Filipendula vulgaris restrains the HIPPO oncogenic axis YAP/TAZ/TEAD in mesothelioma progression

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Abstract

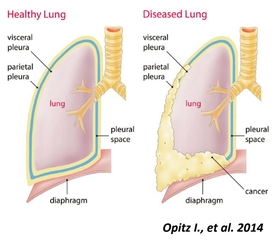
Over the last decade, new therapeutic approaches have not significant improved the survival of Malignant Pleural Mesothelioma (MPM), an aggressive tumor arising from the lining mesothelial surfaces. A limited number of genes are frequently mutated in MPM and some of them are involved in the HIPPO tumor suppressor pathway. This suggests that it might exert a critical role in the development and progression of MPM. Therefore, the therapeutic targeting of the HIPPO pathway holds the potential to improve the clinical management of MPM patients.

Methods: The anticancer effects of Filipendula vulgaris, a natural phytonutrient, were characterized "in vitro" and "in vivo" in MPM cell lines. At the molecular level, we used two genome wide analyses such as metabolomic profiling and phosphoarray analysis. Ubiquitin assay was used to evidence the strong impact of Filipendula vulgaris treatment on the stability of the two key transducers of the HIPPO pathway, YAP and TAZ.

Results: In this study, we found that Filipendula vulgaris significantly impaired mesothelioma tumor progression, at least partially, through the silencing of YAP and TAZ oncogenic activities. This is mainly due to YAP and TAZ ubiquitination induced by Filipendula Vulgaris treatment.

Conclusions: Our findings unveil Filipendula as a promising natural compound targeting the HIPPO pathway that might have important chemo-preventive and anticancer implications for MPM management.

Introduction



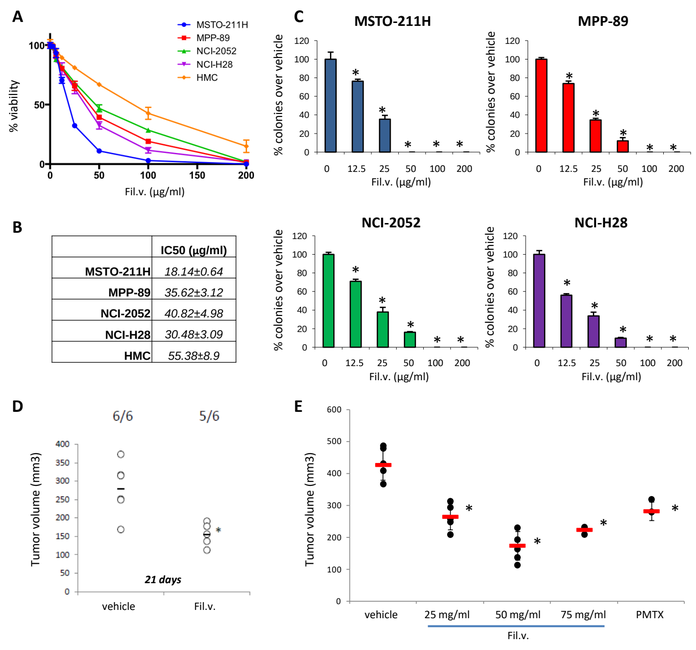
Opitz I., et al. 2014

Malignant pleural mesothelioma (MPM) is a rare and local aggressive tumor arising from lining pleural or peritoneal mesothelial cell surfaces. The chronic inhalation of asbestos nano-particles induces a local inflammatory infiltration of the mesothelial surfaces containing macrophages that internalize them and activate pro-inflammatory responses inducing a release of cytokines and chemokines to pleural and lung tissues. Other factors, including Erionite and radiation exposure, SV40 infection and genetic factors have been considered as concomitant causes of the mesothelioma's development. Platinum based-chemiotherapy combined with the folate antagonist Pemetrexed represent the conventional treatment. Unfortunately, MPM exhibits resistance to this chemotherapy. Treatment of MPM with Cisplatin plus Pemetrexed, in fact, increases patient survival by only a few months. Actually, there is no second line standard therapy for MPM. Consequently, there is an urgent need of new therapeutic options for such an orphan disease, whose peak of incidence is expected to rise in the next decade. Dietary phytochemicals for the absence of adverse events and their ability to target multiple signaling pathways have been considered appealing as coadjutants in anticancer therapies. We and others have previously shown that some phytonutrients can exert anticancer effects in mesothelioma cell lines by interfering with given oncogenic and tumor suppressor pathways. Mesothelioma is one the very few tumors that has also been found associated with different HIPPO mutations. Accordingly to the COSMIC database, the genes that are most frequently mutated in MPM are loss of function mutations of tumor suppressor genes such as cyclin dependent kinase inhibitor 2A gene (CDNKN2A), TP53, Neurofibromin 2 (Merlin) gene (NF2) and BRACA1 associated protein gene 1 (BAP1) . Here we show that Filipendula Vulgaris extract significantly impairs mesothelioma progression "in vitro" and "in vivo", at least partially, through the silencing of YAP and TAZ oncogenic activities.

Filipendula vulgaris Dropwort, Meadowsweet

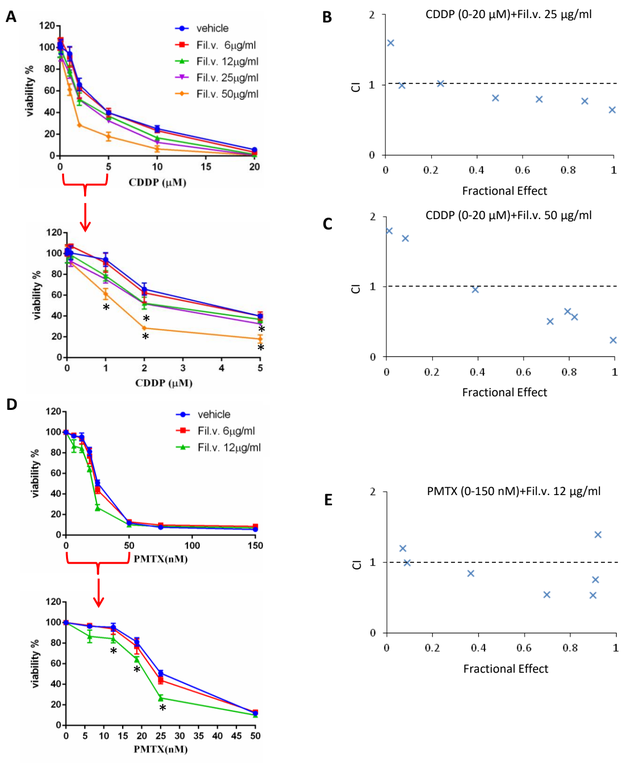
Results

Filipendula vulgaris extract affects in vitro and in vivo mesothelioma tumor growth



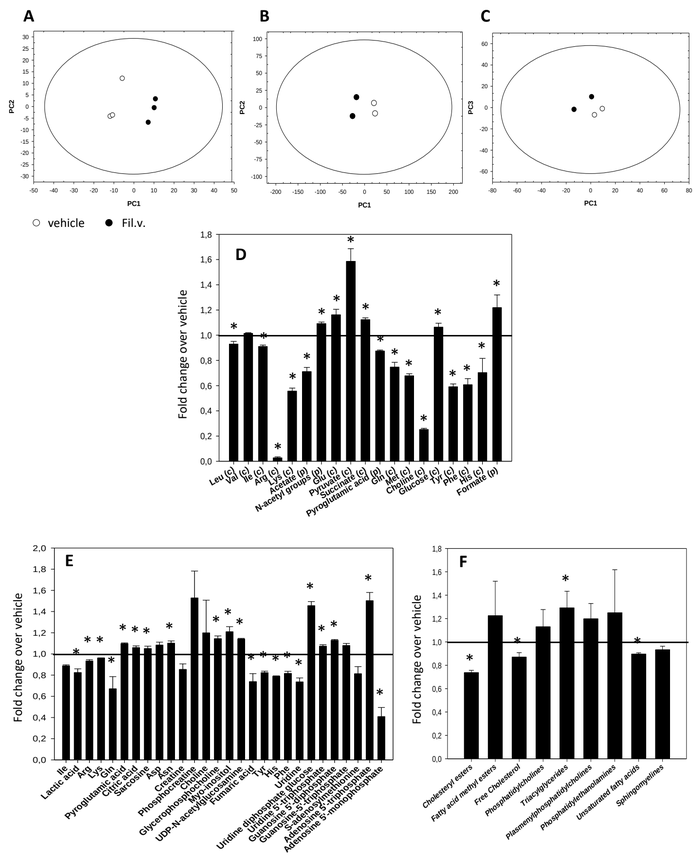
Filipendula vulgaris extract affected MPM cells viability in a dose dependent manner (A). HMC cells were more resistant to the Filipendula vulgaris-induced growth inhibition compared to the MPM cells (A-B); thereby suggesting that the extract is less toxic for mesothelial cells than for its malignant counterparts. Moreover, the natural extract inhibited in a dose dependent manner the capability of MSTO-211H MPP-89, NCI-2052 and NCI-H28 cells to form colonies (C). Filipendula treated cells engrafted less compared to the vehicle treated ones (D). In addition, three weeks of treatment with the Filipendula extract inhibited tumor xenograft growth in a dose dependent manner (E). Mice treated with Pemetrexed showed a tumor growth reduction similar to those treated with the natural compound (E).

Filipendula vulgaris treatment sensitizes MSTO-211H cells to MPM conventional chemotherapy



Pre-treatment of MSTO-211H cells with different doses of Filipendula vulgaris extract (6-50 µg/ml) for 24 hrs sensitized MSTO-211H mesothelioma cell lines to CDDP (0-20 µM) (A-C) or PMTX (0-150 nM) (D-E) -induced cell killing.

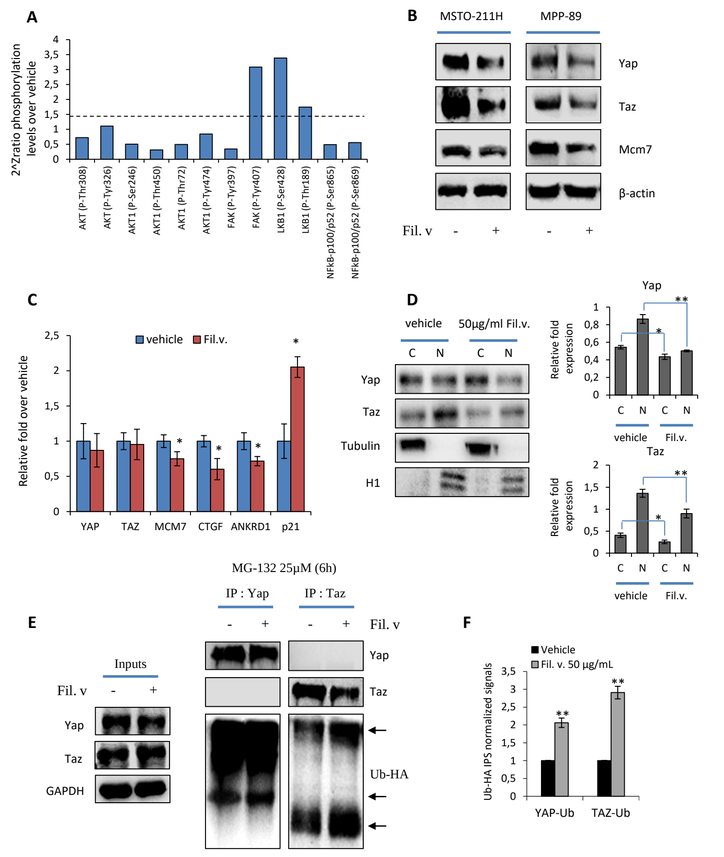
Filipendula extract triggers multiple pathways in mesothelioma cells



The effectiveness of a natural agent resides in its complexity. Therefore, to discover the signaling pathways involved in the Filipendula extract antitumoral activity, we applied two different high-throughput technologies. First, we explored the changes of extracellular and intracellular metabolites in MSTO-211H cells by using ^{1}H-NMR-based metabolomics (A-C). A statistically significant separation between the treated and vehicle samples was observed on PC1 for media samples (p=0.001) and hydrophilic cell extracts (p=0.01) and on PC1/PC3 plane for lipophilic cell extracts (p=0.017). Analysis of the loading plots on the discriminant PCs revealed that Filipendula extract mainly affects the valine, leucine and isoleucine degradation, arginine and proline metabolism, alanine, aspartate and glutamate metabolism, glycine, serine and threonine metabolism, citrate cycle (TCA cycle), oxidative phosphorylation, glutamine and glutamate metabolism, glutathione metabolism, pyruvate metabolism, glycolysis/gluconeogenesis, pentose phosphate pathway, tyrosine metabolism, histidine metabolism, phenylalanine metabolism, purine metabolism, pyrimidine metabolism, carbon metabolism and fatty acid metabolism (D-F).

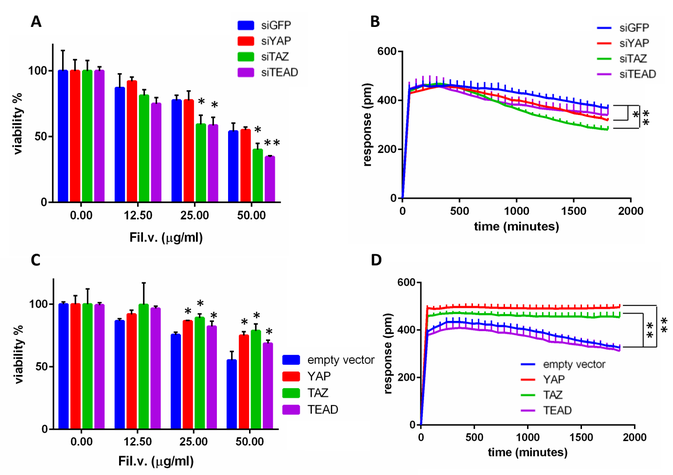
Second, protein lysates of MSTO-211H cell treated either with vehicle or Filipendula extract (50 µg/ml) for 24 hrs were loaded on a phospho-antibody array containing 1318 antibodies representative of over than 30 signaling pathways involved in cancer (A). In silico prediction analysis revealed that the Filipendula extract modified the expression of proteins involved in cancer pathways (B). The mammalian target of rapamycin (mTOR) signaling pathway ranked as most significant compared to the other pathways (C)

Filipendula vulgaris impairs YAP and TAZ activity in MSTO-211H cells



Several evidences indicate that AKT, NF-KB and FAK activate, while LKB1 inhibits, the oncogenic transcriptional co-activators YAP and TAZ, the final effectors of the Hippo signaling transduction pathway. Intriguingly, Filipendula treatment, in agreement with the observed inhibition of mTOR, induced a reduction in the phosphorylation levels of several AKT residues, NF-Kb and FAK (A). Conversely, we observed a strong increase of LKB1 phosphorylation (A). Notably, Filipendula vulgaris treatment did not affect YAP/TAZ transcript levels (C), it did affect YAP and TAZ protein abundance (B) both in the citosol (D) that in the nuclei (D) thereby suggesting a post-translational regulatory mechanism. Strikingly, we observed that Filipendula extract induced an increased ubiquitination and a consequently degradation of both YAP and TAZ (E-F).

YAP/TAZ/TEAD axis favors Filipendula vulgaris extract anticancer activity



Cells expressing low levels of TAZ or TEAD were more prone to Filipendula induced cell killing than control cells (A, B). Conversely, we found that elevated expression of YAP or TAZ or TEAD rendered MSTO-211H cells more resistant to Filipedula extract treatment than control cells (C, D).

Conclusions

Our findings document that Filipendula vulgaris exerts its anticancer effects on MPM, at least in part, curtailing the oncogenic activities of the YAP/TAZ/TEAD Hippo pathway. Both metabolomic that phospho-proteomic analisys, indeed, unveil the role of the PI3K-AKT-mTOR and metabolics pathway in the Filipendula-mediated inhibition of HIPPO oncogenic pathway. Accordingly, Filipendula vulgaris extract might have important chemo-preventive and anticancer implications for MPM management.

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