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Research article

Molecular docking study of natural alkaloids as multi-targeted hedgehog pathway inhibitors in cancer stem cell therapy

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ABSTRACT

Cancer is responsible for millions of deaths throughout the world every year. Increased understanding as well as advancements in the therapeutic aspect seems suboptimal to restrict the huge deaths associated with cancer. The major cause responsible for this is high resistance as well as relapse rate associated with cancers. Several evidences indicated that cancer stem cells (CSC) are mainly responsible for the resistance and relapses associated with cancer. Furthermore, agents targeting a single protein seem to have higher chances of resistance than multitargeting drugs. According to the concept of network model, partial inhibition of multiple targets is more productive than single hit agents. Thus, by fusing both the premises that CSC and single hit anticancer drugs, both are responsible for cancer related resistances and screened alkaloids for the search of leads having CSC targeting ability as well as the capability to modulating multiple target proteins. The *in silico* experimental data indicated that emetine and cortistatin have the ability to modulate hedgehog (Hh) pathway by binding to sonic hedgehog (Hh), smoothened (Smo) and Gli protein, involved in maintenance CSCs. Furthermore, solamargine, solasonine and tylophorine are also seems to be good lead molecules targeting towards CSCs by modulating Hh pathway. Except solamargine and solasonine, other best lead molecules also showed acceptable *in silico* ADME profile. The predicted lead molecules can be suitably modified to get multitargeting CSC targeting agent to get rid of associate resistances.

1. Introduction

Cancer is a major health problem which is responsible for millions of global deaths each year (Raz et al., 2012). It has been estimated that 14.1 million new cases and 8.2 million cancer related death occurred during 2012 (Dosanjh et al., 2014). Moreover, more than five ten thousand cancer related deaths and approximately sixteen ten thousand new cases are predicted to occur only in United State during year 2014 (Siegel et al., 2014). Population throughout the world is suffering from the huge burden of this deadly disease. Due to technical advancements and increased understanding of the disease, cancer statistics have been slightly improved during the time period of the last two decades (Siegel et al., 2014). But still it is associated with a massive death rate, which indicates the lacunas related to its treatment. Among the various aspects resistance towards chemotherapy is the major factor responsible for massive cancer related death rate (Singh and Settleman, 2010). Almost all types of cancer cells have shown varying degree of drug resistance when exposed to chemotherapeutic agents (Luqmani, 2008). Several reports indicated that CSCs are the major factor behind drug resistance and relapse of disease (Bashyal Insan and Jaitak, 2014). CSC is a term used for a subpopulation of cancer cells, which show self renewal capacity, whole tumor regenerating capability, inbuilt resistance to chemotherapeutic agents and can produces a similar kind of tumor when transplanted into immune-compromised animal models (Bashyal Insan and Jaitak, 2014). CSC have been isolated from multiple cancer types such as leukemias, breast, liver, glioblastoma, pancreatic, prostate, head and neck cancers by using specific types of surface marker or cellular activity shown by these cells (Bashyal Insan and Jaitak, 2014). CSCs have stem like characteristics and is maintained significantly by several signaling pathways and among them intensity of hedgehog (Hh) signaling (Fig. 1) was found to impart significant impact on these cells (Bashyal Insan and Jaitak 2014; Zhao et al., 2009).

Hh pathway is a developmental pathway which play an active role in various developmental processes, such as cell fate, proliferation, survival and differentiation (Bashyal Insan and

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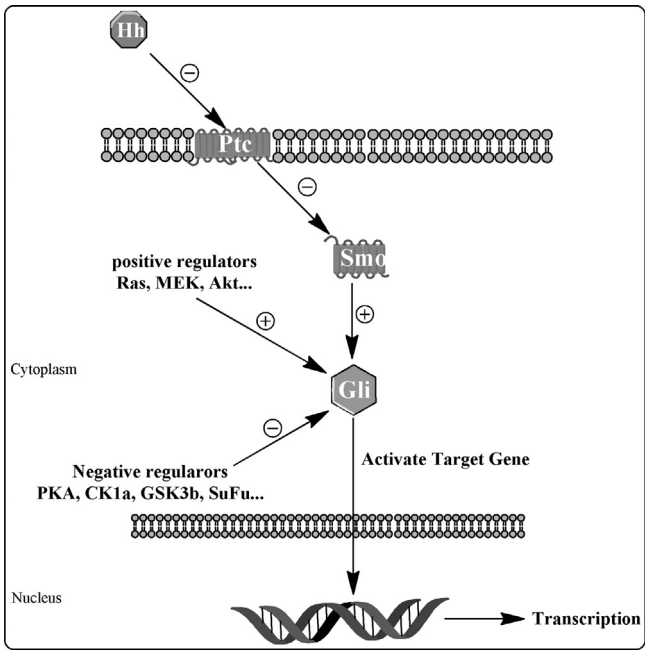

Fig. 1. Hedgehog signaling pathway: Hedgehog protein (Hh) binds to patched (Ptc) and inactivate it. The Ptc is twelve transmembrane glycoprotein components that suppress the activity of Smothened (Smo) protein. When Ptc is inactivated by Hh protein, activation of Smo takes place, which results in increased concentration of Gli. The Gli is a transcription factor that mediate transcription related to cancer and CSCs. Some negative and positive regulators also modify Hh signaling by increasing or decreasing gli mediated activity.

Jaitak, 2014). Up-regulated Hh pathway was found to be significantly associated with multiple forms of cancers along with its involvement in CSC maintenance (Merchant and Matsui, 2010). Thus, various targeting approaches have been implemented to down regulate Hh pathways as a targeting strategy for cancer and CSCs. Hh pathway inhibitors have been developed which can be majorly categorized into inhibitors of Hh protein, Smo inhibitors and inhibitor of Gli proteins mediated activity (Peukert and Miller-Moslin, 2010). Among all categories of inhibitors only Smo inhibitors have gained significant considerations and several compounds such as cyclopamine derivative IPI 926 have entered in clinical trials (Bashyal Insan and Jaitak, 2014). But there is not any inhibitor of Hh and Gli protein is available, which is under clinical consideration (Bashyal Insan and Jaitak, 2014). Because of the availability of only Smo based Hh pathway inhibitors we don't have any alternate drugs to overcome resistance acquired by alteration in Smo protein. Furthermore, Hh pathway involves complex signaling mechanisms where several protein messengers are involved and there exists a complex crosstalk among multiple pathways, which further increase the possibility of drug resistance (Sengupta et al., 2007). Thus, multitargeted CSC therapy seems to be a more reasonable way to overcome possible drug resistance related problems associated with Hh pathway inhibitors (Bashyal Insan and Jaitak, 2014). According to network model partial inhibition of several targets are more efficient than complete inhibition of a single protein (Csermely et al., 2005). Among various drug like molecules, natural products being originated from natural phenomenon, having defined role in the body and thus seems to have strength to modulate several target proteins. Therefore, natural products may have potential for being developed as multitargeting drugs. Thus, taking in consideration the above mentioned facts, the aim of the current study is to investigate the natural drug like molecules that can be developed as multi-targeting inhibitors of Hh pathways. Among various natural products, anticancer potential of alkaloids is well established and thus included in the study. The alkaloids which were chosen are previously reported to have significant anticancer potential and further exploring them for their mechanistic prospective may provide us a good anticancer lead molecule.

2. Material and methods

2.1. Obtaining ligands and protein molecules

The alkaloids selected for the study are shown in Table 1. Alkaloids included in this study have shown promising anticancer potential and can be explored further for searching alternative anticancer lead. Required chemical structures were drawn manually using ChemBioDraw Ultra-12 by carefully considering the chiral centres using wedged/hashed bonds. Crystal structures of Smo, Hh and Gli protein used in this study were downloaded from protein data bank (PDB), have PDB ID 4JKV (Wang et al., 2013), 4C4 M (Whalen et al., 2013) and 2GLI (Pavletich and Pabo, 1993) respectively. Standard drugs used as a control in this study include GDC-0449, Robotnikinin and GANT61 which are the standard inhibitors of Smo, Hh and Gli mediated activity respectively (LoRusso et al., 2011; Pan et al., 2012; Stanton et al., 2009).

2.2. Ligand and protein preparation

For the purpose of ligand preparation all the forty five ligands and three standard drug molecules had been imported to the project table of Schrödinger Maestro 9.6 suite and using the LigPrep wizard application, ligands were made ready for docking (Singla et al., 2015). In ligand preparation step, the raw structures were incorporated with several structural modifications such as integrating hydrogen atom properly according to valency, appropriate fixation of charge as well as orientation of functional groups, bond length, bond angle correction, appropriate stereochemistry as well as tautomeric state generation. In ionization section of ligand preparation step, possible protonated state of ligands at pH range of 7 ± 2 was generatedusing Epik (Shelley et al., 2007). Furthermore generate tautomer command have been implemented while preparing ligands. While executing LigPrep module chiral centres of all the molecules were considered critically by implimenting retain specific chirality option available with the wizard. Maestro is provided with MMFFs and OPLS_2005 forcefield. OPLS_2005 is suitable and thus generally used for biological systems and organic molecules (Singla et al., 2015; Singla et al., 2015). Thus, minimization and then optimization of ligands using OPLS_2005 force field were performed to get one final 3D representative model of all ligands ready to dock. (Naik et al., 2011). For the preparation of protein molecules the crystal structures of protein were imported to the protein preparation wizard application of maestro 9.6 (Naik et al., 2011). The various step involved in protein preparation steps include preprocess, review, modiefy and refinement. During preprocess step basic modifications such as water molecules deletion, addition of hydrogen atoms, bond order assigning, creation of disulfide bonds and zero order bonds to metal were incorporated into the raw PDB structure. Thereafter, generate state option available in review and modiefy tab is executed considering pH range of 7 ± 3. Finally proteins are optimized and minimized with options availale in refine panel of protein preparation wizard. Optimization was done with default settings and then minimization step was executed. During minimization step conserve heavy atom to RMSD cutoff was taken as 30 Å with the implementation of OPLS_2005 force field. We have deleted associated DNA molecule in case of Gli (2GLI) and ligand in case of Hh (4C4 M) during protein preparation.