SUMMARY:

Microtubules are important cellular targets for anticancer therapy because of their key role in mitosis. Microtubule targeting agents (MTAs) such as taxanes, vinca alkaloids, and epothilones stabilize or destabilize microtubules, thereby suppressing microtubule dynamics required for proper mitotic function, effectively blocking cell cycle progression and resulting in apoptosis. In spite of their antitumor activity, innate or acquired drug resistance to MTAs such as the taxanes is common, limiting their overall clinical efficacy. Further insight into the mechanisms of action of microtubule-targeting drugs could lead to the discovery of novel agents that may provide higher efficacy with limited toxicity and help overcome resistance to conventional MTAs. Moreover, continued investigation of the mechanisms of action of microtubule-targeting drugs, and exploring new treatment strategies that reduce side effects and circumvent drug resistance may provide more effective therapeutic options for cancer patients.

The aim of this work was to study the role of p53 in the apoptotic pathways initiated in cancer cells in response to tubulin network disorganization. The available MTAs are known to cause damage to the normal cells and are therefore limited in their use in clinical settings. A novel tubulin interfering agent fenbendazole (methyl-N-(6-phenylsulfanyl-1H-benzimidazol-2-yl) carbamate) has been identified which was found to display mild tubulin interfering activity in mammalian cells as compared to established microtubule binding agents like colchicine, nocodazole and taxol. Fenbendazole (FZ) is a safe, widely used anthelmintic benzimidazole in animals previously known to have tubulin disrupting activity selectively in parasitic cells. Experimental results from this study show that FZ causes an alteration in the mammalian microtubule network and exhibits a potent growth inhibitory activity against cancer cells in vitro and in vivo, partly mediated through the activation of p53 protein, while sparing normal cells. Remarkably, unlike other microtubule depolymerizing agents colchicine, nocodazole and vincristine, FZ did not cause
a loss of acetylated tubulin. Acetylated tubulin has been reported to bind to p53 and result in its enhanced nuclear accumulation in response to stress. Furthermore, FZ treatment caused a marked induction of p53 due to inhibition of its degradation, decreased mitochondrial membrane potential resulting in release of cytochrome c from mitochondria and activation of downstream caspases that eventually led to cell death. Significant inhibition in tumor growth was observed upon oral administration of FZ in a xenograft nude mice model. Using fluorogenic substrates, it was found that FZ treatment leads to the inhibition of proteasomal activity in human lung cancer cell lines. Succinyl-Leu-Leu-Val-Tyr-MCA, Z-Leu-Leu-Glu-7-amido-4-MCA and Boc-Gln-Ala-Arg-7-amido-4-MCA fluorescent derivatives were used to assess chymotrypsin-like, post glutamyl peptidyl hydrolyzing and trypsin-like protease activities respectively. Non small cell lung cancer (NSCLC) cells transiently transfected with an expression plasmid encoding pd1EGFP followed by FZ treatment showed an increased accumulation of the green fluorescent protein in the cells due to an increase in its half-life. In addition to p53, a number of apoptosis regulatory proteins that are normally degraded by ubiquitin-proteasome pathway, like cyclins, p53 and IκBα were found to be accumulated in FZ treated cells. Correspondingly, there was a reduction in NFκB activity as shown by EMSA as well as immunofluorescence experiments. Thus, it appeared that FZ resulted in impairment of proteasome function leading to accumulation of ubiquitinated form of p53 and as a result of increased accumulation of activated p53, cells were susceptible to apoptotic cell death. In addition, distinct ER stress associated genes like GRP78, GADD153, ATF3, IRE1α and NOXA were also induced in these cells. Thus, induction of ER stress was one of the consequences of FZ exposure to cancer cells.

Further, efforts were made to dissect out the mechanism of preferential toxicity of the drug towards cancer cells. Cancer cells are known to have altered metabolism, specifically, they are known to thrive on the glycolytic pathway
even in the presence of aerobic conditions - a phenomenon termed as “aerobic glycolysis” or “Warburg effect”. Taking into consideration this distinguishing feature of cancer cells, experiments were done to determine if FZ had any effect on glucose metabolism of cancer cells. Using a fluorescent derivative of glucose, 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG), it was found that FZ could inhibit glucose uptake and resulted in increased ROS production in NSCLC cells. This was further confirmed by glucose consumption assay following FZ treatment. FZ exposure reduced the expression of Glut transporters as well as hexokinase (HK II), a key glycolytic enzyme. In addition, 24h treatment with FZ resulted in reduced lactate production suggesting that FZ induced cancer cell death may be due to reduced availability of glucose to cells as a result of altered metabolism, which may be linked to p53 induction. Since cancer cells mostly thrive on increased glycolysis for generation of ATP, impairment of this pathway could lead to fatal consequences specifically for these cells.

Altogether, p53 stabilization associated with the activation of other cell signaling pathways following FZ treatment led to preferential apoptosis of cancer cells both in vitro and in vivo. This may have a significant clinical implication.