Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
iRHOM2: A Regulator of Palmoplantar Biology, Inflammation, and Viral Susceptibility

Jennifer Chao-Chu1,2, Stephen Murtough1,2, Najwa Zaman1, Daniel J. Pennington1, Diana C. Blaydon1 and David P. Kelsell1

The palmoplantar epidermis is a specialized area of the skin that undergoes high levels of mechanical stress. The palmoplantar keratinization and esophageal cancer syndrome, tylosis with esophageal cancer, is linked to mutations in RHBDLF2 encoding the proteolytically inactive rhomboid protein, iRHOM2. Subsequently, iRHOM2 was found to affect palmoplantar thickening to modulate the stress keratin response and to mediate context-dependent stress pathways by p63. iRHOM2 is also a direct regulator of the sheddase, ADAM17, and the antiviral adaptor protein, stimulator of IFN genes. In this perspective, the pleiotropic functions of iRHOM2 are discussed with respect to the skin, inflammation, and the antiviral response.

INTRODUCTION

The vast majority of published findings on epidermal biology have been obtained from interfollicular-derived keratinocyte (KC) or cutaneous squamous cancer cell lines. By contrast, there are relatively few cells and molecular studies in palmoplantar (palm and sole) skin, largely owing to reduced consent for palmoplantar biopsies due to the constant use of these body sites during daily tasks. The palmoplantar epidermis is quite different, both structurally and with respect to context-dependent signaling and protein expression, from interfollicular epidermis. The palmoplantar epidermis is a specialized area of the skin that undergoes high levels of homeostatic mechanical stress and consequently displays a broad variety of adaptations. For example, protein expression varies between the load-bearing ridges and the nonload-bearing furrows, suggesting different specialized functions (Figure 1) (Swensson et al., 1998). Biological insights into the palmoplantar epidermis are emerging from genetic studies of the clinically heterogeneous palmoplantar keratodermas (PPKs), inherited skin conditions that are characterized by palmoplantar epidermal thickening (Thomas and O’Toole, 2020). PPK is also commonly acquired in response to inflammatory skin conditions (such as psoriasis and eczema), in response to fungal infection and increased weight bearing with obesity, or owing to drugs or toxins in the environment. PPKs can have a profound effect on an individual’s QOL, with complications including severe pain, blistering, difficulty in walking, and infection. There is currently no effective therapy for PPK and existing treatments are largely palliative (Bodemer et al., 2020; Thomas and O’Toole, 2020).

To date, more than 40 PPK-associated genes have been identified encoding proteins with diverse functions, including channel proteins, components of the desmosome, keratins, and proteases and their inhibitors. Beyond the palmoplantar, human genetic studies in syndromic forms of PPKs have identified genes also associated with hair disorders, hearing loss, cardiomyopathy, and esophageal squamous cell carcinoma (OSCC) (Thomas and O’Toole, 2020).

The focus of this review stems from the discovery that hyperactive RHBDLF2 missense mutations underlie the autosomal dominant PPK syndrome (tylosis with esophageal cancer [TOC], OSCC) (Blaydon et al., 2012). The PPK in this syndrome presents as a focal, nonepidermolytic PPK (Stevens et al., 1996). RHBDLF2 encodes iRHOM2, an inactive homolog of the Rhomboid superfamily of transmembrane serine proteases (Lemberg and Freeman, 2007). It has seven transmembrane domains and a long cytoplasmic amino terminal part where the TOC missense mutations cluster. In this perspective, the pleiotropic functions of iRHOM2 will be discussed.

iRHOM2 and the palmoplantar epidermis

iRHOM2 expression is highly correlated with palmoplantar epidermal thickness, with Rhbd2−/− mice displaying a thin, translucent but physically robust paw epidermis (Maruthappu et al., 2017). To our knowledge, this represents the first time this specific phenotype has been reported in a mouse model, implicating iRHOM2 in palmoplantar (paw) biology. In contrast, the hyperactive dominant TOC mutations lead to significantly
thicker palmoplantar epidermis (Blaydon et al., 2012).

To investigate why iRhom2 has a key role in palmoplantar epidermal homeostasis, a yeast-two-hybrid screen identified an interaction between iRhom2 and the stress keratin, K16 (Maruthappu et al., 2017). K16 is constitutively expressed in the furrows of the palmoplantar epidermis (Figure 1) but is only observed in interfollicular skin during stress situations, such as wound healing (Paladini et al., 1996).

iRhom2 regulates K16 in response to stress, and K16 was strongly downregulated in Rhbd12−/− mouse paws relative to those of the controls (Maruthappu et al., 2017). In contrast, iRhom2 hyperactivity in TOC KCs resulted in the switching of K16’s binding partner from the type II keratin, K6, to iRhom2 and reorganization of K16 filaments around the nucleus. This may facilitate cellular events important in mechanical defense and wound healing.

Recently, iRhom2 was identified as a target gene of p63 (Arcidiacono et al., 2018). The p63-iRhom2 axis differentially regulates cell survival and oxidative stress signaling pathways in normal interfollicular KCs compared with those from hyperproliferative KCs, such as the mouse paw (Arcidiacono et al., 2018). Thus, iRhom2 functions in context-dependent signaling in palmoplantar and interfollicular epithelia. However, the mechanistic basis for this phenomenon is still unclear, and studies are ongoing to understand the molecular processes underpinning this differential signaling.

**iRhom2 and ADAM17**

iRhom2 (and its close relative iRhom1) directly regulates the trafficking and maturation of the major ectodomain sheddase, ADAM17 (also called TNF-α-converting enzyme) (Figure 2a) (Adrain et al., 2012; Brooke et al., 2014; Li et al., 2015; McIlwain et al., 2017). In mammals, the shedding of TNF-α is dependent on ADAM17 activity (Black et al., 1997). ADAM17 cleaves a plethora of ligands, for example, amphiregulin, TGF-α, and heparin-binding EGF-like growth factor in the EGF pathway and Desmoglein 2 of the desmosome (Dulloo et al., 2019). Human TOC KCs (harboring a hyperactive iRhom2 germline mutation) showed a higher expression of mature ADAM17 localized at the cell surface and constitutively high shedding of its substrates, such as TGF-α and amphiregulin, relative to control KCs (Brooke et al., 2014). Thus, iRhom2 regulates EGFR signaling in KCs and subsequently trans-glutaminase 1 activity in epidermal barrier formation (Brooke et al., 2014). Hyperactive iRhom2, owing to TOC missense mutations, also leads to accelerated cutaneous wound healing both in vitro and in vivo, in part, by controlling ADAM17-mediated shedding of EGF ligands (Brooke et al., 2014; Hosur et al., 2017).

The iRhom homology domain, located in loop 1 of iRhom2, is required for immature ADAM17 trafficking from the endoplasmic reticulum to the Golgi apparatus, and the cytoplasmic N-terminal tail is essential for trafficking and stabilizing mature ADAM17 at the cell surface (Li et al., 2017; Vinothkumar et al., 2010). Phosphorylation of iRhom2 at the plasma membrane or TOC-associated missense mutations at the N-terminal tail controls the release of ADAM17 activity–dependent ligands (Brooke et al., 2012; Cavadas et al., 2017; Grieve et al., 2017). A recent study in mouse embryonic fibroblasts showed that iRhom2 stability, but not that of iRhom1, requires the presence of ADAM17 (Weskamp et al., 2020). In support of this finding, we show a reduced iRhom2 expression in the skin from an individual with an inflammatory skin and gastrointestinal disorder linked to biallelic loss-of-function ADAM17 mutations (Figure 2b) (Blaydon et al., 2011), suggesting that the iRhom2-ADAM17 axis has a key role in epidermal biology.

ADAM17 also cleaves IL-6 receptor (IL-6R) from the cell surface, giving rise to soluble IL-6R (Althoff et al., 2000). Soluble IL-6R is able to bind soluble IL-6 and subsequently cell surface gp130, which dimerizes and initiates IL-6 trans-signaling (Schaper and Rose-John, 2015). IL-6R secretion in KCs through ADAM17 activity is iRhom2 dependent (Brooke et al., 2014). TOC KCs secrete high levels of both IL-6 and IL-6R and were more resistant to *Staphylococcus aureus* adherence and infection than control KCs in vitro (Brooke et al., 2014). IL-6/IL-6R

**Figure 1. Differential expression of K16 in palm epidermis.** Immunofluorescent staining of human palm skin using mouse monoclonal anti-K16 antibody (clone: LL025, green). Nuclei counterstained with DAPI (blue). The boundary between stratum granulosum and stratum corneum is indicated by a dashed white line to highlight the Rs and Fs that constitute palmoplantar skin. K16 expression is high in KCs located in the F and greatly reduced or absent in KCs in the R. Bar = 50 µm. F, furrow; K16, keratin 16; KC, keratinocyte; R, ridge.

J Chao-Chu et al.

**Pleiotropic Functions of iRhom2**
signaling also activates the Jak/signal transducer and activator of transcription pathway in inflammatory and autoimmune processes (Heinrich et al., 1998; Romano et al., 1997).

**iRhom2: Esophageal cancer and inflammatory disease**

**RHBDF2** is the only known highly penetrant genetic predisposition to OSCC, although how and why hyperactivity of iRhom2 in TOC leads to OSCC and not to other squamous cancers remains poorly understood.

Our unpublished studies in human TOC esophageal biopsies indicate that many of the findings in the epidermis are mirrored in the esophagus, but which, if any, are driving carcinogenesis remains an unanswered question. Working hypotheses include that hyperactive iRhom2 leads to dysregulated maintenance of the esophageal barrier and that activated p63 signaling and/or constitutively activated ADAM17 leads to upregulated EGFR and inflammatory cytokine signaling.

In addition to iRhom2’s key role in palmoplantar and esophageal homeostasis, emerging evidence from Rhbdf2<sup>-/-</sup> models links the loss of iRhom2 with protection from a diverse range of inflammatory conditions, including experimental arthritis and lupus (Blaydon et al., 2012; Issuree et al., 2013; Qing et al., 2018). Because anti-TNF-α is a highly successful therapeutic in several inflammatory conditions, including psoriasis, bowel disease, and arthritis, this places iRhom2 as a likely tissue-specific...
upstream regulator of TNF-α-mediated inflammation. Both increased TNF-α-mediated shedding by ADAM17 and oxidative stress are also seen in obesity (Furukawa et al., 2004; Serino et al., 2007). Adipose macrophages in obesity are associated with the production of proinflammatory adipokines, for instance, known ADAM17 targets, IL-6R, and TNF-α (Minxuan et al., 2019; Xu et al., 2020). Although two studies were contradictory in their findings, possibly because they used differently derived Rhbd2−/− mouse models, a role for iRhom2 in modulating adipose inflammation, metabolism, and obesity is proposed (Badenes et al., 2020; Skurski et al., 2020).

iRhom2 may participate in the cellular response to viruses
iRhom2 was recently proposed as a mediator of the antiviral response through its regulation of stimulator of IFN genes (STING) in human monocytic THP-1 cells and murine bone marrow–derived macrophages (Luo et al., 2016). STING orchestrates the response to DNA viruses downstream of the cytosolic DNA sensor, cGas (Motwani et al., 2019). There is also emerging evidence that STING has several functions during RNA virus infection. STING has been shown to interact with RIG-I and MAVS of the RIG-I–like receptor signaling pathway (Ishikawa and Barber, 2008; Wu and Chen, 2014; Zhong et al., 2008). Moreover, it has been reported that STING can regulate IFN-1 production independently of cGas in response to RNA viruses such as influenza A (Holm et al., 2016) and STING can restrict the translation of viral and host proteins during RNA virus infection (Franz et al., 2018). Intriguingly, the severe acute respiratory syndrome coronavirus–encoded papain-like protease has been shown to inhibit IFN-1 production by directly interacting with and disrupting the STING-TRAF3-TBK1 complex (Chen et al., 2014). It is interesting that Rhbd2, Adam17, and Tmem173 (encoding STING) are upregulated in murine lung tissue after infection with RNA viruses, influenza A, and respiratory syncytial virus (Figure 2c); yet, the role of iRhom2 in the STING response to RNA virus infection, especially in the epithelium, remains largely unexplored.

CONCLUDING REMARKS
We have described several biological functions that link iRhom2 to key aspects of palmoplantar biology such as the regulation of the cytoskeletal stress response, barrier integrity, and modulating signaling by p63 and ADAM17. Furthermore, iRhom2 is emerging as a key regulator of ADAM17-mediated responses to inflammation and the innate immune response to viruses through its interaction with STING. Further investigation of iRhom2 biology will lead to new insights into its pleiotropic, yet tissue-specific functions, including within the skin, and may lead to the development of novel therapeutic strategies for PPKs, OSCC, and other inflammatory disorders.

REFERENCES
Adrain C, Zettl M, Christova Y, Taylor N, Freeman M. Tumor necrosis factor signaling requires iRhom2 to promote trafficking and activation of TACE. Science 2012;335:225–8.
Althoff K, Reddy P, Voltz N, Rose-John S, Möllberg J. Shedding of interleukin-6 receptor and tumor necrosis factor alpha. Contribution of the stalk sequence to the cleavage pattern of transmembrane proteins. Eur J Biochem 2000;267:2624–31.
Arcidiacono P, Webb CM, Brooke MA, Zhou H, Delaney PJ, Ng KE, et al. p63 is a key regulator of iRhom2 signalling in the keratinocyte stress response. Nat Commun 2018;9:1021.
Badenes M, Amin A, González-García I, Félix I, Burbridge E, Cavadas M, et al. Deletion of iRhom2 protects against diet-induced obesity by increasing thermogenesis. Mol Metab 2020;31:67–84.
Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 2013;41:D991–5.
Black RA, Rauch CT, Kozlowski CJ, Peschon JJ, Slack JL, Wolfson MF, et al. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature 1997;385:729–33.
Blaydon DC, Bianchetti P, Di WL, Plagnol V, Cabral RM, Brooke MA, et al. Inflammatory skin and bowel disease linked to ADAM17 deletion. N Engl J Med 2011;365:1502–8.
Blaydon DC, Etheridge SL, Risk JM, Hennies HC, Gay LJ, Carroll R, et al. RHBD2 mutations are associated with tylosis, a familial esophageal cancer syndrome. Am J Hum Genet 2012;90:340–6.
Bodemer C, Steijlen P, Mazereeuw-Hautier J, O’Toole EA. Treatment of hereditary palmoplantar keratoderma: a review by analysis of the literature (e-pub ahead of print). Br J Dermatol 2020;https://doi.org/10.1111/bjd.19144 (accessed 31 August 2020).
Brooke MA, Etheridge SL, Kaplan N, Simpson C, O’Toole EA, Ishida-Yamamoto A, et al. iRHom2-dependent regulation of ADAM17 in cutaneous disease and epidermal barrier function. Hum Mol Genet 2014;23:4064–76.
Brooke MA, Nitoiu D, Kellsell DP. Cell-cell connectivity: desmosomes and disease. J Pathol 2012;226:158–71.
Cavadas M, Oikonomidi I, Gaspar CJ, Burbridge E, Badenes M, Félix I, et al. Phosphorylation of iRhom2 controls stimulated proteolytic shedding by the metalloprotease ADAM17/TACE. Cell Rep 2017;21:745–57.
Chen X, Yang X, Zheng Y, Yang X, Xing Y, Chen Z. SARS coronavirus papain-like protease inhibits the type I interferon signaling pathway through interaction with the STING-TRAF3-TBK1 complex. Protein Cell 2014;5:369–81.
Dullo I, Muliyil S, Freeman M. The molecular, cellular and pathophysiological roles of iRhom pseudoproteases. Open Biol 2019;9:190003.
Franz KM, Neidermyer WJ, Tan YJ, Whelan SP, Kagan JC. STING-dependent translation inhibition restricts RNA virus replication. Proc Natl Acad Sci USA 2018;115:E2058–67.
Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–61.
Grieve AG, Xu H, Künzel U, Bambrough P, Sieber B, Freeman M. Phosphorylation of iRhom2 at the plasma membrane controls mammalian TACE-dependent inflammatory and growth factor signalling. Elife 2017;6:e23968.

Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J 1998;334:297–314.

Holm CK, Raabek SH, Gad HH, Bak RO, Jakobsen MR, Jiang Z, et al. Influenza A virus targets a cGAS-independent STING pathway that controls enveloped RNA viruses. Nat Commun 2016;7:10680.

Issuree PD, Maretzky T, McIlwain DR, Lang PA, et al. iRhom2 is critical for innate immunity to DNA viruses by mediating trafficking and stabilization of the adaptor STING. Nat Immunol 2015;16:1057–66.

Maruthappu T, Chikah A, Fell B, Delaney PJ, Brooke MA, Levet C, et al. Rhomboid family member 2 regulates cytoskeletal stress-associated keratin 16. Nat Commun 2017;8:14174.

Mcllwain DR, Lang PA, Maretzky T, Hamada K, Ohishi K, Maney SK, et al. iRhom2 regulation of TACE controls TNF-mediated protection against Listeria and responses to LPS. Science 2012;335:229–32.

Motwani M, Pesiridis S, Fitzgerald KA. DNA sensing by the cGAS-STING pathway in health and disease. Nat Rev Genet 2019;20:657–74.

Paladini RD, Takahashi K, Bravo NS, Coulombe PA. Onset of epidermalization after skin injury correlates with a reorganization of keratin filaments in wound edge keratinocytes: defining a potential role for keratin 16. J Cell Biol 1996;133:381–97.

Qing X, Chinenov Y, Redecha P, Madaio M, Singhania A, Graham CM, Gabrylova L, Moreira-Texeira L, Stavropoulos E, Pitt JM, et al. Transcriptional profiling unveils type I and II interferon networks in blood and tissues across diseases. Nat Commun 2019;10:2887.

Skurski J, Penninman CM, Geesala R, Dixit G, Pulipati P, Bhardwaj G, et al. Loss of iRhom2 accelerates fat gain and insulin resistance in diet-induced obesity despite reduced adipose tissue inflammation. Metabolism 2020;106:154194.

Stevens HP, Kehell DP, Bryant SP, Bishop DT, Spur‐RK, Weissenbach J, et al. Linkage of an American pedigree with palmoplantar keratodermia and malignancy (palmoplantar ectodermal dysplasia type III) to 17q24. Literature survey and proposed updated classification of the keratodermas. Arch Dermatol 1996;132:640–51.

Swenson O, Langbein L, McMillan JR, Stevens HP, Leigh IM, McLean WH, et al. Specialized keratin expression pattern in human ridged skin as an adaptation to high physical stress. Br J Dermatol 1998;139:767–75.

Thomas BR, O’Toole EA. Diagnosis and management of inherited palmoplantar keratoderma. Acta Derm Venereol 2020;100:adv00094.

Vinothkumar KR, Strisovsky K, Andreava A, Christova Y, Verhelst S, Freeman M. The structural basis for catalysis and substrate specificity of a rhomboid protease. EMBO J 2010;29:3148–59.

Weskamp G, Tushaus J, Li D, Feederle R, Maretzky T, Swendemann S, et al. ADAM17 stabilizes its interacting partner inactive Rhomboid 2 (iRhom2) but not inactive Rhomboid 1 (iRhom1). J Biol Chem 2020;295:3430–8.

Wu J, Chen ZJ. Innate immune sensing and signaling of cytokine nuclear acids. Annu Rev Immunol 2014;32:461–88.

Xu M, Ge C, Qin Y, Lou D, Li Q, Feng J, et al. Functional loss of inactive rhomboid-like protein 2 mitigates obesity by suppressing pro-inflammatory macrophage activation-triggered adipose inflammation. Mol Metab 2020;34:112–23.

Zhong B, Yang Y, Li S, Wang YY, Li Y, Diao F, et al. The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. Immunity 2008;29:538–50.