INTRODUCTION

Cancer is characterized by uncontrolled cell growth and the spread of these abnormal cells **(Hanahan and Weinberg, 2000**). Over time, carcinogens induce individual cells to acquire genetic and epigenetic changes in signalling pathways that regulate their growth and proliferation. The alterations change normal cells into malignant cells, a process that is referred to as "transformation" (**Heeg *et al.*, 2006**). Transformed cells gain a distinct growth advantage over the neighbours and subsequently out-compete them for space and nutrients. These cells stop responding to natural mechanism of growth regulation and acquire the ability to proliferate independent of their anchorage. Modified genes that can induce cellular transformation are known as oncogenes (**Croce, 2008**).

Retinoic acid (RA) is a critical signaling molecule that regulates gene transcription and the cell cycle. It has been proposed that cytochrome P450 family 26 (CYP26A1) enzymes have a role in determining the cellular exposure to retinoic acid (RA) by inactivating retinoic acid catabolism. The CYP26A1 enzymes have been shown to metabolize RA efficiently and they are also inducible by RA in selected systems. However, their expression patterns in different cell types and a mechanistic understanding of their function is still lacking.

Retinoic acid is the biologically active metabolite of vitamin A (retinol). Naturally occurring derivatives or synthetic compounds that have structures or activities similar to vitamin A are referred to as retinoids (**Tzimas and Nau, 2001)**. Vitamin A (retinol) and its natural and synthetic derivatives (retinoids) are involved in several important physiological processes such as reproduction, cell proliferation, differentiation, vision, and embryonic development **(Gudas *et al.*, 1994).** Vitamin A is stored primarily in liver stellate cells as retinyl esters, which are hydrolyzed in hepatocytes by retinyl ester hydrolases (REH) to retinol **(Ross, 1993)**. Retinol, the precursor of retinoic acid, is the main circulating retinoid and typically circulates at concentrations of 1–3 μM **(Fex and Felding, 1984; Eckhoff *et al.*, 1991).** The circulating concentrations of both endogenous at-RA and 13-cis-RA in human volunteers were 3–13 nM **(Tang and Russell, 1990; Eckhoff and Nau, 1990).**

Tissue concentrations for RA isomers were greater, and ranged from 7–40 nM for at-RA and 1–6 nM for 13-cis-RA. Retinoic acid can chemically exist as several different geometric isomers including: all-trans RA (at-RA), 9-cis-RA, 11-cis-RA, 13-cis-RA, and 9,13-di cis-RA **(Tzimas *et al*., 1996; Blomhoff and Blomhoff, 2006).**

CYP26A1, the gene encoding a Cytochrome P450 enzyme is specifically involved in metabolic inactivation of retinoic acid (RA), the most active vitamin A derivative is highly expressed in liver, pituitary gland, adrenal gland etc. Its highly expressed in 42% of primary breast cancers. When the CYP26A1 enzyme overexpressed the concentration of retinoic acid decreases due to which retinoic acid does not convert into retinol, a natural derivative of Vitamin A and this shows in form of defieciency of Vitamin A. Vitamin A deficiency (VAD) is associated with increased susceptibility to carcinogenesis in animal models and elevated risk for a number of human cancers. Enhanced expression of CYP26A1 suppresses cellular responses to anoikis and consequently promotes anchorage-independent growth. This transformed phenotype was sufficient to markedly increase tumorigenic and metastatic potential. Suppression of CYP26A1 significantly reversed the CYP26A1-mediated oncogenic characteristics, suggesting a direct link between intracellular RA status and tumorigenicity. Strong evidence is available for oncogenic and cell survival properties of CYP26A1 in carcinogenesis, and mechanisms whereby VAD might promote cancer development (**Osanai *et al*., 2010**).

Vitamin A deficiency leads to poor immunity and deaths due to infections **(Ross, 1992**). The World Health Organization (WHO) estimates that approximately 250 million pre-school age children are deficient in vitamin A and that WHO's intervention program, consisting of promotion of breast feeding and vitamin A supplementation, has reduced mortality by 23% overall, and up to 50% for acute measles sufferers **(World Health Organization, 2009).**

In humans CYP26A1 (Cytochrome P450, family 26, subfamily A, polypeptide 1) is expressed in the liver, heart, pituitary gland, adrenal gland, testis, brain and placenta and has been mapped to chromosomes 10q23-q24. It has 7 exons and codes for 2 proteins by alternative splicing. It is thought that the principle role of

CYP26A1 is in retinoic acid metabolism. It acts on retinoids, including all-trans-retinoic acid (atRA) and its stereoisomer 9-cis-RA is capable of both 4-hydroxylation and 18-hydroxylation. It is responsible for generation of several hydroxylated form of RA, including 4-OH-RA, 4-oxo-RA and 18-OH-RA. CYP26A1 is usually membrane bound protein and localized to the inner mitochondrial or endoplasmic reticular membrane. The enzyme, therefore has a protective function, as an important regulator of differentiation and a possible modulator of disease states indirectly by controlling ATRA and other retinoid concentrations. The CYP26 family is now recognized as a major contributor to the oxidative metabolism of RA under nutritional and pharmacologic conditions. The metabolism of retinoic acid occurs in two phases. The phase 1 (oxidation) and phase 2 (conjugation) metabolism of RA, as mediated sequentially by CYP26 metabolize retinoic acid (RA) to 4-hydroxy and 4-oxo metabolites and uridine- 5-diphospho (UDP)-glucuronosyl transferase enzymes, glucuronidation to form water-soluble polar metabolites. CYPs consist of a superfamily of heme-containing monooxygenases that are associated with the metabolism of various substrates, including drugs, environmental pollutants, endogenous substrates, and have broad overlapping specificity **(Danielson, 2002).** NADPH is required as a cofactor and molecular oxygen is used as a substrate. These enzymes are located in the endoplasmic reticulum and are highly expressed in the liver and small intestine.

The precise mechanism of how RA regulates biological processes is not yet fully understood. This enzyme regulates the cellular level of retinoic acid which is involved in regulation of gene expression in both embryonic and adult tissues. Two alternatively spliced transcripts and one alternative catalyst activity variant of this gene, which encode the distinct isoforms have been reported. It has been shown that RA acts as a signaling molecule and regulates gene expression by binding to the nuclear retinoic acid receptors (RARα, RARβ, and RARγ) **(Petkovich *et al.*, 1987)**, which then bind DNA as a heterodimer with the retinoid X receptors (RXRα, RXRβ, and RXRγ). At-RA also activates peroxisome proliferator-activated receptors (PPARβ/δ) (**Mangelsdor and Evan, 1995; Schug *et al.*, 2007).** It is believed that at-RA binds to RAR and 9-cis RA binds to either RAR or RXR (**Mangelsdor and Evan, 1995; Marill *et al.*, 2003**), but the biological role of 9-cis RA in humans is controversial as 9-cis RA has not been detected in human plasma or

tissues but has been detected in locust embryos (**Nowickjy *et al.*, 2008).** Based on the biological effects of deficiency and excess exposure of RA, there is a clear need to regulate the concentrations of RA at both the cellular and total body levels.

Why CYP26A1 overexpressed is not fully understood but its expression is identified by some changes in cell cycle. The cytostatic effects of retinoic acid in CYP26A1-overexpressing cells as measured by changes in cell growth and mitotic index. Overexpressing CYP26A1 gains significant resistance to apoptosis signaling, possibly because of a state of reduced RA bioavailability caused by metabolic inactivation of RA. However, the one possible alternative explanation for the effect of CYP26A1 overexpression on apoptosis is that RA metabolites generated by CYP26A1 may have antiapoptotic activities. Apoptosis is an evolutionarily conserved process in normal embryogenesis, tissue homeostasis, and regulation of the immune system **(Fisher and Vorhees, 1996; White *et al.*, 1996**). Many studies have implicated retinoic acid as an important regulator of this process, and certain types of tumor cells undergo apoptosis after retinoic acid treatment **(Fisher and Voorhees, 1996; White *et al.*, 1996**). Apoptosis pathways can be activated in a cell-specific manner by a diversity of distinct triggers, including death receptor ligands such as tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and TNF-α, retinoids, genotoxins, oxidative stress, nutrient deprivation, and anoikis (anchorage dependence-mediated cell death). RA has been shown to enhance sensitivity of certain cells to a number of proapoptogenic factors, including death receptor ligands such as TRAIL. The RA-metabolizing enzyme CYP26A1 significantly abrogates the proapoptotic activity of RA and its synergistic activity with apoptosis-inducing factors, resulting in the suppression of cellular response to several differently acting triggers of apoptosis.

Retinoic acid (RA) is the active metabolite of vitamin A. RA is also used as a drug, and synthetic retinoic acid analogs and inhibitors of retinoic acid (RA) metabolism have been developed. But design of CYP26A1 inhibitors is hindered by lack of information on CYP26A1 structure and structure-activity relationships of its ligands. The aim of this study was to identify the primary metabolites of retinoic acid formed by CYP26A1 and to characterize the ligand selectivity and ligand interactions of CYP26A1. Resistance to atRA treatment in cancer therapy is attributed to increased

systemic clearance and cellular metabolism of atRA during treatment **(Muindi *et al*., 1992; Van der Leede *et al*., 1997).** To improve therapeutic outcomes, synthetic RA analogs, RAR agonists, and RA metabolism-blocking agents (RAMBAs) have been developed and tested for treatment of cancers and skin diseases **(Njar *et al*., 2006; Altucci *et al*., 2007).** CYP26A1 is also inducible by atRA in human liver **(Tay *et al*., 2010**). However, neither the structure of CYP26A1 nor the structural requirements of potent CYP26A1 binding are well characterized. Three homology models of CYP26A1 have been reported **(Gomaa *et al*., 2006; Ren *et al*., 2008; Karlsson *et al*., 2008)** on the basis of the crystal structures of CYP3A4, CYP2C8, CYP2C9. However, experimental data on ligand overlap, atRA binding orientations, and metabolite identification are not available to support the construction of these models, perhaps because of a lack of a system to screen CYP26A1 ligands. However, questions arise as to how this would interact with screening, which reduces the progress of cancer abnormalities to serious disease. The available forms of treatment for cancer such as surgery, radiation therapy and chemotherapy are all cytoreductive treatment modalities so in addition to killing cancerous cells, healthy cells are also destroyed in the process. Indeed, there is a need to decrease the incidence of cancer and develop better forms for its treatment. So the main aim of this work was to design a potential inhibitor drug moleclue which inhibits the overexpression of CYP26A1 enzyme against the Vitamin A deficiency. In this context, rational drug designing is considered to be highly potential **(Pearce *et al*., 2008).**

Bioinformatics approaches are used to generate novel lead drug candidates. The availability of complete protein sequence of CYP26A1, has paved a new way for the study of various proteins expressed in humans with different form of cancer. The crystallographic 3-dimensional structure of CYP26A1 proteins was not yet available in Protein Data Bank; hence the present work of predicting the 3D model of CYP26A1 proteins was undertaken. Subsequently, modeled 3D-structure of Proteins can be exploited for generation of potential drug candidates. The present research work aimed to identify some of the significant research domains, like identification of paralogous proteins, comparative homology modeling analysis, identification of novel drug target and inhibitor prediction. It also proposes how Bioinformatics and computational biology methods can be used for the analysis of features of CYP26A1 enzyme.

Therefore, the present investigation was carried out to utilize CYP26A1 as a canonically relevant anticancer drug target in cancer therapy with following objectives:

OBJECTIVES

1. To carry out *in silico* proteome analysis of drug target Cytochrome P450 26A1 (CYP26A1) for identification and validation of target receptor.

2. To determine the 3D structure of target protein CYP26A1 and its validation through molecular dynamics.

3. To screen modeled target protein conformation against literature driven drug library by employing high throughput virtual screening and its validation through docking for evaluation of anticancer drug molecule(s).

4. To carry out *in silico* analysis and validation of potent drug molecule(s) for target protein CYP26A1.