Introduction

Prostate cancer is one of the most common cancers diagnosed and the second leading cause of deaths due to cancer in American men. According to the estimation from the American Cancer Society in 2015, approximately 1 in 7 men will be diagnosed with prostate cancer during their lifetime. In men aged over 65, approximately 6 cases in 10 are diagnosed while one in 38 men dies of prostate cancer. Most prostate cancers developed from gland cells and are therefore classified as prostate adenocarcinoma. The growth rate of most prostate cancer is slow, although some are highly malignant and grow and spread quickly. The male hormone androgen is known to promote prostate cancer growth and survival through activation of androgen receptors (AR) (1-4). Therefore, hormone therapy has been used clinically to inhibit prostate cancer growth and spread via androgen deprivation or blockade. However, prostate cancer cells tend to resist this treatment and transform into more aggressive and highly metastatic androgen-independent cells. Unfortunately, the details of this transformation have not been clarified. Nowadays, researchers are trying to find a potential treatment for advanced prostate cancer patients by understanding the detailed molecular mechanisms underpinning androgen-independent prostate cancer cells. Lysophosphatidic acid (LPA) is a simple phospholipid involved in multiple cellular events in almost mammalian cell types. It has been known that LPA binds to LPA receptors and subsequently activates intracellular signaling pathway to regulate prostate cancer cell proliferation (5), survival (6), invasion (7) and migration (8). These functions are dependent on the expression of LPA receptors and activation of downstream signaling transduction pathways. This suggests that LPA...
receptors are critical for prostate cancer progression. Here, we review the functions of LPA as well as its receptors in prostate cancer progression and how LPA signals mediate cellular functions in prostate cancer cells. In addition, our study shows that LPA stimulates the expressions of VEGF-C in prostate cancer cells (9). These effects are mediated through LPA receptors and ROS production (9,10). Our results, therefore, suggest that blocking LPA signals via targeting LPA receptors and downstream effectors may prevent lymphangiogenesis as well as lymphatic metastasis in advanced prostate cancer. The pathological significances of LPA in different prostate cancer cells are summarized in Table 1.

**LPA functions in prostate cancer cell proliferation and survival**

The first study of LPA in prostate cancer was conducted by Qi in 1998. It showed that LPA stimulates cell proliferation of human androgen-insensitive prostate cancer PC-3 cells (5). This mitogenic effect is through phosphorylation of extracellular-signal-regulated kinases (ERKs) (11-13). The upstream regulator has been illustrated that epidermal growth factor receptors (EGFRs) are activated in response to LPA. Similar to the effect of LPA in the fibroblasts, LPA has found to trigger an outside-in signal through LPA receptors, and then inside-out activation of matrix metalloproteinase (MMP) to cleavage extracellular EGF-like ligands. However, this transactivation was not mediated by the shedding of heparin-binding EGF (14,26). Modified EGF-like ligands stimulate the outside-in signaling again via phosphorylate EGFRs and activate downstream intracellular ERKs in prostate cancer cells (14). Conversely, androgen-dependent LNCaP cells do not respond to LPA in terms of cell growth or ERK phosphorylation (13,17). The expression profile of LPA receptors in both cell lines has shown by Guo in 2006 and Figure 1; this may explain

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**Table 1 LPA functions in prostate cancer cells**

<table>
<thead>
<tr>
<th>Cellular events</th>
<th>Cell type</th>
<th>Mechanisms</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation</td>
<td>PC-3</td>
<td>EGFR transactivation/ERK</td>
<td>(5,11-14)</td>
</tr>
<tr>
<td></td>
<td>LNCaP</td>
<td>Pyk2 activation</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KLF4</td>
<td>(16)</td>
</tr>
<tr>
<td>Survival</td>
<td>PC-3</td>
<td>Apoptosis</td>
<td>(13,17)</td>
</tr>
<tr>
<td></td>
<td>LNCaP</td>
<td>No effect</td>
<td>(13)</td>
</tr>
<tr>
<td>Motility</td>
<td>PC-3</td>
<td>Lamellipodia formation</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>LNCaP</td>
<td>No effect</td>
<td>(13)</td>
</tr>
<tr>
<td>Invasion</td>
<td>PC-3</td>
<td>RhoA and NF-κB</td>
<td>(7)</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>PC-3</td>
<td>VEGF-A</td>
<td>(24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PI3K/Akt/mTOR/p70S6K, p42/p44 MAPK, and HIF-1α</td>
<td></td>
</tr>
<tr>
<td>Lymphangiogenesis</td>
<td>PC-3</td>
<td>VEGF-C</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>LNCaP</td>
<td>ROS production/LEDGF</td>
<td>(9,10)</td>
</tr>
</tbody>
</table>

LPA, lysophosphatidic acid; EGFR, epidermal growth factor receptor.
LPA promotes prostate cancer cell migration once cancer develops due to the stimulation of LPA. Besides, LPA inhibits serum deprivation-induced autophagy in PC-3 cells (18). Together, these imply that LPA facilitates prostate cancer development by enhancing cell survivals.

**LPA regulates prostate cancer cell motility**

LPA regulates cell migration in various cancer cells including prostate cancer cells. In PC-3 cells, LPA induces activation of vasodilator-stimulated phosphoprotein (VASP) which subsequently mediates lamellipodia formation to initiate cell motility (19). In addition, the calcium-independent group VIA phospholipase A$_2$ (iPLA$_2$) is required for LPA-induced cell migration and invasion in mouse TRAMP-C1P3 cells (22). LPA also functions in preventing the calpain-mediated proteolysis of focal adhesion kinase (FAK) in PC-3 cells (20). Calcium-dependent calpains modulate cell migration (30,31) in a process that involves degradation of FAK (32). PC-3 cells require FAK for bombesin-induced cell motility (33). Unlike LPA stimulates FAK phosphorylation in PC-3 and DU145 cells, no effect has been shown in LNCaP cells (13). Migration of LNCaP cells does not respond to LPA (23).

The small GTPase Rho is important in cell movement. Activation of Rho mediates actin rearrangements, gene transcription, cell rounding, and smooth muscle contraction. It was reported that LPA stimulates Rho in PC-3 cells via LPA receptors and G$_{i2/13}$ proteins which directly activate PDZ-RhoGEFs that contain a regulator of G protein signaling (RGS) domain (21). These studies indicate that LPA promotes prostate cancer cell migration once cancer
cells have become androgen-independent and highly metastatic.

Since LPA has a role in cell migration, it may have potential roles in promoting cell invasion. For instance, LPA stimulates matrigel invasion through activation of RhoA and NF-κB in PC-3 cells (7). LPA-stimulated RhoA activity leads to morphologic changes, from polygonal to round, of PC-3 cells (34). Moreover, analysis of the profile of gene expression between highly invasive and less invasive PC-3 cell sublines suggests that invasion-related molecules are involved in invasiveness of the prostate cancer (35). For instance, higher activity levels of NF-κB, activator protein 1 (AP-1) and RhoA activities as well as thrombospondin-1, interleukin-7 (IL-7), kallikrein 6, MMP-1 and tissue factor were found in invasive cells and may respond to LPA. Heterodimerization of LPA

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and adaptation-linked G protein-coupled receptor (GPCR) CD97 amplify LPA-initiated Rho-dependent signaling and invasion in PC-3 cells (36). Accordingly, the RhoA signaling cascade is necessary to promote LPA-induced cell invasion in prostate cancer cells (36).

Conversely, PC-3 cells which respond to LPA in three-dimensional culture exhibit signs of epithelial-to-mesenchymal transition (EMT) in contrast to metastable acinar differentiation. LPA promotes acinar morphogenesis and blocks the disintegration of epithelial structures with the basal lamina and formation of invadopodia (37). This mechanism is through LPA

3 Gα12/13/RhoA/ROCK pathway which suppresses invasive properties. Therefore, the functions of LPA in prostate cancer invasion in either 2-D or 3-D culture system need to be clarified in the future.

**LPA mediates tumoral angiogenesis and lymphangiogenesis**

VEGFs are important growth factors for angiogenesis and lymphangiogenesis in prostate cancer progression and metastasis. In PC-3 cells, LPA induces VEGF-A expression through the PI3K/Akt/mTOR/p70S6K and p42/p44 MAPK pathways that are similar in OVCAR-3 ovarian cancer cells (24,38). Moreover, hypoxia-inducible factor 1α (HIF-1α) participates in LPA-induced VEGF-A expression (24). HIF-1 proteins are transcription factors which are induced by hypoxia within the tumor. The HIF-1 complex is a heterodimer composed of HIF-1α and HIF-1β, which is also known as the aryl hydrocarbon receptor nuclear translocator (ARNT) (39). The ARNT binding partner, aryl hydrocarbon receptor (AHR), inhibits LPA-induced VEGF-A (25). AHR is a basic helix-loop-helix transcription factors that function as an environmental sensor binding with dioxin-like compound and leads to nuclear localization. Translocated AHR further heterodimerizes with ARNT and leads to changes in gene transcription. AHR inhibits prostate carcinogenesis and vanadate-induced VEGF-A production in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice (40,41). Together, the inhibitory role of AHR in LPA-induced VEGF-A is due to the sequestering of ARNT from HIF-1α (25), thereby inhibiting tumoral angiogenesis.

VEGF-C is a critical lymphangiogenic factor that secreted by normal human tissues (42) and prostate cancer cells (43,44). The high expression of VEGF-C in prostate cancer results in the formation of lymphatic vessels which have been implicated in lymph node metastasis (43). However, the serum VEGF-C level cannot be a marker for prostate cancer growth because no significant differences between prostate cancer and BPH patients have been found (46). Overexpressing VEGF-C in poorly metastatic LAPC-9 cells induces tumoral lymphangiogenesis and leads to the development of metastatic lesions (47). Reduction of the key mitogenic factor androgen in prostate cancer cells upregulates VEGF-C (48) through ROS production and small GTPase RaA activation (49). In our lab, we found that LPA stimulates VEGF-C mRNA expression through binding to LPA

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B in PC-3 cells (7). LPA-stimulated RhoA activities with ARNT and leads to changes in gene transcription. AHR inhibits prostate carcinogenesis and vanadate-induced VEGF-A production in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice (40,41). Together, the inhibitory role of AHR in LPA-induced VEGF-A is due to the sequestering of ARNT from HIF-1α (25), thereby inhibiting tumoral angiogenesis.

**LPA-related enzymes in prostate cancer**

Due to the production of LPA in ovarian cancer (50), questions arose whether prostate cancer cells also generate and secrete LPA. Prostate cancer cells do produce and utilize LPA for themselves (12). A neuroendocrine peptide bombesin has been shown to stimulate LPA production. Electrospray ionization mass spectrometry showed that 18:1 LPA (Oleoyl-LPA) is the most abundant LPA in the prostate cancer medium. Secreted LPA from prostate cancer cells induces calcium mobilization (12). These results demonstrate that LPA is generated by prostate...
cancer cells and suggest that 18:1 LPA act as an autocrine mediator. LPA activates phospholipase D (PLD) in prostate cancer cells, including PC-3, DU-145, and LNCaP cells, to catalyze phospholipids (PLs) to phosphatidic acid (PA) and further may produce more LPA to stimulate the cellular response. Protein kinase C (PKC) is also involved in the LPA-induced activation of PLD in PC-3 cells. In addition, two important LPA-producing enzymes, LysoPLD (Autotaxin, ATX) and acylglycerol kinase (AGK), were found in prostate cancer samples and seminal plasma. ATX and AGK are both highly expressed at the protein level in prostate cancer cells, including LNCaP, PC-3, and DU-145 cells, compared with non-neoplastic prostate cells, including PrECs and PrSCs. ATX is the major LPA-synthesizing enzyme for extracellular LPA production. Activated platelets are responsible for the increased levels of ATX in serum. However, the activity of ATX in prostate cancer patients has been shown no differences from that in the controls. Besides, there is no correlation between serum ATX activity and serum PSA concentrations. Nevertheless, ATX is not a useful diagnostic marker for prostate cancer patients. Another enzyme, AGK, is an intracellular lipid kinase that localizes to the mitochondria in epithelial cells and fibroblasts and phospholipases monoacylglycerol and diacylglycerol to form LPA and PA, respectively. Prostate cancer patients and PC-3 cells highly express AGK. This increase results in the formation and secretion of LPA, which cross-talk with EGFR resulting in the subsequent activation of ERK1/2. AGK expression enhanced both prostate cancer cell proliferation and migration. Furthermore, the LPA degradation enzyme, prostatic acid phosphatase (PAP), was found in the seminal plasma. PAP is a non-specific phosphomonoesterase that is synthesized and secreted into the seminal plasma under androgenic control. LNCaP cells expressing endogenous PAP show a slow growth rate compared with PC-3 and DU-145 cells that lack PAP expression. Intriguingly, the introduction of PAP into prostate cancer cells results in decreased cell growth. Moreover, the cellular form of PAP is involved in regulating the androgen-stimulated growth of prostate cells. C-33 LNCaP cells that express PAP and AR are responsive to androgen stimulation, whereas C-81 LNCaP and PC-3 cells that express the functional AR but lack PAP expression are androgen-insensitive. Reintroducing cellular PAP expression can restore androgen responsiveness of these cell lines. This suggests that PAP has a role in inhibiting the proliferation of prostate cells by negatively regulating the LPA level. Clinical evidence indicates that AGK was abundantly expressed in the stroma and epithelium while PAP was predominantly localized in the epithelial cells of benign prostatic hyperplasia (BPH). Conversely, ATX is predominantly expressed in the stroma. However, the expression of ATX does not significantly differ between normal tissue, benign gland, and cancer foci. In conclusion, LPA-degrading enzyme PAP, rather than LPA-producing enzyme ATX or AGK, may be the key player to mediate the levels of LPA in prostate cancer, and therefore affect prostate cancer cell behaviors.

General function of the expression of LPA receptors

LPA receptors are GPCRs with seven transmembrane domains that are activated by binding with LPA and subsequently initiate downstream cellular signaling cascades for different biological functions. Six LPA receptors have been identified and classified into two types: LPA1-3 are close relative and initially known as the endothelial differentiation gene (EDG) while LPA4-6 belong to the P2Y purinergic receptor family. LPA1 was the first to be identified and the best studied LPA receptor. LPA1 activation regulates cellular events, such as cell-cell contact alteration, cell proliferation and survival, cell migration and cytokine changes, calcium mobilization, and adenylyl cyclase inhibition. Lpar1/LPAR1 is widely expressed in the organs of adult mice and humans and is enriched in parts of the brain during embryonic development. Lpar1−/− mice were generated and demonstrated clear neurodevelopmental defects. A 50% of perinatal lethality of these mice may be due to olfactory deficits leading to a defect in suckling behavior. Therefore, LPA1 is involved in the nervous system development and function.

In cancer cells, LPA mediates cell motility and metastasis. LPAR2 is highly expressed in the testis and leukocytes while Lpar2 is highly expressed in the kidney, uterus, and testis. LPAR4 is expressed in the heart, testis, prostate, pancreas, lung, ovary, and brain of human and LPA4 is expressed in the frontal cortex, hippocampus, and amygdala. These suggest LPA4 may have significant functions in the brain. However, Lpar3−/− mice are viable and grossly normal in the nervous system. Lpar3−/− mice showed delays in embryo implantation as well as reduced litter size and embryo implantation suggesting a role of LPA signaling in reproduction system. LPA4 negatively regulates cell motility which is notably different from EDG LPA1-3.
receptors that promote cell migration (74). Lpar4 is present in mouse heart, skin, thymus, bone marrow, and embryonic brain (75). Some Lpar4−/− mice show hemorrhage and prenatal lethality during embryonic development (76), but others can grow up normally (75). The abnormal formation of blood during mouse embryogenesis cause prenatal death (76) of mice. However, neither LPA1 nor LPA3 knockout mice have been generated yet. Hypotrichosis patients show LPAR6 mutation (77-79), which suggests that LPA6 is critical for the formation of human hair. The expression of LPA receptors depends on the cell type that will mediate signaling transduction in the cells.

Expression profile of LPA receptors and signaling pathway in prostate cancer

LPA receptors coupled with Gα proteins, including Gαq, Gα12, Gα13, and Gq/11, initiate a variety of signaling cascades. LPA1 interacts with three types of Gα proteins, Gαq, Gαq/11, and G12/13, which leads to the activation of downstream effectors such as mitogen-activated protein kinase (MAPK), phospholipase C (PLC), Akt, and Rho, respectively (80). Like LPA1, LPA2 couples with Gαq, Gq/11, and G12/13, and subsequently initiates downstream effectors such as Ras, MAPK, PI3K, Rac, PLC, diacylglycerol, and Rho (67). LPA2 couples with Gαq and Gq/11 and mediates PLC, adenyl cyclase and MAPK activation (81). LPA3 coupled with G12/13 and Gαq, thereby initiate receptor internalization and elevates intracellular calcium levels (82). LPA4 is involved in cAMP accumulation and Rho-dependent cell morphology alterations through G13 (83). Therefore, the expressions of LPA receptors are important to mediate activation of the downstream signaling pathways as well as cellular events.

Prostate cancer cells highly express LPA receptors, LPA1, LPA3, and LPA6 but not LPA2, LPA4, and LPA5 (Figure 1) (13,17,84). The levels of LPA receptors are different between the androgen-dependent and androgen-insensitive prostate cancer cells (17). The LPA1 gene is dominantly expressed in PC-3 and DU-145, but not LNCaP cells. Stable expressing of LPA1 in LNCaP cells shows a response to LPA-induced cell proliferation in vitro and in vivo. LPA1 may play a role in transducing proliferative signals in prostate cancer by transducing Gα-independent signals to promote AR nuclear localization and cell proliferation (17). LPA2 is expressed in these three types of cells as well as being highly expressed in LNCaP cells (62). LNCaP cells show high levels of LPA2 in comparison to PC-3 cells while DU-145 cells do not express LPA2. Clinical evidence also indicates the importance of LPA receptors in prostate cancer (62). Both high-grade intraepithelial neoplasia (HGPIN) and cancer epithelia displayed significantly decreased levels of LPA2 mRNA compared with the benign glands. Cancer epithelia showed greater expression of LPA2 mRNA compared with the benign glands. The expression of LPA1 is high in epithelium compared to the stroma of prostate when microdissected from benign glands, BPH, high-grade PIN (PIN) and prostate cancer foci in the prostate harboring prostate cancer. However, the expression of LPA2 is not significantly modified when comparing normal tissue, benign gland, and cancer foci. Most papers have demonstrated the functions of LPA1, LPA2, and LPA3 in prostate cancer; however, the pathophysiological roles of LPA4 in prostate cancer remain unclear and needs to be clarified.

Future prospects

Tumor progression is affected by alteration in the surrounding microenvironment. A study has therefore demonstrated the intercellular cross-talk of prostate cancer cells with prostate stromal cells in response to LPA. The co-culture of human prostate stromal PS30 and epithelial LNCaP cells results in the activation of ERK in LNCaP cells and further enhanced the biophysiological activities to LPA stimulation (85). Implantation of a mixture of both cell types into nude mice reveals the physiologic relevance of the interaction between these two cells. Tumors from mice with both kinds of cells are larger compared with only mice implanted with LNCaP cells. The larger tumor is because LPA stimulates synthesis of interleukin 6 (IL-6) in PS30 cells. IL-6 controls the LPA-induced mitogenic ERK and STAT3 signaling and growth of the LNCaP cells. These results suggest that other surrounding cells such as endothelial cells or epithelial cells may also participate in cross-talk with prostate cancer cells and regulate the physiological functions.

The expression and function of LPA receptors are critical for regulating prostate cancer progression and metastasis. Therefore, a selective antagonist for LPA receptors may represent a potential therapy against tumor development. For instance, Ki16425 is a selective antagonist for LPA1 and LPA4 (86). Administering the R-stereoisomer of Ki16425, Debio 0719, into BALB/c mice with orthotopic mouse 4T1 breast cancer inhibits bone and lung metastasis from the primary tumor (53), but not tumor growth and angiogenesis. In prostate cancer, Ki16425 treatment into
nude mice after subcutaneously implanting PC-3 cells inhibits heparin-binding EGF-like growth factor (HB-EGF) secretion by human PC-3 xenograft (84). HB-EGF was therefore identified as a biomarker for LPA1 activation in human prostate cancer in vitro and in vivo (84). However, Ki16425 treatment does not reduce the size of primary tumors in prostate cancer, which is the same result found in the treatment of breast cancer. The expression levels of LPA receptors do not change in tumors with Ki16425 treatment compared with the vehicle-treated group. These suggest that in vivo Ki16425 treatment does not inhibit PC-3 tumor growth or apoptosis. The functions of the blockade of LPA1/3 by Ki16425 should be further analyzed.

Compared with the EDG LPA receptors, little is known about the biological roles of the novel subtype of LPA receptors LPA4-6 in cancer. LPA3 and LPA4 are difficult to detect in prostate cancer, which suggests that expressions of both are repressed in malignant cells. Conversely, LPA6 is highly expressed (84) in prostate cancer, although the role of LPA6 has not been identified. Hence, further study is required on the biological functions of LPA6 in prostate cancer progression.

Conclusions

In this review, we summarize that LPA increases cell proliferation and promotes cell survival in advanced prostate cancer. Cell migration and invasion are also stimulated by LPA, suggesting that LPA mediates prostate cancer metastasis. Moreover, LPA stimulates VEGF-A and VEGF-C expression which may promote tumor angiogenesis and lymphangiogenesis and therefore metastasis. Moreover, these LPA-regulated cell behaviors in prostate cancer are mainly mediated through activating LPA receptors. Interestingly, advanced prostate cancer cells secret LPA and the level of secreted LPA is affected by LPA-degrading enzyme PAP rather than by LPA-producing enzyme ATX. Therefore, clarify the roles of LPA, LPA receptors, and its related enzymes in prostate cancer will help us to identify the pathological functions and molecular mechanisms in prostate cancer progression. Accordingly, targeting the specific effectors of LPA signaling in prostate cancer may contribute to the development of clinical therapeutic strategies for advanced prostate cancer in the future.

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Footnote

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