

SIGNALING CROSSTALKS:
EGFR AND TAZ IN BREAST CANCER

By

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ABSTRACT

Breast cancer is driven by multiple molecular aberrations, transforming benign epithelial cells into metastatic cancer. Identification of key signaling nodes underlying metastasis and resistance to treatment is crucial to improving targeted therapies. This review examines two oncogenes driving epithelial to mesenchymal transition (EMT) and the acquisition of cancer stem cell properties. The epidermal growth factor receptor (EGFR), a well-established oncogene driving migration, survival, and proliferation, activates several downstream signaling cascades including the AKT and MAPK pathways. EGFR overexpression, mutation, and mislocalization are frequently observed in breast cancer. TAZ, transcriptional coactivator with PDZ-binding motif, induces and sustains EMT and is required for the acquisition of breast cancer stem cell traits. The emerging crosstalk between these pathways yields insights into early mammary tumorigenesis, epithelial plasticity, and the metastatic niche, suggesting novel avenues for the development of targeted therapies.

I. Introduction

Breast cancer currently remains the second leading cause of cancer deaths among women in the United States (Siegel, 2017). Progress has been made to improve treatment in recent years largely due to the development of targeted therapies, which interrupt cellular processes with astounding molecular specificity. Current targeted therapies for breast cancer are directed towards oncogenic lesions specific to the cancer cell population, such as the estrogen and progesterone receptors, the HER2 receptor, and the epidermal growth factor receptor (EGFR) signaling pathway. Novel drug targets arise through identification of the molecular pathways critical to cellular transformation and metastasis.

Cancer stem cells (CSC), a subset of the tumor population harboring self-renewal and tumor initiation potential, are believed to underlie resistance to chemotherapy and tumor recurrence in breast cancer (Chaffer and Weinberg 2015). These cells have also been proposed to drive breast cancer metastasis through interaction with the metastatic niche (Bacelli et al., 2013). Mammary epithelial cells exhibit a remarkable plasticity, such that lineage-committed luminal epithelial cells may revert to a progenitor-like state during oncogenic transformation (Skibinski et al., 2014). Compounding evidence implicates TAZ in this transformation towards a mesenchymal, stem-like state through a complex transdifferentiation program known as epithelial to mesenchymal transition (EMT) (Cordenonsi et al., 2011). EMT inherently involves a loss of cell polarity and induction of CSC phenotypes (Chaffer and Weinberg 2015). However, the precise

molecular mechanisms relating EMT, loss of cell polarity, and CSC characteristics during breast cancer progression have yet to be unraveled.

This review will examine two oncogenes driving EMT and the adoption of CSC properties and the intersections between these pathways. The epidermal growth factor receptor (EGFR), a well-established oncogene driving migration, survival, and proliferation, activates several downstream signaling cascades including the AKT survival and MAPK growth pathway. EGFR overexpression, mutation, and mislocalization are frequently observed in breast cancer, particularly in the triple negative breast cancer subtype, for which no targeted therapies currently exist (Lurje et al., 2010). TAZ, transcriptional coactivator with PDZ-binding motif, can induce and sustain epithelial to mesenchymal transition and is required for the acquisition of breast cancer stem cell traits (Cordenonsi et al., 2011; Bartucci et al., 2015). Unraveling these signaling pathways provides insight into mammary tumorigenesis and the acquisition of stem cell properties, suggesting synergistic targets for future breast cancer therapies.

II. TAZ activity correlates with poor prognosis and is required for breast cancer stem cell phenotypes

In breast cancer clinical studies, TAZ activation is correlated with poor prognosis. TAZ expression is reported to be preferentially higher in TNBC than in other BC subclasses (Kim et al., 2015a; Li et al., 2015c; Skibinski et al., 2014). Clinical datasets reveal a correlation between YAP/TAZ gene signatures and histological grade, stem cell signatures, metastasis, and poor outcome (Cordenonsi et al., 2011; Di Agostino et al., 2015; Zanconato et al., 2015). TAZ activation, measured by nuclear staining, indicates

poor clinical prognosis (Oku et al., 2015; Bartucci et al., 2015; Diaz-Martin et al., 2015). Yes-associated protein (YAP), the mammalian paralog of TAZ, carries several overlapping functions; however, in breast cancer there appears to be no correlation between YAP IHC staining and clinical variables (Skibinski et al., 2014). Despite structural similarity, the molecular mechanisms regulating TAZ/YAP and the functional consequences of transcriptional coactivation diverge (Zhou et al., 2016; Zanconato et al., 2016).

TAZ activation is intrinsically linked to the acquisition and maintenance of breast cancer stem cell properties. Within a tumor population, cancer stem cells can be functionally defined on the basis of self-renewal and tumor seeding potential (Cordenonsi et al., 2011). Within this defined CSC population, TAZ is active and required for CSC expansion (Bartucci et al., 2015; Cordenonsi et al., 2011). TAZ plays an essential role in maintaining stem-ness and conferring metastatic capability to breast cancer cells.

YAP/TAZ are also capable of conferring full CSC attributes to differentiated, non-stem tumor cells (Bartucci et al., 2015; Cordenonsi et al., 2011). *In vitro* studies of normal mammary epithelial cells demonstrate how ectopic overexpression of TAZ stimulates proliferation, anchorage-independent growth, reduced cell-contact inhibition, EMT, gain of invasive structures, migration, and EGF-independent growth (Lei et al., 2008, Chan et al., 2008; Yang et al., 2012). Likewise, EMT and loss of cell polarity are known drivers of TAZ activity, suggesting an essential role for TAZ in the early pre-cancerous transformation of breast epithelial cells (Greenwood et al., 2016; Liu et al. 2010).

Interestingly, TAZ but not YAP is capable on its own of switching the differentiation state of mammary epithelial cells. Within the mammary duct, cells are distinguished into two major populations: luminal cells line the ductal lumen and produce milk, while basal/ME cells line the basement membrane and contract to pump milk (Visvader 2009; Skibinski et al, 2014). Skibinski *et al* identified TAZ in a gain-of-function screen of epithelial lineage plasticity. Immunostaining of normal breast terminal duct lobular units (TDLUs) revealed similar levels of TAZ total protein and mRNA, but contrasting YAP/TAZ nuclear localization. Basal/ME cells exhibit nuclear YAP/TAZ localization; whereas luminal cells exhibit diffuse cytoplasmic YAP/TAZ (Skibinski et al., 2014). TAZ's interaction with the SWI/SNF complex allows it to repress luminal differentiation and maintain basal/ME features.

III. TAZ Regulation

TAZ can be thought of as a molecular transducer, capable of sensing a range of upstream signals. As a transcriptional coactivator, TAZ activity depends on its subcellular localization in the cytoplasm or nucleus (Sorrentino et al., 2014). This localization is controlled by the status of phosphorylation. Serine phosphorylation inactivates TAZ through either cytoplasmic retention or ubiquitin-mediated lysosomal degradation. Dephosphorylation of these residues allows TAZ to translocate to the nucleus, where it interacts with TEA domain transcription factors (TEADs) via its transactivation domain (Kanai et al., 2000). TAZ modulates the activity of additional transcription factors, including runt-related transcription factor 2 (RUNX2), paired box-3

(PAX3), PAX8, transcription termination factor-1 (TTF-1), T-box 5 (TBX5), and mothers against decapentaplegic homologs (SMADs) (Bhat et al., 2011, Zhou and Lei, 2016; Varelas et al., 2010). TAZ nuclear translocation dominantly controls SMAD nucleocytoplasmic localization (Varelas et al., 2008). TAZ/YAP also have a cytoplasmic function in regulating Wnt/ β -catenin signaling (Varelas et al., 2010).

TAZ Phosphorylation Sites

TAZ phosphorylation is controlled by several upstream kinases, including casein kinase 1 (CK1), glycogen synthase kinase 3 β (GSK3 β), and large tumor suppressor (Lats) (Huang et al., 2012; Lei et al., 2008; Liu et al., 2010, 2011; Dupont et al., 2011). First identified as a 14-3-3 binding protein, TAZ was discovered to be one of the main effectors of the Hippo tumor suppressor pathway. TAZ activity is regulated largely by the Hippo core kinases Lats1/2. Hippo signaling inactivates TAZ through two mechanisms: localization and stability. Phosphorylation at serine 89 results in cytoplasmic sequestration by 14-3-3 (Chan et al., 2008; Lei et al., 2008). Phosphorylation at serine 311 by Lats1/2 and subsequent phosphorylation at serine 314 by CK1 creates a phosphodegron at the C-terminal. β -TrCP binds to this phosphodegron, leading to polyubiquitylation and degradation via the SCF/CRL1(β -TrCP) E3 ligase complex (Liu et al., 2010). During cell contact inhibition, TAZ is phosphorylated primarily at serine 311 and targeted for degradation (Liu et al., 2010).

TAZ stability is also regulated by a phosphodegron at the N-terminus. Phosphorylation at serine 58 and serine 62 by GSK3 β promotes β -TrCP binding,

polyubiquitylation, and degradation (Huang et al., 2012). This GSK3 β -mediated phosphorylation exclusively regulates TAZ but not YAP.

TAZ is furthermore subject to tyrosine phosphorylation and mitotic phosphoregulation by cyclin-dependent kinase 1 (Cdk1). Under hyperosmotic stress, TAZ undergoes tyrosine phosphorylation at tyrosine 316 by c-Abl kinase. The tyrosine phosphorylated TAZ selectively binds and inhibits nuclear factor of activated T cells 5 (NFAT5) (Jang et al., 2012). During the G2/M phase of the cell cycle, TAZ is phosphorylated by Cdk1 (Zhang et al., 2015). Cdk1 directly phosphorylates TAZ on six sites (serine 90, serine 105, threonine 175, threonine 285, threonine 326, and threonine 346). Mitotically phosphorylated TAZ loses transcriptional and oncogenic activity. In contrast, YAP is positively activated by phosphorylation by the same kinase. Blocking TAZ phosphorylation by Cdk1 induces mitotic defects in MCF10A cells (Zhang et al., 2015).

TAZ Regulatory Inputs

The Hippo-LATS axis was understood for many years to be the primary regulatory module for TAZ (Harvey et al., 2013). However, recent years have elucidated an extensive array of signaling inputs that converge to control TAZ activation. Rather than functioning as an on-off switch, TAZ regulation allows the cell to enact a graded response to disruptions in cell polarity. Here we review the known inputs controlling TAZ function: Hippo signaling, cell mechanotransduction, actin cytoskeletal rearrangement, and signaling crosstalks.

Hippo Signaling

The first and best-understood pathway for TAZ regulation is the Hippo tumor suppressor pathway. The Hippo tumor suppressor pathway is an evolutionarily conserved pathway that functions in organ development, regeneration, and stem cell biology (Harvey et al., 2013; Moroishi et al., 2015). The Hippo core kinase cassette involves STE20-like protein kinase 1 (MST1), MST2, and the large tumor suppressor 1 (LATS1) and LATS2, which combine with activating adaptor proteins salvador family WW domain-containing protein 1 (SAV1), MOB kinase activator 1A (MOB1A), and MOB1B (Moroishi et al., 2015). When LATS is phosphorylated, the Hippo-LATS pathway is “on” and TAZ is retained or degraded. When LATS is not phosphorylated, the Hippo-LATS pathway is “off”, permitting nuclear translocation.

Hippo signaling is modulated by cell polarity, cell-cell adhesion, cell contact inhibition, and mechanotransduction. Cell contacts allow an epithelial cell to maintain apicobasal cell polarity through the formation of adherens and tight junctions (Varelas et al., 2010; Cordenonsi et al., 2011; Greenwood et al., 2016). Deletion of the Crumbs complex, which maintains the apical domain of polarized epithelial cells, results in increased TAZ/YAP nuclear localization (Varelas et al., 2010). Angiomotin (AMOT), a protein that localizes to the Crumbs complex, tightly associates with TAZ/YAP to promote cytoplasmic localization (Varelas et al., 2010). Nf2/Merlin, an upstream Hippo regulator frequently mutated in cancer, also controls TAZ/YAP activity (Harvey et al., 2013). Merlin associates with epithelial tight and adherens junctions and can bind to F-

actin, allowing for signaling in response to cytoskeletal changes (Reviewed by Varelas, 2008). At the adherens junctions, depletion of α -catenin also induces nuclear TAZ/YAP accumulation (Varelas et al., 2010).

The Scribble complex, which defines and maintains basal-lateral domains, regulates TAZ by creating a scaffold for MST, LATS, and TAZ (Greenwood et al., 2016, Cordenonsi et al., 2011). This inactivation requires LATS phosphorylation sites (S89 and S306), pointing to a role upstream of the Hippo pathway (Cordenonsi et al., 2015). Cordenonsi et al found that Scribble does not associate with TAZ after Snail-induced EMT in MII and HMLE cells, suggesting that Scribble-LATS regulation may occur primarily in the initial stages of epithelial to mesenchymal transition.

Cellular polarity is essential for maintaining normal epithelial function and localization of cell surface proteins. Loss of Llg1, a key component of the Scribble complex, correlates with increased TAZ activation, EGFR mislocalization, and the acquisition of stem cell properties, and EGFR-dependent survival in mammospheres (Greenwood et al., 2016). EGFR mislocalization similarly disrupts cell-cell junctions and causes increased TAZ nuclear localization, suggesting a link between these molecular events during EMT (Greenwood et al., 2016; Chung et al., 2014).

Scribble localization is also fundamental for TAZ regulation. Transduction of a mutant Scribble construct (ScribP305L), which does not localize to the cell membrane, is sufficient to induce TAZ activation in MII cells. Conversely, overexpression of a membrane-tethered version of Scribble inhibits TAZ in transformed MII-Snail cells (Cordenonsi et al., 2011). These findings suggest that complete mesenchymalization is not required to activate TAZ; rather, Scribble delocalization within a partial EMT is

sufficient to increase TAZ levels and induce CSC properties. Additional signaling inputs, such as EMT-induced actin cytoskeletal rearrangement, may feed into this observed TAZ activation when cell-cell contacts are disrupted.

Hippo signaling has crosstalks with additional signaling pathways such as the GPCR pathway. Several recent studies have elucidated the potent role of GPCR signaling on TAZ activity. In response to extracellular stimuli (such as estrogen, progesterone, insulin, and glucagon), GPCRs activate RHO GTPases, inducing F-actin polymerization (Cordenonsi et al., 2011). Rho activity and actin cytoskeletal rearrangement inhibit LATS kinase activity, thereby activating YAP/TAZ (Dupont et al., 2011).

Mechanotransduction

Beyond Hippo signaling, TAZ activity is controlled by extracellular matrix stiffness. Dupont et al. (2011) discovered that YAP and TAZ are extremely responsive to mechanical cues, allowing a cell to perceive the ECM rigidity of its microenvironment and the state of compaction. Seeding cells on a stiff extracellular matrix, conducive to cell spreading, leads to TAZ nuclear accumulation. In contrast, cells seeded on a soft extracellular matrix are compacted, leading to cytoplasmic accumulation. Extracellular matrix stiffness regulates TAZ in a dominant manner independently of the Hippo cascade. TAZ mechanotransduction requires Rho GTPase activity and tension of the actomyosin cytoskeleton, however, the complete molecular mechanism leading to TAZ degradation remains elusive (Dupont et al., 2011).

Signaling Crosstalks

Upon activation, AKT phosphorylates and inhibits GSK3B, which directly phosphorylates the N-terminal phosphodegron of TAZ, inducing ubiquitin-mediated degradation. A correlation between AKT phosphorylation status and TAZ activity is observed in mesenchymal stem cells (Feng et al., 2015). This GSK3B-dependent regulation of TAZ occurs independently of LATS phosphorylation, and results in an alteration of the mesenchymal stem cell differentiation program. This pathway was uncovered as a mediator of radiation-induced osteogenic differentiation. (Feng et al., 2015). In stem cells, TAZ activation is known to promote osteogenic over adipogenic differentiation (Hong et al., 2005, Dupont et al., 2011). These findings have led to the hypothesis that growth and differentiation factors present during embryonic development drive TAZ-mediated mesenchymal differentiation through AKT/GSK3B signaling during differentiation stages. It has also been speculated that in breast cancer this signaling pathway may promote a metastatic niche conducive to bone metastasis (Feng et al., 2015). The relative contribution of this pathway during human development and cancer progression, however, remains unclear.

In recent years, exciting progress has been made to uncover additional signaling pathways that potentiate TAZ activity. Outside the scope of this review, these pathways and regulatory inputs include WNT, NOTCH, TGF β , and bone morphogenetic protein signaling, metabolism, cholesterol synthesis, hypoxia, and sodium homeostasis. For further discussion of these pathways, the reader is directed to insightful reviews by Moroishi (2015) and Zhou (2016).

IV. Altered EGFR Dynamics and Epithelial to Mesenchymal Transition

EGFR Structure and Activity

Epidermal growth factor receptor (EGFR) is a member of the ErbB (avian erythroblastic leukemia virus oncogene homolog) or human EGF receptor (HER) family of transmembrane receptor tyrosine kinases. Other members of this family include ErbB-2 (also known as neu, HER2), ErbB-3 (HER3) and ErbB-4 (HER4). EGFR signaling is essential for normal cellular activity during development, wound healing, and tissue regeneration. However, these natural functions are undermined by cancer cells to drive cellular proliferation, migration, and survival (Chung et al., 2014).

EGFR contains both extracellular and cytoplasmic domains. The extracellular domain binds to growth factors, such as epidermal growth factor (EGF), leading to receptor dimerization at the cell surface and transphosphorylation of the cytoplasmic tyrosine kinase domain. The activated cytoplasmic domain orchestrates an extensive signal transduction network resulting in downstream phosphorylation, second messenger activation, and transcription (Lurje et al., 2010). Following ligand binding at the cell surface, EGFR is internalized into endosomes, where it continually activates downstream pathways, such as the AKT and MAPK pathways (Chung et al., 2014). Tyrosine phosphorylated EGFR is subsequently trafficked to the lysosome for ubiquitin-mediated degradation (Haugh et al., 1999).

EGFR Trafficking in Development and Cancer

EGFR activity is both pulsatile and dynamic: the signal initiates at the cell surface and propagates intracellularly as the receptor travels in endosomes. A multitude of factors impact EGFR signaling effects, including the type and number of receptors expressed on the cell, ligand affinity, apicobasal receptor localization, and endosomal trafficking. At least 7 peptide growth factors activate EGFR, including EGF, transforming growth factor- α (TGF- α), heparin-binding EGF (HB-EGF), amphiregulin (AREG), betacellulin, epiregulin and epigen (Zeng and Harris, 2015). These ligands are differentially expressed and activated during tissue development, injury, and pathologic disease response.

During normal mammary development, all ligands are expressed; however, AREG may be uniquely involved in pubertal mammary development. It is the only ligand strongly upregulated at puberty and dramatically downregulated during and after pregnancy, and it is the only ligand uniquely required for ductal outgrowth and lactation (Lee et al. 2007; Schroeder and Lee, 1998). Recent studies suggest that AREG's functional effects on EGFR stability underlie its role in mammary development and tumorigenesis (Fukuda et al., 2015; Willmarth et al., 2008).

EGFR ligands produce distinct functional consequences based on their affinity for EGFR. EGF, a high-affinity ligand for EGFR, interacts persistently with the receptor following endocytosis, promoting continual tyrosine phosphorylation and receptor down-regulation. In contrast, the low-affinity ligands AREG and TGF α dissociate following endocytosis, promoting receptor recycling and evasion of proteolysis (Haugh et al., 1999; Fukuda et al., 2015). In contrast with EGF, AREG promotes EGFR overexpression and

accumulation at the plasma membrane and cell-cell junctions (Fukuda et al., 2015; Kappler et al., 2014; Willmarth et al., 2008).

The functional consequences of AREG activation in comparison to EGF are intriguing. AREG supports mammary epithelial cell proliferation with equal efficiency as EGF at equivalent molarity, despite low-affinity binding (Fukuda et al., 2015). AREG-induced EGFR accumulation has been proposed to explain this phenomenon (Fukuda et al., 2015; Willmarth et al., 2008). AREG-stimulated activation of EGFR stimulates motile and invasive phenotypes in MCF-10A cells, while knock-down of AREG in SUM-149 cells significantly reduces motile and invasive properties (Baillo et al., 2011). Both EGF and AREG drive mesenchymal characteristics in mammary epithelial cells, but to different degrees (Fukuda et al., 2015).

Differential ligand expression may play a role in the development of precancerous hyperplasia. DNA microarray analysis of terminal duct lobular units (TDLUs) and hyperplastic enlarged lobular units (HELUs) reveals a remarkable change in ERBB gene expression (Lee et al., 2007). The expression of ERBB receptors does not change significantly— however, there is a 14-fold downregulation of EGF, a two-fold downregulation of TGF α , a 10-fold up-regulation of AREG, and a 17-fold upregulation of epiregulin in HELUs compared with TDLUs (Lee et al., 2007). While the functional consequences of this transition are unknown, these findings suggest that early precancerous lesions reactivate embryonic differentiation pathways in part through differential EGFR ligand expression. Interestingly, Fukuda and colleagues reported that ligand switching plays a role in epithelial-to-mesenchymal interconversion in mammary epithelial cells. Ligand switching between EGF and AREG alters expression of ZEB1

and its antagonizing microRNAs, miR-205 and miR-200c, thus altering epithelial phenotype in a reversible manner (Fukuda et al., 2015). Taken together, these findings suggest that EGFR ligands may induce cellular transformation by short or long-term changes to EGFR biology.

Beyond ligand activation, EGFR signaling is influenced by a cell's apicobasal polarity. Polarized epithelial cells restrict EGFR to the basolateral domain through the tight maintenance of adherens junctions and tight junctions. When polarity is lost due to EMT or a loss of cell-cell junctions, EGFR becomes dispersed and mislocalized throughout the cell membrane. Mislocalized EGFR can further drive EMT and break down cell-cell junctions (Fan et al., 2013). EGFR mislocalization can be induced by a single proline to alanine point mutation (P667A), redirecting EGFR throughout the membrane and into endosomes (Greenwood et al., 2016; Chung et al., 2014). Mammary epithelial cells transfected with the EGFR P667A construct preferentially activate RAS/MAPK and AKT survival pathways over cells expressing wild type EGFR, suggesting that loss of cellular polarity leads to amplified signal transduction following mislocalization of EGFR (Greenwood et al., 2016).

V. Signaling Crosstalks: EGFR Signaling Promotes TAZ Nuclear Activity

TAZ activity is typically associated with changes in tissue architecture, rather than directly mediating growth factor initiated signaling cascades (Zanconato et al., 2016). However, recent studies suggest a role for EGFR signaling upstream of TAZ in early breast cancer progression and epithelial to mesenchymal transition. In a screen of

small molecule inhibitors of YAP, Yan et al discovered that EGFR signaling can induce rapid dissociation of the core Hippo kinase scaffold. Loss of this scaffold in polarized epithelial cells deactivates LATS1/2, thereby activating YAP (Yan et al., 2013).

Presumably TAZ would be subject to this same activation, although TAZ was not examined in the study. Interestingly, this EGF-induced dissociation of the Hippo complex requires PI3K-PDK1 activity, but occurs independently of AKT activation (Yan et al., 2013). These findings suggests that crosstalk between the EGFR pathway and TAZ may be mediated through antagonistic EGFR-Hippo interactions when mammary cells initially lose polarity.

EGFR may also modulate TAZ through the AKT pathway independent of Hippo signaling. AKT, a downstream effector of the EGFR signaling pathway, plays a central role in survival and proliferation. Huang and colleagues observed a positive correlation between AKT phosphorylation and TAZ expression in multiple breast cancer cell lines. PTEN overexpression or PI3K inhibition reduced AKT phosphorylation and TAZ protein levels, whereas GSK3B inhibition rescued TAZ (Huang et al., 2012).

EGFR mislocalization also accompanies EMT following a disruption of adherens junctions, as evidenced Lgl1 knockdown in normal mammary epithelial cells (Greenwood et al., 2016). EGFR mislocalization conferred by the EGFR P667A point mutation leads to increased AKT and ERK signaling compared to wild type EGFR. Remarkably, this EGFR mislocalization event on its own is sufficient to induce TAZ activation, but not YAP (Greenwood et al., 2016). Receptor localization is intrinsically linked to epithelial polarity and plasticity. The correlation between EGFR mislocalization

and TAZ activation suggests that TAZ may play a novel downstream role when EGFR dynamics are altered.

VI. Signaling Crosstalks: TAZ activation potentiates EGFR signaling

Conversely, TAZ activation and EGFR signaling may synergize predominantly through TAZ-induced activation of the EGFR pathway. In breast cancer and glioma, TAZ has been proposed to drive CSC properties (Bhat et al., 2016; Cordenonsi et al., 2011; Yang et al., 2010). TAZ has also been reported to activate EGFR in breast cancer, lung cancer, and glioblastoma multiforme (GBM), leading to migration and proliferation (Yang et al., 2010; Xu et al., 2015; Yang et al., 2016). Overexpression of TAZ in GBM cells promotes proliferation and tumor formation by potentiating the EGFR/AKT/ERK pathway (Yang et al., 2016). These effects are blocked by the EGFR inhibitor Erlotinib. Erlotinib has no effect on TAZ expression in GBM cells overexpressing TAZ, suggesting that TAZ acts upstream of EGFR in GBM (Yang et al., 2016). TAZ may also play a role in the therapeutic response to EGFR tyrosine kinase inhibition (TKI). Non-small cell lung cancer (NSCLC) cells harboring the EGFR T790M mutation, conferring resistance to gefitinib, have highly activated TAZ. Interestingly, TAZ activation is required for EGFR-induced gefitinib resistance. TAZ knockdown effectively resensitized NSCLC cells to gefitinib *in vitro* and reduced tumor formation and resistance to gefitinib *in vivo* (Xu et al., 2016).

TAZ may act upstream of the EGFR pathway by activating EGFR ligands. Microarray screening of NSCLC tissues with high TAZ expression demonstrates that

EGFR ligands amphiregulin, epiregulin, and neuregulin 1 are downstream targets of TAZ (Noguchi et al., 2014). TAZ is also capable of producing cysteine-rich angiogenic inducer 61 (CYR61) and connective tissue growth factor (CTGF) in several tissue types (Mo et al., 2014). In normal breast epithelial cells, TAZ overexpression drives EMT, invasive structures, EGF-independent growth, and malignant behavior (Yang et al., 2010). Suppression of AREG using an anti-AREG neutralizing antibody completely attenuates EGF-independent growth and reduces migration, presenting a non-cell autonomous mediator of TAZ-induced EGFR signaling in normal mammary epithelial cells. The induction of AREG is dependent on TAZ-TEAD binding. AREG is also a direct transcriptional target of YAP, but to a lesser extent compared to TAZ (Yang et al., 2010). Mammary epithelial cells are capable of producing other growth factors besides AREG. However, in a cytokine antibody array of 41 growth factors, AREG was the only significant TAZ-induced factor produced by MCF10A cells (Yang et al., 2010). Furthermore, Yang et al (2010) analyzed 269 breast cancer patient samples by immunohistochemistry and discovered a significant positive correlation ($p = 0.0147$) between TAZ and AREG. TAZ-induced AREG secretion presents an intriguing window for future studies of autocrine, paracrine, and endocrine effects of TAZ activation in breast cancer development and metastasis.

Conclusions

The theme resonating throughout this review is the cell's ability to sense, transduce, and maintain a transformation from a differentiated epithelial cell towards a

mesenchymal and stem-like phenotype. During EMT, growth factor receptor localization changes concomitantly with apicobasal cell polarity. Implicit in this transition is the change enacted by the mesenchymal, TAZ-activated cell upon its microenvironment. TAZ's paracrine effects on EGFR activity suggest a non-cell autonomous mechanism to alter ErbB biology within the local microenvironment, recapitulating an earlier stage of mammary development.

Alteration of the available growth factors may shape the metastatic or stem cell niche during breast cancer progression. Wider, systemic effects are also plausible, as human breast cancer cells are capable of secreting EGFR ligands via exosomes (Higgenbotham et al., 2011). In fact, AREG exosomes increase the invasiveness of breast cancer cells 4-fold over TGF α or HB-EGF and exhibit significantly greater membrane stability (Higgenbotham et al., 2011). It's tempting to speculate that TAZ-induced AREG secretion may lead to local and systemic alteration of EGFR biology and priming of the metastatic niche.

In tandem, cellular mislocalization of EGFR may feed into further TAZ activation, sustaining the acquired mesenchymal phenotype through an autocrine loop. Previous studies have identified autocrine loops in cervical and breast cancer involving EGFR, Hippo signaling, and YAP-induced AREG secretion (Lee et al., 2015; Zhang et al., 2009). No studies were found explicitly reporting an EGFR-TAZ autocrine loop in breast cancer. This may be explained in part by the redundancy of YAP/TAZ regulatory pathways, although future studies should distinguish YAP and TAZ activation. TAZ's ability to induce AREG transcription and secretion at higher levels than YAP suggests that TAZ plays a stronger role in potentiating EGFR signaling (Yang et al., 2012).

Furthermore, TAZ is uniquely activated by EGFR mislocalization in normal mammary epithelial cells (Greenwood et al., 2016). The correlation between EGFR mislocalization could be accounted for by TAZ-selective Hippo signaling, AKT-induced TAZ activation, actin cytoskeletal rearrangement, or a yet to be discovered regulatory mechanism. Future studies of these upstream regulatory pathways will help to elucidate the differential modulation of TAZ and YAP in breast cancer progression. Understanding the relative contributions of upstream regulators on TAZ activity during breast cancer progression may yield new insights and targets for the treatment and prevention of breast cancer.

An increasing number of studies suggest that TAZ activity drives cancer progression through crosstalk with the EGFR pathway in EGFR-driven cancers, suggesting new strategies for targeted therapy (Yang et al., 2012, Xu 2015, Yang et al., 2016). EGFR inhibition appears to attenuate proliferation induced by TAZ overexpression in mammary epithelial cells (Yang et al., 2012). Blocking TAZ activity has minimal effect on epithelial cells—however, in mesenchymal breast cancer cells, TAZ inhibition reduces proliferation and promotes a transition towards an epithelial-like state (Oku et al., 2015; Skibinski et al., 2014). Targeting both pathways simultaneously may synergistically decrease the overlapping hallmarks of proliferation, migration, and survival with reduced toxicity compared to EGFR inhibition alone.

Furthermore, TAZ-dependent breast cancer cells exhibit decreased sensitivity to chemotherapy and targeted therapies (Oku et al., 2015; Cordenonsi et al., 2011). TAZ inhibition may re-sensitize breast cancer cells to targeted therapies and chemotherapy. TAZ and EGFR signaling function interdependently to drive loss of polarity, EMT, and

metastasis, ultimately leading to the acquisition of cancer stem cell properties.

Unraveling the contributions of these two oncogenes during early cancer progression reveals new strategies for the prevention and treatment of breast cancer. Understanding the mysterious acquisition of stemness in mammary epithelial tissues will help direct therapies against the breast cancer stem cell population, improving treatment and lowering the rates of breast cancer recurrence.

Citations

- Bacelli, I. C. A. *et al.* Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. *Nature Biotechnology*31, 539–544 (2013).
- Baillo, A., Giroux, C. & Ethier, S. P. Knock-down of amphiregulin inhibits cellular invasion in inflammatory breast cancer. *Journal of Cellular Physiology*226, 2691–2701 (2011).
- Bartucci, M. *et al.* TAZ is required for metastatic activity and chemoresistance of breast cancer stem cells. *Oncogene*34, 681–690 (2014).
- Basu, S., Totty, N. F., Irwin, M. S., Sudol, M. & Downward, J. Akt Phosphorylates the Yes-Associated Protein, YAP, to Induce Interaction with 14-3-3 and Attenuation of p73-Mediated Apoptosis. *Molecular Cell*11, 11–23 (2003).
- Beyer, T. C. A. A. *et al.* Switch Enhancers Interpret TGF- β and Hippo Signaling to Control Cell Fate in Human Embryonic Stem Cells. *Cell Reports*5, 1611–1624 (2013).
- Bhat, K. P. L. *et al.* The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma. *Genes & Development*25, 2594–2609 (2011).
- Chaffer, C. L. & Weinberg, R. A. How Does Multistep Tumorigenesis Really Proceed? *Cancer Discovery*5, 22–24 (2015).
- Chan, S. W. *et al.* A Role for TAZ in Migration, Invasion, and Tumorigenesis of Breast Cancer Cells. *Cancer Research*68, 2592–2598 (2008).
- Chen, L. *et al.* Structural basis of YAP recognition by TEAD4 in the Hippo pathway. *Genes & Development*24, 290–300 (2010).
- Chung, B. M. Nexus of signaling and endocytosis in oncogenesis driven by non-small cell lung cancer-associated epidermal growth factor receptor mutants. *World Journal of Clinical Oncology*5, 806 (2014).
- Chung, E., Graves-Deal, R., Franklin, J. L. & Coffey, R. J. Differential effects of amphiregulin and TGF- α on the morphology of MDCK cells. *Experimental Cell Research*309, 149–160 (2005).
- Cordenonsi, M. *et al.* The Hippo Transducer TAZ Confers Cancer Stem Cell-Related Traits on Breast Cancer Cells. *Cell*147, 759–772 (2011).
- Cordenonsi, M. *et al.* The Hippo Transducer TAZ Confers Cancer Stem Cell-Related Traits on Breast Cancer Cells. *Cell*147, 759–772 (2011).
- Dupont, S. *et al.* Role of YAP/TAZ in mechanotransduction. *Nature*474, 179–183 (2011).
- Fan, R., Kim, N.-G. & Gumbiner, B. M. Regulation of Hippo pathway by mitogenic growth factors via phosphoinositide 3-kinase and phosphoinositide-dependent kinase-1. *Proceedings of the National Academy of Sciences*110, 2569–2574 (2013).
- Feng, J., Sun, Q., Liu, L. & Xing, D. Photoactivation of TAZ via Akt/GSK3 β signaling pathway promotes osteogenic differentiation. *The International Journal of Biochemistry & Cell Biology*66, 59–68 (2015).
- Greenwood, E. *et al.* Llg11 prevents metaplastic survival driven by epidermal growth factor dependent migration. *Oncotarget* (2016). doi:10.18632/oncotarget.11320

- Harvey, K. F., Zhang, X. & Thomas, D. M. The Hippo pathway and human cancer. *Nature Reviews Cancer*13, 246–257 (2013).
- Haugh, J. M., Huang, A. C., Wiley, H. S., Wells, A. & Lauffenburger, D. A. Internalized Epidermal Growth Factor Receptors Participate in the Activation of p21ras in Fibroblasts. *Journal of Biological Chemistry*274, 34350–34360 (1999).
- He, C. *et al.* The Hippo/YAP pathway interacts with EGFR signaling and HPV oncoproteins to regulate cervical cancer progression. *EMBO Molecular Medicine*7, 1426–1449 (2015).
- Hippo transducer TAZ promotes epithelial mesenchymal transition and supports pancreatic cancer progression. *Oncotarget* (2015). doi:10.18632/oncotarget.5772
- Hong, J.-H. & Yaffe, M. B. TAZ: A β -Catenin-like Molecule that Regulates Mesenchymal Stem Cell Differentiation. *Cell Cycle*5, 176–179 (2006).
- Huang, W. *et al.* The N-terminal Phosphodegrom Targets TAZ/WWTR1 Protein for SCF -TrCP-dependent Degradation in Response to Phosphatidylinositol 3-Kinase Inhibition. *Journal of Biological Chemistry*287, 26245–26253 (2012).
- Jang, E. J. *et al.* TAZ Suppresses NFAT5 Activity through Tyrosine Phosphorylation. *Molecular and Cellular Biology*32, 4925–4932 (2012).
- Kappler, C. S. *et al.* Oncogenic signaling in amphiregulin and EGFR-expressing PTEN-null human breast cancer. *Molecular Oncology*9, 527–543 (2014).
- Lee, S. *et al.* Alterations of Gene Expression in the Development of Early Hyperplastic Precursors of Breast Cancer. *The American Journal of Pathology*171, 252–262 (2007).
- Liu, C.-Y. *et al.* The Hippo Tumor Pathway Promotes TAZ Degradation by Phosphorylating a Phosphodegrom and Recruiting the SCF -TrCP E3 Ligase. *Journal of Biological Chemistry*285, 37159–37169 (2010).
- Mo, J.-S., Park, H. W. & Guan, K.-L. The Hippo signaling pathway in stem cell biology and cancer. *EMBO reports* (2014). doi:10.15252/embr.201438638
- Moroishi, T., Hansen, C. G. & Guan, K.-L. The emerging roles of YAP and TAZ in cancer. *Nature Reviews Cancer*15, 73–79 (2015).
- Noguchi, S. *et al.* An Integrative Analysis of the Tumorigenic Role of TAZ in Human Non-Small Cell Lung Cancer. *Clinical Cancer Research*20, 4660–4672 (2014).
- Oku, Y. *et al.* Small molecules inhibiting the nuclear localization of YAP/TAZ for chemotherapeutics and chemosensitizers against breast cancers. *FEBS Open Bio*5, 542–549 (2015).
- Platt, M. O., Wilder, C. L., Wells, A., Griffith, L. G. & Lauffenburger, D. A. Multipathway Kinase Signatures of Multipotent Stromal Cells Are Predictive for Osteogenic Differentiation. *Stem Cells*27, 2804–2814 (2009).
- Pobbati, A. V. & Hong, W. Emerging roles of TEAD transcription factors and its coactivators in cancers. *Cancer Biology & Therapy*14, 390–398 (2013).
- Qin, H. *et al.* Transcriptional analysis of pluripotency reveals the Hippo pathway as a barrier to reprogramming. *Human Molecular Genetics*21, 2054–2067 (2012).

- Siegel, R. L., Miller, K. D. and Jemal, A. (2017), Cancer statistics, 2017. CA: A Cancer Journal for Clinicians, 67: 7–30. doi:10.3322/caac.21387
- Silvy, M., Giusti, C., Martin, P.-M. & Berthois, Y. Differential regulation of cell proliferation and protease secretion by epidermal growth factor and amphiregulin in tumoral versus normal breast epithelial cells. *British Journal of Cancer*84, 936–945 (2001).
- Skibinski, A. *et al.* The Hippo Transducer TAZ Interacts with the SWI/SNF Complex to Regulate Breast Epithelial Lineage Commitment. *Cell Reports*6, 1059–1072 (2014).
- Varelas, X. The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease. *Development*141, 1614–1626 (2014).
- Varelas, X. *et al.* The Hippo Pathway Regulates Wnt/ β -Catenin Signaling. *Developmental Cell*18, 579–591 (2010).
- Wang, Z. Transactivation of Epidermal Growth Factor Receptor by G Protein-Coupled Receptors: Recent Progress, Challenges and Future Research. *International Journal of Molecular Sciences*17, 95 (2016).
- Willmarth, N. E. & Ethier, S. P. Autocrine and Juxtacrine Effects of Amphiregulin on the Proliferative, Invasive, and Migratory Properties of Normal and Neoplastic Human Mammary Epithelial Cells. *Journal of Biological Chemistry*281, 37728–37737 (2006).
- Willmarth, N. E. *et al.* Altered EGFR localization and degradation in human breast cancer cells with an amphiregulin/EGFR autocrine loop. *Cellular Signalling*21, 212–219 (2009).
- Xu, W. *et al.* Up-regulation of the Hippo pathway effector TAZ renders lung adenocarcinoma cells harboring EGFR-T790M mutation resistant to gefitinib. *Cell & Bioscience*5, 7 (2015).
- Yang, N. *et al.* TAZ induces growth factor-independent proliferation through activation of EGFR ligand amphiregulin. *Cell Cycle*11, 2922–2930 (2012).
- Yang, R. *et al.* HDAC9 promotes glioblastoma growth via TAZ-mediated EGFR pathway activation. *Oncotarget*6, 7644–7656 (2015).
- Yang, R. *et al.* The Hippo transducer TAZ promotes cell proliferation and tumor formation of glioblastoma cells through EGFR pathway. *Oncotarget* (2016). doi:10.18632/oncotarget.9199
- Yu, Z., Pestell, T. G., Lisanti, M. P. & Pestell, R. G. Cancer stem cells. *The International Journal of Biochemistry & Cell Biology*44, 2144–2151 (2012).
- Zanconato, F., Cordenonsi, M. & Piccolo, S. YAP/TAZ at the Roots of Cancer. *Cancer Cell*29, 783–803 (2016).
- Zhang, J. *et al.* YAP-dependent induction of amphiregulin identifies a non-cell-autonomous component of the Hippo pathway. *Nature Cell Biology*11, 1444–1450 (2009).
- Zhang, Y. *et al.* Suppression of microRNA-125a-5p upregulates the TAZ-EGFR signaling pathway and promotes retinoblastoma proliferation. *Cellular Signalling*28, 850–860 (2016).