epithelial cell cultures leads to a transient activation of MAPK. E: Results suggest that SED1/MFG-E8 is secreted by myoepithelial and/or basally from luminal epithelial cells. The RGD motif within the second EGF repeat binds $\alpha_v\beta_{5/3}$ integrins on myoepithelial cells, whereas adhesion to luminal epithelial cells may be mediated by intercalation of the F5/8C domains into the phospholipid bilayers. Ligation of $\alpha_v\beta_{5/3}$ integrin induces MAPK activation in myoepithelial cells, leading to proliferation of the epithelial compartment, duct elongation and branching. Reprinted from Ensslin and Shur [2007].

secreted onto the extracellular matrix by endothelial cells during embryonic vasculogenesis [Hidai et al., 1998]. In vitro studies suggest that Del1 serves autocrine and paracrine roles by supporting the migration and proliferation of both endothelial cells and vascular smooth muscle cells [Penta et al., 1999; Rezaee et al., 2002]. In this regard, addition of Del1 to chick chorioallantoic membrane assays results in a potent pro-antigenic response suggesting a role in vascularization [Penta et al., 1999]. This is consistent with the ability of Del1 to increase vascular branching in the intestinal mesentery of transgenic mice that over-express Del1 [Hidai et al., 2005].

Further characterization indicates that Del1 serves as a ligand for $\alpha_v\beta5$ integrins, inducing aggregation of focal adhesion proteins and initiating intracellular signaling cascades including MAPK phosphorylation [Hidai et al., 1998; Penta et al., 1999; Rezaee et al., 2002]. Del1 is therefore thought to facilitate vascular wall development and vascular remodeling by directing endothelial angiogenesis, and migration and proliferation of vascular smooth muscle cells, in-part through activation of anti-apoptotic pathways [Penta et al., 1999; Rezaee et al., 2002]. Furthermore, endothelial derived Del1 has been reported to inhibit leukocyte adhesion to the endothelium, suggestive of an anti-inflammatory function for Del1 [Choi et al., 2008].

These findings are supported by the observation that endogenous Del1 is upregulated in ischemic hind limbs [Zhong et al., 2003]. Therapeutic delivery of Del1 to the ischemic tissue, either through protein-soaked implants or gene transfer, enhances disease recovery by increasing vessel formation, capillary density, vascular flow, and muscle function [Zhong et al., 2003; Ho et al., 2004]. Exogenous Del1 appears to first engage $\alpha_v\beta5$ integrins leading to upregulation of the Hox D3 transcription factor and subsequent increased expression $\alpha_v\beta3$ integrins [Zhong et al., 2003]. Activation of $\alpha_v\beta3$ integrins through binding to Del1 or other ligands is then thought to enhance vascular recovery by triggering an increase in endothelial cell proliferation [Zhong et al., 2003].

In an analogous manner, SED1/MFG-E8 may facilitate neovascularization in adult tissue through similar mechanisms. Unlike Del1, SED1/MFG-E8 is expressed in the vasculature of healthy adult tissues including the aorta and hindlimb muscles [Silvestre et al., 2005]. Ectopic expression of VEGF is pro-angiogenic in in vitro assays, and increases capillary densities and angiography scores in an ischemia recovery model in mice [Silvestre et al., 2005]. Importantly, ischemic muscle of SED1/MFG-E8-deficient mice is unaffected by VEGF over-expression, while over-expression of SED1/MFG-E8 in wildtype muscle induces recovery independent of VEGF [Silvestre et al., 2005]. These data suggest SED1/MFG-E8 is a downstream effector of pro-angiogenic VEGF signaling [Silvestre et al., 2005]. Further investigation into this pathway indicates that SED1/MFG-E8 serves as a ligand for $\alpha_v\beta3$ and/or $\alpha_v\beta5$ integrins that in turn elevates AKT phosphorylation, a known mediator of endothelial cell survival and proliferation [Silvestre et al., 2005].

While these data support the substantial potential for Del1 and SED1/MFG-E8 in therapeutic neovascularization, other studies indicate circumstances where inhibition of these proteins may also be desirable. Del1 was found to be misexpressed in naturally occurring adult, human tumor tissues [Aoka et al., 2002]. In a disease model developed to investigate this phenomenon, multiple tumor cell lines transfected with Del1 exhibit increased capillary density and growth, and reduced apoptosis during tumor formation in nude and syngeneic mice [Aoka et al., 2002]. Additional studies indicate that Del1 expression in tumor cells is activated by tumor-derived growth factors, including VEGF, and support a role for Del1 in pathological angiogenesis [Aoki et al., 2005]. Furthermore, SED1/MFG-E8 is found in murine tumor cell lines and is up-regulated in angiogenic islets and tumors of an in vivo model for pancreatic carcinogenesis [Neutzner et al., 2007]. Interestingly, SED1/MFG-E8-deficient mice exhibit only a modest reduction in tumor frequency and survival, and although vascular permeability and proliferation of the tumors are somewhat reduced, other pro-angiogenic signals, including FGF2 and Del1, are elevated suggesting tumorigenesis circumvented the requirement for SED1/MFG-E8 [Neutzner et al., 2007]. These data indicate that SED1/MFG-E8 and Del1 may serve similar roles in embryonic (Del1) and adult (SED1/MGF-E8) vascular modeling, and point to each being a candidate for endovascular enhancement as well as a target for anti-angiogenic/tumorigenic therapies.

FACILITATING EXOSOME-FUNCTION IN DENDRITIC CELLS

An interesting feature of SED1/MFG-E8 secretion in all cell types thus far examined is that it is found associated with exosomes—small membrane-bound vesicles released from the cell upon fusion of multivesicular bodies with the plasma membrane (Fig. 4) [Denzer et al., 2000]. In this regard, SED1/MFG-E8 is found as a predominant component of the mammary fat globule released into milk, as well as exosome-like vesicles called epididymosomes in the epididymis [Gatti et al., 2005]. Early studies of SED1/MFG-E8's association with exosomes suggested it may be important for the formation and/or secretion of exosome vesicles. For example, transfection of COS-7 cells with full-length SED1/MFG-E8 increases exosome secretion three-to fourfold [Oshima et al., 2002]. Moreover, expression of mutant constructs with either C-terminal F5/8C domain deleted abolishes both cell-surface and exosome localizations of the protein. The C2 domain, in particular, was found to be indispensible for SED1/MFG-E8-dependent increase in exosome secretion [Oshima et al., 2002].

Nevertheless, the significance of SED1/MFG-E8 association with exosomes remains obscure. It is presently unclear if SED1/MFG-E8 association with exosomes simply reflects its means of transport to the cell surface, or rather, has additional functional significance. In this regard, epididymosomes are thought to influence sperm maturation by bringing critical proteins in contact with the sperm plasma membrane, where they can transfer from vesicles to the plasma membrane in a zinc and pH-dependent mechanism [Sullivan et al., 2005]. However, most insight into the function of exosome-associated SED1/MFG-E8 has come from studies of dendritic cells.

Dendritic cells (DC) are professional antigen presenting cells that contribute to tolerance and immunity by accumulating antigens from their microenvironment. Circulating (immature), DC are highly phagocytic, engulfing antigens and processing them into peptides that are presented on the cell surface in association with Major Histocompatibility Complexes (MHC). Under homeostatic conditions, immature DC help to preserve tolerance; however, when an antigen of pathologic origin is detected, DC initiate a maturation

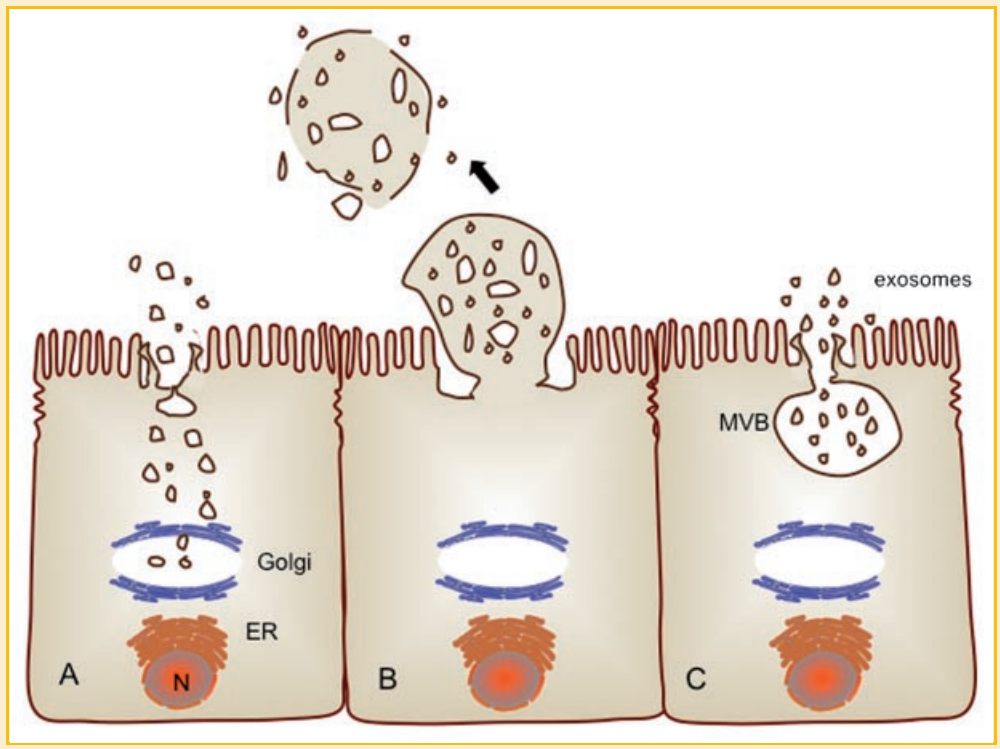

Fig. 4. SED1/MFG-E8 is secreted in association with exosomes. A: SED1/MFG-E8 may be secreted as a soluble protein through classical mechanisms, however SED1/MFG-E8's association with microvesicles suggests it is also secreted through non-classical pathways including (B) apocrine shedding, as occurs in the epididymis, and (C) fusion of multivesicular bodies (MVB) with the apical plasma membrane. Both apocrine shedding and MVB fusion result in the release of microvesicles, called exosomes, into the extracellular milieu.

process. The cell migrates to the lymph node, presenting antigen/MHC and various other co-stimulatory molecules to T cells. Although both immature and mature dendritic cells secrete exosomes, the components and activity of these vesicles are distinct, suggesting they have different functions depending on the cell's maturation state. In fact, exosomes secreted by mature DC are 50- to 100-times more effective at inducing a T cell immune response than are exosomes from immature DC [Segura et al., 2005]. On the other hand, exosomes from immature DC, which are known to carry antigen/MHC complexes, are thought to amplify the immune response by providing both immature and mature DC with additional unique antigens for presentation to T cells [Segura et al., 2005].

Not surprisingly, the discovery that SED1/MFG-E8 is highly expressed by immature DC, but significantly reduced in mature DC, has lead to several investigations into its function [Thery et al., 1999; Miyasaka et al., 2004; Veron et al., 2005]. Given the well-documented adhesive nature of SED1/MFG-E8, investigators initially proposed that exosome-associated protein could facilitate docking or uptake of secreted exosomes to target effector cells [Thery et al., 1999; Miyasaka et al., 2004; Morelli et al., 2004; Veron et al., 2005]. However, exosomes isolated from SED1/MFG-E8-deficient mice are nearly as efficient in transferring antigen/MHC complexes to recipient DC as vesicles isolated from wild-type mice, indicating this is probably not the case [Veron et al., 2005]. Furthermore, SED1/MFG-E8 is not required for mature DC-mediated T cell activation [Segura et al., 2005].

Alternatively, DC are known to phagocytose a wide range of materials including pathogens, dying cells, and in some cases live cells; therefore, a second hypothesis suggests that SED1/MFG-E8 secreted by immature DC may facilitate engulfment through mechanisms similar to its well-characterized role in macrophage clearance of apoptotic cells [Akakura et al., 2004]. One study indicates that SED1/MFG-E8 binds to $\alpha_v\beta5$ integrins on immature DC via its RGD motif, initiating a signal transduction cascade through DOCK180 and Rac1 that potentiates phagocytosis [Akakura et al., 2004]. Conversely, SED1/MFG-E8 and DOCK180 are both down-regulated in mature DC, cells that exhibit reduced phagocytosis relative to their immature counterparts [Akakura et al., 2004]. Importantly, the nature of SED1/MFG-E8 secretion either soluble, or bound to exosomes, was not specifically addressed in this study, and therefore the role of exosomes in this pathway remains somewhat unclear.

CONCLUDING THOUGHTS: SED1/MFG-E8 AS A THERAPEUTIC AGENT

The various roles established for SED1/MFG-E8 immediately suggest a number of potential therapeutic opportunities. Oncological studies indicate that SED1/MFG-E8 is expressed and often up-regulated on the surface of breast carcinoma cells, and antibodies against the protein have met with some success in cancer diagnosis and therapy [Larocca et al., 1991]. More recently,

investigators have been successful in priming cytotoxic T cells to target SED1/MFG-E8-positive breast cancers [Liu et al., 2005]. In this case, adeno-associated virus-based gene loading was used to delivery SED1/MFG-E8 to dendritic cells, which in turn presented the protein to cytotoxic T cells in the form of antigen/MHC complexes [Liu et al., 2005]. Alternatively, fusion of the C-terminal domains of SED1/MFG-E8 to a tumor antigen has been found sufficient to target otherwise soluble antigen to secreted exosome-like vesicles [Zeelenberg et al., 2008]. In this investigation, a vesicle-associated tumor antigen induced a stronger T cell-mediated immune response than did the same antigen secreted as a soluble protein. As a result, tumors secreting vesicle-associated antigens were slower growing, and a DNA-vaccine therapy featuring an antigen-SED1/MFG-E8 fusion construct had a protective effect, limiting tumor size in a mouse tumor progression model [Zeelenberg et al., 2008].

As mentioned above, the clear involvement of SED1/MFG-E8, and its homolog Del1, in angiogenesis raises a number of intriguing possibilities regarding their potential clinical use. Investigators have already applied these observations to enhance recovery of ischemia in a rodent model. Finding that inappropriate activation of this pathway initiates angiogenesis in developing tumors also suggests that SED1/MFG-E8 antagonists could be used to arrest tumor growth. How the SED1/MFG-E8 antagonists will be targeted to the appropriate site is unclear, but workers have suggested that RGD-bearing chemotherapeutic agents could be targeted to the SED1/MFG-E8-dependent phagocytic activity of endothelial cells in angiogenic tumors [Fens et al., 2008].

A number of studies also indicate that SED1/MFG-E8 expression is dramatically reduced during sepsis, and in at least one instance, exogenous administration SED1/MFG-E8 was able to reverse some of the cellular defects associated with sepsis [Bu et al., 2007; Miksa et al., 2007]. The decrease in SED1/MFG-E8 expression during sepsis is also associated with a decrease in clearance of apoptotic cells, an effect that can be partially rescued by the administration of SED1/MFG-E8-containing exosomes collected from immature DC. Exosome administration also leads to increased survival of septic animals [Miksa et al., 2006]. In this regard, administration of the cytokine, fractalkine, to septic animals, induces SED1/MFG-E8 expression and enhances clearance of apoptotic cells [Miksa et al., 2007].

A few other therapeutic applications of SED1/MFG-E8 are less well-defined, but none-the-less bare mentioning. One of the first reported functions for SED1/MFG-E8 in breast milk was to prevent rotavirus infection in breast-fed infants, and the protein has also been found to block enterotoxigenic *E. coli* attachment to the intestinal villi of piglets [Newburg et al., 1998; Kvistgaard et al., 2004; Shahriar et al., 2006]. Together, these data raise the possibility of supplementing formula with SED1/MFG-E8 to boost immunity for formula-fed children. SED1/MFG-E8 has also been utilized similarly to annexin V as a reagent for identifying apoptotic cells, and recent membrane-binding studies reveal that the affinity of SED1/MFG-E8 for phosphatidylserine-containing membranes is more than 100-fold greater than that of Factor VIII C2 [Shi and Gilbert, 2003; Venegas and Zhou, 2007; Shao et al., 2008]. In fact, SED1/MFG-E8 directly competes for the phospholipid binding sites of Factor V and Factor VIII, therefore inhibiting prothrombinase and Xase complexes that are activated during the coagulation cascade [Shi et al., 2008]. These data suggest SED1/MFG-E8, perhaps even the C2 domain alone, could serve as potent anti-coagulant, or alternatively, a targeting molecule directing a therapeutic-of-interest to phosphatidylserine-rich membranes [Shao et al., 2008]. Furthermore, SED1/MFG-E8 plays multiple roles during sperm maturation and fertilization, and therefore opens the possibility of using SED1/MFG-E8 antagonists as potential contraceptive agents. Alternatively, since SED1/MFG-E8 is added to mature sperm post-mieotically, it may be possible to enhance the fertilizing potential of sperm with low fertilizing efficacy by the application of SED1/MFG-E8 during intercourse.

These are just a few of the rich possibilities that present themselves, reflecting the many distinct types of cellular interactions mediated by SED1/MFG-E8. Although all of these potential uses raise many more questions than they answer, it is clear that this unusual multi-domain protein offers much potential for research and therapeutics.

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REFERENCES

Ait-Oufella H, Kinugawa K, Zoll J, Simon T, Boddaert J, Heeneman S, Blanc-Brude O, Barateau V, Potteaux S, Merval R, Esposito B, Teissier E, Daemen MJ, Leseche G, Boulanger C, Tedgui A, Mallat Z. 2007. Lactadherin deficiency leads to apoptotic cell accumulation and accelerated atherosclerosis in mice. Circulation 115:2168–2177.

Akakura S, Singh S, Spataro M, Akakura R, Kim JI, Albert ML, Birge RB. 2004. The opsonin MFG-E8 is a ligand for the alphavbeta5 integrin and triggers DOCK180-dependent Rac1 activation for the phagocytosis of apoptotic cells. Exp Cell Res 292:403–416.

Andersen MH, Berglund L, Rasmussen JT, Petersen TE. 1997. Bovine PAS-6/7 binds alpha v beta 5 integrins and anionic phospholipids through two domains. Biochemistry 36:5441–5446.

Andersen MH, Graversen H, Fedosov SN, Petersen TE, Rasmussen JT. 2000. Functional analyses of two cellular binding domains of bovine lactadherin. Biochemistry 39:6200–6206.

Aoka Y, Johnson FL, Penta K, Hirata Ki K, Hidai C, Schatzman R, Varner JA, Quertermous T. 2002. The embryonic angiogenic factor Del1 accelerates tumor growth by enhancing vascular formation. Microvasc Res 64:148–161.

Aoki M, Kanamori M, Ohmori K, Takaishi M, Huh NH, Nogami S, Kimura T. 2005. Expression of developmentally regulated endothelial cell locus 1 was induced by tumor-derived factors including VEGF. Biochem Biophys Res Commun 333:990–995.

Asano K, Miwa M, Miwa K, Hanayama R, Nagase H, Nagata S, Tanaka M. 2004. Masking of phosphatidylserine inhibits apoptotic cell engulfment and induces autoantibody production in mice. J Exp Med 200:459–467.

Atabai K, Fernandez R, Huang X, Ueki I, Kline A, Li Y, Sadatmansoori S, Smith-Steinhart C, Zhu W, Pytela R, Werb Z, Sheppard D. 2005. Mfge8 is critical for mammary gland remodeling during involution. Mol Biol Cell 16:5528–5537.

Balzar M, Briaire-de Bruijn IH, Rees-Bakker HA, Prins FA, Helfrich W, de Leij L, Riethmuller G, Alberti S, Warnaar SO, Fleuren GJ, Litvinov SV. 2001. Epidermal growth factor-like repeats mediate lateral and reciprocal inter-

actions of Ep-CAM molecules in homophilic adhesions. Mol Cell Biol 21: 2570–2580.

Boddaert J, Kinugawa K, Lambert JC, Boukhtouche F, Zoll J, Merval R, Blanc-Brude O, Mann D, Berr C, Vilar J, Garabedian B, Journiac N, Charue D, Silvestre JS, Duyckaerts C, Amouyel P, Mariani J, Tedgui A, Mallat Z. 2007. Evidence of a role for lactadherin in Alzheimer's disease. Am J Pathol 170: 921–929.

Bu HF, Zuo XL, Wang X, Ensslin MA, Koti V, Hsueh W, Raymond AS, Shur BD, Tan XD. 2007. Milk fat globule-EGF factor 8/lactadherin plays a crucial role in maintenance and repair of murine intestinal epithelium. J Clin Invest 117:3673–3683.

Burgess BL, Abrams TA, Nagata S, Hall MO. 2006. MFG-E8 in the retina and retinal pigment epithelium of rat and mouse. Mol Vis 12:1437–1447.

Choi EY, Chavakis E, Czabanka MA, Langer HF, Fraemohs L, Economopoulou M, Kundu RK, Orlandi A, Zheng YY, Prieto DA, Ballantyne CM, Constant SL, Aird WC, Papayannopoulou T, Gahmberg CG, Udey MC, Vajkoczy P, Quertermous T, Dimmeler S, Weber C, Chavakis T. 2008. Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment. Science 322:1101–1104.

Couto JR, Taylor MR, Godwin SG, Ceriani RL, Peterson JA. 1996. Cloning and sequence analysis of human breast epithelial antigen BA46 reveals an RGD cell adhesion sequence presented on an epidermal growth factor-like domain. DNA Cell Biol 15:281–286.

Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, Geuze HJ. 2000. Exosome: From internal vesicle of the multivesicular body to intercellular signaling device. J Cell Sci 113(Pt 19):3365–3374.

Ensslin MA, Shur BD. 2003. Identification of mouse sperm SED1, a bimotif EGF repeat and discoidin-domain protein involved in sperm-egg binding. Cell 114:405–417.

Ensslin MA, Shur BD. 2007. The EGF repeat and discoidin domain protein, SED1/MFG-E8, is required for mammary gland branching morphogenesis. Proc Natl Acad Sci USA 104:2715–2720.

Ensslin M, Vogel T, Calvete JJ, Thole HH, Schmidtke J, Matsuda T, Tèopfer-Petersen E. 1998. Molecular cloning and characterization of P47, a novel boar sperm-associated zona pellucida-binding protein homologous to a family of mammalian secretory proteins. Biol Reprod 58:1057–1064.

Fens MH, Mastrobattista E, de Graaff AM, Flesch FM, Ultee A, Rasmussen JT, Molema G, Storm G, Schiffelers RM. 2008. Angiogenic endothelium shows lactadherin-dependent phagocytosis of aged erythrocytes and apoptotic cells. Blood 111:4542–4550.

Fuentes-Prior P, Fujikawa K, Pratt KP. 2002. New insights into binding interfaces of coagulation factors V and VIII and their homologues lessons from high resolution crystal structures. Curr Protein Pept Sci 3:313–339.

Gatti JL, Metayer S, Belghazi M, Dacheux F, Dacheux JL. 2005. Identification, proteomic profiling, and origin of ram epididymal fluid exosome-like vesicles. Biol Reprod 72:1452–1465.

Hanayama R, Nagata S. 2005. Impaired involution of mammary glands in the absence of milk fat globule EGF factor 8. Proc Natl Acad Sci USA 102:16886–16891.

Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. 2002. Identification of a factor that links apoptotic cells to phagocytes. Nature 417:182–187.

Hanayama R, Tanaka M, Miyasaka K, Aozasa K, Koike M, Uchiyama Y, Nagata S. 2004. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science (New York, NY) 304:1147–1150.

Hidai C, Zupancic T, Penta K, Mikhail A, Kawana M, Quertermous EE, Aoka Y, Fukagawa M, Matsui Y, Platika D, Auerbach R, Hogan BL, Snodgrass R, Quertermous T. 1998. Cloning and characterization of developmental endothelial locus-1: An embryonic endothelial cell protein that binds the alphavbeta3 integrin receptor. Genes Dev 12:21–33.

Hidai C, Kawana M, Habu K, Kazama H, Kawase Y, Iwata T, Suzuki H, Quertermous T, Kokubun S. 2005. Overexpression of the Del1 gene causes dendritic branching in the mouse mesentery. Anat Rec A Discov Mol Cell Evol Biol 287:1165–1175.

Hidai C, Kawana M, Kitano H, Kokubun S. 2007. Discoidin domain of Del1 protein contributes to its deposition in the extracellular matrix. Cell Tissue Res 330:83–95.

Ho HK, Jang JJ, Kaji S, Spektor G, Fong A, Yang P, Hu BS, Schatzman R, Quertermous T, Cooke JP. 2004. Developmental endothelial locus-1 (Del-1), a novel angiogenic protein: Its role in ischemia. Circulation 109:1314–1319.

Hoffhines AJ, Jen CH, Leary JA, Moore KL. 2008. Tyrosylprotein sulfotransferase-2 expression is required for sulfation of Rnase9 and Mfge8 in vivo. J Biol Chem [Epub ahead of print].

Kvistgaard AS, Pallesen LT, Arias CF, Lopez S, Petersen TE, Heegaard CW, Rasmussen JT. 2004. Inhibitory effects of human and bovine milk constituents on rotavirus infections. J Dairy Sci 87:4088–4096.

Larocca D, Peterson JA, Urrea R, Kuniyoshi J, Bistrain AM, Ceriani RL. 1991. A Mr 46,000 human milk fat globule protein that is highly expressed in human breast tumors contains factor VIII-like domains. Cancer Res 51:4994–4998.

Leonardi-Essmann F, Emig M, Kitamura Y, Spanagel R, Gebicke-Haerter PJ. 2005. Fractalkine-upregulated milk-fat globule EGF factor-8 protein in cultured rat microglia. J Neuroimmunol 160:92–101.

Lin L, Huai Q, Huang M, Furie B, Furie BC. 2007. Crystal structure of the bovine lactadherin C2 domain, a membrane binding motif, shows similarity to the C2 domains of factor V and factor VIII. J Mol Biol 371:717–724.

Liu Y, Chiriva-Internati M, You C, Luo R, You H, Prasad CK, Grizzi F, Cobos E, Klimberg VS, Kay H, Mehta JL, Hermonat PL. 2005. Use and specificity of breast cancer antigen/milk protein BA46 for generating anti-self-cytotoxic T lymphocytes by recombinant adeno-associated virus-based gene loading of dendritic cells. Cancer Gene Ther 12:304–312.

Macedo-Ribeiro S, Bode W, Huber R, Quinn-Allen MA, Kim SW, Ortel TL, Bourenkov GP, Bartunik HD, Stubbs MT, Kane WH, Fuentes-Prior P. 1999. Crystal structures of the membrane-binding C2 domain of human coagulation factor V. Nature 402:434–439.

Miksa M, Wu R, Dong W, Das P, Yang D, Wang P. 2006. Dendritic cell-derived exosomes containing milk fat globule epidermal growth factor-factor VIII attenuate proinflammatory responses in sepsis. Shock 25:586–593.

Miksa M, Amin D, Wu R, Ravikumar TS, Wang P. 2007. Fractalkine-induced MFG-E8 leads to enhanced apoptotic cell clearance by macrophages. Mol Med 13:553–560.

Miyasaka K, Hanayama R, Tanaka M, Nagata S. 2004. Expression of milk fat globule epidermal growth factor 8 in immature dendritic cells for engulfment of apoptotic cells. Eur J Immunol 34:1414–1422.

Morelli AE, Larregina AT, Shufesky WJ, Sullivan ML, Stolz DB, Papworth GD, Zahorchak AF, Logar AJ, Wang Z, Watkins SC, Falo LD, Jr., Thomson AW. 2004. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. Blood 104:3257–3266.

Nandrot EF, Kim Y, Brodie SE, Huang X, Sheppard D, Finnemann SC. 2004. Loss of synchronized retinal phagocytosis and age-related blindness in mice lacking alphavbeta5 integrin. J Exp Med 200:1539–1545.

Nandrot EF, Anand M, Sircar M, Finnemann SC. 2006. Novel role for alphavbeta5-integrin in retinal adhesion and its diurnal peak. Am J Physiol Cell Physiol 290:C1256–C1262.

Nandrot EF, Anand M, Almeida D, Atabai K, Sheppard D, Finnemann SC. 2007. Essential role for MFG-E8 as ligand for alphavbeta5 integrin in diurnal retinal phagocytosis. Proc Natl Acad Sci USA 104:12005–12010.

Neutzner M, Lopez T, Feng X, Bergmann-Leitner ES, Leitner WW, Udey MC. 2007. MFG-E8/lactadherin promotes tumor growth in an angiogenesis-dependent transgenic mouse model of multistage carcinogenesis. Cancer Res 67:6777–6785.

Newburg DS, Peterson JA, Ruiz-Palacios GM, Matson DO, Morrow AL, Shults J, Guerrero ML, Chaturvedi P, Newburg SO, Scallan CD, Taylor MR, Ceriani RL, Pickering LK. 1998. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. Lancet 351:1160–1164.

Ogura K, Nara K, Watanabe Y, Kohno K, Tai T, Sanai Y. 1996. Cloning and expression of cDNA for O-acetylation of GD3 ganglioside. Biochem Biophys Res Commun 225:932–938.

Oshima K, Aoki N, Negi M, Kishi M, Kitajima K, Matsuda T. 1999. Lactation-dependent expression of an mRNA splice variant with an exon for a multiply O-glycosylated domain of mouse milk fat globule glycoprotein MFG-E8. Biochem Biophys Res Commun 254:522–528.

Oshima K, Aoki N, Kato T, Kitajima K, Matsuda T. 2002. Secretion of a peripheral membrane protein, MFG-E8, as a complex with membrane vesicles. Eur J Biochem/FEBS 269:1209–1218.

Penta K, Varner JA, Liaw L, Hidai C, Schatzman R, Quertermous T. 1999. Del1 induces integrin signaling and angiogenesis by ligation of alphaVbeta3. J Biol Chem 274:11101–11109.

Pratt KP, Shen BW, Takeshima K, Davie EW, Fujikawa K, Stoddard BL. 1999. Structure of the C2 domain of human factor VIII at 1.5 A resolution. Nature 402:439–442.

Raymond AS, Shur BD. In press. A novel role for SED1/MFG-E8 in maintaining the integrity of the epididymal epithelium. J Cell Sci.

Reitherman RW, Rosen SD, Frasier WA, Barondes SH. 1975. Cell surface species-specific high affinity receptors for discoidin: Developmental regulation in Dictyostelium discoideum. Proc Natl Acad Sci USA 72:3541–3545.

Rezaee M, Penta K, Quertermous T. 2002. Del1 mediates VSMC adhesion, migration, and proliferation through interaction with integrin alpha(v)-beta(3). Am J Physiol Heart Circ Physiol 282:H1924–H1932.

Segura E, Amigorena S, Thery C. 2005. Mature dendritic cells secrete exosomes with strong ability to induce antigen-specific effector immune responses. Blood Cells Mol Dis 35:89–93.

Shahriar F, Ngeleka M, Gordon JR, Simko E. 2006. Identification by mass spectroscopy of F4ac-fimbrial-binding proteins in porcine milk and characterization of lactadherin as an inhibitor of F4ac-positive Escherichia coli attachment to intestinal villi in vitro. Dev Comp Immunol 30:723–734.

Shao C, Novakovic VA, Head JF, Seaton BA, Gilbert GE. 2008. Crystal structure of lactadherin C2 domain at 1.7A resolution with mutational and computational analyses of its membrane-binding motif. J Biol Chem 283:7230–7241.

Shi J, Gilbert GE. 2003. Lactadherin inhibits enzyme complexes of blood coagulation by competing for phospholipid-binding sites. Blood 101:2628–2636.

Shi J, Heegaard CW, Rasmussen JT, Gilbert GE. 2004. Lactadherin binds selectively to membranes containing phosphatidyl-L-serine and increased curvature. Biochim Biophys Acta 1667:82–90.

Shi J, Pipe SW, Rasmussen JT, Heegaard CW, Gilbert GE. 2008. Lactadherin blocks thrombosis and hemostasis in vivo: Correlation with platelet phosphatidylserine exposure. J Thromb Haemostasis 6:1167–1174.

Shur BD, Ensslin MA, Rodeheffer C. 2004. SED1 function during mammalian sperm-egg adhesion. Curr Opin Cell Biol 16:477–485.

Silvestre JS, Thery C, Hamard G, Boddaert J, Aguilar B, Delcayre A, Houbron C, Tamarat R, Blanc-Brude O, Heeneman S, Clergue M, Duriez M, Merval R, Levy B, Tedgui A, Amigorena S, Mallat Z. 2005. Lactadherin promotes VEGF-dependent neovascularization. Nat Med 11:499–506.

Stubbs JD, Lekutis C, Singer KL, Bui A, Yuzuki D, Srinivasan U, Parry G. 1990. cDNA cloning of a mouse mammary epithelial cell surface protein reveals the existence of epidermal growth factor-like domains linked to factor VIII-like sequences. Proc Natl Acad Sci USA 87:8417–8421.

Sullivan R, Saez F, Girouard J, Frenette G. 2005. Role of exosomes in sperm maturation during the transit along the male reproductive tract. Blood Cells Mol Dis 35:1–10.

Taylor MR, Couto JR, Scallan CD, Ceriani RL, Peterson JA. 1997. Lactadherin (formerly BA46), a membrane-associated glycoprotein expressed in human milk and breast carcinomas, promotes Arg-Gly-Asp (RGD)-dependent cell adhesion. DNA Cell Biol 16:861–869.

Thery C, Regnault A, Garin J, Wolfers J, Zitvogel L, Ricciardi-Castagnoli P, Raposo G, Amigorena S. 1999. Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. J Cell Biol 147:599–610.

Venegas V, Zhou Z. 2007. Two alternative mechanisms that regulate the presentation of apoptotic cell engulfment signal in Caenorhabditis elegans. Mol Biol Cell 18:3180–3192.

Veron P, Segura E, Sugano G, Amigorena S, Thery C. 2005. Accumulation of MFG-E8/lactadherin on exosomes from immature dendritic cells. Blood Cells Mol Dis 35:81–88.

Watanabe T, Totsuka R, Miyatani S, Kurata S, Sato S, Katoh I, Kobayashi S, Ikawa Y. 2005. Production of the long and short forms of MFG-E8 by epidermal keratinocytes. Cell Tissue Res 321:185–193.

Weng S, Zemany L, Standley KN, Novack DV, La Regina M, Bernal-Mizrachi C, Coleman T, Semenkovich CF. 2003. Beta3 integrin deficiency promotes atherosclerosis and pulmonary inflammation in high-fat-fed, hyperlipidemic mice. Proc Natl Acad Sci USA 100:6730–6735.

Yamaguchi H, Takagi J, Miyamae T, Yokota S, Fujimoto T, Nakamura S, Ohshima S, Naka T, Nagata S. 2008. Milk fat globule EGF factor 8 in the serum of human patients of systemic lupus erythematosus. J Leukoc Biol 83:1300–1307.

Zeelenberg IS, Ostrowski M, Krumeich S, Bobrie A, Jancic C, Boissonnas A, Delcayre A, Le Pecq JB, Combadiere B, Amigorena S, Thery C. 2008. Targeting tumor antigens to secreted membrane vesicles in vivo induces efficient antitumor immune responses. Cancer Res 68:1228–1235.

Zhong J, Eliceiri B, Stupack D, Penta K, Sakamoto G, Quertermous T, Coleman M, Boudreau N, Varner JA. 2003. Neovascularization of ischemic tissues by gene delivery of the extracellular matrix protein Del-1. J Clin Invest 112:30–41.