Characterizing the molecular mechanism of the multifunctional antitumor compound withaferin A in a multiple myeloma model

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Despite progress and success in chemotherapy, many types of cancer remain largely incurable. Most often this is due to the development of (multi)drug resistance, a phenomenon characterized by cancer cells who manage to withstand the most powerful chemotherapies available during an acute anticancer response. In this respect, interest has recently focused on multifunctional chemotherapeutic compounds that simultaneously target a series of pathways, and by extend networks, peculiar to cancer development. Withaferin A (WA), a steroidal lactone from the plant *Withania Somnifera*, is such a multifunctional compound with promising antitumor responses. To examine the multifunctional antitumor potential of WA, we used a multiple myeloma (MM) disease model which is characterized by subsequent phases of remission and relapse leading towards (multi)drug resistance and eventually patients death.

A MM1R cell line was SILAC labeled and treated with- or left without WA to examine the WA effects on the cancer proteome. Two main approaches consisted of a differential expression experiment resulting in 121 upregulated and 78 downregulated (≤ 2 fold difference) proteins and a chemoproteomics approach resulting in 199 direct protein-WA interacting partners. Combining these data with the Ingenuity Pathway Analysis (IPA) database resulted in many influenced canonical pathways, molecular functions and diseases including:

- Protein ubiquitination
- NRF2 mediated oxidative stress response
- Waldenstrom's macroglobulinemia

Further validation of the high throughput data was based on these IPA output and confirmed that WA causes an accumulation of ubiquitinated proteins, most likely as a result of targeting the proteasome. Also, the upregulation of the NRF2 stress response gene Heme Oxygenase 1 (HMOX1) after WA treatment was confirmed, suggesting that WA probably puts the already stressed cancer cells under additional (fatal?) stress. Interestingly, 12 proteins were assigned to involve Waldenstrom's macroglobulinemia and 9 out of those 12 were influenced by WA in a way that would be beneficial for the disease outcome. For example, we showed by Western blot that annexin A4 (ANXA4), a calcium/phospholipid-binding protein which promotes membrane fusion, is downregulated after WA treatment, whereas it is seen upregulated in the disease. Other proteins within this Waldenstrom's macroglobulinemia "network" will be validated in the near future, as well as other interesting proteins selected from the IPA analysis, which will hopefully result in an elegant method of circumventing (multi)drug resistance and defeating MM.