

Leucino­statin A inhibits prostate cancer growth through reduction of insulin-like growth factor-I expression in prostate stromal cells

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Targeting stroma in tumor tissues is an attractive new strategy for cancer treatment. We developed *in vitro* coculture system, in which the growth of human prostate cancer DU-145 cells is stimulated by prostate stromal cells (PrSC) through insulin-like growth factor I (IGF-I). Using this system, we have been searching for small molecules that inhibit tumor growth through modulation of tumor-stromal cell interactions. As a result, we have found that leucino­statins and atpenins, natural antifungal antibiotics, inhibit the growth of DU-145 cells cocultured with PrSC more strongly than that of DU-145 cells alone. In this study we examined the antitumor effects of these small molecules *in vitro* and *in vivo*. When DU-145 cells were coinoculated with PrSC subcutaneously in nude mice, leucino­statin A was found to significantly suppress the tumor growth more than atpenin B. The antitumor effect of leucino­statin A *in vivo* was not obtained against the tumors of DU-145 cells alone. RT-PCR experiments revealed that leucino­statin A specifically inhibited IGF-I expression in PrSC without effect on expressions of other IGF axis molecules. Leucino­statins and atpenins are known to abrogate mitochondrial functions. However, when we used mitochondrial DNA-depleted, pseudo-p⁰ cells, we found that one of leucino­statin A actions certainly depended on mitochondrial function, but it actually inhibited the growth of DU-145 cells more strongly in coculture with pseudo-p⁰ PrSC and reduced IGF-I expression in pseudo-p⁰ PrSC. Taken together, our results suggested that leucino­statin A inhibited prostate cancer cell growth through reduction of IGF-I expression in PrSC.

Growing evidence indicates that the stroma plays a critical role in the growth and metastasis of various cancers, including colorectal,¹ breast,^{2,3} pancreatic⁴ and prostate cancer.⁵ The constituents of stroma vary in each tissue, but they generally include fibroblasts, macrophages, endothelial cells and extracellular matrix.^{6,7} Among these components, certain types of fibroblasts appear to enhance tumor growth and others suppress it.^{2,8-10} Fibroblasts that enhance tumor growth are especially referred to as cancer-associated fibroblasts or activated fibroblasts,^{11,12} and have distinct characters from normal fibroblasts as they express both vimentin and smooth muscle α -actin (SM α -actin), indicating a myofibroblast phenotype.⁶ These cells secrete various factors favorable

for tumor cell growth, such as growth factors, cytokines and adhesion molecules.^{6,12} Thus, tumor-stromal cell interactions can promote tumor growth and metastasis through secreted factors and cell-cell adhesion.^{9,10,12,13} Although various kinds of growth factors and cytokines are reported to be involved in tumor-stromal cell interactions, many studies suggest that insulin-like growth factor I (IGF-I) plays an important role in prostate tumor development.^{14,15}

The fact that stromal cells can regulate tumor development positively or negatively drives us to consider the modulation of tumor-stromal cell interactions could be an attractive new strategy for the treatment of cancer.^{16,17} Some growth factors and antibodies actually suppress the growth of some cancers.¹⁸⁻²⁰ However, small molecules that modulate tumor-stromal cell interactions are less reported. We therefore constructed an *in vitro* coculture system of prostate cancer cells and PrSC, designed to mimic characteristics of tumors *in vivo*.^{14,21} Using this system, we have been searching for small-molecule modulators of tumor-stromal cell interactions. As a result, we found that phthoxazolin A inhibits growth of prostate cancer cells through reduction of IGF-I secretion from PrSC by suppressing myofibroblast differentiation of PrSC.²²

Moreover, we have recently found that atpenins along with new congeners inhibit growth of prostate cancer cells possibly through modulation of tumor-stromal cell interactions. Further screening has brought about the finding that leucino­statins, fungal metabolites, have great activities in our

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