Pro-apoptotic and anti-tumoral activities of vinyl disulfides from *Ferula assa-foetida L.* in melanoma

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Melanoma is the most common form of skin cancer and it is responsible for the majority of skin cancer deaths. While early-stage melanomas can usually be effectively treated with surgery, more advanced tumors have a high metastatic potential and are notoriously resistant to conventional cancer therapies such as radiation and chemotherapy. Despite significant advances in understanding of melanoma biology and pathogenesis, and the recent success in developing targeted therapies for melanoma (Shtivelman E. et al. 2014, Tsao H. et al. 2012), the prognosis of the disease remains poor, therefore the search for new agents for its treatment is of great importance.

Ferula assa-foetida L. is a major source of asafoetida, a foul-smelling gum-resin of dietary and medicinal relevance. Asafoetida is characterized by an unpleasant sulfurous odor, reminiscent of garlic, rotten meat and sweat (Mahendra P. and Bisht S. 2012).

In this study, we tested the potential effect of several vinyl disulfide compounds, isolated and purified from asafoetida, on the proliferation of human melanoma cell lines. Toward this goal, the effects of the vinyl disulfides were evaluated *in vitro* on four different human melanoma cell lines: A375, SK-Mel-5, SK-Mel-28 and PES4. We found that all the compounds tested, decreased the proliferation rate of melanoma cells with different potency. Interestingly, the largest degree of cell proliferation inhibition (24%, 57% and 70%, respectively) was observed in melanoma metastatic cells PES43 exposed to increasing doses of RTFA 16C (10-30-100μM) for 72h. Thus, this cell line was selected for the subsequent molecular studies.

Cytofluorimetric analysis with annexin V/PI staining demonstrated that the anti-proliferative effect of RTFA 16C was due to its ability to induce apoptosis in PES43 cells. Western blot analysis further proved that this compound caused a time-dependent activation of Caspase 3 and the cleavage of its substrate poly (adenosine diphosphate-ribose) polymerase (PARP).

The apoptotic machinery can be controlled, at least in part, by NF-κB, which regulates transcription of the Bcl-2 family members (Ben-Neriah and Karin, 2011). Several reports have shown that in melanoma the constitutive activation of NF-κB confers tumor survival capacity and avoidance of apoptosis (Ueda and Richmond, 2006). Thus, we have hypothesized that the RTFA 16C induction of apoptosis was associated with suppression of NF-κB activation. Western blot analysis carried out on the nuclear extracts of PES43 cells incubated with RTFA 16C (30μM) for 3-6-24h revealed a time-dependent reduction of nuclear translocation and activation of p65. Moreover, this effect was also supported by the finding that the treatment with RTFA 16C decreased the expression of the anti-apoptotic proteins c-FLIP, XIAP and Bcl-2, that are transcriptionally regulated by NF-κB. In order to better define the mechanism through which this latter effect is achieved, we investigated the possible involvement of the MAPK/ERK and PI3K/AKT pathways, two of the most frequently deregulated pathways in melanoma (Hodis et al., 2012). Western blot analysis revealed that the treatment of PES43 cells with RTFA 16C (30μM) inhibited the phosphorylation and activation of both AKT and ERK proteins at the time points considered (3-6-24h).

Finally, to corroborate these results obtained in vitro, we injected B16/F10 mouse melanoma cells into tail veins of C57BL/6 mice to induce lung metastasis. In these mice, the RTFA 16C (50mg/kg, orally administrated) significantly reduced metastatic foci of lung surface when compared to control group.

In conclusion, all these findings suggest that RTFA 16C plays an important role in inhibiting the cancer metastasis and could represent an important lead compound for the development of new anti-metastatic agents.