



Cutting down on lung cancer: Ecliptasaponin A is a novel therapeutic agent

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The sacred lotus *Eclipta prostrata* or commonly known as “false daisy”, has long been used in traditional Chinese medicine for a diverse range of ailments, from treating alopecia to liver enlargement or asthma (1). *E. prostrata* contains a multitude of chemical compounds including triterpenoids, steroids, flavonoids, coumestans, saponins and other volatile oils (2). According to Chinese Pharmacopoeia, the primary effect of the dried aerial part of *E. prostrata* can “cool” the blood and serves as a coagulating agent (3). Another reported therapeutic attribute is the anticancer properties of *E. prostrata* extracts (4,5). However, the identification of the key components responsible for this antitumoral activity and mechanism of action remain unclear. A new study from *Annals of Translational Medicine* now reports that one of the extracted compounds, Ecliptasaponin A (ES), has anticancer effects on non-small cell lung cancer (NSCLC) (6).

The pentacyclic triterpenoid ES is structurally identical to Eclalbasaponin II (E-II) (1). E-II has previously shown to exhibit antiproliferative effects in hepatic stellate cells (7). In human ovarian cancer cells, E-II induces autophagic and apoptotic cell death through the regulation of JNK, p38 and mTOR signaling (8). The recently published study in *Annals of Translational Medicine* suggests that ES can be a promising therapeutic agent in lung cancer (6). Through a series of canonical experiments, Han *et al.* demonstrated a potent inhibition in lung cancer cells viability after ES treatment. The paper dug deep into the molecular mechanisms to further elucidate ES-mediated activation of

apoptosis and autophagy, driving cell death.

For their studies, the authors used H460 and H1975 NSCLC cell lines. NSCLC accounts for ~85% of all cases of lung cancer including adenocarcinoma, large-cell carcinoma and squamous-cell carcinoma (9). ES inhibited cell growth of both cell lines in a dose- and time-dependent manner. ES treated cells also exhibited impaired ability to form colonies in soft agar. To test the cancer killing effect of ES *in vivo*, H640 cells were implanted subcutaneously in nude mice. Consistent with the *in vitro* results, ES treatment resulted in reduced tumor burden and tumor weight. Importantly, ES did not affect mice body weight, suggesting the compound is overall well tolerated.

With these data in hand, the authors proceed to identify and characterize the modality of death that lung cancer cells underwent after being treated with ES. Most anticancer drugs currently used in clinical oncology exploit the apoptotic signaling pathways to trigger cancer cell death (10). Consistent with this, Han *et al.* observed the hallmark signs of apoptosis including cell shrinkage, condensed nuclei, and an overall cell number reduction post-treatment. Treatment with ES elicited both the intrinsic and extrinsic apoptotic pathways as evidenced by cleavage of caspase-3, -8 and -9. Fluorescent-based visualization of microtubule-associated protein 1 light chain 3 (LC3) also revealed the existence of autophagic activity. Autophagy manifested with the formation of vesicles (autophagosomes) that engulf cellular macromolecules and organelles and culminates with lysosomal degradation (11).

ES treatment also altered the expression of other autophagy-related proteins such as Beclin-1 and P62/SQSTM1 in both cell lines.

Regulated modes of cell death such as autophagy can act in concert with apoptotic signaling to induce cell death (11). However, autophagy activation preceding cell death does not suggest causality; autophagy can also be a rescue mechanism to escape apoptosis (12,13). That being said, does the ES-mediated autophagy contribute to apoptosis in NSCLC cells or is it just a salvage pathway? Han *et al.* addressed this question by conducting combinatorial treatments of ES with classic autophagy inhibitors: 3-methyladenine (3-MA) or chloroquine (CQ). These drugs inhibit autophagy at different stages: 3-MA blocks autophagy at early phases whilst CQ affects late autophagy (14). Treatment with either inhibitors with ES resulted in a reduction of apoptotic events, confirming that autophagy contributes to ES-induced cell death in NSCLC cells.

Finally, to identify potential signaling pathways involved in ES-induced apoptosis and autophagy, the authors analyze several phospho-kinases belonging or related to the mitogen-activated protein kinase (MAPK) pathway. Apoptosis signal-regulating kinase 1 (ASK1) is a member of the MAPK kinase kinase (MAP3K) family that activates downstream MAPKs, c-Jun N-terminal kinases (JNKs) and p38, and plays a key role in various stress responses, including cell death (15). The authors found that ES treatment induced ASK1, JNK, AKT and p38 phosphorylation whereas p-ERK levels were reduced. They also investigated the JNK signaling pathway as previous literature shows its involvement in regulating multiple modalities of cell death (16). Combinatorial treatment of ES with specific AKS1 and JNK1 inhibitors (GS-4997 and SP600125, respectively) reduced the number of apoptotic events and expression of cleaved caspase-3 compared to ES-treatment alone. The JNK1 inhibitor also prevented ES-induced autophagic activity as shown by LC3 levels. Taken together, these results demonstrate that ES induces apoptosis and autophagy through activation of the JNK pathway.

Conclusions and perspectives

Lung cancer is the leading cause of cancer-related deaths among men and women. In 2017, lung cancer caused more deaths than breast, prostate, colorectal, and brain cancers combined (17). Despite significant

progress in the oncological management of lung cancer in recent years, the clinical outcomes are still not at a satisfactory level and the need for better therapeutic regimens is clear. This study provides a new strategy for lung cancer treatment that involves the use of the phytochemical Ecliptasaponin A. Phytochemicals and derivatives have been widely used in chemotherapy of cancer patients (e.g., taxol analogs, vinca alkaloids, podophyllotoxin analogues) (18) and several of these compounds are being actively evaluated for use as adjuvants in anticancer therapies (e.g., immunomodulation) (19). The study by Han *et al.* opens the door to the evaluation of other anticancer activities ES may have, that could easily expand its cytotoxic effect on cancer cells. Combination therapy involving ES with standard of care chemotherapy drugs should be tested, as ES treatment might sensitize NSCLC cells to other therapy and/or help to overcome drug resistance. The use of omics approaches in future studies will help to get a better insight and accelerate the understanding on ES potential pleiotropy, molecular targets and hierarchical mechanisms.

Low lung cancer survival rates reflect the large proportion of patients (57%) diagnosed with metastatic disease (5-year survival for stage IV is 5%) (20). *E. prostrata* extracts have shown to attenuate migration and invasion of a variety of cancer cell lines and endothelial cells (21,22). Futures studies will need to address whether ES plays any role in cancer metastasis and explore its role in advanced lung cancer. This compound also provides a new opportunity in chemoprevention research. ES has been reported to exert preventive effects in bleomycin-induced pulmonary fibrosis in mice (23). *E. prostrata* components also showed to exhibit protective properties in human bronchial epithelial cells in which oxidative stress injury and inflammation responses are induced by continuous cigarette smoke exposure (24). These data suggest that efforts should be directed towards investigating the role of ES as an agent with the potential of preventing disease development and progression. ES is extracted from what farmers consider a weed and its use in Asian traditional medicine can be tracked back to 3,000 year ago. Modern medicine could now benefit from a weed that grows at our fingertips. Let's teach an old drug new tricks and use it to our advantage.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/atm.2020.04.51>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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