CONCLUSION

Conclusion

Benzo [a] pyrene (BaP) and Nicotine derived nitrosamino ketone (NNK) are the major contributors of lung cancer. BaP and NNK both enter the cellular machinery and hamper its normal functioning leading to various types of cancers. In our current study we have tried to elucidate the mechanism of how these carcinogens perturb the cell cycle regulatory machinery and cause cancer using systems biology approach. We created separate networks of the proteins that get perturbed due to BaP and NNK and then did network analysis to find the most potent biomolecular targets of BaP and NNK. For this we first generated the networks using STRING db. The networks generated had 50-50 connectors in both shells which provide a chance to find a gene that has not yet been reported to interact with these environmental carcinogens. We also kept the confidence score of the network highest which reduced the chances of having any false positive connections in the network. We then used cytoscape software and its various tools and plugins like network analyzer, cytohubba, MCODE and ClueGO to do network analysis and to reduce the noise from the network. With the help of network analyser, we analysed the network on the basis of its various topological properties. Network analysis helped in getting a better understanding about the type of the network. From the topological properties like shortest path length, average clustering coefficient, diameter of the network etc, we can easily predict the networks rewired by BaP and

NNK, both belong to the real world small scale network. The power laws applied to average clustering coefficient graph, node degree distribution graph and neighborhood connectivity distribution graphs of both BaP rewired PPIN as well as NNK rewired PPIN, show that both the networks are weak real world scale free networks.

Next step was the modulation of the networks which was done to reduce the noise from the networks. From modulation process, we generated clusters and found the seed proteins. We also performed the GO enrichment analysis to find the functionally related proteins for all the involved pathways. We then again generated PPIN from the seed proteins obtained for BaP and NNK separately and did topological analysis of all the nodes involved. While making the network, we kept the opportunity of adding connectors which helped in finding novel non reported proteins in the network. On topological analysis of networks, we sorted the proteins on the basis of degree, clustering coefficients, betweenness centrality and bottleneck scores and selected our final list of proteins by applying median. For further refining our results, we performed molecular docking on all the proteins obtained for BaP and NNK respectively. The top three molecules with highest binding affinity were chosen as the best biomolecular targets for BaP and NNK separately. For BaP QSOX1, PTGS2 and NOS2 were the top three biomolecular targets and for NNK, CDK7, CCNA1 and CDKN1B were the top three biomolecular targets.

QSOX1 and NOS2 are not directly involved in cell cycle regulatory machinery but various studies have shown that both these biomolecules play role in development of cancer. PTGS2 has a direct involvement in the regulation of cell cycle. From the time course graphs of PTGS2 inhibition, it becomes clear that when BaP interacts with PTGS2 and hampers its functioning, whole cell cycle gets perturbed. An excessively increased levels of PTGS2 in cells is a hallmark of various types of cancers. From the data obtained from mathematical modeling, we can see that there is a rise in PTGS2 active concentration which falls in line with the earlier studies conducted to find the relation between cancer and PTGS2. From the time course analysis, we can also conclude that when PTGS2 gets hampered, changes are observed in the oscillation patters of Cyc E CDK2 and Cyc B CDK1. Such fluctuations may lead to problems associated with DNA replication in the S phase as well as in the accurate separation of the chromosomes during mitosis.

All the top three biomolecular targets of NNK (CDK7, CCNA1 and CDKN1B) play an important role in the regulation of cell cycle. From these three biomolecular targets of NNK, CCNA1 emerged out as a connector as it was not earlier present in our gene set. From the time course analysis of CCNA1, we can clearly see that the concentrations and wave patterns of Cyc B CDK1 which is the main complex responsible for the transition of cell cycle into the M phase. Hence we can say that due to inhibition of CCNA1 by NNK, the cell may face the problems of improper separation of chromosomes during the cell division process.This gives us an opportunity for further

investigation about the involvement of NNK perturbed CCNA1 in cancer development and progression.

CDK7 emerged out to the main biomolecular target of NNK with the highest binding efficiency. Though the concentrations of CDK7 do not fluctuate much during the whole cell cycle but on mathematical modeling, we can clearly see the impact of its inhibition by NNK. All the major cell cycle regulatory complexes got perturbed which increases the importance of CDK7 in the smooth functioning of the cell cycle regulation.

CDKN1B (p27) is the third top biomolecular target of NNK which on time course analysis showed its impact on Cyc E CDK2 complex. Though the change in concentrations is not much, but we can observe slight fluctuations in the oscillation patterns of Cyc E CDK2 complex which can cause an impact in the transition of cell cycle into the S Phase.

We also tried to investigate the role of carbon based nanoparticles namely, single walled carbon nanotube, multiwalled carbon nanotube and fullerene, as protective agents against these hazardous environmental carcinogens. Earlier studies have proved the scavenging capacities of nanoparticles in environment. Studies have been conducted on the scavenging potentials of TiO2 against BaP and NNK, so in this study we have tried to find the scavenging potentials of carbon based nano particles against BaP and NNK. We designed these carbon nanoparticles using nanotube modeler and then we tried to find the binding affinities and adsorption capacities using material studio. On

analysis, MWCNT showed highest binding affinity as well as highest adsorption capacity for BaP and SWCNT showed the highest binding affinity against NNK. In case of NNK, though SWCNT showed the highest binding efficiency against NNK, MWCNT showed highest adsorption load.

From this study we can conclude that QSOX1, PTGS2 and NOS2 are the most potent biomolecular targets of BaP. PTGS2 is directly involved in cell cycle regulatory machinery and on interaction with BaP, PTGS2 inactivates P27 which further hampers the normal cell cycle progression. QSOX1 and NOS2 have shown their roles in cancer progression but are not involved in the cell cycle regulatory machinery. CDK7, CCNA1 and CDKN1B are the most potent biomolecular targets of NNK. All the three biomolecular targets are directly involved in the cell cycle regulatory machinery. Out of all the three most potent biomolecular targets, interaction of BaP with CDK7 has the most severe impact on the cell cycle.For the protection of cellular components from the hazardous impacts of BaP and NNK, carbon nanoparticles (SWCNTs, MWCNTs and Fullerenes) were used as scavengers using *in silico* techniques. On the basis of molecular docking analysis of all these carbon based nano particles with BaP and NNK, MWCNTs emerged out as the best scavenger against BaP while SWCNT as well as MWCNT, both had better scavenging potential for NNK.