Ezrin and Metastatic Behavior of Common Estrogen Dependent Tumors

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Abstract

Ezrin, a membrane - cytoskeleton linking protein, is a member of ezrin/radixin/moesin (ERM) that contribute to maintain the specific cellular shape and play an important role in cellular motility and cell signaling. Ezrin in this way can be involved in cancer progression and distant metastasis in variable human cancers and the expression of this molecule is under the control of estrogen. Breast, uterus and ovary are well known estrogen dependent organs. Breast cancer and gynecologic cancer cells with high levels of ezrin expression have more invasive, metastatic potential than lower levels of ezrin expressed cancer cells and also related to advanced histological grade and poor outcome. It has being reported that ezrin expression is enhanced and it move from apical to cytoplasm and/or membranous portion in metastatic breast cancer cells and high stage gynecologic tumor cells. Therefore, in the near future, if further investigation is clearly added, ezrin expression with its cellular location might serve as a marker for the metastatic behavior of common estrogen dependent tumors.

Introduction

Worldwide, breast and gynecologic cancers account for approximately 45.9% of all female cancers(1). In 2009, 36% of total women cancer in the United States was diagnosed with a cancer affecting the breast(27%) and reproductive organs(9%), and 23% of women died from common estrogen dependent breast cancer and gynecologic cancer, second only to lung cancer(2). However, the precise mechanisms of progression and distant metastasis of estrogen dependent tumors have not been clearly delineated. Furthermore, in spite of having advanced treatment modalities, a lot of cases of advanced estrogen dependent tumors are resistant to chemotherapy, and there is no outstanding achievement of survival rate in patients with metastatic estrogen dependent tumors(3, 4, 5, 6). So, for the purpose of finding predictable factors for metastasis and improve treatment outcome for advanced estrogen dependent tumors through them, many researchers have focused on the roles of estrogen and its regulating proteins in responsible for the progression and distant metastasis of estrogen dependent tumors. A lot of efforts are taking place to find key proteins that are relevant to tumorigenesis and can better explain the complicated process of distant metastasis of cancer cells(7, 8, 9). ERM proteins are one of the groups of molecules that are receiving the most attention. They can explain the functional and structural communication needed between normal cells and cancerous cells in the sequential process of cancer progression(10, 11, 12, 13). Recently, It also has been reported that high expression of ezrin and its specific cellular location are implicated in high invasiveness of tumor and poor prognosis in several malignancies of animal and humans such as osteosarcoma(14), pancreas(15,16), prostate(17,18), uveal malignant melanoma(19), and astrocytoma(20) involving estrogen dependent tumors. Therefore, in this review, we first describe the ezrin and then evaluate the possible roles of this protein as a marker for the progression and distant metastasis of common estrogen dependent tumors, and we hope to estimate the possibility of anti – estrogen and anti - ezrin can either confer the novel treatment modality for common estrogen dependent tumors or not

Ezrin/Radixin/Moesin (ERM) proteins

Hunter and Cooper first noticed a specific polypeptide of 81 kDa, which rapidly phosphorylated on tyrosine residues in A-431 carcinoma cells when they added epidermal growth factor (EGF)(21). This polypeptide, now referred to as ezrin, was purified, and has been found mainly on cell surface structures, such as microvilli, ruffles border, and filopodia(22). Radixin, one of the ERM proteins, was isolated from rat liver and is known as a barbed end-capping protein because it is highly concentrated in cell to cell adhesion junction site, but not in cell to substrate adherens junctions(23). Moesin, another ERM protein, has been identified as a receptor protein for heparin or heparan sulfate and was isolated from the bovine uterus(24). The ERM family is composed of these three proteins and share approximately 75% of their homology (25,26,27,28,29). N-terminal half domain of ERM proteins are highly conserved and share homology with the membrane binding domain of
Ezrin is composed of 585 amino acids with three distinctive regions. The highly conserved N-terminal ERM associated domain (N-ERMAD) contains 296 amino acid. It is followed by an extended α-helix and a positively charged C-terminal domain (C-ERMAD) that contains 107 amino acids. All ERM proteins have structures similar to ezrin, N-terminal domains that bind to plasma membranes and C-terminal domains that bind to actin filaments. The N-terminal domain of ezrin has 84%, 83%, and 62% homology with other ERM proteins, radixin, moesin and merlin respectively. The N-terminal domain of four. one, ezrin, radixin, moesin (FERM) is globular shape with three subdomains (F1, F2 and F3) that are arranged like a clover leaf. This specific subdomains make ezrin bind to peptide and lipid ligand. C-terminal domain is one β-stranded and 6 helical regions that bind to N-terminal. Since the N-ERMAD of ERM proteins can be associated with the C-ERMAD of ERM proteins, ezrin can exist as one of two forms in the cytoplasm, active or dormant. A majority of ezrin in the cytoplasm exist as monomeric dormant forms with N-terminal is masked by C-terminal and dormant forms are composed of monomer, homodimer, heterodimer and some of oligomer. This form of ezrin is regarded as functionally inactive state. Pearson et al confirmed that it is possible that a conformational change could mask the binding sites of both terminals, which would inactivate the ezrin. However, the active forms of ezrin expose the binding domain of both terminals that bind to specific ligand, and are currently thought to be associated with specific cellular function. This switching action of ezrin is regarded as an essential mechanism for cellular motility, adhesion and signal transduction.

**Ezrin as a cytoskeleton - membrane linker molecule**

Since the concept of a cytoskeleton was first introduced by Paul Intrebert, many studies have examined its roles in cellular function. The cytoskeleton is a dynamic structure that maintains cell shape, protects the cell, enables cellular motion (using structures such as flagella, cilia and ruffles), and plays important roles in both intracellular transport and cellular division. The filamentous type of cytoskeleton, 6nm diameter, is composed of two intertwined actin chains and the cytoskeleton is most concentrated just beneath the cell membrane. Therefore, theoretically, it might be quiet reasonable that there should be exist some linker molecules between them. Ezrin was first identified as a linker protein between the plasma membrane and cytoskeleton. The N-terminal of ERM proteins attach to the C-terminal of integral protein in the plasma membrane directly or indirectly. Well known adhesion molecules that bind to ERM proteins are CD44, ICAM-1, 2, 3 and L-selectin. CD44 is highly expressed in hematopoietic cells, connective tissue such as fibroblasts, and endothelial cells. The level and location of this molecule is controlled by various cytokines, such as IL-4, 7, TNF-α and HGF(54,55,56,57). The main function of CD44 is adhesion during hematopoiesis, monocyte activation and cell migration. It also participate in maintaining homeostasis of water, supporting cellular shape and cell to cell adhesion as a receptor of hyaluronic acid. Ezrin also can directly bind to intercellular adhesion molecule-1 (ICAM-1) that is expressed by the vascular endothelium, macrophages and lymphocytes. ICAM-1 is a member of immunoglobulin superfamily (IgSF) of adhesion molecules, whichis barely detectable in normal...
endothelial cells, but it is enhanced on endothelial cells in response to inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), IL-1β and INF-γ (60), and plays a pivotal role in leukocyte adhesion and subsequent transendothelial migration during inflammation (61,62). In addition to this, endothelial CAM-1 initiates intracellular calcium flux and cytoskeletal signal transduction events, including activation of Rho GTPase and phosphorylation of cofilin, paxillin, p130cas, and focal adhesion kinase (63,64). ICAM-2, another ezrin binding molecule, plays an important role in lymphocyte recirculation and mediates adhesive interactions important for antigen-specific immune responses, such as NK-cell mediated clearance, lymphocyte recirculation, and other cellular interactions important for immune responses and surveillance (65,66). Ezrin, however, does not interact with ICAM-3 (67). Instead, Moesin interacts with ICAM-3, which is constitutively and abundantly expressed by all leucocytes and may be the most important ligand for lymphocyte function-associated antigen-1 (LFA-1) in the initiation of the immune response. ICAM-3 can act not only as an adhesion molecule, but also as a potent signaling molecule (68). Ezrin can also bind to L-selectin. This molecule is a cell adhesion molecule that mediates the initial tethering and subsequent rolling of leucocytes along ligands expressed on endothelial cells. Frequent ezrin binding scaffolding molecules are ERM – binding phosphoprotein 50 (EBP50) /Na+/H+ exchanger–regulatory factor (NHERF). Na+/H+ exchanger (NHE) type 3 kinase A regulatory protein (E3KARP) and syndecan-2. EBP50, an important binding protein of the ERM family, was found in human placenta and the bovine brain by Reczek et al. Shortly after, it was confirmed that this protein is identical to the Na+/H+ exchanger–regulatory factor (NHERF). EBP50 binds tightly to the N-ERMADs of ezrin and it also has two PSD-95/DlgA/ZO-1-like (PDZ) domains that can associate with the C-terminal of integral transmembranous proteins. Therefore, EBP50 can act as a scaffolding molecule between membranous proteins and ERM proteins and ERM proteins can link membranous protein and actin filaments via the EBP50 (69,70). Another protein of this family was cloned and characterized (NHERF2, also called Na+/H+ exchanger (NHE) type 3 kinase A regulatory protein (E3KARP) or tyrosine kinase activator protein 1 (TKA1)) (71,72). E3KARP associates with the actin cytoskeleton by binding ezrin both directly and indirectly via its PDZ domains and it plays as a scaffolding protein to allow complex formation for cellular shape and also in changing rates of regulated endocytosis and/or exocytosis (73). The syndecan-2, a family of heparan sulfate proteoglycans which is considered glue in the adhesion of cells to the extracellular matrix, is known to associate with the actin cytoskeleton. It probably transducing signals from the extracellular matrix and it was observed that the direct association between the amino-terminal domain of ezrin and the cytoplasmic domain of syndecan under the regulation of Rho A (74).

### Activation of ezrin

A large part of ezrin found in the cytoplasm is in its inactive form and must be activated to participate in cellular reactions. Several activation mechanisms have been proposed and confirmed. First, specific residues of the ERM protein, such as Tyr145 and Tyr353, are phosphorylated in vitro and in vivo by the epidermal growth factor (EGF) receptor (75). These sites are also phosphorylated by hepatocyte growth factor (HGF) (76). Second, serine/threonine residues are other important phosphorylating sites of ERM proteins. Dephosphorylation of these sites results in ezrin translocation from the membrane, disappearance of microvilli and disruption of the renal brush border (77). A threonine residue of C-terminal can also be phosphorylated in moesin and radixin (78,79). Binding to phosphatidylinositolinositol is another activation method. ERM can be linked to the cytoplasmic tail of membrane proteins with a single transmembrane domain. Some of these reactions are more easily developed under the phosphatidylinositol 4, 5-bisphosphate (PIP 2) existing condition (80,81,82). This means that PIP2 may be involved in ERM activation and ERM proteins can directly interact with PIP2 containing phospholipids layers. Cytoplasmic domains of CD44 can bind to full length ezrin through the phosphatidylinositol phosphate (PIP) or phosphatidylinositols 4, 5-bisphosphate (PIP2) in vitro (80,83,84). Using internal deletions and COOH-terminal truncations of ezrin, it was confirmed that ezrin contains two PIP2 binding sites on amino acids 12–115 and 233–310 at the N-terminal (85). Therefore, PIP2 is involved in ezrin activation.

### Ezrin as a signal transducer

It is thought that the Rho pathway is one of the main mechanisms of signal transduction for activation of ERM proteins. Rho, a family of small GTP-binding proteins within the Ras superfamily is activated by various internal or external factors. The small GTPases of the Rho family, like RhoA, Rac1, and...
CDC42, act as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state. These Rho GTPases are under the control of guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). GEFs catalyze the conversion to the GTP-bound state and GAPs accelerate the inverse reaction (86, 87). Rho guanine nucleotide dissociation inhibitors (Rho GDI) are also involved in this process. Rho protein causes dormant ERM proteins to become active via phosphatidylinositol-4-phosphate 5-kinase (PIP5K), which increases the amount of phosphatidylinositol (4, 5)-bisphosphate (PIP2). As a result, increased PIP2 binds to N-terminal of ERM proteins, promoting the unfolding of latent ERM proteins and exposure of protein interacting sites (88, 89). Intersteringly, activated ERM proteins participate in activation of Rho pathway. The binding of ERM proteins to Rho dissociation inhibitor (RhoGDI) which is strong negative regulator of Rho activation leads to release of Rho from the RhoGDI complex, after then inactive Rho is activated by GTP (90). P13K/AKT pathway is one of main signal pathway of ERM proteins. Akt is a key factor for cell survival because it takes receives signals from the outside of the cell for the first time and transfers them to the intracellular system. This process involves regulating protein synthesis, apoptotic process, proliferation, glucose metabolism and a lot of other functions needed for cell survival. Akt is activated by phosphoinositide-3 kinase (PI3K) activity, because Akt requires the formation of the phosphatidylinositol 3, 4, 5 trisphosphate (PIP3) molecule in order to be translocated to the cell membrane. With PIP3, Akt can be phosphorylated by another kinase called phosphoinositide dependent protein kinase 1 (PDK1). Class I P13K, among the three different classes of P13Ks, convert phosphatidylinositol 3, 4 bisphosphate (PIP2) to PIP3 which activates Akt. Class I P13Ks are composed of a catalytic subunit known as p110 and a regulatory subunit either related to p85 or p101. The p85 subunits contain src homology 2 (SH2) domain and src homology 3 (SH3) domains (91). In experiments with a kidney-derived epithelial cell line, LLC-PK1, ezrin was found to contain two binding sites for the C-terminal SH2 domain of p85 at the 309 amino acid of amino-terminal domain and the phosphorylated Tyr-353 residue. Through this process, up regulated P13-kinase induces Akt activation (92). Therefore, ezrin is thought to participate in a parts of cellular signal transduction. The small GTPases of the Rho family act as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state, a process that is regulated by guanine nucleotide exchange factor (GEF) and GTPase activating protein (GAP). GEF catalyze the conversion to the GTP-bound state and GAP accelerates the intrinsic rate of hydrolysis of bound GTP to GDP. Rho associated protein kinase (ROCK) can also phosphorylate the sodium–hydrogen exchanger. Sodium-hydrogen exchanger 1 (NHE1), on the other hand, interacts with ezrin-radixin-moesin(ERM) proteins. ERM Proteins can also be activated by Rho via phosphatidylinositol-4-phosphate 5-kinase (PIP5K). Both Rac and Rho bind to and activate PIP5K, which increases the amount of phosphatidylinositol (4, 5)-bisphosphate (PIP2), PIP2 then activates ERM proteins by inhibiting their interdomain interaction, which allows phosphorylation of their carboxy-terminal threonine by some kinases. Threonine-phosphorylated carboxy-terminal of ERM proteins are stable in their activated forms. It functions as an actin filament/plasma membrane cross-linker to form microvilli. Activated ERM proteins are associated directly with adhesion molecules such as CD44 and intercellular adhesion molecule-1,-2(ICAM-1, -2), and indirectly with other integral membrane proteins such as NHE3 through ERM – binding phosphoprotein 50(EBP50)/sodium-hydrogen exchanger regulatory factor (NHERF).

**Importance of ezrin in metastatic behavior of non estrogen dependent tumors**

In general, for the successful metastasis, cancer cells have to break the linkages between neighboring cells and/or between the cells and the matrix, penetrate endothelium, migrate to other sites without mishap and successfully attach to new sites. Ezrin plays specific roles in the adherent junction between transmembranous protein and cytoskeleton within cell and between cells, and act as a signal transducer. In this way, Ezrin is involved in the regulation of phenotypical change of cellular architecture, controlling cellular motility, tethering action between cell to cell and cell to matrix, recognizing and conducting internal and/or external signal. All of these cellular functions are essential for maintaining homeostasis of normal cell as well as entire process of tumorigenesis and distant metastasis (93,94). There are many reports strongly suggest that ezrin can relevant to metastasis of cancer cells. The overexpression of ezrin gene was observed in murine and human osteosarcoma (OSA) cell lines by using...
cDNA microarray and Northern analysis, respectively(95). Furthermore, ezrin was relatively more expressed in highly metastatic murine rhabdomyosarcoma and OSA cell lines than in the cell lines with low metastatic potential. By imaging metastatic cells in the lungs of mice, they observed that ezrin expression provided an early survival advantage for cancer cells that reached the lung, and also observed positive correlation between high levels of ezrin expression and advancing stages of both tumors(96,97). High levels of ezrin mRNA and protein were observed in pancreatic adenocarcinoma cell lines with high metastatic potential (98) and membranous translocation of ezrin might play a role during malignant transformation and disease progression. Pancreatic cystic neoplasms and ductal adenocarcinoma (99). In prostatic cancer study, ezrin was overexpressed in high-grade prostatic intraepithelial neoplasia and prostate cancer compared with adjacent benign prostatic epithelium, and ezrin was expressed in the majority of prostate cancers and correlated with adverse prognostic factors in another study (100,101). In immunocytochemical study for the ezrin in human astrocytes, the intensity of ezrin-staining was strongly and significantly correlated with increasing tumor grades. Ezrin-IR permitted a clear distinction between benign grade II astrocytic tumors and anaplastic grade III astrocytomas. Malignant, grade III and IV astrocytic tumors, showed cytoplasmic localization of ezrin and clearly increased staining intensity compared with benign grade II astrocytomas (102). The presence of ezrin immunoreactivity in uveal malignant melanoma is associated with higher mortality, and the positive staining of ezrin was significantly associated with high microvascular density and presence of number of macrophages. Both of them are crucial factors for metastasis of cancer cell (103). In addition to this, high levels of Ezrin expression is obviously observed in colorectal cancer tissues compared with normal colorectal mucosa, and its level is closely related to the colorectal cancer invasion and metastasis process (104). The expression of ezrin was typically observed in the cytoplasm of the colorectal cancer cells, but it was predominantly localized at the membranous portion of normal colorectal epithelium. The expression was more intense in colon than in rectal carcinomas and increased ezrin expression was associated with adverse outcome (105). In human lung cancer cell, up-regulated ezrin was observed in lung squamous carcinoma cell and in cells of bone metastatic sites (106). Recently, using tissue microarray, ezrin expression was found in most cancers and normal tissues at varying levels of intensity. Specifically ezrin was expressed at higher levels in sarcomas than in carcinomas. By normalizing the expression of ezrin in each cancer using ezrin expression found in the corresponding normal tissue, significant associations between ezrin and advancing histological grade in sarcomas and poor outcome in breast were found (107).

Estrogen and ezrin

It is known that estrogen in particular regulates ezrin expression on the genomic basis and controls functional activity via effects on the synthesis of co-activating proteins. The first evidence of the regulation of ezrin by estrogen (estradiol-17β (E2)) was demonstrated in pituitary GH3 cells by Smith et al. They observed that E2 stimulates ezrin gene expression by using a cDNA expression array and confirmed that ezrin gene expression is regulated pretranslationally by ICI 182780. They also found that E2 elevated ezrin protein levels in whole-cell lysates and in the cytoskeletal-associated, detergent-insoluble fraction. Confocal microscopy revealed that ezrin was associated with free apical membranes of E2-treated cells (108). Using differential display RNA methods, Ediger et al. have identified the human homolog of the NHERF as being under rapid and direct regulation by estrogen in estrogen receptor (ER)-containing breast cancer cells (109). Recently, it was reported that there exist tissue specificity in expression of ezrin and EBP50 by estrogen. Another study noted that ezrin and EBP50 expression is coordinately increased by E2 in GH3 cells and rat pituitary glands. Ezrin levels are repressed by the steroidal antiestrogen, and reversed by E2 and the ERα-specific agonist in GH3 cells, but EBP50 levels remained constant during these treatments. Ezrin and EBP50 did not display extensive colocalization. In juvenile female rats, E2 injections increased ezrin expression in the pituitary and uterus, but increased EBP50 expression was observed only in the uterus (110). Another study revealed that NHERF expression was markedly increased in the cytoplasm, luminal membrane of glandular epithelium and stromal cells of proliferative endometrium, but only weakly expressed in the secretory endometrium. Furthermore, estrogen receptor status and NHERF expression correlates closely in breast carcinoma specimens (111). Therefore, estrogen is involved with ezrin gene expression and activation directly or indirectly. It also affects where ezrin is located in the cell. The ezrin controlled by estrogen also could be involved in the process of metastasis via various mechanisms such
as, influencing intercellular or intracellular linkers, signal transducers, oncogene activation and suppression of tumor suppression genes. As a result of this relationship between estrogen and ezrin, ezrin, in part, can act critical role in metastasis of estrogen dependent tumors and estrogen also participate in the process via regulating ezrin.

**Ezrin and metastatic behavior of breast cancer**

In the study for ezrin expression in human cancer and normal tissue using tissue microassay, it was conformed that Ezrin is highly expressed in breast cancer tissues(112). Recent paper provides strong evidence that ezrin is required for the metastasis of breast cancer cells. They used pcB6 vector that cDNA of either wild-type ezrin or its NH2-terminal domain (amino acids 1–309constructs coding) fused at the COOH terminus to oligonucleotides encoding the 11–amino acid COOH terminus of the VSVG, which allowed discrimination between the transfected and endogenous ezrins. In this study, they observed that highly metastatic cancer cell lines with or without overexpressing wild-type ezrin were also strongly metastatic, but in amino-terminal ezrin injected mice groups showed a marked reduction in metastases. Moreover, they found that overexpression of wild-type ezrin does not augment metastasis of parental cell lines. Furthermore, no increase in expression of endogenous ezrin was observed in the metastatic cells compared with the low potential metastatic parental cell lines. These findings imply that overexpression of ezrin alone is not sufficient to induce metastasis in this tumour model, suggesting that multiple pathways are involved in the metastatic cascade(113). In another study, the abnormal expression rates of Ezrin were significantly higher in the lymphatic metastasis of invasive ductal breast cancer than in the cases without metastasis, and ezrin can be closely related to invasion and metastasis of ductal breast cancer(114). In one study for ezrin localization in the breast cancer cell and breast carcinoma tissues, ezrin was concentrated at the apical surface in Estrogen receptor (ER)-positive, non-invasive and non-tumorigenic cell lines, whereas ezrin was localized in motile structures (membrane ruffles and filopodia) and had more diffuse cytoplasmic staining in invasive cell lines. In tissues, Ezrin staining in normal breast epithelium localized at the apical, whereas, in most breast tumor cases, it localized in the cytoplasm or membranous staining. There were significant positive associations between cytoplasmic and/or membranous ezrin localization and adverse tumor characteristics such as high grade, high level of Ki-67 expression, hormonal-receptor negativity, and lymph-node metastases. Apical ezrin staining was associated with favorable clinicopathological features and node-negative tumors (115). Another strong compelling evidence for a metastasis promoting function of ezrin was reported in breast cancer cell lines. Li Q et al. observed that up-regulated ezrin expression was and its translocation from cell membrane to cytoplasm, whereas E-cadherin expression decreased in highly metastatic human breast cancer cell lines and in the lymphatic metastases cells. After using ezrin small hairpin RNAs (ezrin shRNAs) to block ezrin in this breast cancer cells with the highest levels of ezrin mRNA and protein, the abilities of cell motility and invasiveness were obviously inhibited. The levels of E-cadherin expression was increased and the levels of phosphorylation of beta-catenin was decreased by inhibiting phosphorylation levels of c-src, and decreased ezrin expression by shRNA reversed metastatic behaviors of human breast cancer cells by inducing c-src-mediated E-cadherin expression. This may suggest that ezrin can a something role in the lymphatic metastasis of breast cancer(116). Therefore, on the basis of above studies, ezrin expression may have potential values as a determinant of metastasis of breast cancer and its cellular translocation from apical to cytoplasm or membranous portion may be a strongly suggesting marker for metastasis.

**Ezrin and metastatic behavior of ovarian cancer**

Since it was first reported that ezrin is implicated in the invasion of endometrial cancer cells(117), many researchers have continued to examine the role of ezrin in the tumor invasion of many other cancer cells. In one study of ezrin expression and translocation in ovarian cancer, investigators observed the changes of ezrin distribution in the cells and its phenotypical change after IL-1α or EGF treatment in a normal ovary, primary epithelial ovarian carcinoma (OVCA), metastatic OVCA tissue and three different ovarian cancer cell lines. They confirmed that ezrin expression is increased in metastatic OVCA and ascites cells, primary OVCA, and normal ovaries. The highest levels of ezrin were observed in the metastatic tissue and cells. In the IL-1α and EGF untreated SKOV3 cells, the cells were smooth and rounded without membrane ruffling, had very few protrusions, and ezrin was distributed evenly in cytoplasm and along the margin
of the cells. However, IL-1α and EGF also enhanced OVAC cell proliferation and induced ezrin translocation, tyrosine phosphorylation, process formation, and membrane ruffling in SKOV3 cells. Nevertheless, all of these effects induced by IL-1α and EGF were abolished by Genistein, a specific protein tyrosine kinase (PTK) inhibitor(35). Another experimental study was conducted to evaluate the reciprocal action of estrogen and ezrin on ovarian cancer cells. The researchers administrated 17β estradiol (E2) to SKOV3 (estrogen receptor α dominant) and DOV13 OVCA (estrogen receptor β dominant) cell lines. They found that E2 induced ezrin over-expression and an invasive phenotype where ezrin was translocated to cell edges, pseudopodia and membrane ruffles. E2, in a dose-related manner, also increased cell number and enhanced OVCA cell proliferation, motility and increased its ability to penetrate Matrigel. In addition to these effects, they observed that there exists a clear positive correlation between ezrin expression and matrigel penetration ability. However, raloxifene or tamoxifen, an estrogen receptor blocker, blocked all of these estrogenic effects. Therefore, these experiments indicate that the effects of estrogen on OVCA growth and phenotypic changes are in part due to the induction of ezrin over-expression, a process estrogen receptors are also strongly involved in(118). Since ezrin expression is increased with cancerous cellular severity, its location moves to membranous portion and it is accompanied by proper phenotypical changes that allow for successful metastasis, ezrin levels and its location could be used to monitor the process of metastasis.

**Ezrin and metastatic behavior of endometrial cancer**

Although the ezrin encoding gene is up regulated in many estrogen dependent tumors, until recently there has been no precise evidence that ezrin is involved in tumor progression or invasion of commonly estrogen dependent gynecologic tumors. Only recently was a study published that reported that ezrin contributes to at least the migration and invasiveness of endometrial cancer cells. The researchers investigated ezrin expression and its invasive ability in two human endometrial cancer cell lines (Ishikawa, low-metastatic endometrial cancer cell line, and its subclone (mEIIL) with high-metastatic activity and higher ezrin expression). Using the matrigel invasion assay, they estimated the change of cellular invasiveness after treating cells with ezrin antisense phosphorothioate oligonucleotids (ePONs) for the purpose of blocking ezrin, moesin, and other related proteins such as, spectrin, α-catenin, even though only ezrin was selectively affected by ePONs. In both groups, matrigel penetrated cells were significantly decreased after treatment with ePONs, but cell proliferation was not affected and ezrin expression was inhibited by ePONs at the protein level. mEIIL cell lines with high ezrin expression were better able to migrate through the Matrigel membrane compared with Ishikawa cell lines(117). In addition, another study provided direct evidence of ezrin expression in normal human endometrial tissues and in endometrial adenocarcinoma. It evaluated the expression of ezrin according to the series of development of endometrioid adenocarcinoma progression, from normal to hyperplasia and adenocarcinoma, involving metastatic tumor. Ezrin expression was progressively increased according to aggressive disease progression. Ezrin was expressed abundantly in atypical endometrial hyperplasia and adenocarcinoma compared with normal endometrium, simple endometrial hyperplasia and complex endometrial hyperplasia. Furthermore, ezrin was expressed significantly more in metastatic lesions compared with primary lesions. These results are consistent with a previous report that ezrin expression was higher in strong metastatic potential cell lines than in weak metastatic potential endometrial cancer cell lines. Cell distribution of ezrin was analyzed by immunohistochemistry. Ezrin was localized in the membranous portions of metastasized cancer cells, whereas ezrin was mainly distributed in the cytoplasm of most cancer cells and some endometrial hyperplasia cells. Similarly, western blots showed that ezrin was distributed throughout the membranous portion more in cancer cells than in hyperplasia. Membranous distribution of ezrin also was observed in all the metastatic lesions (25). These results are consistent with a previous cellular based study; the movement of ezrin from the cytoplasm to the membranous portion, such as microvilli, membrane ruffles, cellular extension and foot processes is commonly observed in the highly metastatic cancer cell and is thought to play an important role in the early stages of tumor invasion(119,120). In addition to contributing to metastatic behavior of cancer cells, ezrin expression is also relevant to the prognosis of cancer. A recent report showed that ezrin expression is related to poor prognosis in FIGO stage I endometrioid carcinomas. In this study, they also observed a shift of ezrin expression from the apical membrane to cytoplasmic distribution in carcinomas when compared with normal endometrium. The cytoplasmic distribution of ezrin was also observed in
free floating cells detached from papillae and in cells at the tumor–host interface. This ezrin shift seemed to loosen the cell to cell adhesion. The study also noted that strong ezrin expression was correlated with reduced overall survival in univariate survival analysis(37). A similar finding was observed in another recent study. Researchers identified ezrin expression on the basal, lateral an apical aspect of the epithelial cells in normal tissue, but ezrin was patchy and uniformly distributed in the cytoplasm of cancer cells(38). This may mean that the anchoring or tethering effects of ezrin is decreased in cancer cells, specifically in the apical portion, which may promote movement of cancer cells from their original sites to other sites. All of these findings support that ezrin involves in the process of cancer metastasis. Therefore, monitoring the level of ezrin in cancer cells may help predict whether or not the endometrial cancer will spread.

Conclusion

Ezrin was at first known as a small 81kDa cell surface protein, but now it has become one of the most focused molecules for the parts of metastatic behavior of cancer cells. A lot of evidences exist that high levels of ezrin expression strongly suggest metastatic behavior of variable animal cancer cells and human cancer cells. Furthermore, high levels of ezrin expression are more observed in high potential metastatic cancer cell than low potential metastatic cancer cells. The reasons that ezrin has being focused molecule are its versatile abilities, such as enhancing cellular invasiveness, controlling other tethering molecules and activation of metastasis relating proteins. In addition, it is predisposed to move from the apex to the cytoplasmic and/or membranous portion more in cancer cells than in normal cells. These facts imply that ezrin may play a role in cancer progression. Since ezrin expression is controlled by estrogen, especially, it has become one of the main focusing molecules related to metastasis of breast cancer and gynecologic cancer. Until now, all the reporting data for the correlation between ezrin and metastasis strongly imply that ezrin may play a pivotal role in distant metastasis and their survival in common estrogen dependent cancer cells. It was already reported that ezrin expression is related to poor prognosis in low stage ovarian and endometrial carcinoma. In breast cancer, RNA interference (RNAi) using ezrin small hairpinRNAs(ezrin shRNAs) lead to decrease the abilities of cell motility and invasiveness, and ezrin anti-sense made decreased matrigel membrane penetration by endometrial cancer. Recently, decreased invasiveness of cancer cells were reported in ovarian cancer, prostate and hepatocellular cells by using small interfering RNA (siRNA). Furthermore, Anti-estrogen decreased the invasiveness of ovarian cancer cells via decreased levels of ezrin. All these data imply that anti-ezrin agents can be as a new treatment modality for estrogen dependent tumors. Given that there are limited reports that evaluated the roles of ezrin in breast and gynecologic cancer, further studies are needed to confirm the precise roles of ezrin in the process of breast cancer and gynecologic cancer metastasis. Nevertheless, it is likely that ezrin levels and its cellular position will begin to be used as a metastatic marker and novel treatment modality for common estrogen dependent tumors.

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