SUMMARY

Prostate cancer is a common malignancy in men and to date no adequate therapy is available for the disease. Steroid hormones, testosterone and Dihydrotestosterone (DHT), are known to be involved in the normal growth and development of the prostate gland in men. Now it is believed that the testosterone-DHT pathway mediated through 5 alpha reductase pathway perhaps plays a significant role in the progression and aggressiveness of the prostate cancer.

As implied earlier, high level of testosterone drives the prostate cancer cells to grow. Conversion of testosterone to DHT could be blocked by reduced level of SRD5A2 or by blocking the step that make testosterone available to SRD5A2. I have identified (FZ), a novel non-toxic compound, already known for its anthelmints activity, has its affinity for testosterone as indicated by in silico study. To validate this observation, mice were treated with the compound orally and their serum testosterone level was examined. Treatment with this drug resulted in an increased accumulation of serum testosterone in these animals. Interestingly, the expression of SRD5A2 was reduced considerably in our in vitro cell culture system after this drug treatment. It was further investigated whether the effect of this drug was toxic to the normal cells. To our surprise we found that the drug was not toxic to normal cells obtained from primary cell culture from human or mouse tissues.

Consequently, treatment of the cancer cells with FZ resulted in severe growth inhibition both in vitro and in vivo. An effort has been directed to understand the molecular mechanism of this drug action using molecular and in silico tools and techniques.

In our in silico studies FZ displayed a binding affinity for testosterone. A drug approved by the FDA known as Finastride is being used in the clinic to treat BPH or the cancer of prostate has a strong affinity for SRD5A2. However, efficacy of the drug in the disease control is controversial. The mechanism of the drug action is apparently inhibition of the SRD5A2 enzyme activity thereby reduced synthesis of DHT, a more potent androgen whose level is increased in many advanced stages of prostate cancer. On the other side, we found that FZ has a binding tendency toward testosterone as a
result availability of testosterone for DHT synthesis become limited. At the same time the expression of SRD5A2 is also inhibited to a great extent. It is possible that there is a feedback loop present and due to reduced availability of the testosterone the SRD5A2 expression diminishes. It is possible that FZ targets testosterone and as a consequence of reduced SRD5A2 DHT production is limited. We have validated our in silico data with in vivo experiments in mice. Mice were fed with FZ for two weeks and the serum testosterone was measured. Results indicated significant reduction of serum testosterone in FZ treated mice.

FZ elicited growth inhibition by triggering apoptosis in cancer cells. A dose and time dependent experiments indicated that FZ inhibited the DU145 and PC3 cell growth in culture. Interestingly when the primary cultured cells were exposed to this drug it had virtually no effect in indicated time points. It indicate that FZ has differential effect on normal and cancer cells. Twenty four hours after FZ treatment the morphology of cells get altered and a significant number of cells stated showing signal for cell death when visualized under phase contrast microscope. Molecular signaling pathway of the drug action was further evaluated through various experiments. Cell cycle analysis was performed using FACS. FZ treated cells displayed a large number of sub-G1 population generally indicating apoptotic cell death. This data was further confirmed by DNA laddering and TUNEL assays. A panel of marker genes responsible for growth inhibition (SRD5A2), apoptosis (Bcl2 and Bcl-XI) and anti-apoptotic (BAK and BAX) activities were examined. Results indicated that FZ treated cells undergone mitochondrial pathway of apoptosis. FZ treatment led to the mitochondrial membrane depolymerization as a result cytochrome c was released and apoptosis process was initiated. SRD5A2 expression in DU145 and PC3 cell lines was examined by RT PCR a significant reduction in SRD5A2 mRNA level was noticed. Real time PCR analysis indicated about three fold reduction in SRD5A2 mRNA level after FZ treatment. Western blot analysis was performed but we were unable to detect the SRD5A2 protein in gel with the antibody. Perhaps the antibody could not detect SRD5A2 protein in denatured SDS gel.
FZ showed a significant antitumor activity both in vitro and in vivo. Twenty four hour FZ or DMSO treated cells were trypsinized and seeded at a very low dilution. Colony forming ability of these cells were examined after 15 days. FZ had remarkably reduced the colony forming ability of these cancer cell lines. That prompted us to study the effect on mouse xenografts. For mouse experiments, nu/nu mice were injected with prostate cancer cell line, subcutaneous tumors were developed. Mice with tumors were either fed with FZ or olive oil (mock) orally every otherday for two weeks. FZ inhibited the growth of tumor cells in vivo significantly. Tumor markers together with anti-angiogenic marker genes (TSG101, CD31, vWF, hemoglobin etc.) were examined at the RNA level. It appeared that FZ acted as a potent anti-tumorigenic compound.

As implied from these studies that FZ was not toxic to the cells and to the animals, it may have a therapeutic implication. Although we documented the antitumor activity of the FZ both in vitro and in vivo but the molecular mechanism was not clear. In order to understand the role of SRD5A2 gene in prostate carcinogenesis, we used antisense method of gene inhibition. Complete cDNA of the SRD5A2 was made and subcloned into pCDNA3 mammalian expression vector in antisense orientation. Prostate cancer cell lines were transfected with either vector alone or antisense construct and stable G418 resistant clones were selected and amplified. Antisense clones displayed a strong growth advantage. Both cell invasion and cell motility was enhanced in these antisense clones. Therefore, together with all these experiments it suggests that SRD5A2 may be considered as a tumor suppressor gene.