SUMMARY AND CONCLUSIONS

Myriad number of intercalated factors play a pivotal role in maintaining normal physiological conditions. Malfunctioning of any one of these might result in pathophysiological conditions, which is deleterious to life. Cancer- as a diseased state is one such a culmination of more than a factor, where in the normal cells are irreversibly converted to a state where the neoplastic cells loose the potential to control the proliferation. Some of these factors are either toxic electrophiles generated endogenously or xenobiotics to which one is exposed. Cellular system, however, are equipped with detoxification systems, among which glutathione S-transferases constitutes a primary pathway. Preclinical studies have correlated enhanced metabolism of electrophiles with increased levels of GST isoenzymes within various tissues. Expression of GSTs in an individual can therefore provide an indicator about the metabolic potential of their tissues and possible deficiencies in the susceptibility to dietary or environmental carcinogens. GSTs are overexpressed in certain tumor types, therefore measurement of GST and their subunits in serum or in pathological specimens can be used as diagnostic markers for certain types of cancer. Also overexpression of GSTs have been implicated for the development of drug resistance during the course of treatment of cancers. Therefore, measurement of GSTs can be used to follow the course of disease and to monitor the success of intervention. The
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present study in an attempt to isolate and characterize GSTs expressed in rapidly proliferating normal and cancerous tissues. In order to develop rapid assay methods to identify individual GST subunits such as ELISA and Western blot studies, GSTs were purified from human uterus, individual subunits separated on RP-HPLC and polyclonal antibodies were raised. The salient findings of the present study are summarized below.

1) Human Uterine GSTs were purified to electrophoretic homogeneity by GSH-Affinity chromatography.

2) SDS-PAGE of affinity purified cytosolic uterine GSTs resolved into four bands with relative molecular weights of 23, 26, 27 and 28.5 kDa respectively.

3) Further separation of affinity purified cytosolic uterine GSTs on RP-HPLC showed four peaks with retention times of 15.2, 16.1, 28.4 & 36.6 min respectively.

4) Based on the order of elution on RP-HPLC and immunoblot analysis, the four peaks obtained on RP-HPLC were identified as $\mu_1$ (15.2 min), $\mu_2$ (16.1 min), $\eta$ (28.4 min) and $\alpha$ (36.6 min).

5) Polyclonal antibodies were raised in rabbits to different subunits of human uterine GSTs separated on RP-HPLC.

6) In order to understand the role of GSTs in colon cancer, GSTs were purified and characterized from colon cancer
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tissues. Using the class specific polyclonal antibodies and RP-HPLC data it is identified that there is an increase in \( \mu_2 \) (7%) and \( \pi \) (38%) and decrease in \( \alpha \) (28%) in adenocarcinoma of colon. These results suggest that \( \alpha \) and \( n \) subunits of GSTs can be used as potential biomarkers for detection of colon cancer.

7) Similar studies on squamous cell carcinoma of cervix, penis and uterine endometrium revealed that \( n \) form of GST is the major subunit. Also no significant difference was observed in the levels of different subunits of squamous cell carcinoma tissues.

8) In order to study the involvement of GSTs in actively proliferating normal tissues, GSTs were purified from fetal livers at different stages of development. These studies revealed that \( \alpha \) class GSTs are the only type of GSTs expressed in fetal livers. Fetal liver is conspicuous in the absence of \( n \) and \( \mu \) class of GSTs. These studies on fetal livers thus revealed that normal proliferative cells do not mimic the expression of GSTs in cancerous tissues i.e. abnormal proliferative cells. Further it can be suggested that different regulatory mechanisms are controlling the expression of
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GSTs in cancer (abnormal proliferating) fetal (normal proliferating) tissues.