

Sortilin as a new membrane inhibitor of EGFR trafficking for overcoming resistance to EGFR inhibitors in non-small cell lung cancer

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Provenance: This is an invited Editorial commissioned by the Section Editor Long Jiang (Department of Thoracic Oncology, Second Affiliated Hospital, Institute of Respiratory Diseases, Zhejiang University School of Medicine, Hangzhou, China).

Comment on: Al-Akhrass H, Naves T, Vincent F, *et al.* Sortilin limits EGFR signaling by promoting its internalization in lung cancer. *Nat Commun* 2017;8:1182.

Submitted Jul 16, 2018. Accepted for publication Jul 31, 2018.

doi: 10.21037/jtd.2018.08.25

View this article at: <http://dx.doi.org/10.21037/jtd.2018.08.25>

Non-small cell lung cancer (NSCLC) accounts for about 85% of lung cancer, which is the most common and leading cause of cancer-related death in the United States and worldwide (1,2). The clinical benefit of cytotoxic chemotherapy doublets reached a plateau of median overall survival of 7–8 months and a 5-year survival rate of <5% in molecularly unselected patients with metastatic NSCLC in 2000 (3). Advances in cancer biology research and genomics technology enable the current era of precision oncology to treat an individual's cancer based on the unique genetic and immune biomarkers (4,5). Drugs targeting the epidermal growth factor receptor (EGFR), by either small-molecule tyrosine kinase inhibitors (TKIs) or neutralizing monoclonal antibodies (mAbs), are by far the most successful molecularly targeted therapy developed in NSCLC, which have significantly improved the overall survival over chemotherapy in patients with metastatic NSCLC since 2004 (6).

EGFR is expressed on the surface of epithelial carcinoma cells and plays an essential role in the tumorigenesis, proliferation, differentiation, migration, anti-apoptosis, angiogenesis, and metastasis (7). Several mechanisms, such as EGFR overexpression, autocrine ligand stimulation, or constitutively activated mutant receptor, can lead to

abnormal receptor activation, resulting in a variety of pathophysiological diseases and promoting oncogenesis or cancer development. In addition, EGFR could be transactivated in the absence of a specific ligand through G protein-coupled receptor activation. The presence of gain-of-function somatic mutations in the tyrosine kinase domain of the *EGFR* gene in NSCLC tumors defines the first molecular subset of 10–15% of Caucasian patients and 30–40% of East Asian patients who have a response rate of 60–80%, a median progression-free survival of 9–19 months and a median overall survival of 18–36 months to first-line EGFR TKIs (i.e., erlotinib, gefitinib, afatinib, dacomitinib, osimertinib) (6). The clinical benefit of EGFR TKIs in *EGFR*-mutant NSCLC cells is mainly due to its cytotoxic effects by induction of apoptosis (8,9), while their mechanism of action in *EGFR*-wild type NSCLC cells is mainly cytostatic by induction of G1 arrest and inhibition of tumor growth (10,11). However, the magnitude of tumor regression is often variable and transient (12). Mechanisms of primary resistance to EGFR-TKIs include in-frame insertion mutation in *EGFR* exon 20, *de novo* *EGFR* T790M mutation, *KRAS* mutations, loss of *PTEN*, and *MET* proto-oncogene amplification. Almost all patients with *EGFR*-mutant NSCLC eventually develop acquired resistance to

the EGFR TKIs, which include the detection of a second-site mutation in the *EGFR* gene (such as T790M, V769M, L747S) (~50% of cases), *MET* pro-oncogene amplification (20%), or other molecular mechanisms such as upregulation of bypass RTK function (13). For patients with metastatic squamous NSCLC with *EGFR*-wild type gene, second generation EGFR TKI afatinib and second generation EGFR mAb necitumumab in combination with gemcitabine and cisplatin have been approved for second- and first-line treatment, respectively (14,15). Many strategies have been attempted to overcome primary and acquired resistance to EGFR-targeting therapy (6). One of the novel strategies for overcoming resistance to EGFR inhibitors is to inhibit EGFR trafficking as shown in this referenced paper (16).

EGFR functions as a receptor tyrosine kinase (RTK) localized on the plasma membrane with a transmembrane domain and is activated upon extracellular ligand binding to transduce information from the microenvironment into the cell and activate homeostatic downstream signaling pathways (6,7). EGFR consists of an extracellular domain (ECD), a transmembrane lipophilic segment, and an intracellular domain (ICD) containing a tyrosine kinase domain. At least six EGFR ligands have been identified, including epidermal growth factor (EGF), heparin binding-EGF, amphiregulin (ARG), and transforming growth factor (TGF)- α . Upon the ligand binding to the ECD, dimerization of the transmembrane EGFR induces autophosphorylation at distinct tyrosine residues of ICD, mediating several major signaling pathways, including the RAS/RAF/MEK/ERK pathway, PI3K/AKT/mTOR, and JAK/STAT pathways, for cell proliferation, survival, invasion, migration, anti-apoptosis, and pro-angiogenesis. Internalization and degradation of EGFR after ligand binding limits the intensity of proliferative signaling, which is a crucial step for signal termination and maintenance of cell integrity. In cancer cells, dysregulation of EGFR trafficking contributed to uncontrolled cell proliferation and survival. However, the selection of additional therapies increasingly depends on the molecular composition of the tumor and the mechanism of resistance.

Sortilin, encoded by the *SORT1* gene on chromosome 1 at the band 1p13.3 in human, is a type I membrane glycoprotein in the vacuolar protein sorting 10 (VPS10) protein family of sorting receptors (17). Sortilin is ubiquitously expressed in many human tissues and shuttles between the plasma membrane, subcellular compartments such as endosomes, lysosomes, and the trans-Golgi network

(TGN) (*Figure 1*). Sortilin acts as a multifaceted sorting receptor, sortilin facilitates the transportation of many intracellular proteins involved in many critical physiological processes such as lipid and glucose metabolism, neural development and cell death, as well as several major human diseases such as cardiovascular disease, Alzheimer's disease, type 2 diabetes mellitus, and most recently cancer (19,20). Following their previous work showing sortilin is important for transporting and loading EGFR into extracellular vesicles via endocytosis (21), Al-Akhrass *et al.* determined the role of sortilin in regulating EGFR intracellular trafficking in this paper (16). They showed that sortilin regulated EGFR activity by inhibiting its internalization from the plasma membrane, thereby limiting proliferative signaling driving tumor aggressiveness. Sortilin exhibits its inhibitory effect on EGFR via a ligand independent mechanism, i.e., an independent mechanism of EGF-induced EGFR phosphorylation and endocytosis. Loss of sortilin in tumor cells promoted cell proliferation and accelerated tumor growth by sustaining EGFR signaling on the cell surface. In lung cancer patients, sortilin expression was correlated with high pathologic grade and poor overall survival, especially in patients with high EGFR expression. Sortilin acts as a tumor suppressor inhibiting tumorigenesis in the *EGFR*-mutant lung cancers. In contrast, sortilin acts as an oncogene promoting malignant behavior in *EGFR*-wild type lung cancers (22).

There are several clinical implications of this study. First, targeting EGFR trafficking by modulating sortilin expression is a novel strategy to overcome primary or acquired resistance to EGFR TKIs in *EGFR*-mutant NSCLC. Second, targeting EGFR trafficking by modulating sortilin expression might be particularly important for NSCLC and other types of EGFR-expressing tumors, such as glioblastomas, colorectal cancer, and head and neck cancers, that are not driven by the gain-of-function mutations in tyrosine kinase domains. Third, sortilin regulates ligand-independent EGFR or other RTK signaling which is important for regulating the tumor microenvironment, immunity, inflammation, and tissue repair (23). Nevertheless, there are several questions that remain to be answered before clinical translation. First, the prevalence of sortilin expression in EGFR-expressing NSCLC. Second, the development of therapeutics targeting sortilin.

It is worthy to mention that membrane proteins are an important class of proteome encoded by about 30% of the

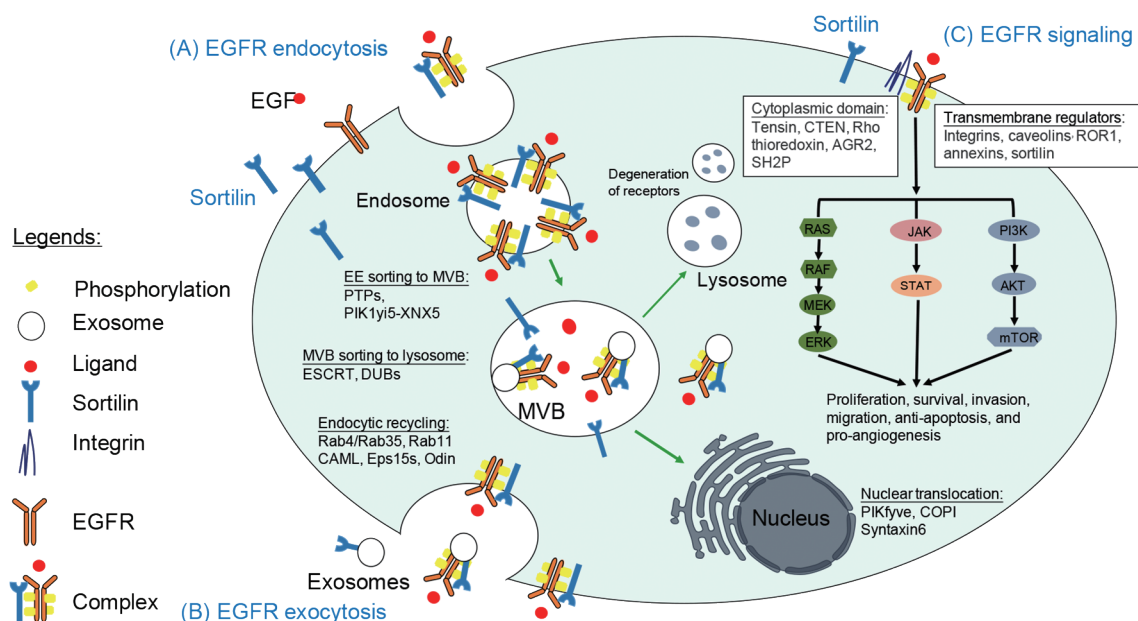


Figure 1 A schema of sortilin regulating EGFR trafficking (18). Sortilin is mainly localized at the plasma membrane and the trans-Golgi network (TGN). (A) Endocytosis: under normal circumstances, sortilin is essential for EGFR sorting in EGFR endocytosis (B) Exocytosis: upon ligand binding, cell surface EGFRs are activated and internalized, and then either sorted at the early endosome (EE) or cycled continually between the surface of plasma membrane and the TGN via endocytosis and exocytosis. (C) EGFR signaling: the most known EGFR ligand is EGF, which activates the phosphorylation of EGFR tyrosine kinases and downstream signaling pathways, including the Ras/Raf/MEK-ERK/MAP kinase, PI3K/AKT/mTOR, and JAK/STAT pathways, for cell growth, survival, migration, anti-apoptosis, and promoting angiogenesis. Loss of sortilin expression results in failure of EGFR receptor internalization which leads to prolonged or continuous activation of EGFR signaling cascade, enhanced cell proliferation and/or survival, invasion, metastasis, and poor clinical outcomes. The fate of EGFR has important consequences for biological cell outputs: the recycling pathway favoring cell proliferation while the degradative pathway controlling normal cellular homeostasis. Several reported key regulators for each process were listed within this figure (18). Additionally, EGFR may translocate to the nucleus and mitochondria although the transport mechanisms remain to be elucidated. EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ESCRT, endosomal sorting complex required for transport; MVBs, multivesicular bodies; TGN, the trans-Golgi network.

human protein coding genes (24) and represent about 70% of known clinical drug targets (25). Many of the membrane proteins have been explored as important targets for cancer biomarker discovery and drug development. *Table 1* summarizes several key membrane-related proteins that have been associated with regulating EGFR trafficking through a variety of mechanisms, including (I) cytoplasmic regulators, such as tensin, C-terminal tensin-like (CTEN), Rho, thioredoxin (TRX), anterior gradient homolog 2 (AGR2), and Src homology 2 phosphotyrosine (SH2P); and (II) transmembrane regulators such as integrins, caveolins, RTK like orphan receptor 1 (ROR1), annexins, and sortilin. Further mechanistic studies are needed to elucidate the interaction between these membrane proteins

in regulating the function of EGFR in the context of other RTKs that are involved in initiation and progression of lung adenocarcinoma and develop therapeutic strategies to improve the efficacy of EGFR inhibitors.

In conclusion, sortilin has been identified as a new negative membrane regulator for inhibiting EGFR intracellular trafficking in NSCLC. Sortilin expression is a favorable prognostic marker for patients with lung adenocarcinoma, independent of the mutation status in the EGFR tyrosine kinase domain. Further studies are needed to investigate the role of targeting sortilin and other EGFR-membrane associated proteins as a novel therapeutic strategy to improve EGFR-targeting therapy in NSCLC and other types of EGFR-expressing cancers.

Table 1 Summary of key regulatory proteins for EGFR trafficking

Protein	Function	Regulating EGFR	Author (year)
(I) Cytoplasmic regulators			
Tensin	Focal adhesion molecules link integrin receptors to actin cytoskeletons and regulate cell adhesion and migration	Tensin regulates the response of EGFR inhibitor by targeting EGFR for degradation	Vivanco [2010] (26)
CTEN	A protein containing the Src homology 2 and phosphotyrosine-binding domains that are similar to the COOH termini of tensin molecules. Up-regulated CTEN enhances cell invasion, epithelial mesenchymal transition (EMT), and colony formation activities of cancer cells	CTEN reduces ligand-induced EGFR degradation by a SH2-dependent binding to c-Cbl E3 ligase and modulates RTK and NF- κ B signaling pathways	Hong [2013] (27)
Rho/ROCK	Members of the Ras super-family of small GTP-binding protein. Rho-associated coiled-coil containing protein kinase (Rho-kinase/ROCK) regulates cell proliferation and migration	ROCK is activated by EGF ligand and turns off the activated EGFR pathway via a negative feedback system	Ridley [1997] (28); Zhao [2010] (29); Nakashima [2011] (30)
Thioredoxin (TRX)	TRX and the TRX-related molecules constitute a cellular redox regulation system, which provides cytoprotective action against oxidative stresses. Overexpression of TRX proteins are seen in many cancers and other disorders caused or complicated by oxidative stresses	Regulates transactivation of EGFR and activation of NF- κ B by lysophosphatidic acid	Chuang [2009] (31); Hirota [2001] (32)
AGR2	Induces expression of EGFR ligand amphiregulin	Reduced AGR2 protein level decreases cell surface EGFR and other signaling molecules expression	Dong [2015] (33)
SH2P	SH2P is the most prevalent phosphotyrosine-binding protein that regulates cellular RTK signaling pathways	SH2P prolongs Src activity following EGF stimulation via stabilizing the Src kinase in its active conformation	Dülk [2018] (34)
(II) Transmembrane regulators			
Integrins	Mediates cell adhesion and regulates cellular responses, such as proliferation, survival and cross-talk between different cellular signaling pathways	Regulates EGFR signaling through the activation of a protein tyrosine phosphatase	Mattila [2005] (35); Saxena [2017] (36)
Caveolins	Acts as scaffolding proteins mediating receptor-independent endocytosis and signal transduction	Modulates drug sensitivity. Knockdown of Cav-1 dramatically enhances the sensitivity to EGFR-TKIs by down-regulating EGFR	Cui [2018] (37)
ROR1	Sustains the balance between pro-survival and proapoptotic signaling through stabilization of EGFR-ERBB3 and c-Src activation	High ROR1 expression is associated with short PFS in erlotinib-treated patients with T790M mutations. Knockdown of ROR1 inhibits the growth of lung cancer with different acquired resistance mechanisms via RTKs such as MET and IGF-IR	Karachaliou [2014] (38)
Annexins	Regulates membrane trafficking and mediates critical physiological processes including proliferation, differentiation, inflammation and cell migration in tumor cells and tumor microenvironment	Regulates EGFR transport and degradation, either directly or indirectly, for EGFR activity	Woś [2014] (39)
Sortilin	Regulates membrane trafficking and mediates the function of signaling transduction and cellular communication in tumor microenvironment	Inhibits tumor growth by promoting receptor internalization and EGFR intracellular trafficking. Loss of sortilin promotes tumor growth by sustaining EGFR signaling at the cell surface	Wilson [2014] (21); Al-Akhrass [2017] (16)

EGFR, epidermal growth factor receptor; AGR2, anterior gradient homolog 2; ROCK, Rho-associated coiled-coil containing protein kinase; SH2P, SH2-containing protein.

Acknowledgements

Funding: This work was supported by “Novel Treatment Strategies for Adenocarcinomas” (UCD grant #49873) to T Li. Dr. Q Li was also supported by a research and training scholarship from Shanghai Jiaotong University Affiliated Sixth People’s Hospital.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Li Q, Ma W, Li T. Sortilin as a new membrane inhibitor of EGFR trafficking for overcoming resistance to EGFR inhibitors in non-small cell lung cancer. *J Thorac Dis* 2018;10(Suppl 26):S3186-S3191. doi: 10.21037/jtd.2018.08.25