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Research Article

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Computational systems pharmacology and docking explored diosgenin targets causal and effectors pathogenic signalling network of vascular calcification

Jeganathan Manivannan[a], TR Barath Kumar[b], Pandiyan Arunagiri[a] and Elumalai Balamurugan[*a]

[a]*Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar, Tamilnadu, India*
[b]*Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamilnadu, India*

ABSTRACT

The Objective of this study is to investigate the multi-target potential of diosgenin on pathogenic network of vascular calcification due to the osteogenic transdifferentiation of vascular smooth muscle cell (VSMC). Target analysis of diosgenin was done via a 'reverse pharmacophore mapping' by PharmMapper server and the target network was constructed by STRING database. In this study we discovered compound-target associations, compound-pathway connections and disease-target interactions for reconstructing the biologically meaningful networks. In this study we found, diosgenin can potentially target many proteins. Among them, diosgenin targets seven important signaling and executor proteins of vascular calcification related transdifferentiation pathogenic network. This study proved that, due to the multi target potential, diosgenin may be used for vascular calcification in chronic renal failure patients in future.

Keywords: Vascular calcification, VSMC, Transdifferentiation, , Systems pharmacology, Diosgenin

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INTRODUCTION

Systems pharmacology is an emerging integrated discipline that combines many features of systems biology and pharmacology and emerged as a promising subject to overcome pharmacological challenges by providing powerful new tools and conceptions. Systematic identification of protein-drug interaction networks is crucial to correlate complex modes of drug action to clinical indications. Since kinase signalling cascades are complex and interconnected, it is thought that simultaneous inhibition of several signalling 'nodes' is needed to prevent the rapid emergence of resistance to targeted agents [1]. Polypharmacology, which focuses on designing therapeutics to target multiple receptors, has emerged as a new paradigm in drug discovery. Ultimately, to predict a drug response phenotype, it is necessary to understand the change in information flow through cellular networks resulting from dynamic drug-target interactions and the impact that this has on the complete biological system [2]. Network models suggest that partial inhibition of a surprisingly small number of targets can be more efficient than the complete inhibition of a single target. Development of a multi-target drug is likely to produce a drug that interacts with lower affinity than a single-target drug because it is unlikely that a small, drug-like molecule will bind to a variety of different targets with equally high affinity [3].

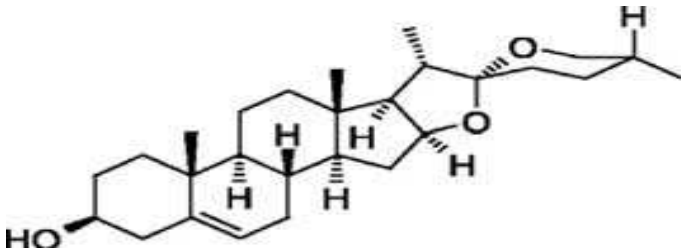
The molecular mechanisms governing vascular calcification overlap substantially with the mechanisms of bone formation. In the setting of chronic kidney disease, diabetes, aging, inflammation, and multiple other toxins, VSMC can dedifferentiate or transform into osteo/chondrocytic-like cells by upregulation of transcription factors critical for normal bone development and thus their upregulation in VSMC is indicative of a phenotypic switch. These osteo/chondrocytic-like VSMC then become calcified in a process similar to bone formation [4].

Diosgenin (Figure 1) is a naturally occurring steroidal saponin present in a variety of plants including fenugreek (*Trigonella foenum graecum*) and roots of wild yam (*Dioscorea villosa*) [5]. Diosgenin has been used in traditional medicine as an antihypercholesterolemia, antihypertriacylglycerolemia, antidiabetes and antihyperglycemia agent [5-8]. Another finding suggest that diosgenin could have a beneficial role against aortic damage induced by oxidative stress in diabetic state, which was evidenced by the propensity of diosgenin to modulate the antioxidant defence and to decrease the lipid peroxidation in aorta [9].

In silico drug target identification with docking is usually used to finding the best interaction mode between the potential target candidates and small molecule probes. Pharmacophore, which is the spatial arrangement of features essential for a molecule to interact with a specific target receptor, is an alternative method for achieving this goal apart from molecular docking method. PharmMapper server is a freely accessed web server designed to identify potential target candidates for the given small molecules (drugs, natural products or other newly discovered compounds with unidentified binding targets) using pharmacophore mapping approach [10].

From this point of view, the present study was intended to discover the potential targets of diosgenin on human protein targets and find out the polypharmacological potential of diosgenin on vascular calcification related pathogenic network.

Figure 1. Structure of diosgenin



EXPERIMENTAL SECTION

PharmMapper Target identification

The targets of diosgenin were searched by PharmMapper Server [10], which is designed to identify potential target candidates for the given small molecules via a 'reverse' pharmacophore mapping approach. The model is supported by a large repertoire of pharmacophore database composed of more than 7,000 receptor based pharmacophore models that are extracted from various database. A strategy algorithm of sequential combination of triangle hashing and genetic algorithm optimization is designed to solve the molecule pharmacophore best fitting task. In this work, the number of the reserved matched targets is defined as 300. The target set is only limited to the human targets (2214); and all parameters were kept as default. Fit score equal or more than 3.8 was considering as more potential targets.

Drug targeted network

The Potential Targets of diosgenin were searched against the STRING database version 9 [11] for the construction of protein-protein interaction network using a STRING confidence score set to ≥ 0.4 (medium confidence).

RESULTS

Table 1. indicates the top ranked more potential targets of diosgenin with equal or more than 3.8 fit score. Table.2 demonstrates the important potential targets that involved in pathogenic signaling of vascular calcification collected from literatures which were supported in the following discussion part. The protein interaction network of potential targets constructed by STRING which shows the confidence based interaction among them (Figure 2). The network shows multiple potential target molecules are interacted with each other, where as the calcification related Vitamin D receptor/Retinoic acid receptor (VDR/RXR) and mitogen activated protein kinase (MAPK) signaling molecules is network together which were the potential targets of diosgenin. Figure.3 summarizes the targets of diosgenin on vascular calcification programme. This supports diosgenin with multi target capacity can efficiently stop vascular calcification.

Table 1.Top rank target proteins of diosgenin (potential targets)

Gene symbol	Protein name
CRABP2	Cellular retinoic acid-binding protein 2
VDR	Vitamin D3 receptor
HSD17B1	Estradiol 17-beta-dehydrogenase 1
TTR	Transthyretin
RBP4	Retinol-binding protein 4
MAPK14	Mitogen-activated protein kinase 14
TTPA	Alpha-tocopherol transfer protein
NR1I2	Nuclear receptor subfamily 1 group I member 2
DHODH	Dihydroorotate dehydrogenase, mitochondrial
SHBG	Sex hormone-binding globulin
RARG	Retinoic acid receptor gamma
MAOB	Amine oxidase [flavin-containing] B
SULT1E1	Estrogen sulfotransferase
PDPK1	3-phosphoinositide-dependent protein kinase 1
CA2	Carbonic anhydrase 2
MMP12	Macrophage metalloelastase
ALB	Serum albumin
RORA	Nuclear receptor ROR-alpha
HGFR	Hepatocyte growth factor receptor
SULT2A1	Bile salt sulfotransferase
HSD11B1	Corticosteroid 11-beta-dehydrogenase isozyme 1
BRAF1	B-Raf proto-oncogene serine/threonine-protein kinase
SEC14L2	SEC14-like protein 2
TRAPPC3	Trafficking protein particle complex subunit 3
BACE1	Beta-secretase 1
F2	Prothrombin
RXRB	Retinoic acid receptor RXR-beta
RBP4	Retinol-binding protein 4
MEK1	Dual specificity mitogen-activated protein kinase kinase 1
AR	Androgen receptor
EPCR	Endothelial protein C receptor
CYP2C8	Cytochrome P450 2C8
ADH5	Alcohol dehydrogenase class-3
RXRA	Retinoic acid receptor RXR-alpha
SULT2B1	Sulfotransferase family cytosolic 2B member 1
GLO1	Lactoylglutathione lyase
CDK2	Cell division protein kinase 2
ALDR1	Aldose reductase
MMP2	72 kDa type IV collagenase, Matrix metalloprotease 2
PPP1CC	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit
ESR1	Estrogen receptor

Table 2. Potential targets of diosgenin associated with osteogenic transdifferentiation programme of VSMC and osteogenic differentiation.

Protein	Role in osteogenic transdifferentiation of VSMC
VDR	Induction of osteogenesis related gene expression
MAPK14	Involves in osteogenic signal transduction , activated during oxidative stress and increases alkaline Phosphatase (ALP) activity
MEK1	ERK activator kinase , important activator of ERK during VSMC calcification
BRAF	Upstream activator of ERK signaling
MMP2	elastin degradation mediated vascular calcification
RXRA	Partner of VDR and Co-activator of VDR responsive osteogenic gene expression
CDK2	involved in osteogenic differentiation

Figure 2. Protein – protein interaction network of the potential targets of diosgenin

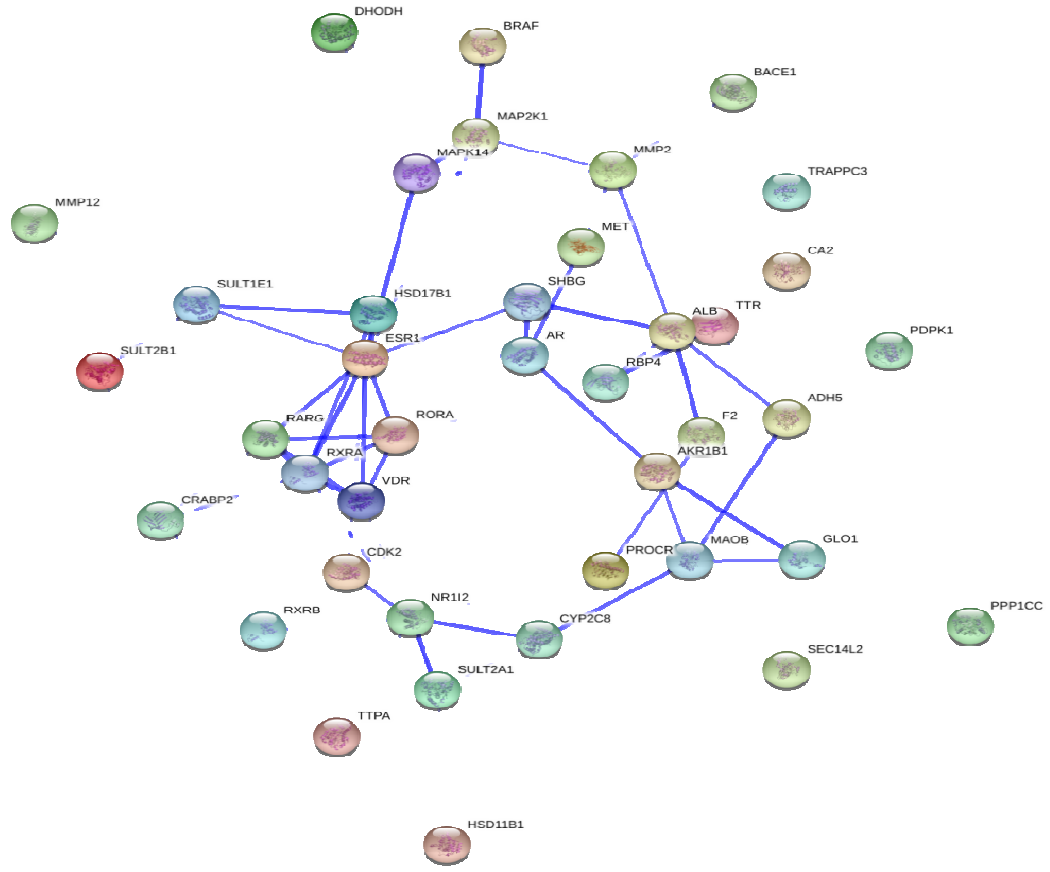
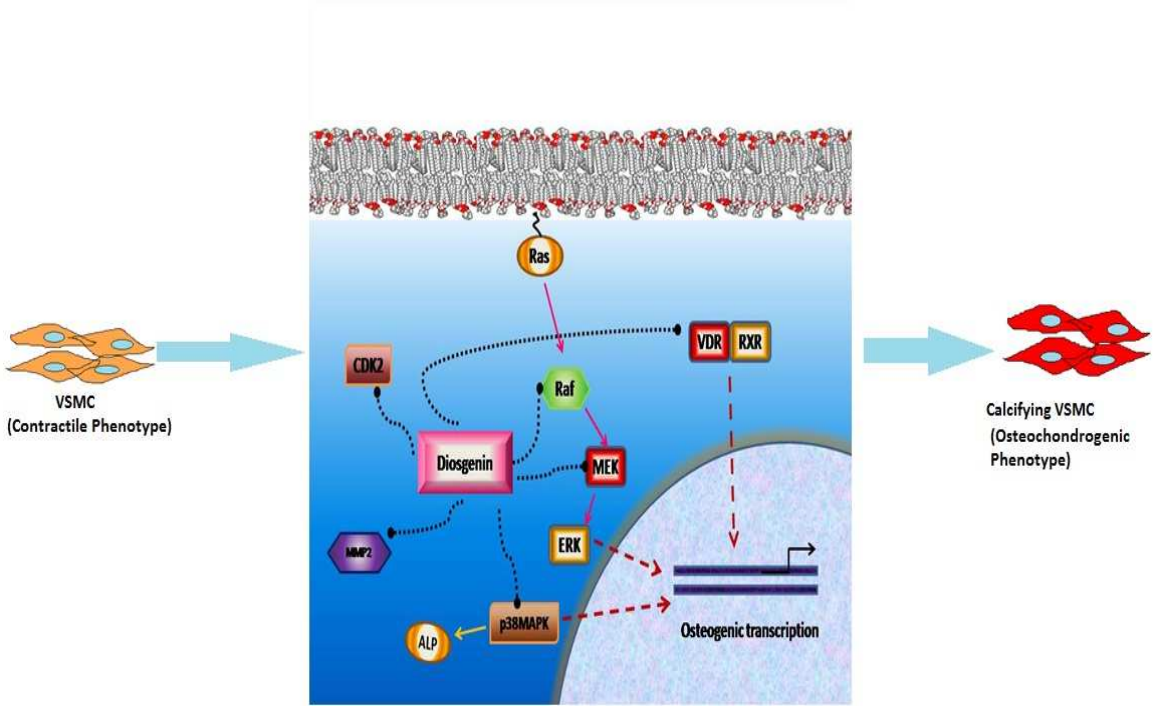


Figure 3. Targets of diosgenin on the pathogenic signalling of vascular smooth muscle cell osteogenic transdifferentiation and vascular calcification



DISCUSSION

Cardiovascular disease has been becoming a leading contributor to mortality in all over the world [12]. Currently, only a small number of proteins have been demonstrated as CVD targets for those approved drugs despite more than 230 proteins are confirmed related to the CVD [13]. Drugs that target the phosphorous homeostasis such as phosphate binders are the only remedy for the renal failure related calcifications. This study found that, diosgenin with multiple targets in the pathogenic signaling network will be the good remedy for vascular calcification other than phosphate binders.

In this work the chemical-protein interactome of diosgenin on human targets was predicted for the identification of active targets. In this work, a pharmacophore modeling technique was applied to search potential molecular targets. In order to find as many as possible targets, the proteins whose fit score displayed more than 3.8 in the top 300 high-ranking protein targets of diosgenin was considered as 'more potential drug targets'. The protein–protein interaction network was constructed by STRING, which helps to discover the importance of target protein in the pathogenic network. Related literature evidences in parallel to these aforementioned results provides strong basis for possible anti vascular calcification potential of diosgenin based on the targets.

One of the highest fit targets is vitamin D receptor (VDR) which is an important regulator of vascular calcification. A previous study demonstrated for the first time that elevated phosphorus affects VDR-mediated gene expression in human coronary artery smooth muscle cells and the effect is not limited to VDR in smooth muscle cells [14]. Moreover, reports have shown that VDR activation by calcitriol increased calcification of VSMCs cultured in calcification media [15]. Further, suppression of vitamin D receptor (VDR)-mediated transcription by suppressing the ligand-activated VDR/RXR heterodimer is expected be of therapeutic value in Paget's disease, [16]. Biological effects of vitamin D are known to be elicited by binding of vitamin D to the ligand binding domain (LBD) of vitamin D receptor (VDR), a member of the nuclear receptor (NR) superfamily. This binding result in heterodimer formation with retinoid X receptor (RXR), which enables high-affinity binding to the vitamin D-responsive element (VDRE) sequence within, and subsequent transcription of vitamin D target genes, including the genes encoding bone proteins [16].

This study discovered that VDR and RXR system that includes RXR-Alpha, RXR-beta, and RXR-gamma are the potential targets of the diosgenin. The protein-protein interaction network shows that all these proteins form a tight connection between them. This indicates diosgenin could efficiently inhibit this VDR/RXR transcription complex which in turn may inhibit VSMC transdifferentiation to osteogenic phenotype. Thereby diosgenin may have the ability to prevent vascular calcification.

Conserved signaling pathways that activate the mitogen-activated protein kinases (MAPKs) are involved in relaying extracellular stimulations to intracellular responses. The MAPKs co-ordinately regulate cell proliferation, differentiation, motility, and survival [17]. The important noteworthy signaling target of diosgenin with high fit score is MAPK14 (p38MAPK). p38 Mitogen-activated protein kinase (p38 MAPK) is a conserved subfamily of MAPK involved in inflammatory response, stress response, cell growth and survival, as well as differentiation . A previous report has shown that, elevated oxidative stress associated vascular calcification has co-occurred with MAPK14 activation [18]. Another study shows H_2O_2 activated the phosphorylation of ERK1/2, JNK and p38 MAPK, but only the last two were associated with the alkaline Phosphatase (ALP) activity [19]. Moreover, another study has shown that genistein can stimulates osteoblastic differentiation via p38 MAPK–Cbfa1 pathway in bone marrow culture [20]. Thus, the inhibition of this kinase with diosgenin will be a potent way to inhibit calcium accumulation of vascular smooth muscle cell.

The extracellular signal-regulated kinases is at the heart of signalling networks that govern cell proliferation, differentiation and cell survival [21].Activation of extracellular signal-regulated kinases (Erks) has been involved in vascular calcification ; Mouse aortic medial cells treated with high phosphate demonstrate increase of phosphorylated Erk1/2 levels along with increase of runt-related transcription factor-2 (Runx2/Cbfa1), prior to loss of VSMC lineage markers in conjunction with VSMC osteochondrogenic transdifferentiation. Inhibition of ERK phosphorylation by the ERK activator kinase (MEK) inhibitor U0126 prevented upregulation of Runx2 and promoted VSMC lineage markers [22]. Diosgenin targets MEK which is the activator of ERK the important signaling component of ERK signaling. Therefore, this can be taken as proven indication that diosgenin can modulate the ERK signaling which in turn prevent vascular calcification.

Another important target of diosgenin on ERK signaling is B-RAF. Mammals possess three Raf proteins: Raf-1, A-Raf and B-Raf. [21]. Though the exact mechanism of B-Raf in calcification was not clear, it is an upstream kinase

which activates MEK, this in turn may probably induce calcification program as described above. Thus, this evidence also supports that diosgenin nearly targets the major components of ERK signaling pathway.

The protein-protein interaction network of this study has shown that MAPK signaling components were highly interactive and perturbation of any one of these components in this network may modulate the MAPK pathway. Hereby, nearly four signaling components were predicted to be the potential targets of diosgenin. Therefore diosgenin can be considered as an effective molecule to inhibit vascular calcification. Further getting deep in search of strong evidences with regard to these strong predicted results, a previous study on mouse vascular smooth muscle cells reported that, TNF-α-induced adhesion molecule expression was inhibited by diosgenin via down regulation of the MAPK, Akt and NF-κB signaling pathways. Diosgenin abrogated TNF-α induced production of intracellular reactive oxygen species (ROS) and phosphorylation of p38 MAPK, ERK, JNK and Akt [23].

Another important target of diosgenin was matrix metalloproteinase 2 (MMP-2). Recent studies demonstrated a correlation between MMP-mediated elastin degradation and aortic calcification. Inhibiting elastin degradation with aluminum ions prevented calcification in the aortas of rats after calcium chloride–mediated injury; and mice deficient in MMP-2 and MMP-9 did not develop calcification in a similar model [24]. Recent study demonstrate that, doxycycline, an antibiotic that inhibits MMP activity and reduces MMP levels, and GM6001, a synthetic selective MMP inhibitor, can inhibit arterial calcification both in organ culture and in vivo [25]. These evidences again pinpoint that diosgenin could protect VSMC from calcification through inhibit the matrix remodelling.

As one of the stimulators on bone formation, osteogenic growth peptide (OGP) increases both proliferation and differentiation of the osteoblasts *in vitro* and *in vivo*, particularly osteoprotegerin (OPG) has been suggested to be involved. A study suggested that OGP may increase the bone formation in OPG-deficient mice by stimulating MSC proliferation rather than differentiation probably by triggering CDK2/cyclin A pathway [26]. The effect of CDK2 on calcification is not clear, but the above statement shows that CDK2 may be directly involved in osteogenic differentiation. Inhibition of CDK2 by diosgenin may inhibit osteogenic differentiation of vascular smooth muscle cell.

These integrated approaches provide a novel and efficient way to connect the multiple targets of diosgenin in a network level understanding. The potential targets of diosgenin are the major components of the vascular calcification pathogenic program. Drugs with multiple targets might have a better chance of affecting the complex equilibrium of whole cellular networks than drugs that act on a single target. This study proved that diosgenin consistent with the above statement. Moreover, experimental testing of these targets will be required to support further assessment of potential preclinical study and clinical applications of diosgenin on vascular calcification.

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