Published in final edited form as:

Adv Exp Med Biol. 2006 ; 572: 499–503. doi:10.1007/0-387-32442-9_69.

MERTK ACTIVATION DURING RPE PHAGOCYTOSIS *IN VIVO* REQUIRES $\alpha V\beta 5$ INTEGRIN

Silvia C. Finnemann^{1,2} and Emeline F. Nandrot^{1,*}

¹Department of Ophthalmology, Weill Medical College of Cornell University, Box 233, 1300 York Avenue, New York, NY10021, USA

²Department of Cell and Developmental Biology, Weill Medical College of Cornell University, Box 233, 1300 York Avenue, New York, NY10021, USA

*Margaret M. Dyson Vision Research Institute, Weill Medical College of Cornell University, Box 233, 1300 York Avenue, New York, NY10021, USA

1. INTRODUCTION

Daily phagocytosis of shed photoreceptor outer segment fragments (POS) is a key task of the retinal pigment epithelium (RPE) in the retina. Lack or inefficiency of daily POS clearance causes early onset, rapid, and complete retinal degeneration in experimental animals and likely contributes to human blinding diseases such as retinitis pigmentosa and age-related macular degeneration (Dowling and Sidman, 1962, Gal et al., 2000). The phagocytic mechanism of the RPE belongs to a group of conserved non-inflammatory clearance pathways that mediate recognition and engulfment of apoptotic cells in both non-professional and professional phagocytic cells, such as fibroblasts and macrophages, respectively (Finnemann and Rodriguez-Boulant, 1999). These pathways share the use of phagocyte cell surface receptors such as the lipid scavenger receptor CD36 (Ryeom et al., 1996), the integrin adhesion receptor $\alpha V\beta 5$ (Finnemann et al., 1997; Miceli et al., 1997; Lin and Clegg, 1998) and the receptor tyrosine kinase Mer (MerTK) (D'Cruz et al., 2000; Nandrot et al., 2000). *In vitro* phagocytosis assays studying primary or permanent RPE in culture fed with isolated POS suggest that CD36 and MerTK participate in the engulfment step of the phagocytic process (Chaitin and Hall, 1983; Finnemann and Silverstein, 2001), while $\alpha V\beta 5$ integrin promotes POS recognition/binding and initiates a downstream cytoplasmic signaling cascade in the RPE (Finnemann et al., 1997). However, the precise function of these receptors and their roles in the intact retina are so far only poorly understood. Most recently, we have begun to study phagocytosis and receptor activity in animal models that lack $\alpha V\beta 5$ integrin or MerTK to determine how these different plasma membrane receptors of the RPE functionally interact to coordinate particle uptake.

2. ROLE OF MERTK ACTIVATION IN RPE PHAGOCYTOSIS

Activity of the Mer tyrosine kinase receptor MerTK is essential for efficient engulfment of POS by RPE *in vivo* and *in vitro* (Mullen and LaVail, 1976; Edwards and Szamier, 1977). Despite its importance, mechanisms of MerTK activation and MerTK downstream signaling target proteins in RPE are still largely obscure. Retinal ligands of MerTK have not yet been conclusively identified. Moreover, we still do not know which RPE proteins serve as substrates for MerTK's kinase activity during RPE phagocytosis. However, both endogenous and overexpressed MerTK reveal a striking redistribution to the sites of internalized POS in *in vitro* phagocytosis assays suggesting that MerTK receptors may be components of the phagocytic machinery of the RPE (Feng et al., 2002; Finnemann, 2003). Furthermore, challenge with isolated POS causes increased phosphorylation at tyrosine

residues of MerTK in RPE in culture (Feng et al., 2002; Finnemann, 2003). Although their mutual dependence has not been demonstrated directly, levels of MerTK tyrosine phosphorylation commonly serve to assess the extent of MerTK activity.

3. FOCAL ADHESION KINASE SIGNALING ACTIVATES MERTK DURING RPE PHAGOCYTOSIS

Our previous studies on phagocytic signaling in RPE suggest an important role for focal adhesion kinase (FAK) in MerTK activation. FAK is a cytoplasmic non-receptor tyrosine kinase that colocalizes with integrin receptors at focal contacts where it commonly transduces signaling pathways downstream of activated integrins (for a recent review on FAK see (Parsons, 2003)). Reversible activation of FAK is critical for integrin functions that involve cytoskeletal reorganization (Ilic et al., 1995). We expressed a C-terminal fragment of FAK that competes with full-length endogenous FAK for cytoskeletal anchorage. This fragment has been shown to act as a dominant-negative inhibitor of endogenous FAK abrogating FAK downstream signal transduction. We showed that the rat derived RPE-J cell line, like primary wild-type rat RPE, utilizes endogenous MerTK to engulf POS. Importantly, expression of the dominant-negative FAK C-terminal fragment in RPE-J cells inhibited POS engulfment (Finnemann, 2003). Furthermore, it eliminated the increase in MerTK tyrosine phosphorylation that is elicited by phagocytic challenge of RPE cells in culture (Finnemann, 2003). On the contrary, RPE cultures derived from RCS rats retained similar FAK activation as wild-type Long Evans rat RPE cultures in response to OS challenge. These results identify a novel signal transduction pathway in which FAK acts upstream of MerTK to stimulate the internalization machinery of the RPE.

4. α v β 5 SIGNALING VIA FOCAL ADHESION KINASE ACTIVATES MERTK DURING RPE PHAGOCYTOSIS *IN VIVO* AND *IN VITRO*

Previous studies have shown that phosphorylation of FAK at tyrosine residue 861 promotes direct binding of FAK to the cytoplasmic face of α v β 5 integrin receptors (Eliceiri et al., 2002). In our studies, we observed increased levels of FAK in α v β 5 protein complexes isolated by immunoprecipitation from RPE-J cells during the early POS binding phase but loss of FAK from the integrin complex during the later POS internalization phase (Finnemann, 2003). Residence of FAK in the complex correlated well with elevated phosphorylation of tyrosine 861, while phosphorylation of other tyrosine residues that indicate FAK enzymatic activity persisted beyond the time of FAK in the integrin complex. These results suggest that RPE cells activate FAK recruited to its apical α v β 5 surface receptors in response to POS phagocytic challenge *in vitro*.

To directly determine whether α v β 5 integrin receptors were required for FAK and MerTK activation in RPE, we tested FAK and MerTK activation during RPE phagocytosis by RPE cells of β 5 integrin knockout mice that lack all α v β 5 integrin receptors. β 5 null RPE cells in culture largely fail to phagocytose isolated POS (Nandrot et al., 2004). β 5 null retina lacks the synchronized burst of RPE phagocytosis that characteristically follows early morning rod shedding in rodent retina (Nandrot et al., 2004). The detrimental effects of this abnormal timing of phagocytosis on retinal function in β 5 integrin null mice of age are discussed in more detail in the chapter by Nandrot and Finnemann in this volume.

When we fed isolated POS to wild-type mouse RPE, we found robust FAK and MerTK activation confirming our earlier results using stable and primary rat RPE (shown for MerTK in Figure 1a, β 5+/+). In contrast, β 5 null RPE cells in primary culture did not increase tyrosine phosphorylation of either FAK or MerTK in response to POS, although they expressed both proteins at normal levels (shown for MerTK in Figure 1a, β 5-/-).

–). Furthermore, *in vivo* phagocytic signaling via FAK and MerTK was strongly and transiently stimulated following light onset in wild-type mouse retina but was absent in $\beta 5$ knockout mouse retina (shown for MerTK in Figure 1b). These data provide conclusive evidence that $\alpha v \beta 5$ integrin signaling regulates rhythmic activation of FAK and MerTK during RPE phagocytosis in the intact retina.

5. PERSPECTIVE

The results discussed here are the first to describe signaling activities by phagocytic receptors in intact retina that precisely correlate temporally with POS shedding and uptake by RPE cells. The late onset retinal dysfunction of the $\beta 5$ knockout mouse as a consequence of lack of such phagocytic signaling emphasizes the importance of precise temporal regulation of POS phagocytosis by the RPE. In future studies, we will use similar experimental approaches exploring *in vivo* signaling in normal and mutant animal models to identify further components of the RPE phagocytic mechanism and to unravel their functional interactions.

Acknowledgments

This work was supported by NIH grants EY13295 and EY14184, by a Karl Kirchgeßner research grant, and by the Irma T. Hirschl/Monique Weill-Caulier Trust.

REFERENCES

- Chaitin MH, Hall MO. Defective ingestion of rod outer segments by cultured dystrophic rat pigment epithelial cells. *Invest. Ophthalmol. Vis. Sci.* 1983; 24:812–820. [PubMed: 6345445]
- D'Cruz PM, Yasumura D, Weir J, Matthes MT, Abderrahim H, LaVail MM, Vollrath D. Mutation of the receptor tyrosine kinase gene *Mertk* in the retinal dystrophic RCS rat. *Hum. Mol. Genet.* 2000; 9:645–651. [PubMed: 10699188]
- Dowling JE, Sidman RL. Inherited retinal dystrophy of the rat. *J. Cell Biol.* 1962; 14:73–109. [PubMed: 13887627]
- Edwards RB, Szamier RB. Defective phagocytosis of isolated rod outer segments by RCS rat retinal pigment epithelium in culture. *Science.* 1977; 197:1001–1003. [PubMed: 560718]
- Eliceiri BP, Puente XS, Hood JD, Stupack DG, Schlaepfer DD, Huang XZ, Sheppard D, Cheresch DA. Src-mediated coupling of focal adhesion kinase to integrin $\alpha v \beta 5$ in vascular endothelial growth factor signaling. *J. Cell Biol.* 2002; 157:149–160. [PubMed: 11927607]
- Feng W, Yasumura D, Matthes MT, LaVail MM, Vollrath D. *Mertk* triggers uptake of photoreceptor outer segments during phagocytosis by cultured retinal pigment epithelial cells. *J. Biol. Chem.* 2002; 277:17016–17022. [PubMed: 11861639]
- Finnemann SC. Focal adhesion kinase signaling promotes phagocytosis of integrin-bound photoreceptors. *EMBO J.* 2003; 22:4143–4154. [PubMed: 12912913]
- Finnemann SC, Bonilha VL, Marmorstein AD, Rodriguez-Boulan E. Phagocytosis of rod outer segments by retinal pigment epithelial cells requires $\alpha v \beta 5$ integrin for binding but not for internalization. *Proc. Natl. Acad. Sci. U.S.A.* 1997; 94:12932–12937. [PubMed: 9371778]
- Finnemann SC, Rodriguez-Boulan E. Macrophage and retinal pigment epithelium phagocytosis: apoptotic cells and photoreceptors compete for $\alpha v \beta 3$ and $\alpha v \beta 5$ integrins, and protein kinase C regulates $\alpha v \beta 5$ binding and cytoskeletal linkage. *J. Exp. Med.* 1999; 190:861–874. [PubMed: 10499924]
- Finnemann SC, Silverstein RL. Differential roles of CD36 and $\alpha v \beta 5$ integrin in photoreceptor phagocytosis by the retinal pigment epithelium. *J. Exp. Med.* 2001; 194:1289–1298. [PubMed: 11696594]
- Gal A, Li Y, Thompson DA, Weir J, Orth U, Jacobson SG, Apfelstedt-Sylla E, Vollrath D. Mutations in *MERTK*, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nat. Genet.* 2000; 26:270–271. [PubMed: 11062461]

- Ilic D, Furuta Y, Kanazawa S, Takeda N, Sobue K, Nakatsuji N, Nomura S, Fujimoto J, Okada M, Yamamoto T. Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. *Nature*. 1995; 377:539–544. [PubMed: 7566154]
- Lin H, Clegg DO. Integrin $\alpha v \beta 5$ participates in the binding of photoreceptor rod outer segments during phagocytosis by cultured human retinal pigment epithelium. *Invest. Ophthalmol. Vis. Sci*. 1998; 39:1703–1712. [PubMed: 9699560]
- Miceli MV, Newsome DA, Tate DJ Jr. Vitronectin is responsible for serum-stimulated uptake of rod outer segments by cultured retinal pigment epithelial cells. *Invest. Ophthalmol. Vis. Sci*. 1997; 38:1588–1597. [PubMed: 9224287]
- Mullen RJ, LaVail MM. Inherited retinal dystrophy: primary defect in pigment epithelium determined with experimental rat chimeras. *Science*. 1976; 192:799–801. [PubMed: 1265483]
- Nandrot E, Dufour EM, Provost AC, Pequignot MO, Bonnel S, Gogat K, Marchant D, Rouillac C, Sepulchre de Conde B, Bihoreau MT, Shaver C, Dufier JL, Marsac C, Lathrop M, Menasche M, Abitbol MM. Homozygous deletion in the coding sequence of the c-mer gene in RCS rats unravels general mechanisms of physiological cell adhesion and apoptosis. *Neurobiol. Dis*. 2000; 7:586–599. [PubMed: 11114258]
- Nandrot EF, Kim Y, Brodie SE, Huang X, Sheppard D, Finnemann SC. Loss of synchronized retinal phagocytosis and age-related blindness in mice lacking $\alpha v \beta 5$ integrin. *J. Exp. Med*. 2004; 200:1539–1545. [PubMed: 15596525]
- Parsons JT. Focal adhesion kinase: the first ten years. *J. Cell Sci*. 2003; 116:1409–1416. [PubMed: 12640026]
- Ryeom SW, Sparrow JR, Silverstein RL. CD36 participates in the phagocytosis of rod outer segments by retinal pigment epithelium. *J. Cell Sci*. 1996; 109:387–395. [PubMed: 8838662]

Figure 1.

MerTK activation requires $\alpha v \beta 5$ integrin. a. Primary RPE in culture from $\beta 5^{+/+}$ or $\beta 5^{-/-}$ mice received isolated POS (POS) or assay medium alone (m) for 1.5 hours before lysis of cells. b. Eyecups were harvested from 3 week old strain-matched $\beta 5^{+/+}$ and $\beta 5^{-/-}$ mice at different times of day as indicated and protein lysates prepared immediately. a and b. Lysates were analyzed by SDS-PAGE and immunoblotting for MerTK protein and tyrosine-phosphorylated MerTK (PY-MerTK). Band intensities were quantified to calculate relative levels of MerTK phosphorylation (= activation). Bars represent means $\pm$ SD, $n=3$. Significant differences between equivalent $\beta 5^{+/+}$ and $\beta 5^{-/-}$ values were determined by Student's *t*-test and are indicated by asterisks ($P < 0.001$ for a, $P < 0.05$ for b). Modified from Nandrot et al., 2004, with permission from Rockefeller University Press.