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REVIEW

TAK1 mediates convergence of cellular signals for death and survival

Sabreena Aashaq[1] · Asiya Batool[1] · Khurshid I. Andrabi[1]

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Abstract
TGF-β activated kinase 1, a MAPK kinase kinase family serine threonine kinase has been implicated in regulating diverse range of cellular processes that include embryonic development, differentiation, autophagy, apoptosis and cell survival. TAK1 along with its binding partners TAB1, TAB2 and TAB3 displays a complex pattern of regulation that includes serious crosstalk with major signaling pathways including the C-Jun N-terminal kinase (JNK), p38 MAPK, and I-kappa B kinase complex (IKK) involved in establishing cellular commitments for death and survival. This review also highlights how TAK1 orchestrates regulation of energy homeostasis via AMPK and its emerging role in influencing mTORC1 pathway to regulate death or survival in tandem.

Keywords Apoptosis · Autophagy · Cytokine · Inflammatory · Smad

TAK1, a multifunctional kinase

Transforming growth factor-β is a versatile cytokine, regulating a wide variety of intracellular signaling pathways. The Smad dependent signaling pathway is conventionally acknowledged as the traditional pathway promoted by TGF-β1 [1]. However, the Smad dependent signaling pathway does not unfold the myriad functions of TGF-β1. Accumulating evidence suggests that the TGF-β1 multifunctionality is associated with the activation of diverse Smad independent pathways that may or may not involve crosstalk with Smads [2, 3]. TGF-β1, TGF-β2, and TGF-β3 are the three mammalian isoforms of TGF-β of which TGF-β1 represents the predominant isoform and the epitome of the TGF-β superfamily. TGF-β1 regulates a wide array of cellular functions including cell growth, differentiation, wound healing and apoptosis. TGF-β1 is also a puissant inducer in ECM synthesis [4, 5].

TGF-β1 signals originate with the interaction of type I and type II TGF-β receptors to activate distinct intracellular pathways [6]. Apart from the canonical Smad dependent pathways, the non-canonical, Smad independent pathways are directly activated by ligand-occupied receptors to regulate a wide array of downstream cellular responses [2, 7, 8]. Various branches of MAP kinase pathways including the extracellular signal regulated kinase (Erk) ½ [3, 9], p38 MAPK [10, 11], c-Jun N-Terminal kinase (JNK) [12, 13], phosphatidylinositol-3-kinase/AKT pathway [14, 15] and Rho-like GTPase [16, 17] signaling pathways are included among the Smad independent pathways. TGF-β-activated kinase 1 (TAK1) has emerged as an indispensable signaling molecule in Smad-independent TGF-β-induced signaling pathways. TAK1 is also a prime upstream molecule in TGF-β1 induced expression of fibronectin and type I collagen via activation of MKK4-JNK and MKK3-p38 signaling pathways respectively [18–20].

TAK1, a serine/threonine kinase, was first discovered as a member of the MAPK kinase kinase (MAP3K) family, named as MAP3K7, and is activated by TGF-β1 [21]. TAK1 is an extensively expressed kinase, which, as the name implies, was originally spotted as a TGFβ-activated enzyme [22, 23]. In addition to TGF-β1, TAK1 can be activated by various other stimuli encompassing lipopolysaccharides [24], pro-inflammatory cytokines like interleukin (IL)-1 [25], tumor necrosis factor (TNF)-α [26] and environmental stress [27]. TAK1 was originally recognized as a kinase in the TGF-β pathway by complementation and rescue of MAPKK mutant of yeast. TAK1 has been identified by a cDNA library screening and protein-fragment complementation assay in yeast for its tendency to substitute for the MAPKKK Ste11p in the yeast pheromone-induced MAPK

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PROSPECTS

Eukaryotic initiation factor 4E (eIF4E): A recap of the cap-binding protein

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Abstract

Eukaryotic initiation factor 4E (eIF4E), a fundamental effector and rate limiting element of protein synthesis, binds the 7-methylguanosine cap at the 5′ end of eukaryotic messenger RNA (mRNA) specifically as a constituent of eIF4F translation initiation complex thus facilitating the recruitment of mRNA to the ribosomes. This review focusses on the engagement of signals contributing to growth factor originated maxim and their role in the activation of eIF4E to achieve a collective influence on cellular growth, with a key focus on conjuring vital processes like protein synthesis. The review invites considerable interest in elevating the appeal of eIF4E beyond its role in regulating translation viz a viz cancer genesis, attributed to its phosphorylation state that improves the prospect for the growth of the cancerous cell. This review highlights the latest studies that have envisioned to target these pathways and ultimately the translational machinery for therapeutic intervention. The review also brings forward the prospect of eIF4E to act as a converging juncture for signaling pathways like mTOR/PI3K and Mnk/MAPK to promote tumorigenesis.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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Eukaryotic Initiation Factor 4E (eIF4E) sequestration mediates 4E-BP1 response to rapamycin

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ABSTRACT

The cap dependent translation initiation is a tightly controlled process of cooperative ternary complex formation by 4E-BP1, eIF4E and the 5′ cap of eukaryotic mRNA in response to environmental cues like glucose, nutrients and growth factor levels. Based on the well-described effects of mTORC1,rapamycin complex on 4E-BP1 phosphorylation s, it is generally accepted that rapamycin is a global inhibitor of cap-dependent translation. We have previously shown that 4E-BP1 resistance to rapamycin was overcome by the stoichiometric abundance of S6K1. Now we present evidence that the TOS-bearing amino terminal domain of S6K1 is sufficient to relieve the rapamycin resistance of 4E-BP1 as TOS deleted variants of S6K1, active or inactive with regard to S6K1 activity failed to bring about relief of 4E-BP1 resistance to rapamycin. We also show that the reciprocal inactivation of S6K1 by abundance of 4E-BP1 gets accomplished only with intact TOS motif in the protein. The data presented in this study identifies eIF4E and not Raptor as a cellular factor responsible to regulate rapamycin sensitivity of 4E-BP1 suggesting that the phosphorylation dynamics and rapamycin sensitivity of 4E-BP1 and S6K1 are regulated independently.

1. Introduction

mTOR (mammalian target of rapamycin), a member of PI3K (phosphatidyl inositol-3-kinase, is a highly conserved atypical ser/thr kinase with an outsized role in regulating major cellular processes like homeostasis, organismal growth and various pathological conditions including cancer, neurodegeneration, obesity and Type 2 diabetes [1]. Work in the last decade clearly demonstrates that mTOR controls protein synthesis through a stunning number of downstream targets especially S6K1 and 4E-BP1 whose activity is regulated by post-translational modifications like phosphorylation, inhibitory proteins and proteolytic cleavage [2,3].

The emerging picture is that the activation of mTOR contributes to the phosphorylation of 4E-BP1 and S6K1 by interacting through a regulatory Raptor binding TOS motif [2,4] however, there are inconsistencies in literature about the molecular mechanism that underlie the process. Phosphorylation of mTORC1 substrates is subject to various feedforward and feedback loops that encompass far beyond the sequence motifs of each phosphorylation site [5–8]. 4E-BP1, being observed as the point of convergence of various signaling conduits, can be phosphorylated by alternative kinases like GSK3, P^{38} MAPK, ATM, CDC2/CDK1, LRRK2 etc.: in conjunction or independent of mTORC1 [9], thereby challenging the conventional mTOR/4E-BP1 cascade. Nevertheless, in our previous studies, we have shown abysmal correlation between Raptor binding and state of 4E-BP1 phosphorylation [10] abrogating the role of Raptor, though paradoxically advocated to be the major interacting partner for all mTOR mediated phosphorylations. There is also a potential disconnect between rapamycin response of 4E-BP1 and its phosphorylation turn over [11–16]. It is well documented that rapamycin treatment affects Ser65 and Thr70 residues of 4E-BP1 which are less effectually phosphorylated by mTORC1 in-vitro [13] whereas, Thr37 and Thr46 that are well-identified mTORC1 sites in vitro are not rapamycin sensitive in vivo [13]. Equally credible work has demonstrated the unconventional response of 4E-BP1 towards rapamycin in glioma cells [17], myoblasts [18] and during AML [19].

In addition to this, there are no universal opinions regarding the importance of individual phosphorylation sites that dictate the release of eIF4E from 4E-BP1. T37 and T46 phosphorylations have been suggested to have either little or no effect on binding to eIF4E [14,20]. While some studies suggest that phosphorylation of Ser-65 residue alone is insufficient to release eIF4E from 4E-BP1, other studies highlight that the substitution of S65 alone drastically reduces the interaction by 100-fold [21]. Moreover, in sea-urchin, a hyperphosphorylated 4E-BP1 variant does not release eIF4E from 4E-BP1 [22]. In agreement with this, we have previously shown that 4E-BP1 phosphorylation is dispensable for its binding to eIF4E [10], therefore signifying that alternative mechanisms other than phosphorylation at canonical sites might play a possible role in regulating 4E-BP1 binding to eIF4E.

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Bioinformatics and Medicinal Plant Research: Current Scenario

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Abstract

Bioinformatics being a multidisciplinary data-driven field has revolutionized several aspects of life sciences research, and area of drug development through medicinal plants is no exception. Medicinal plants have been known to play a major role in the primary healthcare system of several communities across the globe since ancient times. They continue to provide a multitude of pharmacologically active compounds. Now, to increase the utility of medicinal plants for drug discovery, bioinformatics plays a major role in replacing the conventional expensive, time-consuming and sluggish methods of drug development through high-throughput computational approaches. In this chapter, we attempt to present the comprehensive and updated summary on the role of bioinformatics in the area of medicinal plant research through the development of plant-based drugs. We need to understand the role of different bioinformatics approaches in medicinal plant research as it could serve as harbinger for the discovery of new therapeutic potential leads against various

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Influence of processing methods and storage on phenolic compounds and carotenoids of apricots

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Procyanidin B_2 (PubChem CID: 122738)
β-carotene (PubChem CID: 5280489)
α-carotene (PubChem CID: 6419725)
β-cryptoxanthin (PubChem CID: 5281235)
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ABSTRACT

Apricots are a rich source of phytochemicals such as carotenoids and phenolic compounds which contribute to their health promoting properties. Apricots are processed by several methods which include thermal treatment, drying etc. However, processing results in significant changes in the chemical composition of foods. This study was carried out to evaluate the effect of different processing methods and storage on the phytochemical profile of apricots. Three apricot varieties (CITH-1, CITH-2, New Castle) from Kashmir were subjected to different processing treatments (freezing, canning and drying). Processed apricots were stored at ambient temperatures for 12 months and evaluated at 4 months interval. The processed samples were analyzed for various phenolic compounds (chlorogenic acid, neochlorogenic acid, catechin, kaempferol, quercetin, procyanidin B_2) by HPLC during the storage period. Freezing and canning increased the phenolic content of processed samples while as drying resulted in a decline compared to fresh samples. Evaluation of carotenoids by HPLC showed that highest β-carotene, α-carotene, β-cryptoxanthin, and zeaxanthin contents were observed in canned apricots followed by frozen and dried apricots ($P < 0.05$). These compounds showed a decline with respect to storage time.

1. Introduction

Fruits and vegetables are rich in bioactive compounds and offer protection against degenerative diseases of ageing, such as heart disease, cardiovascular disease, Alzheimer's disease, cataracts and several forms of cancer (; ; ;). Apricots (*Prunus armeniaca* L.) are rich in bioactive compounds such as carotenoids and phenolic compounds. Carotenoids present in apricots mainly include β-carotene, β-cryptoxanthin, lutein and γ-carotene. Phenolic characterization of the apricot fruits shows that the major phenolic compounds in these fruits are chlorogenic acid, neochlorogenic acid, protocatechuic acid, (+)-catechin, 3′-caffeoylquinic (or chlorogenic) acid, (−)-epicatechin, naringenin-7-glucoside (or prunin), quercetin-3-glucoside, quercetin-3-rhamnoglucoside (or rutin), and kaempferol-3-rutinoside (; ; ;).

As known, the fruit of apricot besides being consumed fresh is also used to produce a variety of processed products which involve different processing methods. However, these processing methods affect the physiochemical, functional, nutritional, safety and sensory quality of the fruits. These methods may have a positive or negative influence on the stability of phytochemicals. In fruits and vegetables, phytochemicals can be bound in the plant cell membranes or exist as free compounds. Food processing such as heating or freezing can disrupt the cell membrane leading to the release of membrane-bound phytochemicals, which implies higher bioaccessibility (;). Canning does not affect the β-carotene and antioxidant activity of apricots however, total phenol content increased with canning ( ). discovered a slight increase in total phenols and anthocyanins following canning of cherries, which they attributed to increased phenolic extraction due to

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CHAPTER 4

LONG NON-CODING RNAS IN INFECTION AND IMMUNITY

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Abstract

Immune responses combat various infectious agents by inducing inflammatory responses, antimicrobial pathways, and adaptive immunity. The polygenic responses to these external stimuli are regulated temporally and in coordination. Specific lncRNAs are induced to modulate innate and adaptive immune responses, which can function through various target interactions, like RNA-DNA, RNA-RNA, and RNA-protein interaction and hence affect the immunogenic regulation at various stages of gene expression. LncRNA are found to be present in various immune cells, like monocytes, macrophages,

CHAPTER 3

LONG NON-CODING RNAS IN MULTIPLE SCLEROSIS: AN UPDATE

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Abstract

Multiple sclerosis (MS) is a long-term immune-mediated progressive demyelinating disease of the central nervous system (CNS) that has become

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Bioactive supra decorated thiazolidine-4-carboxylic acid derivatives attenuate cellular oxidative stress by enhancing catalase activity†

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A pharmacophoric motif decorated with supramolecular functionalities (TZT) was designed for potential interaction with biological targets. Main insights of this work include the correlation of supra functionalities of TZT with its binding ability to proteins leading to the modulation of their structure and bioactivity as a promising perspective in the field of cellular protection from oxidative stress. To investigate the role of TZT in obliterating oxidative stress at a molecular level, its binding propensity with bovine serum albumin (BSA) and bovine liver catalase (BLC) was characterized using various biophysical methods. The binding constants of TZT with BSA (K_b = 2.09 × 10^5 M^{-1}) and BLC (K_b = 2.349 × 10^5 M^{-1}) indicate its considerable interaction with these proteins. TZT efficiently triggers favourable structural changes in BLC, thereby enhancing its enzyme activity in a dose dependent manner. The enzyme kinetics parameters of TZT binding to BLC were quantified using the Michaelis–Menten model. Both *in silico* and experimental results suggest that an increased substrate availability could be the reason for enhanced BLC activity. Furthermore, physiological relevance of this interaction was demonstrated by investigating the ability of TZT to attenuate oxidative stress. Treatment with TZT was found to mitigate the inhibition of A549 cell proliferation in the presence of high concentrations of vitamin C. This finding was confirmed at a molecular level by PARP cleavage status, demonstrating that TZT inhibits apoptotic cell death induced by oxidative stress.

Introduction

Supramolecular chemistry, which involves molecular aggregation *via* non-covalent interactions, represents an attractive approach for understanding and targeting biological processes.[1] Indeed, the binding of bioactive molecules to their biological targets occurs primarily through supramolecular interactions,[1–5] thus offering routes for the modulation of target-based drug development. In continuation of our interests in chemical biology,[6–9] this work presents a comprehensive biophysical investigation of a synthesised pharmacophoric motif decorated with various supramolecular functionalities. Thiazolidine derivatives are remarkable compounds with a plethora of biological activities[10–12] that can be decorated with functionalities favouring supramolecular forces as potential ligands for biotargets.[2] With this motivation we synthesised (2*S*,4*R*)-3-(*tert*-butoxycarbonyl)-2-(2-hydroxyphenyl) thiazolidine-4-carboxylic acid referred to as the TZT molecule. The potential supramolecular interaction sites in the TZT molecule are depicted in Scheme 1. Main physical insights of this work are the ability of TZT to restructure proteins through complexation, thus enhancing enzyme activity and showing promising perspectives in the field of cellular protection from oxidative stress.

The increase in life expectancy is accompanied by a higher frequency of crippling degenerative diseases, thus triggering the need for new drugs. However, interactions with model proteins have to be considered while designing pharmacophoric molecules. Serum albumins are vital drug carriers determining

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Eukaryotic initiation factor 4E is a novel effector of mTORC1 signaling pathway in cross talk with Mnk1

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Abstract

Cellular signals that influence Cap-dependent translation have assumed significant relevance in the backdrop of their enforced dysregulation during oncogenesis. Eukaryotic initiation factor 4E(eIF4E), the mRNA cap-binding protein, has emerged as a key player to facilitate tumor progression through upregulated cap-dependent translation synchronized with enhanced cell division. We provide evidence that eIF4E phosphorylation is regulated by mTORC1 by virtue of its interaction with Raptor through a novel *TPTPNPP* motif and consequent phosphorylation invitro and in vivo in a Rapamycin-sensitive manner. While we show that phosphorylation pattern of eIF4E responds faithfully to Rapamycin inhibition, the prolonged exposure to Rapamycin rescues the loss of eIF4E phosphorylation through Mnk1 activation. We also present evidence that eIF4E interacts with the amino terminal domain of S6K1 in a phospho-dependent manner, and this interaction is instrumental in overriding Rapamycin inhibition of S6K1. The data endorses eIF4E as a regulatory subunit that modulates the functional attributes of mTOR effectors to synchronize cap-dependent translation with growth assertion.

Keywords mTORC1 · eIF4E · 4E-BP1 · Mnk1 · Rapamycin · Translation

Introduction

Eukaryotic initiation factor 4E (eIF4E), a 25-kDa phosphoprotein, is a pivotal effector and rate-limiting determinant of protein translation that specifically binds the 7-methylguanosine cap at the 5′ end of eukaryotic mRNA as a component of eIF4F translation initiation complex thereby mediating the recruitment of mRNA to the ribosomes [1, 2]. Engagement of signals that subscribe to growth factor initiated maxim transduce into the activation of eIF4E to achieve a cumulative influence on cellular growth, with prime focus on evoking vital processes like protein translation [3]. The activation of eIF4E is controlled by either phosphorylation at Ser209 by upstream signaling pathways or sequestration by the well-established regulator 4E-BP1 [4]. Ser209, an activating signature phosphorylation of eIF4E, is reported to be catalyzed by mitogen-activated protein kinase (MAPK) interacting serine/threonine kinase (Mnk) [5–7], a substrate of Erk1/2 and p38 MAPK [8, 9]. The more prolific mode of regulation of eIF4E is via a stringently regulated phenomenon dictated by the binding of 4E-BP1 that binds eIF4E and prevents its incorporation into the initiation complex [10].

Over the past decade, a wealth of experimental evidence has assumed significance in light of documenting the oncogenic stature of eIF4E [11, 12]. The concerted upregulation of eIF4E in various cancers with prospective involvement in metastasis has been evidenced by numerous studies underlining the contribution of eIF4E in malignant transformation and progression [13–18].

4E-BP1 has been identified as an important regulator of overall translation levels in cells that bind and impair eIF4E in its dephosphorylated state [19]. However, we have previously confronted the very basis of the dogma that the phosphorylation status of 4E-BP1 is discordant for its binding to eIF4E [20]. It has been well documented that the phosphorylation of eIF4E at Ser209 is crucial for its tumorigenic potential [21, 22]. However, the functional consequence of

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Eukaryotic Initiation Factor 4E phosphorylation acts a switch for its binding to 4E-BP1 and mRNA cap assembly

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ABSTRACT

Translational regulation has invited considerable interest consequent of its circumstantial dysregulation during cancer genesis. eIF4E (Eukaryotic Initiation Factor 4E) has been identified as an important factor involved in tumor progression by way of instrumenting the convergence of oncogenic signals for up-regulation of Cap-dependent translation. In the backdrop of dramatic eIF4E over-expression in a large population of human cancers, we suggest that the tumorigenic property of eIF4E is strictly attributed to its phosphorylation state. We provide evidence that while phosphorylated eIF4E fails to be sequestered by 4E-BP1, its dephosphorylated form shows overwhelming binding with 4E-BP1 without any consideration to the state of 4E-BP1 phosphorylation to suggest that eIF4E-4EBP1 binding is governed by eIF4E phosphorylation instead of 4E-BP1. We also show that eIF4E engages in Cap-assembly formation preferably in a phosphorylation-dependent manner to suggest that eIF4E phosphorylation rather than 4E-BP1 regulates its availability for Cap-assembly.

1. Introduction

eIF4E is a fundamental component essential for the translation of all capped mRNAs [1,2]. Complete loss of eIF4E results in embryo death within 6.5 embryonic days as seen in eIF4E−/− mice [3]. This importance is vested upon the ability of eIF4E to recognize the m7GpppN cap moiety at the 5′ end of eukaryotic mRNA, thereby initiating the cap-dependent translation [4,5].

Conceptual progress in the last decade has added to the oncogenic stature of eIF4E, wherein a lot of studies suggest its abundance as a means to bring about cellular transformation and tumorigenesis [6–10]. This attribute is associated with the ability of eIF4E to modify the translational dynamics in a way that favors the survival and proliferative necessities of cancer cells [11]. Also, potential of 4E-BP1 to act as a tumor-suppressor has gained profound insights [12–14].

The activity of eIF4E is regulated either by sequestration by its well-established regulator 4EBP1 or by Ser209 phosphorylation via upstream signalling pathways [15,16]. 4E-BP1 has been recognized as a critical regulator of overall translation levels in cells by way of binding and impairing eIF4E in its dephosphorylated state [17,18].

The availability of free eIF4E virtually wholly ascribed to the state of 4EBP1 phosphorylation by mTORC1, has not survived up in faith as an exclusive factor in light of the recalcitrance of 4EBP1 to rapamycin action in vivo [19]. Evidence supporting kinases other than mTORC1 as physiological prospect for 4EBP1 phosphorylation [20–22], seems to shift the onus of 4E availability on factors other than 4EBP1 phosphorylation.

However, we have previously shown the discordance of 4E-BP1 phosphorylation in binding to eIF4E [23]. To address this question, we have extended the study here to evaluate the possible role of eIF4E in dictating this binding. We herein compare the capacity of 4E-BP1 to be dephosphorylated in response to rapamycin in different cell types upon manipulation of eIF4E-4EBP1 stoichiometry.

2. Materials and methods

2.1. Antibodies and reagents

NIH-3T3 and HEK293 were kindly gifted by Dr. Fayaz Malik (CSIR-IIIM Jammu). HCT, A549, SW480 MCF-7, MDA-MB, and HELA cells were procured from NCCS, Pune-India. Lipofectamine reagent (Invitrogen), Rapamycin (Calbiochem, USA), Torin (Tocris) PVDF

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Review

Structure-functional implications of longevity protein p66Shc in health and disease

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ABSTRACT

ShcA (Src homologous- collagen homologue), family of adapter proteins, consists of three isoforms which integrate and transduce external stimuli to different signaling networks. ShcA family consists of p46Shc, p52Shc and p66Shc isoforms, characterized by having multiple protein-lipid and protein-protein interaction domains implying their functional diversity. Among the three isoforms p66Shc is structurally different containing an additional CH2 domain which attributes to its dual functionality in cell growth, mediating both cell proliferation and apoptosis. Besides, p66Shc is also involved in different biological processes including reactive oxygen species (ROS) production, cell migration, ageing, cytoskeletal reorganization and cell adhesion. Moreover, the interplay between p66Shc and ROS is implicated in the pathology of various dreadful diseases. Accordingly, here we discuss the recent structural aspects of all ShcA adaptor proteins but are highlighting the case of p66Shc as model isoform. Furthermore, this review insights the role of p66Shc in progression of chronic age-related diseases like neuro diseases, metabolic disorders (non-alcoholic fatty liver, obesity, diabetes, cardiovascular diseases, vascular endothelial dysfunction) and cancer in relation to ROS. We finally conclude that p66Shc might act as a valuable biomarker for the prognosis of these diseases and could be used as a potential therapeutic target.

1. Overview of ShcA family of adaptor proteins

ShcA adaptor protein family is a group of three different members which are produced from the same gene locus. The ShcA gene locus maps to the chromosome 1q21, containing 13 exons that are translated into three proteins of about 46, 52, and 66 kDa known as p46Shc, p52Shc and p66Shc respectively (Pelicci et al., 1992). The protein products of all three isoforms are produced by either RNA splicing or by alternative translational initiation (Ravichandran, 2001). The longest isoform p66Shc is composed of 583 amino acids, produced from exon 2–13 of ShcA mRNA whereas the other two shorter isoforms p52Shc and p46Shc are composed of 474 and 429 amino acids respectively, generated from exon 1, a fragment of exon 2(2a) and exon 3–13(2) of ShcA mRNA (Kumar, 2019b; Wright et al., 2018). The other mechanism involved in production of these isoforms occurs by the utilization of alternative in-frame translational start codons. The longest isoform translation uses three in-frame ATGs while as p52shc/p46shc isoforms translation is driven by the use of two ATGs which are in frame. Each member of Shc A family share similar core domain organization containing N-terminal phosphotyrosine binding domain (PTB), C-terminal sarcoma homologous type 2 domain (SH2) and a proline rich linker collagen homology 1 (CH1) domain that links PTB domain with SH2 domain. In addition, a cytochrome C binding region is found in p66Shc and p52Shc isoforms towards N-terminal side end (Ahmed and Prigent, 2017). Unlike other two Shc A isoforms, p66Shc contains an extra fragment joined to N-terminus side of PTB domain known as collagen homology 2 (CH2) domain, making it the longest isoform of this family. The two shorter isoforms, p52Shc and p46Shc are found in amphibians, mammals, fishes, insects (D. Melanogaster), nematodes (C. Elegans), and yeasts while as p66Shc is the most recent evolved isoform of this family and is specifically found in vertebrates, but not in yeasts, nematodes, and insects (Galimov, 2010). (Fig. 1)

1.1. Regulation of ShcA expression and localization

ShcA isoforms exhibit differential expression pattern which is ascribed to its posttranscriptional and posttranslational control system (Kumar, 2019b). Among the three isoforms, p66Shc is restrictedly expressed in most of cells excluding hematopoietic lineage whereas the p52Shc and p46Shc isoforms are expressed in all cells (Bhat et al., 2015).

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RESEARCH ARTICLE-BIOLOGICAL SCIENCES

Flavonoid Treatment of Breast Cancer Cells has Multifarious Consequences on Alpha-1-Syntrophin Expression and other Downstream Processes

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Abstract
Alpha-1-syntrophin (SNTA1) is emerging as an important regulator of cell signaling. SNTA1 forms a part of the dystrophin glycoprotein complex (DGC) and takes part in signaling pathways via the DGC. SNTA1 has been seen to be over expressed in various carcinomas, including breast, esophageal and colon cancer. In this study, we analyze the effect of anti-cancer compounds vis-à-vis quercetin and kaempferol on the expression of SNTA1 protein, reactive oxygen species (ROS) production and cell migration in breast cancer cells. Quercetin as well kaempferol treatment leads to a decrease in the expression of SNTA1 protein in HBL100 cells as well as a decrease in both ROS production and cell migration. The multipartite effects of the flavonoids in HBL100 cells can pave the way for further studies where SNTA1 is targeted for drug therapy in cancer.

Keywords Breast cancer · Alpha-1-syntrophin · Quercetin · Kaempferol · ROS · Cell migration

1 Introduction

Syntrophins are a family of scaffolding adaptor proteins that were first identified in postsynaptic membrane of Torpedo electric organ. Syntrophins are biologically diverse group of ~ 59KDa cytosolic peripheral membrane proteins that are characterized by the presence of the N-terminal PH1 (pleckstrin homology 1) domain that is split by a PDZ (postsynaptic density protein-95/disc large/zona occludens-1), followed by a second PH2 (pleckstrin homology 2) domain and a C-terminal SU (syntrophin unique) domain. Syntrophins serve to the internal cellular components with the dystrophin glycoprotein complex (DGC) by directly associating with dystrophin and dystrophin-related proteins like utrophin and dystrobrevin. These interactions with DGC are primarily mediated by the split PH1 domain and the SU domain of syntrophin [1–6]. The syntrophin family consists of five different homologous isoforms vis-à-vis, alpha-1-syntrophin, beta-1-syntrophin, beta-2-syntrophin, gamma-1-syntrophin and gamma-2-syntrophin [4, 7, 8]. Alpha-1-syntrophin (SNTA1) is a 59 kDa membrane associated adaptor protein. SNTA1 is primarily expressed in skeletal muscles and also in tissues like heart, breast, brain etc. [8, 9]. SNTA1 acts as a link between the extracellular matrix and the internal cell signaling apparatus via the DGC [10]. SNTA1 plays an important role in the internal machinery of the cell, by interacting with proteins like stress-activated protein kinase 3 (SAPK), calmodulin, actin, G-proteins, neuronal nitric oxide synthase (nNOS), growth factor receptor bound 2 (Grb2) and other signaling proteins in the cell [11]. SNTA1 binding to the SH2 domain of Grb2 leads to the activation of Rac1 protein, followed by the rest of the signaling pathway [12–15]. SNTA1 via its interaction with the DGC regulates the SNTA1-Grb2-Sos1-Rac1-PAK1-JNK signaling pathway. Any change in the binding of laminin leads to conformational changes in SNTA1 that disrupts its binding to SH2 domain of Grb2 and lead to disruption of the entire signaling pathway [15–17].

Any disruption in these signaling pathways can cause abnormalities in the cell and lead to pathologies like cancer [18]. We have previously shown that SNTA1 is over expressed in breast cancer tissue, when compared to adja-

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RESEARCH PAPER

mTORC1 induces eukaryotic translation initiation factor 4E interaction with TOS-S6 kinase 1 and its activation

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ABSTRACT

Eukaryotic translation initiation factor 4E was recently shown to be a substrate of mTORC1, suggesting it may be a mediator of mTORC1 signaling. Here, we present evidence that eIF4E phosphorylated at S209 interacts with TOS motif of S6 Kinase1 (S6K1). We also show that this interaction is sufficient to overcome rapamycin sensitivity and mTORC1 dependence of S6K1. Furthermore, we show that eIF4E-TOS interaction relieves S6K1 from auto-inhibition due to carboxy terminal domain (CTD) and primes it for hydrophobic motif (HM) phosphorylation and activation in mTORC1 independent manner. We conclude that the role of mTORC1 is restricted to engaging eIF4E with S6K1-TOS motif to influence its state of HM phosphorylation and inducing its activation.

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KEYWORDS
mTOR; S6 Kinase 1; eIF4E

Introduction

Eukaryotic translation initiation factor 4E (eIF4E) is a potent oncogene whose expression is elevated in diverse range of cancers [1–6]. While eIF4E overexpression promotes CAP-dependent translation during tumor development [7], several reports highlight the role of eIF4E phosphorylation at S-209 in facilitating tumor formation [8–10]. Pertinently, for sustaining tumorigenesis, enhanced CAP dependent translation due to increased eIF4E expression/phosphorylation must correlate with other growth promoting functions. Incidentally, increased expression and activation of ribosomal protein S6 kinase 1 (S6K1), a major downstream effector of mTORC1, required for ribosome biogenesis and cell cycle progression, is a frequent feature associated with tumorigenesis [11–13]. Therefore, it is logical to assume that signals regulating eIF4E expression/phospho dynamics must synchronize with the ones that influence S6K1 activation. Accordingly, MAP kinase interacting kinase (MNK) pathway, believed to govern the state of eIF4E phosphorylation in eIF4G-dependent manner [14,15], has been shown to be related to the state of mTORC1-S6K1 regulation and its response to rapamycin. Although the role ascribed to eIF4E phosphorylation does endorse the crosstalk between MNK1 and mTORC1 pathways [14], the mechanistic basis of its relation with S6K1 regulation remains unclear. Interestingly, our recent observations that identify eIF4E as mTORC1 substrate [16] and as a potential to influence cellular response to rapamycin [17] was suggestive of its prospect as an intermediate in propagating mTORC1 response. Here, we report that eIF4E in its phospho form interacts with TOS motif of S6K1. This interaction relieves CTD-mediated auto-inhibition of S6K1 and primes it for T412 phosphorylation at HM site and subsequent activation in mTORC1 independent manner.

Materials and methods

Cell lines and culture conditions

HEK293 were a gift from Dr. Tyagi JNU-India. HEK293T and NIH3T3 were gifted by Dr. Fayaz Malik and Dr. Jamal respectively (CSIR-IIIM

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REVIEW ARTICLE

Expansion of the CRISPR/Cas Genome-Sculpting Toolbox: Innovations, Applications and Challenges

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Abstract
The emergence of the versatile gene-editing technology using programmable sequence-specific endonuclease system (CRISPR-Cas9) has instigated a major upheaval in biomedical research. In a brief span of time, CRISPR/Cas has been adopted by research labs around the globe because of its potential for significant progress and applicability in terms of efficiency, versatility and simplicity. It is a breakthrough technique for systematic genetic engineering, genome labelling, epigenetic and transcriptional modulation, and multiplexed gene editing, amongst others. This review provides an illustrative overview of the current research trends using CRISPR/Cas technology. We highlight the latest developments in CRISPR/Cas technique including CRISPR imaging, discovery of novel CRISPR systems, and applications in altering the genome, epigenome or RNA in different organisms. Finally, we address the potential challenges of this technique for its future use.

Key Points

In the past decade, the exploitation of the CRISPR/Cas technique has swiftly eclipsed the ZNF and TALEN gene-editing tools.

CRSIPR/Cas may result in transformative therapies based on the creation of permanent and inheritable changes in the human genome.

The varying functionality of CRISPR systems have allowed researchers to pursue the therapeutic potential of molecular genetics in relation to disease progression.

1 Introduction

The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) system, found in 90% of archaea and 40% of bacteria, forms part of the bacterial adaptive immunity against extraneous genetic elements, such as bacteriophages, plasmids and RNA in some cases [1, 2]. This microbial-based RNA-guided endonuclease system has been utilized to engineer biological systems of diverse species, such as zebrafish [3, 4], Drosophila [5, 6], Bombyx mori [7], Caenorhabditis elegans [8], rat [9], mouse [10] and humans [11].

CRISPRs were initially described by a team of Japanese scientists in 1987 as stretches of direct repeats in *Escherichia coli* [12], and later also described in different bacteria [13]. In the mid-2000s, microbiologists determined that CRISPR/Cas is a pre-existing bacterial defence system that uses antisense RNA as the memory signature of a previous intrusion [14]. This was experimentally confirmed in 2007 using lytic phages and streptococcus thermophilus (lactic acid bacterium) [15]. A significant insight was provided in 2012 from the discovery that *Streptococcus pyogenes* and *S. thermophilus* Cas9-crRNA complexes can act as RNA-guided endonucleases in vitro [16]. These findings, along with previous observations, suggested that the complex of Cas9-crRNA can be utilized as a targeted genome-engineering technology for making precise double-stranded breaks. Moreover, newer studies continually aim at optimizing and modifying

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REVIEW ARTICLE

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TGF-β signaling: A recap of SMAD-independent and SMAD-dependent pathways

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Abstract

Transforming growth factor-β (TGF-β) is a proinflammatory cytokine known to control a diverse array of pathological and physiological conditions during normal development and tumorigenesis. TGF-β-mediated physiological effects are heterogeneous and vary among different types of cells and environmental conditions. TGF-β serves as an antiproliferative agent and inhibits tumor development during primary stages of tumor progression; however, during the later stages, it encourages tumor development and mediates metastatic progression and chemoresistance. The fundamental elements of TGF-β signaling have been divulged more than a decade ago; however, the process by which the signals are relayed from cell surface to nucleus is very complex with additional layers added in tumor cell niches. Although the intricate understanding of TGF-β-mediated signaling pathways and their regulation are still evolving, we tried to make an attempt to summarize the TGF-β-mediated SMAD-dependent andSMAD-independent pathways. This manuscript emphasizes the functions of TGF-β as a metastatic promoter and tumor suppressor during the later and initial phases of tumor progression respectively.

KEYWORDS
angiogenesis, chemoresistance, metastasis

1 | TGF-β—A VERSATILE CYTOKINE

Transforming growth factor-β (TGF-β) superfamily includes a variety of conserved growth factors, each controlling a wide network of cellular tasks encompassing cellular differentiation, proliferation, cell death, cell adhesion, motility, lineage determination, adult tissue homeostasis, and embryogenesis. TGF-β and its structurally related polypeptide factors are expressed in a complex tissue-specific and temporal manner and play an important part in repair during tissue damage and organismal homeostasis in all organisms from Drosophila to humans. These polypeptide factors conjointly comprise a significant proportion of cellular signals that dictate cell fate (Hata & Chen, 2016; Massagué, 1998; Tzavlaki & Moustakas, 2020). Dysregulation of TGF-β signaling results in the development of various diseases like cancer and fibrotic diseases and also leads to various developmental defects (Rik Derynck & Budi, 2019). TGF-β signaling emerged in the early metazoan evolution. A few elements of the pathway are also present in the existent non-metazoans, pointing to the advent of this pathway as a major upheaval in switching to multicellularity by the early metazoans. Although the SMAD homologs were also detected in the choanoflagellates, there is no evidence of TGF-β receptors and ligands outside the metazoans (Pang et al., 2011). As many as 30 members of the TGF-β superfamily are reported in humans and many orthologs are described in xenopus, mouse, and other vertebrates (Massagué et al., 2000). Seven members of the family are reported in *Drosophila melanogaster* (Raftery & Sutherland, 1999) and seven in *C. elegans* (Padgett et al., 1998). The members of the TGF-β superfamily are generally divided into two categories that include the bone morphogenetic protein (BMP)/growth and differentiation factor (GDF) and TGF-β/

diagnostics

MDPI

Article

Ensemble Machine Learning Model to Predict SARS-CoV-2 T-Cell Epitopes as Potential Vaccine Targets

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Abstract: An ongoing outbreak of coronavirus disease 2019 (COVID-19), caused by a single-stranded RNA virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused a worldwide pandemic that continues to date. Vaccination has proven to be the most effective technique, by far, for the treatment of COVID-19 and to combat the outbreak. Among all vaccine types, epitope-based peptide vaccines have received less attention and hold a large untapped potential for boosting vaccine safety and immunogenicity. Peptides used in such vaccine technology are chemically synthesized based on the amino acid sequences of antigenic proteins (T-cell epitopes) of the target pathogen. Using wet-lab experiments to identify antigenic proteins is very difficult, expensive, and time-consuming. We hereby propose an ensemble machine learning (ML) model for the prediction of T-cell epitopes (also known as immune relevant determinants or antigenic determinants) against SARS-CoV-2, utilizing physicochemical properties of amino acids. To train the model, we retrieved the experimentally determined SARS-CoV-2 T-cell epitopes from Immune Epitope Database and Analysis Resource (IEDB) repository. The model so developed achieved accuracy, AUC (Area under the ROC curve), Gini, specificity, sensitivity, F-score, and precision of 98.20%, 0.991, 0.994, 0.971, 0.982, 0.990, and 0.981, respectively, using a test set consisting of SARS-CoV-2 peptides (T-cell epitopes and non-epitopes) obtained from IEDB. The average accuracy of 97.98% was recorded in repeated 5-fold cross validation. Its comparison with 05 robust machine learning classifiers and existing T-cell epitope prediction techniques, such as NetMHC and CTLpred, suggest the proposed work as a better model. The predicted epitopes from the current model could possess a high probability to act as potential peptide vaccine candidates subjected to in vitro and in vivo scientific assessments. The model developed would help scientific community working in vaccine development save time to screen the active T-cell epitope candidates of SARS-CoV-2 against the inactive ones.

Keywords: COVID-19; SARS-CoV-2; T-cell epitope; peptide-based vaccines; machine learning; random forest; ensemble learning; voting ensemble

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1. Introduction

An infection outbreak caused by a novel coronavirus has proliferated rapidly around the world. The World Health Organization (WHO) designated the disease as COVID-19 [1,2]. The pathogen was named SARS-CoV-2 by the Coronaviridae Study Group (CSG) [3]. The pathogen has resulted in 225,488,491 COVID-19 cases and 4,644,376 deaths worldwide as of September 13, 2021, posing a significant challenge to public health worldwide [4]. Furthermore, because SARS-CoV-2 keeps on circulating, the chances of mutations in the virus also increases. The recent delta variant with *Pango lineage* as AY.1, AY.2, AY.3,

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Research Article

Machine Learning-Based Ensemble Model for Zika Virus T-Cell Epitope Prediction

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Zika virus (ZIKV), the causative agent of Zika fever in humans, is an RNA virus that belongs to the genus *Flavivirus*. Currently, there is no approved vaccine for clinical use to combat the ZIKV infection and contain the epidemic. Epitope-based peptide vaccines have a large untapped potential for boosting vaccination safety, cross-reactivity, and immunogenicity. Though many attempts have been made to develop vaccines for ZIKV, none of these have proved to be successful. Epitope-based peptide vaccines can act as powerful alternatives to conventional vaccines due to their low production cost, less reactogenic, and allergenic responses. For designing an effective and viable epitope-based peptide vaccine against this deadly virus, it is essential to select the antigenic T-cell epitopes since epitope-based vaccines are considered safe. The in silico machine-learning-based approach for ZIKV T-cell epitope prediction would save a lot of physical experimental time and efforts for speedy vaccine development compared to *in vivo* approaches. We hereby have trained a machine-learning-based computational model to predict novel ZIKV T-cell epitopes by employing physicochemical properties of amino acids. The proposed ensemble model based on a voting mechanism works by blending the predictions for each class (epitope or nonepitope) from each base classifier. Predictions obtained for each class by the individual classifier are summed up, and the class with the majority vote is predicted upon. An odd number of classifiers have been used to avoid the occurrence of ties in the voting. Experimentally determined ZIKV peptide sequences data set was collected from Immune Epitope Database and Analysis Resource (IEDB) repository. The data set consists of 3,519 sequences, of which 1,762 are epitopes and 1,757 are nonepitopes. The length of sequences ranges from 6 to 30 meter. For each sequence, we extracted 13 physicochemical features. The proposed ensemble model achieved sensitivity, specificity, Gini coefficient, AUC, precision, F-score, and accuracy of 0.976, 0.959, 0.993, 0.994, 0.989, 0.985, and 97.13%, respectively. To check the consistency of the model, we carried out five-fold cross-validation and an average accuracy of 96.072% is reported. Finally, a comparative analysis of the proposed model with existing methods has been carried out using a separate validation data set, suggesting the proposed ensemble model as a better model. The proposed ensemble model will help predict novel ZIKV vaccine candidates to save lives globally and prevent future epidemic-scale outbreaks.

1. Introduction

ZIKV is an enveloped virus that belongs to the genus *Flavivirus* and the family Flaviviridae. It is almost similar to dengue fever and the West Nile virus because of its propagation through infected mosquito stings [1]. The World Health Organization (WHO) declared the outbreak a "public health emergency of international concern" in February 2016. To date, the shreds of evidence of ZIKV disease have been reported from 86 countries and territories

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Review article

Epidermal growth factor receptor and integrins meet redox signaling through P66shc and Rac1

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Reactive Oxygen Species

ABSTRACT

This review examines the concerted role of Epidermal Growth Factor Receptor (EGFR) and integrins in regulating Reactive oxygen species (ROS) production through different signaling pathways. ROS as such are not always deleterious to the cells but they also act as signaling molecules, that regulates numerous indespensible physiological fuctions of life. Many adaptor proteins, particularly Shc and Grb2, are involved in mediating the downstream signaling pathways stimulated by EGFR and integrins. Integrin-induced activation of EGFR and subsequent tyrosine phosphorylation of a class of acceptor sites on EGFR leads to alignment and tyrosine phosphorylation of Shc, PLCγ, the p85 subunit of PI-3 K, and Cbl, followed by activation of the downstream targets Erk and Akt/PKB. Functional interactions between these receptors result in the activation of Rac1 via these adaptor proteins, thereby leading to Reactive Oxygen Species. Both GF and integrin activation can produce oxidants independently, however synergistically there is increased ROS generation, suggesting a mutual cooperation between integrins and GFRs for redox signalling. The ROS produced further promotes feed-forward stimulation of redox signaling events such as MAPK activation and gene expression. This relationship has not been reviewed previously. The literature presented here can have multiple implications, ranging from looking at synergistic effects of integrin and EGFR mediated signaling mechanisms of different proteins to possible therapeutic interventions operated by these two receptors. Furthermore, such mutual redox regulation of crosstalk between EGFR and integrins not only add to the established models of pathological oxidative stress, but also can impart new avenues and opportunities for targeted antioxidant based therapeutics.

1. Introduction

Oxidative stress is a phenomenon of elevated intracellular levels of reactive oxygen species (ROS) causing damage to lipids, proteins and DNA. Oxidative stress has been linked to a number of pathologies. However, elevated ROS are also signaling molecules maintaining various physiological functions [1]. Mitochondria in metazoan cells are not only one of the principle cell organelles involved in energy production in the form of ATP but also a significant source of ROS generation [2-4]. Within the mitochondria the primary reactive oxygen species produced is superoxide, a large part of which is converted to hydrogen peroxide by the action of superoxide dismutase. The production of superoxide by mitochondria has been dedicated to several enzymes of the electron transport chain, including Complexes I and III and glycerol-3-phosphate dehydrogenase via the process of oxidative phosphorylation (OXPHOS) [5-8]. $O_2^{\cdot}$ is most commonly the result of the spillage of electrons from the mitochondria [9]. It is produced from the one-electron reduction of molecular oxygen (O_2) and, is rapidly converted to H_2O_2 by superoxide dismutases 1 and 2 (SOD 1 and 2) [10]. The superoxide produced as such causes protein oxidation, DNA damage and lipid peroxidation in a variety of cells. H_2O_2 also shows damaging effect on cells until degraded to H_2O by catalaze enzyme [11]. Such mitochondrial ROS promotes apoptotic cell death [12] apart from being a signaling molecule for tumor necrosis factor-α (TNF-α) activation [13], hypoxia [14], and integrin activation [15]. Superoxide anions, may also be generated enzymatically by NADPH (Nicotinamide adenine dinucleotide phosphate) oxidase activity, another hotspot for ROS production and prostanoid metabolism through cycloxygenase and

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Review

β-Glucan: A dual regulator of apoptosis and cell proliferation

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ABSTRACT

β-Glucans are polysaccharides generally obtained from the cell wall of bacteria, fungi, yeasts, and aleurone layer of cereals. β-Glucans are polymers, with β-1,3 glucose as core linear structure, but they differ in their main branch length, linkages and branching patterns, giving rise to high and low-molecular-weight β-glucans. They are well-known cell response modifiers with immune-modulating, nutraceutical and health beneficial effects, including anticancer and pro-apoptotic properties. β-Glucan extracts have shown positive responses in controlling tumor cell proliferation and activation of the immune system. The immunomodulatory action of β-glucans enhances the host's antitumor defense against cancer. In consonance with the above, many studies have shown that β-glucan treatment leads to the induction of apoptotic death of cancer cells. The ability of β-glucans to stimulate apoptotic pathways or the proteins involved in apoptosis prompting a new domain in cancer therapy. β-glucan can be a potential therapeutic agent for the treatment of cancer. However, there is a need to legitimize the β-glucan type, as most of the studies include β-glucan from different sources having different physicochemical properties. The body of literature presented here focuses on the effects of β-glucan on immunomodulation, proliferation, cell death and the possible mechanisms and pathways involved in these processes.

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1. Introduction

β-Glucans are β-1,3 D-glucose polymers naturally occurring in cereals [1], bacteria [2], yeast [3] and fungi [4] with significantly different physicochemical properties depending on the source. They are heterogeneous non-starch polysaccharides, present in the cell wall of some microorganisms, particularly fungi. The sources from where β-glucans have been extracted include yeasts, algae, protists, fungi and cereals. Other sources include seaweeds [5] and various medicinal mushrooms like *Shiitake*, *Lingzhi*, *Chaga*, *Maitake* having potential immunomodulatory and antitumor effects [6,7]. Glucan is mainly composed of linear chain of β-1, 3 D-glucose units and vary with respect to side branching, solubility, viscosity, gelation properties, glycosidic linkages and molecular weight, for example, Lentinan (triple helix, β-1, 6 branched, β-1, 3 glucan, 400–800 kDa), Schizophyllan (reversible coiled helix or random coil, β-1, 6 branched, β-1, 3 glucan, 10^2–10^4 kDa), Maitake D fraction (triple helix, 800 kDa), cereal β-glucan (mixed linkage β-1, 3, β-1, 4 glucan, 130–410 kDa MW etc.) [7–9]. β-Glucan with β-1, 3 D-glucose units as a core linear structure is the most common form. Yeast and fungal β-glucan contain 1, 6 side branches, while as cereal β-glucan contains 1, 4 side branches. β-Glucan exists in both soluble and insoluble forms. Commercially available β-glucan is derived from yeast and is generally insoluble and structurally it is β-1, 3 D-glucose

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Synthesis, surface activity, self-aggregation and cytotoxicity of ruthenium(II) and Oxovanadium(IV) based metallo-surfactants

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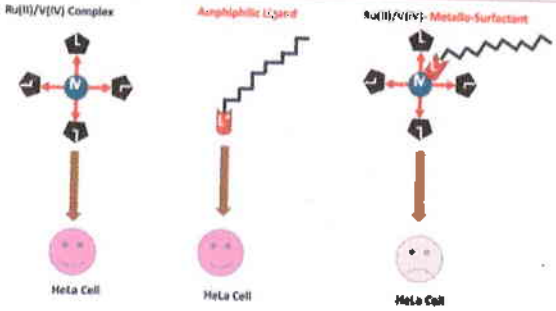
Abstract

Synthesis, surface activity, self-aggregation and cytotoxicity studies of a series of amphiphilic Ruthenium (II) and Oxovanadium (IV) metal complexes are presented. A series of Ruthenium (II) and Oxovanadium (IV) based metallo-surfactants was synthesized and characterized using UV–Visible, ^{1}H NMR and infrared spectroscopy. The synthesized metallo-surfactants were analysed for their surface activity and self-aggregation properties through tensiometric and conductometric investigations. The surface activity as well as the self-aggregation tendency of the as synthesized amphiphilic Ru (II) and Oxovanadium (IV) based amphiphilic metal complexes was observed to be very sensitive to their overall geometry, hydrophobicity and counter ion polarizability. The metallo-amphiphiles were tested for their potential role in cell growth inhibition. MTT assay results establish that the amphiphilic Ruthenium and Vanadium complexes significantly inhibit the proliferation of human cervix carcinoma, HeLa cell line. The amphiphilic phendione complexes of both Ruthenium (II) and Oxovanadium (IV) exhibited an excellent cytotoxicity with an IC50 value of 57 μM.

Graphical abstract

Transforming Ru(II) and Oxovanadium (IV) based metal complexes into metallo-surfactants via insertion of octadecylpyridine-2-yl methyl amine (L_{py}^{A}) into their ligand sphere significantly enhances their cytotoxicity toward human cervix carcinoma.



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Introduction

The micro-environment specific redox activity, variable coordination ability and reactivity exhibited by the transition metal ions and their complexes are very crucial for various biochemical and biophysical processes [1]. This uniqueness of these metal ion/metal complexes has been attracting a considerable attention of researchers since last many decades. The main focus of the so far published research works in this regard has been to explore and understand the role of metal ions/complexes in biochemical processes and the exploitation of their synthetic analogues as catalytic substitutes for various biochemical/chemical transformations [2]. Of late, the

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Jasplakinolide Attenuates Cell Migration by Impeding Alpha-1-syntrophin Protein Phosphorylation in Breast Cancer Cells

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Abstract

Background Alpha-1-syntrophin (SNTA1) is emerging as a novel modulator of the actin cytoskeleton. SNTA1 binds to F-actin and regulates intracellular localization and activity of various actin organizing signaling molecules. Aberration in syntrophin signaling has been closely linked with deregulated growth connected to tumor development/metastasis and its abnormal over expression has been observed in breast cancer. In the present work the effect of jasplakinolide, an actin-binding cyclodepsipeptide, on the SNTA1 protein activity and SNTA1 mediated downstream cellular events was studied in MDA-MB-231 breast cancer cell line.

Methods SNTA1 protein levels and phosphorylation status were determined in MDA-MB-231 cells post jasplakinolide exposure using western blotting and immunoprecipitation techniques respectively. MDA-MB-231 cells were transfected with WT SNTA1 and DM SNTA1 ($Y^{215/229}$ phospho mutant) and simultaneously treated with jasplakinolide. The effect of jasplakinolide and SNTA1 protein on cell migration was determined using the boyden chamber assay.

Results Jasplakinolide treatment decreases proliferation of MDA-MB-231 cells in both dose and time dependent manner. Results suggest that subtoxic doses of jasplakinolide induce morphological changes in MDA-MB-231 cells from flat spindle shape adherent cells to round weakly adherent forms. Mechanistically, jasplakinolide treatment was found to decrease SNTA1 protein levels and its tyrosine phosphorylation status. Moreover, migratory potential of jasplakinolide treated cells was significantly inhibited in comparison to control cells.

Conclusion Our results demonstrate that jasplakinolide inhibits cell migration by impairing SNTA1 functioning in breast cancer cells

Keywords Jasplakinolide · Breast cancer · Actin polymerization · Alpha-1-syntrophin · Migration

1 Introduction

Cancer cells are known to adopt various strategies to evade the normal regulatory checkpoints and progress into an advanced aggressive states; this may involve an increase in cell motility. There are various mechanisms that could lead to increased cell motility in breast cancer cells and one such mechanism is cytoskeleton remodelling. As a result, proteins involved in cytoskeleton remodelling are crucial for breast cancer metastasis [1, 2]. In recent years, research regarding the role of cytoskeleton remodelling proteins in breast cancer cell metastasis, SNTA1 an adaptor protein has emerged as a novel modulator of the actin cytoskeleton in response to various stimuli [3–6]. SNTA1 is a 59 KDa multi-domain dystrophin-associated scaffolding membrane protein that coordinates the assembly of signaling molecules into various intracellular signaling events [7, 8]. SNTA1 has not

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A simple, economical and environmental-friendly method for staining protein gels using an extract from walnut-husk

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ABSTRACT

We wish to present a simple, rapid, cost-effective and environmentally safe method for staining proteins in polyacrylamide gels, using aqueous-based natural extracts from fresh green walnut (*Juglans regia*) hulls/husks. The technique takes not more than 10 min for staining and is comparable in sensitivity to the most commonly used Coomassie R-250 staining method when applied to different concentrations of Bovine Serum Albumin (BSA) and various amounts of *E. coli* extracts. The protein (BSA) band (~0.5 μg) and *E. coli* extract comprising ~25 μg total protein can be visualized on polyacrylamide gels. Compared to both Coomassie and Ponceau S staining, the current method displayed more intense bands when proteins are transferred to polyvinylidene fluoride (PVDF) membrane. Although the walnut-dye (WD) method does not require a time-consuming destaining step, excess background stain can simply be removed by washing in water. Extract from old dried black husks and extract from fresh green husks kept for a year was also effective. Using LC-MS, Myricetin and/or Kaempferol were found to be active compounds responsible for staining proteins. Compared to traditional Coomassie method, the inclusion of expensive and toxic solvents (methanol and acetic acid) is completely avoided resulting in positive health, environmental and economic benefits. In view of all these advantages, the WD method has immense potential to replace currently used protein staining techniques.

1. Introduction

The top three all-time cited papers deals with the methods involving proteins that include detection and quantification (Lowry and Bradford methods) and separation by polyacrylamide gel electrophoresis, PAGE [1]. Consequently, the visualization of proteins on PAGE gels is one of the most familiar and widely used techniques in the field of life sciences that demands a cheap, rapid, non-toxic, non-pungent and environmentally safe staining method [2].

Presently, Coomassie Brilliant Blue R-250 in an aqueous mixture of methanol and acetic acid is routinely used to visualize protein bands on gels and membranes followed by destaining in the same solution lacking the dye [3,4]. The method can detect ~0.1 μg of protein per band is being lengthy and involves toxic, irritating and pungent solvents such as methanol and acetic acid. Apart from being expensive, methanol is very harmful by all routes of exposure-ingestion, inhalation, and skin absorption. Acetic acid is an irritant and contact by liquid or vapours can cause burns and damage to the eyes and respiratory tract [5]. Although the silver staining method is more sensitive (detection limit ~ 1 ng), it suffers from being tedious, and is not compatible with mass-spectrometry due to protein cross-linking in the presence of toxic formaldehyde [4]. Other methods include *in situ* protein staining by crystal violet that is messy and requires destaining [6].

The whole process is environmentally safe as the natural product (dye) is obtained directly in water from the green husk of walnuts and does not require any harsh and toxic chemicals at any step from

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Advances in pullulan production from agro-based wastes by *Aureobasidium pullulans* and its applications

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ABSTRACT

Pullulan is an important exopolysaccharide commercially obtained from ubiquitous fungi *Aureobasidium pullulans*. Pullulan has unique physicochemical properties, due to which it has wide applications in the food, pharmaceutical and biomedical industries. Various synthetic media and agro-industrial wastes have been utilized for the production of pullulan. Agro-industrial waste has become a preferred substrate for biotransformation due to its economical aspect and nutritional attributes. Pullulan is one of the valuable products produced by the biotransformation of agro-based wastes. Numerous factors which influence the pullulan production are the type of substrate, the addition of carbon source, nitrogen source, pH of the medium, aeration/ agitation speed and temperature. The cost, productivity of the product and efficiency depends on the selection of raw material, type of fermentation (bioprocess), type of strain and downstream processing (recovery). This review focuses on pullulan production from agro-based wastes, the influence of factors and its applications.

1. Industrial applications

Pullulan has unique physicochemical properties, due to which it has various industrial applications. Pullulan has wide applications in the food, pharmaceutical and biomedical industries. Pullulan is used in baked foods, confectioneries and beverages as stabilizer, intensifier, adhesive and low viscosity filler. Pullulan is used in cosmetic products as a moisturizing, stabilizing and coating agent. Considering the film-forming ability of pullulan, it has a promising future in food packaging and targeted delivery of biomolecules, vaccines and drugs. Pullulan blended films/coatings have improved the shelf life and quality of fruits and other food products. Pullulan being water-soluble and biocompatible has vast applications in drug delivery, tissue engineering, wound healing and blood plasma substitute. Pullulan has ability to form hydrogels, it is conjugated with nanogels to act as molecular chaperons. Electrospinning nanofibers have paved the way for new unexplored applications of pullulan in nanotechnology.

2. Introduction

Pullulan is an exopolysaccharide commercially produced by *Aureobasidium pullulans*. Exopolysaccharides are produced by microorganisms as an extracellular or surface material in the form of a slime layer (Sutherland, 1998). They protect the cell from biotic and abiotic stress (Barcelos, Vespermann, Pelissari, & Molina, 2020). Pullulan is a linear glucan composed of repeating maltotriose units linked by α-1,6 linkage and maltotriose units are composed of α-1,4 linked glucose units. However, maltotetraose units may also occur occasionally (Catley, Ramsay, & Servis, 1986; Catley & Whelan, 1971). Pullulan production was first reported by (Bauer, 1938) during fermentation by *Aureobasidium pullulans* and its structure was elaborated by (Bender, Lehmann, & Wallenfels, 1959) Characterization of the pullulan recovered from culture broths of *A. pullulans* was first done in 1958 (Bernier, 1958).

A. pullulans is a ubiquitous microbe and widespread oligotroph found on rocks, wooden surfaces, moist temperate regions and has been isolated mainly from the leaves of different plants (Peterson, Manitchotpisit, & Leathers, 2013; Prasongsuk, Lotrakul, Ali, Bankeeree, & Punnapayak, 2018). *Aureobasidium pullulans* a saprotrophic fungus grows on the dead and decaying matter in a temperate phylloplane and results in the blackening of surfaces due to melanin production. *A. pullulans* have also been isolated from extreme conditions such as glacial and subglacial ice (Zalar et al., 2008) and hypersaline waters (Gunde-Cimerman, Zalar, De Hoog, & Plemenitaš, 2000).

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RESEARCH ARTICLE

WILEY

Delineating binding potential, stability of Sulforaphane-N-acetyl-cysteine in the active site of histone deacetylase 2 and testing its cytotoxicity against distinct cancer lines through stringent molecular dynamics, DFT and cell-based assays

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Abstract

Histone deacetylase 2 (HDAC2), an isozyme of Class I HDACs has potent imputations in actuating neurodegenerative signaling. Currently, there are sizeable therapeutic disquiets with the use of synthetic histone deacetylase inhibitors in disease management. This strongly suggests the unfulfilled medical necessity of plant substitutes for therapeutic intervention. Sulforaphane-N-acetyl-cysteine (SFN-N-acetylcysteine or SFN-NAC), a sulforaphane metabolite has shown significantly worthier activity against HDACs under *in vitro* conditions. However, the atomistic studies of SFN-NAC against HDAC2 are currently lacking. Thus, the present study employed a hybrid strategy including extra-precision (XP) grid-based flexible molecular docking, molecular mechanics generalized born surface area (MM-GBSA), e-Pharmacophores method, and molecular dynamics simulation for exploring the binding strength, mode of interaction, e-Pharmacophoric features, and stability of SFN-NAC towards HDAC2. Further, the globally acknowledged density functional theory (DFT) study was performed on SFN-NAC and entinostat individually in complex state with HDAC2. Apart from this, these inhibitors were tested against three distinct cancer cell models and one transformed cell line for cytotoxic activity. Moreover, double mutant of HDAC2 was generated and the binding orientation and interaction of SFN-NAC was scrutinized in this state. On the whole, this study unbosomed and explained the comparatively higher binding affinity of entinostat for HDAC2 and its wide spectrum cytotoxicity than SFN-NAC.

KEYWORDS

DFT, E-Pharmacophores method, HDAC2, MM-GBSA, molecular docking, molecular dynamics, MTT assay, mutation, neurodegenerative disorders, SFN-NAC, Sulforaphane

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RESEARCH ARTICLE

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Evaluation of 17 genetic variants in association with leukemia in the north Indian population using MassARRAY Sequenom

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Abstract

Leukemia is a heterogeneous disorder, characterized by elevated proliferation of white blood cells. In this study, we explored the association of 17 genetic variants with leukemia patients in the Jammu and Kashmir region of north India. The variants were genotyped by using a high-throughput Agena MassARRAY platform in 758 individuals (166 cases and 592 controls). Of the 17 single-nucleotide polymorphisms (SNPs) studied, five SNPs were showing significant association with the high risk of leukemia in the north Indian population, which includes rs10069690 of telomere reverse transcriptase (*TERT*) with OR = 0.34 (95% CI, 0.20–0.58; $p = .0008$), rs2972392 (*PSCA*) with OR 1.86 (95% CI, 1.04–3.81; $p = .035$), rs4986764 (*BRIP1*) with OR 1.34 (95% CI, 1.00–1.80; $p = .04$), rs6990097 (*TNKS*) with OR 1.81 (95% CI, 1.2–2.6; $p = .001$) and rs12190287 (*TCF21*) with OR 2.87 (95% CI, 1.72–4.7; $p = .0001$) by allelic association using Plink and analyzed by SPSS. This is the first study to explore these variants with leukemia in the studied population.

KEYWORDS

Jammu and Kashmir, leukemia, multifactor dimensionality reduction, north India, single-nucleotide polymorphism

1 | INTRODUCTION

Leukemia is the heterogeneous group of hematopoietic disorders that multiply and gather in the bone marrow, which results in the uncontrolled proliferation of white blood cells[1–3] Globally leukemia is ranked eighth for being the most frequently detected cancer with 60 300 new cases and 24 370 deaths in 2020[1,3] In India, leukemia ranked 7th for being the most frequently identified cancers with 42 055 new cases and 32 471 deaths in 2019[1,3] According to the population-based cancer registries in India, males are typically affected more as compared to females with a ratio of 1.2[1,4] Among north Indian populations, the people of Jammu and Kashmir are found to be at greater risk and have a higher mortality rate associated with different cancers[1,5] The incidence of leukemia has rapidly shot up in males (2.62%) as well as in females (2.45%) from the Jammu and Kashmir region of north India[1,5] About 65% population of Jammu and Kashmir practice endogamy, thus preserving the gene pools resulting in higher rate of homozygosity. This influence has been predictable as an inherited genetic factor related to the etiology of leukemia[1,6] Leukemia is multifactorial in origin, which can be caused by both genetic as well as nongenetic factors[1,7] Some molecular modifications moreover proto-oncogenes or tumor suppressor genes can be one of the risk factors responsible for the development of leukemia. Several genetic distinctions, including genetic polymorphism resulting in mutation in proto-oncogenes as well as inactivating tumor suppressor

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ORIGINAL ARTICLE

Genetic analysis of colorectal carcinoma using high throughput single nucleotide polymorphism genotyping technique within the population of Jammu and Kashmir

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Abstract

Background SNP genotyping has become increasingly more common place to understand the genetic basis of complex diseases like cancer. SNP-genotyping through MassARRAY™ is a cost-effective method to quantitatively analyse the variation of gene expression in multiple samples, making it a potential tool to identify the underlying causes of colorectal carcinogenesis.

Methods In the present study, SNP genotyping was carried out using Agena MassARRAY™, which is a cost-effective, robust, and sensitive method to analyse multiple SNPs simultaneously. We analysed 7 genes in 492 samples (100 cases and 392 controls) associated with CRC within the population of Jammu and Kashmir. These SNPs were selected based on their association with multiple cancers in literature.

Results This is the first study to explore these SNPs with colorectal cancer within the J&K population. 7 SNPs with a call rate of 90% were selected for the study. Out of these, five SNPs rs2234593, rs1799966, rs2229080, rs8034191, rs1042522 were found to be significantly associated with the current study under the allelic model with an Odds Ratio OR = 2.981(1.731–5.136 at 95% CI); p value = 4.81E-05 for rs2234593,OR = 1.685(1.073–2.647 at 95% CI);; p value = 0.02292 for rs1799966, OR = 1.5 (1.1–2.3 at 95% CI), p value = 0.02 for rs2229080, OR = 1.699(1.035–2.791 at 95% CI); p value = 0.03521 for rs8034191, OR = 20.07 (11.26–35.75); p value = 1.84E-34 for rs1042522 respectively.

Conclusion This is the first study to find the relation of Genetic variants with the colorectal cancer within the studied population using high throughput MassARRAY™ technology. It is further anticipated that the variants should be evaluated in other population groups that may aid in understanding the genetic complexity and bridge the missing heritability.

Keywords Colorectal cancer · Single nucleotide polymorphism (SNPs) · MassARRAY™ · Jammu and Kashmir

Introduction

Colorectal cancer (CRC) is the third most common type of cancer worldwide, resulting in 1–2 million new cases each year (1). In 2020, it was estimated that nearly 10% of all cancer incidences were reported to be of CRC [1]. The incidence of CRC has been associated with obesity, red meat consumption, and physical inactivity [2, 3]. In addition, the genetic factors and epigenetic changes also play a key role in the initiation and progression of CRC [3, 4]. Delay in the diagnosis of CRC is a major hurdle in the management of CRC, which is evident by the rise in new cases each year. Therefore, it is critical to identify markers that may help in the early prognosis and development of therapeutic interventions accordingly

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JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS

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Dep domain containing mTOR interacting protein (DEPTOR) has critical implications in the development and progression of human malignancies. Increased expression of DEPTOR promotes the growth of tumor cells by inhibiting the mTORC1, which alleviates the negative feedback inhibition by mTORC1 downstream target S6Ks on PI3K/AKT pathway thereby promotes cell survival and prevents apoptosis. This clearly suggests that targetting DEPTOR-mTOR interactions through small molecules may prove as an effective strategy for circumventing distinct cancers. In this study, we employed a top-down approach for finding three novel molecules which may prove effective in disrupting Deptor-mTOR interaction. Following DEPTOR modelling and validation we performed grid-directed structure based by specifying the residues of DEPTOR known to interact with mTOR. A library of 10,000 protein-protein disrupting molecules was screened against the defined region of DEPTOR. From the screened molecules, 30 molecules with highest binding affinity were chosen for molecular docking. Thirty (30) extra precision molecular docking experiments and 30 molecular mechanics generalized born surface area (MMGBSA) assays were performed. Following this top 10 molecules in terms of binding affinity were selected and the interaction profile of their corresponding docked files was generated. The top three molecules were finally selected after taking all the three parameters including docking score, binding energy value and interaction profile into consideration. For atomistic insights regarding DEPTOR-topmost hit interactions, molecular dynamics was performed for 100 ns. This molecule after further evaluation may prove as promising candidate for anticancer therapy.

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DEPTOR; mTOR; cancer; structure-based virtual screening; molecular docking; MM-GBSA; molecular dynamics

1. Introduction

The mechanistic target of rapamycin (mTOR), which forms two distinct complexes mTORC1 and mTORC2 is an evolutionary conserved kinase that belongs to the Phosphoinositide 3 kinase (PI3K) related kinase family (Caron et al., 2018). mTOR functions as a central regulator molecule that integrates signals from diverse sources to regulate metabolism, growth, proliferation, survival, autophagy, cytoskeletal organization and mobility (Wang et al., 2012). Hence, deregulation of the mTOR pathway has been linked to the pathogenesis of various diseases including cancer, diabetes, obesity and Alzheimer's disease (Li et al., 2014). Abnormal activation of the mTOR pathway is mainly due to mutations of its upstream pathways including the PI3K/AKT and RAS/RAF/MEK/ERK which plays a pivotal role in the progression and metastasis of various cancers (Saxton & Sabatini, 2017). Therefore, targeting mTOR pathway through mTOR inhibitors may be a viable therapeutic strategy to limit the growth and progression of various diseases including cancer.

DEPTOR an mTOR binding partner has been identified as an endogenous inhibitor of mTOR that inhibits the activities of both mTORC1 and mTORC2 (Peterson et al., 2009). Normally, DEPTOR acts as a tumor suppressor by inhibiting mTOR which is hyper activated in majority of human cancers. Indeed, DEPTOR has been found to be downregulated in majority of cancers (Peterson et al., 2009). However, under certain circumstances, interestingly, DEPTOR could act as an oncogene (Zhao et al., 2011). The oncogenic properties of DEPTOR has been mostly attributed to its feedback activation of mTOR PI3K/AKT pathway in various cancers including multiple myeloma, cervical squamous cell carcinoma,

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BMC Research Notes

RESEARCH NOTE Open Access

Isolation of high-quality RNA for high throughput applications from secondary metabolite-rich *Crocus sativus* L.

Umer Majeed Wani†, Zubair Ahmad Wani†, Aabid M. Koul, Asif Amin, Basit Amin Shah, Faizah Farooq and Rares A. Qadri*

Abstract

Objective: Isolating high quality RNA is a basic requirement while performing high throughput sequencing, microarrays and various other molecular investigations. However, it has been quite challenging to isolate RNA with adequate purity from plants like *Crocus sativus* that are rich in secondary metabolites, polysaccharides, and other interfering compounds which often irreversibly co-precipitate with the RNA. While many methods have been proposed for RNA extraction including CTAB, Tr Zol, and SDS based methods, which invariably yield less and poor quality RNA and hence necessitated the isolation of high quality RNA suitable for high throughput applications.

Results: In the present study we made certain adjustments to the available protocols including modifications in the extraction buffer itself and the procedure employed. Our method led to the isolation of clear and intact pure total RNA with an RNA Integrity Number (RIN) value greater than 7.5. The quality of the RNA was further assessed by qPCR based amplification of mRNA and mature miRNAs such as Cs-MIR166c and Cs-MIR396a

Keywords: Crocus sativus, RNA integrity number, High throughput sequencing, microRNA, Secondary metabolites

Introduction

Crocus sativus is a perennial geophyte with crimson trifid stigmas. The stigmas contain apocarotenoids such crocin, picrocrocin, and safranal, which give saffron its distinctive color, taste, and perfume [1]. It is one of the world's most expensive spices due to its unique organoleptic properties and difficulty in cultivation, processing, and harvesting [2, 3]. Moreover, there is compelling evidence that supports the therapeutic potential of saffron [4], and due to the growing demand, it becomes important to devise a strategy to improve the quality and quantity of saffron [5].

The current scientific evidence supports the significance of microRNAs in regulating essential plant developmental processes as leaf morphogenesis, polarity [6, 7], floral differentiation, and development [8, 9]. Moreover, the microRNAs identified hitherto, have 50% targets as transcription factors out of which many miRNAs target mRNAs encoding transcription factors that regulate development [10, 11].

A stepping stone in the direction of molecular analysis involves the high-quality RNA [12] to synthesize cDNA for performing quantitative PCR, and various other experiments that rely on cDNA as a starting material. Moreover, isolating high-quality RNA becomes important while carrying out post-transcriptional studies involving microRNAs, as the preparation of a microRNA library entails meeting a specific RIN value for the RNA. To isolate RNA from different tissues of *Crocus sativus*, it is important to ensure that there is no interference of

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RESEARCH ARTICLE

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Lung cancer cell-derived EDA-containing fibronectin induces an inflammatory response from monocytes and promotes metastatic tumor microenvironment

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Abstract

Tumor-associated macrophages (TAMs) play a pivotal role in facilitating tumor growth and metastasis. This tumor-promoting propensity of TAMs sets in as a result of their complex cross-talk with tumor cells mediated primarily by tumor cell-secreted proteins in the tumor microenvironment. To explore such interactions, we employed an immunoscreening approach involving the immunization of Balb-c mice with model human lung carcinoma cell line, A549. From serological examination combined with mass spectrometric analysis, EDA-containing fibronectin (EDA_{FN}) was identified as a conspicuous immunogenic protein in A549 cell secretome. We showed that A549 secreted EDA_{FN} engages TLR-4 on THP-1 monocytes to drive the proinflammatory response via NF-κB signaling cascade. Conversely, A549 derived EDA_{FN} potentiates their metastatic capacity by inducing epithelial-mesenchymal transition through its autocrine activity. In conclusion, the study proposes a possible mechanism of cellular cross-talk between lung cancer cells and associated monocytes mediated by lung cancer-derived EDA_{FN} and resulting in the establishment of proinflammatory and metastatic tumor microenvironment.

KEYWORDS

fibronectin, inflammation, metastasis, secretome, tumor microenvironment

1 | INTRODUCTION

Cancer cell-secreted proteins function as communicating signals in the tumor microenvironment to mediate the heterotypic interactions among tumor and other recruited cell populations which contributes to acquisition and maintenance of cellular competence necessary for tumor growth and metastasis. Immune cells are considered pivotal to such intricate cellular interactions and engage in a comprehensive interplay with tumor cells.

Infiltration by immune cells is one of the typical hallmarks of most solid tumors. A variety of leukocytes, most populously monocytes infiltrate tumor stroma and tend to differentiate into macrophages often referred to as tumor-associated macrophages or TAMs. TAMs play a key role in mediating functional interplay between a solid tumor and immune system, maintaining supportive milieu to the former by facilitating the recruitment of other inflammatory leukocytes via secretion of proinflammatory cytokines, chemokines, and growth

pathogens

MDPI

Review

Machine Learning Techniques for the Prediction of B-Cell and T-Cell Epitopes as Potential Vaccine Targets with a Specific Focus on SARS-CoV-2 Pathogen: A Review

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Abstract: The only part of an antigen (a protein molecule found on the surface of a pathogen) that is composed of epitopes specific to T and B cells is recognized by the human immune system (HIS). Identification of epitopes is considered critical for designing an epitope-based peptide vaccine (EBPV). Although there are a number of vaccine types, EBPVs have received less attention thus far. It is important to mention that EBPVs have a great deal of untapped potential for boosting vaccination safety—they are less expensive and take a short time to produce. Thus, in order to quickly contain global pandemics such as the ongoing outbreak of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), as well as epidemics and endemics, EBPVs are considered promising vaccine types. The high mutation rate of SARS-CoV-2 has posed a great challenge to public health worldwide because either the composition of existing vaccines has to be changed or a new vaccine has to be developed to protect against its different variants. In such scenarios, time being the critical factor, EBPVs can be a promising alternative. To design an effective and viable EBPV against different strains of a pathogen, it is important to identify the putative T- and B-cell epitopes. Using the wet-lab experimental approach to identify these epitopes is time-consuming and costly because the experimental screening of a vast number of potential epitope candidates is required. Fortunately, various available machine learning (ML)-based prediction methods have reduced the burden related to the epitope mapping process by decreasing the potential epitope candidate list for experimental trials. Moreover, these methods are also cost-effective, scalable, and fast. This paper presents a systematic review of various state-of-the-art and relevant ML-based methods and tools for predicting T- and B-cell epitopes. Special emphasis is placed on highlighting and analyzing various models for predicting epitopes of SARS-CoV-2, the causative agent of COVID-19. Based on the various methods and tools discussed, future research directions for epitope prediction are presented.

Keywords: machine learning; antigenic determinant; antigen; antibody; immune-relevant determinants; epitope-based peptide vaccine; SARS-CoV-2; COVID-19; epitopes; ensemble model

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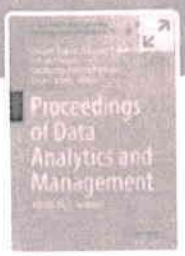
1. Introduction

An antigenic determinant (AD) is a portion of an antigen molecule known as an epitope that is recognized by the human immune system, specifically by antibodies or T and B cells [1]. Recognition of epitopes is considered important in EBPV design to contain pandemics, epidemics, and endemics due to the outbreak of infectious diseases. The ongoing COVID-19 pandemic due to the SARS-CoV-2 outbreak is the latest among the



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A Novel Ensemble Machine Learning Model for Prediction of *Zika Virus* T-Cell Epitopes

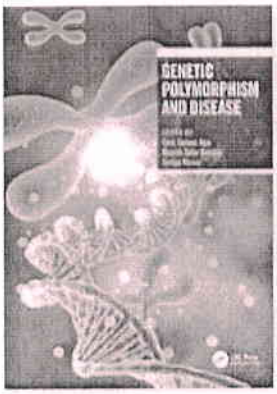
Syed Nisar Hussain Bukhari, Amit Jain & Ehtishamul Haq

Conference paper | First Online: 22 November 2021

Part of the Lecture Notes on Data Engineering and Communications Technologies book series (LNDECT,volume 91)

Abstract

Zika virus belongs to the genus Flavivirus and causes Zika fever in humans. World Health Organization (WHO) declared its outbreak as Public Health Emergency of International Concern in 2016. Currently, there is no approved vaccine for clinical use to combat the *Zika Virus* infection and its epidemic. The *in-silico* approach to T-cell epitope prediction of Zika virus is useful to save biologist's time and efforts for vaccine development. The authors have proposed a novel ensemble machine learning model to predict Zika virus T–cell epitopes using physicochemical properties of amino acids. The model has been designed by fusing the top two performing classifiers from among the six machine learning classifiers (base classifiers). The peptide sequences consisting of experimentally determined T-cell epitopes and non-epitopes of Zika virus were collected from Immune Epitope Database and Analysis



Chapter

Genetic Polymorphisms in Matrix Metalloproteinase (MMP) Genes and Cancer

By Mujeeb Zafar Banday, Saniya Nissar, Syed Sameer Aga, Ehtishamul Haq, Sobhiya Majid

Book Genetic Polymorphism and Disease

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ABSTRACT

Matrix metalloproteinases (MMPs) constitute an important family of endopeptidases in humans and other primates. MMPs together process or cleave almost all the components of the basement membrane (BM) and the extracellular matrix (ECM) and various non-matrix bioactive substrates. Most of the MMP-processed ECM components and soluble protein mediators play crucial roles in numerous cellular signaling pathways and, therefore, regulate a multitude of physiological processes that depend on these cellular signaling pathways. Consequently, dysfunction or dysregulation of MMPs promote various pathophysiological conditions that often manifest as varied diseases or disorders including cancers of almost all the types. MMPs, predominantly through the proteolysis of diverse substrates, play a critical role in almost all the important pro-tumorigenic processes including tumor growth and progression, apoptosis, angiogenesis, activation and promotion of tissue invasion and metastasis and escape of tumor cells from different immunesurveillance mechanisms. Various single nucleotide polymorphisms (SNPs) in MMP genes have the potential to modulate gene transcription, 140expression and subsequently serum levels and enzyme activity of MMPs in an allele-specific manner. The allele-specific differences that result in increased or decreased physiological levels and functions of MMPs may possibly modulate the risk of various cancers. In this chapter, we briefly discuss the

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Cell Communication and Signaling

REVIEW Open Access

Scavenger receptors in host defense: from functional aspects to mode of action

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Abstract

Scavenger receptors belong to a superfamily of proteins that are structurally heterogeneous and encompass the miscellaneous group of transmembrane proteins and soluble secretory extracellular domain. They are functionally diverse as they are involved in various disorders and biological pathways and their major function in innate immunity and homeostasis. Numerous scavenger receptors have been discovered so far and are apportioned in various classes (A-L). Scavenger receptors are documented as pattern recognition receptors and known to act in coordination with other co-receptors such as Toll-like receptors in generating the immune responses against a repertoire of ligands such as microbial pathogens, non-self, intracellular and modified self-molecules through various diverse mechanisms like adhesion, endocytosis and phagocytosis etc. Unlike, most of the scavenger receptors discussed below have both membrane and soluble forms that participate in scavenging; the role of a potential scavenging receptor Angiotensin-Converting Enzyme-2 has also been discussed whereby only its soluble form might participate in preventing the pathogen entry and replication, unlike its membrane-bound form. This review majorly gives an insight on the functional aspect of scavenger receptors in host defence and describes their mode of action extensively in various immune pathways involved with each receptor type.

Keywords: Scavenger receptors, Immunity, PAMPs, Signalling pathways, ACE-2

Background

Scavenger receptors (SRs) were shown for the first time on macrophages to function in endocytosis and degradation of modified (acetylated) low-density lipoproteins (LDLs) [1]. SRs are a structurally heterogeneous superfamily of proteins that belong to different classes with very little or no structural resemblance. The only characteristic that designates various classes is their competence to bind mutual ligands. SRs show interactions with modified self-molecules, damage-associated molecular patterns (DAMPs), non-self molecules like preserved pathogen-associated molecular patterns (PAMPs) on microbial pathogens (lipopolysaccharide (LPS) and lipoteichoic acid (LTA)). They also recognize unmodified endogenous proteins, lipoproteins, apoptotic cells and polyionic ligands such as carbohydrates, proteoglycans, cholesterol ester and phospholipids etc. Host cells are effective guardians of the immune response through the expression of complex surveillance systems, including the Pattern Recognition Receptors (PRRs) [2]. Scavenger receptors are membrane-associated pattern recognition receptors (PRRs) [3–5] that act as phagocytic receptors mediating direct non-opsonic uptake of pathogenic microbes and/or their products. SRs may partner with other PRRs like TLRs (Toll-like receptors) or multimolecular complexes on various cell types and participate in diverse functions like signalling other than scavenging. Recognition of pathogens by SRs

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Materials Advances

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Prospectus of advanced nanomaterials for antiviral properties

Tabinda Showkat Patoo,[a] Firdous Khanday*[a] and Ahsanulhaq Qurashi *[b]

Viral hazards have suddenly increased in the form of the century's biggest pandemic through COVID-19. However, viruses are also associated with other human diseases, the severity of which range from the mild common cold to deleterious cancers and HIV. Conventional anti-viral therapies that have been developed to mitigate deleterious viral effects have not stood the test of time owing to their numerous limitations. This has burdened the research community worldwide with the challenging task of discovering advanced anti-viral strategies to overcome the limitations being faced. In this regard, fortunately, metal and inorganic nanoparticles offer respite as they exhibit tremendous anti-viral potential and are considered a powerful weapon against viral intrusions. Metal nanoparticles of various metals such as silver, gold, and copper have not only successfully attenuated the infectivity of malignant viruses (HIV, HSV, HINI, etc.) in *in vitro* conditions and *in vivo* conditions (mainly silver and zinc oxide nanoparticles) but have also successfully overcome the limitations faced by conventional anti-viral therapies. Acting in a resistance insensitive, age and co-morbidity independent and low cytotoxic manner, metal nanoparticles can successfully inhibit viral entry and other viral development processes. In the light of the mechanisms and advantages offered by metal nanoparticles, it is suggested to consider their usage in actual clinical practice rather than as an alternate therapy. Further, considering the mechanisms exhibited by metal nanoparticles to deprive the viral load, we anticipate that the current pandemic (COVID-19) can be treated to some extent *via* the aid of metal nanoparticles. The successful implication of the hypothesized mechanisms can offer abating strategies to combat the current pandemic and open new avenues to cope with future pandemics. In this prospective, we provide the frontiers and current scenario of various classes of nanoparticles being explored for antiviral activities.

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1. Introduction

Viruses are intracellular pathogens capable of capturing host cells to increase their number, thus converting them into factories for their mass production and re-infection.[1] The phases of viral infection include attachment to the host cell,[2,3] synthesis, assembly of mature virus particles,[4] and finally their release.[5,6] All these phases succeed through the orchestrated aid of viral proteins as well as the host-cell machinery.[1,4,7] To evade viral destruction and confer protection to living beings, researchers have been devising anti-viral strategies for a long time. The conventional anti-viral strategies developed thus far include anti-viral drugs (therapeutics), vaccination and the recent si-RNA-based anti-viral approach. However, due to disadvantages such as resistance, sensitivity,[8–10] narrow specificity,[11] low therapeutic index of anti-viral drugs,[12,13] vaccination failure due to host insensitivity,[14] genetic predisposition,[15] as well as age and co-morbidities,[16,17] the development of more promising anti-viral strategies is necessary. With regards to this, the use of metal and inorganic nanoparticles for advanced anti-viral approaches is promising. The use of nanoparticles in the field of virology is not a novel idea. Nanomaterials have already proved themselves to be efficient nano-sensors, tracking viruses with great efficacy and sensitivity on account of their phenomenal conductivity and photo-electrochemical properties, making them an ideal choice for diagnostic purposes.[18–20] Various novel approaches have been developed to investigate advanced nanomaterials for antiviral applications. Besides, nanoparticles, both organic and inorganic, owing to their characteristics properties such as quantum size effect, high surface-to-volume ratio,[21] tunable surfaces and easy functionalization with desired chemical moieties have already proven their value as excellent conventional anti-viral therapy carriers.[22] In addition, the biomimetic properties of metal and inorganic nanoparticles have enabled researchers to explore their intrinsic anti-viral activity.[23] Besides anti-viral activity, metal nanoparticles also possess intrinsic anti-fungal, anti-bacterial and other anti-microbial properties.[24,25] It is noteworthy to mention that

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Pro-oxidant vitamin C mechanistically exploits p66Shc/Rac1 GTPase pathway in inducing cytotoxicity

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ABSTRACT

P66Shc is the master regulator of oxidative stress whose pro-oxidant functioning is governed by ser36 phosphorylation. Phosphorylated p66Shc via Rac1 GTPase activation modulates ROS levels which in turn influence its pro-oxidative functions. Vitamin C at higher concentrations exhibits cytotoxic activity in various cancers, inducing ROS mediated cell death via pro-apoptotic mechanisms. Here we show a novel role of p66Shc in mediating pro-oxidant activity of vitamin C. Effect of vitamin C on the viability of breast cancer and normal cells was studied. High doses of vitamin C decreased viability of cancerous cells but not normal cells. Docking study displayed significant binding affinity of vitamin C with p66Shc PTB domain. Western blot results suggest that vitamin C not only enhances p66Shc expression but also induces its ser36 phosphorylation. Vitamin C at high doses was also found to activate Rac1, enhance ROS production and induce apoptosis. Interestingly, ser36 phosphorylation mutant transfection and pretreatment with antioxidant *N*-acetylcysteine results indicate that vitamin C induced Rac1 activation, ROS production and apoptosis is p66Shc ser36 phosphorylation dependent. Overall, results highlight that vitamin C mechanistically explores p66Shc/Rac1 pathway in inducing apoptosis and thus can pave a way to use this pathway as a potential therapeutic target in breast cancers.

1. Introduction

ShcA adaptor proteins are the family of three splice variant isoforms including p46shc, p52shc and p66Shc [1]. All the isoforms of ShcA consist of three similar domains Collagen homologue 1(CH1), phosphotyrosine binding domain (PTB) and Src homologue 2 (SH2) domain. P66Shc the longest isoform, contains an extra domain CH2, attributing to its structural and functional diversity [2]. The p52Shc isoform is usually involved in propagating mitogenic signals while as p46Shc besides transducing mitogenic signals is involved in inhibition of lipid oxidation [3,4]. The longest isoform p66Shc plays a dual role in cell growth propagating both mitogenic as well as apoptotic signals [5]. In response to growth factor stimuli ShcA proteins get tyrosine phosphorylated, resulting in activation of Ras/MAPK cell proliferation pathway, however, under oxidative stress conditions, p66Shc gets phosphorylated at serine36 residue that inhibits mitogenic signaling pathway and promotes oxidative stress responses [3,6]. Besides, p66Shc is a well-established regulator of life span and apoptosis [7]. Genetic deletion of p66Shc in mice showed significant resistance towards oxidative stress and apoptosis [8]. Under oxidative stress conditions, p66Shc was found to induce reactive oxygen species (ROS) mediated DNA damage and cell death in B-lymphocytes [9]. Moreover, elevated p66Shc expression and activation was linked to cell cytotoxicity of the central nervous system (CNS) [10]. We have also earlier shown the interplay between MKK6 and p66Shc leads to ROS induced cell death of neuronal cells in response to beta amyloid exposure [11]. The ability of p66Shc to induce apoptosis under oxidative stress is governed by the phosphorylation status of CH2 domain Ser36 amino acid residue [12]. Excessive oxidative stress in cells has been found to activate various kinases including JNK, PKC-β and MKK6 [11,13–15]. These kinases then phosphorylate Ser36 and trigger apoptotic responses, facilitated through different ways including, ROS scavenging enzymes production inhibition, the release of cytochrome C from mitochondrial membrane and activation of Rac1/ NADPH Oxidase pathway [16–18].

Rac1 the member of the Rho GTPase family has been well implicated in the regulation of ROS production in a variety of cells [19]. Being a member of NADPH oxidase, Rac1 in GTP bound form promotes assembly of NADPH Oxidases and triggers the production of ROS. Rac1 acts as the downstream target of p66Shc and is implicated in maintaining p66Shc stability and oxidative stress responses [17,20]. Moreover, p66Shc

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RESEARCH ARTICLE

CELL BIOCHEMISTRY & FUNCTION WILEY

Ala307Thr variation modulates FSHR structure and impairs its binding affinity for FSH: Implications in polycystic ovarian syndrome

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Abstract

Follicle-stimulating hormone receptor (FSHR) belongs to the family of G-protein coupled receptors and acts as a cognate receptor for follicle-stimulating hormone (FSH). Among the various polymorphic changes reported in FSHR, rs6165 polymorphism leading to Ala307Thr variation in the extracellular domain of the FSHR ($FSHR_{ECD}$) is widely reported. Therefore we attempted to evaluate the functional implications of this variation by studying its effects on $FSHR_{ECD}$ structure as well as FSH binding. Our atomic-scale investigations reveal that the hinge region, a key hormone interaction site in the extracellular domain of Wt FSHR, exhibits significantly more flexibility compared with the variant structure. Moreover, the Wt receptor in complex with FSH was observed to form a pocket-like structure in its hinge region whereas such a structure was not detected in the variant. The study further reveals that the key residue, sTyr335, required for FSH recognition and FSHR activation, exhibits lower binding free energy in the variant structure as compared to the Wt. In conclusion, our results point out that Ala307Thr variation leads to structural and conformational anomalies in $FSHR_{ECD}$ which may alter its FSH binding and affect its activation.

KEYWORDS
Ala307Thr genotype, FSHR, polycystic ovarian syndrome, premature ovarian failure

1 | INTRODUCTION

The follicle-stimulating hormone receptor (FSHR) is a member of the highly conserved subfamily of the G protein-coupled receptor (GPCR) superfamily. It is positioned on chromosome 2 in the p21–p16 region and comprises 10 exons with 9 intervening introns. The protein architecture includes a large NH_2-terminal extracellular domain, a transmembrane region and an intracellular domain. Most of the extracellular domain is encoded by the first 9 exons, whereas exon 10 encodes the C-terminal end of the extracellular domain, transmembrane domain and the intracellular domain.[1,2] FSHR engages its cognate ligand, follicle-stimulating hormone (FSH) through the extracellular domain which induces conformational changes in the transmembrane domain ultimately transducing hormone signals to the cell interior through the intracellular domain resulting in the follicular development and diverse ovarian functions.[3] The extracellular domain has been postulated to contain two functional subdomains including the hormone-binding domain responsible for high-affinity ligand recognition and the hinge or hinge domain required for signal specificity.[4,5] The latter contains a sulfated Tyr335 residue which represents a key residue responsible for hormone interaction.

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REVIEW ARTICLE

Cellular Physiology WILEY

Shielding and nurturing: Fibronectin as a modulator of cancer drug resistance

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Abstract

Resistance to chemotherapy and targeted therapies constitute a common hallmark of most cancers and represent a dominant factor fostering tumor relapse and metastasis. Fibronectin, an abundant extracellular matrix glycoprotein, has long been proposed to play an important role in the pathobiology of cancer. Recent research has unraveled the role of Fibronectin in the onset of chemoresistance against a variety of antineoplastic drugs including DNA-damaging agents, hormone receptor antagonists, tyrosine kinase inhibitors, microtubule destabilizing agents, etc. The current review summarizes the role played by Fibronectin in mediating drug resistance against diverse anticancer drugs. We have also discussed how the aberrant expression of Fibronectin drives the oncogenic signaling pathways ultimately leading to drug resistance through the inhibition of apoptosis, promotion of cancer cell growth and proliferation.

KEYWORDS

apoptosis, chemoresistance, DNA damaging drugs, extracellular matrix, fibronectin, tumor microenvironment

1 | INTRODUCTION

The extracellular matrix (ECM) is a complex and extensive network composed of proteoglycans, glycoproteins and fibrous proteins that maintains tissue architecture and dynamics (Karamanos et al., 2021). The molecules of ECM and the underlying cells are in continual reciprocal cross-talk initiating various biochemical and biomechanical cues that influence cellular phenotype and behavior. The ECM is constantly remodeled and reshaped which modulates the signaling cascades responsible for a cell's proliferation, motility, and differentiation pattern (Sainio & Järveläinen, 2020). Changes in ECM composition and structure are closely associated with the development and progression of several physiological and pathologic conditions.

Fibronectin is the most abundant disulfide-linked heterodimeric ECM glycoprotein with a wide variety of physiological functions and is produced by many cell types, including fibroblasts, endothelial cells, chondrocytes, synovial cells and myocytes. Fibronectin is assembled to produce a scaffold that is used by cancer cells to spread and invade the local stroma (Patten & Wang, 2021). Fibronectin plays a deterministic role in development, cellular growth and differentiation, adhesion, migration, and wound healing (T.-C. Lin et al., 2021; Rick et al., 2019). Apart from its physiological functions, Fibronectin has been implicated in many disease processes including cancer

Abbreviations: 5-FU, 5-fluorouracil; Akt, protein kinase B; ATF2, activating transcription factor 2; BCL-2, B-cell lymphoma 2; BCL-xl, B-cell lymphoma-extra large; CAM-DR, cell adhesion-mediated drug resistance; cFN, cellular fibronectin; dCK, deoxycytidine kinase; dTMP, deoxythymidine monophosphate; ECM, extracellular matrix; EDA, extra Domain A; EDB, extra Domain B; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ER, estrogen receptor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FDA, Food and Drug Administration; FdUMP, fluorodeoxyuridine monophosphate; FN, fibronectin; hENT-1, human nucleoside transporter-1; IGF-1, insulin-like growth factor 1; ILK, integrin linked kinase; IMG1, inhibitor of growth 1; mAbs, monoclonal antibodies; MAPK, mitogen-activated protein kinase; MCL-1, proteins myeloid cell leukemia-1; MEK, mitogen-activated protein kinase kinase; MRP, multidrug resistance-associated protein; NF-κb, nuclear factor kappa B; NSCLC, nonsmall cell lung cancer; PARP, Poly (ADP-ribose) polymerase; pFN, plasma fibronectin; PI3K, phosphatidylinositol 3-kinase; RAF, rapidly accelerated fibro sarcoma; RAS, rat sarcoma virus; RB, retroblastoma; RR, ribonucleotide reductase; SERM, selective estrogen receptor modulator; SH2, Src homology domain 2; TGF, transforming growth factor; TKIs, tyrosine kinase inhibitors; Wnt, wingless-related integration site.

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A multiepitope vaccine candidate against infectious bursal disease virus using immunoinformatics-based reverse vaccinology approach

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Infectious bursal disease virus is the causative agent of infectious bursal disease (Gumboro disease), a highly contagious immunosuppressive disease of chicken with a substantial economic impact on small- and large-scale poultry industries worldwide. Currently, live attenuated vaccines are widely used to control the disease in chickens despite their issues with safety (immunosuppression and bursal atrophy) and efficiency (breaking through the maternally-derived antibody titer). To overcome the drawbacks, the current study has, for the first time, attempted to construct a computational model of a multiepitope based vaccine candidate against infectious bursal disease virus, which has the potential to overcome the safety and protection issues found in the existing live-attenuated vaccines. The current study used a reverse vaccinology based immunoinformatics approach to construct the vaccine candidate using major and minor capsid proteins of the virus, VP2 and VP3, respectively. The vaccine construct was composed of four $CD8^+$ epitopes, seven $CD4^+$ T-cell epitopes, 11 B-cell epitopes and a Cholera Toxin B adjuvant, connected using appropriate flexible peptide linkers. The vaccine construct was evaluated as antigenic with VaxiJen Score of 0.6781, immunogenic with IEDB score of 2.89887 and non-allergenic. The 55.64 kDa construct was further evaluated for its physicochemical characteristics, which revealed that it was stable with an instability index of 16.24, basic with theoretical pI of 9.24, thermostable with aliphatic index of 86.72 and hydrophilic with GRAVY score of −0.256. The docking and molecular dynamics simulation studies of the vaccine construct with Toll-like receptor-3 revealed fair structural interaction (binding affinity of −295.94 kcal/mol) and complex stability. Further, the predicted induction of antibodies and cytokines by the vaccine construct indicated the possible elicitation of the host's immune response against the virus. The work is a significant attempt to develop next-generation vaccines against the infectious bursal disease virus though further experimental studies are required to assess the efficacy and protectivity of the proposed vaccine candidate *in vivo*.

KEYWORDS
IBDV, immunoinformatics, B cell epitopes, T cell epitopes, molecular dynamics simulations

viruses

MDPI

Article

An Investigation of the Antiviral Potential of Phytocompounds against Avian Infectious Bronchitis Virus through Template-Based Molecular Docking and Molecular Dynamics Simulation Analysis

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Abstract: Vaccination is widely used to control Infectious Bronchitis in poultry; however, the limited cross-protection and safety issues associated with these vaccines can lead to vaccination failures. Keeping these limitations in mind, the current study explored the antiviral potential of phytocompounds against the Infectious Bronchitis virus using in silico approaches. A total of 1300 phytocompounds derived from fourteen botanicals were screened for their potential ability to inhibit the main protease, papain-like protease or RNA-dependent RNA–polymerase of the virus. The study identified Methyl Rosmarinate, Cianidanol, Royleanone, and 6,7-Dehydroroyleanone as dual-target inhibitors against any two of the key proteins. At the same time, 7-alpha-Acetoxyroyleanone from *Rosmarinus officinalis* was found to be a multi-target protein inhibitor against all three proteins. The potential multi-target inhibitor was subjected to molecular dynamics simulations to assess the stability of the protein–ligand complexes along with the corresponding reference ligands. The findings specified stable interactions of 7-alpha-Acetoxyroyleanone with the protein targets. The results based on the in silico study indicate that the phytocompounds can potentially inhibit the essential proteins of the Infectious Bronchitis virus; however, in vitro and in vivo studies are required for validation. Nevertheless, this study is a significant step in exploring the use of botanicals in feed to control Infectious Bronchitis infections in poultry.

Keywords: infectious bronchitis virus; natural antiviral; pharmacokinetic; molecular docking; molecular dynamics simulation

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1. Introduction

Infectious Bronchitis (IB) is a highly contagious disease with significant economic implications for the global chicken industry. First documented in 1931, IB is mainly linked to respiratory, reproductive, digestive, and renal disorders in domestic chickens and various avian species [1,2]. Infectious bronchitis virus (IBV) replicates primarily in the epithelial cells of the respiratory tract, resulting in respiratory problems [3]. The epithelial cells in the oviduct and the kidney are also susceptible to IBV infection, impairing the quality and production of eggs and causing nephritis. IB infections can cause a mortality rate of 20–30% [4,5], increasing significantly with secondary infections in infected flocks [6,7]. The IBV is an enveloped positive-sense single-stranded RNA virus (+ssRNA) that belongs to the genus Gammacoronavirus of the *Coronaviridae* family [8]. The IBV

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Original Research

Combination of Caffeic Acid Phenethyl Ester and Crocin Realign Potential Molecular Markers in U87-MG Glioma Cells

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ABSTRACT

Background: Glial tumors are the most common primary malignant central nervous system tumors. They are hard to treat, not only because of the deregulation in multiple pathways but also because they are not contained in a well-defined mass with clear borders. The use of a single therapeutic agent to target gliomas has yielded unsatisfactory results.
Objective: A combination of molecules targeting multiple pathways may prove to be a better alternative.
Methods: The effect of caffeic acid phenethyl ester and crocin on the proliferation and death of U87-MG cells over a concentration range was analyzed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and lactate dehydrogenase assays. A colony formation assay was used to measure the effect of caffeic acid phenethyl ester and crocin on contact inhibition and anchorage independence ability of U87-MG cells. Furthermore, apoptosis in U87-MG cells was analyzed by propidium iodide assay. Real-time polymerase chain reaction and Western blotting were performed to determine the expression level of p53, epidermal growth factor receptor, and proliferating cell nuclear antigen.
Results: Caffeic acid phenethyl ester and crocin when used in combination present an anticancer potential for glioma. These molecules, in combination, inhibit proliferation and induce apoptosis in U87-MG glioma cells. Our results provide evidence that combination treatment realigns the expression paradigm of p53, epidermal growth factor receptor, and proliferating cell nuclear antigen in cotreated U87-MG cells.
Conclusions: The combination of caffeic acid phenethyl ester and crocin led to inhibition in glioma cell proliferation and might prove to be an effective adjunct to the therapies in vogue.

Introduction

Brain tumors are the most common form of solid tumor among children younger than age 20 years. These tumors are now the second leading cause of cancer-related deaths, following leukemia. Because brain tumors are located at the control center for thought, emotion, and movement, their effects on an individual's physical and cognitive abilities are adverse.[1,2] There are more than 120 different types of brain tumors, which make their effective treatment very complicated. Complete surgical resection of a high-grade glial tumor is extremely difficult owing to the heterogeneity of tumor cells, diffuse infiltrating nature, and high proliferative potential.[3-5] Adjuvant therapy, radiation, and chemotherapy after surgery can only extend the survival time of these patients for up to 9 months to a year. As a result, most targeted agents like monotherapies have failed to provide survival benefits in malignant gliomas. In addition, resistance to chemotherapy occurs often, which leads to tumor recurrence and treatment failure.[6] Gliomas are among the most widespread primary tumors of the brain arising from gluey or supportive tissue of the brain, representing 31% of all brain tumors and 80% of all malignant tumors. Gliomas result in more years of life lost than any other tumors.[1,7] On the basis of different histopathological characteristics, gliomas are graded from I to IV as per the 2016 World Health Organization classification and grade IV glioma or glioblastoma multiforme has a median overall survival of merely 14.6 months.[4,8] Mutations in epidermal growth factor receptor (EGFR), isocitrate dehydrogenase; and deletion of the long/short arm of chromosome 19/chromosome 1, respectively, as well as methylation of O6-methylguanine-DNA methyltransferase

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Cell Communication and Signaling

RESEARCH Open Access

Scavenger receptor B1 facilitates the endocytosis of *Escherichia coli* via TLR4 signaling in mammary gland infection

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Abstract

SCARB1 belongs to class B of Scavenger receptors (SRs) that are known to be involved in binding and endocytosis of various pathogens. SRs have emerging role in regulating innate immunity and host–pathogen interactions by acting in co-ordination with Toll-like receptors.Query Little is known about the function of SCARB1 in milk-derived mammary epithelial cells (MECs). This study reports the role of SCARB1 in infection and its potential association in TLR4 signaling on bacterial challenge in Goat mammary epithelial cells (GMECs). The novelty in the establishment of MEC culture lies in the method that aims to enhance the viability of the cells with intact characteristics upto a higher passage number. We represent MEC culture to be used as a potential infection model for deeper understanding of animal physiology especially around the mammary gland. On *E.coli* challenge the expression of SCARB1 was significant in induced GMECs at 6 h. Endoribonuclease-esiRNA based silencing of SCARB1 affects the expression of TLR4 and its pathways i.e. MyD88 and TRIF pathways on infection. Knockdown also affected the endocytosis of *E.coli* in GMECs demonstrating that *E.coli* uses SCARB1 function to gain entry in cells. Furthermore, we predict 3 unique protein structures of uncharacterized SCARB1 (*Capra hircus)* protein. Overall, we highlight SCARB1 as a main participant in host defence and its function in antibacterial advances to check mammary gland infections.

Keywords Scavenger receptor B1, TLR4, *Escherichia coli*, Goat mammary gland, Infection

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Pullulan production by *Aureobasidium pullulans* MTCC 1991 from apple pomace and its characterization

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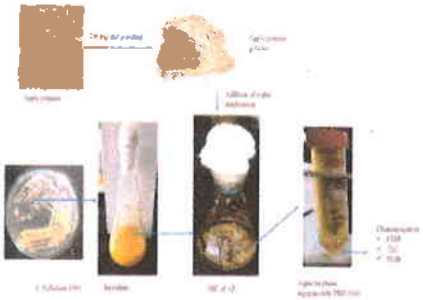
Abstract

Apple pomace, a by-product of the apple juice processing industry, is significant agro-based waste in the Union Territory of Jammu & Kashmir, India. A considerable amount of apple is processed for juice production, resulting in a surplus of apple pomace, which is generally underutilized. This study utilized apple pomace as a substrate matrix for producing pullulan by applying Aureobasidium pullulans MTCC 1991. Solid-state fermentation was done at a lab scale in Erlenmeyer flasks to produce this exopolysaccharide. The suitable conditions for solid-state fermentation of apple pomace were: a solid-liquid ratio of 1:4, inoculum volume of 3 mL, pH 6 and incubation time of 14 days. A yield of 42 mg/g dw was obtained in the control sample without adding any other nutrients or chemicals. The addition of yeast extract at a concentration of 1% (w/w) and sucrose (5% w/w) significantly ($p<0.05$) increased the yield to 62 mg/g dw and 75 mg/g dw, respectively. The purified pullulan had similar characteristics to that of standard pullulan, as confirmed by ATR-FTIR, H NMR and TLC techniques. Pullulan production from apple pomace can be a better way to utilize this by-product from the apple juice industry. It will be a cost-effective technique for pullulan production as an inexpensive substrate and an environmentally friendly approach are also used.

Graphical abstract



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Introduction

Pullulan is an exopolysaccharide produced during fermentation by *Aureobasidium pullulans*, known as black yeast. Many microbes have been reported to produce pullulan as an exopolysaccharide, but *A. pullulans* is commercially used for pullulan production. *A. pullulans* is a ubiquitous yeast-like saprotrophic fungus found on leaves, moist surfaces, trees, etc. This organism is known to exist in a plethora of temperatures and natural habitats. *A. pullulans* is known to produce a variety of compounds, including exopolysaccharides pullulan and aureobasidins, enzymes and poly malic acid (Bozoudi & Tsaltas, 2018). Pullulan is an α-glucan composed of repeating maltotriose units composed of α-glucose molecules linked together with α-1,4 glycosidic bonds and these units are, in turn,

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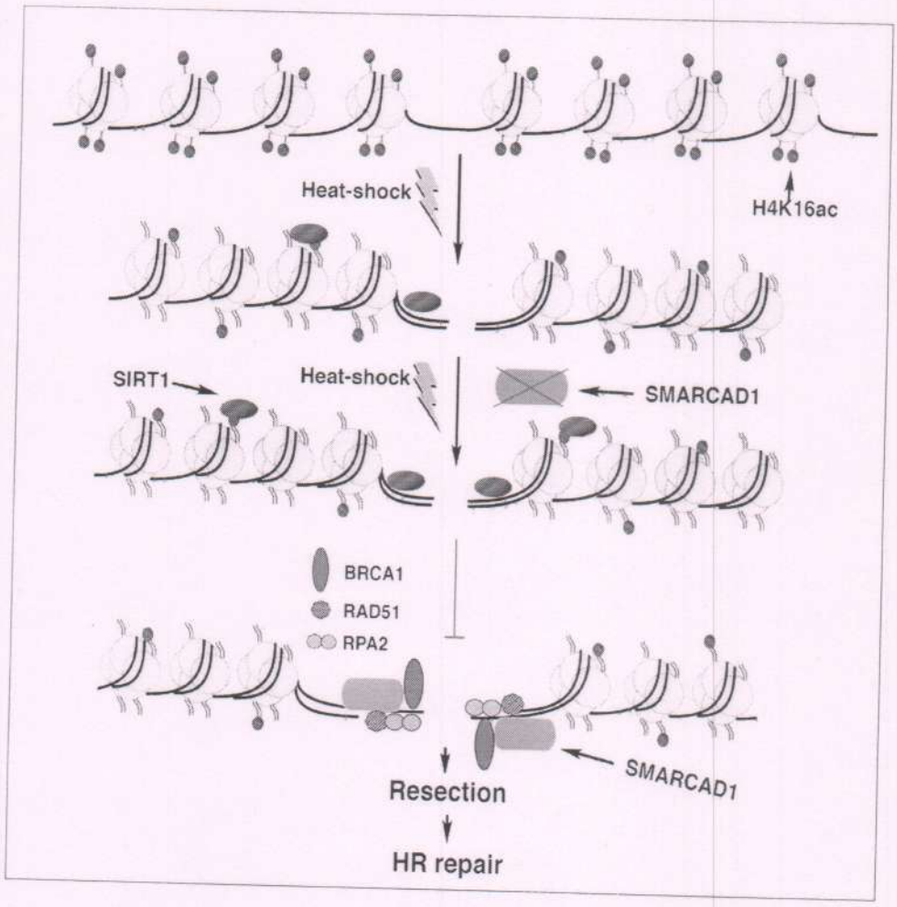
Article

Heat-induced SIRT1-mediated H4K16ac deacetylation impairs resection and SMARCAD1 recruitment to double strand breaks



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Highlights

H4K16ac deacetylation during hyperthermia is conserved in human, *Drosophila*, and yeast

Dynamic regulation of the chromatin functions during hyperthermia is SIRT1-dependent

SIRT1 function is negatively impacted by SMARCAD1

Hyperthermia increases replication stress and impacts DNA resection, impairing DSB repair

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Research Article

Some important biochemical changes orchestrating flower development and senescence in *Nicotiana plumbaginifolia* Viv. and *Petunia hybrida* Vilm. flowers

Shaziya Nisar, Riyaz Ahmad Dar, Altaf Ahmad Bhat, Zeenat Farooq & Inayatullah Tahir

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ELSEVIER

2021

Review Article

Role of histone acetyltransferases MOF and Tip60 in genome stability ☆, ☆☆

Ulfat Syed Mir [a], Audesh Bhat [b], Arjamand Mushtaq [a], Shruti Pandita [c], Mohammad Altaf [a d] ✉, Tej K. Pandita [e] ✉

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PLOS GENETICS 2021

RESEARCH ARTICLE

Antagonistic relationship of NuA4 with the non-homologous end-joining machinery at DNA damage sites

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Abstract

The NuA4 histone acetyltransferase complex, apart from its known role in gene regulation, has also been directly implicated in the repair of DNA double-strand breaks (DSBs), favoring homologous recombination (HR) in S/G2 during the cell cycle. Here, we investigate the antagonistic relationship of NuA4 with non-homologous end joining (NHEJ) factors. We show that budding yeast Rad9, the 53BP1 ortholog, can inhibit NuA4 acetyltransferase activity when bound to chromatin *in vitro*. While we previously reported that NuA4 is recruited at DSBs during the S/G2 phase, we can also detect its recruitment in G1 when genes for Rad9 and NHEJ factors Yku80 and Nej1 are mutated. This is accompanied with the binding of single-strand DNA binding protein RPA and Rad52, indicating DNA end resection in G1 as well as recruitment of the HR machinery. This NuA4 recruitment to DSBs in G1 depends on Mre11-Rad50-Xrs2 (MRX) and Lcd1/Ddc2 and is linked to the hyper-resection phenotype of NHEJ mutants. It also implicates NuA4 in the resection-based single-strand annealing (SSA) repair pathway along Rad52. Interestingly, we identified two novel non-histone acetylation targets of NuA4, Nej1 and Yku80. Acetyl-mimicking mutant of Nej1 inhibits repair of DNA breaks by NHEJ, decreases its interaction with other core NHEJ factors such as Yku80 and Lif1 and favors end resection. Altogether, these results establish a strong reciprocal antagonistic regulatory function of NuA4 and NHEJ factors in repair pathway choice and suggests a role of NuA4 in alternative repair mechanisms in situations where some DNA-end resection can occur in G1.

Author summary

DNA double-strand breaks (DSBs) are one of the most harmful form of DNA damage. Cells employ two major repair pathways to resolve DSBs: Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ). Here we wanted to dissect further the role played by the NuA4 (Nucleosome acetyltransferase of histone H4) complex in the

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RESEARCH ARTICLE

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Autism-Associated Vigilin Depletion Impairs DNA Damage Repair

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ABSTRACT Vigilin (Vgl1) is essential for heterochromatin formation, chromosome segregation, and mRNA stability and is associated with autism spectrum disorders and cancer: vigilin, for example, can suppress proto-oncogene *c-fms* expression in breast cancer. Conserved from yeast to humans, vigilin is an RNA-binding protein with 14 tandemly arranged nonidentical hnRNP K-type homology (KH) domains. Here, we report that vigilin depletion increased cell sensitivity to cisplatin- or ionizing radiation (IR)-induced cell death and genomic instability due to defective DNA repair. Vigilin depletion delayed dephosphorylation of IR-induced γ-H2AX and elevated levels of residual 53BP1 and RIF1 foci, while reducing Rad51 and BRCA1 focus formation, DNA end resection, and double-strand break (DSB) repair. We show that vigilin interacts with the DNA damage response (DDR) proteins RAD51 and BRCA1, and vigilin depletion impairs their recruitment to DSB sites. Transient hydroxyurea (HU)-induced replicative stress in vigilin-depleted cells increased replication fork stalling and blocked restart of DNA synthesis. Furthermore, histone acetylation promoted vigilin recruitment to DSBs preferentially in the transcriptionally active genome. These findings uncover a novel vigilin role in DNA damage repair with implications for autism and cancer-related disorders.

KEYWORDS vigilin, DNA repair, Rad51, homologous recombination, histone acetylation, replicative stress, autism-related disorders, cancer

The vigilin-coding gene, a high-density lipoprotein binding protein gene (*HDLBP*), has been identified as one of >350 genes associated with cancer and autistic spectrum disorder (ASD) susceptibility (1–6). In mouse models, predisposition to ASD has been linked to haploinsufficiency in ASD susceptibility genes such as *Nuak* (7), *Nbea* (8), and *Sh3rf2* (9), and it has been postulated that *HDLBP* mutant proband haploinsufficiency also predisposes to ASD (1). Functional clustering of the >350 mutated autism target genes reveals strong enrichment for genes related to fragile X mental retardation protein (FMRP) and β-catenin/chromatin remodeling networks (2, 5). As a multi-KH domain-containing protein, FMRP shares structural similarities with vigilin, and its loss-of-function mutations lead to fragile X syndrome, a mental retardation condition that, like ADS, displays deregulation of synaptic pathways (10). As with FMRP, vigilin is involved in mRNA transport to the cytoplasm (11), suggesting that the association between *HDLBP* haploinsufficiency and ASD may stem in part from altered mRNA transport.

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Open Access Review

Role of Histone Methylation in Maintenance of Genome Integrity

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REVIEW

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Therapeutic strategies against hDOT1L as a potential drug target in MLL-rearranged leukemias

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Abstract

Therapeutic intervention of proteins participating in chromatin-mediated signaling with small-molecules is a novel option to reprogram expression networks for restraining disease states. Protein methyltransferases form the prominent family of such proteins regulating gene expression via epigenetic mechanisms thereby representing novel targets for pharmacological intervention. Disruptor of telomeric silencing, hDot1L is the only non-SET domain containing histone methyltransferase that methylates histone H3 at lysine 79. H3K79 methylation mediated by hDot1L plays a crucial role in mixed lineage leukemia (MLL) pathosis. MLL fusion protein mediated mistargeting of DOT1L to aberrant gene locations results in ectopic H3K79 methylation culminating in aberrant expression of leukemogenic genes like HOXA9 and MEIS1. hDOT1L has thus been proposed as a potential target for therapeutic intervention in MLL. This review presents the general overview of hDOT1L and its functional role in distinct biological processes. Furthermore, we discuss various therapeutic strategies against hDOT1L as a promising drug target to vanquish therapeutically challenging MLL.

Keywords: Chromatin, Histone methyltransferases, Histone methylation, DNA repair, Mixed lineage leukemia, Gene expression

Introduction

The assembly of eukaryotic genome into a highly complex nucleoprotein structure, called chromatin, controls all DNA-mediated functions of the cell. The fundamental building block of chromatin is the nucleosome core particle, which contains 146 bp of DNA wrapped around an octamer of four core histones namely H2A, H2B, H3, and H4 [1, 2]. The packaging of eukaryotic DNA into chromatin not only facilitates the accommodation of large eukaryotic genome in the small nuclear space, but also blocks access of various enzymes and factors that facilitate DNA-mediated processes like transcription, repair replication and recombination. Several mechanisms operate within the cell to facilitate access to DNA, these include (i) sliding/eviction of nucleosomes by ATP dependent chromatin remodelers, (ii) modifications of protruding histone tails, and (iii) incorporation of variant histones [3–6]. Histone proteins that form an integral part of chromatin contain two domains, a globular domain responsible for histone–histone interactions and a highly dynamic N-terminal tail rich in basic amino acids. Histone tails undergo a number of post-translational modifications which including methylation, acetylation, phosphorylation, ubiquitylation, and ribosylation. These histone modifications either directly alter the chromatin structure or they can serve as binding sites for various transacting factors, which in turn elicit changes in the structure and functionality of the chromatin fiber [1, 3, 4, 7–12]. Histone methylation among the various post-translational modifications

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2019

JBC ARTICLE

Vigilin protein Vgl1 is required for heterochromatin-mediated gene silencing in *Schizosaccharomyces pombe*

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Heterochromatin is a conserved feature of eukaryotic genomes and regulates various cellular processes, including gene silencing, chromosome segregation, and maintenance of genome stability. In the fission yeast *Schizosaccharomyces pombe*, heterochromatin formation involves methylation of lysine 9 in histone H3 (H3K9), which recruits Swi6/HP1 proteins to heterochromatic loci. The Swi6/HP1–H3K9me3 chromatin complex lies at the center of heterochromatic macromolecular assemblies and mediates many functions of heterochromatin by recruiting a diverse set of regulators. However, additional factors may be required for proper heterochromatin organization, but they are not fully known. Here, using several molecular and biochemical approaches, we report that Vgl1, a member of a large family of multiple KH-domain proteins, collectively known as vigilins, is indispensable for the heterochromatin-mediated gene silencing in *S. pombe*. ChIP analysis revealed that Vgl1 binds to pericentromeric heterochromatin in an RNA-dependent manner and that Vgl1 deletion leads to loss of H3K9 methylation and Swi6 recruitment to centromeric and telomeric heterochromatic loci. Furthermore, we show that Vgl1 interacts with the H3K9 methyltransferase, Clr4, and that loss of Vgl1 impairs Clr4 recruitment to heterochromatic regions of the genome. These findings uncover a novel role for Vgl1 as a key regulator in heterochromatin-mediated gene silencing in *S. pombe*.

The eukaryotic genome is spatially segregated in the nucleus into discrete structural and functional chromatin domains (1–5). The genome is organized into euchromatin and heterochromatin distinguished on the basis of their appearance, organization, localization, function, and the type of histone modifications on nucleosomes (6, 7). Heterochromatin is a typically condensed chromatin structure refractory to transcriptional machinery and is important for various cellular processes, including regulation of gene expression, chromosome segregation, dosage compensation, and maintenance of genome stability by inhibiting unwanted recombination between repetitive DNA elements (8, 9). Across species, assembly of heterochromatin involves coordinating activities of small noncoding RNAs associated with the RNA interference (RNAi) pathway and chromatin-modifying machinery (10–13). In fission yeast, *Schizosaccharomyces pombe* heterochromatin is found at the telomeres, the mating-type and ribosomal DNA loci, and the pericentromeric region. The centromeres are composed of a central core region, which is excluded from heterochromatin-specific factors, and modifications flanked by innermost repeat (imr)[3] and outer (otr) repeat, called dg and dh repeats (14–19). These repeats are transcribed into dsRNAs by RNA pol II and are further processed into siRNAs by RNAi factors, including Argonaute (Ago1), Dicer (Dcr1), and RNA-dependent RNA polymerase (Rdp1). RNA transcripts act as a scaffold for the assembly of RNAi and chromatin-modifying factors that initiate the formation of heterochromatin (20–23). Deletion of fission yeast RNAi proteins like Dcr1, Rdp1, and Argonaute results in loss of heterochromatin gene silencing (24–27). Histone H3 lysine 9 methylation (H3K9me), mediated by a conserved Clr4 methyltransferase, is specifically localized in heterochromatic regions of the genome. H3K9 methylation creates binding sites for HP1 proteins Swi6 and Chp2, which are critical for heterochromatic gene silencing (28–36). Nucleation and spreading of H3K9 methylation depends on two distinct processes: an RNAi-independent pathway mediates low levels of H3K9me, and an RNAi-dependent mechanism boosts this methylation. In the absence of RNAi, H3K9me fails to spread, and silencing of reporter genes inserted within the centromeres is abrogated (28, 37, 38). In addition to RNAi, H3K9me, and Swi6, other additional factors have also been associated with heterochromatic gene silencing (39, 40). Recently, we have reported that Lem2–Nur1 inner nuclear membrane complex is essential for heterochromatin gene silencing (41). Tandem affinity purification of Lem2 or Nur1 identified Vgl1, a predicted heterochromatin protein, as an interacting partner. Vgl1 is an evolutionarily-conserved protein from the yeast *Saccharomyces cerevisiae* (Scp160) to *Drosophila* (DDP1) and vertebrates (Vigilin) and belongs to a large family of multiple KH-domain proteins, collectively known as vigilins (42, 43). KH domains are involved in protein–protein and protein–nucleic acid interactions. Several KH domain-con-

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[3] The abbreviations used are: imr, innermost repeat; otr, outer; qRT-PCR, quantitative RT-PCR; pol, polymerase; TBZ, thiabendazole; KH, K homology; otr, outer repeat element; FOA, 5-fluoroorotic acid; ChIP, chromatin immunoprecipitation.

Research Article

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Disruption of polyhomeotic polymerization decreases nucleosome occupancy and alters genome accessibility

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Chromatin attains its three-dimensional (3D) conformation by establishing contacts between different noncontiguous regions. Sterile Alpha Motif (SAM)–mediated polymerization of the polyhomeotic (PH) protein regulates subnuclear clustering of Polycomb Repressive Complex 1 (PRC1) and chromatin topology. The mutations that perturb the ability of the PH to polymerize, disrupt long-range chromatin contacts, alter Hox gene expression, and lead to developmental defects. To understand the underlying mechanism, we combined the experiments and theory to investigate the effect of this SAM domain mutation on nucleosome occupancy and accessibility on a genome wide scale. Our data show that disruption of PH polymerization because of SAM domain mutation decreases nucleosome occupancy and alters accessibility. Polymer simulations investigating the interplay between distant chromatin contacts and nucleosome occupancy, both of which are regulated by PH polymerization, suggest that nucleosome density increases when contacts between different regions of chromatin are established. Taken together, it appears that SAM domain–mediated PH polymerization biomechanically regulates the organization of chromatin at multiple scales from nucleosomes to chromosomes and we suggest that higher order organization can have a top–down causation effect on nucleosome occupancy.

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Introduction

Chromatin within the cell nucleus is organized in a complex, nonrandom 3D conformation. A generic feature of chromatin folding, well accepted, is its hierarchical nature (1, 2, 3). The organizational complexity increases from nucleosomes to the formation of simple chromatin loops and topologically associating domains (TADs), which form because of preferential contacts within a genomic region as compared with neighboring regions. TADs of the same type aggregate and result in the formation of A and B compartments. This type of hierarchy seems to continue up to the scale of entire chromosome via formation of meta-TADs of increasing size (4, 5). These organizational features have been observed in different organisms and cell types, implying the existence of fundamental underlying principles governing the architecture of chromatin. 3D chromatin organization is linked to the regulation of chromatin-associated processes like gene expression, replication, and repair which occur at the nucleosome level (5, 6, 7, 8, 9), but, the mechanistic details of how higher order chromatin folding exerts its effects at the level of nucleosomes is not well understood.

3D organization of chromatin is shaped by biochemical and by biomechanical mechanisms. The polymeric nature of chromatin, nuclear confinement, and the nuclear lamina impart mechanical constraints which can influence the 3D folding of chromatin (10, 11, 12, 13, 14). 3D folding of chromatin is achieved by formation of contacts between different noncontiguous regions mediated by protein–protein interactions. Crosslinking density in the case of synthetic polymers has been shown to modulate various properties like stiffness, volume, temperature dependence, etc. (15, 16, 17). Another mechanical property affected by crosslinking is polymer chain dynamics, which decreases with increasing crosslinking (17). Chromatin, as a polymer (18) can also possess these properties. For example, the number and strength of chromatin contacts can influence the properties of the chromatin chain composed of nucleosomes.

Folding of chromatin is driven by many non-histone chromatin-associated proteins like CCCTC binding factor (CTCF), cohesin, Polycomb Group (PcG) proteins, etc. (19, 20, 21, 22, 23, 24). PcG proteins, conserved from *Drosophila* to humans, modulate chromatin organization either biochemically by modifying histones or biomechanically by physically constraining and compacting chromatin

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Non-canonical DNA/RNA structures associated with the pathogenesis of Fragile X-associated tremor/ataxia syndrome and Fragile X syndrome

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Fragile X-associated tremor/ataxia syndrome (FXTAS) and fragile X syndrome (FXS) are primary examples of fragile X-related disorders (FXDs) caused by abnormal expansion of CGG repeats above a certain threshold in the 5′-untranslated region of the fragile X mental retardation (FMR1) gene. Both diseases have distinct clinical manifestations and molecular pathogenesis. FXTAS is a late-adult-onset neurodegenerative disorder caused by a premutation (PM) allele (CGG expansion of 55–200 repeats), resulting in FMR1 gene hyperexpression. On the other hand, FXS is a neurodevelopmental disorder that results from a full mutation (FM) allele (CGG expansions of ≥200 repeats) leading to heterochromatization and transcriptional silencing of the FMR1 gene. The main challenge is to determine how CGG repeat expansion affects the fundamentally distinct nature of FMR1 expression in FM and PM ranges. Abnormal CGG repeat expansions form a variety of non-canonical DNA and RNA structures that can disrupt various cellular processes and cause distinct effects in PM and FM alleles. Here, we review these structures and how they are related to underlying mutations and disease pathology in FXS and FXTAS. Finally, as new CGG expansions within the genome have been identified, it will be interesting to determine their implications in disease pathology and treatment.

KEYWORDS

fragile X-associated tremor/ataxia syndrome (FXTAS), fragile X syndrome (FXS), FMR1, R-loop, hairpin

Introduction

CGG repeats are a type of microsatellite or short tandem repeat (STR) found in the human genome, with the majority located in the 5′-untranslated regions (5′-UTRs), suggesting that they may play a role in transcriptional regulation or translation initiation (Bagshaw, 2017). Abnormal expansion of CGG repeat tracts above a certain threshold

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MINI REVIEW
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Non-coding RNAs in the Pathogenesis of Multiple Sclerosis

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Multiple sclerosis (MS) is an early onset chronic neurological condition in adults characterized by inflammation, demyelination, gliosis, and axonal loss in the central nervous system. The pathological cause of MS is complex and includes both genetic and environmental factors. Non-protein-coding RNAs (ncRNAs), specifically miRNAs and lncRNAs, are important regulators of various biological processes. Over the past decade, many studies have investigated both miRNAs and lncRNAs in patients with MS. Since then, insightful knowledge has been gained in this field. Here, we review the role of miRNAs and lncRNAs in MS pathogenesis and discuss their implications for diagnosis and treatment.

Keywords: multiple sclerosis, central nervous system, microRNA, long noncoding RNA, neurodegeneration

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INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating neurodegenerative disease of the central nervous system (CNS) (Thompson et al., 2018). It mainly affects young adults, with onset between the ages of 20 and 40 years, and is predominant in women (Thompson et al., 2018). The pathological hallmark of MS is the accumulation of focal plaques, which are areas of demyelination along with infiltration of immune cells found throughout the CNS (Mahad et al., 2015).

The clinical manifestations and course of MS vary and are broadly divided into three types: relapsing-remitting MS (RRMS), primary progressive MS (PPMS), and secondary progressive MS (SPMS). Almost 85% of patients typically present with RRMS, which is characterized by episodes of disability, followed by a period of recovery (Confavreux and Vukusic, 2014; Mahad et al., 2015; Thompson et al., 2018). Approximately 10–15% of patients exhibit PPMS, which is characterized by a slow progression of disease from the beginning without remission (Confavreux and Vukusic, 2014; Thompson et al., 2018). Over 10 years, roughly half of RRMS patients progress to the SPMS stage characterized by chronic inflammation, sclerosis, and brain atrophy with few or no periods of remission (Confavreux and Vukusic, 2014; Thompson et al., 2018).

The pathophysiological mechanism of MS is heterogeneous and is thought to involve complex gene-environment interactions (Mahad et al., 2015). However, the major cause of MS development is a pro-inflammatory response. Immune cells such as $CD4^+$ and $CD8^+$ T cells, B cells, macrophages, and other cells infiltrate the CNS through a disrupted blood-brain barrier (BBB) (Mahad et al., 2015). These cells, together with resident activated microglia and astrocytes, damage oligodendrocytes and myelin through contact-dependent mechanisms and the secretion of cytokines and chemokines (Mahad et al., 2015).

In the initial stages of MS development, $CD4^+$ T helper type 1 (Th1) and $CD4^+$ T helper type 17 (Th17) are autoreactive to myelin and have therefore been intensively investigated

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General Article

GENERAL ARTICLE

Fragile X premutation rCGG repeats impair synaptic growth and synaptic transmission at *Drosophila* larval neuromuscular junction

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Abstract

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disease that develops in some premutation (PM) carriers of the *FMR1* gene with alleles bearing 55–200 CGG repeats. The discovery of a broad spectrum of clinical and cell-developmental abnormalities among PM carriers with or without FXTAS and in model systems suggests that neurodegeneration seen in FXTAS could be the inevitable end-result of pathophysiological processes set during early development. Hence, it is imperative to trace early PM-induced pathological abnormalities. Previous studies have shown that transgenic *Drosophila* carrying PM-length CGG repeats are sufficient to cause neurodegeneration. Here, we used the same transgenic model to understand the effect of CGG repeats on the structure and function of the developing nervous system. We show that presynaptic expression of CGG repeats restricts synaptic growth, reduces the number of synaptic boutons, leads to aberrant presynaptic varicosities, and impairs synaptic transmission at the larval neuromuscular junctions. The postsynaptic analysis shows that both glutamate receptors and subsynaptic reticulum proteins were normal. However, a high percentage of boutons show a reduced density of Bruchpilot protein, a key component of presynaptic active zones required for vesicle release. The electrophysiological analysis shows a significant reduction in quantal content, a measure of total synaptic vesicles released per excitation potential. Together, these findings suggest that synapse perturbation caused by riboCGG (rCGG) repeats mediates presynaptically during larval neuromuscular junction development. We also suggest that the stress-activated c-Jun N-terminal kinase protein Basket and CIDE-N protein Drep-2 positively mediate Bruchpilot active zone defects caused by rCGG repeats.

Introduction

The fragile X mental retardation 1 (*FMR1*) gene normally harbors a highly polymorphic trinucleotide repeat sequence (CGG) within its 5′ untranslated region (5′ UTR). The normal allele of the *FMR1* gene typically has 5–40 CGG repeats. Abnormal alleles include the full mutation (>200 CGG repeats), premutation (PM) (55–200 CGG repeats) and gray zone mutation (45–54 CGG repeats). Carriers of full mutation develop fragile X syndrome (FXS; OMIM: 300624), the most common inherited form of neurodevelopment and intellectual disability (ID) disorder, occurring in 1 in 4000 to 1 in 7000 people (1–4). On the other hand, PM carriers account for a variety of phenotypes that are found frequently in the population, with an estimated prevalence of 1:259 in females and 1:813 in males (5,6). A proportion of these PM carriers, about 40% of males and 16% of females develop a

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