CHAPTER - 6

SUMMARY AND CONCLUSION

Summary and Conclusion:

Prostate cancer (1.46 million cases) is ranked as the second most common cancer following lung cancer (1.57 million cases). Various treatments have been devised for prostate cancer, including androgen blockers, stimulators, and other broad-spectrum chemotherapy drugs. Therapy-induced changes in the behavior of PCa causes gradual cellular adaptation, resulting in reliance on alternative signaling pathways, such as nonspecific hormonal induction to ensure cell survival. Thus, novel therapy had been our prime focus for resistant (CRPC/AR independent) cancer.

Metformin has received considerable attention as a potential anti-cancer agent as its exhibits a strong and consistent anti-proliferative action on several cancer cell lines. Deregulation of energetic and cellular metabolism is a signature of cancer cells its high energy demand and redox imbalance is an inherent property. Hence, in the present thesis we tried to evaluate growth inhibitory property of metformin in AR heterogenic cell lines like AR negative(PC3),AR positive(Lncap) and AR splice variant(22RV1) cell lines. Further, we evaluated cell death mechanism of metformin by analyzing autophagy and apoptotic parameters. we also evaluated another candidate molecule swertiamarin which shares many properties with metformin,

- In objective one, we studied the growth inhibition of different AR heterogenic cell lines in the absence and presence of R1881 induction, using clonogenic and migration assays, as well as evaluating AR/ARV7 transcriptional and translational expression. Additionally, we studied the mRNA expression of PSA.
    - We observed that the growth inhibition of AR heterogenic cells depends on AR/ARV7 expression, as R1881 induction alters the efficacy in relation to AR presence in respective cell lines.
    - Clonogenic and migration inhibition was observed following metformin treatment which indicates that it has the potential to halt cell survival and metastasis. The observed inhibition of AR expression after treatment, shows that metformin can degrade Androgen Receptor. Moreover, the inhibition of ARV7 mRNA expression indicates that metformin can effectively inhibit CRPC.
    - AR-negative cells are also inhibited by metformin, indicating that metformin inhibits prostate cancer by regulating AR-independent cell death pathways such as autophagy and apoptosis.

- The presence of wild-type p53 or mutated p53, also affects metformin effectiveness. Cell responsiveness to metformin decreases in cells featuring nonfunctional or mutated p53 status. Possession of wild-type p53 makes LNCaP cells the most sensitive to metformin among the three cell lines

- In objective 2, we performed Western blot analysis to evaluate the expression of the autophagy marker LC3II and the apoptotic markers- cleaved caspase-3 and cleaved PARP1
  - Metformin increases LC3II (an autophagic marker), indicating it can enhance autophagy. However, this effect depends on AR expression. PC3 cells were most sensitive in terms of autophagy due to the lack of AR. In contrast, LNCaP and 22RV1 cells only showed a significant increase in LC3II with different treatment conditions compared to the control.
  - The introduction of R1881/EBSS causes less autophagy and apoptosis compared to media (in the case of CRPC), leading to the conclusion that splice variant activity increases under stress conditions and negatively regulates p53 functions (autophagy and apoptosis)
  - C-caspase 3 expression increased only in the androgen-sensitive cell line (LNCaP), but it cannot actively confer the effect by cleaving C-PARP1, a later step of apoptosis, which is overcome by autophagy. In the case of CRPC splice variants, they promote apoptosis by cleaving PARP1, while inhibiting autophagy. Where as the AR-independent cell line(PC3) is prone to both autophagy and apoptosis simultaneously
  - The ROS levels resulting from metformin treatment were examined under stress conditions induced by EBSS treatment and R1881, along with the growth inhibition caused by metformin treatment
  - Stress condition created by EBSS treatment and R1881 induction creates a chaos in cancer cells which was handled by metformin until stress condition is under control. For instance 10% EBSS makes cells sensitive to metformin, resulting in both increased ROS and decreased viability..But while conditions became excessively over stressed(90%EBSS), metformin lose control over cell death pathway and starts to promote proliferation instead of cell inhibition.

- Swertiamarin also exhibits anticancer effects, which we studied through growth inhibition, clonogenic and migration inhibition assays, and by evaluating cell death mechanisms through C-caspase and C-PARP1.
    - Swertiamarin was more effective on androgen-independent and CRPC cell lines, respectively.
    - Swertiamarin inhibited CRPC cells more effectively under androgenic induction and other known nonspecific hormonal inductions.
    - Swertiamarin did not show inhibition of clonogenic and migratory properties of PCa cells.
    - Swertiamarin decreased AR expression in androgen-sensitive cell lines.
    - Swertiamarin significantly increased cleavage of Caspase 3 and PARP1 in androgen sensitive cell lines.

Thus over all conclusion from this study reveals that although, metformin exhibits anticancer activity, its efficacy gets altered in AR heterogenic and EBSS stress conditions suggesting causious use in various clinical status of prostate cancer.

Swertiamarin another bioactive compound explored in present study, demonstrated more potent growth inhibitory property in AR independent and CRPC cells compare to metformin. Since, this is the prelimnory observation it needs to be explored further.