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Development of Anticancer Agents - What Assessment Do We Schedule for Mechanism Study

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Malignancy is the leading cause of death in Taiwan and a lot of developed and developing countries. Our lab is devoted to preclinical development of anticancer agents. We have set up a screening system, sulforhodamine B assay (SRB assay) according to National Cancer Institute in USA, and have been doing a large scale of screening tests to discover potential agents against cancers. In recent years, we have screened more than 3000 samples from both chemically synthetic compounds and natural products. The mechanisms of anticancer abilities of the effective compounds were studied in this program. The detection of cell-cycle progression is the next step to SRB assay since numerous cellular stresses may cause an arrest of cell-cycle progression. From the abnormal signals in checkpoint arrest, the primary insult by anticancer agents can be traced. Take antroquinonol as an example. Antroquinonol that was isolated from *Antrodia camphorate*, a well-known Traditional Chinese Medicine for treatment of liver diseases, displayed effective anticancer activity against numerous HCC cell lines. Antroquinonol arrested cells at G1 phase associated with down-regulation of G1-regulator proteins. Antroquinonol induced the assembly of tuberous sclerosis complex (TSC)-1/TSC2, leading to the blockade of cellular protein synthesis through inhibition of protein phosphorylation including mTOR (Ser^{2448}), $p70^{S6K}$ (Thr^{421}/Ser^{424} and Thr^{389}) and 4E-BP1 (Thr^{37}/Thr^{46} and Thr^{70}). Furthermore, the AMPK activity was elevated by antroquinonol. Compound C, a selective AMPK inhibitor, significantly reversed antroquinonol-mediated effects suggesting the crucial role of AMPK. In summary, the data suggest that antroquinonol displays anticancer activity against HCCs through AMPK activation and inhibition of mTOR translational pathway, leading to G1-arrest of the cell-cycle and subsequent cell apoptosis.

The mechanisms that act on tubulins play a major part in our discovered potential compounds. The compounds, in spite of diverse structures, are capable of blocking tubulin polymerization. The revealed common signaling pathway is the induction of Cdk1 activity in mitotic arrested cells, leading to phosphorylation and degradation of anti-apoptotic Bcl-2 family protein members, including Bcl-2, Bcl-xL and Mcl-1. Accordingly, it induces the loss of mitochondrial membrane potential, formation of apoptosome, activation of caspase-9 and downstream executor caspase-3, and an ultimate destiny - apoptosis. A niche of mechanism study is the discovery of potential cellular targets that can be directed for further approaches.