



Ezrin Mediates Invasion and Metastasis in Tumorigenesis: A Review

Yanan Song^{1†}, Xiaokun Ma^{2†}, Miao Zhang¹, Menghan Wang², Guoyu Wang², Ying Ye^{1*} and Wei Xia^{2*}

¹ Central Laboratory, The Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine, Shanghai, China, ² Department of Nuclear Medicine, The Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine, Shanghai, China

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*Correspondence:

Wei Xia
awingxia@163.com
Ying Ye
yy49453324@163.com

[†] These authors have contributed
equally to this work and share first
authorship

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Ezrin, as encoded by the EZR gene, is a member of the Ezrin/Radixin/Moesin (ERM) family. The ERM family includes three highly related actin filament binding proteins, Ezrin, Radixin, and Moesin. These three members share similar structural properties containing an N-terminal domain named FERM, a central helical linker region, and a C-terminal domain that mediates the interaction with F-actin. Ezrin protein is highly regulated through the conformational change between a closed, inactivate form and an open, active form. As a membrane-cytoskeleton linker protein, Ezrin facilitates numerous signal transductions in tumorigenesis and mediates diverse essential functions through interactions with a variety of growth factor receptors and adhesion molecules. Emerging evidence has demonstrated that Ezrin is an oncogene protein, as high levels of Ezrin are associated with metastatic behavior in various types of cancer. The diverse functions attributed to Ezrin and the understanding of how Ezrin drives the deadly process of metastasis are complex and often controversial. Here by reviewing recent findings across a wide spectrum of cancer types we will highlight the structures, protein interactions and oncogenic roles of Ezrin as well as the emerging therapeutic agents targeting Ezrin. This review provides a comprehensive framework to guide future studies of Ezrin and other ERM proteins in basic and clinical studies.

Keywords: Ezrin, cancer, migration, invasion, metastasis

INTRODUCTION

Cancer is one of the most debilitating diseases worldwide. The molecular mechanisms of carcinogenesis provide essential implications for potential prevention and treatment of cancers. Extensive studies have been conducted on tumor invasion and metastasis, and multi-step processes have been described. Previous research has shown that cell adhesion, migration, and morphogenesis regulate tumor invasion and metastasis (Janiszewska et al., 2020). However, adhesion complexes, reorganization of the cytoskeleton, and their underlying molecular mechanisms are still poorly defined.

The Ezrin/Radixin/Moesin (ERM) family proteins regulate cell networks through linking actin cytoskeleton to the cell membranes (Kong et al., 2013). ERM family members, actin cytoskeleton and the cell membranes form highly dynamic domains including lamellipodia and filopodia

(Baumgartner et al., 2006). ERM family proteins switch between a closed (inactive) and an open (active) conformation to work with their interacting partners, which is tightly regulated by phosphorylation through different kinases (Matsui et al., 1998).

Ezrin, a member of ERM family, is phosphorylated by threonine and tyrosine kinases (Srivastava et al., 2005). Ezrin is a highly conserved protein through evolution, suggesting the same regulatory mechanisms between organisms (Fouassier et al., 2000). Ezrin mediates signal transduction, coordinates dynamic cellular processes, and acts through cytoskeletal reorganization (Bretscher et al., 2002). Genetic ablation experiments have confirmed the pleiotropic effects of Ezrin including cell polarity, adhesion, and invasion (Clucas and Valderrama, 2014). Ezrin controls signaling transduction by interacting with adhesion molecules and various growth factor receptors (Khanna et al., 2004; Auvinen et al., 2013). In this review, we focus on Ezrin's distinct roles in tumor growth, metastasis, and morphogenesis in cancer biology, because increased Ezrin expression is correlated with poor prognoses in various cancers. In addition, we address Ezrin's signaling pathways in cancer development and prognosis.

STRUCTURE, FUNCTION, AND SIGNALING PATHWAYS

Structure and Activation

Encoded by the EZR gene that locates at chromosome 6q25.2-q26, the Ezrin protein is the most studied member of the Ezrin/Radixin/Moesin (ERM) family, containing an FERM domain (band 4.1 protein, Ezrin, Radixin, Moesin), a central helical linker region and an ERM-associated domain (Figure 1A; Yin et al., 2018).

The conformational change in Ezrin determines its activity. When the NH₂- and COOH-terminal bind to each other, full length Ezrin is in a closed inactive form. The abolition of the intramolecular head-to-tail interaction is required to expose the actin binding sites, since the F-actin binding site at the C-terminal domain is normally masked in the full length Ezrin (Gary and Bretscher, 1995; Roy et al., 1997). Therefore, full length Ezrin is inactive and cannot interact with actin (Fehon et al., 2010). Activated Ezrin directly binds F-actin through a C-terminal domain (Bretscher et al., 1997). Direct binding of F- and G-actin occurs at the Ezrin N-terminal domain between residues 281 and 333 (Roy et al., 1997).

Ezrin dimers and higher oligomers present as inactive, and monomers are considered active (Gautreau et al., 2000). Multiple sites in ERM family proteins can be phosphorylated by several kinases and exhibit various biological functions (Table 1). Specifically, phosphorylation of the C-terminal threonine residue (Thr567) is the key step to activate Ezrin, which allows the actin filament binding domains to interact with other proteins and break head-to-tail associations (Figure 1B; Matsui et al., 1998). The threonine phosphorylation is a Rho-dependent activation of Ezrin (Chen et al., 2011). Besides threonine phosphorylation, tyrosine phosphorylation in Ezrin (Tyr353) is linked to p85 interaction and Akt overexpression (Cui et al., 2010). Together with Thr567, Tyr353 regulates Ezrin's

transition to its active form (Jin et al., 2014). Phosphorylation contributes to a plasma membrane mediated transition from Ezrin oligomers and monomers *in vivo* (Gautreau et al., 2000). The regulation of Ezrin phosphorylation is complex (McClatchey, 2003). It is also reported that *in vivo* phosphorylation of Ezrin is required in its binding and recruiting to the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) (Fievet et al., 2004; Hao et al., 2009). Ezrin interacts with other kinases, including myotonic dystrophy kinase-related Cdc42-binding kinase (Nakamura et al., 2000). Additionally, Src kinases and RhoA/Rho kinase activities are required for ERM activation, a key step in the growth of cone filopodia for axon outgrowth (Antoine-Bertrand et al., 2011). Interestingly, Ezrin mediates focal adhesion kinase activation independently from external stimuli (Poullet et al., 2001). Although phosphorylation of Ezrin is the most studied post-translational regulation, the biological effects of the phosphorylation sites are largely unexplored (Michie et al., 2019).

As discussed earlier, unphosphorylated/inactive Ezrin remains in a folded conformation through head-to-tail interaction, masking binding sites for other molecules. Phosphorylation on the conserved threonine residue T567 causes conformational changes, unmasking binding sites (Matsui et al., 1998; Figure 1B). T567 keeps Ezrin open and active, and prolongs its lifetime (Prag et al., 2007). Phosphorylated Ezrin is involved in fiber formation, adhesion, and migration (Shiue et al., 2005; Viswanatha et al., 2012; Antelmi et al., 2013). To regulate cytoskeleton dynamics, the ERM family proteins directly interact with actin filaments to link the cytoskeleton to the plasma membrane (Figure 1C). The FERM domain is essential for Ezrin's binding partners including intercellular adhesion molecules (ICAMs) 1–3, CD43/44, and NHE-1 (Denker et al., 2000; Ivetic and Ridley, 2004). Importantly, anti-metastatic small molecules NSC30587 and NSC668394 were identified that directly target Ezrin T567 phosphorylation and inhibit Ezrin's actin binding (Bulut et al., 2012). Therefore, targeting Ezrin phosphorylation and actin binding activity provides a new therapeutic direction for clinical cancer interventions.

Ezrin's Function Physiological Roles

In normal cells, Ezrin protein is known to contribute to epithelial morphogenesis, adhesion, and migration (Figure 2). Under physiological conditions, Ezrin maintains the cytoskeleton and normal shapes of epithelial cells. It mediates signaling pathways to maintain an apical–basal cellular polarity, as well as normal cell morphology, and binds to actin filaments to keep consistent cell–cell contact. In cancer cells, Ezrin is significantly activated, phosphorylated, and elevated, enhancing cancer cells' invasive abilities (Figure 2).

Cancer-Promoting Roles

During cancer development, the relative membrane localization of Ezrin proteins is increased and cell–cell contact is disrupted. Therefore, the activation of Ezrin proteins facilitates the process of tumor progression and invasion. The effect of enhanced Ezrin proteins in cancer metastasis takes different forms in

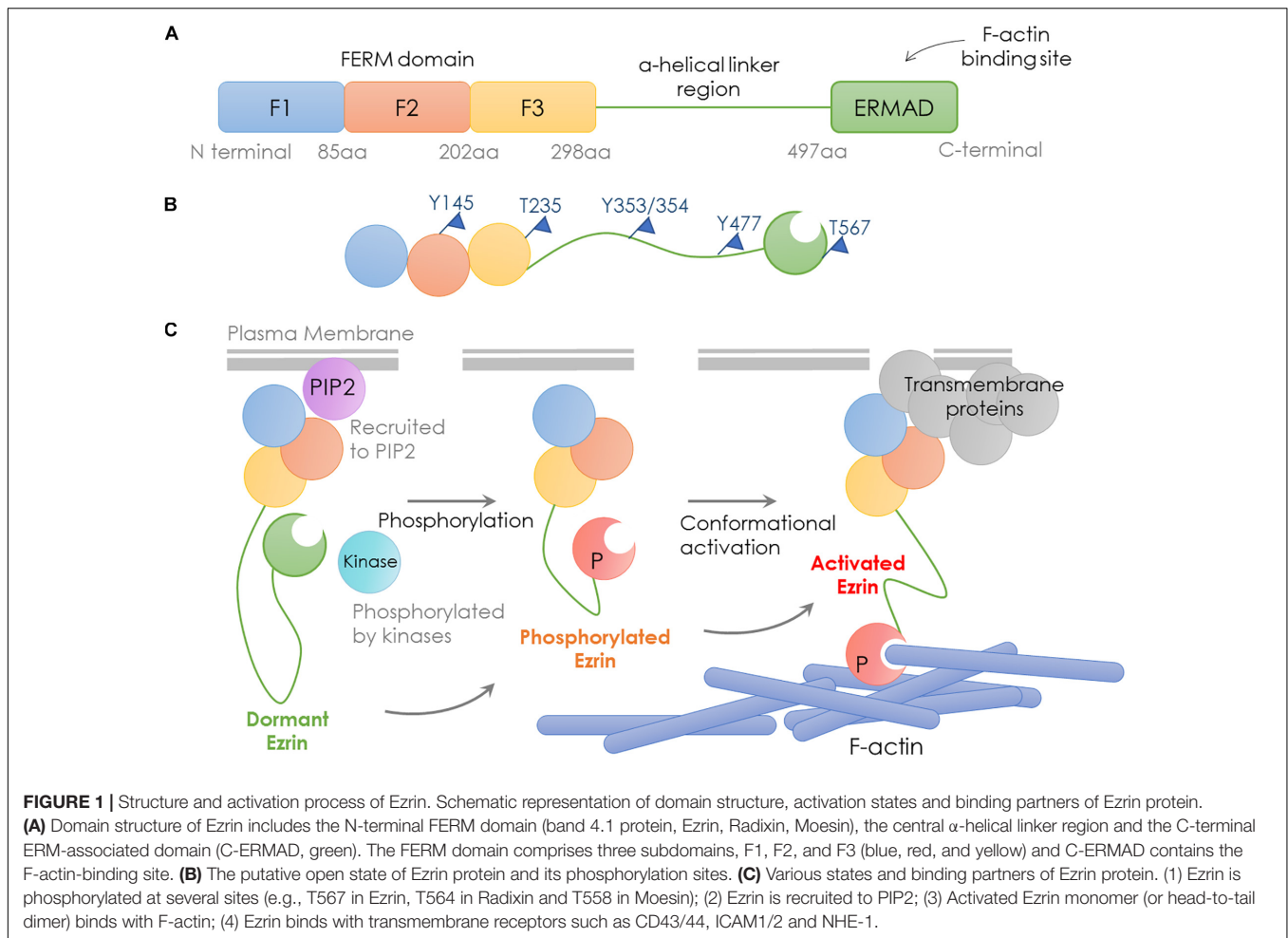
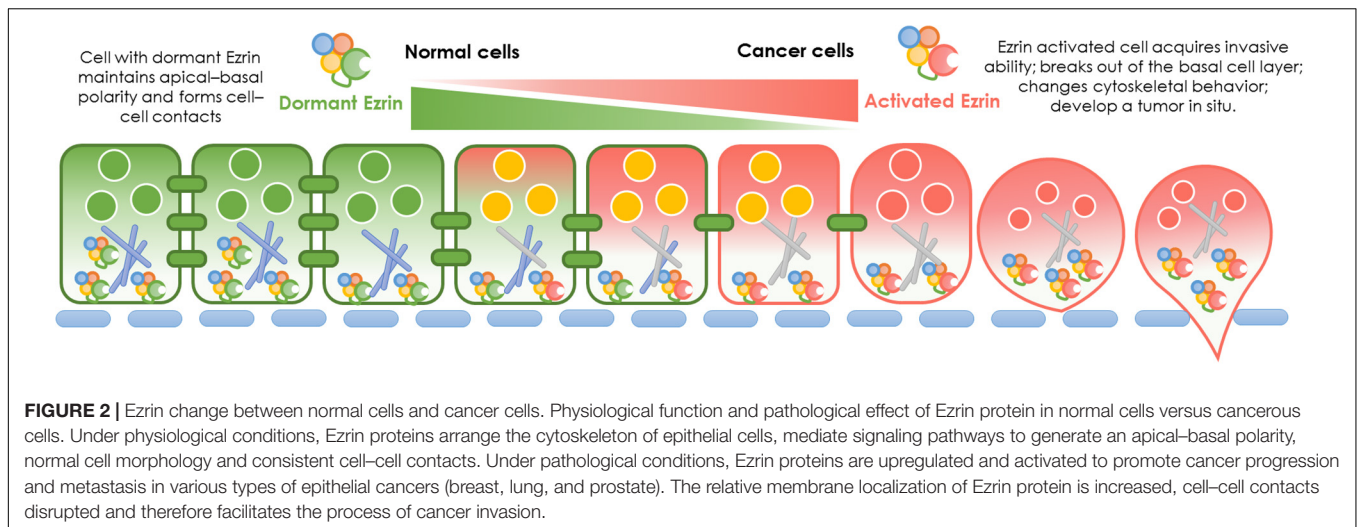


TABLE 1 | Ezrin phosphorylation sites and kinases.

Phosphorylation sites	Kinases	Functions	References
Y145	Hepatocyte growth factor (HGF) receptor, Lck	Activate Ezrin, enhances migration and tubulogenesis; T cell activation	Crepaldi et al., 1997; Autero et al., 2003
T235	Cyclin-dependent kinase 5	Induce the release of Rho GDP dissociation inhibitor, increase interaction with Rac1	Yang and Hinds, 2006
Y353/354	Hepatocyte growth factor receptor	Activate Ezrin, enhances migration and tubulogenesis; Responsible for the interaction with p85, required for PI3-kinase and Akt activation mediated cell survival; Nuclear localization	Crepaldi et al., 1997; Gautreau et al., 1999; Di Cristofano et al., 2010
Y477	Src	Not related to head-to-tail conformational opening, is associated with kelch-repeat superfamily protein; Regulates invasion and metastasis	Heiska and Carpen, 2005; Mak et al., 2012
T567 in Ezrin (T564 in Radixin and T558 in Moesin)	Rho-associated kinase, protein kinase B2/Akt2, atypical protein kinase C-iota (aPKC ι), mammalian Sterile 20 (Ste20)-like kinase-4 (Mst4), lymphocyte-oriented kinase (LOK) and Ste20-like kinase (SLK)	Interferes with the intermolecular head-to-tail association, activates Ezrin and is positively associated with invasive growth	Matsui et al., 1998; Shiue et al., 2005; Wald et al., 2008; Gloerich et al., 2012; Viswanatha et al., 2012; Antelmi et al., 2013

various cancers. For example, in osteosarcoma, Ezrin allows metastatic tumor cells to overcome a number of stresses as cells from the primary lesion are able to break loose and effectively initiate the growth of secondary lesions by generating additional

ATP from a variety of sources (Ren and Khanna, 2014; Zhang et al., 2014). In pancreatic cancer cells, Ezrin translocates into plasma membranes, binds to increasing amounts of cortactin, and forms a highly ordered structure called a podosomal rosette,



which enables epithelial cancer cells to adhere to the underlying substrate and modify their cytoskeletal behaviors (Kocher et al., 2009). Moreover, Ezrin is responsible for cellular polarization in pancreatic cancer associated macrophages (Chang et al., 2020). Enriched Ezrin expression has been detected in salivary gland carcinomas, which was significantly correlated with the levels of other cancerous molecular markers such as Ki67, HER2, p53, male sex, high-grade histopathology, and distant tumor metastasis (Hashimoto et al., 2017). Additionally, in lung cancer cells, activated Ezrin facilitates mechanical transduction from the cytoskeleton to the membrane and regulates the malignant process in a tension-dependent manner (Zhang et al., 2019).

Ezrin's Signaling Pathways

Ezrin Interacts With Multiple Signals Through Spatial and Temporal Regulation

Activated Ezrin regulates key events and interacts with different proteins in a variety of cancer types. The precise spatial and temporal activation of Rho GTPases establishes cell polarity and morphology (Haga and Ridley, 2016). The antagonistic relationships between different Rho GTPases regulate migration and adhesion, consistent with their opposing effects on ERM family proteins (Ivetic and Ridley, 2004). Ezrin recruits Cdc42, and the conformational active (phosphorylated) Ezrin brings Rho/Cdc42 specific guanine nucleotide exchange factor Dbl to the membrane. A precise spatial Dbl activated Cdc42 is crucial for directional cell migration in breast cancer cells (Prag et al., 2007). Dysfunction and loss of cell adhesion has been recognized as a pro-tumorigenic step, which enables the cancer cell to migrate and metastasize.

Ezrin Mediates E-Cadherin-Catenin Complex Maintenance

The E-cadherin-catenin adhesion complex maintains tissue architecture and is critical for intercellular adhesiveness. Ezrin suppression promotes the expression of E-cadherin and β -catenin. Both E-cadherin and β -catenin play a key role in epithelial cell adhesion. Co-precipitation experiments suggests

Ezrin associates with E-cadherin and β -catenin (Hiscox and Jiang, 1999). The modulation between Ezrin and E-cadherin is mediated by IL-1 β and TGF- β 1, suggesting that cytokine regulation in tumor invasion is governed by alteration in cell-cell interactions (Karmakar and Das, 2004).

Other Signaling Molecules

Ezrin mediates cell growth and survival through Akt signaling, but not the mitogen-activated protein kinase (MAPK) pathway in certain cancers, which is essential for cancer proliferation, invasion, migration and survival (Krishnan et al., 2006; Hu et al., 2016; Quan et al., 2019). Ezrin is correlated with poor prognoses in these cancer patients (Quan et al., 2019). In addition, Ezrin is associated with the p85 subunit, activating phosphatidylinositol 3-kinase (PI3K)/Akt in regulating tumorigenesis, metastasis, cell survival, and invasion in epithelial cells (Gautreau et al., 1999; Cui et al., 2010).

EZRIN'S ROLES IN CANCERS

As an important member of the ERM family of proteins, Ezrin has been well studied in many cellular events. As summarized earlier, Ezrin plays a vital role in molecular signaling, including cell proliferation, cell polarity establishment, cell motility, and cell adhesion (Ren and Khanna, 2014; Kawaguchi et al., 2017). Since these processes are crucial in invasion, and metastasis in a variety of solid tumors, the pathophysiological roles of Ezrin protein were extensively studied and discussed (Kawaguchi et al., 2017). Although Ezrin is known associated with poor prognosis in several cancers, the predictive value of Ezrin and its relationships with clinicopathological features or prognostic parameters remain controversial (Cihan, 2018). It is interesting to note that the Ezrin expression was associated with bad prognosis in a cancer type-specific manner (Li et al., 2015). In few cases such as bladder cancer, higher Ezrin expression indicates better prognosis rather than worse. In order to draw a most recent conclusion from the up-to-date work, below we summarized

the specific roles of Ezrin in various cancers, highlighting the special signaling cascades and pathophysiological roles (Figure 3 and Table 2).

Ezrin in Lung Cancer

Highly expressed Ezrin has been detected in lung cancer cell lines and primary lung cancer tissues. Ezrin has been found to be primarily distributed in the cytoplasm of lung cancerous tissue and metastatic foci (Wang et al., 2008;

Lee et al., 2012; Zhang et al., 2012; Jin et al., 2014; Kolegova et al., 2020). Ezrin expression correlates to the degree of lymphatic metastasis, malignant phenotype, and advanced TNM staging of lung cancer patients significantly (Lee et al., 2012; Li et al., 2012). Not surprisingly, downregulation of Ezrin was observed to reverse these aggressive biological behaviors (Chen et al., 2012; Lee et al., 2012). The underlying molecular mechanism of Ezrin activation in lung cancer involves Ezrin modifications (such as phosphorylation and

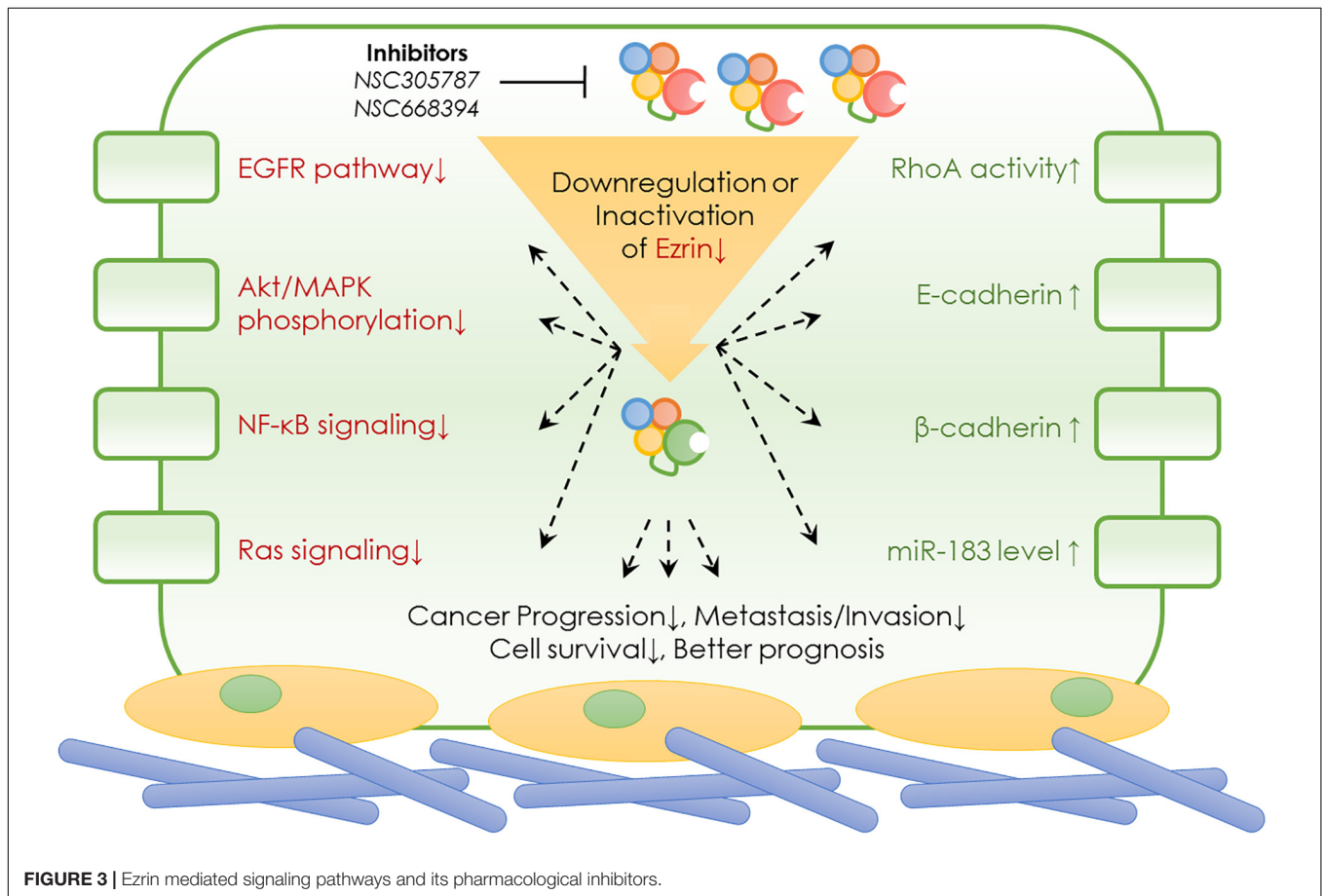


FIGURE 3 | Ezrin mediated signaling pathways and its pharmacological inhibitors.

TABLE 2 | Selective Ezrin interacting proteins in various cancers.

Cancers	Interacting proteins	Roles	References
Lung cancer	EGFR, ROCK1/2, RhoA, Akt signaling	Promotes cell migration and invasion	Lee et al., 2012; Li et al., 2012; Chen et al., 2013; Hata et al., 2016; Saygideger-Kont et al., 2016; Moodley et al., 2020
Breast cancer and ovarian cancer	miR-183, Akt signaling	Promotes cancer progression and enhances metastasis	Lowery et al., 2010; Horwitz et al., 2016; Ghaffari et al., 2019; Li et al., 2019
Cervical cancer	LGALS1, Galectin-3, E-cadherin, MAPK JNK/p38 and Akt/ERK1/2 signaling	Controls cell adhesion and enhances invasion. Serves as the cervical cancer marker for non-invasive detection	Elliott et al., 2005; Saito et al., 2013; Kong et al., 2016; Fadiel et al., 2017; Li et al., 2017; Zacapala-Gomez et al., 2018; Chetry et al., 2020
Gastric cancer	miR-183	Antagonizes mi-183 actions, and is correlated with tumor size, invasion lymph node and metastasis	Li et al., 2011; Cao et al., 2014
Osteosarcoma	miR-183, Akt and MAPK, Akt/mTOR	Promotes tumor metastasis. High expression is correlated with poor prognosis	Khanna et al., 2004; Krishnan et al., 2006
Hepatocellular cancer	Rho kinase, cytokeratin 19	Positive expression is correlated with a smaller tumor size and higher frequency of tumor dedifferentiation	Okamura et al., 2008; Yeh et al., 2009

S-nitrosylation), epidermal growth factor receptor (EGFR), and EGFR-mediated signaling pathways in non-small cell lung cancer (NSCLC) cells (Saygideger-Kont et al., 2016; Zhang et al., 2019). Downregulation of Ezrin in lung cancer cells has resulted in actin cytoskeleton rearrangements, reduced EGFR activity and phosphorylation levels of downstream signaling pathways, as well as a substantial reduction in cell migration and invasion (Chen et al., 2013; Saygideger-Kont et al., 2016). Ezrin also mediates downstream signaling pathways, including the activation of RhoA-GTPase and the signaling of ROCK1/2 and Akt in lung adenocarcinoma (Hata et al., 2016; Moodley et al., 2020). Interestingly, Ezrin serum levels were negatively correlated with serum IL-13 levels (which are believed to play an important role in lung function) (Jia et al., 2019).

Ezrin, Breast Carcinoma, and Ovarian Carcinoma

Similar to observations in other carcinomas, Ezrin is elevated in breast carcinoma and ovarian carcinoma. Ezrin plays a critical role in extracellular matrix remodeling and tumor dissemination in a 3-dimensional model (Horwitz et al., 2016). Since both breast and ovarian carcinomas exhibit a similar ability to disseminate due to malignant effusion formation, the significant increase of Ezrin serves as a future therapeutic intervention target. Ezrin mediates cell migration and invasion in lung and breast cancers that can be inhibited by the overexpression of miR-183 (Lowery et al., 2010). Ezrin promotes breast cancer progression and enhances metastasis through Akt signaling (Li et al., 2019). Elevated Ezrin expression increases the risk of relapse in node-positive and high-risk node-negative breast cancer patients. Pharmacological inhibition of Ezrin has significantly reduced cancer cell migration and invasion into the lymph nodes and lungs *in vivo* in real time (Ghaffari et al., 2019).

Ezrin in Cervical Cancer

Cervical cancer is the fourth most common cause of cancer-causing death in women. Cervical cancer originates from an epithelial neoplastic transformation in the uterine cervix. Cervical cancer is generally caused by an infection of the human papillomavirus (HPV) (Saavedra et al., 2012). As a migration-related protein, Ezrin is upregulated in cervical cancer (Zacapala-Gomez et al., 2018; Chetry et al., 2020) and its expression level is associated with advanced metastasis and poor prognosis. Specifically, Ezrin is increased in cervical cancer cells (SiHa and C33A) when Galectin-1 (LGALS1) is overexpressed. Ezrin expression is significantly suppressed when LGALS1 is downregulated. LGALS1 belongs to the carbohydrate-binding protein family and exhibits a high affinity for β -galactoside-containing glycol-conjugates (Chetry et al., 2020). Although multiple signaling pathways linked to LGALS1 have been reported, the underlying mechanisms of how LGALS1 affects Ezrin levels have not been fully elucidated. LGALS1 may interact with Ezrin through the MAPK, JNK/p38, and Akt/ERK1/2 pathways in the regulation of invasion and migration (Elliott et al., 2005; Chetry et al., 2020). Another lectin family member, Galectin-3, is also overexpressed along with Ezrin in cervical

cancer and both are predictors of poor prognosis in cervical cancer patients (Li et al., 2017). Ezrin down-regulation induces Akt phosphorylation, and Ezrin regulates both epithelial-mesenchymal transitions and metastasis in cervical cancer (Kong et al., 2016). Ezrin promotes cell proliferation through phosphorylation on residue Y145 (Gautreau et al., 1999; Saito et al., 2013), cell mobility, and migration in cervical cancer cells (Kong et al., 2016).

Ezrin plays a key role in cervical cancer invasion and is a potential prognostic immunomarker. Interestingly, Ezrin expression is correlated with HPV associated lesions (Auvinen et al., 2013; Zacapala-Gomez et al., 2018), suggesting that Ezrin can be used to distinguish between transient and persistent HPV integration. More than 80% of cervical cancer samples exhibit high Ezrin expression and a decrease in E-cadherin levels, which can be detected using immunohistochemistry and cervical smears (Zacapala-Gomez et al., 2018). Consistent with this study, the overlapping of Ezrin and estrogen receptor expression during cervical carcinogenesis raises the possibility that Ezrin is associated with the penetration of the basement membrane (Fadiel et al., 2017). Therefore, Ezrin controls adhesion and the invasiveness of cancer cells through the interactions between cell adhesion molecules, suggesting a role in developing cervical neoplasia and cancer. Due to the high expression of Ezrin, a non-invasive testing method can serve as a milestone for cervical cancer detection, which is crucial for early treatment and a better prognosis in patients with squamous intraepithelial lesions. In addition to cervical cancer, enhanced Ezrin expression is a new, independent prognostic marker in endometrioid carcinoma and is correlated with endometrioid carcinoma stages (FIGO) (Kobel et al., 2006).

Ezrin and Gastric Cancer

Gastric cancer is the second most prevalent cause of cancer death. The Ezrin protein is up-regulated in gastric cancer lesions. Ezrin expression is correlated with tumor size, tumor location, lymph node invasion and metastasis, and shortened survival in stages I, II, and III (Li et al., 2011). Specifically, Ezrin can be used as an early diagnostic marker and to predict later metastasis in gastric cancer using meta-analysis (Jin et al., 2012; Liang et al., 2017). Decreased miR-183 and elevated Ezrin have been reported in gastric cancer cells and tissues. The 3'UTR region of Ezrin's mRNA is a direct target of miR-183. miR-183 antagonizes Ezrin and acts as a tumor suppressor in gastric cancer (Cao et al., 2014).

Ezrin and Osteosarcoma

Dysregulation of miR-183 through Ezrin targeting promotes osteosarcoma tumor metastasis. Ezrin is required for metastasis in osteosarcoma and its high expression is associated with poor outcomes in pediatric osteosarcoma patients. Khanna et al. (2004) has reported that suppression of Ezrin decreases Akt and MAPK phosphorylation, but Ezrin induced metastatic survival is mediated partially by MAPK instead of Akt. Interestingly, Khanna et al. (2004) have reported that Ezrin-mediated growth and survival in Ewing sarcoma is dependent on Akt/mTOR, but not MAPK (Krishnan et al., 2006), suggesting that Ezrin acts through different signaling pathways in different cancers.

Ezrin's roles in Ewing sarcoma are distinct from its roles in other sarcomas. A majority of Ewing sarcoma samples express Ezrin, but the intensity and expression pattern of Ezrin is not correlated with clinical characteristics. In contrast to Ezrin's roles in promoting carcinogenesis, Ewing sarcoma patients with high Ezrin intensity had a superior 5-year event-free survival compared to patients with low or no Ezrin expression (Cash et al., 2017). However, other factors, including diagnosis time, tumor size, therapeutic treatment, and larger sample size should be considered to validate the correlation between Ezrin and Ewing sarcoma clinical outcomes.

Ezrin and Hepatocellular Cancer

As discussed earlier, Ezrin phosphorylation regulation contributes to Ezrin's molecular plasticity. Hyperphosphorylation at the C-terminal threonine residue (T567) is significantly correlated with an invasive clinical hepatocellular carcinoma (HCC) (Chen et al., 2011). Therefore, blocking Rho kinase-mediated Ezrin phosphorylation can inhibit liver tumor metastasis. Ezrin staining in HCC is dramatically associated with cytokeratin 19 expression. Ezrin-positive patients had increased serum α -fetoprotein, shortened recurrence-free periods, and shortened overall survival (Okamura et al., 2008). Ezrin is expressed in hepatic progenitor cells, and some cases of HCC are derived from hepatic progenitor cells. Ezrin overexpression is involved in the dedifferentiation and invasion of hepatitis B virus-associated HCC (HBV-HCC). Surprisingly, patients with positive Ezrin expression had smaller tumor sizes and a higher frequency of tumor dedifferentiation and vascular invasion. Ezrin expression is independently associated with tumor size, poor differentiation, and vascular invasion in HBV-HCC (Yeh et al., 2009).

Ezrin and Bladder Cancers

Inconsistent with most of cancers mentioned above, membranous expression of Ezrin is significantly lower in high grade bladder cancer and significantly associated disease-specific overall survival (Palou et al., 2009; Athanasopoulou et al., 2013). Ezrin is an independent predictor of muscularis propria invasion and increased progression. Unlike its role in other cancers, reduced membranous Ezrin expression is related with unfavorable clinicopathological characteristics and an impaired survival (Andersson et al., 2014). Although these reports collectively suggested the prognostic value of Ezrin in bladder cancer, its immunohistochemical expression level failed to predict therapy effect (Malmstrom et al., 2017).

Ezrin and Other Cancers

Ezrin expression negatively correlated with renal cell carcinoma (RCC) metastasis, and the inhibition of Ezrin expression suppressed the invasive abilities of RCC cells (Yu et al., 2015). Using immunohistochemical approaches, Ezrin reactivity was observed mainly in conventional, papillary, and mucinous tubular spindle cell carcinoma subtypes of RCC, suggesting that the Ezrin protein might be beneficial as an additional diagnostic marker in the differential diagnosis of RCC subtypes (Tuna et al., 2009).

In colorectal cancer (CRC), Ezrin binds with a cell-neural adhesion molecule (L1CAM) and mediates the phosphorylation of NF- κ B as well as the activation of NF- κ B signaling (Gavert et al., 2010). It has also been reported that increased expression of Ezrin (phosphorylated on T567) was seen in liver metastasis in an insulin-like growth factor type 1 receptor (IGF1R)-dependent CRC xenograft model as compared to primary CRC. The Ezrin protein induces CRC cell survival through the modulation of apoptosis protein inhibitor XIAP, which was dependent on T567 (Leiphrakpam et al., 2014). Several studies have confirmed that Ezrin may serve as a promising biomarker in estimating the prognosis, outcome, and differential status of CRC patients (Patara et al., 2011; Lin and Chen, 2013; Fathi et al., 2017; Slik et al., 2017; Aikawa et al., 2019).

In glioblastoma, Ezrin interacts with and delocalizes the cytoskeletal-related protein neurofibromatosis type 2 (NF2), which carries out opposite activities in tumor growth (Morales et al., 2010). Notably, Ezrin, in a complex with NF2, enhances glioblastoma growth independent of its molecular conformation or subcellular localization. Using medulloblastoma cell lines and athymic mice as models, a study reported that Ezrin is localized to filopodia in medulloblastoma cells and promotes filopodia formation as well as *in vitro* invasion in medulloblastoma (Osawa et al., 2009).

In primary melanomas of the skin and metastatic tumors, Ezrin expression correlates with tumor progression and suggests worsening clinical disease behaviors. The molecular mechanism involves molecules related to metastatic functions such as CD44, merlin, and Ras signaling (Ilmonen et al., 2005; Federici et al., 2009; Riecken et al., 2016). Consistent with findings in osteosarcoma, Ezrin was found to be highly expressed in pancreatic cancer tissues and to positively regulate cell proliferation and invasion through the activation of the Akt/mTOR pathway (Meng et al., 2010; Quan et al., 2019; Chang et al., 2020). Ezrin and Rho-A expressions in squamous cell carcinoma suggest a cooperative participation of these proteins in cell movement and invasion (Assao et al., 2017). A tumorigenic role of Ezrin in skin cancer has also been demonstrated using immunohistochemical staining specimens from epithelial skin tumors, together with squamous carcinoma cell lines (Abdou et al., 2011; Wu et al., 2011). A similar result was seen in nasopharyngeal carcinoma as phosphorylated Ezrin expression was dependent on increased Rho kinase and protein kinase C activity (Tang et al., 2011). The oncogenic role of Ezrin is not limited to solid tumors as it has also been seen in blood cancers, such as diffuse large B-cell lymphoma, where the knockdown of Ezrin attenuated chemotherapy resistance (Pore et al., 2015; Sun et al., 2018).

EZRIN AS A PHARMACOLOGICAL TARGET

The above sections are not meant to recap all the latest important findings in Ezrin research but rather to provide an overview of the evidence showing the oncogenic roles and prognostic value of Ezrin in a wide range of cancer types. One of the questions that

remain to be answered is what the clinical implication of Ezrin is. As described above, high levels of Ezrin are observed in many cancers with lung metastasis, indicating poor survival and bad prognoses. Ezrin as an essential prognosis predictor of various cancers has been demonstrated to be a key modulator of tumor metastasis. All the existing studies, taken together, highlighted the fact that Ezrin may serve as a potential therapeutic target in cancer (Hoskin et al., 2019). This prompts the next question whether or not pharmacological regulators with a high affinity to Ezrin would exhibit encouraging results for cancer treatment. Despite various downstream pathways (Figure 3) of Ezrin been identified in cancers, it is expected that identification of small molecule inhibitors of Ezrin would lead to the discovery of anti-metastatic and anti-invasion drugs.

Small Molecular Inhibitors

Over the past decade, many studies have attempted to develop targeted cancer treatment strategies using small molecule inhibitors of Ezrin (Table 3). For the first time, Bulut et al. (2012) identified two compounds (NSC305787 and NSC668394) from small molecule libraries, which can directly bind to Ezrin, reduce phosphorylation on T567 and block its functional activity. These two inhibitors effectively reduced tumor metastasis in lung cancer and osteosarcoma (Celik et al., 2015, 2016). Following that, more and more studies attempted to extend the anti-metastatic activity of these two small molecule inhibitors in other cancers. Surprisingly, although Ezrin showed widely pro-metastatic capacity in many cancers, the anti-metastatic effect of its inhibitors was only seen in a few cancer types (Table 3). To date, NSC305787 and NSC668394 are undergoing investigation through animal models but not yet included in any clinical trials.

Activator

Because of the oncogenic role of Ezrin, studies investigating Ezrin activator are rare. Ezrin activation has been linked to CDK5 in the senescent phenotype as CDK5 is able to activate

Ezrin by phosphorylating T235 of Ezrin (Yang and Hinds, 2003). Interestingly, CDK5 mediated activation of Ezrin prevents the intermolecular interactions with/within cell membranes and cooperative with phosphorylation of another site T567, allowing Ezrin to participate in cytoskeleton-related signaling.

CONCLUSION AND FUTURE DIRECTION

In the literature, the oncogenic roles of Ezrin were intensively studied but there are a limited number of studies investigating the predictive performance of Ezrin expression level. In this review, we summarized not only the oncogenic roles of Ezrin but also its pathophysiological roles and potential pharmacological regulators in a wide range of cancer types. Our understanding of Ezrin as a potential drug target is strongly influenced by the idea that Ezrin is commonly proved to promote tumor metastasis and predicts poor prognosis in different types of cancers. Therefore, direct inactivation of Ezrin by the small molecule inhibitors should provide a new strategy for metastatic treatment in many cancers. While this hypothesis is indeed supported by a few lines of evidence in a couple of cancer types such as lung cancer, this rule seems failed to expand in many other cancer types.

Many fundamental questions in the roles of Ezrin remain to be answered. From this work, some basic understanding of Ezrin protein may be challenged. For example, the expression level of Ezrin in bladder cancer is reduced while it is commonly up-regulated in many other cancer types. The predictive value of Ezrin in bladder cancer is also found opposite to the other cancer types. Given metastasis is a complicated process that involves many steps that are poorly understood at this time, some of which may include tissue type-specific mechanism involving Ezrin. This mechanism may not be shared within ERM family proteins as the other ERM protein Mosin was found enriched in bladder cancer and consistent with its oncogenic role in invasion process. Future

TABLE 3 | Pharmacological inhibitors and activators targeting Ezrin.

Name	Target site and modification	Experiment models	References
<i>Inhibitor</i>			
NSC668394 and NSC305787	Inhibition of T567 phosphorylation	Zebrafish, osteosarcoma cell culture, Xenopus embryonic development, mouse lung organ culture and <i>in vivo</i> lung metastasis models	Bulut et al., 2012
Compounds 21k and 21m, as analogs of NSC668394	Inhibition of T567 phosphorylation	<i>In vitro</i> binding assays	Paige et al., 2014
NSC668394 and NSC305787	Inhibition of T567 phosphorylation	Mouse lung metastasis cell culture model	Celik et al., 2015, 2016
NSC668394, drug-like compounds MMV020549 and MMV666069	Inhibition of T567 phosphorylation	Zebrafish, osteosarcoma cell culture, and Xenopus embryonic development models	Celik et al., 2015
NSC668394	Inhibition of T567 phosphorylation	Diffuse large B-cell lymphoma cell line and tumor Xenografts mice models	Pore et al., 2015
NSC668394	Inhibition of T567 phosphorylation	Tumor-bearing lymphatic reporter mice model	Ghaffari et al., 2019
NSC305787	Inhibition of T567 phosphorylation	Lung cancer cell model	Moodley et al., 2020
NSC668394	Inhibition of T567 phosphorylation	Japanese encephalitis virus mouse infection model	Liu et al., 2020
<i>Activator</i>			
Cyclin-dependent kinase 5, CDK5	Activation of T235 phosphorylation	RB-transfected osteosarcoma cell model	Yang and Hinds, 2003

work is needed to uncover new pharmacological inhibitors and to explore the *in vivo* activity of the existing small molecule inhibitors as potential tools in cancer therapeutics.

AUTHOR CONTRIBUTIONS

WX and YY designed the manuscript. YS and XM wrote and revised the manuscript. MZ searched the references. MW and GW drafted the figures. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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