

## DOCTORAL THESIS

### Exploration of the novel anticancer mechanisms of medicinal compounds involving calpain and S100A4 in the treatment of colon cancer

Wang, Yue

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## ABSTRACT

The incidence of colon cancer in Hong Kong and worldwide is on a rising trend, while its metastatic development is the leading cause of cancer-related deaths. Understanding the molecular mechanisms of how tumors progress and metastasize to secondary sites, at both biological and genetic levels, could enable us to identify potential molecular targets in drug development. In the present study, we explored how manipulation of signaling pathways by targeting calpains and S100A4 could facilitate the development of anti-tumor and anti-metastatic drugs.

With respect to the targeting on calpains, it was discovered that total Astragalus saponins (AST) and cryptotanshinone (CPT) are effective anti-cancer agents that elicit the endoplasmic reticulum (ER) stress response. They act by upregulating the expression of glucose-regulated protein (GRP) 78, leading to the initiation of apoptosis when the ER recovery process begins to fail. In particular, CPT caused rapid and sustained increase in cytosolic calcium in colon cancer cells that was accompanied by early GRP78 overexpression. The increase in cytosolic calcium was blocked by pre-treatment of BAPTA-AM through depletion of the ER calcium store. In consistent with these, we also confirmed that CPT significantly increased calpain activity, which could be blocked by calcium chelator or calpain inhibitors. Furthermore, a dynamic interaction between GRP78 and calpain under ER stress was unveiled during AST or CPT exposure. The degree of

association was increased following prolonged ER stress, and suppressed either as the ER recovery process failed or with the presence of calpain inhibitors. Besides, inhibition of calpain activity suppressed NF- $\kappa$ B activation (a consequence of ER stress) and substantially enhanced the effects of CPT to promote apoptosis. More importantly, it was confirmed that the effects of calpain inhibitors to sensitize colon cancer cells to ER stress-associated apoptosis are p53-dependent. The anti-tumorigenic effects of CPT were further demonstrated *in vivo* in xenografted nude mice by targeting calpains and in combination with calpain inhibitors.

The study investigating drug targeting on S100A4 in both *in vitro* and *in vivo* models had shown that the pharmacological store-operated calcium channel blocker would suppress S100A4-mediated migration by weakening extracellular S100A4-mediated calcium responses. The effects on S100A4-induced metastasis formation were confirmed *in vivo* with reduced splenic tumor volume and decreased number of liver metastases. These results have provided new insights to correlate between S100A4 and calcium signaling, making an important step forward in characterizing the dependence of calcium homeostasis in the process of metastasis, providing a novel strategy for S100A4-mediated metastasis.

In summary, this thesis has explored the anti-cancer mechanisms of novel medicinal compounds via targeting calpains or S100A4 in the treatment of colon cancer, which could facilitate future establishment of effective

medicinal compounds in the treatment of metastatic colon cancers with known molecular targets.

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