To Study the Molecular Mechanism(s) Involved In Cigarette Smoke (CS) Induced Cellular Pathogenesis

Abstract

Cigarette smoke (CS), a very rich source of free radicals, is the etiological agent for various human pathological conditions, such as emphysema, chronic bronchitis, different types of cancers including lung, larynx, oral cavity, esophagus etc and cardiovascular diseases like atherosclerosis. Emphysema is a highly prevalent airway disease that causes serious morbidity and mortality. It is characterized by chronic inflammation, and loss of structural integrity throughout the lung from conducting airways, to the alveolar walls. CS-induced cellular death plays vital role for the development of this disease. Cigarette smoking directly induces apoptosis of airway epithelial cells. In addition to apoptosis, a number of studies present evidence for the occurrence of autophagic cell death, also known as type II cell death, in the lungs of both emphysema patients and mouse exposed to CS. Hence, CS-induced cellular death is a complex process that involves multiple mechanisms and the underlying regulatory events are not yet fully understood. Towards understanding the underlying mechanism(s) of CS-extract (CSE) induced cellular death effort were targeted towards the identification of new gene(s) through multicopy suppressor analysis using *Saccharomyces cerevisiae* as the model system. The result of the multicopy suppressor analysis showed that genes *BAP2* and *TAT1*, that encode branched chain amino acid transporters impart resistance to CSE-induced cellular death.

In agreement with this, treatment of alveolar epithelial A549 cells with CSE also showed reduced expression of branched chain amino acid transporter LAT1. Towards understanding the underlying mechanism related with cell death and downregulation of LAT1, it was observed that by downregulation of LAT1, CS regulates one of the vital cellular signaling components, namely mammalian target of rapamycin (mTOR). mTOR is a central element in the signaling pathways involved in the control of cell growth and proliferation. Besides it was observed that the downregulation of LAT1 by CSE was mediated by a known transcriptional repressor Mad1. Hence, CSE causes an upregulation of Mad1 which in turn acts as a transcriptional repressor for the transporter LAT1 and ultimately leads to mTOR inactivation. This mTOR inactivation was associated with CSE-induced cell death. Another important observation of this study was the capability of excess leucine and overexpression of LAT1 to combat CSE-induced cell death in A549 cells. Interestingly validation of this observation in animal model confirmed the preventive role of leucine in development of CS-induced emphysema. Moreover it was also observed for the
first time that leucine can also revert preexisting emphysematous changes in CS-exposed guinea pig lung.

Cigarette smoke mediated oxidative stress is a prime reason for the development of different pathological conditions and inflammatory diseases wherein transcription factor NF-κB plays an important role. Therefore reducing the CS-induced ROS level can be an effective measure in preventing the initiation and progression of these CS-induced diseases. Hence, it is likely that an antioxidant like vitamin C, which can effectively scavenge a wide array of ROS and free radicals, can be used to inhibit CS-induced NF-κB activation. Vitamin C is a strong antioxidant that maintains a balance of ROS within the cell. However, the importance of vitamin C lies with the fact that this antioxidant is water soluble and it can be easily manipulated by dietary supplementation. Towards this end efforts were directed towards the investigation of the effect of vitamin C on CS-induced NF-κB activation. The results showed that vitamin C treatment along with CS-exposure was capable of preventing CS-induced NF-κB activation in guinea pig lung by interfering with the nuclear translocation of cRel, thereby making it a potent agent for preventing CS-induced inflammatory diseases related to NF-κB activation.