IQGAP1 and IQGAP3 Serve Individually Essential Roles in Normal Epidermal Homeostasis and Tumor Progression

Christine L. Monteleon[1], Andrew McNeal[1], Elizabeth K. Duperret[1], Seung J. Oh[1], Emily Schapira[1] and Todd W. Ridky[1]

IQ motif–containing GTPase-activating protein (IQGAP) scaffolding proteins regulate many essential cellular processes including growth factor receptor signaling, cytoskeletal rearrangement, adhesion, and proliferation and are highly expressed in many cancers. Using genetically engineered human skin tissue *in vivo*, we demonstrate that diminished, sub-physiologic expression of IQGAP1 or IQGAP3 is sufficient to maintain normal epidermal homeostasis, whereas significantly higher levels are required to support tumorigenesis. To target this tumor-specific IQGAP requirement *in vivo*, we engineered epidermal keratinocytes to express individual IQGAP protein domains designed to compete with endogenous IQGAPs for effector protein binding. Expression of the IQGAP1-IQ motif decoy domain in epidermal tissue *in vivo* inhibits oncogenic Ras-driven mitogen-activated protein kinase signaling and antagonizes tumorigenesis, without disrupting normal epidermal proliferation or differentiation. These findings define essential non-redundant roles for IQGAP1 and IQGAP3 in the epidermis and demonstrate the potential of IQGAP antagonism for cancer therapy.

Journal of Investigative Dermatology (2015) **135,** 2258–2265; doi:10.1038/jid.2015.140; published online 14 May 2015

INTRODUCTION

The progression of invasive neoplasia frequently involves dysregulation of cellular signals that are integral to normal processes. Therapeutics against common oncogenic drivers often directly interfere with central components of core signaling pathways, and their clinical utility is limited by toxicity to normal cells. In this regard, blocking cell-signaling scaffolding molecules, in addition to oncogenic drivers, may be a promising strategy. As modulators of EGFR and mitogen-activated protein kinase (MAPK) signaling, IQ motif–containing GTPase-activating proteins (IQGAPs) may be useful therapeutic targets.

IQGAPs are a family of ubiquitously expressed scaffolding proteins that coordinate signaling pathways by facilitating physical interactions between effector proteins to enhance signal transduction (Brown and Sacks, 2009). The IQGAP family is comprised of three isoforms (IQGAP1, IQGAP2, and IQGAP3), which each contain multiple protein recognition domains: the F-actin-binding calponin homology domain; a polyproline-binding region (WW) that binds ERK 1/2; an IQ motif (IQM) that binds to MEK1/2, Raf, EGFR, and Calmodulin; the Ras GAP–related domain that binds to Rac1 and Cdc42; and the C-terminal domain that interacts with E-cadherin, ß-catenin, APC, and Clip-170 (Figure 1a) (Fukata *et al.*, 2002; Johnson *et al.*, 2009; White *et al.*, 2009). Through these interactions, IQGAPs regulate growth factor receptor signaling, MAPK flux, cell–cell adhesion, cell cycle regulation, and cytoskeletal rearrangements (Briggs and Sacks, 2003; White *et al.*, 2012)—all critical cellular processes that are tightly coordinated both spatially and temporally for proper epidermal growth and differentiation.

Despite the role that IQGAP scaffolds have in supporting these key cell functions, IQGAP1-null mice develop normally (Li *et al.*, 2000). The lack of an overt phenotype suggests the possibility of functional rescue by another IQGAP isoform(s) in mice. In this regard, IQGAP3 seems to be the most likely candidate, as it shares 60% homology to IQGAP1 and has a relatively broad expression pattern (White *et al.*, 2012). Although IQGAP3-null murine tissue has not been generated, RNA interference studies in the mouse epithelial cell line Eph4 demonstrate that, like IQGAP1, IQGAP3 also facilitates signaling through MAPK (Nojima *et al.*, 2008). IQGAP2 expression is much more limited and expressed predominantly in the liver, gastro-intestinal tract, and platelets (Nojima *et al.*, 2008). IQGAP2 is not expressed in the skin. The effects of acute IQGAP loss in adult murine or human tissue have

[1]*Department of Dermatology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, Pennsylvania, USA*

Correspondence: Todd W. Ridky, Department of Dermatology, University of Pennsylvania, Perelman School of Medicine, 421 Curie Boulevard—Biomedical Research Building #1010, Philadelphia, Pennsylvania 19104, USA. E-mail: ridky@mail.med.upenn.edu

Abbreviations: cDNA, complementary DNA; IQGAP, IQ motif–containing-GTPase activating protein; MAPK, mitogen-activated protein kinase; pERK, phospho ERK; SCC, squamous cell carcinoma

Received 11 December 2014; revised 20 February 2015; accepted 26 March 2015; accepted article preview online 7 April 2015; published online 14 May 2015

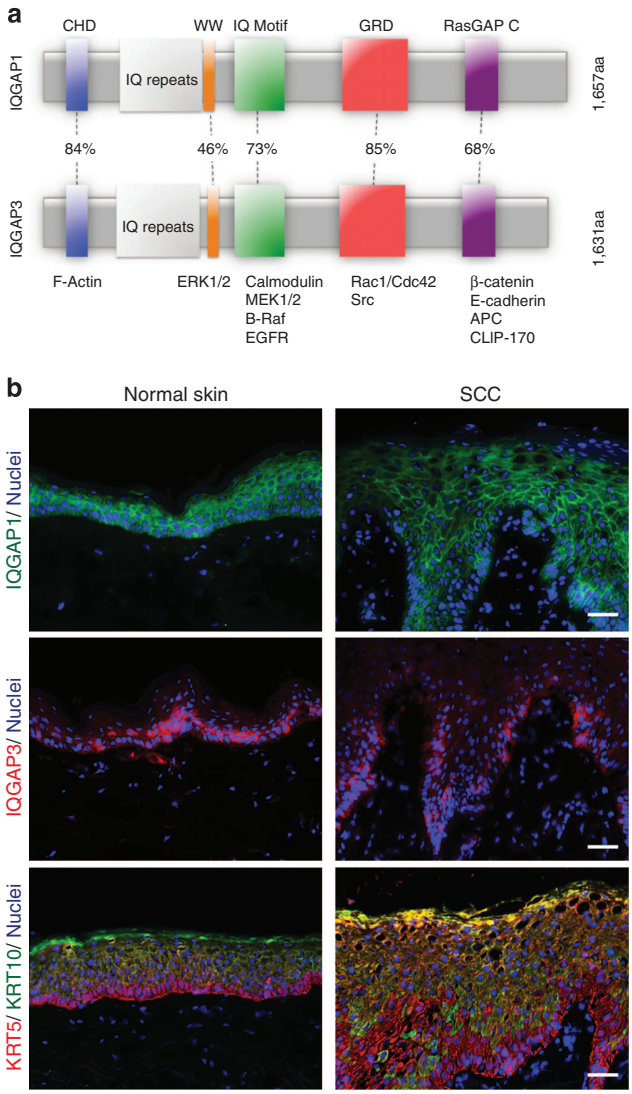

Figure 1. IQGAP1 and IQGAP3 are highly expressed in normal human epidermis and epidermal squamous cell carcinoma. (**a**) IQGAP1 and IQGAP3 are multidomain scaffolding proteins with highly homologous structural motifs and the ability to bind a range of cell signaling and cytostructrual molecules. (**b**) Cryosections of human normal and SCC tissue were prepared for immunofluorescence microscopy of IQGAP1 (green), IQGAP3 (red), and nuclei (blue). Bar = 100 μm. IQGAP1 is expressed throughout the epidermal compartment of both normal and cancer tissue. IQGAP3 expression is concentrated within the proliferative basal layer. Keratin 5 (red) and Keratin 10 (green) differentiation markers are also shown in both normal and SCC tissue. Bar = 100 μm. CHD, calponin homology domain; IQGAP, IQ motif–containing GTPase-activating protein; SCC, squamous cell carcinoma.

not been studied but are potentially important to examine as phenotypes resulting from protein loss in developed tissues can vary significantly from those observed with loss during embryogenesis. Further, mouse and human tissues may differ with regard to their specific IQGAP requirements.

IQGAP1 is upregulated in many human cancers including lung, colon, liver, breast, glioblastoma, and melanoma (Clark *et al.*, 2000) and regulates core cellular processes critical for malignant progression including proliferation, adhesion, and migration. As progression to invasive neoplasia frequently involves dysregulation of normal cellular processes rather than activation of pathways completely lacking in the normal tissue, targeting central components of dysregulated pathways often results in high toxicity. As modulators rather than required core elements of key oncogenic signaling pathways, IQGAP1 or IQGAP3, may be uniquely poised therapeutic targets.

In this study, we define the roles of IQGAP1 and IQGAP3 in both human epidermal homeostasis and invasive epithelial cancer, by using genetically defined human skin tissues maintained in their native, orthotopic location *in vivo* (Ridky *et al.*, 2010). Tissues were generated using primary keratinocytes expressing short hairpin RNAs against IQGAP1 or IQGAP3. We show that the effects of IQGAP loss vary with the extent of the knockdown, as partial knockdown (50–85%) of either IQGAP1 or IQGAP3 was well tolerated in normal tissues, whereas more complete knockdown (85–95%) resulted in marked proliferation arrest and tissue failure *in vivo* We also engineered human tissue grafts to express tumor-associated oncogenes sufficient to drive squamous cell carcinoma (SCC) and demonstrate a tumor-specific requirement for increased IQGAP1 and IQGAP3 in this cancer context. Finally, we developed an interfering decoy peptide based on the IQGAP-IQM domain that selectively inhibits oncogenic Ras activity in tissue. These findings establish the requirements of IQGAP1 and IQGAP3 in epidermal homeostasis and cutaneous tumor progression and establish the potential therapeutic utility of targeting IQGAPs in human cancer.

RESULTS

IQGAP1 and IQGAP3 are expressed in normal human epidermis and in epidermal SCC

Both normal human skin and spontaneous human SCC tumor tissues display robust IQGAP1 and IQGAP3 expression (Figure 1b). IQGAP1 is located throughout all layers of the epidermal compartment, whereas IQGAP3 expression is concentrated within the proliferative basal cells and is relatively absent in the post-mitotic suprabasal layers (Figure 1b and Supplementary Figure S1 online). Within keratinocytes, IQGAP1 and IQGAP3 are localized throughout the cytoplasm and are also concentrated at the plasma membrane. This expression and cellular distribution reflects the diversity of scaffolding activities and interacting effector proteins and is consistent with IQGAP expression patterns in other tissues (Nabeshima *et al.*, 2002; Berglund *et al.*, 2008). We were unable to detect IQGAP2 protein in skin tissue or cultured primary keratinocytes (data not shown), consistent with its previously established limited tissue distribution (White *et al.*, 2009, 2012).

IQGAP1 and IQGAP3 are both required for human epidermal keratinocyte proliferation

To determine the degree to which IQGAP1 and IQGAP3 are required for normal epidermal function, we generated stable populations of primary, early passage human IQGAP1 or IQGAP3 knockdown (IQGAP1i or IQGAP3i) keratinocytes utilizing lentiviral transduction to drive constitutive short