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MODELLING STUDIES ON MOLECULAR PATHWAYS RELATED TO HYPOXIA IN SOLID TUMOR GROWTH

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Background
Hypoxia, a frequent effect of solid tumor growth, serves to generate a cascade of molecular pathways which include angiogenesis, glycolysis and various cell-cycle control proteins.[1] Hypoxia creates conditions that, on one hand, are conducive to the accumulation of extracellular adenosine and, on the other, stabilize hypoxia-inducible factors, such as HIF-1α.[2]

Aims
The aim of this study was to use combined homology modeling, molecular dynamics, 3D-QSAR, and molecular docking studies to identify leads with inhibitory activity towards HIF-1α and A3 adenosine receptors. Their functions can be specifically inhibited by:
1) blocking protein-protein interaction between C-terminal transactivation domain (CTAD) of HIF-1α and p300;
2) hypoxia response elements (HRE) at the DNA consensus sequence binders;
3) A3 receptor antagonists.

Methods
HIF-1α homology model was built with I-TASSER based on multiple-threading alignments (LOMETS/iterative TASSER assembly simulations). To screen within ZINC-DB small molecules, that might be inhibitors, HIF-1α/HRE complex and NMR structure of HIF-1α/p300 were used in docking procedure. The identified molecules were redocked into target using InducedFit.[3] Dynamic simulation of the complex was performed by DESMOND.[3] Homology model of the A3 receptor was constructed. Phospholipid bilayer was built around the receptor, and molecular dynamic simulations were carried out with DESMOND. Pharmacophore model, developed by PHASE,[3] was used to search 3D-databases to identify additional molecules possessing a higher affinity.

Results
Since a X-ray HIF-1α crystal structure was not reported yet, a homology model using multiple templates was built (Fig. 1). Quality of the obtained model was higher in the PAS regions of the HIF protein, which has highly conserved amino acid sequence and structure. Ramachandran plot analysis showed that 87.7 % of residues were in allowed region. HIF-1α/p300 complex (PDB code:1L3E) was used in ZINC database to search novel potential inhibitors. Identified inhibitors were docked into the protein target. To keep the receptor flexible during the docking procedure, Induced Fit Docking (IFD) protocol was used. Key interactions between protein and ligands were further analyzed. Ligands establish hydrogen bonding interactions with Asp331, Ser395, Gln398, Gln342, and His402. IFD model confirms the observations emerged from flexible docking. Low RMSD values and similar interactions were observed.

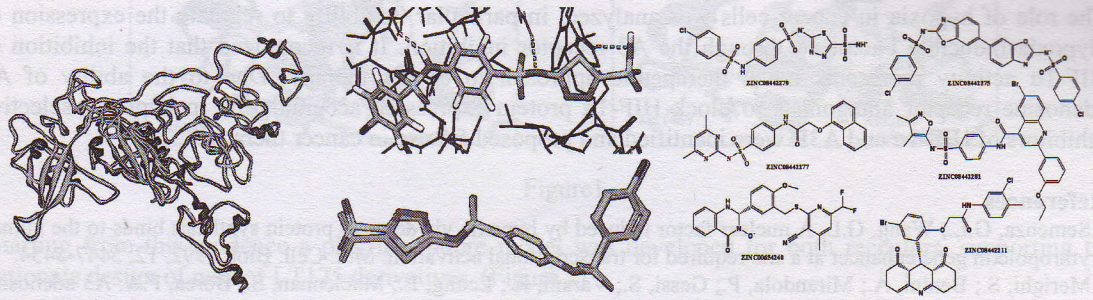

Fig. 1

Furthermore, molecular docking studies on the HRE fragment (PDB code: 1D7G) were performed, using a large library of compounds selected from NCI database with the virtual screening workflow of GLIDE.

The best docked compounds obtained are all heterocyclic molecules, anthracycline analogues with peptide linkers of different lengths. Thus, they can intercalate between the DNA stacking bases (Fig. 2).

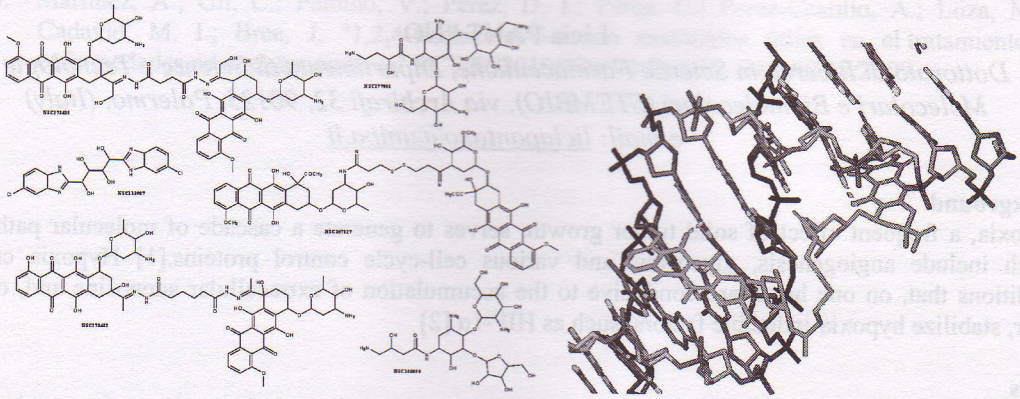

Fig. 2

The recently published structures of hA2AR provide a new template for A3R modeling. A new model of the hA3R was built using this crystal structure as template (Fig. 3). The validation of the obtained structure model was performed by inspecting the Ramachandran plot. Molecular dynamics, in a phospholipid bilayer, was be used to understand the plausible binding modes and interactions of A3R. A training data set of 122 A3R active and selective antagonists were docked into the obtained model using IFD. Then, pharmacophore models based on common chemical features of molecule with inhibitory activity towards A3R were generated with PHASE. The resulting pharmacophore model contained an aromatic ring, a hydrogen-bond donor, and three hydrophobic sites. To each pharmacophore feature site was first assigned an energetic value equal to the sum of the GLIDE XP contributions of the atoms included in the site. This procedure allows sites to be quantified and ranked on the basis of these energetic terms. Using these pharmacophore features, a large public library of compounds (ZINC lead-like) was screened, to identify new hits that could inhibit the A3 receptor.

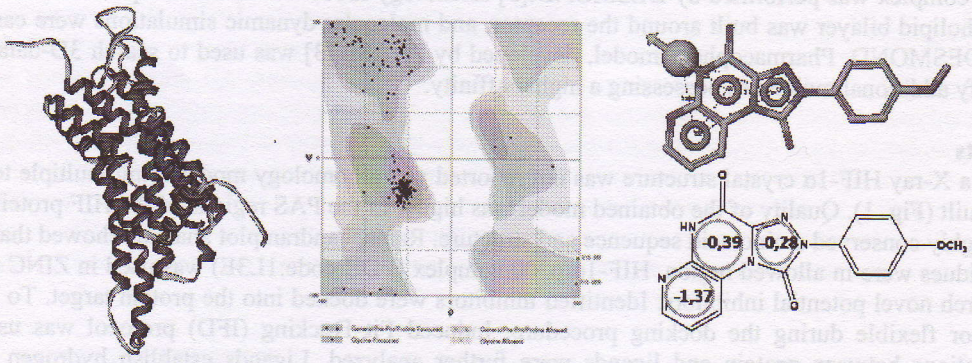

Fig. 3

Conclusion

The role of hypoxia in cancer cells was analyzed, in particular its ability to regulate the expression of Hypoxia-Inducible Factor-1α through the A3 receptor inhibition. It is recognized that the inhibition of HIF-1α activity represents novel therapeutic approach to cancer therapy. Due to the ability of A3 adenosine receptor antagonists to block HIF-1α protein expression accumulation in hypoxia, selective inhibitors of HIF-1α and A3R were identified and proposed for use in cancer therapy.

References

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