CHAPTER - 2

AIM AND OBJECTIVES

2. Aim and Objectives

2.1Aim and Rationale of the study:

Prostate cancer recurrences are very complicated. Antiandrogen therapy and the inhibition of androgen biosynthesis inhibition becomes ineffective in many cases with increased Androgen Receptor expression and constitutive amplification of downstream genes in AR-depleted condition called androgen-independent prostate cancer. AR mutation phenomena reduce the specificity for androgen, allowing other ligands such as progesterone, to bind to the AR. Non ligand therapy like Taxanes causes BRCA2 mutation and create resistance in some incidences. [(Nientiedt et al., 2017)]. Both radiation therapy and chemotherapy lose effectiveness against progressing prostate cancer. To enhance drug sensitivity, drug combination approach is being considered, which focuses on targeting cell death, inflammation, ROS, DNA repair and immunomodulatory functions with AR signaling and effectively addressing resistance.

Metformin, a widely recognized medication, effectively regulates energy metabolism through the AMPK pathway, exhibiting anti-cancer properties. Additionally, it induces an anti-inflammatory and immunomodulatory impact, leading to changes in the tumor microenvironment that disrupt the signaling support for cancerous cells. Metformin exhibits fewer side effects in comparison to other anticancer drugs. It can also suppress Androgen Receptor (AR) expression. By inhibiting the mTOR pathway, metformin enhances autophagy. However, it's important to note that autophagy can either lead to the death of cancer cells or support their growth by providing raw materials for cellular expansion.

The effectiveness of metformin has been assessed in various cancers, including prostate cancer. Several clinical trials have been undertaken, revealing mixed outcomes. Some trials have demonstrated promising results (NCT01627067), while others have deemed metformin ineffective as an anticancer drug. For instance, the Phase II findings of clinical trial NCT01654185 indicated no enhanced efficacy with the addition of metformin to aromatase inhibitor treatment. Similarly, the results from clinical trial NCT01677897's Phase II stage showed no clinical benefit with combination therapy. [(Skuli et al., 2022)].

The ambiguity stems from the heterogeneity within the tumor environment and the tumor itself. A primary factor contributing to this uncertainty is the presence of circular androgen produced by both cancer and stromal cells. Circular androgens, also known as intracrine androgens, are a type of androgen hormone that is produced within cancer cells through a process called intracrine metabolism. Unlike traditional androgens, such as testosterone and dihydrotestosterone (DHT), which are primarily produced by the testes and adrenal glands, circular androgens are synthesized locally within the cancer cells themselves. Additionally, consistently elevated mRNA levels of Androgen Receptor (AR) are observed after reaching the androgen-refractory stage, and antiandrogen drugs exhibit agonistic effects [(C. D. Chen et al., 2004)]. This type of microenvironment can significantly influence Androgen Receptor (AR) heterogeneity. Cancer cells face nutrient limitations, leading to the initiation of the autophagic process within the tumor microenvironment (TME). which influences Androgen Receptor (AR) signaling through p53 and PTEN. Establishing the presence of circular androgen and assessing the autophagic effect in an *in vitro* model with genomic heterogeneity in Androgen Receptor (AR), The presence and absence of AR and ARV7, and the evaluation of molecular markers of cellular death may potentially provide a more scientific approach to assess the efficacy of metformin.

It is well-documented that, besides the androgen receptor, Estrogen Receptor alpha levels also play a crucial role in prostate cancer development in old age, and metformin's inhibitory effect on this receptor adds to its advantages. The impact of glucose and other nutrients on metformin-mediated inhibition is noteworthy; for example, in renal cell cancer, a low glucose environment can reverse the effect and promote cancer cell growth. From this perspective, an autophagic microenvironment (involving amino acid, androgen, and glucose starvation) is deemed necessary [(Aljofan & Riethmacher, 2019)].

In India, there remains a substantial mortality rate (60%) among treated patients, despite the Asian population generally exhibiting a favorable response to antiandrogen therapy. The cost factor also comes into play, as most anti-cancer drugs are more expensive than metformin, making metformin a more favorable option. Given its widespread acceptance and prescription for diabetes management, introducing metformin into cancer therapy makes sense, aiming to reach a larger

population. Metformin treatment offers the advantage of a selectively inhibitory effect on cancerous cells Examining the impact of metformin on diverse Androgen Receptor (AR) heterogenic in vitro models under various AR and autophagic conditions provides more pertinent insights than solely assessing cytotoxic effects in nutrient-rich media. Inducing AR can offer insights into the circular androgen-activated signaling by both cancer and stromal cells. Furthermore, to assess the autophagic effect in the *in vitro* model with genomic heterogeneity in androgen receptor will provide better insight. The induced autophagy condition created by amino acid starvation, mimicking the tumor microenvironment (TME), restricts the nutrient availability. Evaluating the expression of molecular markers associated with cell death pathways under such conditions potentially may offer a conclusive understanding of metformin's efficacy in prostate cancer.

Apart from Metformin, another bioactive drug is swertiamarin (a seco glycoside) exhibits antidiabetic efficacy in Type II diabetes by inhibiting the AKT pathway. Also there are few report where its anticancer property has been evaluated, Hence in present study anticancer properties of Swertiamarin have been evaluated for the prostate cancer In cases where metformin treatment lacks significant impact, combining it with Swertiamarin could be a viable alternative.

Following are Major Objectives of the present study:

1. To explore the role of metformin on different prostate cancer cell lines
2. To Study mechanism of action of metformin on cell death.
3. To modulate the efficacy of Metformin with bio actives