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Thesis title: Studies on the role of phytocompounds in targeting breast cancer metabolism
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Reprogrammed glucose metabolism is considered as the hallmark of cancer with therapeutic implications. Phytocompounds have potential to inhibit cancer metabolism. In our study, we tested the ability of Withaferin A (WA), a withanolide derived from Withania somnifera, in modulating cancer metabolism. We assessed the effect of WA on aerobic glycolysis in breast cancer cell lines and showed that WA decreases the glucose uptake, lactate production and ATP generation by inhibiting the expression of key glycolytic enzymes i.e., GLUT1, HK2 and PKM2. We next identified that WA induced inhibition of cancer glycolysis by targeting c-myc as validated by silencing experiments followed by metabolic readouts. Decreased glycolysis resulted in reduced cell viability, biomass and colony forming ability of breast cancer cells. To further validate our in vitro findings in breast cancer patients, we analyzed 90 metabolic pathways in ~2000 breast tumors and observed that glycolysis is most deregulated in patient tumors. Furthermore, deregulated glycolysis predicted poor patient outcome in breast cancer. Moreover, patient data revealed a correlation of c-myc expression with glycolysis deregulation in breast cancer patients. Taken together, our results the highlight role of WA in inhibiting breast cancer metabolism via c-myc/glycolysis axis.
Next, we checked whether WA targets Hexokinase 2 (HK2) or not. As HK2 catalyzes the first committed step of the glycolysis for trapping glucose by converting it into glucose 6 Phosphate. The HK2 isoform of hexokinase is highly upregulated in cancer, and the druggability of HK2 opens the window in search of natural compound inhibitors for anticancer therapy development. Here, we have employed structure-based virtual screening using in-house library to identify potential phytoconstituents which could inhibit the HK2 activity. The initial hits were selected based on their binding affinity towards HK2 using the molecular docking approach. Subsequently, the filters for physicochemical properties, PAINS patterns and PASS evaluation were applied to find potential hits against HK2. Finally, we have identified epigallocatechin gallate (EGCG) and quercitrin, two natural compounds with appreciable binding affinity, efficiency and specificity towards the HK2 binding pocket. Both compounds were found to be binding preferentially to the HK2 active site and showed a decent set of drug-like properties. All-atom molecular dynamics (MD) simulations for 100 ns were carried out to see the conformational dynamics, complexes stability and

interaction mechanism of HK2 with EGCG and quercitrin. MD simulation results showed that HK2 forms stable protein-ligand complexes with EGCG and quercitrin with consistency throughout the trajectory. Overall, these findings suggest that EGCG and quercitrin might be further exploited as promising scaffolds in the drug development process against HK2.