Identification and Biological Evaluation of Small Molecules as Sirtuin Inhibitor against Breast Cancer

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By

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5 Summary, conclusions and scope for further work

5.1 Summary and conclusion:

The family of nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases are known as mammalian sirtuins (SIRT1–7) controls a variety of biological functions. These include aging, DNA damage repair, cellular senescence, cell viability, survival, cellular differentiation, cell proliferation, cellular apoptosis, cellular senescence, and the cellular response to stress [60]. Overall, our main finding reveals that sirtuins involves and facilitates cancer progression. Additionally, we also observed higher expression levels of sirtuins in breast cancer associated poorer prognosis and shorter overall treatment outcome [135]. Therefore, targeting and poring over sirtins improved breast cancer treatment. We evaluated the anticancer activity of phytoestrogen coumestrol and 2-(diarylalkyl)aminobenzothiazole derivatives **7**ab and **7**ba and inhibiting SIRTs in human breast cancer cell lines. Subsequently, as we discussed in each chapter that coumestrol and molecules **7**ab and **7**ba producing cytotoxic effect via reactive oxidative species mediation, SIRT inhibition, autophagy induction and apoptotic cell death. Consequently, all results states that coumestrol and molecules 7ab and 7ba cytotoxic properties against breast cancer.

Protein–ligand complex formation is one of the most significant molecular biological processes [240]. Determining the structure and fundamental interactions of specific protein–ligand complexes is crucial because proteins must correctly recognize, bind, and discriminate among a vast array of potential ligands in order to carry out their functions [241]. In this work we performed *In*-silico studies mainly molecular docking and molecular dynamic simulations as well as *in*-vitro studies like cell cytotoxicity assay, flow cytometric analysis, metabolomics and western blot analysis. Insilico studies revels that coumestrol and molecules 7ab and 7ba observed higher binding energies and stable

binding interactions with sirtins. Further, invitro studies reveals that coumestrol inhibits sirtuins and induces reactive oxidative species.

Lipid metabolism changes are a major factor in the onset and progression of many diseases including breast cancer [242]. There are considerable changes in the absorption, synthesis, and catabolism of lipids in breast cancer cells [243]. The most notable changes are in the metabolism of fatty acids, cholesterol, sphingolipids, and glycolipids [244]. The up-regulation of fatty acid oxidation and the increased absorption and production of fatty acids and cholesterols are closely linked to the growth, progression, metastasis, and medication resistance of breast cancer cells [245]. In this study we observed coumestrol involves in fatty acid metabolism alteration and cholesterol alteration linked to reduction in growth of breast cancer cell lines.

SIRT1 high expression is linked to oncogenic transformation, cancer cell survival, and treatment resistance [246]. Numerous cells death and cell cycle progression were known to be regulated by p53, and SIRT1 was found to directly target p53 acetylation [247]. SIRT1 primarily targets p53, and when DNA damage and stress occur, deacetylated p53 inhibits the p53-mediated cell death pathway. SIRT1 decreased p53-dependent apoptosis, however, by deacetylating and inactivating the p53 protein [248].

The main outcomes of this work are coumestrol and molecules 7ab and 7ba inhibiting SIRTs expression in breast cancer. Additionally, Molecules 7ab and 7ba causes autophagy and reactive oxidative species dependent apoptotic cell death and inhibition of sirtuins. Molecules 7ab and 7ba also activates the p53 upon acetylation of p53 followed by inhibiting sirtuins.

Major findings of the present research work are as follows:

- Phytoestrogens were proven as an anticancer agent and effective sirtuin inhibition observed in breast cancer cells.
- Coumestrol regulate the cholesterol and fatty acid metabolism in breast cancer.
- 2-(diarylalkyl)aminobenzothiazole derivatives induce autophagy and apoptotic death through SIRT inhibition and p53 activation in MCF7 breast cancer cells.

5.2 Scope for further work:

Phytoestrogen coumestrol and molecules 7ab and 7ba significantly inhibited sirtuins followed by autophagy and apoptotic death via p53 activation in breast cancer cell lines. Additionally, phytoestrogen coumestrol involves in few metabolic pathway alterations leads to changes in metabolite signatures in breast cancer cell lines.

In future work, we evaluate the phytoestrogen coumestrol and molecules 7ab and 7ba in cancer models especially *in* vivo breast cancer models. We also evaluate the molecules in combinational therapies with known potent anticancer drug in both *in*vitro and *in* vivo models. Additionally, we also interested to evaluate phytoestrogen coumestrol with immunotherapy cancer models of breast cancer. However, multiple targets are further warranted and more validation to our studies are required. All of these model and studies will able to produce complete information of anticancer cancer activity of coumestrol and 7ab and 7ba. Further, these can be useful in order to improve the clinical and treatment outcomes.