SUMMARY AND CONCLUSION

Globally, lung cancer ranks first in both incidence and mortality rates, which indicates the essentially fatal condition; and in lung cancer, non-small cell lung cancer (NSCLC) incidence rates are the highest. Also, NSCLC is the only cancer associated with non-smoking, which means apart from established risk factor “smoking”, other potential risk factors (environmental or genetic) needs to be determined. Plausible causative environmental factors such as organophosphate pesticides (OPPs) viz. chlorpyrifos (CP) and monocrotophos (MCP) exposure may be involved in the process of lung carcinogenesis. CP and MCP are mostly used OPPs in agriculture, industries, public health and household, owing to their efficiency, affordable price and easy availability.

Some studies have demonstrated association between lung cancer risk and OPPs, but results are conflicting. Studies concerning the genotoxic potential of CP and MCP have shown to induce oxidative stress and DNA damage globally in in vitro and in vivo models. But we don’t have enough evidence on the oxidative DNA damage to establish the role of CP and MCP. Therefore, the present study is an attempt to gather substantial evidence regarding altering the pathway-associated with DNA base damage repair and activation of cell survival mechanisms in cancer cells after exposure to CP and MCP. We used A549, NCI-H23 and NCI-H1299 cells as our experimental cell culture model, where they were exposed to a range of concentration of OPPs CP and MCP for different time periods.

The aim was to determine whether OPPs CP and MCP were able to induce oxidative stress and oxidative DNA damage to elicit cellular responses in NSCLC cells. The results showed that CP and MCP increased reactive oxygen species (ROS) and oxidative DNA damage apurinic/apyrimidinic (AP) sites in response to which the
cellular responses resulted in alterations in mRNA and protein expression of BER-pathway enzymes viz. APE1, OGG1, PARP1, XRCC1, DNA pol β and DNA ligase III α, and pro-survival signalling pathways transcription factors (TFs) viz. c-jun, p-c-jun, NF-κB and Nrf2, that may leads to increased DNA repair, cell survival, proliferation and promotion of carcinogenic events in A549 cells. Increase in protein expression of inducible nitric oxide synthase (iNOS/NOS2) in response to CP-mediated oxidative stress was also observed which further explains the possibility of oxidative damage and inflammation via NO signalling pathway. The OPPs were not found to change the mRNA expression of apoptosis-related factors Bax and bcl-2, indicating no change in basal apoptosis rate. However, marked decrease in pro-apoptotic factor caspase-3 protein expression was observed even in the presence of increased caspase-9 expression, advocating for suppression of caspase-dependent apoptosis which may be due to intervention of other anti-apoptotic factors frequently present in cancer cells.

As observed in our results, APE1’s mRNA and protein expression was significantly upregulated, which reflects its increased repair and redox functions. In repair function, apart from AP endonuclease activity, another important role of APE1 is coordination of BER-pathway through protein-protein interaction with BER proteins. Also, in redox function, APE1 reductively activates the TFs or act as co-activator of TFs directly through physical protein-protein interactions or indirectly by acting as a redox chaperone. The ability of APE1 to bind various proteins over a small interface indicates the presence of flexible disordered protein regions; also, emerging evidences have shown that intrinsically disordered (ID) protein regions plays a crucial role in protein-protein interactions. Therefore using STRING database, we first analyzed the known and predicted protein-protein interaction network of APE1 and, then using PONDOR bioinformatics tool, we determined the ID regions in APE1, AP-1, NF-κB and Nrf2. ID regions were identified in N-terminal of APE1; it is well known that N-terminal region of APE1 is responsible for its redox-regulatory activity. Next, ID regions were also found in middle and C-terminal segments of the transcription
factors AP-1, NF-κB and Nrf2. The average PONDR prediction score, which corresponds to extent of disordered structure, was found moderate for APE1 and NF-κB, but highest for AP-1 and Nrf2, which advocates for the potential protein-protein interactions between APE1 and TFs AP-1, NF-κB and Nrf2.

Subcellular distribution of proteins and their colocalization pattern determines their functions and possible physical interactions, respectively. Changes in APE1’s subcellular localization in response to low and moderate concentrations of CP and MCP-mediated oxidative stress were first determined. It was found that an early CP and MCP treatment period increases cytoplasmic distribution which may corresponds to mitochondrial or ER or ribosomal association; whereas, 24 hr treatment period resulted in exclusive nuclear localization, which indicates repair and redox-regulatory role of APE1.

For NF-κB and c-jun (AP-1) to be activated to confer pro-survival advantages, they must be translocated from cytoplasm to nucleus, and for APE1-regulated activation of NF-κB and c-jun (AP-1), they must show nuclear colocalization with APE1. Therefore, to determine this, we performed colocalization studies using confocal laser scanning microscopy (CLSM) technique. Positive nuclear colocalization was observed for APE1 and c-jun that advocates for the APE1-mediated redox regulation of c-jun. However, no significant nuclear or cytoplasmic colocalization was observed for p-c-jun and APE1, which indicates that p-c-jun, may act as a switch to fall off from the redox-complex after the regulation or APE1 has no role in JNK-mediated phosphorylation of c-jun in response to oxidative stress. Notable APE1 and NF-κB nuclear colocalization was observed for an early MCP treatment time period; whereas, slight increase in APE1 and NF-κB nuclear colocalization was observed for both CP and MCP treatments at 24 hr time period. Moderate level of Nrf2 and APE1 nuclear colocalization was also observed in CP and MCP-treated NCI-H1299 cells. Results suggest that CP and MCP-mediated oxidative stress
moderately mediates APE1’s regulation of NF-κB and Nrf2. Further immunoblotting analysis of immunoprecipitated APE1 with an anti-Nrf2 and anti-c-jun antibody maintained the supposition that APE1 interacts with TFs upon MCP and CP treatments in A549 cells.

The present study suggest that low and moderately high concentration of OPPs CP and MCP-mediated oxidative stress and oxidative DNA damage may act as a driving force to lung cancer initiation and progression via modulation of DNA BER-pathway and cell survival signalling pathways. Besides of this, APE1 act as a critical regulator of pro-survival TFs in CP and MCP-induced oxidatively-stressed lung cancer cells that can potentially add to the process of lung cancer progression.

**Future Perspectives**

We have shown the role of APE1 in the process of lung cancer progression. However, as a multifunctional enzyme capable of various protein-protein interactions, APE1 can have a variety of other roles that may affect the lung cancer risk. Therefore, to search for its potential interacting protein partners, we could perform the pull-down assay. Also, as APE1 is speculated to be involved in various canonical and non-canonical protein-protein interactions, further proteomic studies are needed to determine the specific APE1 protein-protein interactions underlying significant biological pathways, might be involved in oxidative stress-mediated pathogenesis of other human diseases. As recently shown by a study, post-translational modification acetylation can modulate the repair function of APE1. Therefore, future investigations elucidating the effect of other post-translational modifications regulating the repair and redox functions of APE1 are required to be performed both *in vitro* and *in vivo* model studies.
Various population-based studies have suggested for the role of BER-enzymes mutant variants in cancer susceptibility. APE1 variants such as D148E, L104R, D283G and R237C, have been observed in the human populations. Studies regarding association of these variants with human pathologies have not been performed at biochemical and molecular level. However, the existing literature indicates that APE1 SNPs may affect its enzymatic activities or its interaction with specific protein partners that may ultimately affect the risk for human pathology such as cancer. Hence, further studies can be carried out in this direction too.