Clinical Cancer Research

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

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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 TGFβ 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 mechanical 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 TNFα-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 TNFα, and growth factors, including TGFα. ADAMs additionally cause the α-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 TGFα and human epidermal growth factor receptor 2 (HER2/ERBB2) have oncogenic potential. Both *ADAM10*$^{-/-}$ and *ADAM17*$^{-/-}$ 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*$^{-/-}$ and *ADAM17*$^{-/-}$ 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-NRG1 (3, 4). Co-localization 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 α-HER2 monoclonal antibody (mAb) trastuzumab (Herceptin; Genentech; FDA approved for HER2^{+} 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

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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 PLCγ/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, γ-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-κB. Following these functions, $MerTK^{-/-}$ 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 erythematosus (SLE). Yet, surface receptor levels actually decrease on circulating monocytes of patients with SLE (20). Genetically engineered mice with MerTK either knocked-out ($MerTK^{-/-}$), or mutated at its ADAM17 proteolysis site to confer cleavage resistance ($MerTK^{CR}$), provide strong causal evidence that MerTK shedding downregulates its activity (21). In a model of ischemia/reperfusion-induced lung injury, $MerTK^{-/-}$ mice exhibited increased inflammation and injury, whereas the $MerTK^{CR}$ 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 TGFβ receptor (TβRIII) presents TGFβ ligand to enhance prometastatic signaling. Transgenic studies used TβRIII that had been mutated to either have more or less affinity for proteolysis. Results show that enhanced shedding decreases TGFβ-related signaling, decreases metastasis, and enhances survival in a xenograft model (27). Other receptors with compelling evidence that shedding downregulates their activity include TβRI (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 $p95^{HER2}$. Anti-HER2 antibody therapies such as trastuzumab cannot bind $p95^{HER2}$, 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 aderbasib (INCB7839; Incyte Corp.) was tested in $HER2^{+}$ breast cancer phase I/II trials in combination with

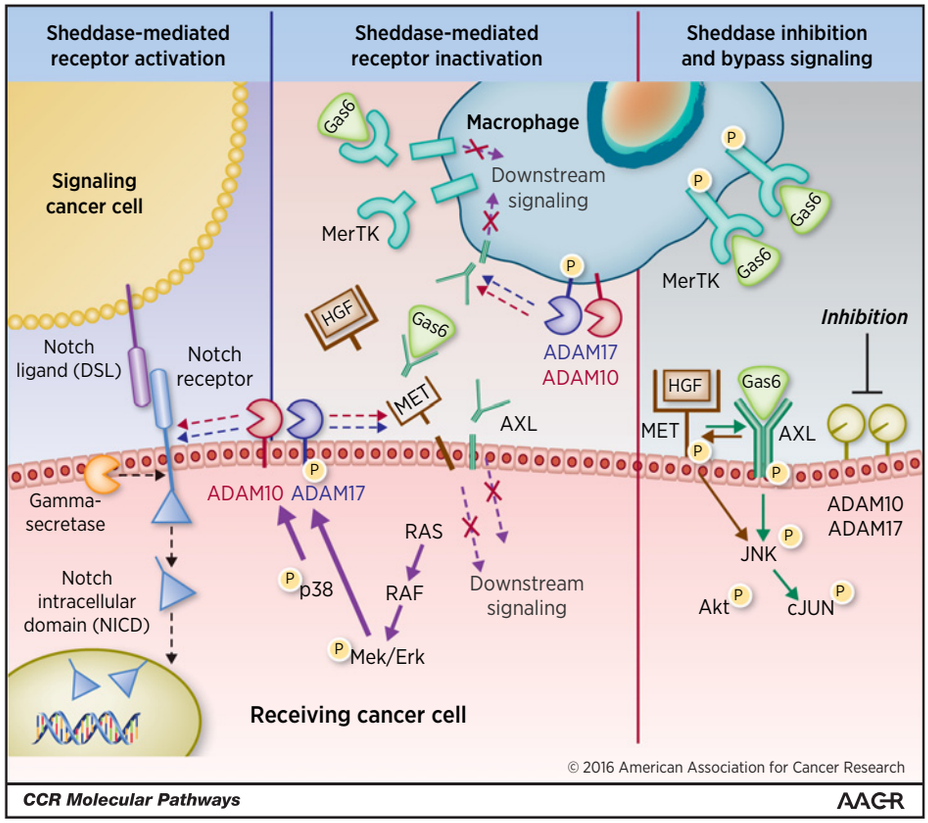

Figure 1.
Modes of receptor ectodomain shedding. Left, as an example of activation, ADAM-mediated proteolysis of the Notch receptor is followed by regulated intramembrane proteolysis to enable nuclear translocation and activity. Center, as an example of receptor downregulation, the proteolytic cleavage 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 aderbasib 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 $p95^{HER2}$ 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 TGFα, 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 ($p110^{EGFR}$) 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 α-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 α-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 $HER2^+$ breast cancer) in an $HER2^+$ 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 $p95^{HER2}$ 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 α-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 phosphosignaling 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 nonmalignant 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 FcγRIIIa (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 are 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 aderbasib (INCB7839) in patients with $HER2^{+}$ 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
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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
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.A. Miller, R.J. Sullivan
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