INTRODUCTION

Introduction

Cancer is an umbrella term that covers various diseases having uncontrolled cell growth. Lots of experiments have been conducted till date to study the causes of various types of cancers. Studies have been conducted to understand how carcinogens affect various pathways of normal cells and convert them into cancerous cells. The aim of the current work is to pin-point the biomolecular targets of two most potent environmental carcinogens, namely Benzo alpha pyrene (BaP) and Nitotine derived Nitrosamine Ketones (NNK), using various tools of systems biology. In this study, we also aim to check the metabolic pathways which get perturbed due to these carcinogens. Apart from these, cell cycle regulatory machinery will be designed and simulated to visualize the impact of these carcinogens on the biomolecular targets involved in cell cycle. In the end, with the help of carbon nanoparticles, we will try to find the scavenging capacity of single walled carbon nanotube (SWNCT), multiple walled carbon nanotube (MWCNT) and fullerenes on these carcinogens using *in silico*approaches.

1.1) Brief of Cancer and environmental carcinogens:

Human body is composed of trillions of cells and each cell in itself holds various genes and proteins. With the help of these proteins and the organelles present inside the cell, it performs various functions to sustain the life of human body. Any deviation in the cellular processes are harmful and may lead to various diseases that can be life

threatening. In our day to day we face various chemicals that can easily enter our body and can pose a risk of cellular process deviation and lead to cancer.

Cancer is the second deadliest disease which claims approximately 9.6 billion deaths in 2018 (Bray *et al*., 2017). Among various cancers, lung cancer is a major cause of cancer related deaths followed by breast cancer and head and neck cancer. Main reason of the rapid increase in the incidences of lung and head and neck cancer is the rise in the consumption of tobacco in either chewable forms or smoking. These environmental carcinogens disrupt the normal cell cycle by causing mutation in the genes that encode various growth receptors and also leads to an increased copy number of such genes causing over-expression of receptors on the tumor cells. Cancer is considered as an umbrella term for many diseases having certain features like uncontrolled cell division, ability to escape the immune system and metastasis, induction of angiogenesis and resistance towards apoptotic mechanism(Tang*et al*, 2013).

International Agency for Research on Cancer (2017) reports that cigarette smoking causes approximately 90% of all deaths caused by cancer making lung cancer the most common type of cancer globally (Hetch, 2003). Various studies have proved that smoking also causes other cancers like head and neck cancer, pancreatic cancer, renal cancer and bladder cancer. More than 60 carcinogenic compounds have been reported in cigarette smoke(Hetch, 2003) and around 30 carcinogenic compounds are

found in smokeless tobacco that includes tobacco specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), aldehydes and metals (Boffetta *et al,* 2008).

Main chemical compounds present in environment and cigarette smoke that are categorized as carcinogens are polycyclic aromatic hydrocarbons like benzo(a)pyrene (BaP) andtobacco specific nitrosamines like NNK. These are potent mutagens and play a significant role in carcinogenesis. The main contributors of BaP are the vehicle exhausts, industrial chimney exhausts, forest fires, volcano eruptions, grilled & smoked food and cigarette smoke to name few. Studies on BaP suggest that BaP, though a very potent and strong environmental carcinogen is found in small traces in unburned fuels and unburnedcigarettes(Hetch, 2003). NNK on the other hand is another extremely potent and hazardous environmental carcinogen is reported to be present in both, burned cigarettes as well as unburned tobacco products like chewing tobacco products, snuffs and smokeless tobacco products. These carcinogens bind with various proteins of the cell cycle regulatory machinery and DNA leading to severe mutations and perturbations in the cell cycle. They bind with DNA and form DNA adducts which can lead to miscoding by DNA polymerase causing permanent conversion of base pairs (Hetch, 2002). Fig 1 represents the chemical structure for BaP and NNK.

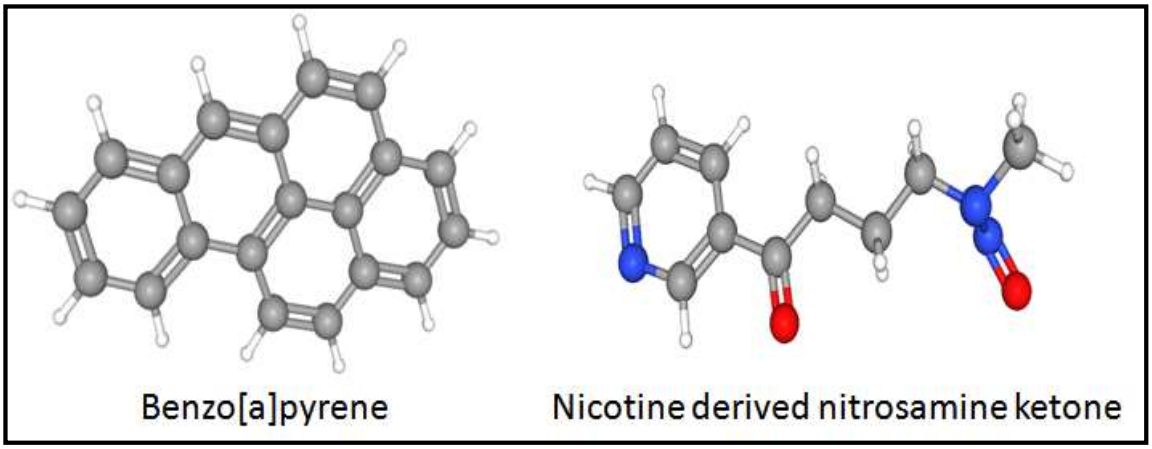

Fig 1: Chemical structures of BaP (CID: 90044747) and NNK (Compound ID: 47289)

1.2) Brief introduction of human cell cycle:

Human cell cycle, just like any other eukaryotic cell cycle, is tightly interlinked and extremely complex system, involving many genes and proteins and various organelles. There are many internal and external factors that determine whether the cell will divide or not. Along with these factors there are certain check-points that determine the movement of the division cycle from one phase to another. Any perturbation in the genes and proteins regulating these check-points leads to harmful consequences as the cell loses its regulatory mechanisms and divides vigorously causing tumors. Environmental carcinogens are one of the major contributors that cause such hazardous perturbations in the cell cycle regulatory machinery. Various polycyclic aromatic hydrocarbons added in the environment by various human activities like burning of fuel, incomplete combustion of wood, from vehicle exhausts and industrial chimneys, welding of iron, cigarette smoking etc. Along with PAHs, NNKs are also a major contributor of carcinogens in environment whose major source is burning of tobacco. It

hampers the cell cycle not only as an environmental carcinogen but on directly consuming it in chewable forms of tobacco and also by inhaling it in form of snuffs.

1.3) Introduction to systems biology:

Till date huge numbers of experiments have been performed by scientists explaining the functions of various cellular components which have generated huge data related to cells, theirmolecular components and their involvement in any processes or diseases. Systems biology aims at combining all this valuable knowledge and making networks that will help in understanding how these molecules interact with each other enabling any system to work properly.

Systems biology is an interdisciplinaryfield that deals with the study of such highly complex biological networks using mathematical, physical and chemical laws using computer softwares and bioinformatics tools. Its origin dates back to 1969 when Ludwig von Bertalanffy gave “systems theory” (Junker, 2008). This field provides an opportunity to view any pathway or process as a complexsystems and helps in understanding the complex interactions between all the molecular components taking part in that system or process. This has also opened up a different perspective for treatment of various diseases. We can now try to pin point the proteins which act as hub proteins and have the capacity to perturb whole network.

For the first time the concept of networks in biological systems was introduced by Albert Barabasi. The study of complex network called graph theory, which is the area of a branch of discrete mathematics. The first case study was done by Swiss mathematician Leonhard Euler in 1736, when he published the solution to the Königsberg bridge problem. Now current scenario has become the witness of the origin of a new movement of research in the study of complex networks. These networks can be physical objects in the euclidean space, like power grids, transportation networks, phone call networks, and internet web, social media networks, scientific authorship-citation networks, and also involved in biology and medicine, as neural networks or genetic, metabolic and protein interaction networks (PIN).The ongoing trends of research are very progressive and dynamic, in the term of regular development of newer, efficient and high throughput techniques. The consequence of this progress is generating the huge scientific information on the pubmed and other scientific data warehouses. This huge information is enriching the pubmed like a precious treasure of scientific data and here the "system biology" can play a role as a key to this treasure. Fig 2a and 2b are the pictorial representation of systems biology approach against the molecular approach.

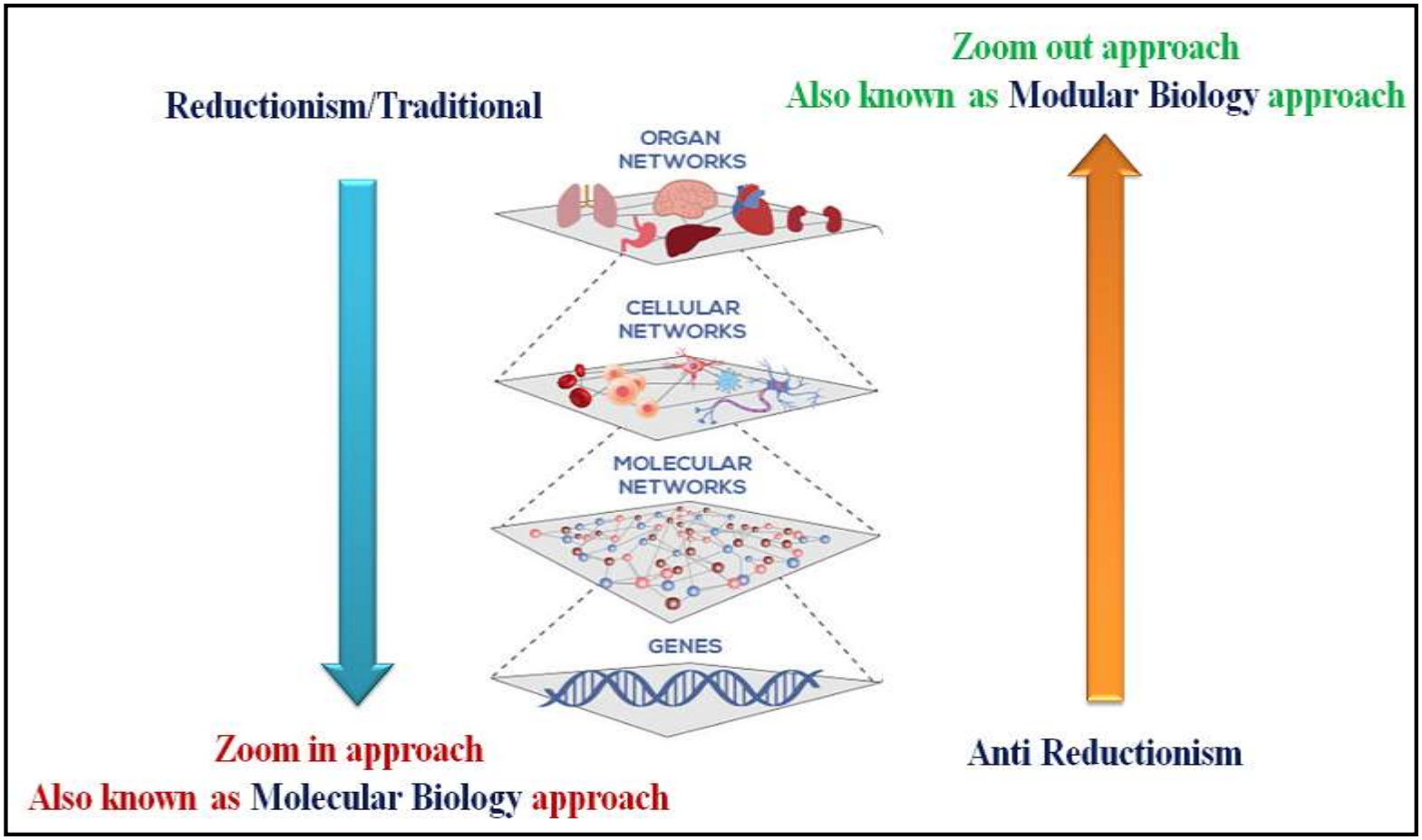

Fig2a: Pictorial representation of difference between reductionist approach and anti reductionist approach

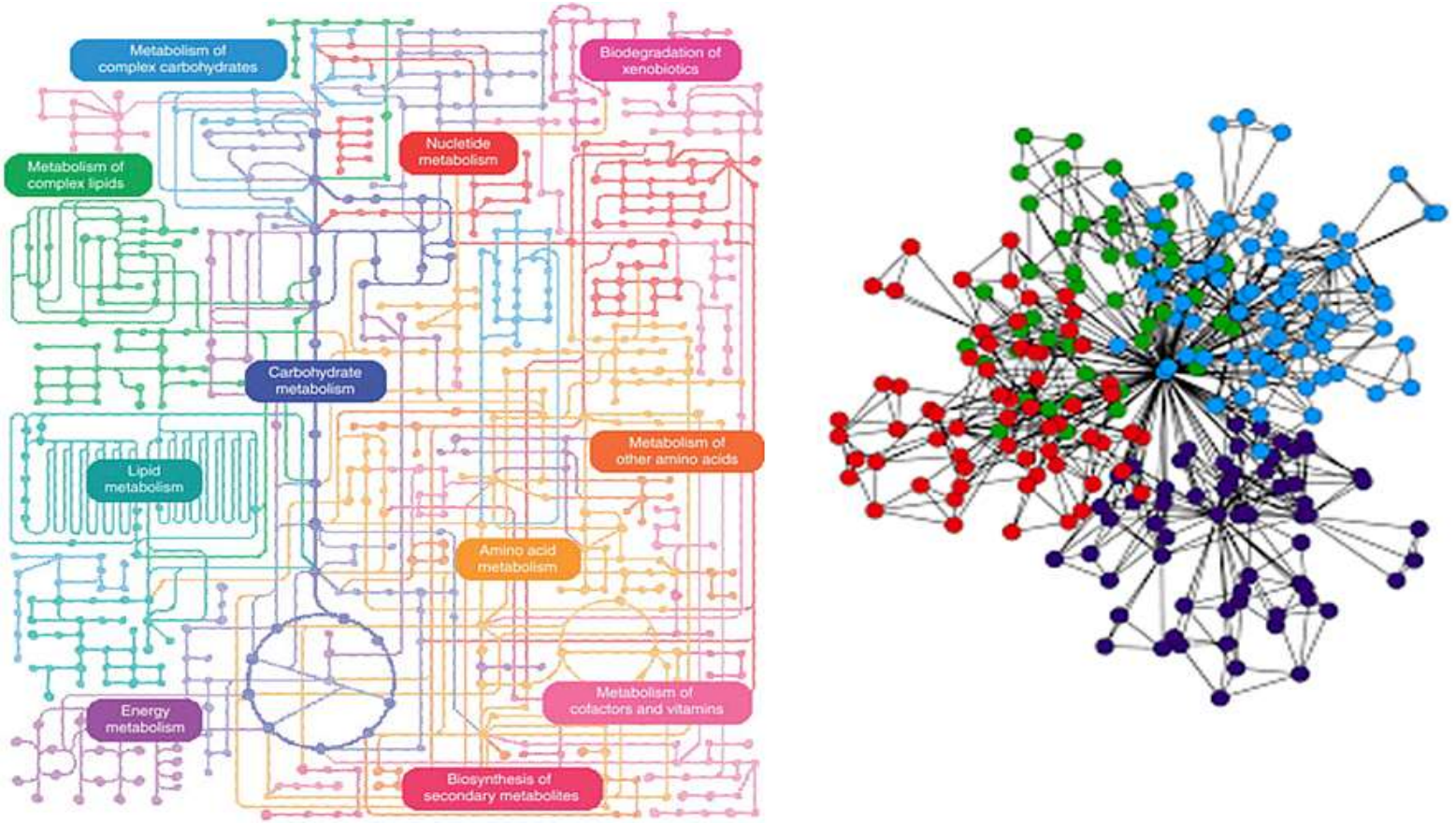

Fig 2b: Circuit & Network/Interactome Study of Bio-molecules: Systems biology aims to spot which biomolecules are most important and key regulatory proteins/genes in the circuit or networkwhich govern whole communication path of metabolic signals.

1.4) Introduction to bio-kinetics of cell cycle:

Mathematical modeling is the term which is generally used when a complex system is analyzed and solved with the help of mathematical equations using computers. Differential equations, statistical models and dynamical systems are various forms of a mathematical model. Creating models for biological systems is an important part of systems biology. To get a thorough understanding of how systems are working and how diseases are caused, focus of researchers is now making using mathematical models. Mathematical modeling is also playing an important role in pharmaceutical industry by assessing suitable drug targets and in other drug discovery processes (Fischer, 2008).By the help of mathematical or computational models of complex biological processes, new and precise drugs can be predicted. Various approaches have been designed till date which are based on the data generated by high through put techniques and their analysis by modeling methods that have opened a new era of designing new and precision drugs for the treatment of highly complex diseases like cancer, Alzheimer's and many more (Ji *et al.*, 2017 and Colijn *et al*., 2017).

In the current study we have tried to find the impact of the perturbations caused by BaP and NNK (environmental carcinogens) on the cell cycle regulatory transcriptome. With the help of time course analysis, we have tried to understand the rate of change of concentrations of various proteins present in cell cycle regulatory machinery when no environmental carcinogen is present and also when the carcinogens are interacting with their biomolecular targets.

1.5) Introduction of protective potential of carbon based nanoparticles:

In our study we have used tried to investigate the use of carbon nanoparticles as a protective agent against environmental carcinogens (BaP and NNK) inside the human body with the help of computational thermodynamics methods. Nanoparticles are extremely small particles that range from 1 to 100 nanometers in size. They have novel properties owing to its extremely small size due to which these particles have found their use in various field. Single -multiple walled carbon nanoparticles and fullerenes have already been used as scavengers of pollutants from environment (Guerra *et al*., 2018) and also as drug delivery system (Mohajeri*et al*., 2018). In previous studies conducted by Dhasmana A *et al*. 2014, TiO2 has been used as a protective agent against BaP induced alterations in cell cycle. In this study we have used carbon nanoparticles which have been used for drug delivery (Elhissi *et al* 2011). We have designed single walled carbon nanotubes, multi walled carbon nanotubes and fullerenes and have tried to evaluate their binding efficiencies and adsorption loads with BaP and NNK.

Objectives:-

- Identification of most probable biomolecular targets of environmental carcinogens (BaP& NNK) among the cell cycle regulatory proteome once they enter the biological system.
- Construction of network system for functional coverage of most potent biomolecular target of environmental carcinogens among the cell cycle regulatory proteome.

- Designing of cell cycle regulatory bio-model along with its kinetics and determining the impact of environmental carcinogens on normal cell cycle regulation.

- Determination of the binding efficiencies of environmental carcinogens with carbon-based nanoparticles, environmental carcinogens adsorption load over nanoparticles and comparison of binding efficiencies of carcinogens with their biomolecular targets and nanoparticles.