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Research Article

In silico screening of cancer-associated mutations in the HSA domain of BRG1 and its role in affecting the Arp-HSA sub-complex of SWI/SNF

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ABSTRACT

SWI/SNF (SWItch/Sucrose Non-Fermentable) complexes regulate the gene expression programs by remodeling the nucleosome architecture of the chromatin functional elements. These large multi-component complexes comprise eight or more subunits and are conserved from yeast to human. Noticeably, nuclear actin and actin-related proteins (Arps) are an integral part of these complexes and are known to directly interact with the helicase-SANT-associated (HSA) domain of ATPase subunit. Recently, SWI/SNF subunits are gaining importance because of the prevalence of cancer-causing mutations associated with them. The functional characterization of the mutations in the SWI/SNF subunits is important for understanding their role in tumorigenesis and identifying potential therapeutic strategies. To study the actin-related complex of human SWI/SNF and the cancer-associated mutations interfering Arp assembly with the ATPase subunit, we modelled the structure of the β-actin-BAF53A-HSA complex based on the yeast Arp-HSA complex (PDB ID: 4I6M). Seven deleterious mutations in the HSA domain of BRG1 were identified based on the functional screening of cancer-associated mutations in the COSMIC database. Detailed structural analysis of the six mutations (R466H, R469W, Y489C, K502N, R513Q and R521P) based on molecular dynamics (MD) simulations reveal the distinct effect of each mutation in destabilizing the structure of the Arp-HSA complex. Predominantly we could notice the long-range effect of the HSA mutations in influencing the dynamics of the Arp subunits.

1. Introduction

The SWI/SNF family of remodeling complexes were the first identified family of remodeling complexes originally in yeast as regulators of mating type switching (SWI) or sucrose fermentation (SNF) (Winston and Carlson, 1992). A multi-subunit complex of ~*2* MDa formed by the SWI/SNF subunits are recruited to the target nucleosomes, and it utilizes the energy derived from ATP hydrolysis for the remodeling activity (Hirschhorn et al., 1992). There exist subfamilies in SWI/SNF complex, and the BAF (BRG1-Associated Factors) and PBAF (Polybromo-associated BAF) are the two major subfamilies in human. The ATPase subunit is the main catalytic subunit in the complex, and in yeast, it is known as Snf2. In higher eukaryotes, it can be either Brahma-Related Gene 1(BRG1) (*SMARCA4*, gene name of the human subunit is mentioned in the parenthesis in italics throughout the manuscript) or Brahma (BRM) (*SMARCA2*). The ATPase subunit, together with the Brahma-associated factor(BAF) subunits, BAF47 (*SMARCB1*), BAF155 (*SMARCC1*) and BAF170 (*SMARCC2*) form the core complex which is the minimized complex for the remodeling activity (Phelan et al., 1999). Along with this core complex, the accessory subunits (which help in targeting or regulation of the complex) and signature subunits (which are specific to SWI/SNF subfamilies) are recruited to form the full functional SWI/SNF complex. The accessory subunits include BAF60(*SMARCD1/2/3*), BAF57(*SMARCE1*), β-actin(*ACTB*), Actin-related proteins(Arps)(BAF53A/B(ACTL6A/B)) and the sub-complex specific signature subunits include ARID domain-containing subunits BAF250A/B(*ARID1A/B*) or BAF200(*ARID2*), bromodomain containing protein BRD9(*BRD9*) or BRD7(*BRD7*), BAF45B/C/D(*DPF1/3/2*) or BAF45A(*PHF10*) and other sub-complex specific subunits form the full functional SWI/SNF complex (Mohrmann and Verrijzer, 2005; Mani et al., 2017) (Fig. 1A). The modulation of gene expression mediated by the SWI/SNF remodeling factors was known to influence a wide variety of cellular processes such as cell differentiation, proliferation, DNA repair and stress response, etc. (Euskirchen et al., 2011).

Interestingly, β-actin and two Arps BAF53A and BAF53B are the accessory subunits of the human SWI/SNF complex. BAF53A and BAF53B are paralogous subunits present in higher eukaryotes, and either one of them can be incorporated in the complex along with β-actin

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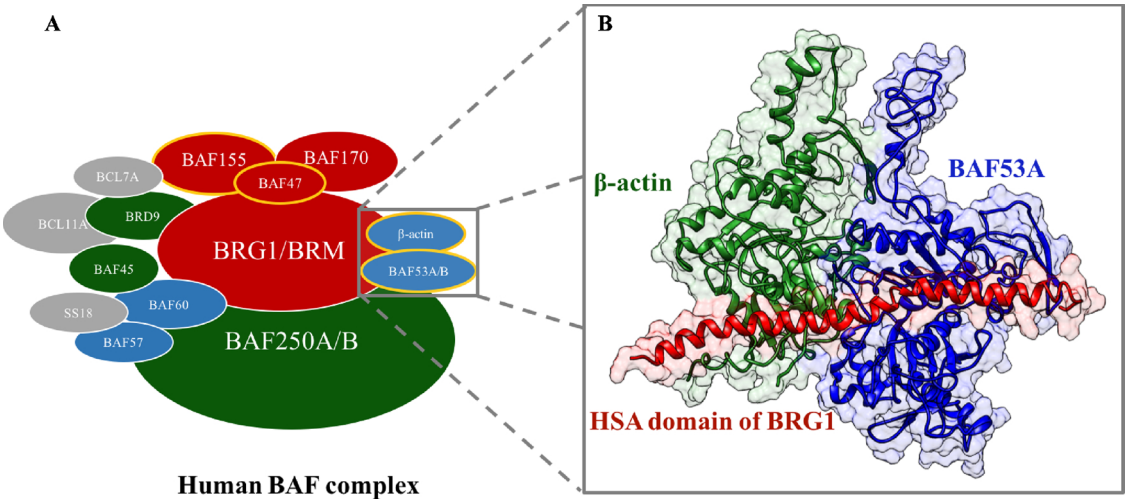

Fig. 1. (A) Schematic diagram representing the composition of Human BAF complex (a subfamily of human SWI/SNF complex). The core, accessory and signature subunits are represented in red, blue and green color respectively. Other BAF specific subunits are represented in gray color. The high-resolution structure of the whole SWI/SNF complex is not solved so far, and the solved interface of subunits is highlighted with yellow lines (B)Cartoon representation of the human Arp-HSA sub-complex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and found to play a role in the development and tissue-specific gene regulation. The BAF53B subunit is neuron specific and is replaced with BAF53A during neuronal differentiation (Lessard et al., 2007; Vogel-Ciernia et al., 2013). The Arps present in yeast SWI/SNF complex are Arp7 and Arp9. It is known that the ATPase subunit Snf2 in yeast interacts with the dimer of actin-related proteins, Arp7 and Arp9 via the helicase-SANT–associated (HSA) domain (Schubert et al., 2013). The 3D electron microscopy reconstruction of yeast SWI/SNF by Zhang et al. suggest that the Arp module might perform its regulatory role by initiating the nucleosome binding event for the catalytic activity of the ATPase subunit (Zhang et al., 2018).

Recent large-scale tumor genome sequencing projects have highlighted the somatic mutation in genes encoding subunits of chromatin-remodeling complexes as common drivers of tumorigenesis (Tyagi et al., 2016). It has been noted that 20% of human cancers are caused due to the loss of function mutation in this family of remodeling complex (Shain and Pollack, 2013; Wang et al., 2013). The most frequently mutated subunits of SWI/SNF include BRG1, ARID1A, and PBRM1 (Oike et al., 2013). From the earlier studies, mostly loss-of-function mutations were identified in the ATPase subunit, BRG1 and thus considered as a tumor-suppressor protein. But, elevated levels of BRG1 expression in multiple tumor types and the influence of BRG1 on cell proliferation and survival mechanism via invoking different signaling pathways indicates its oncogenic potential as well (Wu et al., 2017).

Owing to the presence of a large number of cancer-associated mutations and the cancer driver status of the subunit such as ATPase, it is important to study the structural and functional consequences of the associated mutations. Identification of pathogenic variants and understanding the mechanism of pathogenicity are an important step for the cancer precision medicine (Dimitrov et al., 2015). All the reported mutations may not impose a deleterious effect on the protein functions and experimentally investigating the impact of mutations will be time-consuming and costly. In this scenario, in silico screening of cancer-associated mutations will be an effective strategy to identify pathogenic mutations. Detailed insights on the severity of the amino acid substitution into the structure and dynamic behavior of proteins can be obtained by molecular dynamics (MD) simulation studies. Such computational approach has been exploited in obtaining mechanical insight on the key mutational events in cancer-associated proteins including PIK3CA (Gkeka et al., 2014), AKT1 (Kumar and Purohit, 2013), FANCD1/BRCA2 (Doss and Nagasundaram, 2014), K-Ras4B (Lu et al., 2016), and EZH2 (Aier et al., 2016).

The HSA domain of the ATPase subunit is an important connecting domain of ATPase with the Actin-related dimer and thus forms the Arp-HSA sub-complex. We could identify around 12 cancer-associated missense mutations in the ~75 residue long HSA domain of the human ATPase subunit, BRG1. In the present study, we investigate the impact of missense mutations in the HSA domain on the structural assembly and dynamic behavior of Arp-HSA sub-complex (consists of HSA domain of BRG1, β-actin and BAF53A) using computational mutation screening followed by MD simulation studies. Collectively, our investigation identifies seven deleterious mutations (R466H, R469W, Y489C, K502N, R513Q, R521W and R521P) on the HSA domain of BRG1 and the MD simulations of the wt and six selected mutations indicate the distinct effect of each mutation in destabilizing the Arp-HSA sub-complex.

2. Materials and methods

2.1. Homology modeling of human β-actin-BAF53A-HSA complex

The structure of human Arp-HSA complex was obtained by homology modeling approach based on the yeast Arp-HSA complex (PDB ID: 4I6M) (Schubert et al., 2013). The ortholog assignment of human β-actin and BAF53A with yeast Arp proteins, Arp7 and Arp9, respectively were done based on interaction analysis using Protein Interaction Calculator (PIC) (Tina et al., 2007) and residue conservation status. Ten models for the human β-actin-BAF53A-HSA complex were generated using Modeller v9.16 (Sali and Blundell, 1993) and, the best model was selected based on modeler DOPE score and validation analysis using RAMPAGE and Verify3D (Lovell et al., 2003; Bowie et al., 1991; Lüthy et al., 1992).

2.2. Identification of cancer-associated mutations in the HSA domain of BRG1

The COSMIC v81 database was used to identify the cancer-associated mutations in the HSA domain of human BRG1 (Forbes et al., 2017). Functional effects of the mutations were predicted using PredictSNP, which is a consensus classifier based on eight prediction tools (MAPP, nsSNPAnalyzer, PANTHER, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT and SNAP) (Bendl et al., 2014). Conservation status of the mutated residues was analysed based on multiple sequence alignment (Sievers et al., 2011).