Computational Studies and Molecular Dynamics of the Potent Biochemical and Molecular Markers in Relevance to Oral Cancer

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Abstract: Oral cancer is the uncontrolled tissue growth observed in the oral cavity. It may arise as a consequence of metastasis and/or by extension from a neighboring anatomic structure, such as the nasal cavity or the maxillary sinus. In view of putative biochemical exploration on oral carcinoma and development of certain nutraceuticals viz. omega 3 versus 6 fatty acids, cancer protective vitamins, probiotics, antioxidants, oral enzymes and mineral mainly selenium based on the hypothesis projected by non-conventional laboratory study, the present work on molecular dynamics of oral cancer was undertaken. The data thus obtained provide new insights into exploring major biochemical and/or molecular markers probably involved in diagnostics as well as therapeutics concerned with the oral carcinoma.

Keywords: Bioinformatics, Neoplasia, Oncogenes, Oral cancer, Papillomavirus, Modeling, Docking.

INTRODUCTION

Oral cancer is mainly recognized as any indeterminate and asymmetrical tissue growth located in the oral cavity. It may arise as a primary lesion originating in any of the oral tissues either by metastasis from a distant site of origin; or through further extension from a neighboring anatomic structure(s) such as the nasal cavity or the maxillary sinus. Oral cancers may get initiated in any of the tissues of the mouth, and be observed as the varied histological types: teratoma, adenocarcinoma derived from a major or minor salivary gland, lymphoma from tonsillar or other lymphoid tissue, or melanoma from the pigment producing cells of the oral mucosa. Far away the most common oral cancer is squamous cell carcinoma, originating in, and spreading throughout, the tissues lining the mouth and lips [1]. Oral or mouth cancer has been observed to most frequently involve the tissue of the lips and/or tongue. It may also occur on the floor of the mouth, cheek lining, gingiva (gums) or palate. Most of the oral cancers are known as squamous cell carcinoma [1, 2], which are malignant and liable to expand rapidly. Oral cancer incidence ranks fifth in the global cancer burden, and a 2- to 3-fold mortality increase has been recorded in eastern and central European countries in the last three decades [2]. In India, oral cancer, ranks first among all cancer cases in males and is the third most common among females in many regions [3-5]. The major high- risk factors associated with oral cancer are: (a) smoking and use of other tobacco products; (b) alcohol use; (c) infection with human papillomavirus (HPV), particularly type 16 [6]. The mode of HPV infection has been documented [7]. There is evidence from certain laboratory studies, prospective cohort studies, and mechanistic studies showing that vitamin B-12 is an important nutrient for genetic stability, DNA repair, carcinogenesis, and cancer therapy [8, 9]. Cravo *et al.,* [10] used 5 mg of folic acid a day (a supra physiological dose) in a prospective, controlled, cross-over study of 20 patients with adenoma polyps. They found that the folic acid could reverse DNA hypomethylation in 7 of 12 patients who had only one polyp. Several prospective studies of vitamin D and cancer have also shown a protective effect of vitamin D [11]. It could be that sunshine and vitamin D are protective factors for carcinoma, which can convert 25(OH) D into 1, 25(OH) D2. Certain antioxidants (α- and β-Carotenes) have been studied vigorously to see if these colorful compounds can decrease cancer risk [12]. Besides, there are many more substances that will have some benefit for cancer therapy. Most of these substances are found in foods, but their effective doses for therapy are much higher than the normal concentration in the food. For example, grape seed extract contains proanthocyanidin, which shows anticarcinogenic properties [13]. Also, green tea contains a flavanol, epigallocatechin-3-gallate (EGCG) that can inhibit metallo-proteinases, among several possible other mechanisms [14]. And there are claims for various other herbal substances and extracts that might be of benefit, which are beyond the scope of awareness in the relevant area till date.

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Various biochemical and molecular studies appear to reflect the gene(s) probably being associated with cellular growth and differentiation [7]. Oncogenes, leading to mutations of highly regulated normal cellular counterparts, are responsible for involvement in the initiation and succession of oral neoplasia [7, 15]. Biochemical and molecular mechanisms of activation of the cellular oncogenes were monitored to ascertain point mutations and DNA re-arrangements [16, 17]. Several of these cellular oncogenes were established as homologs of retroviral oncogenes [18]. In contrast, tumor suppressor genes or anti-oncogenes have been recognized which confer potent negative regulatory controls that are not evident due to chromosomal alterations during tumor development. Functional loss of multiple tumor suppressor genes is believed to be the major event leading to the development of malignancy [19, 20]. Concurrently, the genetic polymorphism of three major free radical scavenging enzymes, namely, Glutathione-S-tranferase, Glutathione peroxidase and Glutathione reductase has been studied [21-23] in relevance to proteomics of oral carcinoma. Thus, in view of further biochemical exploration on oral carcinoma and development of nutraceuticals based on the hypothesis projected by non-conventional laboratory study, the present study on bioinformatics and molecular dynamics of oral cancer was undertaken.

MATERIALS AND METHODS

Retrieval of Marker Enzyme Proteins

The sequences of the enzyme-proteins viz. Glutathione-s-transferase, Glutathione reductase and Glutathione peroxidase were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and the respective template structures (Glutathione-S-transferase: PDB_ID:3IE3, Glutathione reductase: PDB_ID:3H4K, Glutathione peroxidase: PDB_ID:2WGR) were chosen using PSIBLAST search with PDB.

Modeling of Proteins and Ligands

The templates of all above three marker enzyme-proteins were aligned with respect to respective target sequences, and after successful examination for potential alignment errors, the automated comparative protein modeling program MODELLER 8v2 [24] on SGI Insight II was used to build-up the specific model(s). Further, the ligands were designed with the aid of the canvas module of Schrodinger software [25] followed by the energy minimization using discovery studio 2.5 [26]. Protein structure prediction was accomplished with the aid of HB (hydrogen bonding) plot and Ramachandran plot employing the Virtuadrug server [27] and RAMPAGE evaluation server [28], respectively.

Docking

The enzyme- proteins, namely, Glutathione-S-transferase, Glutathione reductase and Glutathione peroxidase were docked sequentially with ligands such as DTT, EDTA and iodoacetamide using Autodock 4.2 [29]. Besides, certain other putative tools, namely, ARGUS LAB (http://www.arguslab.com/arguslab.com/ArgusLab.html) and DrugBank's Chem Query tool (http://www.drugbank.ca/chemquery/smiles) were also incorporated herein for revalidation of the docking results.

RESULTS AND DISCUSSION

The theoretical models of marker enzyme- proteins, namely, glutathione peroxidase, glutathione-S-transferase and glutathione reductase were generated with the help of MODULLER 8v2 as shown in Fig. (**1**). Among the twenty models generated from SGI Insight-II for each of the enzyme- proteins, the lowest energy structures were illustrated in Fig. (**1**).

Successful execution of the binding of ligands to these putative macromolecules of relevant interest was accomplished with an aid of the docking server online, showing the optimized affinity of the ligands viz. Iodoacetamide, beta-mercaptoethanol and DTT. . The desired enzyme model was selected and validated on the basis of estimated free energy of binding, inhibition constant (Ki), van der Waals resultants, electrostatic energy, total intermolecular energy (data not shown). In addition, rather more advanced studies regarding projection of HB plot results (Fig. **2**) and Ramachandran plot (Fig. **3**) were undertaken and analyses reflected that all models with specific chain conformation, and relating to the allowed region for all the three enzymes (Fig. **1**), were observed to have binding of 92%, 93% and 89 %, for glutathione peroxidase, glutathione S-transferase and glutathione reductase respectively. Interestingly, HB Plot represented the well-defined structure of marker enzyme

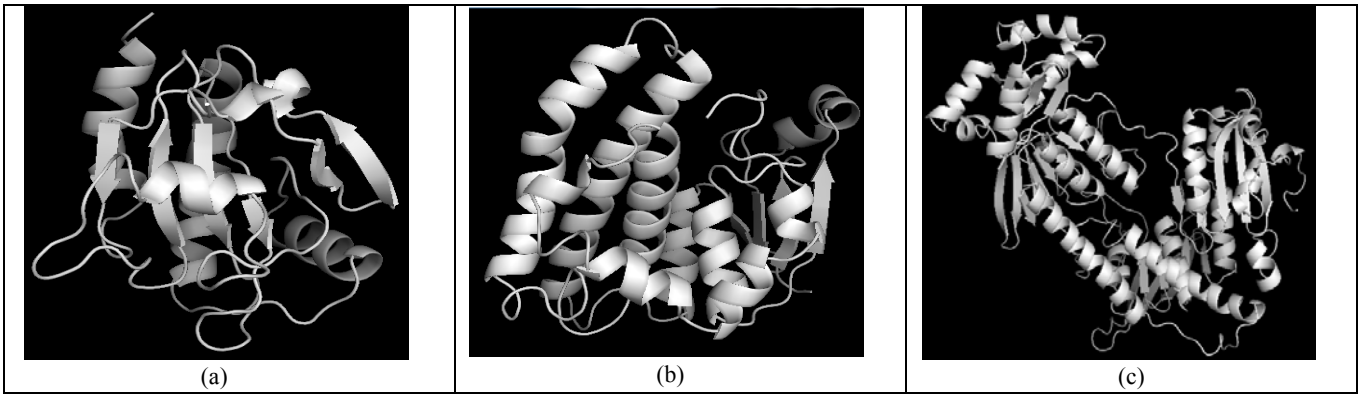

Fig.(1). Modelled enzymes using Modeller 8v2 on SGI Insight II (**a**) Glutathione peroxidase; (**b**) Glutathione-S-transferase; (**c**) Glutathione reductase.

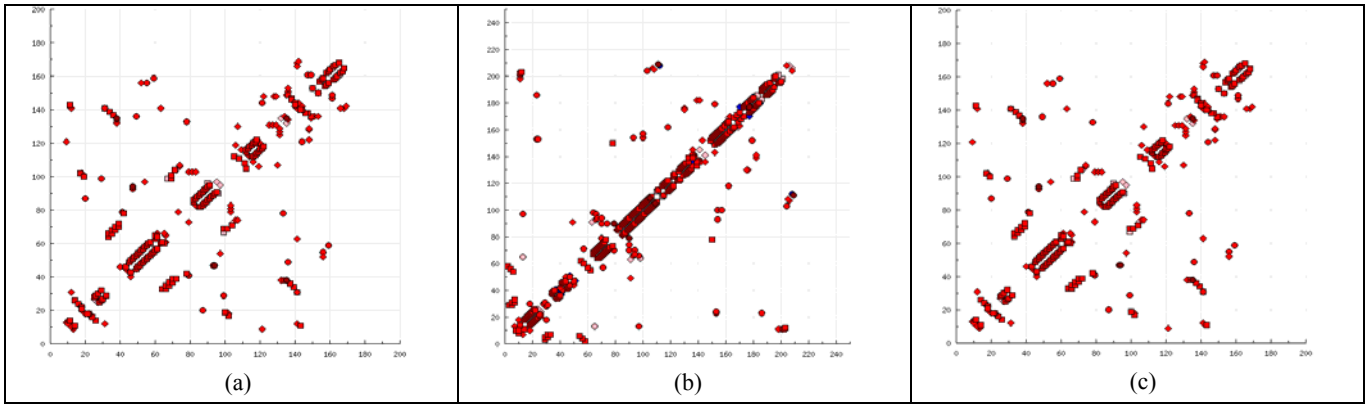

Fig. (2). HB plot detection using virtuadrug server for (**a**) Glutathione peroxidase; (**b**) Glutathione-S-transferase and (**c**) Glutathione reductase.

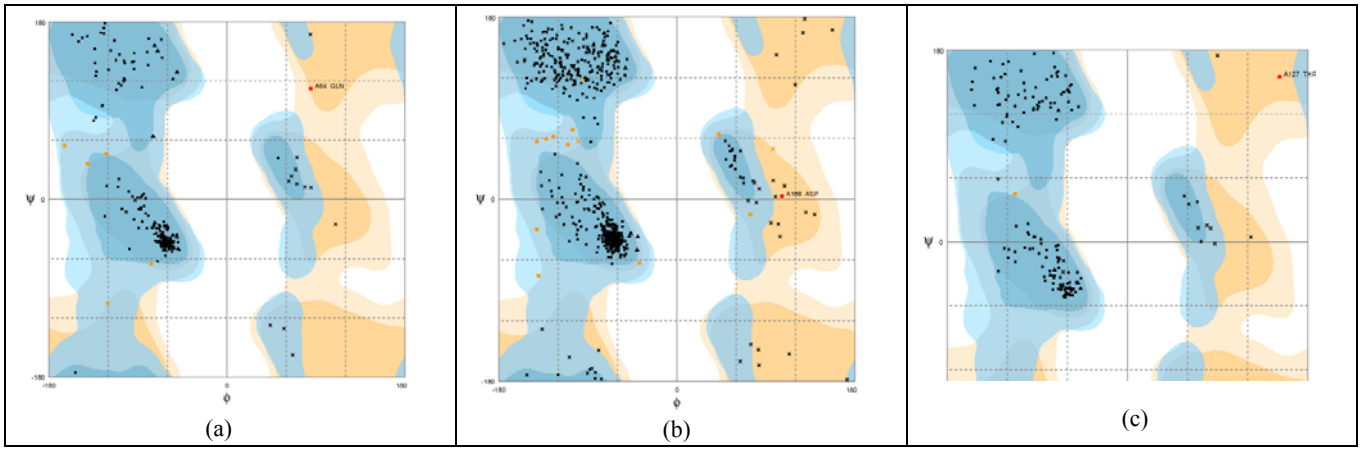

Fig. (3). Ramachandran plot for modelled enzymes using RAMPAGE evaluation server for (**a**) Glutathione-S-transferase, (**b**) Glutathione reductase, (**c**) Glutathione peroxidase.

protein containing parallel and antiparallel beta sheets. Glutathione peroxidase was shown to be comprised of a similar number of β strands and α helices i.e. 6. Glutathione-S-transferase was noticed to have 4 β strands and 10 α helices, while Glutathione reductase has 24 β strands and 19 α helices. The designing of ligands of interest as shown in Fig. (**4**) was carried out, and followed by competent docking to various marker enzyme-proteins. Specific binding sites invoke the enzyme-protein(s) functional activity with respect to diagnosis as well as in clinical practices relevant to oral cancer. Fig. (**5**) shows that the docking of glutathione peroxidase with iodoacetamide (IA) reflects a putative interaction, mainly, hydrogen bonding with specific amino acids (ASP94, ARG160) at certain specific binding (H-H, N-H and O-H) sites for regulating the downstream path of apoptosis ultimately leading to necrosis and thus oral carcinoma. Affinity of glutathione peroxidase with beta-mercaptoethanol (BME) and dithiothreitol (DTT) was observed more or less in similar fashion in terms of binding pattern as well as site(s) of interaction of amino acids (THR30, ARG33 and ASP34), although this conjugation (H-H, N-H and O-H) was much stronger as compared to that with iodoacetamide. On the basis of H- bonding the best interaction could be concluded in case of Glutathione peroxidase. The authenticated reason for the distinction in the mode of action and reaction relevant to these putative biochemical markers is still obscure and further exploration along this avenue is in progress in our laboratory. As a preliminary conclusion, Glutathione-S-transferase has been observed to show rather different bridging with iodoacetamide, beta-mercaptoethanol and dithiothreitol in such a way to specify a kind of reversal mechanism of spreading and/or checking oral carcinoma at cellular/molecular level. Indeed, ligand binding with iodoacetamide interacting (N-H) at specific amino acids (GLU101, THR104) and interaction (H-H, O-H) of beta-mercaptoethanol and dithiothreitol at specific amino acids (Tyr7, HIS108, ASP162) may probably be helpful for identifying the homologous molecules and their specific derivatives in various herbs likely to provide the base of significant nutraceutical edibles. This important area was explored and the approach attempted to resolve certain unanswered facts using specific bioinformatics tools, which were rather cheaper and convenient as compared to enzymatic studies in the chemical and reagent-based laboratory. Further interaction (N-H, O-H, and H-H) of glutathione reductase with ligands iodoacetamide, beta-mercaptoethanol and dithiothreitol at specific amino acids (SER81, SER96, GLY204, ASP97, GLU119) was noticed to be poorer than glutathione peroxidase and transferase (data not shown). These differential binding affinities of three specific ligands with specific enzyme-proteins, putative biochemical and/or molecular markers, reflect variable mode(s) of interaction(s) in oral cancer initiation, propagation and/or preventing/curing the cancer from the grass-root level.

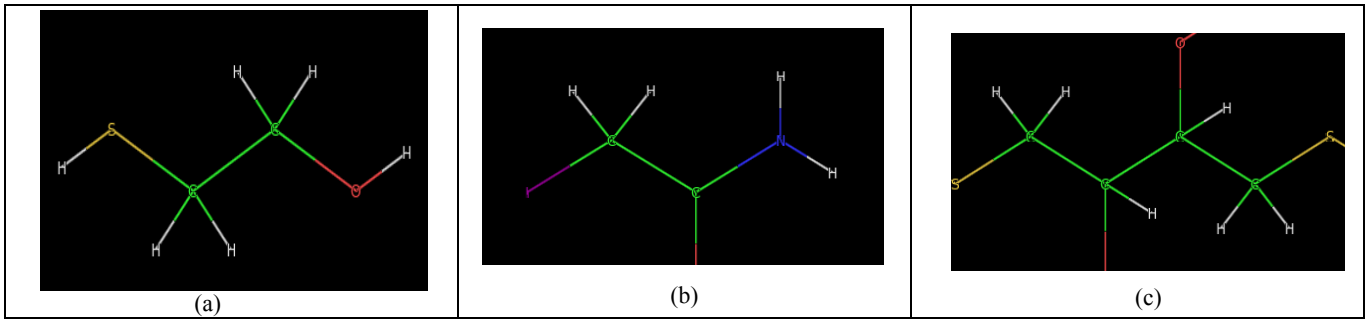

Fig. (4). The ligands β-Mercaptoethanol, iodoacetamide and dithiothreitol, were designed with the help of canvas module of Schrodinger software.

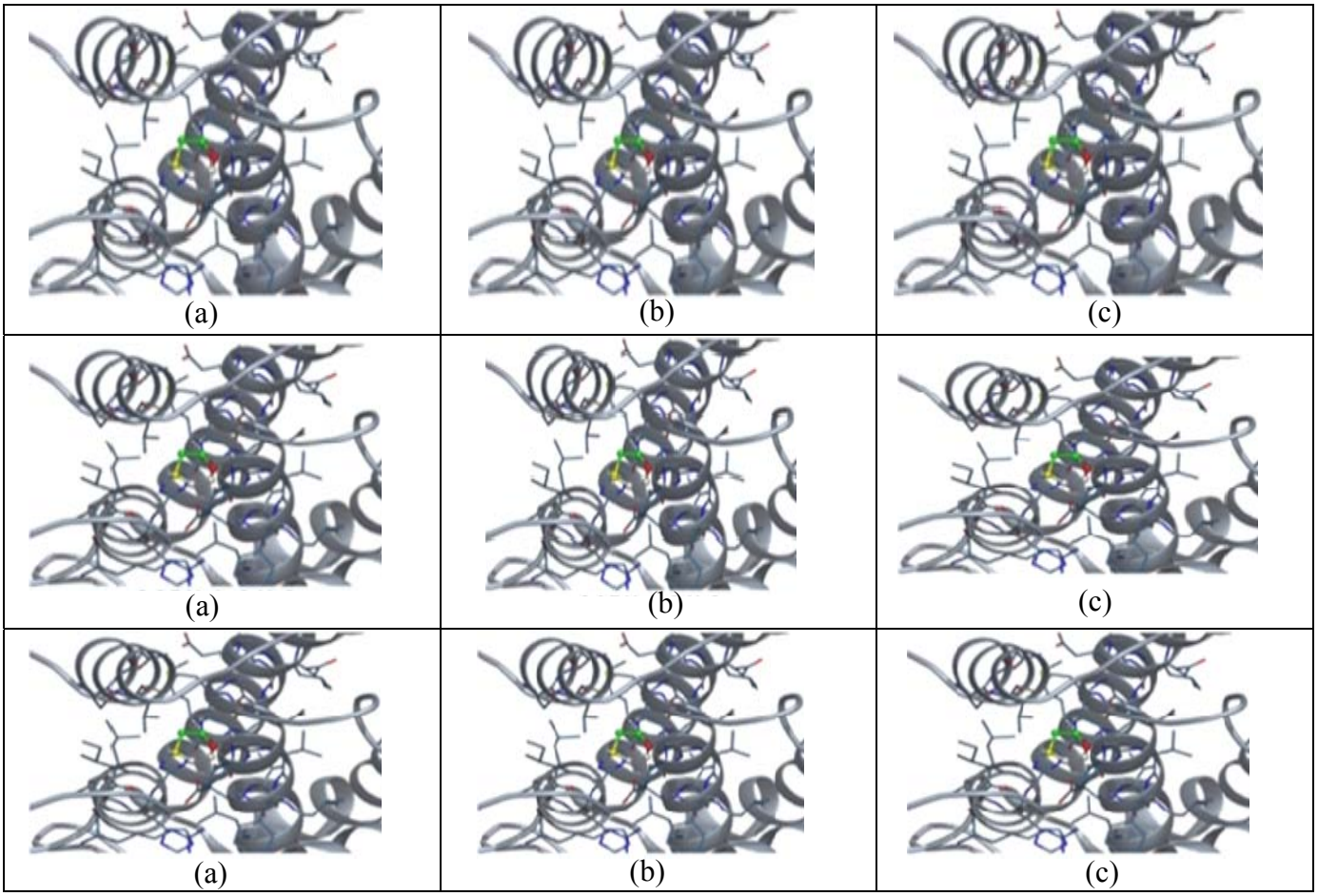

Fig. (5). Interaction between DTT, BME and IA and the modelled enzymes Glutathione peroxidase, Glutathione-S-transferase and Glutathione reductase respectively. 'Interacting amino acid residues' has been produced by Discovery studio 2.5.

Lastly, the authors attempted to comprehend the studies from a viewpoint of activation as well as inhibition of enzymes' pathway with an expression of regulation of state(s) causing oral cancer. The results obtained so far may provide new insights into studies on both of the aspects so as to explore a platform for the development and projection of certain hypotheses for scientists working in the area of biochemical and molecular biology themes for oral cancer. The docking results of DTT and BME are more or less identical, reflecting their possessiveness to keep the enzymes intact and prevent them from oxidation. A good number of hydrogen bonds was observed to be formed between the lead and the target, and showed their complex highly stable and thus binding being fairly specific. The low MolDock score of certain selective nutraceuticals, namely, Cyclocurcumin (-599.644), Curcumin (-587.426) obtained for the lead-target protein complex (data not shown) was observed to be a favorable one as it depicts that the ligands have sufficient affinity and specificity towards the target protein. The degree of average MolDock score were varying among the natural ligands considered in the present study. Further comprehension of the relevant bioinformatics studies is on the way to overview knowledge on oral cancer validating the studies concerning with (a) malnutrition and malfunctioning of glu-

cose metabolism [30]; (b) omega 3:6 ratio imbalance [31, 32]; (c) over consumption of energy [33, 34]; (d) selenium, a mineral with anti-cancer properties [35]; (e) cancer protective vitamins [9, 36, 37]; (f) antioxidants [14, 38]; (g) probiotics [39-43]; (h) oral enzymes [44-47]. Besides, certain specific phosphatases and/or kinases [48, 49] taken together with data obtained from the present study may probably be of great interest with respect to regulation and/or prevention/cure of oral carcinoma.

CONCLUSION

The data obtained from the present study provide clues for exploring major biochemical and/or molecular markers probably involved in diagnostics as well as therapeutics concerning with the oral carcinoma. Further, the hypothesis originated from the present study would certainly be of value in drug design and targeting oral carcinoma.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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