論　文　の　要　旨

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論　文　題　目　Pharmacological studies on the anti-cancer effect of *Perilla frutescens*-derived methoxyflavanone, and its application to a combination therapy with tyrosine kinase inhibitors
（*Perilla frutescens* 由来メトキシフラバノンの抗癌作用に関する薬理学的研究およびそのチロシンキナーゼ阻害剤併用療法への応用）

Chapter 1. General introduction

Cancer is the second leading cause of death after cardiovascular diseases. About one third of all cancer-related deaths are due to lung cancer, which accounts for more deaths each year than breast, prostate, and colon cancer combined. Nearly 60,000 patients in Japan died of lung cancer in 2004, and the mortality rate is still increasing. Chemotherapy for advanced lung cancer is often considered ineffective or excessively toxic, mandating us to search for new agents for advanced lung cancer treatment. Flavonoids are natural phytochemicals containing in the human diet. Flavonoids exhibit many benefits for human health including anti-cancer properties that have recently received an increasing interest, since they can suppress cancer initiation and progression steps. Recently, a new methoxyflavanone derivative (8-hydroxy-5,7-dimethoxyflavanone, named *Perilla*-derived methoxyflavanone; PDMF) has been discovered from the Asian dietary herb, *Perilla frutescens*. PDMF directly suppresses allergic inflammation by inhibiting the serine/threonine kinase Akt, that also is a critical regulator of cell proliferation and cancer development. The targeted action of PDMF to Akt has prompted me to investigate whether PDMF is also effective for tumor suppression. I also have tried to apply PDMF to a combination therapy with anti-cancer tyrosine kinase inhibitors.

Chapter 2. Prominent anti-cancer activity of PDMF on A549 human lung adenocarcinoma and elucidation of its tumor-suppressive mechanisms

In this chapter, I tested whether stimulation with PDMF showed anti-cancer effect on human lung adenocarcinoma A549. BrdU incorporation assay revealed that treatment with PDMF significantly inhibited cell proliferation and decreased viability of A549 cells. Flow cytometric analysis indicated that treatment with PDMF triggered cell cycle arrest at G_2/M phase, and induced apoptosis. PDMF stimulation induced phosphorylation of tumor suppressor p53 on Ser15, and increases its protein amount in conjunction with the up-regulation of downstream cyclin-dependent kinase (CDK) inhibitor $p21^{Cip1/Waf1}$. I also found that PDMF activated proapoptotic caspases-9 and 3, both of which were critical for the p53-dependent proapoptotic pathway. Strikingly, small interfering RNA knockdown of p53 completely abolished the PDMF-driven G_2/M cell cycle arrest, and substantially

abrogated its proapoptotic potency. Taken together, my data suggest that PDMF shows anti-cancer activity on A549 human lung cancer cells through p53-driven G_2/M cell cycle arrest and apoptosis.

Chapter 3. Application of PDMF to a combination therapy with anti-cancer tyrosine kinase inhibitors

Anti-cancer tyrosine kinase inhibitors (TKIs) are effective in lung cancer treatment, while appearance of TKI-resistant tumors suggests a need for the development of their potentiation strategies. In the previous chapter, I showed the prominent anti-cancer activity of PDMF against A549 human lung adenocarcinoma cells. Here, I tested whether PDMF was also applicable to a combination therapy with anti-cancer TKIs on A549 cells. To evaluate whether combination of PDMF and TKIs presents additive, synergistic, or antagonistic effects on A549 cells, I stimulated those cells with sub-optimal doses of PDMF (10-125 μM) and sub-optimal doses of TKIs (nilotinib, bosutinib, dasatinib and ponatinib; 1, 4, 0.05, 0.5 μM, respectively). I found that the combination regimens synergistically suppressed cell proliferation of A549 cells, and that nilotinib showed the most potent cytostatic action upon co-stimulation with PDMF. Flow cytometric analysis revealed that co-stimulation with nilotinib and PDMF induced G_2/M cell cycle arrest in sub-optimal PDMF doses (10-50 μM), and that this combination newly triggered G_1 cell cycle arrest in higher PDMF dosages (50-125 μM). Mechanistically, co-stimulation with a low dose (10 μM) of PDMF with nilotinib synergistically decreased protein levels of CDK1 and cyclin B1, that form a hallmark cyclin/CDK complex involved in the G_2/M cell cycle progression, whereas a high dose (125 μM) of PDMF synergized with nilotinib to induce CDK inhibitor $p21^{Cip1/Waf1}$, a possible negative regulator of the G_1 cell cycle checkpoint. I also found that co-administration with nilotinib and PDMF significantly suppressed *in vivo* tumorigenicity of A549 cells in athymic nude mice. These results suggest that co-treatment with PDMF and anti-cancer TKIs shows a synergistic tumor suppressive potency, and that the combination regimen would be useful for the development of new therapeutic strategies against lung cancers.

Chapter 4. General conclusion

In the present study, I have shown that a new *P. frutescens*-derived methoxyflavanone (PDMF) represents a useful tumor preventive phytochemical that triggers G_2/M cell cycle arrest and apoptosis in a p53-dependent manner. Furthermore, I also have demonstrated that co-administration of PDMF and anti-cancer TKIs achieves a prominent tumor suppressive potency *in vitro* as well as *in vivo*. This study not only confirms the beneficial use of plant-derived flavanone derivatives in cancer chemoprevention, but also provides insight into their clinical applications.