

# Molecular Pathways: Receptor Ectodomain Shedding in Treatment, Resistance, and Monitoring of Cancer

Miles A. Miller<sup>1</sup>, Ryan J. Sullivan<sup>2</sup>, and Douglas A. Lauffenburger<sup>3</sup>

## Abstract

Proteases known as sheddases cleave the extracellular domains of their substrates from the cell surface. The A Disintegrin and Metalloproteinases ADAM10 and ADAM17 are among the most prominent sheddases, being widely expressed in many tissues, frequently overexpressed in cancer, and promiscuously cleaving diverse substrates. It is increasingly clear that the proteolytic shedding of transmembrane receptors impacts pathophysiology and drug response. Receptor substrates of sheddases include the cytokine receptors TNFR1 and IL6R; the Notch receptors; type-I and -III TGF $\beta$  receptors; receptor tyrosine kinases (RTK) such as HER2, HER4, and VEGFR2; and, in particular, MET and TAM-family RTKs AXL and Mer (MerTK). Activation of receptor shedding by mechan-

ical cues, hypoxia, radiation, and phosphosignaling offers insight into mechanisms of drug resistance. This particularly holds for kinase inhibitors targeting BRAF (such as vemurafenib and dabrafenib) and MEK (such as trametinib and cobimetinib), along with direct sheddase inhibitors. Receptor proteolysis can be detected in patient fluids and is especially relevant in melanoma, glioblastoma, lung cancer, and triple-negative breast cancer where RTK substrates, MAPK signaling, and ADAMs are frequently dysregulated. Translatable strategies to exploit receptor shedding include combination kinase inhibitor regimens, recombinant decoy receptors based on endogenous counterparts, and, potentially, immunotherapy. *Clin Cancer Res*; 23(3); 623–9. ©2016 AACR.

## Background

### ADAM sheddases and proteolytic regulation

ADAM sheddases proteolytically cleave the extracellular domain (ectodomain) of hundreds of transmembrane proteins from the cell surface, allowing them to transport in soluble form to neighboring cells. The sheddases ADAM10 and ADAM17 (also known as TNF $\alpha$ -converting enzyme, TACE) are of central importance. They have traditionally been known for facilitating cell signaling through the pro-protein cleavage of inflammatory cytokines, such as TNF $\alpha$ , and growth factors, including TGF $\alpha$ . ADAMs additionally cause the  $\alpha$ -secretase cleavage of peptides such as amyloid precursor protein (APP). As a third class of substrates, ADAMs shed numerous receptors for cytokines, growth factors, adhesion molecules, and lipoproteins. Although ligand and peptide shedding are crucial to understanding sheddase biological functions, and many reviews extensively discuss the topic, here we instead focus on the increasingly appreciated proteolysis of receptors themselves.

ADAM10 and ADAM17 are structurally similar to other transmembrane ADAMs. They contain a catalytic metalloproteinase

domain related to that of matrix metalloproteinases (MMP), a disintegrin domain important in cell adhesion, and a C-terminal cytoplasmic tail involved in activity regulation. ADAM10 and ADAM17 share common substrates, yet nevertheless, display unique and context-dependent catalytic preferences. Other proteases can function as sheddases, including MMPs such as MT1-MMP; nonetheless, ADAMs are typically more prominent. Sheddases frequently become overexpressed, along with many of their substrates in various cancers and precancerous lesions. Furthermore, sheddase substrates such as TGF $\alpha$  and human epidermal growth factor receptor 2 (HER2/ERBB2) have oncogenic potential. Both *ADAM10*<sup>-/-</sup> and *ADAM17*<sup>-/-</sup> mice are not viable, underscoring their central role in development. Knockout mouse phenotypes suggest defects in signaling pathways that canonically depend upon ADAM-mediated proteolysis. In particular, *ADAM10*<sup>-/-</sup> and *ADAM17*<sup>-/-</sup> mice exhibit impaired developmental signaling in the Notch and EGFR pathways, respectively (1).

Proteolytic ectodomain shedding is regulated at the level of both the sheddases as well as their individual substrates. In general, regulation of the latter offers more selective control over otherwise promiscuous enzymes. Examples include substrate dimerization (2, 3) and intracellular domain phosphorylation of the sheddase substrates CD44 and pro-NGF (3, 4). Colocalization of ADAM17 and its substrates, particularly in lipid rafts, also regulates activity (5). Therapeutics may impact sheddase activity at the substrate level; for instance, the  $\alpha$ -HER2 monoclonal antibody (mAb) trastuzumab (Herceptin; Genentech; FDA approved for HER2<sup>+</sup> breast cancer) blocks HER2 shedding (6).

Regulation of proteases themselves has been extensively studied. The four Tissue Inhibitor of Metalloproteinases (TIMP1–TIMP4) are the key endogenous regulators of ADAMs

<sup>1</sup>Center for Systems Biology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. <sup>2</sup>Division of Medical Oncology, Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, Massachusetts. <sup>3</sup>Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

**Corresponding Author:** Douglas A. Lauffenburger, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139. Phone: 617-252-1629; Fax: 617-258-0204; E-mail: lauffen@mit.edu

doi: 10.1158/1078-0432.CCR-16-0869

©2016 American Association for Cancer Research.

and other metalloproteinases, including MMPs. Tumor cells and associated stromal populations frequently overexpress TIMPs. TIMPs generally restrict protease activity by directly binding to the catalytic domain. ADAM17 undergoes regulated homodimerization, and these dimers complex with TIMP3 (7). Consequently, catalytic site access is dynamically regulated on the cell surface (8). C-terminal ADAM17 phosphorylation at Thr735 is associated with activation in some cases (9). A wide range of phosphosignaling cues influence ADAM17-mediated ectodomain shedding, including through the RAS/RAF/MEK, Jnk/cJUN, p38, and PLC $\gamma$ /PKC/mTORC1 pathways (4, 9–12). Upstream of these pathways, myriad stimuli including osmotic stress, hypoxia, ER stress, and activity of RTKs, cytokine receptors, and G-protein coupled receptors (GPCR) may influence sheddase activity (13). Compared with ADAM17, the activity of ADAM10 is often considered more constitutive. Nonetheless, ADAM10 similarly undergoes dimerization and dynamic association with TIMP1, and has regulated activity from signaling cues (7, 11).

### Sheddase-mediated receptor activation

The downstream effects of receptor ectodomain shedding vary drastically and can either downregulate activity or perform a critical step in receptor activation. As an example of the latter, ADAM-mediated proteolysis is required for Notch receptor activation. After receptor engagement with Delta-like ligand, ADAM10 cleaves the Notch ectodomain. Following ectodomain loss, the remaining Notch receptor fragment is processed by regulated intramembrane proteolysis (RIP), which is distinct from sheddase processing. During RIP, the multi-subunit protease complex,  $\gamma$ -secretase, proteolytically releases the receptor C-terminus from the cell membrane. Free C-terminal fragment then translocates to the nucleus and influences transcriptional processes (Fig. 1, left; ref. 1). Similar RIP has been described for ErbB4/HER4 (14), the Ephrin receptor EphB2 (15), and the p75 neurotrophin receptor (16), among others. However, not all nuclear translocation involves RIP, including for EGFR (17). Besides RIP, receptor shedding may activate signaling by allowing the soluble receptor ectodomain to form a signaling-competent complex with co-receptors and ligand on other cells, as has been found with IL6R (18). Nonetheless, RIP remains the most common and important mechanism for cleavage of receptors such as Notch to drive their activity.

### Sheddase-mediated downregulation

In contrast, receptor shedding may attenuate activity (Fig. 1, center) through both downregulation on the cell surface and through competitive ligand binding of the released ectodomain. The Mer proto-oncogene tyrosine kinase (MerTK) exemplifies such behavior. Related to other TAM receptors AXL and Tyro3, MerTK is highly expressed on some cancers (for instance, in melanoma) and on most macrophage populations, including tumor-associated macrophages. In the latter, MerTK guides phagocytic clearance of apoptotic bodies (efferocytosis). This occurs via a bridged connection between externalized phosphatidylserine on apoptotic bodies and the MerTK ligands, Gas6 and protein S (which are not metalloproteinase substrates). Ligand engagement with MerTK activates efferocytosis and causes anti-inflammatory signaling by suppressing NF- $\kappa$ B. Following these functions, *MerTK*<sup>-/-</sup> mice accumulate residual apoptotic bodies in tissue and develop autoimmune phenotypes (19). Soluble, circulating levels of MerTK and AXL ectodomain are elevated in patients with the autoimmune disorder systemic lupus erythe-

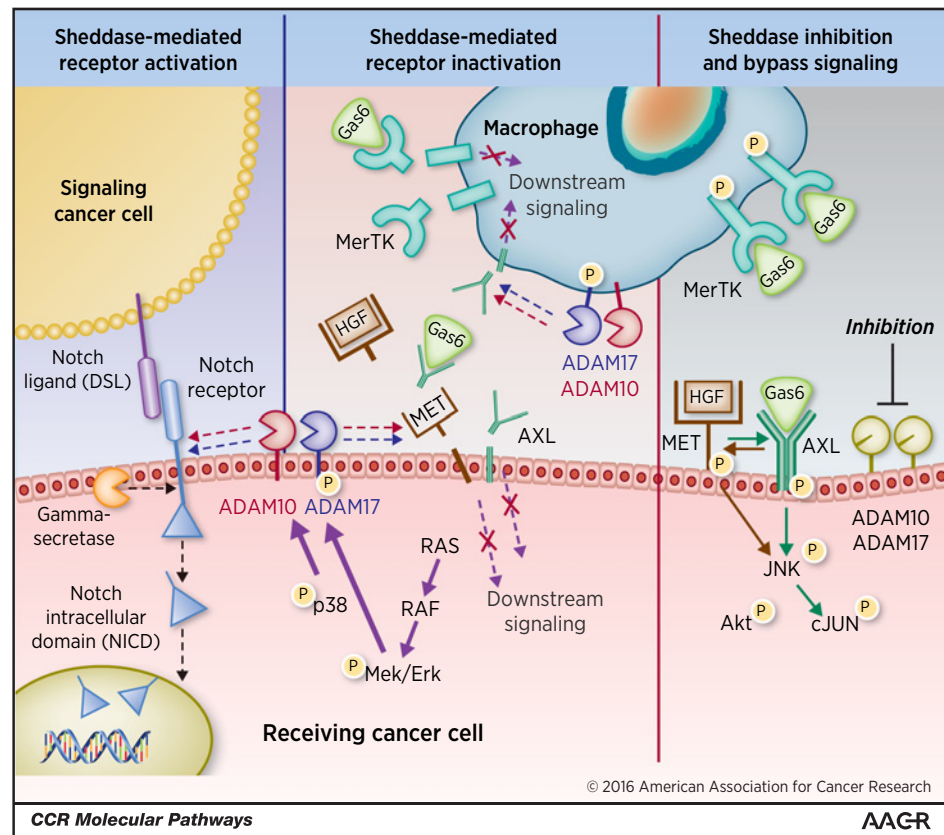
matusus (SLE). Yet, surface receptor levels actually decrease on circulating monocytes of patients with SLE (20). Genetically engineered mice with MerTK either knocked-out (*MerTK*<sup>-/-</sup>), or mutated at its ADAM17 proteolysis site to confer cleavage resistance (*MerTK*<sup>CR</sup>), provide strong causal evidence that MerTK shedding downregulates its activity (21). In a model of ischemia/reperfusion-induced lung injury, *MerTK*<sup>-/-</sup> mice exhibited increased inflammation and injury, whereas the *MerTK*<sup>CR</sup> mice showed the opposite. As further evidence, recombinant soluble receptor ectodomains reveal the specific effect of the ectodomain cleavage product. Fc-fusion with MerTK ectodomain binds Gas6, limits Akt signaling downstream of endogenous MerTK, and reduces efferocytosis (22). Studies using ADAM10 or ADAM17 perturbation allow for investigation of sheddase activity on receptor signaling but are more difficult to interpret due to the many affected sheddase substrates. Nonetheless, ADAM17, but not ADAM10, silencing decreases soluble MerTK accumulation (12). Furthermore, pharmacologic metalloproteinase inhibition increases surface MerTK engagement and enhances MerTK phosphorylation in a model of retinopathy (23).

Ectodomain shedding downregulates the activity of several other receptors, although MerTK is exceptional in having a cleavage-resistant mutant mouse model as evidence. For instance, the closely related AXL expressed on cancer cells is cleaved by ADAM10 and ADAM17; AXL surface levels, phosphorylation, and coimmunoprecipitation with other ADAM substrates, including HER2 and MET, increase with protease inhibition; and direct sheddase inhibition affects cell signaling and mitosis in an AXL-dependent manner (11). Likewise, evidence for ADAM-mediated AXL shedding has been found in macrophages and B cells of lupus-prone mice (24). The hepatocyte growth factor receptor (HGF-R/MET) also exhibits decreased biological activity after ectodomain shedding. For instance, high levels of endogenous TIMP1 block ADAM10 activity in liver metastases. Consequently, surface MET accumulates and responds to its ligand HGF, which is not a sheddase substrate (25, 26). Similarly, in a model of the invasive disease endometriosis, metalloproteinase inhibition stimulates surface accumulation of MET, increases its phosphorylation, and activates downstream phosphosignaling pathways in a MET-dependent manner (10). The type III TGF $\beta$  receptor (T $\beta$ RIII) presents TGF $\beta$  ligand to enhance prometastatic signaling. Transgenic studies used T $\beta$ RIII that had been mutated to either have more or less affinity for proteolysis. Results show that enhanced shedding decreases TGF $\beta$ -related signaling, decreases metastasis, and enhances survival in a xenograft model (27). Other receptors with compelling evidence that shedding downregulates their activity include T $\beta$ RI (28), TNFR1 (29), and vascular endothelial growth factor receptor 1 (VEGFR1/FLT1; ref. 30).

Some receptors are proteolytically shed from the cell surface, but the consequent biological impact remains uncertain. For many cases, experiments involving genetic mutation at the receptor cleavage site have not been performed. HER2 serves as one example. ADAM10 sheds HER2 in breast cancer, producing the membrane-bound HER2 cleavage fragment p95<sup>HER2</sup>. Anti-HER2 antibody therapies such as trastuzumab cannot bind p95<sup>HER2</sup>, because the binding site has been proteolytically shed. Consequently, it was hypothesized that HER2 shedding caused trastuzumab resistance, which could be blocked by cotreatment with a sheddase inhibitor. The ADAM10/ADAM17 small-molecule inhibitor aderasib (INCB7839; Incyte Corp.) was tested in HER2<sup>+</sup> breast cancer phase I/II trials in combination with

**Figure 1.**

Modes of receptor ectodomain shedding. Left, as an example of activation, ADAM-mediated proteolysis of the Notch receptor is followed by regulated intramembrane proteolysis to enable nuclear translocation and activity. Center, as an example of receptor downregulation, the proteolytic cleavage of MET, AXL, and MerTK decreases surface levels while simultaneously producing soluble decoy receptors that sequester cognate ligands HGF and Gas6. Right, by directly inhibiting sheddases or by targeting the signaling pathways that regulate their activity, previously cleaved receptors accumulate on the cell surface, where they signal through compensatory pathways. Decreased levels of soluble decoy receptors allow cognate ligands to activate surface receptors.



trastuzumab (NCT01254136). Despite promising initial results and evidence that aderbasis blocks HER2 shedding (31, 32), the trial failed and development was discontinued (33). Subsequent work has revealed that HER2 shedding may actually reduce its signaling in some contexts (10, 34), and recent analysis of p95<sup>HER2</sup> shows ambiguous prognostic value (35).

ErbB4/HER4 is another complex example, given that its C-terminal fragment translocates both to the nucleus, where it influences transcription (14), and to the mitochondria, where it elicits proapoptotic responses (36). Moreover, the activity of the HER4 ectodomain itself has not been fully investigated. Some evidence suggests that blocking HER4 shedding correlates with increased phosphorylation in some, but not most, cell types (10, 11). Other receptors such as VEGFR2 (37) and low-density lipoprotein receptor (LDLR; refs. 11, 38) have been demonstrated as sheddase substrates, or implicated as possible substrates in the case of Tyro3 (11) and IGF-1R (39), but the impacts of shedding on receptor activity remain relatively unexplored.

#### Alternative pathways

Mitogenic, prosurvival, and prometastasis EGFR activity depends greatly on the proteolytic shedding of its ligands, including TGF $\alpha$ , heparin-binding EGF (HB-EGF), amphiregulin (AREG), and EGF. Shedding allows soluble ligands to diffuse, bind, and activate receptors on the same or neighboring cells in an autocrine or paracrine manner, respectively. However, the degree of EGFR proteolysis itself is less certain and less significant. In some contexts, metalloproteinase inhibition has no impact on the minimal accumulation of soluble EGFR ectodomain in supernatant (10, 11). Consequently, sheddase inhibition effectively

blocks EGFR signaling when that activity is ligand dependent, by blocking ligand release without directly influencing receptor levels (10, 32, 40). In other instances, when EGFR expression is high (e.g.,  $10^6$  receptors per cell), soluble EGFR ectodomain (p110<sup>EGFR</sup>) can be detected in patients and cell culture supernatants. Moreover, its release can be partially blocked by non-specific metalloproteinase inhibitors (41, 42). Interestingly, one group has reported a role for membrane-anchored serine proteases including hepsin (43) and the matriptase-prostasin proteolytic cascade (44) in shedding EGFR. Despite these reports, in the context of EGFR signaling, the vast majority of evidence focuses on shedding EGF ligands rather than the receptor itself.

Proteolytic shedding is not the only mechanism by which soluble receptor ectodomain can be released from cells. Many transmembrane proteins, including receptors, are packaged into extracellular vesicles such as exosomes, which serve as promising biomarkers for cancer detection and monitoring (45). For non-canonical sheddase substrates such as EGFR, a significant fraction of receptor released from the cell surface is actually associated with extracellular vesicles; in contrast, most extracellular levels of quintessential sheddase substrates AXL and MET are not bound to membranous vesicles in some instances (11). Alternative RNA splicing also accounts for ectodomain release, as noted for HER2 in particular (46).

## Clinical-Translational Advances

### Treatment strategies

Some of the most successful biologic therapeutics use the ligand-trap strategy comprising a receptor ectodomain Fc-fusion,

which has been applied exclusively to sheddase substrates among FDA-approved compounds. For instance, aflibercept (Eylea/Zaltrap; Regeneron Pharmaceuticals/Sanofi-Aventis; FDA approved for metastatic colorectal cancer and wet macular degeneration) consists of IgG1 Fc region fused with the mixed ligand-binding domains of VEGFR1 and VEGFR2, both of which are ADAM17 substrates (30, 37, 47). As a decoy receptor, aflibercept exhibits two orders of magnitude higher affinity to VEGF-A than the antibody-based anti-VEGF treatments bevacizumab and ranibizumab. Furthermore, aflibercept demonstrates therapeutic superiority in certain retinopathy populations (48). Other ligand-trap treatments are based on the ADAM17 substrates IL1R [ref. 49; rilonacept/Arcalyst; Regeneron Pharmaceuticals; FDA approved for Cryopyrin-Associated Periodic Syndromes (CAPS)] and TNFR1 (etanercept/Enbrel; Amgen; FDA approved for rheumatoid arthritis and others). Among preclinical compounds, a recombinant AXL decoy receptor was engineered to have 80-fold greater affinity to its ligand Gas6, and the resulting Fc-fusion limited metastasis in a xenograft model (50). Other preclinical Fc-fusions have been developed for MerTK (22), IGF1R (51), FGFR (52), and EGFR/HER4 (53). In a similar vein, lentiviral approaches deliver soluble decoy receptors for MET (54) and AXL (55). Such approaches exhibit efficacy in xenograft models through ligand sequestration and interference with receptor homodimerization on the cell surface (54).

Beyond mimicking ectodomain shedding directly, other therapeutics may activate proteolysis of the endogenously expressed protein. The preclinical  $\alpha$ -MET mAb DN30 activates MET shedding (56, 57), and the clinical AXL kinase inhibitor R428/BGB324 (phase IIb/II melanoma, NCT02872259) stimulates AXL proteolysis (11). Perturbation of protease activity itself has proven a challenging strategy given the many pleiotropic effects. Nonetheless, a TIMP1-neutralizing antibody has shown some efficacy through activating receptor ectodomain shedding in a xenograft model (11).

Inhibitors of metalloproteinases specific to ADAM10, ADAM17, and other sheddases, including ADAM12, have been developed as anticancer and anti-inflammatory agents based on the prominent role the enzymes play in releasing growth factors and cytokines from the cell surface. With the exception of the aforementioned ADAM10/ADAM17 inhibitor aderbasib (INCB7839), these agents have been limited to preclinical testing and include small molecules (GI254023, INCB3619, INCB7839), antibodies such as  $\alpha$ -ADAM17 D1(A12) (ref. 40), and recombinant ADAM-9, -10, and -12 pro-domains (58). Early metalloproteinase inhibitors exhibited poor selectivity and high toxicity. Yet, even second-generation drugs with specificity toward ADAM10 and ADAM17 have failed in clinical trials. Aderbasib specificity is reportedly similar to the related compound INCB3619, showing a 50- to 100-fold specificity for ADAM10 and ADAM17 over ADAM9, but still displays potent inhibition of MMP-2, -12, and -15. Preclinically, aderbasib exhibited synergistic efficacy when combined with the EGFR/HER2 kinase inhibitor lapatinib (Tykerb; Novartis; FDA approved for HER2<sup>+</sup> breast cancer) in an HER2<sup>+</sup> breast cancer xenograft model. However, in its clinical trial (NCT01254136), patients receiving 100 to 300 mg aderbasib combined with trastuzumab ( $n > 40$ ) and, in some cases, trastuzumab with docetaxel ( $n > 10$ ) showed a response rate that was not significantly improved over historical data. Plasma HER2 extracellular domain substantially decreased during treatment, indicating aderbasib successfully reduced HER2 shedding in

patients. However, patient response was unpredictably heterogeneous, despite a subgroup analysis of tumor p95<sup>HER2</sup> and plasma HER2 levels. Such mixed clinical outcomes may be attributed to the pleiotropic sheddase roles and, possibly, to off-target drug effects. Encouragingly, an  $\alpha$ -ADAM17 mAb shows efficacy in tumor models that depend on ligand-mediated EGFR signaling (59). Most likely, it will be necessary to first analyze expression of multiple protease substrates, including both EGF ligands along with receptors such as MET and AXL, when selecting patient populations for future sheddase inhibitor trials.

### Overcoming resistance

Sheddase activity responds to the activity of multiple phospho-signaling pathways, and small-molecule kinase inhibitors impact sheddase activity. Furthermore, changes in sheddase activity may serve as an early adaptive response to kinase inhibition and promote therapeutic resistance. Most prominently, MAPK pathway inhibition using BRAF and/or MEK inhibitors in patients with melanoma downregulates shedding of RTKs, including AXL and MET. Consequently, these receptors accumulate in the tumor (Fig. 1, right) and elicit drug resistance through signaling pathways, such as Jnk/cJUN and PI3K/Akt, that can bypass the RAF/RAS/MEK/ERK pathway (11). Noninvasively measured AXL and MET shedding in plasma samples predicts kinase inhibitor resistance, and such resistance mechanisms can be overcome using combination regimens of MAPK and AXL/MET kinase inhibitors (11). Similar AXL- and/or MET-driven resistance to kinase inhibition exists in *Kras*-mutant colorectal cancer, EGFR-dysregulated lung cancer, triple-negative breast cancer, and glioblastoma, among others (60). Thus, receptor ectodomain shedding is altered in the context of kinase inhibition, which may drive adaptive therapeutic resistance in some instances. Targeting this mechanism of resistance (e.g., increased MET or AXL signaling) by developing two- and three-drug regimens, given either simultaneously or in direct sequence, is, thus, a logical next step to be explored in clinical trials. Importantly, plasma monitoring of ectodomain shedding is feasible, may be an important predictive marker of patient outcome to kinase inhibitor treatment, and may help identify which patients do or do not need to be treated with multidrug regimens.

### Microenvironmental context and immunotherapy

Modulation of sheddase activity influences malignant and non-malignant elements of the tumor microenvironment. Innate immune cells, including natural killer (NK) cells, dendritic cells, and tumor-associated macrophages, are particularly important, as they frequently express high levels of sheddases and their substrates. In addition to cytokine and TAM receptors, other immunologically significant substrates include the co-stimulatory molecule CD40-L (61), the IgE receptor CD23 (62), and the immune checkpoint TIM3 (63). MAPK inhibition may exert significant effects on immune cells as well, particularly for BRAF inhibitors (e.g., vemurafenib, which is FDA approved for melanoma) that paradoxically activate MAPK signaling in BRAF wild-type cells. For MEK inhibitors (e.g., trametinib, which is FDA approved for melanoma) or combined BRAF/MEK inhibitor regimens (which limit paradoxical activation), sheddase downregulation may lead to unwanted decreases in leukocyte-derived MerTK/AXL decoy receptor and increased tumor-supporting TAM-receptor signaling in associated macrophages and NK cells (64). In other contexts, decreased sheddase activity may be beneficial. For instance, blocked cleavage of the Fc-receptor Fc $\gamma$ RIIIa (CD16) can improve

NK cell effector activity during antibody therapy (65). With the emergence of immune checkpoint inhibition and the development of mAbs targeting programmed death 1 (PD1; nivolumab and pembrolizumab, which are FDA approved for melanoma, non-small cell lung cancer, and others) and its ligand (PDL1; e.g., atezolizumab, which is FDA approved for urothelial bladder cancer), it is critical to understand the effects of kinase inhibitors on the tumor microenvironment to better inform combinatorial regimens. Currently, there are a number of ongoing studies combining MAPK pathway inhibitors with PD1/PDL1 inhibitors. It will be crucial in these clinical trials to perform carefully planned correlative work to gain a deeper understanding about the influence of therapy on sheddase activity. Such work may help optimize combination treatments and predict their efficacy.

#### Monitoring ectodomain shedding for personalized treatment

Noninvasive monitoring of receptor shedding has the potential to identify patients likely to respond to a given therapeutic strategy. Soluble receptors, proteases, and catalytic sheddase activities (66) are readily detectable in the blood or other fluids of patients with cancer or inflammatory pathologies. Such measurements correlate with disease state or outcome in several cases (10, 67–71). However, high interpatient heterogeneity, coupled with detectable ectodomain shedding even in healthy individuals, presents a challenge to using soluble receptors as a standalone diagnostic. Nonetheless, monitoring dynamic response in ectodomain shedding to a given therapy may prove more valuable than mere assessment of pretreatment levels in predicting outcome to therapy. In addition, incorporating a signature of multiple sheddase substrates, as well as combining sheddase data with other blood-based molecular analyses (e.g., circulating tumor cell, exosomes, and circulating free DNA), may prove useful. To date, there are only a few clinical efforts exploring the utility of sheddase monitoring, but the results are encouraging. For example, in the phase I/II trial of the sheddase inhibitor aderbisib (INCB7839) in patients with HER2<sup>+</sup> breast cancer, circulating levels of HER2 ectodomain were noninvasively monitored and observed to decrease with inhibitor treatment, thus allowing assessment of pharmacodynamics (31). In patients with BRAF-

mutant melanoma, plasma levels of seven RTK sheddase substrates, including MerTK, AXL, and MET, decreased in a subset of patients treated with combination BRAF and MEK inhibition. Although this was an expected finding, given the reliance of sheddase activity on MAPK signaling, not all patients showed this pattern. Furthermore, the patients with decreased RTK levels had poorer outcomes compared with patients who had no change or an increase in RTK levels. Thus, this blood-based biomarker could potentially be used to select which patients are appropriate for standard-of-care combined BRAF/MEK inhibitor therapy and which should be considered for enrollment in a clinical trial of BRAF/MEK-targeted therapy plus a MET or AXL inhibitor (11).

Overall, appreciation of the pleiotropic effects of receptor ectodomain shedding, coupled with an ability to noninvasively and longitudinally monitor its activity in patients, holds promise in identifying mechanisms of resistance, prescribing optimized combination therapies, and monitoring disease progression.

#### Disclosure of Potential Conflicts of Interest

R.J. Sullivan is a consultant/advisory board member for Novartis. D.A. Lauffenburger is a consultant/advisory board member for Merrimack Pharmaceuticals. No potential conflicts of interest were disclosed by the other author.

#### Authors' Contributions

**Conception and design:** M.A. Miller, R.J. Sullivan  
**Development of methodology:** M.A. Miller, R.J. Sullivan  
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** M.A. Miller, R.J. Sullivan  
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** M.A. Miller, R.J. Sullivan  
**Writing, review, and/or revision of the manuscript:** M.A. Miller, R.J. Sullivan  
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** M.A. Miller, R.J. Sullivan  
**Study supervision:** M.A. Miller, R.J. Sullivan, D.A. Lauffenburger

#### Grant Support

The study was supported by NIHNCIK99-CA207744, NIHNCIR01-CA96504, NIHNCIU54-CA112967.

Received August 22, 2016; revised November 1, 2016; accepted November 1, 2016; published OnlineFirst November 28, 2016.

#### References

- Saftig P, Reiss K. The "A Disintegrin And Metalloproteases" ADAM10 and ADAM17: novel drug targets with therapeutic potential. *Eur J Cell Biol* 2011;90:527–35.
- Hartmann M, Parra LM, Ruschel A, Lindner C, Morrison H, Herrlich A, et al. Inside-out regulation of ectodomain cleavage of cluster-of-differentiation-44 (CD44) and of neuregulin-1 requires substrate dimerization. *J Biol Chem* 2015;290:17041–54.
- Parra LM, Hartmann M, Schubach S, Li Y, Herrlich P, Herrlich A. Distinct intracellular domain substrate modifications selectively regulate ectodomain cleavage of NRG1 or CD44. *Mol Cell Biol* 2015;35:3381–95.
- Dang M, Armbruster N, Miller MA, Cermeño E, Hartmann M, Bell GW, et al. Regulated ADAM17-dependent EGF family ligand release by substrate-selecting signaling pathways. *Proc Natl Acad Sci U S A* 2013; 110:9776–81.
- Tellier E, Canault M, Rebsomen L, Bonardo B, Juhan-Vague I, Nalbano G, et al. The shedding activity of ADAM17 is sequestered in lipid rafts. *Exp Cell Res* 2006;312:3969–80.
- Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. *Cancer Res* 2001;61:4744–9.
- Xu P, Liu J, Sakaki-Yumoto M, Derynck R. TACE activation by MAPK-mediated regulation of cell surface dimerization and TIMP3 association. *Sci Signal* 2012;5:ra34.
- Le Gall SM, Maretzky T, Issuree PD, Niu XD, Reiss K, Saftig P, et al. ADAM17 is regulated by a rapid and reversible mechanism that controls access to its catalytic site. *J Cell Sci* 2010;123:3913–22.
- Xu P, Derynck R. Direct activation of TACE-mediated ectodomain shedding by p38 MAP kinase regulates EGF receptor-dependent cell proliferation. *Mol Cell* 2010;37:551–66.
- Miller MA, Meyer AS, Beste MT, Lasisi Z, Reddy S, Jeng KW, et al. ADAM-10 and -17 regulate endometriotic cell migration via concerted ligand and receptor shedding feedback on kinase signaling. *Proc Natl Acad Sci U S A* 2013;110:E2074–83.
- Miller MA, Oudin MJ, Sullivan RJ, Wang SJ, Meyer AS, Im H, et al. Reduced proteolytic shedding of receptor tyrosine kinases is a post-translational mechanism of kinase inhibitor resistance. *Cancer Discov* 2016;6:382–99.
- Thorp E, Vaisar T, Subramanian M, Mautner L, Blobel C, Tabas I. Shedding of the Mer tyrosine kinase receptor is mediated by ADAM17 protein through a pathway involving reactive oxygen species, protein kinase C $\delta$ , and p38 mitogen-activated protein kinase (MAPK). *J Biol Chem* 2011;286:33335–44.

13. Inoue A, Ishiguro J, Kitamura H, Arima N, Okutani M, Shuto A, et al. TGF $\alpha$  shedding assay: an accurate and versatile method for detecting GPCR activation. *Nat Methods* 2012;9:1021–9.
14. Ni CY, Murphy MP, Golde TE, Carpenter G. gamma -Secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase. *Science* 2001;294:2179–81.
15. Litterst C, Georgakopoulos A, Shioi J, Ghersi E, Wisniewski T, Wang R, et al. Ligand binding and calcium influx induce distinct ectodomain/gamma-secretase-processing pathways of EphB2 receptor. *J Biol Chem* 2007;282:16155–63.
16. Forsyth PA, Krishna N, Lawn S, Valadez JG, Qu X, Fenstermacher DA, et al. p75 neurotrophin receptor cleavage by  $\alpha$ - and  $\gamma$ -secretases is required for neurotrophin-mediated proliferation of brain tumor-initiating cells. *J Biol Chem* 2014;289:8067–85.
17. Lo HW, Ali-Seyed M, Wu Y, Bartholomeusz G, Hsu SC, Hung MC. Nuclear-cytoplasmic transport of EGFR involves receptor endocytosis, importin beta1 and CRM1. *J Cell Biochem* 2006;98:1570–83.
18. Garbers C, Jänner N, Chalaris A, Moss ML, Floss DM, Meyer D, et al. Species specificity of ADAM10 and ADAM17 proteins in interleukin-6 (IL-6) transsignaling and novel role of ADAM10 in inducible IL-6 receptor shedding. *J Biol Chem* 2011;286:14804–11.
19. Cohen PL, Caricchio R, Abraham V, Camenisch TD, Jennette JC, Roubey RA, et al. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. *J Exp Med* 2002;196:135–40.
20. Ballantine L, Midgley A, Harris D, Richards E, Burgess S, Beresford MW. Increased soluble phagocytic receptors sMer, sTyro3 and sAxl and reduced phagocytosis in juvenile-onset systemic lupus erythematosus. *Pediatr Rheumatol Online J* 2015;13:10.
21. Cai B, Thorp EB, Doran AC, Subramanian M, Sansbury BE, Lin CS, et al. MerTK cleavage limits proresolving mediator biosynthesis and exacerbates tissue inflammation. *Proc Natl Acad Sci U S A* 2016;113:6526–31.
22. Sather S, Kenyon KD, Lefkowitz JB, Liang X, Varnum BC, Henson PM, et al. A soluble form of the Mer receptor tyrosine kinase inhibits macrophage clearance of apoptotic cells and platelet aggregation. *Blood* 2007;109:1026–33.
23. Law AL, Parinot C, Chatagnon J, Gravez B, Sahel JA, Bhattacharya SS, et al. Cleavage of Mer tyrosine kinase (MerTK) from the cell surface contributes to the regulation of retinal phagocytosis. *J Biol Chem* 2015;290:4941–52.
24. Orme JJ, Du Y, Vanarsa K, Mayeux J, Li L, Mutwally A, et al. Heightened cleavage of Axl receptor tyrosine kinase by ADAM metalloproteases may contribute to disease pathogenesis in SLE. *Clin Immunol* 2016;169:58–68.
25. Kopitz C, Gerg M, Bandapalli OR, Ister D, Pennington CJ, Hauser S, et al. Tissue inhibitor of metalloproteinases-1 promotes liver metastasis by induction of hepatocyte growth factor signaling. *Cancer Res* 2007;67:8615–23.
26. Schelter F, Grandl M, Seubert B, Schaten S, Hauser S, Gerg M, et al. Tumor cell-derived Timp-1 is necessary for maintaining metastasis-promoting Met-signaling via inhibition of Adam-10. *Clin Exp Metastasis* 2011;28:793–802.
27. Elderbroom JL, Huang JJ, Gatz CE, Chen J, How T, Starr M, et al. Ectodomain shedding of T $\beta$ RIII is required for T $\beta$ RIII-mediated suppression of TGF- $\beta$  signaling and breast cancer migration and invasion. *Mol Biol Cell* 2014;25:2320–32.
28. Liu C, Xu P, Lamouille S, Xu J, Derynck R. TACE-mediated ectodomain shedding of the type I TGF-beta receptor downregulates TGF-beta signaling. *Mol Cell* 2009;35:26–36.
29. Murthy A, Defamie V, Smookler DS, Di Grappa MA, Horiuchi K, Federici M, et al. Ectodomain shedding of EGFR ligands and TNFR1 dictates hepatocyte apoptosis during fulminant hepatitis in mice. *J Clin Invest* 2010;120:2731–44.
30. Raikwar NS, Liu KZ, Thomas CP. Protein kinase C regulates FLT1 abundance and stimulates its cleavage in vascular endothelial cells with the release of a soluble PlGF/VEGF antagonist. *Exp Cell Res* 2013;319:2578–87.
31. Newton RC, Bradley EC, Levy RS, Doval D, Bondarde S, Sahoo TP, et al. Clinical benefit of INCB7839, a potent and selective ADAM inhibitor, in combination with trastuzumab in patients with metastatic HER2+ breast cancer. *J Clin Oncol* 28:15s, 2010 (suppl; abstr 3025).
32. Witters L, Scherle P, Friedman S, Fridman J, Caulder E, Newton R, et al. Synergistic inhibition with a dual epidermal growth factor receptor/HER-2/neu tyrosine kinase inhibitor and a disintegrin and metalloprotease inhibitor. *Cancer Res* 2008;68:7083–9.
33. Incyte. UBS Global Life Sciences Conference; New York, NY. Sept. 19th, 2011. <http://phx.corporate-ir.net/External.File?item=UGFyZW50SUQ9NDQw-NjgzfENoaWxkSUQ9NDYyNTAzfFR5cGU9MQ==&t=1>. Retrieved Aug 2016.
34. Ghedini GC, Ciravolo V, Tortoreto M, Giuffrè S, Bianchi F, Campiglio M, et al. Shed HER2 extracellular domain in HER2-mediated tumor growth and in trastuzumab susceptibility. *J Cell Physiol* 2010;225:256–65.
35. Scaltriti M, Nuciforo P, Bradbury I, Sperinde J, Agbor-Tarh D, Campbell C, et al. High HER2 expression correlates with response to the combination of lapatinib and trastuzumab. *Clin Cancer Res* 2015;21:569–76.
36. Vidal GA, Naresh A, Marrero L, Jones FE. Presenilin-dependent gamma-secretase processing regulates multiple ERBB4/HER4 activities. *J Biol Chem* 2005;280:19777–83.
37. Swendeman S, Mendelson K, Weskamp G, Horiuchi K, Deutsch U, Scherle P, et al. VEGF-A stimulates ADAM17-dependent shedding of VEGFR2 and crosstalk between VEGFR2 and ERK signaling. *Circ Res* 2008;103:916–8.
38. Kuhn PH, Colombo AV, Schusser B, Dreymueller D, Wetzel S, Schepers U, et al. Systematic substrate identification indicates a central role for the metalloprotease ADAM10 in axon targeting and synapse function. *Elife* 2016;5:pii: e12748.
39. Hoa N, Tsui S, Afifyan NF, Sinha Hikim A, Li B, Douglas RS, et al. Nuclear targeting of IGF-1 receptor in orbital fibroblasts from Graves' disease: apparent role of ADAM17. *PLoS One* 2012;7:e34173.
40. Caiazza F, McGowan PM, Mullooly M, Murray A, Synnott N, O'Donovan N, et al. Targeting ADAM-17 with an inhibitory monoclonal antibody has antitumor effects in triple-negative breast cancer cells. *Br J Cancer* 2015;112:1895–903.
41. Sanderson MP, Keller S, Alonso A, Riedle S, Dempsey PJ, Altevogt P. Generation of novel, secreted epidermal growth factor receptor (EGFR/ ErbB1) isoforms via metalloprotease-dependent ectodomain shedding and exosome secretion. *J Cell Biochem* 2008;103:1783–97.
42. Perez-Torres M, Valle BL, Maible NJ, Negron-Vega L, Nieves-Alicea R, Cora EM. Shedding of epidermal growth factor receptor is a regulated process that occurs with overexpression in malignant cells. *Exp Cell Res* 2008;314:2907–18.
43. Chen M, Chen LM, Lin CY, Chai KX. Hepsin activates prostaticin and cleaves the extracellular domain of the epidermal growth factor receptor. *Mol Cell Biochem* 2010;337:259–66.
44. Chen M, Chen LM, Lin CY, Chai KX. The epidermal growth factor receptor (EGFR) is proteolytically modified by the Matriptase-Prostaticin serine protease cascade in cultured epithelial cells. *Biochim Biophys Acta* 2008;1783:896–903.
45. Im H, Shao H, Park YI, Peterson VM, Castro CM, Weissleder R, et al. Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. *Nat Biotechnol* 2014;32:490–5.
46. Koletsas T, Kostopoulos I, Charalambous E, Christoforidou B, Nenopoulou E, Kotoula V. A splice variant of HER2 corresponding to Herstatin is expressed in the noncancerous breast and in breast carcinomas. *Neoplasia* 2008;10:687–96.
47. Rahimi N, Golde TE, Meyer RD. Identification of ligand-induced proteolytic cleavage and ectodomain shedding of VEGFR-1/FLT1 in leukemic cancer cells. *Cancer Res* 2009;69:2607–14.
48. The Diabetic Retinopathy Clinical Research Network, Wells JA, Glassman AR, Ayala AR, Jampol LM, Aiello LP, et al. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *N Engl J Med* 2015;372:1193–203.
49. Uchikawa S, Yoda M, Tohmonda T, Kanaji A, Matsumoto M, Toyama Y, et al. ADAM17 regulates IL-1 signaling by selectively releasing IL-1 receptor type 2 from the cell surface. *Cytokine* 2015;71:238–45.
50. Kariolis MS, Miao YR, Jones DS, Kapur S, Mathews II, Giaccia AJ, et al. An engineered Axl 'decoy receptor' effectively silences the Gas6-Axl signaling axis. *Nat Chem Biol* 2014;10:977–83.
51. Wang N, Reyes RF, Elahi SM, Lu Y, Hancock MA, Massie B, et al. The IGF-Trap: novel inhibitor of carcinoma growth and metastasis. *Mol Cancer Ther* 2015;14:982–93.
52. Li D, Wei X, Xie K, Chen K, Li J, Fang J. A novel decoy receptor fusion protein for FGF-2 potentially inhibits tumour growth. *Br J Cancer* 2014;111:68–77.
53. Lindzen M, Carvalho S, Starr A, Ben-Chetrit N, Pradeep CR, Köstler WJ, et al. A recombinant decoy comprising EGFR and ErbB-4 inhibits tumor growth and metastasis. *Oncogene* 2012;31:3505–15.



54. Michieli P, Mazzone M, Basilico C, Cavassa S, Sottile A, Naldini L, et al. Targeting the tumor and its microenvironment by a dual-function decoy Met receptor. *Cancer Cell* 2004;6:61–73.
55. Rankin EB, Fuh KC, Taylor TE, Krieg AJ, Musser M, Yuan J, et al. AXL is an essential factor and therapeutic target for metastatic ovarian cancer. *Cancer Res* 2010;70:7570–9.
56. Petrelli A, Circosta P, Granziero L, Mazzone M, Pisacane A, Fenoglio S, et al. Ab-induced ectodomain shedding mediates hepatocyte growth factor receptor down-regulation and hampers biological activity. *Proc Natl Acad Sci U S A* 2006;103:5090–5.
57. Schelter F, Kobuch J, Moss ML, Becherer JD, Comoglio PM, Boccaccio C, et al. A disintegrin and metalloproteinase-10 (ADAM-10) mediates DN30 antibody-induced shedding of the met surface receptor. *J Biol Chem* 2010;285:26335–40.
58. Miller MA, Moss ML, Powell G, Petrovich R, Edwards L, Meyer AS, et al. Targeting autocrine HB-EGF signaling with specific ADAM12 inhibition using recombinant ADAM12 prodomain. *Sci Rep* 2015;5:15150.
59. Rios-Doria J, Sabol D, Chesebrough J, Stewart D, Xu L, Tammali R, et al. A monoclonal antibody to ADAM17 inhibits tumor growth by inhibiting EGFR and non-EGFR-mediated pathways. *Mol Cancer Ther* 2015;14:1637–49.
60. Van Schaeybroeck S, Kalimutho M, Dunne PD, Carson R, Allen W, Jithesh PV, et al. ADAM17-dependent c-MET-STAT3 signaling mediates resistance to MEK inhibitors in KRAS mutant colorectal cancer. *Cell Rep* 2014;7:1940–55.
61. Yacoub D, Benslimane N, Al-Zoobi L, Hassan G, Nadiri A, Mourad W. CD154 is released from T-cells by a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) and ADAM17 in a CD40 protein-dependent manner. *J Biol Chem* 2013;288:36083–93.
62. Weskamp G, Ford JW, Sturgill J, Martin S, Docherty AJ, Swendeman S, et al. ADAM10 is a principal 'shedase' of the low-affinity immunoglobulin E receptor CD23. *Nat Immunol* 2006;7:1293–8.
63. Möller-Hackbarth K, Dewitz C, Schweigert O, Trad A, Garbers C, Rose-John S, et al. A disintegrin and metalloprotease (ADAM) 10 and ADAM17 are major sheddases of T cell immunoglobulin and mucin domain 3 (Tim-3). *J Biol Chem* 2013;288:34529–44.
64. Paolino M, Choidas A, Wallner S, Pranjic B, Uribesalago I, Loeser S, et al. The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. *Nature* 2014;507:508–12.
65. Wiernik A, Foley B, Zhang B, Verneris MR, Warlick E, Gleason MK, et al. Targeting natural killer cells to acute myeloid leukemia in vitro with a CD16 x 33 bispecific killer cell engager and ADAM17 inhibition. *Clin Cancer Res* 2013;19:3844–55.
66. Chen CH, Miller MA, Sarkar A, Beste MT, Isaacson KB, Lauffenburger DA, et al. Multiplexed protease activity assay for low-volume clinical samples using droplet-based microfluidics and its application to endometriosis. *J Am Chem Soc* 2013;135:1645–8.
67. Athauda C, Giubellino A, Coleman JA, Horak C, Steeg PS, Lee MJ, et al. c-Met ectodomain shedding rate correlates with malignant potential. *Clin Cancer Res* 2006;12:4154–62.
68. Jurisic D, Erjavec I, Trkulja V, Dumic-Cule I, Hadzibegovic I, Kovacevic L, et al. Soluble type III TGF  $\beta$  receptor in diagnosis and follow-up of patients with breast cancer. *Growth Factors* 2015;33:200–9.
69. Barisione G, Fabbi M, Gino A, Queirolo P, Orgiano L, Spano L, et al. Potential role of soluble c-Met as a new candidate biomarker of metastatic uveal melanoma. *JAMA Ophthalmol* 2015;133:1013–21.
70. Reichl P, Fang M, Starlinger P, Stauer K, Nenutil R, Muller P, et al. Multicenter analysis of soluble Axl reveals diagnostic value for very early stage hepatocellular carcinoma. *Int J Cancer* 2015;137:385–94.
71. Wader KF, Fagerli UM, Holt RU, Børset M, Sundan A, Waage A. Soluble c-Met in serum of patients with multiple myeloma: correlation with clinical parameters. *Eur J Haematol* 2011;87:394–9.