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The response rates of extensively used chemotherapeutic drugs are relatively disappointing because of considerable side effects associated with their high-dose regimen and due to the lack of specificity towards their respective molecular target. Aim of the present study is to offer a better and more rational approach to cancer chemotherapeutic treatment. Hence the primary objective of present study is to identify some compounds/drugs, which show additive or synergistic cytotoxic effects or enhance the drug uptake in combination with other conventional cytotoxic drugs to facilitate reduction of drug dosage. In addition to investigate the functions of some important regulatory proteins, present study also aims to explore novel targets of drug(s) and their involvement in the induction of cell death initiated by different chemotherapeutic drugs in breast cancer cells.

One of the objectives of the present study was to enhance the efficacy of low concentrations of Carboplatin (Carb) or 5-Fluorouracil (5-FU). Here, we investigated the effect of Methyl-β-cyclodextrin (MCD), a cholesterol depleting agent on efficacy of Carb or 5-FU in MCF-7 and MDA MB-231 breast cancer cell lines, as MCD is known to alter cell membrane function. Data presented here demonstrates that, as compared to drug alone treated cells, MCD pretreated cells showed a significant increase in sensitivity to the drugs even at the low dose treatment. In addition, molecular analysis revealed that MCD mediated increase in susceptibility of breast cancer cells to Carb or 5-FU. At molecular level it can also be partly correlated with de-phosphorylation of Akt, inhibition of NF-κB activity and down-regulation of anti-apoptotic protein, Bcl-2. Further, we observed that MCD pretreatment enhanced the intracellular accumulation of the drugs, which may be one of the reasons for the increased cytotoxicity of these drugs at low dose
treatment. In conclusion, our data provides a biological basis for the potential therapeutic application of MCD in cancer chemotherapy in combination with other conventional cytotoxic drugs. These findings are further validated in animal model system utilizing two different mice strains; nude and C-57 strains. Our results collectively indicate that MCD and 5-FU combination not only regressed tumor regression but also improved survival rate in most of the animals in comparison to 5-FU treated mice. Interestingly, these observations are independent of type of cancer model or mice strains used in present study (tumor induced by breast cancer cells in nude mice and also tumor generated by mouse melanoma cells in C-57 mice). Taken together, our data provides the basis for potential therapeutic application of MCD in combination with other conventional cytotoxic drugs in cancer treatment.

On the way of finding novel targets of chemotherapeutic drugs, here we demonstrate that neuronal cell specific cyclin-dependent kinase 5 (Cdk5) which is a known regulator of neurodegenerative disorders, such as Alzheimer’s and Parkinson’s disease, plays an important role in breast cancer MCF-7 and MDA MB-231 cells proliferation. Cdk5 is functionally involved in chemosensitivit y as well as in cell death pathways induced by anti-cancer drug, Carb. Here we report that Carb induced Cdk5 activation, promotes MCF-7 and MDA MB-231 cell death, under positive regulation of ERK. Basal activity of both ERK and Cdk5 was found to be involved in breast cancer cell proliferation. DNA-damage stress induces ERK activity which plays a central role in Carb mediated cell death in both MCF-7 and MDA MB-231 and utilizes Cdk5 as one of its downstream targets for the execution of death signal. Additionally, present data clearly indicates that Cdk5 modulates p53 promoter transcriptional activity in MCF-7 cell line. However in p53 mutant MDA MB-231 cells Cdk5 mediated cell death seems to be p53 independent. Collectively, our findings not only draw attention to the
extra-neuronal functions of Cdk5 but also propose Cdk5 as a novel and potential therapeutic target of chemotherapeutic drug.

Last part of the thesis underscore the importance of Caveolin-1 (Cav-1) in drug induced cell death. Cholesterol enriched invaginations of plasma membrane regulates cellular signaling by their structural gene Cav-1. Cav-1 is a 21-24 kDa protein and work as an inhibitory clamp and entraps many important signaling molecules through its scaffolding binding domain. Present data indicates that chemotherapeutic drugs Carb or Vinblastine (Vin) upregulate Cav-1 protein expression and phosphorylation in MCF-7 cells. Carb or Vin treatment increases p53 protein expression and p38 phosphorylation, which are well known stress activated proteins. Next we investigated that p53 and p38 positively regulate Carb or Vin induced phosphorylation of Cav-1 protein. Moreover our data indicates that p53 is involved in chemosensitivity of Carb or Vin in MCF-7 cells. Drug induced activation of p38 also contributes partially to Carb or Vin induced cell death as well as it regulate Cav-1 phosphorylation. Further, we investigated that Cav-1 is functionally involved in Carb or Vin mediated cytotoxicity in MCF-7 cells. About 20% more cell survival was observed in Carb or Vin treated MCF-7 cells transfected with Cav-1 SiRNA, in comparison to either of the drug treated MCF-7 cells transfected with control SiRNA. Collectively, results provide evidences suggesting the direct involvement of Cav-1 gene in Carb or Vin mediated cell death signaling and chemosensitivity.

Taken together, present study, is a cumulative effort towards understanding the molecular targets and related signaling pathways of the conventional chemotherapeutic drugs and propose a combination treatment approach to cut down the drug dosage in patients to offer better treatment with minimum side effects.