

Review Article

Epithelial protein lost in neoplasm (EPLIN) and prostate cancer: lessons learned from the ARCaP model

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Abstract: Confucius said, “Good tools are prerequisite to the successful execution of a job”. Among his many groundbreaking achievements, Dr. Leland W. K. Chung established several widely used prostate cancer (PCa) cell lines, including C4-2, C4-2B, and ARCaP. These cellular models have been pivotal tools to enhance our understanding of the biology of PCa progression and assist in the discovery of new strategies to treat metastatic, castration-resistant PCa. Recent studies in the ARCaP PCa progression model uncovered epithelial protein lost in neoplasm (EPLIN), an actin-binding protein with an indispensable role in the maintenance of epithelial structures, as a negative regulator of epithelial-mesenchymal transition. Clinical evidence further supports the potential role of EPLIN in controlling metastasis in PCa and other solid tumors. In this article, we review the current understanding of the biology of EPLIN and the ARCaP model in the discovery of new agents for the prevention and treatment of PCa metastasis.

Keywords: EPLIN, EMT, PCa, metastasis, chemoresistance

Introduction

This article is a part of a special issue of the AJCEU dedicated to Dr. Leland W. K. Chung. The selection of the ARCaP epithelial-mesenchymal transition (EMT) model for this article is particularly poignant because the story of its discovery was one of Dr. Chung’s favorites, and in our opinion, a prime example of his brilliant and unconventional thinking. The original ARCaP cells were actually an unexpected by-product from an effort to collect soluble factors in ascites fluid. “When we passed by the sink and saw several centrifuge tubes containing the precipitates, a thought hit us”, recalled Dr. Chung, with a satisfactory smile. “Why not give it a try”? Thus was the first step to isolating the unique ARCaP cells. Dr. Chung’s team further established ARCaP variants with distinct metastatic capabilities and tissue preference. Among them, ARCaP_E and ARCaP_M have been the most widely used for their representative

EMT and neuroendocrine characteristics. To this day, the treasure box of ARCaP cells as a new tool in prostate cancer research remains an area for exploration.

Prostate cancer metastasis and EMT

At the cellular and molecular levels, prostate cancer (PCa) metastasis can be driven by a combination of hereditary and somatic gene alterations in a variety of signaling pathways [1-3]. EMT is a major mechanism by which epithelial cells acquire plasticity and stemness [4-11]. Complete conversion to a mesenchymal phenotype can lead to enhanced migratory and invasive capacities and allow cancer cells to migrate away from their original epithelial layer. Therefore, EMT may play a critical role in the initial stages of metastasis [12, 13]. Understanding the biological alterations during EMT could provide insight into the mechanisms underlying PCa metastasis and therapeutic resistance.

ARCaP cells provide a relevant preclinical model for the study of EMT and PCa metastasis

A major limitation in the field of PCa research is that, despite numerous attempts to establish immortalized cell lines from human tumor tissues, relevant models that can closely recapitulate the diverse molecular subtypes of PCa, particularly metastatic and therapeutic-resistant diseases, are still lacking [14-16]. Among the currently available human PCa cell lines, DU145 and PC-3 have been widely used in the study of EMT and metastasis. However, both cell lines express only low levels of epithelial markers, such as E-cadherin, and do not exhibit a morphology typical of epithelial-like cells [17, 18]. In addition, although the two cell lines can form bone metastases following intracardiac injection, they mainly cause osteolytic lesions in mouse skeletons [19].

The ARCaP (androgen-repressed prostate cancer) cell line was established by Drs. Haiyen E. Zhou and Leland W. K. Chung's group from the ascites fluid of a patient with metastatic PCa [20, 21]. ARCaP cells are tumorigenic with 100% incidence in intact or castrated athymic male mice. When the cells were implanted orthotopically, metastases were observed in multiple organs, including lymph nodes, lung, kidney, pancreas, liver, and bones. Unlike DU145 or PC-3, ARCaP cells elicit both osteoblastic and osteolytic responses in mouse bones, mimicking a distinct pathological feature of PCa metastasis [22]. Drs. Zhou and Chung's team further established multiple ARCaP sublines from metastatic tissues following the intracardiac injection of parental ARCaP cells in athymic nude mice. These subclones exhibit similar cytogenetic patterns but distinct morphology; they also differ in their growth rates, migratory, invasive, and metastatic potentials, and drug sensitivity. Similar to the establishment of C4-2 and C4-2B lines, the isolation and identification of multiple ARCaP sublines from metastatic tissues highlighted an important role of tumor-microenvironment interaction in the acquisition of tissue tropism in metastatic PCa cells. Two sublines, i.e., ARCaP_E and ARCaP_{M'}, display the most striking differences. ARCaP_E cells display typical epithelial-like morphology and have a relatively low metastatic propensity (12.5%) after intracardi-

ac injection. The lineage-derived ARCaP_M cells recovered from mouse bone have spindle-shaped fibroblastic morphology associated with increased expression of vimentin and reduced expression of epithelial markers. Importantly, the switch in morphology and gene expression in ARCaP_M cells is associated with increased metastatic propensity to skeletal (100%) and soft tissues [23]. ARCaP_E/ARCaP_M cells provide an excellent model that can undergo spontaneous and predictable bone metastasis *in vivo* and exhibit the molecular and behavioral characteristics of EMT *in vitro*. Since the first publication in 2006 describing the two cell lines, at least 40 published studies used ARCaP_E/ARCaP_M cells and identified several novel factors involved in the control of EMT, cellular plasticity, and metastasis [8, 24-31]. In this review, we discuss epithelial protein lost in neoplasm (EPLIN) and its potential function in EMT, tumor progression and therapeutic resistance.

Biological function of EPLIN

EPLIN was initially identified as a cytoskeletal protein in epithelial cells [32]. The human EPLIN gene is located on chromosome 12q13.12. Two EPLIN isoforms, the 600-amino-acid EPLIN- α and 759-amino-acid EPLIN- β , are generated from alternative RNA processing: the β isoform consists of all 11 exons, and the EPLIN- α mRNA consists of exons 4-11 [33]. Both EPLIN isoforms contain a single, centrally located LIM domain, allowing the protein to dimerize with itself or associate with other proteins. At least two actin-binding domains are present at both the amino- and carboxyl-termini, which allow EPLIN to effectively cross-link and bundle actin filaments and lower the monomer dissociation rate constant at the pointed end of actin filaments and stabilize them from depolymerization. Additionally, EPLIN- α can inhibit Arp2/3 complex-mediated actin nucleation by increasing the lag at the outset of polymerization. Therefore, the inhibitory effect of EPLIN on reorganization of the actin cytoskeleton can be two-fold, i.e., affecting both assembly (nucleation) and disassembly (stability) of filamentous actin [34, 35].

EPLIN plays an indispensable role in the maintenance of epithelial phenotypes, directly binding α -catenin and connecting the E-Cadherin/ β -

Catenin- α -Catenin complex and the actin filaments in the epithelium. These physical connections are critical to stabilization of the circumferential actin belt at adherens junctions (AJs) and regulation of apical-basal polarity in epithelial cells, including mechanosensitivity [36, 37]. In vascular endothelium, EPLIN exerts a similar function by directly interacting with α -catenin anchored to the endothelial-specific VE-cadherin- β -catenin complex and promoting vinculin junctional recruitment, thereby reinforcing the cohesion of cell-cell junctions and stabilizing the capillary structures [38-40]. Interestingly, it appears that EPLIN- β is highly expressed in aortic and shear stress-loaded endothelial cells to induce and stabilize stress fibers, whereas EPLIN- α is increased in growing and migrating cells to control protrusion dynamics of actin-driven membrane protrusions, specifically classical lamellipodia (cLP) and junction-associated intermittent lamellipodia (JAIL). The distinct functions of EPLIN isoforms in the fine-tuning of actin dynamics are consistent with a lower EPLIN- β turnover rate compared to EPLIN- α [41].

EPLIN depletion induces cytokinesis failure and formation of multinucleation and aneuploidy [42], suggesting that EPLIN may be involved in the regulation of cytokinesis and genomic stability. During membrane ingression and cleavage furrow formation, EPLIN interacts with at least two cytoskeletal systems, i.e., the actin-myosin II contractile ring and septin filaments, for a complete cytokinesis. EPLIN is required for the recruitment of RhoA, Cdc42, and ACAT-related protein required for viability 1 (Arv1) to the cleavage furrow. Although both EPLIN- α and - β localize to the cleavage furrow during cytokinesis, EPLIN- β may have a stronger interaction with Arv1 via the amino terminus [43].

Zhang et al. recently reported an unexpected role of EPLIN in lipid regulation. A causal single-nucleotide variant of the EPLIN gene was identified in a remote Chinese family with inherited low low-density lipoprotein cholesterol (LDL-C) and reduced cholesterol absorption. In a mouse model, EPLIN protein is highly expressed in the brush border of the small intestine and binds two proteins essential for cholesterol absorption, i.e., NPC1-like intracellular cholesterol transporter 1 (NPC1L1) and myosin Vb. EPLIN knockout mice displayed reduced cholesterol absorption and low LDL-C levels [44, 45].

The evolving role of EPLIN in cancer progression

EPLIN was initially identified as a protein that is highly expressed in normal epithelial cells but its expression is frequently reduced in human cancer cells and primary tumors. Ectopic overexpression of EPLIN- α or - β suppresses the *in vitro* and *in vivo* growth of certain cancer cell lines, including MDA-MB-231 and PC-3 [32, 46-48]. These observations suggested that EPLIN may serve as a tumor suppressor and play a role in the early stages of tumor progression, such as tumorigenesis and primary tumor growth. The underlying mechanism of EPLIN as a tumor suppressor remains largely unknown. Recent work from the Fujita group showed that EPLIN may be directly involved in a cancer preventive process, i.e., the apical elimination of transformed cells from the epithelial layer. In RasV12-transformed cells, EPLIN is upregulated and interacts with caveolin-1, plectin and paxillin, thereby promoting microtubule acetylation and facilitating the apical extrusion of RasV12 cells [49, 50].

To gain unbiased insight into the molecular mechanisms underlying PCa EMT, we performed quantitative proteomic analyses to profile protein expression patterns in the ARCaP EMT model. Among 76 unique proteins differentially expressed in ARCaP_E and ARCaP_M cells, EPLIN- β was found to be markedly reduced in ARCaP_M cells. Consistently, immunohistochemical expression of EPLIN was high in ARCaP_E tumors but significantly reduced in ARCaP_M tumors. These data suggested a potential connection between EPLIN, EMT and invasive phenotypes in PCa cells. A similar correlation between EPLIN expression and invasiveness was observed in other experimental models of PCa and squamous cell carcinoma of the head and neck (SCCHN) [11].

To determine the function of EPLIN in PCa EMT and invasiveness, we established ARCaP_E cells with temporary or permanent knockdown of EPLIN, which demonstrated spindle-shaped and mesenchymal-like morphology. EPLIN depletion caused the disassembly of cellular stress fibers with a concomitant gain of actin foci and formation of prominent membrane ruffles. These data indicated that the loss of EPLIN expression may allow PCa cells to undergo dynamic remodeling of the actin cytoskele-

ton and to acquire migratory and invasive capabilities. EPLIN knockdown led to similar phenotypic changes in other cancer lines, such as LNCaP, PC-3, and MCF-7. Taken together, these results provided the first observation that EPLIN may be a negative regulator of EMT and invasiveness in human cancer cells [11]. This hypothesis was further supported by several studies from other groups [48, 51-56].

At the molecular level, EPLIN regulates a subset of genes involved in EMT, stemness, actin cytoskeleton remodeling, invasion and metastasis, adhesion and extracellular matrix remodeling, and growth factor signaling. EPLIN depletion in ARCaP_E cells led to a remarkable increase in the subpopulation carrying the CD44^{high}/CD24^{negative} marker [11]. In contrast to previous observations that EPLIN depletion usually resulted in increased proliferation in certain human cancer cell lines [32, 46-48], EPLIN shRNA-expressing ARCaP_E cells (ARCaP_E-shEPLIN) have slower *in vitro* proliferation and *in vivo* tumor growth than the control cells (ARCaP_E-shCtrl) ([11] and unpublished results). Another unexpected consequence of EPLIN depletion is that ARCaP-shEPLIN cells display significantly higher resistance to chemotherapeutics, including docetaxel and doxorubicin (by ~8-fold and ~4.4-fold, respectively) [11]. These results indicated that in addition to acting as a tumor suppressor, EPLIN may also play an important role in the acquisition of stemness and therapeutic resistance.

Expression of EPLIN in human cancers

In the first study of EPLIN expression in human cancer specimens, the Jiang group found that compared with normal tissues, EPLIN- α expression is reduced in low-grade breast cancer, and is further decreased in high-grade tumors. EPLIN- α downregulation is associated with poor prognosis and increased incidence of recurrence and mortality [46]. Subsequent studies observed a similar expression pattern of EPLIN- α in other cancer types [48, 54]. Reduced EPLIN- α expression is associated with local advanced esophageal cancer as well as lymphatic metastasis [48]. EPLIN transcripts are decreased in colon, lung and breast cancers compared with corresponding normal tissues [53]. In gastric cancer, EPLIN expression was significantly lower in the T3 + T4 stage

group than in the combined T1 + T2 stage group and could be an independent prognostic factor of disease-free survival and overall survival. EPLIN expression is also significantly associated with responsiveness to neoadjuvant chemotherapy [57]. In a cohort of oral tongue squamous cell carcinoma (OTSCC) patients, high expression of EPLIN protein was significantly associated with longer survival [58]. Low EPLIN expression in melanoma was correlated with increased invasion and metastases [51].

We explored the clinical significance of EPLIN in human cancer progression. In a published study, analyses of four independent sets of microarray data on clinical PCa revealed that EPLIN transcripts were expressed at a similar level in primary tumors and normal prostatic tissues, but were remarkably reduced in metastatic tumors [11]. We further analyzed the expression of EPLIN transcripts using PCa Transcriptome Atlas (PCTA), a database comprised of 1,321 clinical specimens from 38 PCa cohorts [59]. EPLIN expression in primary tumors was significantly reduced when compared with that of benign prostatic glands (fold change = -0.142; $P < 0.001$), and further reduced in metastatic, castration-resistant PCa (fold change = -0.357; $P < 0.001$) (**Figure 1**).

Immunohistochemistry (IHC) expression of EPLIN was evaluated in matched pairs of tumor tissue specimens from primary tumors and lymph node metastases from PCa, breast cancer, colorectal cancer and SCCHN. EPLIN expression was markedly reduced in lymph node metastases compared to their matched primary tumors [11]. In another study, EPLIN expression was evaluated in a prostate tissue microarray (TMA) composed of 69 primary PCa cases with variable Gleason scores (grade groups) and 10 cases composed of benign prostatic glands and stroma (**Figure 2A**). EPLIN protein was highly expressed in all the examined benign prostate tissues (60.0% 3+, 40.0% 2+). The overall positive rate of EPLIN was 94.2% in cancer tissues (43.5% 3+, 23.2% 2+, 27.5% 1+). The IHC score for EPLIN expression in high-grade tumors (Gleason scores 8-10/grade groups 4-5; 1.82 ± 0.15) was significantly lower than in benign prostatic glands (2.6 ± 0.16 ; $P = 0.02$) or low-grade tumors (Gleason score ≤ 7 /grade groups 1-2; $2.43 \pm$

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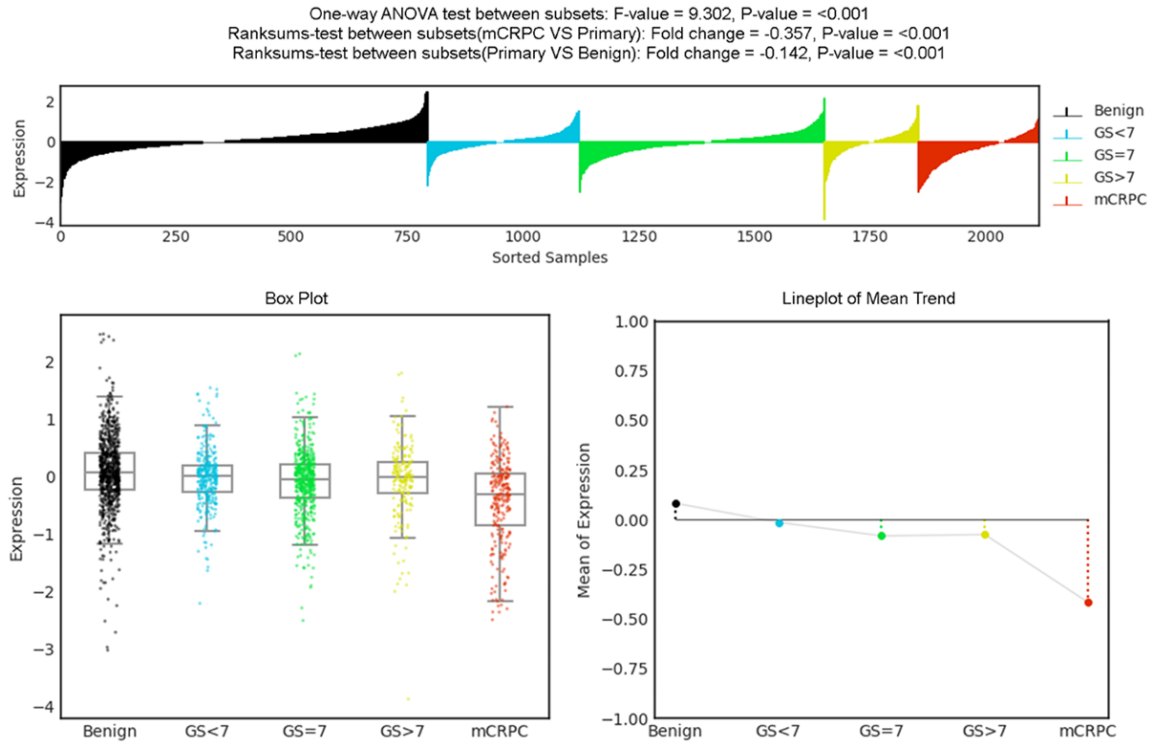


Figure 1. EPLIN transcript profile in benign prostatic glands, primary PCA and metastatic, castration-resistant PCA.

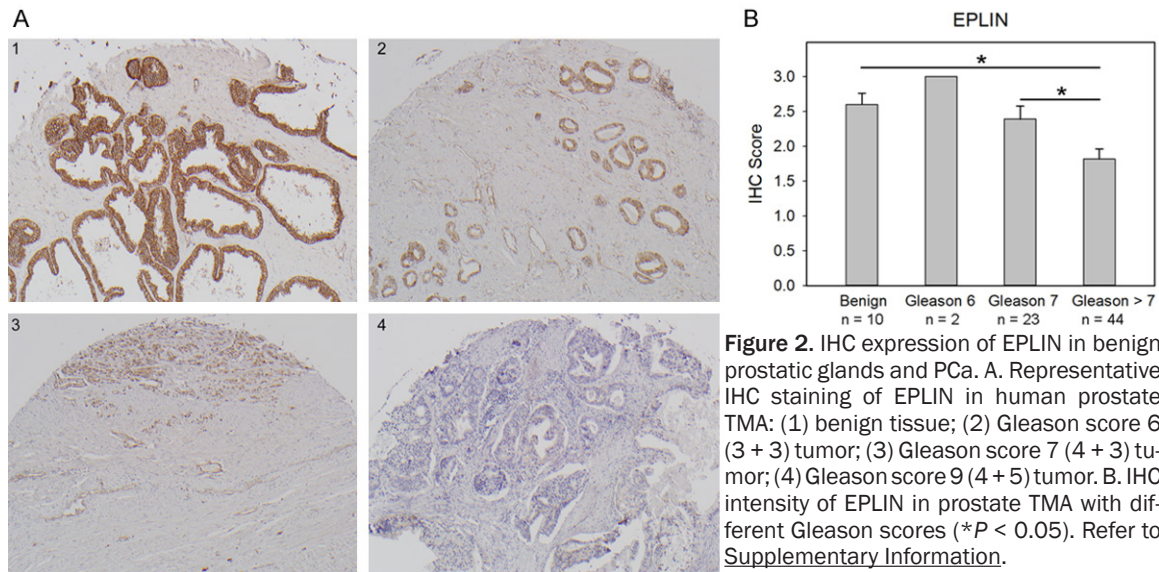


Figure 2. IHC expression of EPLIN in benign prostatic glands and PCA. A. Representative IHC staining of EPLIN in human prostate TMA: (1) benign tissue; (2) Gleason score 6 (3 + 3) tumor; (3) Gleason score 7 (4 + 3) tumor; (4) Gleason score 9 (4 + 5) tumor. B. IHC intensity of EPLIN in prostate TMA with different Gleason scores (*P < 0.05). Refer to [Supplementary Information](#).

0.19; $P = 0.01$). There was no statistically significant difference between EPLIN expression in benign prostatic glands and low-grade PCA ($P = 0.59$) (**Figure 2B**). Collectively, EPLIN expression at both the RNA and protein levels is downregulated in PCA, and its reduction is further associated with high-grade and metastatic, castration-resistant PCA. These results

suggested that EPLIN downregulation may serve as a predictor of tumor aggressiveness and poor prognosis in PCA patients.

EPLIN signaling in human cancer cells

The underlying mechanisms by which EPLIN regulates the proliferation, migration, and inva-

sion in cancer cells remain largely unknown. In melanoma cells, EPLIN downregulation leads to activation of insulin-like growth factor 1 receptor (IGF-1R)-Akt/signal transducer and activator of transcription 3 (Stat3) signaling, followed by an increase in Slug and consecutive downregulation of E-cadherin [51]. EPLIN is capable of regulating the expression and phosphorylation of paxillin, FAK and Src in PCa cells, thereby affecting their aggressive characteristics [56].

Our recent studies have uncovered a novel function of EPLIN in the regulation of chemoresistance. EPLIN depletion in ARCaP_E cells activates noncanonical EZH2 signaling, consisting of Stat3, S-phase protein kinase 2 (SKP2), ATP-binding cassette B1 (ABCB1, p-glycoprotein), and survivin, that allows PCa cells to evade chemotherapeutics. C4-2B-TaxR cells, a highly docetaxel-resistant PCa mimicking acquired chemoresistance [60], appear to activate this common pathway for their survival. However, how EPLIN regulates EZH2 signaling is not clear.

Regulation of EPLIN expression

Several regulatory mechanisms of EPLIN transcription have been identified. A fragment within the first intron ranging from position -43,729 to -43,233 upstream of the EPLIN- α transcriptional start site may be responsible for its induction by wild-type p73 isoforms. p73-dependent EPLIN transcription can be completely inhibited by co-expression of transactivation-deficient N-terminally truncated isoforms of p73 (DNp73) [51]. Two consensus p53-binding motif sequences have been identified in the 4th intron and downstream region of the EPLIN gene, respectively. Consistently, the EPLIN mRNA level in breast and colorectal cancers containing mutant p53 is significantly reduced than that in cancers containing wild-type p53 [53]. The 3'-untranslated region (UTR) of EPLIN mRNA has the targeting sequence of the seed region of an oncogenic miRNA, miR-93-5p; EPLIN has been experimentally validated as a target of miR-93-5p and mediates the oncogenic effect of miR-93-5p in endothelial cells [61]. The human EPLIN- α promoter contains a serum response factor (SRF) binding site, and the EPLIN- β promoter has putative binding sites for Oct-1, Sp1, and AP1. Consistently, serum stimu-

lation activates SRF and induced the transcription of EPLIN- α but not EPLIN- β [33].

We reported that epidermal growth factor (EGF) promotes robust EMT in ARCaP_E cells by activating extracellular signal-regulated kinase 1/2 (ERK1/2)-dependent phosphorylation, ubiquitination, and degradation of EPLIN protein. Two serine residues (serines 362 and 604) were further identified as the ERK1/2 phosphorylation sites [62]. A later study found that hCDC14A, a key regulator of cell migration and adhesion, counteracts EGF-induced rearrangements of actin cytoskeleton by dephosphorylating EPLIN at serines 362 and 604, thereby maintaining the expression of E-cadherin and α/β -catenin at cell-cell adhesions. hCDC14A-mediated EPLIN dephosphorylation could reduce actin dynamics and restrict tumor malignancy [63].

We predicted a putative PEST sequence (RASSLSESSPPK) within the EPLIN protein that may be involved in its post-translational regulation [62]. Supporting this model, a chimeric fusion apoptosis inhibitor 2 (API2)-mucosa-associated lymphoid tissue translocation gene 1 (MALT1) protein cleaves EPLIN- α at the major cleavage site arginine 206 and at the minor cleavage site lysine 289, thereby disrupting the tumor suppressor function of EPLIN- α in B cells. Intriguingly, API2-MALT1 paracaspase-mediated EPLIN- α proteolysis produces a LIM domain-only (LMO)-containing fragment with oncogenic properties in lymphomagenesis [64].

EPLIN was identified as a Rab40b-Cullin5-specific substrate in breast cancer cells. EPLIN binding with GTP-bound Rab40b-CRL5 via the Rab40b SOCS box leads to EPLIN ubiquitylation, disassociation of EPLIN of Rab40b and EPLIN complex, and eventual degradation by the proteasome. Rab40b-Cullin5-mediated EPLIN degradation decreases its subcellular localization at the leading edge of lamellipodia, allowing EPLIN accumulation at stress fibers and invadopodia [65].

EPLIN and the ARCaP model in the discovery of new agents for the prevention and treatment of PCa metastasis

Epidemiological studies support that soy-rich diets could play an important role in the modulation of PCa incidence and mortality [66-68]. A

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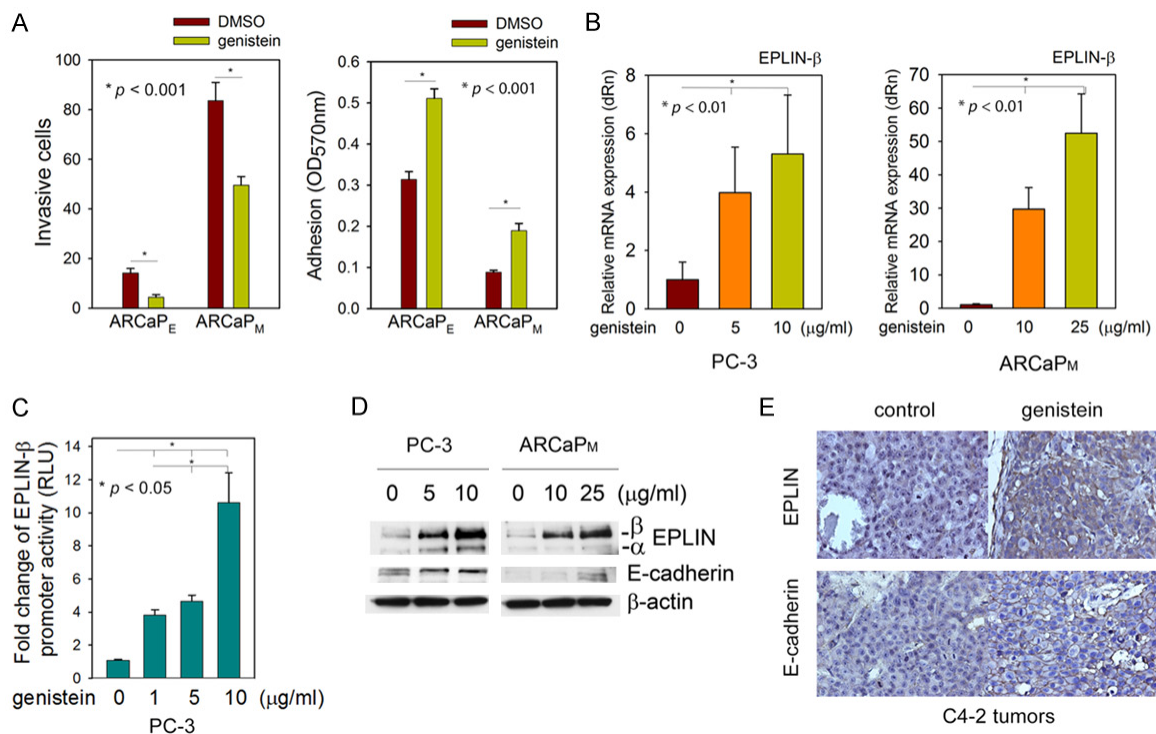


Figure 3. EPLIN is a molecular target of genistein in metastatic PCA cells. A. Genistein inhibits the *in vitro* invasion in transwell experiments (18 h) and increases the *in vitro* adhesion on fibronectin-coated plates (6 h) in ARCaP_E and ARCaP_M cells. B. Genistein treatment (48 h) induces EPLIN- β mRNA in PC-3 and ARCaP_M cells, as determined by qRT-PCR. C. Genistein activates the human EPLIN- β promoter in a dose-dependent manner (48 h). D. Genistein dose-dependently induces EPLIN protein expression (72 h). E. Genistein (50 mg/kg, intraperitoneal injection, 4 times per week, 4-week treatment) increases EPLIN and E-cadherin in C4-2 subcutaneous tumors (n = 6 per group). Refer to [Supplementary Information](#).

meta-analysis of five cohort studies and eight case-control studies demonstrated an inverse relationship between PCa mortality and dietary consumption of genistein (4',5,7-trihydroxyisoflavone), a major isoflavone in soy products [69]. In ARCaP_E and ARCaP_M cells, genistein effectively inhibited their *in vitro* invasion through Boyden chambers and enhanced the adhesion on fibronectin-coated plates (**Figure 3A**). Intriguingly, genistein markedly activated EPLIN- β transcription in a dose-dependent manner in both PC-3 and ARCaP_M cells (**Figure 3B, 3C**), as confirmed at the protein level (**Figure 3D**). Consistently, administration of genistein via the intraperitoneal route significantly increased both EPLIN and E-cadherin at the tissue level in C4-2 tumors (**Figure 3E**). These data revealed EPLIN as a novel molecular target of genistein that may contribute to the inhibitory effect of genistein on PCa metastasis in preclinical models [67, 70-75].

Docetaxel resistance is a major obstacle in the treatment of metastatic, castration-resistant

PCa [76-81]. To discover novel drug candidates for targeting chemoresistant PCa, which is highly heterogeneous, we developed a sequential phenotypic screening platform that consists of two independent cellular models of chemoresistant PCa, i.e., ARCaP_E-shEPLIN and C4-2B-TaxR [82]. We proposed that ARCaP_E-shEPLIN represents a subpopulation of cancer cells that gain stemness and exhibit intrinsic chemoresistance via EMT [11], and that C4-2B-TaxR cells (developed by Dr. Allen C. Gao's group) mimic the progression of acquired docetaxel resistance [60]. Small-molecule inhibitors of chemoresistant cancer were analyzed through this screening platform based on their capability of selectively inducing cell death only in ARCaP_E-shEPLIN and C4-2B-TaxR cells but not in their parental counterparts.

LG1980, an aminobisphosphonate-conjugated compound, was identified as a specific and effective inhibitor of chemoresistant PCa cells. In a recent report [82], we described the *in vitro* and *in vivo* activities of LG1980 against chemo-

resistant PCa. Mechanistic studies uncovered LG1980 as a novel inhibitor of embryonic ectoderm development (EED) that interrupts EED-EZH2 interactions and induces degradation of key PRC2 components, including EZH2, EED and SUZ12, subsequently inhibiting noncanonical EZH2 signaling. As a lead compound, LG1980 is well-tolerated in mice and has an excellent safety pharmacology profile. In several xenograft models, LG1980 effectively suppresses the *in vivo* growth of chemoresistant PCa and synergistically enhances the efficacy of docetaxel chemotherapy.

Using LG1980 as a positive control, we recently conducted a larger-sized screening of potential lead compounds against chemoresistant PCa cells. At least nine FDA-approved, non-oncology drugs were found to have high selectivity and potency in ARCaP_E-shEPLIN and C4-2B-TaxR cells. Most of these candidates effectively inhibited the *in vivo* growth of chemoresistant PCa xenografts and demonstrated excellent safety profiles in animals (data not shown). These drugs are well positioned to be repurposed as novel treatments for metastatic and chemoresistant PCa.

Concluding remarks

Since the discovery of EPLIN as a cytoskeletal protein about two decades ago, there has been greatly increased interest in the biology and clinical implications of EPLIN. EPLIN is not only well recognized as an indispensable component of cytoskeleton dynamics and cell-cell adhesions in various cell types, but also as a potential signaling molecule that plays important roles in human physiology and pathology, including cancer. The role of EPLIN in cancer progression has expanded from that of a tumor suppressor, which may mainly function at early disease stages, to a potential suppressor of metastasis, acting at later stages. Currently available clinical data, although still limited, points to a common pattern that low EPLIN expression is associated with advanced disease, worse response to chemotherapy and poor prognosis in multiple cancer types. While most mechanistic research has focused on the well-acknowledged role of EPLIN in the remodeling of the cytoskeleton and cell-cell adhesion, a few recent studies, including ours, demonstrated that EPLIN may affect multiple signaling

pathways implicated in EMT, stemness, and metastasis. A key remaining question is how and when EPLIN exerts these diverse functions in different phases of tumor progression. There is an expanding list of EPLIN-interacting proteins that have been discovered during the past decade [52], which may provide important clues to the underlying mechanisms by which EPLIN controls tumorigenesis and progression towards metastasis and therapeutic resistance.

For translational science, understanding the regulatory mechanism of EPLIN expression could identify novel agents (such as genistein) to upregulate EPLIN in cancer cells and prevent or delay the metastatic process. Our recent work demonstrated the use of the ARCaP EMT model in the discovery of a novel function of EPLIN in cancer progression and new therapeutic agents that selectively and potently target chemoresistant PCa. Elucidation of the mechanisms of action of these drug candidates obtained from the ARCaP phenotypic screening platform could reveal a cancer-relevant EPLIN signaling network and identify new “druggable” therapeutic targets for the treatment of metastatic PCa.

Tribute to Leland W. K. Chung, PhD

Daqing Wu, PhD: Dr. Leland W. K. Chung was my dear mentor and friend. Leland was an outstanding educator who thoughtfully nurtured the minds of his students, fully supporting the growth and development of their careers as future scientists. He taught us how to see the hidden meanings and connections in seemingly random and messy results. Whether arriving in his office with “good” data or “bad” data, we came out with big smiles, as his trust in our potential and talents was unwavering, and he always encouraged us to strive for the highest standards possible. Leland’s open-mindedness and ability to synthesize information and exude positivity was greatly inspiring.

Throughout his career, Leland chose to be affiliated with clinical departments, and the reason was simple: “As a cancer researcher, you need to follow real-world problems and find ways to help patients who are dying every day”. His quest to find a cure for cancer was fueled by his dedication, his innovative methods, and his ability to build research teams and collaborate

with others. Leland was a great leader and a true visionary. The breadth of his personal and professional networks demonstrated how scientists, engineers, and clinicians must together with industry work closely to address not only cancer but broader societal issues as well. As a cancer survivor himself, Leland positioned himself as an entrepreneur at the end of his career and put enormous energy and effort into his start-up company, DaZen (“Great Kindness”) Theranostics. When we last spoke, he clearly articulated his vision for DaZen’s future and his dream of finding a cure for cancer, with his characteristic enthusiasm and optimism. Yes, this was Leland.

On the wall in Leland’s office was a framed picture of the space shuttle *Columbia*. Leland and his wife Haiyen witnessed *Columbia* blasted off from the Kennedy Space Center on January 16, 2003 carrying an experiment designed by Leland and his team. The experiment was to test an innovative idea about how prostate cancer cells, as an “organoid”, behave in a three-dimensional setting under zero gravity. However, on February 1, 2003, when making re-entry, the shuttle was lost, with all seven dedicated astronauts whom Leland got to know from their close collaboration. There was a deep sadness and admiration when he talked about these people and their brave adventures into the unknown. Today, we share the same feelings when we reflect on Leland and his legacy. Like the *Columbia* crew, Dr. Leland W. K. Chung was here to show what a person with curiosity, talent, a passion for the common good, an infectious smile, and a warm heart could bring to this world. We miss this gentleman, and we are grateful to him for enriching our lives.

Adeboye O. Osunkoya, MD: Leland Chung, PhD will always have a very special place in my memory for the inspiration he gave me during the early phase of my academic career, prior to his departure from Emory. He was one of those that I met during the interview process for my first and current Faculty position here at Emory back in 2006. Apart from the obvious great potential collaborations we would have, his very warm personality and constant smile definitely put me at ease during the interview, and I was convinced that we would have a great collaborative experience together at Emory. Within

the first couple of weeks of my arrival at Emory as a newly minted Faculty in July 2007, Leland had already reached out to me to set up a research meeting with his wife and research partner Haiyen E. Zhau, PhD to discuss further about some of their research projects and potential areas of collaboration. The rest as they say is history. I know so many others had a similar experience with him, because he was constantly surrounded by mentees. I will definitely miss him. Rest in peace my great friend/collaborator and “gentle giant of urology research”.

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Disclosure of conflict of interest

None.

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Supplementary Information

Materials and methods

Cell culture

Human PCa cell lines ARCaP_E, ARCaP_M, PC-3, and C4-2 were routinely maintained in T-medium (ThermoFisher, Carlsbad, CA) with 5% fetal bovine serum (FBS). Genistein was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO).

Quantitative PCR (qPCR)

Total RNA was prepared with Qiagen RNeasy Kit (Valencia, CA). The first-strand cDNA was synthesized using SuperScript[®] III First-Strand Synthesis System (ThermoFisher). Quantitative PCR was performed by the LightCycler 480 system (Roche Applied Science) using a Brilliant[®] SYBR[®] Green QPCR Master Mix (Stratagene) according to the manufacturer's instructions. The specific primer pairs for human EPLIN- β and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were described previously [62].

Western blot analysis

Total cell lysates were prepared using radioimmunoprecipitation (RIPA) buffer (Santa Cruz Biotechnology). Immunoblotting analysis followed standard procedures. The following antibodies were used: EPLIN (NB100-2305, Novus Biologicals), E-cadherin (610181, BD Transduction Laboratories), and β -actin (A5316, Sigma-Aldrich).

EPLIN report assay

Human EPLIN- β reporter was obtained from SwitchGear Genomics (Menlo Park, CA). pTK-RL plasmid was purchased from Promega (Madison, WI). Plasmid transfection and reporter luciferase were conducted as we described previously [62]. 24 h after the transfection, cells were treated with DMSO or genistein at the indicated concentrations for 48 h, luciferase activities were measured using a Dual-Luciferase Reporter Assay system (Promega). Relative Luciferase Units (RLU) were expressed as firefly luciferase intensity normalized to Renilla luciferase activity.

Animal study

A total of 12 athymic nude mice (Harland; 6-week-old) fed with a standard diet were used. C4-2 cells (2×10^6) per 100 μ l per site were mixed with Matrigel (HC) at the ratio of 1:1 and injected subcutaneously using a previously established procedure. One week later, tumor-bearing mice were randomly divided into two groups ($n = 6$ per group) and treated with vehicle control (100% DMOS) or genistein (50 mg/kg) via the intraperitoneal route, 4 times per week, respectively. The treatment lasted for 4 weeks. Animal protocols were approved by Emory University Institutional Animal Care and Use Committee (IACUC).

Immunohistochemistry (IHC) analysis

EPLIN expression in a human prostate tumor array (TMA), and the expression of EPLIN and E-cadherin in C4-2 xenograft tumor tissues were analyzed by IHC staining following our published procedures [11]. The antibodies used were EPLIN (612115) and E-cadherin (610181, BD Transduction Laboratories).

Statistical analysis

Treatment effects were evaluated using a two-sided Student's *t* test. All data represent three or more experiments. Errors are S.E. values of averaged results, and values of $P \leq 0.05$ were taken as a significant difference between means.

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Supplementary Figure 1. Dr. Leland W. K. Chung and his Molecular Urology and Therapeutics team at Emory University (2001-2009).