SUMMARY AND CONCLUSION

Aging population, stressfully transforming lifestyle and perturbed urological physiology contributed to the recent anxious rise of incidence of prostate cancer (PCa) across the globe, including India. Despite of advancing knowledge in both medical diagnostic technology and curative interventions; and decades of applied urological research, the existing insights are insufficient to curb the menace. This very situation intrigued us to investigate and identify the differential and clinically relevant prognostic serum based biomarkers in induced animal model of PCa with an intention to contribute an insight in early and accurate detection and additive avenues for effective clinical case management. CA+TP+MNU (Cyproterone acetate plus Testosterone propionate plus N- nitroso N Methyl Urea) induced rat prostate cancer was developed using a well – established animal model. For proteome analysis serum samples from CA+TP+MNU-treated animals and healthy control were collected at different time intervals. These samples were processed further which enabled us to study the differentially expressed proteins during early stages of prostate carcinogenesis. We employed 2-DE and peptide mass fingerprinting based on MALDI-TOF-MS techniques adopting proteomics approach, tissue diagnosis for pathophysiological characterization and disease differentiation were assessed by histopathology and to corroborate the sustainable evidences of proteomics observations and to ascertain the functional association and regulation of corresponding genes; transcriptional genomics were performed using qRT-PCR.

MRPL15, DCAF6, SRCIN1, PTP4A2, PDLIM5, ZFP663, RASA3, MPP2, and CSK serum proteins were potentially prominent in chemically induced progression of prostatic malignancy as compared to non-induced tissue. These proteins are actively engaged in cellular death processes, autophagy, downstream signaling cascade, orchestrating cellular energy and
metabolism, regulation of tumour suppressor genes, and mediating various other cellular machineries in normal and augmented cells. In view of the identified cellular components and in order to understand the functional expression and impact of these proteins on its regulation in augmented cellular process i.e. during the progression of malignancy; we analyzed the transcriptional expression of MRPL15, SRCIN1, PTP4A2 and DCAF6 genes, using RNA from MNU induced prostatic dorsolateral lobe tissue of adult Wistar rats in time dependent stratified group of animals as compared to non-induced control group of animals by employing qRT-PCR. We distinctly observed MRPL15, DCAF6, SRCIN1, and PTP4A2 novel and under investigated serum markers in protein profiling and time dependent differentially expressed patterns of respective genes in Wistar rat PCa tissues. We found significantly upregulated expression of MRPL15 and quite early pronounced shift (4.9 fold), suggesting specificity and efficiently early tumour inducer candidate marker; whereas DCAF6, though showed overall upregulation, but pronounced and noticeable shift (7.8 fold) was observed quite late; suggesting time dependent sensitivity as marker’s characterization of late tumour inducer candidate. Further, SRCIN1 were down regulated irrespective of time course, whereas PTP4A2 were also down regulated but quite late and marginally showed non-significant upregulation (<0.5 fold); suggesting specificity of SRCIN1 and PTP4A2, as tumour suppressor marker. However, PTP4A2 may have dichotomic potential as quite late phase tumour inducer marker candidate as well.

Human prostate specific gene-1 (HPG-1), a novel gene has been found to have potential involvement in prostate carcinogenesis. Expressed exclusively in prostate tissue it is a recently discovered gene that is localized at the 3q26 chromosomal locus. It is a membrane-anchored/attached protein having a molecular mass of 14.8 kDa and is involved in prostate carcinogenesis (Herness et al, 2003). The absence of the crystal structure of HPG-1 prompted us
to generate a 3D model for docking studies. Five models of HPG-1 (M1-M5) were built using I-TASSER. Based on the DOPE score (-13020.55) the best four models (M4) were selected. The overall G-factor was calculated to be 0.04 (>0.50) of the M4 model, which indicated the acceptance of the model quality. The potential active site residues of HPG-1 were screened by the 3DLigandSite and ConCavity web server. A total of 11 active site residues (namely Thr^{40}, Gln^{44}, Trp^{52}, Pro^{53}, Asp^{87}, Cys^{114}, Arg^{115}, Ile^{116}, Lys^{117}, Ile^{120}, and Phe^{122}) were considered for further analysis. The prominent amino acid residues such as Asp^{87}, Lys^{117} and Ile^{120} were predicted by both servers. Two β sheets (β1 and β2) were present on either side of Asp^{87}. Other residues, Lys^{117} and Ile^{120}, were found to be present in the functional loop. All these active site residues of HPG-1 seem to be involved in protein-ligand interaction and therefore were used for the screening of anti-HPG-1 compounds. The predicted active site, as discussed previously, was used to dock all the molecules to estimate the site of interaction of the ligand molecules with the target protein. Docking calculations for the P dataset revealed two anticancer compounds i.e. Nilutamide (DB00665) and Buserelin (DB06719); with dock scores of 49.47 and 48.79, respectively. We performed five independent MD simulations for the protein alone, the protein in complex with known inhibitors (Nilutamide and Buserelin), and the protein in complex with hit compounds (compound-1 and compound-2) for 100 ns simulation. Similarly, we have observed that the protein in complex with known inhibitors (Nilutamide and Buserelin) showed a significantly different pattern until 50 ns, and there were minor differences in the trajectory leading to a stable equilibrium through to the end of the simulation. This result depicts that the protein’s unbound forms have slightly higher RMSD values than those of protein-compound-1 and protein-compound-2 complex achieves stability. The secondary structure assessment of both complexes during the MD simulation was found to be strongly conserved during the course of
MD simulations: reflecting the stability of secondary structural elements of the model in the aqueous environment. In order to crack the differences in the binding patterns of protein in a complex with known inhibitors (Nilutamide and Buserelin) and a protein in a complex with hit compounds (compound-1 and compound-2), the free energy of binding for all the complexes was calculated using snapshots from MD trajectories through the MM/PBSA method. We have calculated the free energy of binding for the known inhibitors, Nilutamide (-55.2) and Buserelin (-53.6), in the complex with the HPG-1 protein. As compared to the compound-1 complex (-59.3), the compound-2 complex showed a higher negative binding free energy (-69.2). This compound-2 may be further developed as an anti-cancer drug against prostate cancer. Analysis of the 100ns MD simulation revealed that compound-2 stably bound to the active site pocket of the HPG-1 protein playing a major role in the binding of the compound and represents the massive hydrophobic interactions between receptor-ligand complexes: thereby stabilizing the protein-substrate complexes.

Prediction of a robust homology model of HPG-1 protein in combination with docking and MD simulation results in valuable structural insights for ligand binding. Importance of key residues such as Asp^{87}, Arg^{115} and Lys^{117} were elucidated for a stable ligand binding. Specific role of Lys^{117} was highlighted, which is crucial for designed ligand molecules. The said contact is believed to be favorable via cation -π interaction and can be useful for designing selective inhibitors against HPG-1 protein. Docking and simulation studies revealed ethyl 5-[(4-[(4-(ethoxycarbonyl)-1,2,3-thiadiazol-5-yl)amino]butyl)amino]-1,2,3-thiadiazole-4-carboxylate and ethyl 5-[(3-[(4-(ethoxycarbonyl)-1,2,3-thiadiazol-5-yl)amino]propyl)amino]-1,2,3-thiadiazole-4-carboxylate as possible inhibitors of HPG-1 protein. All these pieces of information are believed to be useful for rational design of prospective novel anti-HPG-1 protein compounds.
In conclusion, by developing a well-established Wistar rat prostate cancer model, we analyzed the differential protein expression pattern of treated group or induced group in comparison to control group and identified novel set of proteins at very early stages of prostate carcinogenesis by proteome analysis using 2-DE and MS techniques. To the best of our knowledge, the proteins reported in our study have not been previously reported to be differentially expressed during very early stages of prostate carcinogenesis. These proteins may therefore possess a potential candidature in improving PCa diagnosis in its early stage. Further functional and clinical validation studies of these cancer-associated proteins are necessary to elucidate their precise role in the process of prostate carcinogenesis. Moreover, the results from our study may provide useful addition to the growing knowledge in the pathogenesis of prostate cancer. Ultimately, such proteins (biomarkers) would aid clinicians in diagnosing PCa during the early stages and preventing from unnecessary biopsy and overtreatment.