Identification of De Novo drug candidate for skin cancer using cheminformatics method

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Abstract
Since 2010, skin cancers result in 80,000 deaths a year, 49,000 of which are due to melanoma and 31,000 of which are due to non-melanoma skin cancers. In this paper, we focus on MCR1 protein which acts as a potential target for skin cancer. We identify the sequence and model the protein and finally, we deliver the novel drug candidate using advanced cheminformatics protocols. In our results, we explain the drug protein inhibition with the help of molecular visualization tools. We chose plant growth hormone and anti-oxidant chemicals, combined the molecules and introduced them to the modelled protein. Thus the molecular docking results show high affinity of MCR1 protein. Our research would be useful for Clinical oncologists and Pharmacoinformaticians. The de novo drug would act as a potential therapeutic agent for various types of skin cancers.

Keywords: Pharmacoinformaticians, MCR1 protein and cheminformatics

INTRODUCTION
Skin cancers result in 80,000 deaths a year as of 2010, 49,000 of which are due to melanoma and 31,000 of which are due to non-melanoma skin cancers. This is up from 51,000 in 1990 [1]. In the US in 2008, 59,695 people were diagnosed with melanoma, and 8,623 people died from it. In Australia more than 12,500 new cases of melanoma are reported each year, out of which more than 1,500 die from the disease. Australia has the highest per capita incidence of melanoma in the world.

More than 3.5 million cases of skin cancer are diagnosed annually in the United States, which makes it the most common form of cancer in that country. According to the Skin Cancer Foundation, one in five Americans will develop skin cancer at some point of their lives. The most common form of skin cancer is basal-cell carcinoma, followed by squamous cell carcinoma. Although the incidence of many cancers in the United States is falling, the incidence of melanoma keeps growing, with approximately 68,729 melanomas diagnosed in 2004 according to reports of the National Cancer Institute.

Skin cancer (malignant melanoma) is the fifth most common cancer in the UK (around 13,300 people were diagnosed with malignant melanoma in 2011), and the disease accounts for 1% all cancer deaths (around 2,100 people died in 2012) [2]. The survival rate for people with melanoma depends upon when they start treatment. The cure rate is very high when melanoma is detected in early stages, when it can easily be removed surgically. The prognosis is less favorable if the melanoma has spread to other parts of the body [3].

METHODOLOGY
Protein modelling
The retrieved gene MC1R coded protein sequences was applied into Swiss model server [4] in order to predict the 3 Dimensional structures of the protein sequences.

Structure validation
The after converting the amino acids sequences into 3D structures, the modelled structures was validated through RAPPER Server [5] in order to identify the structural quality based on the assessment of Ramachandran plot values.

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Protein Structure Visualization
The modelled protein 3D structure was viewed with the help of advanced molecular visualization software called Discovery Studio in order to identify the structural regions and to classify the entire 3D structure elements.

CHEMINFORMATICS
Drug selection and identification
The potential chemical inhibitors were selected using NCBI Pubchem [6] chemical compound database related to Skin cancer.

Drug designing and validation
For the entire drug designing and validation, Cheminformatics software called Chemaxon [7] was employed.

Protein-drug docking
The modelled protein MC1R and designed de novo drug was docked using an automated molecular drug docking server called PatchDock server [8].

RESULTS AND DISCUSSION
Molecular and predictive epidemiology play an important role in cancer prevention. With the recognition of early biomarkers such as changes in oncogene and tumor suppressor gene expression, tumor development can be monitored and the efficiency of therapy and primary preventive intervention can be followed.

Multiple genes or proteins need to be targeted concomitantly to achieve the desired outcome with minimal side effects. The identified target gene MC1R- melanocortin-1-receptor (alpha melanocyte stimulating hormone receptor) is present in the 16th chromosome in human. The various literature studies clearly indicated that the gene is directly involved in human skin cancer. Current drug discovery is impossible without sophisticated modelling and computation. The potential of this direction is highlighted by the profound link between the genetic basis of disease and the biology of drug action. ProtParam is one of the best tools for analyzing the primary structure of a protein. ProtParam computes various physico-chemical properties that can be deduced from a protein sequence. The protein can be specified in the form of a raw sequence. The results of the primary sequence analysis shows the complete Physio chemical properties of the target protein sequence. The ProtParam result shows that MC1R protein contains 317 amino acids. The theoretical pI is 8.78. Total number of atoms is 4989 and molecular weight of amino acid is 34705.5. In this study, the MC1R protein sequence shows a high percentage of amino acids (Leu: 17.4 %). The retrieved sequences were converted into 3D structure using Swiss Model server. The structure was downloaded and viewed with the help of advanced visualization tool namely Discovery Studio Software.

The modelled structure was validated using RAPPER server and assessment of Ramachandran plot value shows (94.4%) . In drug designing, using NCBI-PUBCHEM compound databases, the following existing drugs (2-Mercaptobenzimidazole + Abscisic Acid) were chosen and combined (Table:1). Chemaxon’s www.Chemicalize.org was used for the complete chemical structure validation, Tautomerization and Topology analysis. The 3 D structure of designed de novo drug visualized using Discovery studio software. In Molecular drug studies, PatchDock server was used. The modelled target protein, melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor) and the designed drug candidate were docked. PatchDock server employs this technique (FIG:2). The best Protein-ligand interaction was found to be in the de novo drug (5-(1-hydroxy-6,6-dimethyl-4-oxo-2-(2-sulfanylidene-2,3-dihydro-1H,1,3-benzodiazol-1-yl)methyl)cyclohex-2-en-1yl)-3-methylpentac-2,4-dienoicacid)chemaxon with MC1R -melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor), The binding value is -358.68. Table 2.

Cancer is the result of multiple alterations in the processes that control cell proliferation, invasion, and spread. Nearly all cancers result from multiple factors that influence these processes over an extended time. In their recent update on the hallmarks of cancer, describes the multistep development of human tumors and the current understanding of the complex biology of cancer. Although the biology of cancer is still not completely understood, present scientific findings point to multiple cellular pathways by which different cancer risk factors could affect the multistep evolution of normal cells into cancer cells during a person’s lifespan. Many physical and chemical substances that cause cancer in humans act through genetic changes that lead to downstream changes in RNA and protein processing. The most well-studied and common genetic alterations in cancer include mutations in...
oncogenes and tumor-suppressor genes. MC1R
genetic variants play a role in development of skin
cancer beyond their influence on pigmented
phenotypes.

Skin color is mainly determined by the mix of
carotenoids, oxy-/deoxy-hemoglobin and most
importantly, different types of melanin and also the
way that melanin is packaged and distributed in
melanosomes. The production of melanin takes
place in specific ovoid organelles known as
melanosomes which are produced in dendritic
melanocytes that account for only 1% of
epidermal cells. The naturally occurring drug
abscisic acid (ABA) which is a natural plant hormone
with known beneficial properties for the treatment
of cancer disease, helps fight inflammation was
proved at the Virginia Bioinformatics Institute at
Virginia Tech (1976). It is a phytohormone
regulating fundamental physiological functions in
plants. ABA is also an endogenous hormone in
humans, regulating different cell responses and
functions, including activation of innate immune
cells and stimulation of insulin release and glucose
uptake showed that ABA stimulates glucose uptake
by rodent adipocyte and myoblast cell lines.

The compound, 2-mercaptopbenzimidazole, which
belongs to a new group of pharmaceuticals, the
actoprotectors, showed a strong antimutagenic
effect. In vivo investigations 2-
mercaptopbenzimidazole reduced over expression of
oncogenes and tumor suppressor genes in a short-
term test. Pharmacologically active 2-
mercaptopbenzimidazole (bemitil, tomerzole,
afobazole) derivatives are able to reduce the
mutagenic effects of chemical prooxidants by
inhibition of free radical oxidation induced
endogenous mutagen formation. A chromosome
aberration assay with bone marrow cells of C57Bl/6
mice has shown that 2-mercaptopbenzimidazole
prevented manifestations of the clastogenic effects
of dioxidine, reduced its cytogenetic effect and
caused a statistically significant decrease in
cyclophosphamide damage. The coincidence
between the antimutagenic and anticancer effects
of a chemopreventive agent is almost 90%.
The most effective suppressor effect was observed
48 hour after 2-mercaptopbenzimidazole
treatment. A slight increase of expression of the two
genes was evident again 72 hours after the
administration indicating a decrease of the
chemopreventive effect of 2-
mercaptopbenzimidazole.

Recent developments suggest that using
computation extensively to link disparate data and
support integrative models could broaden our
vision of biological and social processes. The
present study of docking of 2-mercaptop
benzimidazole and Abscisic acid concluded that the
experimental procedures make this methodology a
better modesty for the synthesis of the titled
compounds for possible chemopreventive activity.
All the tested compounds with structural
modifications exhibited promising
chemopreventive activity. From these findings, it
can be suggested that the designing of new
chemical analogues with 2-mercaptopbenzimidazole
and Abscisic acid could lead the necessity for the
development of further research. Table 1.

Table 1. Cheminformatics – Chemicalize results

| IUPAC: | 5-(1-hydroxy-6,6-dimethyl-4-oxo-2-[(2-sulfanylidene-2,3-dihydro-1H-1,3-
benzodiazol-1-yl)methyl]cyclohex-2-en-1-yl}-3-methylpenta-2,4-dienoic acid |
| SMILES | CC(C=CC1(O)C(CN2C(=S)NC3=CC=CC=C23)=CC(=O)CC1(C)C)=CC(O)=O |
| Formula: | C_{22}H_{24}N_{2}O_{4}S |
| Mol.weight | 412.502 |
| pH | 7.4 |
| logP | 3.41 |
| Isotope composition: | C (64.06%), H (5.86%), N (6.79%), O (15.51%), S (7.77%) |

Table 2. Drug docking – Binding Affinities Score

<table>
<thead>
<tr>
<th>TARGET PROTEIN</th>
<th>DE NOVO DRUG</th>
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| MC1R | (5-[1-hydroxy-6,6-dimethyl-4-oxo-2-[(2-sulfanylidene-2,3-dihydro-1H-1,3-
benzodiazol-1-yl)methyl]cyclohex-2-en-1-yl]-3-methylpenta-2,4-dienoic acid) -358.68 |
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**Fig 1. 3D structure of De Novo drug**

![3D structure of De Novo drug](image)

Stick model view /colors atoms: Red – Oxygen, Blue-Nitrogen, Yellow-Sulphur and Grey -Carbon

5-{1-hydroxy-6,6-dimethyl-4-oxo-2-[2-sulfanylidene-2,3-dihydro-1H-1,3-benzodiazol-1-yl]methyl[cyclohex-2-en-1-yl]-3-methylpenta-2,4-dienoic acid

**Fig 2. Drug Docking Protein –Ligand Binding Sites Prediction**

![Drug Docking Protein –Ligand Binding Sites Prediction](image)

Drug –protein interaction : pose view – ligand(5-{1-hydroxy-6,6-dimethyl-4-oxo-2-[2-sulfanylidene-2,3-dihydro-1H-1,3-benzodiazol-1-yl]methyl[cyclohex-2-en-1-yl]-3-methylpenta-2,4-dienoic acid) green stick model and protein(MC1R) yellow surface model with amino acids labels.

**CONCLUSION**

In this *Insilico* research investigation, protein modelling and drug designing studies was done using advanced *Insilico* tools.

Two chemical molecules, namely, 2-Mercaptobenzimidazole and Abscisic acid were chosen for the study. 2-Mercaptobenzimidazole naturally has chemopreventive and anti-oxidant properties. Abscisic acid, having anti-cancer activity, is basically a plant hormone.

Two molecules were combined based on Cheminformatics protocols. In summary, two of the
well-established hallmarks of cancer are resistance to apoptosis/cell death and sustained proliferation melanoma cells carrying MC1R variants would have less of both, and additionally poorer DNA repair and therefore the patients would have better survival.

Our research results clearly elucidated that the identified *de novo* drug (5-{1-hydroxy-6,6-dimethyl-4-oxo-2-[2-sulfanylidine-2,3-dihydro-1H-1,3-benzodiazol-1-yl]methyl[cyclohex-2-en-1-yl]-3-methylpenta-2,4-dienoic acid) is potential inhibitor of MC1R (melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor). The protein-ligand interactions was clearly analysed. Drug discovery hold great promises, especially building molecular network models of disease, mining the biomedical literature and patient records and harnessing computation to drive discovery of new targets, multidrug cocktails and novel purposes for existing drugs. Hence it is suggested that this drug molecule can be applied in clinical pharmacological studies in future and could act as a potential therapeutic agent for various types of skin cancer.

**REFERENCES**