（ 続紙 1 ）

京都大学	博士（ 農 学 ）	氏名	Lucia TOMIYAMA
論文題目	The role of Dlg5 in the progression of human prostate cancer （ヒト前立腺がんの進行における Dlg5 の役割）		

（論文内容の要旨）

Prostate cancer is the most common cancer and a leading cause of cancer deaths among men in the Western world. A key turning point in the course of cancer progression is the development of metastatic potential as most prostate cancer related deaths are not due to the primary tumor, but rather to metastasis. A better understanding of molecular mechanisms that underlie the process of prostate cancer metastasis will develop better therapeutic and diagnostic interventions for the disease.

Discs large homolog 5 (Dlg5) belongs to the membrane associated guanylate kinase family of adaptor and scaffold proteins. Dlg5 was shown to colocalize with vinexin and β-catenin, major adherence junction proteins, at sites of cell-cell contact. Increasing evidence has shown that Dlg5 participates in cancer progression. For example, Dlg5 expression is downregulated in breast and pancreatic cancers, leading to a more malignant phenotype. Prostate is one of the tissues expressing Dlg5 at the highest level, but the roles played by this molecule in prostate cancer are largely unknown.

In Chapter 1, the author investigated the role of Dlg5 in the progression of prostate cancer. Immunohistochemical analysis on tissue microarray was performed. The normal prostate tissues were all positive for Dlg5 and most of them showed moderate-strong staining (71%). On the contrary, more than 90% of low-grade tumors and 78% of high-grade tumors showed negative-weak staining. High expression of Dlg5 was also detected in some prostate tumors, particularly in high-grade tumors. Consistent with immunohistochemical analysis of tissues, a high level of Dlg5 expression was detected in normal prostate epithelial cells by using Western blotting. The downregulation of Dlg5 was recapitulated in the low metastatic prostate cancer cell line. In agreement with the increased Dlg5 expression in some high-grade prostate tumors, a higher level of Dlg5 expression was found in highly metastatic PC3 cells. Due to the highest expression of Dlg5 in PC3 cells among all tested prostate cancer cell lines, the author selected these cells as a cell model for loss-of-function studies.

The author then examined the potential role of Dlg5 in prostate cancer progression by establishing Dlg5-depleted PC3 cells. The author showed that depletion of Dlg5 significantly enhanced cell migration as indicated by the increase in the cell index of real-time chemotaxis system that measures a relative changes in the electrode impedance caused by migrating cells. Similar results were obtained upon transient Dlg5 knockdown in another prostate cancer cell line. This enhanced cell migration was dependent on PI3K/Akt signal pathway. The author then indicated that Dlg5 knockdown significantly enhanced the invasiveness by using *in vitro* reconstituted basement membrane. In addition, the author found that Dlg5-depleted cells

proliferated slower than the control cells over a 3-day time course. From these findings, the author concluded that Dlg5 may have dual functions in prostate cancer, which can promote tumor progression by enhancing cell proliferation but suppress it by inhibiting cell migration and invasion.

The author next investigated which molecules mediate the effect of Dlg5 depletion on cell migration and identified Girdin, an actin-binding protein that regulates migration of various cells, as a candidate for a Dlg5-interacting protein. Endogenous interaction was confirmed by reciprocal coimmunoprecipitations of these proteins. Dlg5 and Girdin were colocalized in migrating PC3 cells. Akt-mediated phosphorylation of Girdin at Ser-1416 is known to increase the motility of cancer cells. The author revealed that the Dlg5-depleted cells displayed higher phosphorylation of Girdin than control cells after serum stimulation. In addition, treatment with wortmannin, a specific inhibitor for PI3K/Akt pathway, suppressed the Girdin phosphorylation in Dlg5-depleted cells as well as control cells. Girdin siRNA-transfected cells displayed reduced cell migration compared with control siRNA-transfected cells. From these observations, the author concluded that Girdin phosphorylation has a central role in Dlg5-regulated prostate cancer cell migration.

In Chapter 2, the author examined the interaction of Dlg5 with Girdin more closely. The structure of Girdin can be divided into three different regions; an N-terminal region that seems to facilitate the formation of a dimer, a central large coiled-coil domain, and the C-terminal region (CT) that includes an Akt phosphorylation site. In order to elucidate the mechanism by which Dlg5 inhibits Akt-mediated Girdin phosphorylation, it is important to map the domain necessary for Dlg5-Girdin interaction. To determine the important region of Girdin for Dlg5 binding, glutathione *S*-transferase (GST) pull-down assays were performed using lysates from HEK293T cells stably expressing Dlg5 and transiently expressing each domain of Girdin. The results showed that both CT domains and the half of the central region of Girdin have the potential to bind Dlg5. The CT domain showed stronger binding to Dlg5 than the latter. Using smaller constructs to further narrow down the binding region, the author found that the N-terminal half of CT domain is necessary for Dlg5 interaction. It has been previously shown that this region contains the binding site for Akt. Thus, the finding in this study indicates that Dlg5 and Akt bind to the same region of Girdin, raising the possibility that Dlg5 competes with Akt for the binding site. This would explain how Dlg5 suppresses phosphorylation of Girdin.

In summary, these findings support the notion that Dlg5 regulates migration of prostate cancer cells through the modulation of PI3K/Akt/Girdin signaling pathway, providing insight into potential therapeutic targets to treat prostate cancer.

注)論文内容の要旨と論文審査の結果の要旨は１頁を３８字×３６行で作成し、合わせて、３，０００字を標準とすること。
論文内容の要旨を英語で記入する場合は、４００～１，１００wordsで作成し
審査結果の要旨は日本語５００～２，０００字程度で作成すること。

（続紙 ２ ）

（論文審査の結果の要旨）

前立腺がんは日本人男性における罹患率第３位のがんであり、生活習慣の欧米化によりその罹患率は上昇を続けている。このため、前立腺がんの新たな治療法の開発が望まれている。本研究で対象としたDlg5は、細胞-細胞間接着領域に局在するアダプタータンパク質であり、クローン病の発症との関連が示唆されている。また、最近では乳がんや膵臓がんの悪性化との関連が示唆されてきている。正常組織においては前立腺がDlg5の発現量の最も高い組織であるにもかかわらず、Dlg5の前立腺がんにおける役割については未解明の部分が多い。前立腺がんにおけるDlg5の機能を明らかにすることは、前立腺がん悪性化のメカニズム解明や新しい治療法の開発につながると期待される。本研究は、前立腺がんにおけるDlg5の役割を解析したものであり、評価すべき点は以下のとおりである。

１．正常前立腺組織ではDlg5の高い発現がみられるのに対し、多くの前立腺がん組織では低い発現しか示さないことを明らかとした。一方、一部の悪性前立腺がんでは非常に高いDlg5の発現がみられるものもあることを示した。
２．Dlg5は、前立腺がん細胞の細胞遊走と浸潤を抑制する機能をもち、一方で細胞増殖を促進する機能も有することを明らかとした。
３．Dlg5がアクチン制御因子Girdinと前立腺がん細胞内で複合体を形成していること、Dlg5がAktに依存したGirdinのリン酸化を抑制することを明らかにした。
４．Dlg5による前立腺がん細胞の遊走の制御にはGirdinの発現が必要であることを明らかにした。
５．Girdin のDlg5に対する結合領域を特定し、それがGirdinのAkt結合領域と重なり合うことを明らかとした。

以上のように、本論文は前立腺がん細胞の細胞遊走と浸潤をDlg5が抑制すること、およびDlg5の機能発現の分子メカニズムの一部を明らかにしたものであり、分子細胞生物学、細胞生化学、基礎生理学に寄与するところが大きい。

よって、本論文は博士（農学）の学位論文として価値あるものと認める。

なお、平成26年4月14日、論文並びにそれに関連した分野にわたり試問した結果、博士（農学）の学位を授与される学力が十分あるものと認めた。

また、本論文は、京都大学学位規程第１４条第２項に該当するものと判断し、公表に際しては、当該論文の全文に代えてその内容を要約したものとすることを認める。

注）論文内容の要旨、審査の結果の要旨及び学位論文は、本学学術情報リポジトリに掲載し、公表とする。

ただし、特許申請、雑誌掲載等の関係により、要旨を学位授与後即日公表することに支障がある場合は、以下に公表可能とする日付を記入すること。

要旨公開可能日：　　年　　月　　日以降（学位授与日から３ヶ月以内）