Summary and Conclusion

Glutathione (GSH) is a tripeptide thiol present in almost every living organism and at relatively high intracellular concentration. As a carrier of an active thiol group in the form of a cysteine residue, it acts as an antioxidant either directly by interacting with reactive oxygen/nitrogen species and electrophiles or by operating as a cofactor for peroxidase enzyme. With Glutathione-S-transferase, GSH participates in detoxification and disposal of xenobiotics or their electrophilic metabolites and also in cell signaling system. Along with this GSH is known to work as (i) a reservoir of cysteine, (ii) a store and transporter of nitric oxide, (iii) participant in metabolism of estrogens, leukotrienes and prostaglandins, formation of deoxyribonucleotides, the maturation of iron-sulfur clusters of diverse proteins, (iv) redox regulation of certain transcription factors (particularly those involved in redox signalling), and (v) the detoxification of many endogenous compounds and xenobiotics. Most of these function are dependent on or mediated by GSH in concert with GR,GST,GSH-SS-transhydrogenase and GSH-Peroxidase.

An escalation in GSH content is noted in cells in the proliferation phase. Cytoplasmic GSH migrates to nucleus for maintaining the protein thiols in highly reduced form to help DNA replication. GSH in cancer cells is particularly relevant in regulation of: carcinogenesis, sensitivity against cytotoxic drugs or ionizing radiation, some cytokine function, DNA synthesis and cell proliferation. GSH plays an important role in determining the sensitivity of cells to radiation and drug-induced cytotoxicity. Chemical
carcinogenesis in experimental test systems gives an insight into the changes in the cells during transformation from normal phenotypic characters into the transformed cell characters. Multidrug and radiation resistance of many tumors, as compared with normal tissues, appears to be associated with higher GSH levels in the cancer cells.

An impairment of GSH uptake by mitochondria (or direct mtGSH depletion) appears a useful mechanism to sensitize malignant cells to molecular effectors (e.g., oxidative stress inducers and/or cytotoxic drugs) capable of activating the mitochondrion-based cell death mechanism. Mitochondrial dysfunction is a common event in the mechanisms leading to cell death. GSH, is not synthesized by mitochondria but is taken up from the cytosol through a multicomponent transport system.

Our aim was to examine the regulatory role of GSH mediated redox reactions in chemical carcinogenesis and to elucidate the underlying molecular mechanisms. We hypothesis that alterations in genomic profile of chemically transformed cells may be the molecular basis of carcinogenesis and their response to manipulation of GSH content could demonstrate the regulatory role of GSH mediated redox reactions in chemical carcinogenesis. The proposed objectives were as follows:

- Study regulatory significance of cellular GSH depletion in experimental carcinogenesis using C3H10T1/2 and BALB/c 3T3 fibroblast cell lines.
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- Elucidate its molecular mechanism using microarray approach and validate the altered gene expression using qPCR.
- Identify functional relevance of critically altered genes/pathway for their regulatory role in toxicity-carcinogenesis.

The summary of the study as follows:

- In two stage cell transformation assay using C3H10T1/2 and BALB/c cell line, the exposure to MCA+TPA resulted in the formation of transformed foci, colonies in soft agar culture and an increase in GSH content in both the transformed cell lines (C3H10T1/2 and BALB/c cells).

- The global gene expression profile of MCA+TPA transformed cells growing in constant communication with surrounding non-invasive cell colonies was considerably altered; the trend was relatively more toward downregulation. The change was grossly convergent to pathways of cancer, phagosome activity, and tumor cell microenvironment information processing inclusive of neuroactive ligand–receptor interaction, actin cytoskeleton regulation, tight junction, axon guidance, and cell adhesion molecules. The participant genes were functionally relevant mostly to signal transduction and information processing, described to be important for the interaction of tumor cells with the microenvironment.

- The non-cytotoxic dose of DEM and PHO resulted in GSH depletion, increase in ROS generation followed by the impairment of cell cycle. GSH depletion induced the apoptosis, decreased the transformed cell viability and the anchorage independent tumor cell growth or colony formation. The strategically altered global gene expression profile signified the inhibition of
transformed cell proliferation. The results could be validated in the transformed BALB/c cells.

- DEM exposure and consequential GSH depletion altered considerably the global gene expression profile of the transformed C3H10T1/2 cells. The statistically significant upregulation of only glutathione metabolism pathway and the statistically significant downregulation of several important pathways occurred. These included the pathways of Cancer, cytokine-cytokine receptor interactions, chemokine signaling, melanogenesis, focal adhesion, renal cell carcinoma, axon guidance, gap junction, arachidonic acid metabolism, TGF-beta signaling, cardiac muscle contraction, retinol metabolism, arrhythmogenic right ventricular cardiomyopathy, vascular smooth muscle contraction, RIG-I-like receptor signaling pathway, graft-versus-host disease. GSH depletion downregulated the expression of those genes that otherwise strengthened the functioning of tumor cell characteristics like sustained signaling for proliferation, replicative immortality, evasion of growth suppressors, resistance to cell death, angiogenesis, and activation of unrestricted migration, invasion, and metastasis in extracellular connective tissues. Specifically the genes DAPK2, FGF18, HCK, and EPHB6 found to be upregulated in transformed C3H10T1/2 cells showed downregulation after GSH depletion by DEM.

- The GSH depletion after PHO exposure also resulted in similar alterations of global gene expression profile in the transformed C3H10T1/2 cells. Data showed the statistically significant upregulation of three pathways namely the metabolic pathway, glutathione metabolism pathway and focal adhesion
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pathway; and the statistically significant downregulation of several pathways namely cancer, actin cytoskeleton, calcium signaling pathway, cytokine-cytokine receptor interaction, Wnt signaling pathway, GnRH signaling pathway, chemokine signaling pathway, arachidonic acid metabolism, drug metabolism - cytochrome P450, RIG-I-like receptor signaling pathway and Renal cell carcinoma. Specifically few genes (DAPK2, FGF18, CAMK2B, PDE1B, BST1 and ART3) otherwise found to be upregulated in transformed C3H10T1/2 cells showed downregulation after GSH depletion by PHO. In a comparative analysis, GSH depletion in the transformed cells by DEM or PHO upregulated commonly the genes GSTA1, GSTA3, GCLM, GCLC, GSR, GSS, GSTA3 and GSTA2. These are related to the glutathione metabolism. GSH depletion by PHO or DEM downregulated commonly the expression of FGF18, IGF1, HGF, DAPK2, TGFB2, LAMA1, PDGFB, EPAS1, FGFR2, LAMA4, CCL9, EDAR, PF4, CXCL12, C3, CCL20, CXCL5, TNFRSF11B, PDGFD, CXCL1, CCL7, HCK, ADCY1, GNG7, CBR2, GGT6, CYP2J9, DHX58, ISG15 and DDX58. Which were related to cancer, cytokine-cytokine receptor interaction, chemokine signaling, arachidonic acid metabolism, RIG-I-like receptor signaling pathways and renal cell carcinoma pathways.

On the basis of above results it is concluded that the complex process of chemical carcinogenesis illustrated by the cardinal cellular changes and by the dysregulation of strategically related key pathways and gene in the transformed cells respond to GSH manipulations and therefore are redox
regulated. The new knowledge of commonly dysregulated genes can be exploited as novel targets for designing new anticancer drugs and for use in diagnosis and prognosis of cancer.