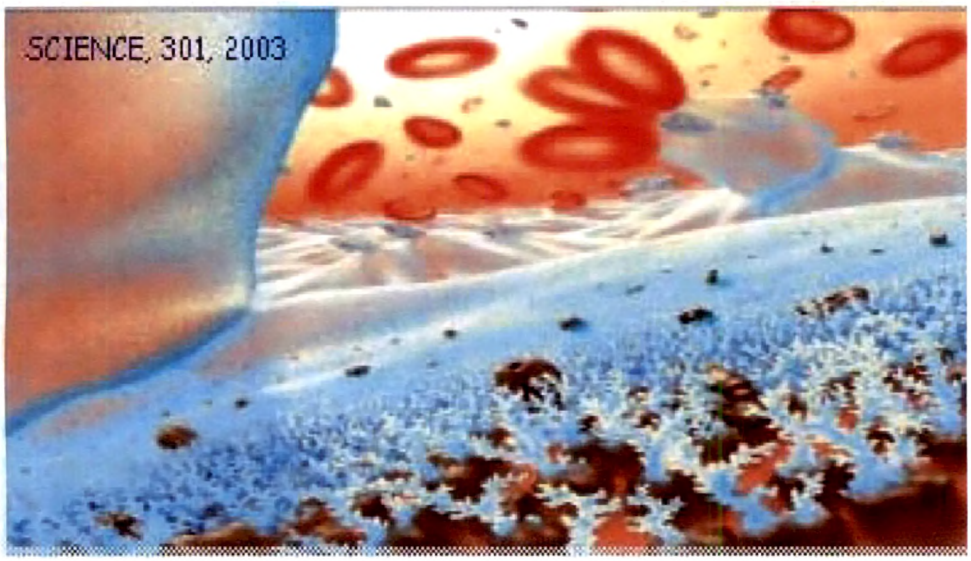
Epilogue



The "Sugars", Biology's next sweet spot for the researchers, can be significantly useful to address current clinical problems of "Cancer"

6. EPILOGUE

Oral cancer is the "new epidemic" in India due to predominance of tobacco habits. Late presentation, low overall survival and spread of the disease are the major clinical challenges. The incidence of OPC continues to increase among the young population. Several genotypic and phenotypic changes play a major role during carcinogenesis. Altered terminal glycosylation and loss of cell adhesion are among the vital phenotypic changes associated with malignant transformation.

The study presented in this thesis center around terminal glycoprotein changes in oral cancer which included blood samples obtained from healthy individuals (n=100), patients with OPC (n=75) and untreated oral cancer patients (n=130) as well as post-treatment follow-ups (n=75) of oral cancer patients. Detailed information of tobacco habits, clinical history, and socioeconomic status of the subjects was gathered. Malignant (n=75) and OPC (n=10) tissues and their adjacent normal tissues were also collected. The terminal sugars, sialic acid and fucose as well as enzymes and proteins changes associated with their metabolism were analyzed. The experimental approach included various techniques viz. spectrophotometry, spectrofluorimetry, ELISA-based solid-phase assay, dot blot, lectin-affinity chromatography, 2D-PAGE, Western blot, etc. The serum levels of the markers were compared between healthy individuals, OPC and oral cancer patients. Further, the markers were compared in oral cancer patients before and during/ after anticancer therapy to assess usefulness of this marker panel in monitoring treatment response. Expression of cell surface antigen SLe^X and transmembrane cell-adhesion glycoprotein E-cadherin were studied from malignant, precancerous and adjacent normal tissues. The statistical significance of the data was evaluated using different statistical methods. The noteworthy observations of the study were as follows:

6.1: SUMMARY

- More than 97% of the patients with OPC and oral cancer patients were tobacco habituates.
- Serum TSA, TSA/TP, fucose and fucose/TP were significantly higher in untreated oral cancer patients as compared to the patients with OPC and controls. Also, elevations in serum TSA, TSA/TP, fucose and fucose/TP in patients with OPC were significant as compared to the controls.
- Serum α2,6-sialoproteins was significantly high in oral cancer patients as compared to the controls and the patients with OPC. Mean density of α2,6-sialoproteins was also higher in patients with OPC than controls.
- Serum fucoproteins were significantly higher in oral cancer patients as compared to controls as well as OPC. Also, increased LTA reactivity was found in patients with OPC as compared to the controls. The electrophoretic patterns showed higher fucosylation of ~43 kD and ~66 kD proteins.
- Serum α2,6-SiT activity was significantly elevated in oral cancer patients as compared to patients with OPC and controls. However, serum α2,3-SiT did not show significant difference among the three groups.
- Serum sialidase activity was significantly high in oral cancer patients as compared to the patients with OPC.
- Patients with OPC and oral cancer showed significant elevations in serum fucosidase activity as compared to the controls.
- Multivariate analysis revealed that serum α2,6-SiT activities were significantly associated with tumour differentiation and nuclear grade of the tumours. Serum α2,6-sialoproteins levels and α2,6-SiT activity were significantly higher in the advanced disease stage. Mean TSA, TSA/TP and α2,6-sialoproteins were higher in poorly differentiated tumours as compared to well and moderately differentiated tumours. Alterations in serum fucose and fucose/TP levels were significantly correlated with tumour differentiation.

- Serum fucosidase activity was significantly associated with the stage of the disease.
- TSA, α2,6-sialoproteins, α2,3-sialoproteins, α2,6-SiT, α2,3-SiT and sialidase levels were significantly higher in malignant tissues as compared to adjacent normal tissues. Also, high α2,6-sialoproteins and α2,3-sialoproteins levels and sialidase activity was seen in precancerous tissues as compared to adjacent normal tissues. α-L-fucosidase activity was significantly higher in malignant tissues than the adjacent normal tissues. OPC tissues also showed increased α-L-fucosidase as compared to adjacent normal tissues.
- α2,6-SiT and α2,3-SiT activities in malignant tissues were increased as the stage of the disease advanced, whereas, sialidase activity was decreased. α2,3-SiT activities as well as α2,3-sialoproteins and α2,6-sialoproteins in malignant tissues were higher in poorly differentiated tumours as compared to well and moderately differentiated tumours.
- Serum levels of TSA, TSA/TP, fucose, fucose/TP, fucoproteins as well as activity of α2,6-SiT, α2,3-SiT and fucosidases were significantly decreased in CR as compared to their pretreatment levels. Serum levels of these markers were increased with disease progression and recurrence.
- Mean serum TSA, α2,6-SiT, fucose and fucose/TP were significantly increased in NR as compared to their pretreatment levels. Mean α2,3-SiT, sialidase and fucoproteins were higher in NR as compared to pretreatment.
- Serum protein profiling revealed remarkable presence of an unusual serum protein band in the post beta region. Presence of extra protein band was seen at a higher frequency in cancer patients (72%) and patients with OPC (75%) as compared to the controls (24%). The unusual protein band was glycoprotein in nature.
- 2D-PAGE analysis revealed two distinct protein spots in serum sample positive for the unusual protein band. Further, separation of the protein elute on SDS-PAGE revealed three distinct bands. The molecular weight

determination of extra protein subunits revealed 54.95, 79.43 and 120 kD apparent molecular weights of three bands.

- Paired t-test demonstrated significant over expression of SLe^X expressions in malignant and OPC tissues as compared to their adjacent normal tissues. Expressions of $E\text{-}cad^{120}$ were comparable between malignant/OPC and adjacent normal tissues. While, truncated $E\text{-}cad^{97}$ expression and ratio $E\text{-}cad^{97}$:$E\text{-}cad^{120}$ were increased in tumour and OPC tissues as compared to their adjacent normal tissues.
- ROC curve indicated good discriminatory efficacy of SLe^X, $E\text{-}cad^{97}$ and $E\text{-}cad^{97}$:$E\text{-}cad^{120}$ between malignant and adjacent normal tissues.
- Over expression of SLe^X, $E\text{-}cad^{97}$ and $E\text{-}cad^{97}$:$E\text{-}cad^{120}$ in malignant tissues were positively associated with nuclear grade, tumour differentiation, lymphnode metastasis and advanced stage of the disease.

6.2: CONCLUSION

Glycobiology has enormous role in cancer development and progression. Hence, the glycobiology studies can significantly contribute in the field of cancer research. Current study demonstrated a number of important aspects of terminal glycoprotein changes in oral cancer. The inclusion of OPC group established the connecting link between normal and malignant conditions. The results suggested that sialylation and fucosylation changes are dominant in oral carcinogenesis. Serum and tissue sialylation and fucosylation changes in patients with OPC indicate early events and thus may be helpful for identification of high-risk group for cancer development. The alterations in serum and tissue marker levels could provide information about the aggressiveness of tumour at the time of diagnosis. Evaluation of the serum markers during post-treatment follow-up could be helpful in treatment monitoring. The markers could be useful for predicting relapse free survival, recurrence and progression of the disease. Further characterization of the unusual serum protein may give clue about the early changes taking place during oral cancer progression. Significantly increased expressions of SLe^X

and truncated E-cad^{97} are found to be associated with alterations in cell adhesion and in-turn invasion and/or metastatic properties of cancer cells. Inactivation of E-cadherin can possibly be due to aberrant translational regulation or dysfunction in posttranslational events, which would be a remarkable approach to predict the prognosis of the patients. Overexpression of SLe^X and E-cad^{97} in oral tumours may also be useful to predict disease progression and lymphnode metastasis. Thus, this study supported the hypothesis that threshold high electro-negative potential on cell surface due to hypersialylation aid cell-cell repulsion and functional loss of E-cadherin may permit the cancer cell detachment from local tumour environment. These phenomena may increase invasiveness and metastatic potential of cancer cells. Further, increased fucosylation and SLe^X expressions may facilitate these cells to migrate to distant sites through binding endothelial cell selectins. The uniqueness of the present findings strengthens the significance of glycobiology in oral cancer in current era of Glycomics. It is prudent to state that the study could contribute to build-up the vital information about malignant transformation and terminal glycosylation changes in oral cancer.

6.3: CONCLUDING REMARKS

Together with nucleic acids and proteins, carbohydrates represent the third important dimension of molecular biology. Thus, glycobiology, the carbohydrate biochemistry and cancer biology are two important complementary areas for cancer research. Understanding the terminal glycosylation changes in cancer is a challenging task that can improve the knowledge of this dynamic and fascinating field of research. The present approach may assist to design the glycosylation related inhibitors or carbohydrate based mimetics in targeted therapy directed against specific steps of cancer progressions to prevent invasiveness of primary tumours and metastasis. In furtherance,

- Proteomics and glycomics approach to the purified unusual serum protein band could be useful.

- Study of different isoforms of fucosyltransferase may help to explore its role in synthesis of SLeX in oral cancer.
- Soluble form of E-cadherin may exist in serum, which can indicate metastatic potentials of the tumours and could be useful as a prognosticator. Glycosylation pattern of E-cadherin and its role in MMP activation may be useful to understand metastatic cascade of oral cancer.
- Study of interactions between β-catenin with E-cadherin truncation and other extracellular matrix components can further explain their role in metastasis.
- It would be of great interest to accomplish and target anti-metastatic pathways for drug discovery to improve treatment response in cancer patients.