

## **ANTI-CANCER PROPERTIES OF ERUCIN, AN ISOTHIOCIANATE PRESENT IN MANY EDIBLE PLANTS OF BRASSICACEAE FAMILY, ON HUMAN PANCREATIC ADENOCARCINOMA CELLS (ASPC-1): IS H<sub>2</sub>S THE HIDDEN PLAYER?**

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Erucin (4-methylthiobutyl isothiocyanate) derives from the enzymatic hydrolysis of the glucosinolate glucoerucin present in *Eruca sativa* Mill. seeds belonging to the Brassicaceae family. Erucin shows close structural analogies with sulforaphane (SFN), an isothiocyanate derived from glucoraphanin, a glucosinolate present at low amount also in the *Eruca* genus and in other Brassicaceae, and well-known for its chemopreventive properties. Indeed, it is well established that isothiocyanates exert anti-cancer properties on many tumor types (liver, breast, bladder, lung and pancreas)(Fofaria et al., 2015) and in particular SFN is actually employed in a pilot randomized controlled clinical trial in advanced pancreatic cancer (Lozanovski et al., 2014). In this work, the anti-cancer properties of Erucin on human pancreatic adenocarcinoma cells (AsPc-1) were examined and the possible role of its hydrogen sulfide (H<sub>2</sub>S)-releasing properties (Citi et al., 2014) was investigated. Indeed, also H<sub>2</sub>S is recognized as anti-proliferative agent in melanoma and intestinal cancer (Ianaro et al., 2016). Methods: Natural Erucin was obtained directly from glucoerucin purified from *E. sativa* seeds and its identity was checked in GC-MS using a commercial standard; its purity was established in GC-FID. Anti-proliferative effect at 72h on AsPc-1 viability was evaluated; then, erucin H<sub>2</sub>S-donor properties were also verified both in cell-based and cell-free assays. Moreover, the erucin effects on cell cycle, mitochondrial potential and caspase 3/7 were investigated. Finally, an evaluation of a possible involvement of the mitogen-activated protein kinase (MAPK) pathway, and in particular of ERK1/2, was carried out. Results: Erucin inhibited AsPc-1 viability with a potency index (IC<sub>50</sub>) of about 30μM and its ability to release H<sub>2</sub>S was evident both in an in vitro assay buffer and on the AsPc-1 cells. Moreover, Erucin evoked a reduction of cell cycle G<sub>0</sub>/G<sub>1</sub> phase, due to an enlargement of both S and G<sub>2</sub>/M phases, and increased the number of cells exhibiting depolarization of mitochondrial potential as sign of early apoptosis. These effects were coupled with an increase of the number of apoptotic cells, recorded by the evaluation of Caspase3/7, as marker of more advanced stage of apoptosis, in AsPc-1 treated with erucin. Finally the investigation of a possible mechanism of action highlighted a significant reduction of p-ERK1/2 activation suggesting an involvement of MAPK pathway in Erucin anti-proliferative effect on AsPc-1. Conclusion: This study demonstrated that Erucin was able to inhibit AsPc-1 cell viability altering cell cycle, inducing mitochondrial depolarization and apoptosis. A significant decrease of p-ERK1/2 activation accounted for an involvement of MAPK pathway in Erucin anti-cancer effect. Moreover, the H<sub>2</sub>S-donor property of Erucin may represent a reliable explanation of its anti-cancer activity that is worthy to be further investigated.

### References

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