Sam domains as drug targets in multiple diseases

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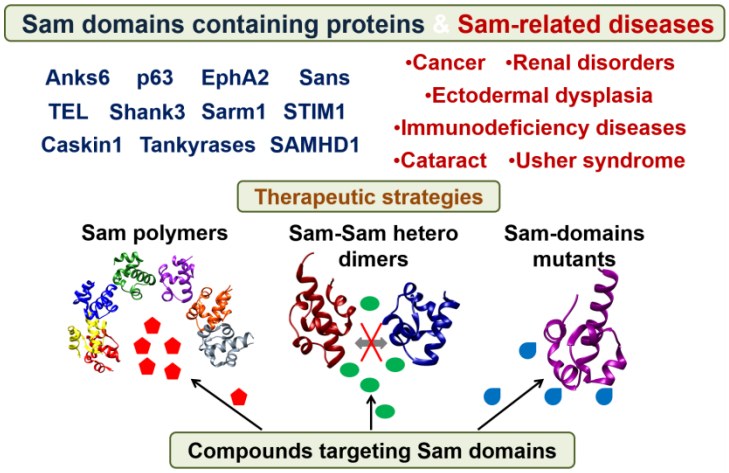

Graphical abstract

Sam domains appear to be linked, directly or indirectly, to many diseases. Small molecules or peptides targeting these domains may represent the pillars to build novel therapeutic strategies.

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Background: The sterile alpha motif (Sam) domain is a small helical protein module able to undergo homo- and hetero-oligomerization as well as polymerization thus forming different types of protein architectures. A few Sam domains are involved in pathological processes and consequently, they represent valuable targets for the development of new potential therapeutic routes. This study intends to collect state-of-the-art knowledge on the different modes by which Sam domains can favor disease onset and progression. ***Methods***: This review was build up by looking throughout the literature for: a) the structural properties of Sam domains, b) interactions mediated by a Sam module, c) presence of a Sam domain in proteins relevant for a specific disease. ***Results***: Sam domains appear crucial in many diseases including but not limited to cataract, cancer, renal disorders. Often pathologies are linked to mutations directly positioned in the Sam domains that alter its stability and/or affect interactions that are crucial for proper protein functions. In only a few diseases the Sam motif plays a kind of "side role" and cooperates to the pathological event by enhancing the action of a different protein domain. ***Conclusion***: Considering the many roles of the Sam domain into a significant variety of diseases, more efforts and novel drug discovery campaigns need to be engaged to find out small molecules and/or peptides targeting Sam domains. Such compounds may represent the pillars on which to build novel therapeutic strategies to cure different pathologies.

Keywords: Sam domains, drug discovery, structural biology, protein-protein interactions, diseases, therapeutic routes

1. INTRODUCTION

In the last two decades many scientists have striven to clarify as much as possible the mechanism of action of the sterile alpha motif (Sam) domain [1-6]. The reason lies on the many different functions that this protein module can play [2, 5, 7]. Furthermore, uncorrected expression and activity of proteins containing Sam domains have been correlated to diverse types of diseases such as cataract, cancer, hematological malignancies, neurological disorders [7-11]. Within this review, we focused our attention on pathologies that are reported as critically dependent on this domain. The aim of this work is to collect state-of-the-art knowledge about the different modes by which Sam domains can favor disease onset and progression. These data are pivotal for the development of new potential therapeutic routes targeting Sam domains.

1.1 Sam domains structural features

Sam domains are small protein interaction modules present in most of eukaryotic genomes (ranging from yeast to human)[2, 6]. A recent review reports on Sam domains from plants and on how their functions are starting to be elucidated [1].

The Sam domain owes its name to the presence in yeast proteins linked to sexual differentiation and to the high helical content [5]. Sam domains are considered rather versatile as concerning their interaction preferences and consequently their functions [2, 6]; their structures are composed by approximately seventy amino acids mostly organized in a five helix bundle, with the α3 helix shorter, as revealed by NMR and crystallographic studies [2, 4, 12-13] (Fig. 1A). The Sam domains of the tumor suppressors DLC1 (Deleted in Liver Cancer 1) [14] and DLC2 (Deleted in Liver Cancer 2) [15] constitute two exceptions by folding in a four-helix bundle (Fig. 1B, C).

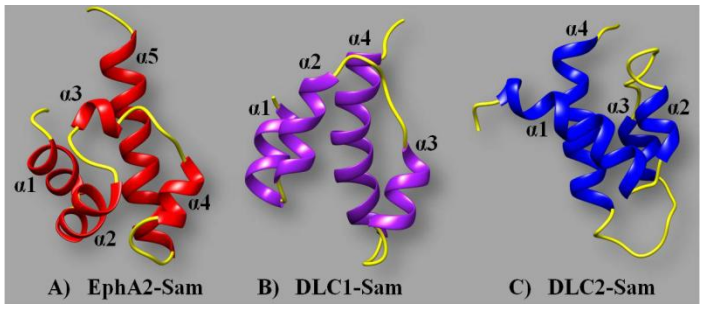

Fig. (**1**). Examples of Sam domain fold with a five (A) or four (B, C) helix bundle: (A) EphA2-Sam (Protein Data Bank (PDB) entry: 2E8N by RIKEN Structural Genomics/Proteomics Initiative 2007/2008). (B) DLC1-Sam (PDB entry: 2KAP [14]), (C) DLC2-Sam (PDB entry: 2H80 [15]).

1.2 Sam-Sam interactions

Sam domains are known for their ability to form homo- and hetero-oligomers or polymers with different structural arrangements [3-4, 13, 16-17].

Tail-to-tail and head-to-head kind of interactions have been rarely described for Sam-Sam associations [17]. The most common Sam-Sam structural arrangement is indeed the head-to-tail one [18], also known as Mid-Loop(ML)/End-Helix(EH) model [4]. In a ML/EH complex, the ML surface,

including the middle portion of one Sam domain, and the EH surface, composed by the C-terminal α5 helix and adjacent loop regions in another Sam domain, bind each other [19-22]. A few examples of different proteins provided with dissimilar functions whose Sam domains associate according to a head-to-tail structural model are given below.

Ankyrin repeat and Sam domain-containing 3 (Anks3) is a protein linked to renal development which contains a Sam domain close to the C-terminus [23-24]. Anks3-Sam domains interact homo-typically by forming a polymer with a ML/EH architecture [25]. The crystal structure of the Anks3-Sam polymer shows a structural helical topology composed of eight Sam domains per turn [25]. Moreover, Anks3-Sam interacts hetero-typically with the Sam domain from the protein Anks6 by adopting the ML/EH interaction mode (Fig. 2) [25]. In detail, the EH surface of Anks3-Sam binds the ML surface of Anks6-Sam (Fig. 2). This Sam-Sam heterotypic interaction has been correlated with Polycystic Kidney Disease (PKD), consisting on development and growth of multiple cysts within the kidney [26-27].

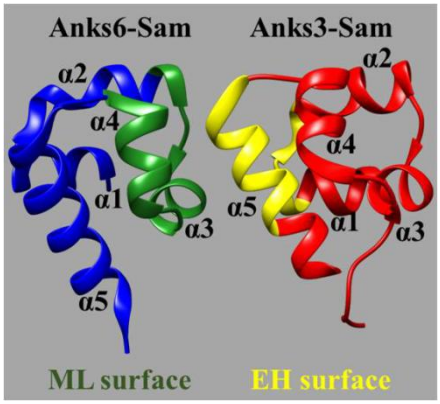

Fig. (**2**). Crystal structure of the Anks6-Sam/Anks3-Sam complex (PDB entry: 4NL9 [25]). The Sam domains of Anks6 and Anks3 are colored blue and red, respectively. The ML surface of Anks6-Sam (residues from Glu27 to Gly47) is highlighted in dark green whereas the EH interface of Anks3-Sam (residues from Gln17 to Lys22 and from Leu52 to Ser61) is reported in yellow.

The neuronal scaffolding protein AIDA-1 contains two Sam domains in tandem which associate according to the ML/EH model, and possible disengage from each other to allow protein translocation to the nucleus [28].

The Sam domain of the receptor tyrosine kinase EphA2 (Erythropoietin-Producing Hepatocellular receptor tyrosine kinase class A2) binds the Sam domain of the lipid phosphatases Ship2 (Src homology 2 domain-containing phosphoinositide-5-phosphatase 2) [19, 29] and the first Sam domain of the adaptor protein Odin [21] by forming dimers with the ML/EH topology in which the receptor provides a positively charged EH interface. The interaction between EphA2-Sam and Ship2-Sam is important to regulate receptor endocitosys and degradation [30] and has a relevance in anticancer drug-discovery [29]. Similarly the Sam domain of the PI3K (Phospatidyl-Inositol 3 Kinase) effector protein Arap3 (Arf GAP, Rho GAP: Ankyrin repeat and PH domain 3) possesses a Sam domain that is also able to interact with Ship2-Sam [20] and Odin-Sam1 [22] by forming discrete dimers in solution with a similar ML/EH organization: the C-terminal α5 helix of Arap3-Sam and adjacent loops constitute the EH interface.

Another well-studied example arises from the Sam domain of TEL (Translocation Ets Leukemia), a protein relevant in human leukemias [31]. Its Sam domain forms a helical polymer with repetitions of ML/EH arrangements [32].

Very recently a new mode of interaction has been revealed by NMR and crystallographic studies related to the oligomers of the neuronal scaffolding protein caskin2 [33]. Two Sam domains in tandem are present in caskin2; the minimal interaction unit is a dimer and all intra- and inter-molecular contacts follow the ML/EH topology of binding [33].

1.2.1 Sam interactions with RNA and lipids

The Smaug protein from Drosophila melanogaster and its homolog Vts1p from Saccharomyces cerevisiae are characterized by a Sam domain with the ability to bind particular RNA hairpins with specificity and high affinity and, therefore acting as post-transcriptional regulators [34-38].

A few Sam domains possess lipid binding properties [39-42]. This is the case of KIAA0725p [40], a protein belonging to the intracellular phospholipase A1 family whose Sam domain has ability to bind phosphoinositides. Another interesting example is given by p73 and p63 that are members of the p53 gene family of transcription factors [43]. The α variants of p63 and p73 have a Sam domain able to interact with monosialotetrahexosylganglioside (GM1) micelles and artificial lipid membranes, respectively [39, 42]. P73 binds both anionic lipids like PA (phosphatidic acid) and zwitterionic lipids such as PC (phosphatidylcholine) [39].
Similarly, binding between the Sam domain of the protein DLC2 with 1,2-dimyristoyl-sn-glycerol-3-phosphatidylglycerol (DMPG) phospholipids and micelles made up of sodium dodecyl sulfate (SDS) was also proved by NMR (Nuclear Magnetic Resonance) and CD (Circular Dichroism) thus suggesting that the Sam domain may be important to target DLC2 to membranes [41].

1.3 Sam domains and diseases

As described in the previous paragraphs, Sam domains act significantly in different biological contexts. The Smaug family of RNA interaction proteins modulates mRNA translation and degradation by targeting specific mRNAs sequences [34]. Furthermore, a Sam domain is important for the initiation of Store-Operated Calcium Entry (SOCE)[44]. This last mechanism is a necessary step to regulate luminal Ca^{2+} levels and is mediated by the Sam domain of STIM1 (Stromal interaction molecule-1)[44].

It is also well known that an incorrect activity or abnormal expression levels of proteins containing Sam domains are often associated with several routes of pathogenesis. This is

the case of TEL whose Sam domain-mediated polymerization is essential for cell transformation in many hematological malignancies [45].

Most neurodegenerative disorders are characterized in their early features by axon degeneration that can also be provoked by nerve injury through a mechanism called "Wallerian degeneration" [46]. The pro-degenerative molecule Sarm1 (sterile and HEAT/Armadillo motif containing protein 1) is required for fast Wallerian degeneration [11, 47] and contains a Sam domain, that plays a pivotal function in axon degeneration [11].

The Sam domain of the EphA2 receptor tyrosine kinase (EphA2-Sam) is possibly related to cancer through its interaction with the Sam domain of the lipid phosphatase Ship2 (Ship2-Sam) [7, 19]. Moreover, mutation in the Sam domain of EphA2 have been also related to eye disorders (i.e., cataracts)[9-10]. A few Sam domains have been linked to kidney pathologies (Anks6, Anks3, Bicc1)[48].

Due to their connections to a vast array of diverse diseases, Sam domains are protein modules with high potentials in drug-discovery.

2. RENAL DISORDERS

Inherited cystic kidney diseases represent a group of disorders with connected but distinct pathogenesis, characterized by the development of renal cysts along with several extrarenal symptoms [49]. Among them, the Autosomal Dominant Polycystic Kidney Disease (ADPKD) constitutes the most common pathology [50-51]. ADPKD is a Mendelian autosomal dominant disease [52] characterized by formation of fluid-filled cysts in both kidneys, ultimately causing kidney failure [51]. ADPKD can be also manifested by cysts in the liver, seminal vesicles, pancreas, and arachnoid membrane, and accompanied by disorders such as intracranial aneurysms, aortic root dilatation, mitral valve prolapse and abdominal wall hernias [50-51].

Recently the protein Anks6 has been implicated in human renal diseases [53]. Human Anks6 is composed by 871 amino acids organized in 11 ankyrin repeats at its N-terminus and a Sam domain near its C-terminus [25]. The Sam domain appears indeed crucial for Anks6 normal functions in both rats and humans [25]. Studies in the PKD/Mhm(cy/+) rat model pointed out that ADPKD is linked to the Arginine (R) 823 to Tryptophan (W) point mutation [25, 54]. The R823W mutation is positioned in the Sam domain of Anks6 [26, 54].

The Sam domain of the protein Anks3 has been identified as a direct binding partner of the Anks6 Sam domain [24-26].

Mutations in Anks3 have been seen in human patients affected by autosomal recessive laterality defects, that represent hallmarks of many ciliopathies [23].

Anks3-Sam can polymerize whereas Anks6-Sam binds to one side of the polymer [25] (Fig. 2).

Computational studies and molecular dynamic simulations indicate that the R823W point mutation destabilizes the Sam domain of Anks6 and negatively influences its association with Anks3 [54].

Bicc1 (Bicaudal C homolog 1) is an RNA-binding protein containing three KH (K-Homology) and two KH-like domains, as well as a C-terminal Sam domain [48]. Similarly to Anks6, polycystic kidneys have been related to mutations in the Sam domain of Bicc1 [53, 55-56].

Homopolymers are made up spontaneously by the recombinant Sam domain of Bicc1 [3] and Sam domain mediated polymerization has been observed also *in vivo* for the full-length Bicc1 protein [57]. Very recently, the crystal structure of the Bicc1 Sam polymer has been reported along with interaction studies with Anks3, and Anks6 [48]. Commonly to other Sam polymers, homotypic Sam-Sam interactions in Bicc1 follow the ML/EH model; a large structural flexibility can be notices in the polymer with uncommonly high dissimilarities in orientation between the different subunits [48] (Fig. 3).

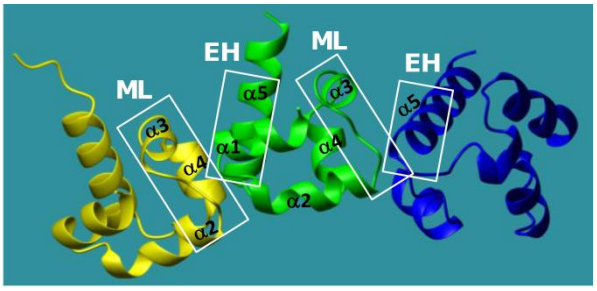

Fig. (**3**). Crystal structure of the Bicc1 Sam Domain R924E mutant (PDB entry: 4RQM[48]). The mutation destabilizes self association and allows to study in detail Bicc1 oligomers. The ML and EH interaction interfaces between different Sam subunits are highlighted.

Moreover Bicc1-Sam and Anks3-Sam associates [48]. Strong interactions occur also between Anks3-Sam and Anks6-Sam [48]. In sharp contrast, Bicc1-Sam, Anks6-Sam or their full length proteins, bind each other only poorly [48].

In this scenario, the three proteins, Bicc1, Anks3 and Anks6 together in a cooperative manner, form gigantic macromolecular complexes where Anks6 is engaged to Bicc1 through Anks3 [48].

It still remains to be investigated how the Bicc1-Anks3-Anks6 complexes and their scaffolding properties intervene exactly to modulate specific signaling pathways.

It would be interesting to develop inhibitors of homotypic or heterotypic Sam-Sam interactions involving Anks3, Anks6, and Bicc1 to study their outcomes in a cellular environment and better analyze their connection to ADPK or other kidney related diseases.

3. CATARACT

Cataract consists in the formation of clouds in the eyes lens with a consequent vision alteration. Cataract is known as an age-related disease that typically affects >50 years olders. In spite of enormous improvements in surgical treatment, cataract is still a primary cause of adult visual impairment (17%-33%) and blindness (33%-51%) worldwide [58-61].

Different genetic studies have shown the relationship between mutations in EphA2 and cataracts [9, 62-70]. EphA2 belongs to the ephrin family of receptors, i.e., the largest subfamily of receptor protein tyrosine kinases (RTKs) [71]. EphA2 is widely expressed in epithelial tissues and is surprisingly abundant in the plasma-membrane of the ocular lens, where it plays a pivotal function in lens cell migration and organization [72-73].

EphA2, along with other Eph receptors, possess in their cytoplasmic portion, at the C-terminus, a highly conserved Sam domain with a relatively poor tendency to self-associate [74](Fig. 4). However, this Sam domain utilizes the End-Helix interface in heterotypic Sam-Sam interactions [19, 21]. The End-Helix Interface in EphA2 is composed by residues in the $\alpha1\alpha2$ loop and by the N-terminal region of the $\alpha5$ helix (Fig. 4) [19, 21].

Interestingly, several cataract related mutations are positioned within or C-terminally to the Sam domain of EphA2 (Fig. 4). A few mutations are directly located in the End-Helix interface (Fig. 4) thus possibly interfering with recognition processes in which EphA2-Sam intervenes [10, 62, 70]. These mutations may also likely destabilize the 3D fold of EphA2-Sam [9, 62], however, to date detailed structural studies of mutant Sam domains have not been reported possibly due to difficulties connected in producing large amounts of stable proteins.

In detail (See Fig. 4), the c.2819C>T (or p.T940I) consists in replacement of Threonine (T) 940 by an Isoleucine (I) [70]; c.2915-2916delTG (or p.V972GfsX39) [70] includes mutation of Valine (V) 972 to Glycine (G), the deletion of the PDZ (Post synaptic Density disc-large Zo-1) binding site and insertion of a novel C-terminal polypeptide made up of 39 residues; c.2826-9G>A [70] is a splicing mutation also known as p.D942fsXC71, characterized by deletion of the C-terminal region in EphA2 (residues from Aspartic (D) 943 till I 976) and insertion of a novel 71 amino acids long C-terminal tail; the c.2842G>T mutation [75] consists in replacement of G948 by a Tryptophan (W) and finally the c.2875G>A [62] mutant (or p.A959T) is characterized by substitution of Alanine (A) 959 with T. The latter mutation is located in the EphA2-Sam EH interface and indeed included in the "KRIAY" motif, at the N-terminal of $\alpha5$ helix, that is important for its interaction with Ship2-Sam [76]. Similarly, the p.D942fsXC71 and the p.V972GfsX39 mutations highly affect the End-Helix Interface in EphA2-Sam and in addition, by destroying the PDZ binding motif of EphA2, may also impair EphA2 interactions in which the "IGI" motif at the C-terminal is involved [10, 62, 70].

Park and co-workers analyzed in details the functional effects of a few different mutations: c.2915-2916delTG, c.2819C>T, c.2842G>T, c.2826-9G>A [9] (Fig. 4). These mutant EphA2 proteins show a decrease in expression levels in HEK(Human Embryonic Kidney)293T and αTN4-1 cells, reduction of protein stability, and an increased propensity to aggregate respect to the wild-type EphA2 [9]. It has been speculated that these differences might be linked to a distorted conformation connected to malfunctioning protein synthesis or maturation [9]. The rapid degradation of EphA2 mutants is correlated to the ubiquitin-proteasome pathway [9]. In addition, following stimulation with an ephrin-A5 ligand, mutants and wild-type EphA2 exhibit comparable tyrosine phosphorylation although mutant proteins show a reduced capacity to activate Akt and induce ligand-independent cell migration [9].

In addition, in our laboratories we focused on a detailed structural investigation of the c.2915_2916delTG (p.V972GfsX39) EphA2 mutant [10]. Park and collaborators first analyzed GST-fused p.V972GfsX39 Sam mutant revealing its poor solubility compared to the wild-type protein [9]. We worked with a His-tagged Sam mutant construct and expressed it as recombinant protein in *E. coli* [10]. Purification trials indicated a clear propensity of the mutant protein to be proteolytically degraded starting from the novel C-terminal region [10]. Bioinformatics tools were employed to analyze the amino acid sequence of the EphA2 mutant. Molecular modeling along with molecular dynamics simulations and conformational NMR studies of peptides reproducing the whole or a fragment of the novel C-terminal extension in the (p.V972GfsX39) mutant, were conducted as well [10]. Results of these studies let speculate that the decrease of stability reported for the mutant protein [9], could be linked to the disorder introduced by the C-terminal extension [10]. The latter is predicted to possess a reduced number of residues belonging to ordered secondary structure elements, and a very low number of amino acids capable of playing a stabilizing role of the whole 3D structure [10].
Experimental and computational evidences indicate that the tail should not directly affect the conformation of the End-Helix interface and interfere with EphA2-mediated Sam-Sam interactions [10].

Further studies focused on the subcellular localization of the mutant proteins in polarized epithelial cells and revealed that diverse mechanisms of action are adopted by the mutants to induce cataract formation [62]. *In vivo* EphA2 is concentrated to the cell membrane in lens fiber and epithelial cells [62]. The p.T940I and p.D942fsXC71 mutants, -ectopically expressed in both MDCK and Caco-2 epithelial cells- mislocalized to the perinuclear space and colocalized within the cis-Golgi apparatus, thus letting speculate an altered folding or glycosylation pattern [62]. Nevertheless, mislocalization of these mutant EphA2 proteins likely inhibit or retard their engagement to the cell membrane and harmfully affect intercellular contacts in the lens epithelium ultimately causing cataract [62]. On the contrary, the p.A959T, and p.V972GfsX39 mutant EphA2 proteins, similarly to the wild-type, concentrate to the cell periphery or cytoplasm [62] and thus, may be linked to cataract through a completely different pathway respect to the other mutants.

In the next few years much efforts need to be devoted to clarify in detail all the structural features characterizing these EphA2-Sam mutants as they may open a window of opportunities for novel cataract treatments.

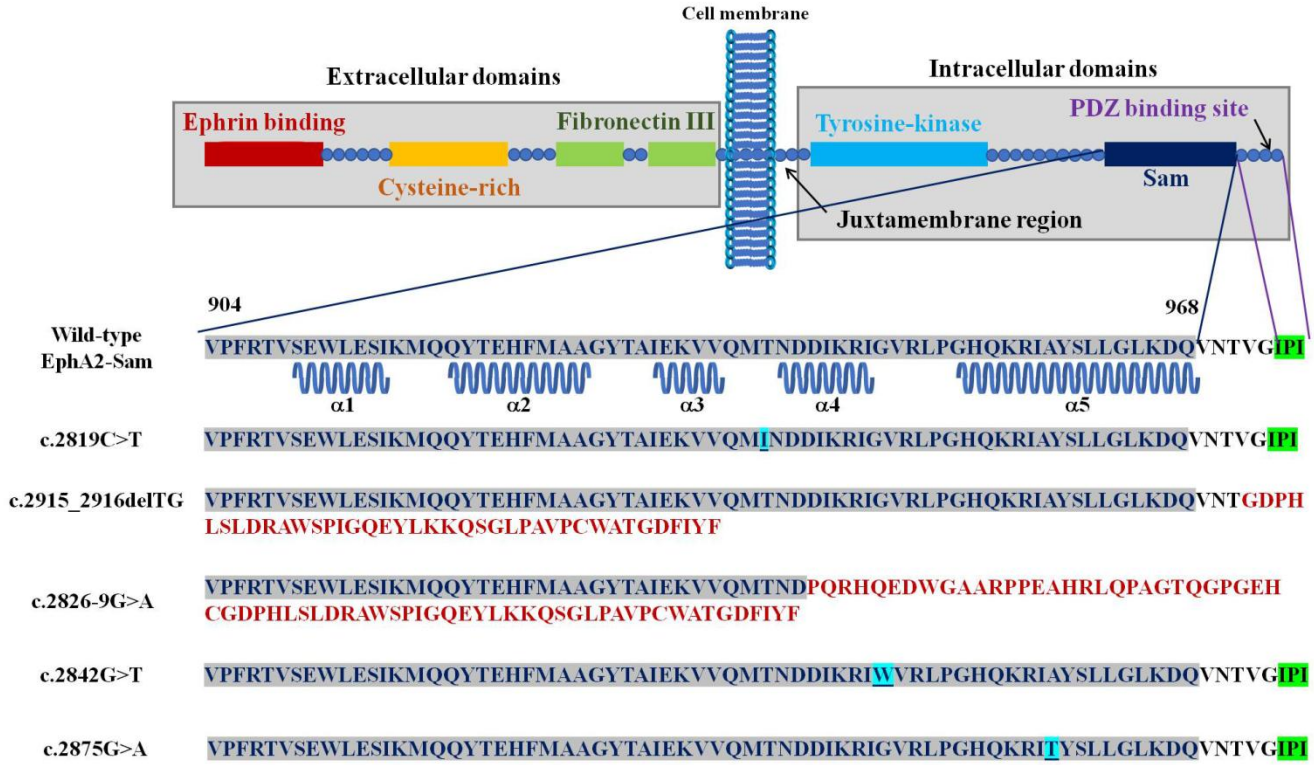

Fig. (**4**). Schematic representation of EphA2 domains (top) followed by EphA2-Sam wild-type amino acid sequence (UniprotKB[77] entry P29317 for human EphA2 receptor - residues 904-976 -) with its secondary structure elements (helices were positioned according to EphA2-Sam NMR structure pdb code: 2E8N). Examples of human cataract causing mutations in the EphA2 gene are shown as well. Single amino acid substitutions are highlighted with a cyan background. The Sam domain in each sequence is highlighted with a light gray background; novel C-terminal sequences are in red and the PDZ binding motif is highlighted green.

4. CANCER

Nowadays cancer represents one of the leading cause of mortality worldwide. The American Cancer Society estimated that -in 2018- 609,640 cancer deaths will arise in the United States while 1,735.350 novel cancer cases will be diagnosed.

Cancer occurs when cells start to reproduce abnormally and migrate to surrounding tissues. When cancer grows, old and damaged cells are not destroyed by the body but survive while new cells originate even if not needed. Cancer can start in every place in the body and can be represented by mass of tissues (i.e., solid tumors) or leukemias (i.e., blood cells cancers). Cancer is somehow accompanied by malignancy. Malignant tumors invade nearby tissues and a few cancer cells can even detach and use the blood or the lymph system to move towards remote places in the body where they can give rise to novel tumors (From the National Cancer Institute web pages at https://www.cancer.gov/about-cancer/understanding/what-is-cancer).

The connection between Sam domains and cancer has been extensively studied because of the various roles that this domain can play in biological processes critical for the rising and progression of this challenging pathology. In this section we will focus our attention on Sam domains that could be considered potentially novel targets to develop possible anticancer agents.

4.1 TNKS1/2 Sam domains

Tankyrase-1 (TNKS1) and its analogue TNKS2 are poly(ADP-ribose) polymerases (PARP) [78]. Tankyrases participate in a number of disease-relevant processes including pathways crucial for cancer cell growth [79].

PARP enzymes use NAD+ (Nicotinamide Adenine Dinucleotide +) as a substrate to generate ADP-ribose polymers on protein acceptors thus marking them for proteasomal degradation [80]. ADP-ribosylation regulates various distinct cellular events such as mitosis [81], glucose metabolism [82], DNA strand break repair [83], and Wnt (Wingless-related integration site) signaling [84]. The Wnt represents an old and evolutionarily conserved pathway

related to cell migration and polarity, cell fate determination, as well as neural patterning and organogenesis [85-86]. The Wnt/β-catenin pathway represents the signaling route regulated by TNKS1 and TNKS2 [87]. These two enzymes modulate axin levels; axin is a protein belonging to the β-catenin destruction complex, that induces degradation of the transcriptional coactivator β-catenin and enhancement of Wnt signaling [88]. Most of colorectal cancers are induced by dysregulation of the β-catenin destruction complex function [87].

Tankyrases possess a Sam domain, close to the catalytic domain, that oligomerizes *in vitro* and *in vivo* (Fig. 5) [89]. A structural model (supported by mutagenesis and NMR analysis) along with crystallographic studies of the Sam domain-mediated oligomerization of tankyrase were recently reported [89-90]. These structural studies show that TNKS-Sam domains self-associate giving rise to a helical head-to-tail polymer (Fig. 5) [89-90].

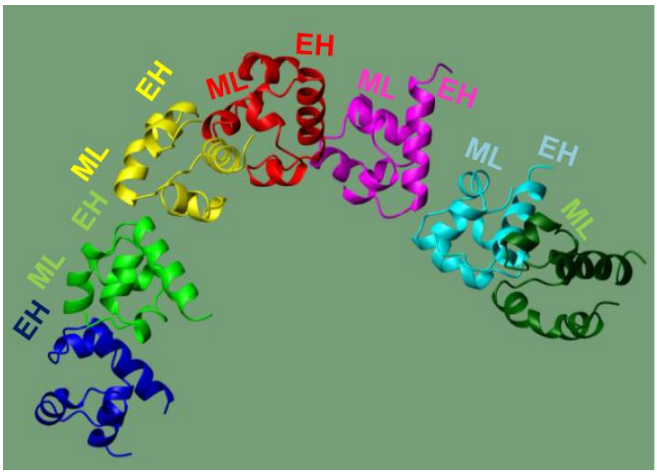

Fig. (**5**). Crystal structure of the human TNKS1 Sam Domain (PDB entry: 5KNI [90]). Seven Sam subunits are present in the asymmetric unit and made one full turn of the polymer. Each subunit interacts through ML and EH interfaces.

Moreover, TNKS1 and TNKS2 can form Sam-Sam hetero-oligomeric structures [89]. Many different cancer cells are characterized by abnormal levels of β-catenin due to an increased activity of TNKS1/2 [90]. A fundamental step for TNKS1/2 to play this action, is represented by its Sam-domain mediated polymerization that influences TNKS catalytic activity and opens access to cytoplasmic signaling complexes [89].

For this reason, Sam domains of tankyrase could be considered an interesting target for the development of novel therapeutics. In fact, molecules able to interfere with tankyrase polymerization may potentially result active against Wnt-dependent cancers.

4.2 TEL Sam domain

Hematologic malignancies are the so called "liquid tumors" and are pathologies that affect blood cells [91]. Blood cells are characterized by two lineages: 1) lymphoid cells producing T, NK, and B cells and 2) myeloid cells producing red blood cells, megakaryocytes, macrophages, neutrophils, basophils, and eosinophils [91]. The whole process of formation of cells in these two lineages is defined hematopoiesis [92]. These cells come up from pluripotent stem cells that, in turn, derive from differentiation of hematopoietic stem cells (or "blast cells") [92]. Each lineage generates related neoplasms. Indeed, there are lymphoid neoplasms, such as Acute Lymphocytic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL) and Hairy Cell Leukemia (HCL) [93-94], and myeloid neoplasms like Acute Myeloid Leukemia (AML), Acute Promyelocytic Leukemia (APL) and Chronic Myeloid Leukemia (CML) [95]. In general, leukemia begins in the bone marrow and moves from there to organs and tissues circulating through blood vessels [91]. Furthermore, leukemia has been associated to an accumulation of blast cells that were unable to differentiate and become functional cells [91].

Different hematological malignancies are characterized by chromosomal translocations that stimulate anomalous fusions of the TEL (Translocation, Ets, Leukemia) protein to a multiplicity of other proteins, including many tyrosine kinases and transcription factors [32, 45, 96-97]. TEL is a transcriptional repressor containing a Sam domain at the N-terminal end, a co-repressor binding domain in the central region and a DNA-interacting domain at the C-terminal side [45]. In chimera proteins, in which TEL is fused with kinase domains, Sam domain oligomerization leads to constitutive activation of the tyrosine kinase function and to cell transformation [45]. The transcriptional activator AML1 (Acute Myelogenous Leukemia1) when fused with TEL becomes a transcriptional repressor, and this function is determined by TEL-Sam oligomerization [45]. From this the interest on TEL-Sam as possible therapeutic target against hematological malignancies. The structural details behind TEL oligomerization were clarified a few years ago [32, 45]. TEL-Sam forms a helical polymer with an open-ended structure. The asymmetric unit of the crystal structure contains three TEL-Sam units (Fig. 6A). TEL-Sam monomers possess the helix bundle fold canonical of Sam domains; different units bind each other through the ML/EH model (Fig. 6B, C) [32, 45]. Many hydrophobic interactions occur at the interface between monomers in the more inner part of the structure whereas, salt-bridges can be found more externally [45]. It has been speculated that TEL polymerization -driven by the Sam domain- may represent a route for spreading repression along a wide chromatin segment through linkage of several DNA interaction modules together [45].

Structural studies revealed as well that the interacting Sam-Sam surfaces in TEL polymers lack deep pockets where to locate small compounds [32]. Therefore, although it seems very appealing to target TEL-Sam with small molecules in order to inhibit polymerization and eventually discover therapeutics against hematological malignancies, this might be a very challenging task.

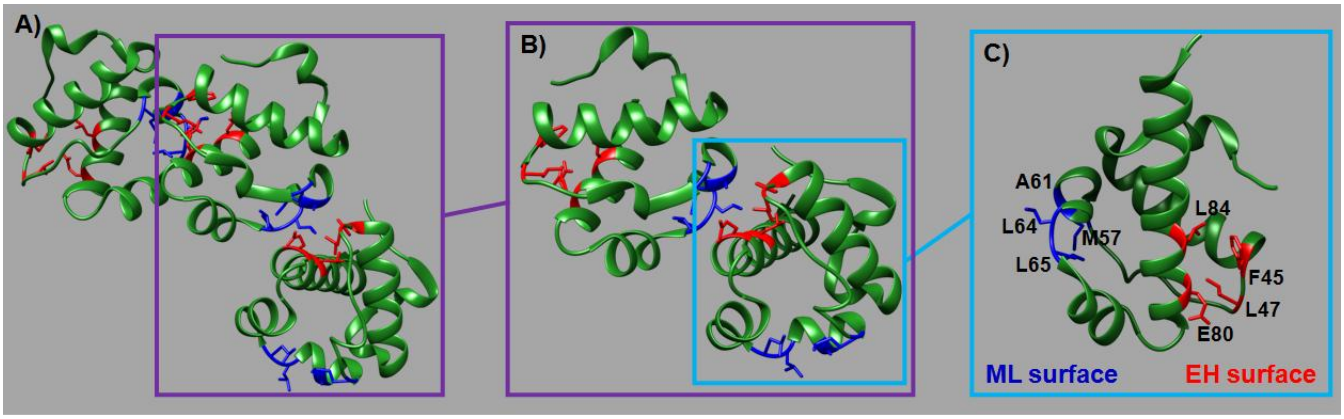

Fig. (**6**). Structural studies of TEL-Sam. (**A**) Crystal structure of the TEL-Sam polymer (PDB entry: 1JI7 [45]). To obtain a soluble form of the protein V80 in the wild-type sequence was mutated to E (Glutammic acid). (**B**) One dimeric unit in the polymer. (**C**) TEL-Sam monomer. A few residues belonging to the EH region and providing interactions at the Sam-Sam interfaces are highlighted in red (F45, L47, E80, L84); similarly, residues belonging to the ML surface and participating in the inner hydrophobic core are reported in blue (M57, A61, L64 and L65).

4.3 EphA2 Sam domain

The receptor tyrosine kinase EphA2 contains at the C-terminus a Sam domain with a canonical five helix bundle fold [7] (Fig. 1). The role of EphA2-Sam within receptor signaling has only recently started to be clarified and revealed its possible correlation to tumor progression and metastatic spread [98-99]. EphA2 levels are elevated in many solid tumors [100-102] such as glioblastoma [103], melanoma [104], breast [100], ovary [105], lung [106], prostate [107] and kidney [108] cancers. Nevertheless, EphA2 role in cancer is controversial as it can either induce and inhibit tumor progression [109-110] through a fine tuning of ligand-dependent and ligand-independent pathways [111]. In cancer cells the levels of ephrin ligands are often reduced [112-113] and EphA2 stimulates cell migration though a route linked to Akt (protein kinase B) [111, 114]. On the contrary, following interaction with an ephrin ligand, EphA2 activation suppresses cell migration [111]. It is well known that upon binding to an ephrin ligand, receptors of the Eph family like EphA2, clusterize, bringing their kinase domains in close contact and auto activating each other [113]. Auto-activation leads to tyrosine phosphorylation and consequent initiation of direct and/or reverse signaling [115].

Although it is clear that Sam domains often drive self-association through homotypic Sam-Sam interactions, this is not the case of Eph receptors for which a low aggregation tendency has been reported [74]. In fact, Eph signaling is not inhibited by deletion of the Sam domain [116] thus letting speculate that Sam domains are not responsible alone for receptor clustering [74]. Indeed, studies in different cancer cell lines have recently also demonstrated that the Sam domain in EphA2 decreases oligomerization and inhibits kinase activity [98-99].

EphA2-Sam acts as docking site for other proteins provided with Sam domains like the lipid phosphatase Ship2 [19] and the adaptor protein Odin [21].

In malignant MDA-MB-231 breast cancer cells the lipid phosphatase Ship2 inhibits EphA2 receptor endocytosis and consequent degradation upon ligand stimulation [30]. Ship2 contains a C-terminal Sam domain and exerts its regulatory role towards EphA2-Sam endocytosis through an heterotypic Sam-Sam interaction with the receptor [30]. Ship2 enzymatic activity is involved in regulation of EphA2 endocytosis and this function is related to Rac1 (Ras-related C3 botulinum toxin substrate 1) GTPase activity and modulation of PIP3 (Phosphatidylinositol(3,4,5)-trisphosphate) cellular levels [30]. Heterotypic association between Ship2-Sam and EphA2-Sam has been widely characterized by several biophysical techniques [19, 29]. The two proteins interact with low micromolar affinity and form a dimer with a highly dynamic ML/EH structural topology (Fig. **7**) [19, 29]. EphA2-Sam forms a positively charged EH Interface while Ship2 contributes a negatively charged ML interaction surface (Fig. **7**) [19]. Interestingly, in HEK293 cells an EphA2-Sam mutant unable to interact with Ship2-Sam is hypersensitive to ligand-stimulation, is rapidly phosphorylated and presents highly improved degradation rate with respect to wild-type EphA2 [29]. In contrast, an EphA2-Sam mutant with improved binding affinity for Ship2-Sam respect to the wild-type protein possesses lower degradation rate connected to the higher stability of the EphA2-Sam/Ship2-Sam complex [29]. *In vitro* cell studies demonstrated also the ability of Ship2 to enhance the ligand-independent migration property of EphA2 [29]. As ligand-independent EphA2 signaling represents a pro-oncogenic route [111], pro-oncogenic effects in cancer cells should be expected as a result of the Ship2-Sam/EphA2-Sam association [29]. In this contest, compounds capable of breaking the Ship2-Sam/EphA2-Sam complex should likely be provided with anticancer therapeutic properties.

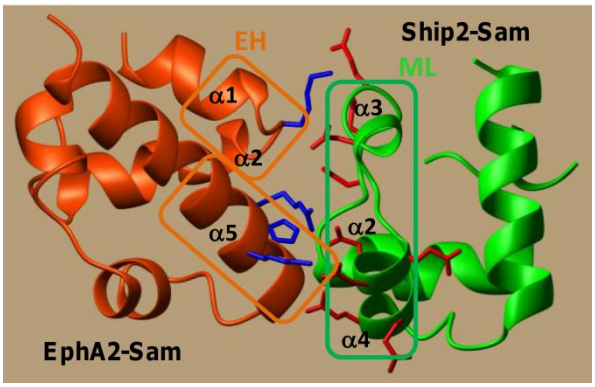

Fig. (**7**). NMR structure of the EphA2-Sam/Ship2-Sam complex (PDB entry: 2KSO[29]). The EH Interface in EphA2-Sam includes the N-terminal region of the α5 helix and the α1-α2 loop. The ML surface in Ship2-Sam includes the α3 and α4 helices, partially the C-terminal region of α2 and the corresponding interhelical loops. Side chains of positively and negatively charged residues in the EH interface of EphA2-Sam and ML interface of Ship2-Sam are colored blue and red respectively.

In addition, EphA2-Sam interacts with the protein Grb7 (Growth factor receptor-bound protein-7) [117]. Grb7 plays a role in integrin signaling pathway and cell migration [118]. In major details, studies with synthetic EphA2-Sam domains, containing phosphorylated tyrosines, indicate that the SH2 (Src Homology 2) domain of Grb7 may be engaged by the tyrosine phosphorylated EphA2-Sam in proximity of its Ship2-Sam binding site [117]. Thus, if Grb7 levels in cell are elevated, Ship2-Sam could be pulled away from EphA2-Sam thus ending its inhibitory action towards receptor endocytosis and consequent degradation [117].

Arap 3(Arf GAP, Rho GAP, Ankyrin repeat and PH domain 3) is a PI3K effector protein acting in cell spreading, formation of lamellipodia and modulation of the actin cytoskeleton [119-120]. Arap3 contains a Sam domain able to associate with Ship2-Sam through a heterotypic ML/EH Sam-Sam interaction [20]. EphA2 and Arap3 compete indeed for the same interaction interface of Ship2-Sam [20]. To date it's unclear if in a cellular context, Arap3 may also pull Ship2-Sam away from EphA2-Sam thus hampering Ship2-mediated inhibition of EphA2 endocytosis.

Odin is a protein belonging to the ANKS (Ankyrin repeat and Sam domain containing) family and has two tandem Sam domains. Sam domains from Odin are involved in increasing EphA2 receptor stability, by possibly interacting with ubiquitinated receptor [121]. A more recent work reports an interaction between the EphA2 kinase domain and Ankyrin repeats from Odin [122]. This study highlights that Odin plays a role in breast tumorigenesis by supporting the exit of the EphA2/ErbB2 *(*avian erythroblastosis oncogene B2) complex from the ER (Endoplasmic Reticulum) [122]. In our laboratories, *in vitro* studies with isolated Sam domains, revealed that the first Sam domain of Odin (Odin-Sam1), which has relatively high sequence homology with Ship2-Sam, interacts with EphA2-Sam by forming a ML/EH dimer with identical structural topology as the Ship2-Sam/EphA2-Sam complex [21]. The biological relevance of the Odin-Sam1/EphA2-Sam association in cancer cells is presently not established and it is worth noting that, when dealing with full length proteins, Odin-Sam1 could even bind the adjacent Odin-Sam2 domain rather than EphA2-Sam.

In the past few years attempting to discover novel anticancer therapeutics, we designed different peptide inhibitors of heterotypic Sam-Sam interactions involving EphA2-Sam. We first adopted protein dissection strategies and thus, analyzed isolated peptide fragments, of different length, encompassing the reciprocal interaction regions of EphA2-Sam, Ship2-Sam and Odin-Sam1 [76, 123-124]. We also focused on helical peptides either enriched with charged residues and amino acids with helical propensities [125] and stapled peptides. Our studies stressed out the challenges related to discover an efficient inhibitor of Sam-Sam interactions endowed with selectivity and high binding affinity. However, recently we identified a penta-amino-acid motif in EphA2-Sam EH interface that, when repeated three-times in tandem, gives appreciable binding to Ship2-Sam and is more cytotoxic to prostate cancer cells (PC3) than to normal dermal fibroblasts [76].

It is also worth noting that a search in the cosmic database (catalogue of somatic mutations in cancer; http://cancer.sanger.ac.uk/cosmic/) evidenced a few mutations in the Sam domain of EphA2 related to cancer [126].

5. ECTODERMAL DYSPLASIA

Ectodermal Dysplasia (ED) includes a group of more than 192 different syndromes characterized by abnormalities of the ectoderm [127]. The ectoderm is one of the three main germ layers that are formed during the early stages of embryogenesis [128]. The ectoderm gives origin to [128]:

- Epidermis and its appendages (hair and nails);
- Tooth enamel;
- Sensory epithelia of the eye, ear, and nose;
- Central nervous system (spine and brain) and peripheral nervous system;
- Mammary glands, hypophysis and subcutaneous glands.

Ectodermal dysplasias are characterized by defects in the development and function of two or more ectodermal-derived structures [129].

In general, all ectodermal dysplasias are a consequence of mutations or deletions into precise genes located on different chromosomes [130]. For example, mutations in the TP63 gene are responsible for EEC (Ectrodactyly–Ectodermal dysplasia–Cleft) [131], AEC (Ankyloblepharon-Ectodermal dysplasia-and Cleft lip/palate) [132] and Rapp-Hodgkin syndromes [133], which are among the most common forms

of EDs. The TP63 gene is also linked to CHANDS (Curly Hair-Ankyloblepharon-Nail Dysplasia Syndrome) or Baughman syndrome that represents a subtype of AEC [134]. EEC is characterized by several symptoms like limb malformations and orofacial clefting [135]. AEC syndrome can be represented by strings of tissue entirely or partly fused between the upper and lower eyelids, sparse wiry hair, skin alterations, nail changes, dental modifications, and subjective decrease in sweating capacity [134]. It is also associated with cleft on lip and/or into palate [134]. Furthermore, different kinds of craniofacial modifications are described in patients affected by AEC syndrome [134]. Concerning the Rapp-Hodgkin and Baughman syndromes, there is an overlapping of clinical manifestations if compared to AEC syndrome [136].

As introduced before, mutations in the TP63 gene have been described as responsible for EEC, AEC, Rapp-Hodgkin and CHANDS syndromes. This gene encodes for the p63 protein that together with p73 [138] and p53 [139] constitute the p53 family of transcription factors.

The p63 protein is involved in the regulation of different processes such as the initiation of epithelial stratification from the undifferentiated embryonic ectoderm [137], in adult stem/progenitor cell regulation [137], apoptosis [138] and control of oocyte integrity [139].

P63 is present in different isoforms with molecular weights ranging from 44 to 77 kDa [140-141]. The p63 isoforms are characterized by different numbers of domains (Fig. 8) [140]. Diverse isoforms possess a transactivation (TA) domain or its truncated version (ΔN variants) at the N-terminus, a DNA Binding Domain (DBD) and an Oligomerization Domain (OD) [140] (Fig. 8). The C-terminal end is more variable as a result of an intricate alternative splicing (Fig. 8). In fact, isoforms may be characterized or not by the presence of other two domains: a sterile alpha motif (Sam) and a transcription Inhibitory Domain (ID) (Fig. 8) [142].

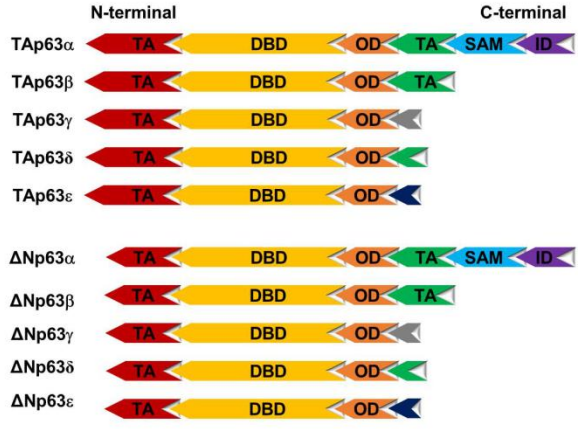

Fig. (**8**). Human p63 isoforms. The first transactivation domain (TA) is colored red. The ΔN forms present an N-terminal truncated TA domain. The DNA binding domain (DBD) is reported in yellow and the oligomerization domain (OD) in orange. The second TA - in its complete as well as shorter variants - is shown in green. Grey and blue are instead used for C-terminal regions in two different splicing variants of p63. The sterile alpha motif (Sam) and inhibitory (ID) domains in both α isoforms (TA and ΔN types) are reported in cyan and violet respectively.

AEC and Rapp-Hodgkin syndromes are correlated to mutations in TP63 gene portion encoding for the Sam domain of p63α [143]. Sathyamurthy and collaborators conducted structural studies on the wild-type and mutant Sam domains of p63α thus analyzing the effects of mutations found in patients affected by AEC on the Sam domain fold and stability [144] (Fig. 9A,B).

Mutations in p63-Sam, that are linked to AEC, can be subdivided into two major groups [144] (Fig. 9C). The first group includes single point mutations affecting residues presented in the hydrophobic core of the Sam domain (i.e., I549T, F552S, L553F, L553V, C561G, C561W, F565L, I576T, L584P and I597T) (Fig. 9C) [144]. The second group comprises mutations of solvent-exposed residues (G557V, G569V, T572P, Q575L, S580F, S580P, S580Y, D583C, D583V, D583Y, P590L, F593S, R594P, G600V and G600D) (Fig. 9C)[144]. L553F, C561G, C561W, G569V, Q575 and I576T mutations seem to be more destabilizing for the protein [144]. As concerning L553F, it has been supposed that the novel phenylalanine (F553) in the hydrophobic core becomes too close to F552, probably causing a severe steric clash [144]. Similarly, the C561W mutation may destabilize the Sam domain through a steric clash in between the newly inserted tryptophan (W) and F593 [144]. Regarding the C561G mutant, the loss of a bulky thiol group leads to the creation of a hydrophobic cavity that, in turn, would potentially cause some rearrangement of the hydrophobic core of the Sam domain and consequently instability [144]. In the G569V mutant, destabilizing outcomes may derive by the insertion of a valine assuming an unfavorable conformation in a loop region (Fig. 9A) [144]. The mutation Q575L is located on the solvent-exposed surface and seems to cause destabilization as a leucine (L), in contrast to the wild-type glutamine (Q), is unable to form a few hydrogen bonds with the main-chain amides of both T571 and T572 [144]. It has also been pointed out that the side chain of T572 is involved in an N-cap hydrogen bond with the backbone amide of Q575 and thus, the substitution of this residue with a proline (i.e., T572P mutation) would destroy this interaction [144].

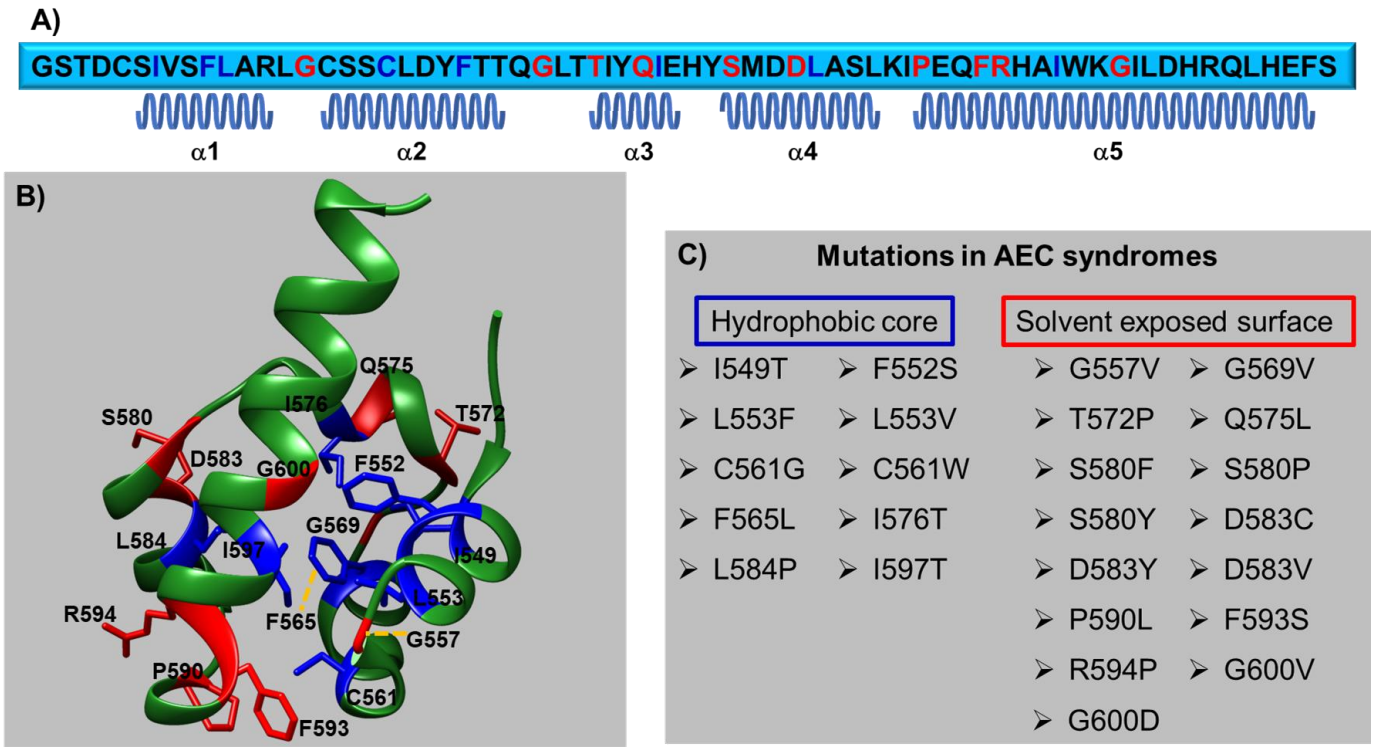

Fig. (**9**). p63-Sam and mutations identified in AEC affected patients. (**A**) Primary sequence of p63-Sam domain (from PDB entry: 2Y9U [144]). Amino acids are reported with the one letter code and position of the helices is indicated. (**B**) Crystal structure of p63-Sam domain (PDB entry: 2Y9U [144]). The side chains of residues substituted by mutations are colored blue -if belonging to the hydrophobic core- and red -if present on the exposed surface-. (**B**) List of mutations that can be associated with AEC syndrome [144-145]. Residue numbering is the same of the correspondent reference structure (PDB entry: 2Y9U [144]).

Another valuable example is given by the missense mutation c.1748A > T (named as c.1631A > T in the ΔN variant of α isoform) which produces the substitution of D583 (equivalent to D544 in the ΔN variant) by a valine [145]. In this case the mutation, found in a patient affected by a mild form of ED, causes disruption of a hydrogen bond network that stabilizes the conformation of the protein [145].

It is clear that most of the mutations in p63-Sam, that are related to the AEC syndrome, are perturbing the 3D fold of the Sam domain [144] and consequently producing pathological outcomes.

The search of small molecules able to bind p63-Sam mutant forms and stabilize its structure could be an interesting route to pursuit in the search of novel drugs against AEC syndrome.

6. X-LINKED MENTAL RETARDATION (XLMR)

X-linked mental retardation (XLMR) is an intellectual disability that consists in intelligence below average level and absence of essential abilities needed in everyday life such as communication, self-care, socialization skills, functional academic skills, work, leisure, health, and safety [146-147]. XLMR seems to be associated to mutations on the X chromosome because affects males more than females [147]. XLMR diseases can be classified into two groups: S-XLMR (or syndromic XMLR) that comprises all forms with the mental retard accompanied by dysmorphic features, malformations or neurological abnormalities; NS-XLMR (or non-syndromic XMLR) group which is manifested just with mental retard [147].

The CASK gene, which encodes for the CASK (Calcium/Calmodulin-dependent Serine Kinase) protein, plays a role in XLMR and in fact, in humans, many different CASK mutations have been reported and linked to mental retardation [148].

CASK is a member of membrane-associated guanylate kinase (MAGUK) protein family. MAGUK family intervenes in diverse processes including synapse generation, brain development, and the regulation of epithelial cell polarity [149]. To achieve these different roles, CASK contributes to a vast array of protein-protein interactions [148].

CASK for example binds caskin1 (CASK-interacting protein 1) forming complexes that are involved in the organization of active zones of neural synapses, i.e. regions of neurotransmitters release [12]. Caskin1 is a large multi-domain protein that comprises six ankyrin repeats, an SH3 domain, two Sam domains in tandem (Sam1 and Sam2) and a proline-rich domain [150-151].

In addition, the CASK Interaction Domain (CID) in caskin1 is located between the SH3 and Sam1 [148]. In caskin1 the two tandem Sam domains associate intra-molecularly with a ML-EH topology where Sam1 and Sam2 provide the ML

(colored magenta in Fig. 10A) and EH (colored yellow in Fig. 10A) surfaces respectively. In addition, two other surfaces in each Sam domain may associate inter-molecularly -adopting again a ML-EH structural arrangement- leading to a caskin1 helical polymer (Fig. 10B, C). This helical polymeric organization includes four caskin1 tandem Sam domains (= a total number of eight Sam domains) per turn [12].

In the polymeric structure of caskin1 the positively charged surface of Sam1 (including K54, P55, G56, H57, K59, and K60, blue in Fig. 10B, C) interacts with the negatively charged surface of Sam2 (including D100, N101, G102, Y103, F108, E104, D116 and E119, red in Fig. 10B, C) of a second caskin unit [12].

Caskin1 polymerization enhances the interaction with CASK as each monomeric caskin entity may be "ornated" by CASK molecules [12]. Thus, caskin1 polymerization -mediated by its tandem Sam domains- represents a crucial step to support the construction of the presynaptic cytomatrix in the active zone [12]. To date still too little is known about caskin1. The design of molecules potentially able to either inhibit or favor Sam domain polymerization and modulate neurotransmitters release in patients affected by XLMR may represent a tool to clarify more precisely the link between caskin1 and this disease.

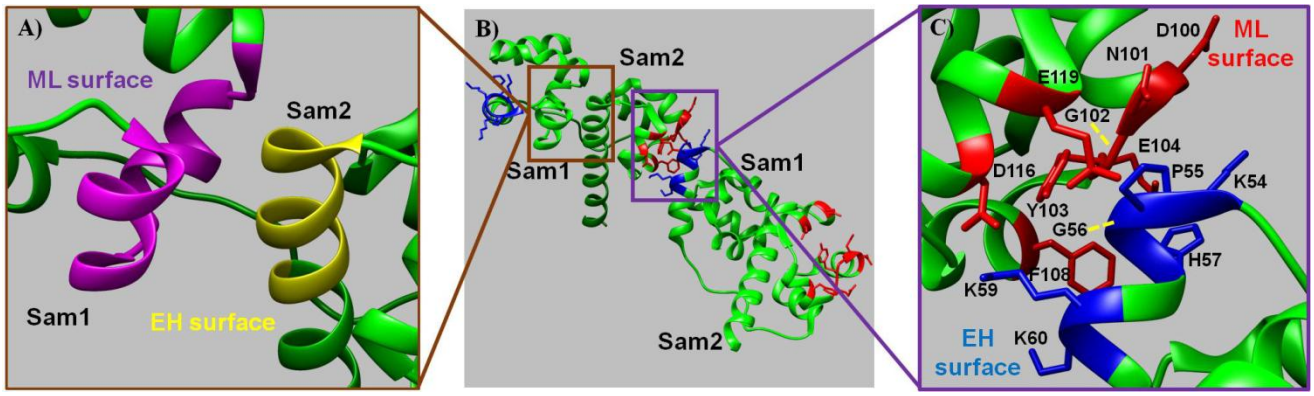

Fig. (**10**). Structural studies of polymers composed by the caskin1 tandem Sam domains (PDB entry code: 3SEN [12]). (**A**) Intramolecular ML-EH interaction between Sam1 and Sam2: the ML surface is colored in magenta and the EH surface is in yellow. (**B**) Caskin1 polymers with intramolecular and intermolecular interactions between Sam1 and Sam2 domains highlighted in the brown and violet rectangles respectively. (**C**) Intermolecular ML-EH like contacts into two adjacent caskin1 subunits: the side chains of residues contributing to the ML and EH interfaces in the caskin1 polymer are colored in red and blue respectively.

7. WALLERIAN-LIKE DEGENERATION

The axon is a part of a nerve cell responsible for the transmission of electrical impulses (or action potentials) from the nerve cell body of a neuron to other neurons, muscles, and gland [152-153]. Axon degeneration represents a highly specialized self-destructive pathway that, similarly to cell death, plays positive roles during development and in reaction to an injury [11]. Nevertheless, axon degeneration represents an important early hallmark of many neurodegenerative diseases [154]. The Wallerian degeneration, occurs when a nerve fiber is crushed/cut and the axon segment, that is far from the nerve's body, degenerates, while the part between the position of injury and the cell body (=proximal segment) remains intact [153].
Different diseases of the nervous system are characterized by an unusual or absent Wallerian-like degeneration with a consequent dysfunction of neuronal connectivity [155].
It has been demonstrated that the protein Sarm1 (Sterile alpha and TIR motif containing) plays a fundamental role in the activation of Wallerian degeneration [11]. Sarm1 belongs to the Toll-like receptor adaptor protein family [11], and contains a N-terminal peptide sequence by which associates with neuronal mitochondria, and three protein–protein interaction modules including two Sam domains (Sam1 and Sam2) and a Toll-interleukin-1 receptor (TIR) domain [11]. Mutational analyses, revealed that both Sam and TIR domains are necessary to promote elimination of injured axons [11]. In major details, it has been established that: Sarm1 multimerizes through its Sam domains; promotion of Wallerian degeneration requires intact Sam domains; the TIR domain is crucial to form functional complexes but, it is not necessary for multimerization [11]. These evidences lead to hypothesize that cooperation of Sam and TIR domains is required for Sarm1 activity in axon degeneration [11].
To date, at the best of our knowledge, no 3D structures of Sam domains from Sarm1 are deposited in the Protein Data Bank. More efforts are needed to gain a detailed structural knowledge of Sam domains in Sarm1 and fully understand the features essential for Sarm1 multimerization. Structural insights will be essential to fully understand how to target Sam domains of Sarm1 in pathological axon degeneration.

8. AICARDI-GOUTIÈRES SYNDROME

Aicardi-Goutières Syndrome (AGS) is a rare genetically determined disorder which is characterized by early-onset progressive encephalopathy, calcifications into basal ganglia and white matter as well as leukoencephalopathy and an elevated level of interferon-α (IFN-α) in the cerebrospinal fluid [156]. Children affected by AGS are subjected to progressive lost of psychomotor skills that subsequently lead to a vegetative state and death [156]. Studies conducted on

AGS revealed that the disease is largely due to the presence of mutations in precise genes (TREX1, RNASEH2A, RNASEH2B, RNASEH2C, and SAMHD1) which encode for nucleases or other proteins involved in the processing of specific DNA and RNA fragments [157-159]. The last gene, SAMHD1, encodes for the protein SAMHD1 (Sterile Alpha Motif and Histidine-Aspartate Domain 1) which cleaves ssDNA, and ssRNA [160]. In addition SAMHD1, which is a Mg^{2+}-dependent tetrameric enzyme, possesses also dNTPs (deoxynucleotide triphosphates) triphosphatase activity [161]. SAMHD1 plays a fundamental role in the pathway of innate immunity system in which it acts as restriction factor by contrasting replication of the HIV-1 genome in myeloid cells [162]. SAMHD1 is a 626 residues long protein with two domains: a Sam domain at the N-terminus and an HD domain at the C-terminus [160]. Studies on the nuclease activity of the entire human SAMHD1 protein and its isolated domains were carried out and coupled to large mutagenesis analysis [160]. The results of these studies revealed that only the HD domain but not the Sam domain alone possesses nuclease and dNTP triphosphatase activity [160].

Interestingly, the Sam domain has no conserved residues but substitution of semi-conserved Arginines within the exposed surface of the Sam domain (i.e., R69, R97, and R106 in pdb entry code: 2E8O) with Alanines is associated with a significant nuclease activity of the full length protein and ssDNA binding capacity, thus indicating that the HD domain may be involved in nucleic acid binding too [160].

Nevertheless, very recent structural studies on full-length mouse-SAMHD1 revealed prominent differences between human- *vs* mouse-SAMHD1 [163]. Nucleotides allosterically activate both mouse-SAMHD1 and human-SAMHD1 however, the mechanisms of active tetramers assembly of the two proteins follow different routes [163]. The Sam domain plays diverse roles in mouse-SAMHD1 with respect to human-SAMHD1[163]. In agreement with previous studies [160] the Sam domain in human-SAMHD1 is dispensable for dNTP hydrolysis and viral restriction, whereas it is needed for these functions of mouse-SAMHD1[163].

Differently from mouse-SAMHD1, human-SAMHD1 does not need the Sam domain for assembling a stable tetrameric catalytic core. In humans, the HD domain presents higher activity with respect to the full-length SAMHD1, thus indicating that the Sam domain act as a sort of inhibitor on the catalytic activity of human-SAMHD1[163].

Interestingly, most AGS-related mutations regard conserved amino-acids located in the HD domain. The AGS-linked mutations R143H, Q149A, M385V, and Q549A, induce a decrease of nuclease activity [160]. In AGS SAMHD1 works as an inhibitor of the innate immune response thus obstructing the immune response to endogenous nucleic acids [164].

In this intricate scenario, it may be fascinating to target the Sam-domain of SAMHD1 in AGS mutated forms with peptides and small molecules and analyze if the activity of the full length protein may somehow be modulated by Sam-domain ligands.

9. AUTISM SPECTRUM DISORDERS

The proper functioning of brain depends dramatically on creation and upkeep of synaptic contacts and is also strongly affected by synaptic plasticity [8, 165]. In this context the postsynaptic density (PSD), an intricate proteins assembly plays a fundamental role [166-167]. The PSD can be described as a disk-shaped dense ensemble containing more than 100 proteins, positioned on the postsynaptic side of neuronal synapses, that link glutamate receptors to different signaling components as well as members of the cortical cytoskeleton [166, 168]. Following precise neural activity, PSD varies its composition and structure to allow proper signaling towards cytoplasmic proteins [166].

Autism Spectrum Disorders (ASD) consist in a group of neuro-developmental diseases which are characterized by complex behavioral and cognitive deficits and are correlated with mutations of many PSD proteins [169]. Among the different components of PSD, Shank (or ProSAP) family is one of the higher order organizing elements [170]. In fact, Shank proteins work to assemble and organize the PSD by mutually linking different receptors and coupling them also to the cytoskeleton [171-172]. Shank proteins are positioned at excitatory synapses; in mouse, three genes belonging to the Shank family can be found (i.e., Shank1, Shank2 and Shank3)[173]. The genes encoding for Shank family members can be considered autism risk genes [169].

The Shank3 protein is the most widely studied family member [173]. Shank3 is characterized by different domains involved in protein-protein interactions: a DUF535 (Domain of Unknown function 535) module followed by an ankyrin repeat domain with six ankyrin repeats at the N-terminal end; a Src homology 3 (SH3) and a PDZ (Postsynaptic density protein 95 (PSD95)/discs large homologue 1/zonula occludens 1) domains in the central region; a proline-rich and a Sam domains at the C-terminus [173].

It has been demonstrated that Shank3 localization at PSD depends on its C-terminal end, that contains the Sam domain [167]. Moreover, it has been found that localization of Shank3 at PSD requires polymerization of its Sam domain into helical parallel and antiparallel fibers which form large sheets by a side by side stacking and by mediation of Zn^{2+} ions [166]. In detail, Zn^{2+}, which is abundant in PSD, stabilizes salt bridges between antiparallel Shank3 fibers (Fig. 11) [166]. The importance of these different fiber layers for the Shank3 localization at PSD was proved by introducing mutations in the Sam domain able to hamper sheet assembly (i.e., W5E, F8E, H22A, M56E and double mutations W5E/F8E, W5E/M56E, L47E/M56E, Fig. 11).

Helical fibers allow Shank3 to interact with both PSD proteins and cytoskeletal components, thus suggesting the importance of its Sam-domain in PSD assembly [8]. Different works have determined a correlation between mutations in Shank3 and Autism Spectrum Disorders (ASDs) but, most of these modifications are not located into the Sam domain [174-177]. However, interestingly, a ASD-linked mutation in Shank3 (i.e., InsG3680) seems to hamper

protein PSD localization by enconding for a Sam-domain deleted Shank3 protein [8, 178].

Again, molecules able to stabilize the Shank3-Sam multimeric assembly may be a valuable tool to better investigate its role in ASDs and clearly establish it as a therapeutic target.

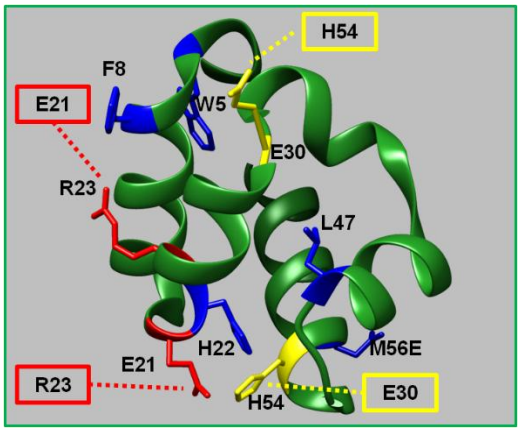

Fig. (**11**). Crystal structure of Shank3-Sam domain (PDB entry: 2F3N [166]). The mutation M56E allows protein crystallization. Residues fundamental for sheet assembly are reported in blue. Residues forming the intra- and inter-polymeric salt bridges stabilized by Zn^{2+} (ions not shown) are reported in yellow and red respectively.

10. SEVERE COMBINED IMMUNODEFICIENCY DISEASE (SCID)

Severe combined immunodeficiency disease (SCID) consists in a group of rare inherited pathologies characterized by a reduced or totally absent immunity system. SCID is associated to an improper development and/or function of the specialized white blood cells (B, T and natural killer cells) with a consequent increase in the chances to acquire an infection by viruses, bacteria and fungi [179]. Immunodeficiency is sometimes characterized by defects in Ca^{2+}-linked signaling in lymphocytes [180]. In fact, the proper activation of lymphocytes requires the influx of Ca^{2+} from the extracellular compartment, which is triggered by the release of Ca^{2+} from intracellular stores, a process that is mediated by Store-operated Ca^{2+}entry (SOCE) channels [180]. In the case of T cells, these channels are the "Ca^{2+}-release–activated Ca^{2+}" (CRAC) channels which are characterized by high Ca^{2+} selectivity and by an opening/closing mechanism regulated by the reduction of Ca^{2+} stores in the endoplasmic reticulum (ER) [180]. CRAC channels and the SOCE through them, are crucially regulated by two molecules: STIM1 (Stromal Interaction Molecule-1) and ORAI1 [180].

ORAI1 represents the pore-forming subunit of CRAC channels in the plasma membrane while, STIM1 perceives Ca^{2+} level in the ER and works as activator of ORAI1-CRAC channels [181]. Mutations in ORAI1/STIM1 weaken or annul CRAC channel function and provoke CRAC channelopathy and, from a clinical point of view, severe combined immunodeficiency, autoimmunity, muscular hypotonia, and ectodermal dysplasia [182].

STIM1 is a type-I transmembrane protein with 685 residues distributed among three regions: a N-terminal portion in the ER lumen which includes an ER signal peptide, two EF-hand domains and a Sam domain; a single transmembrane domain; a cytosolic region including coiled-coil domains (CC1, CC2 and CC3), a Pro/Ser-rich domain and a Lys-rich domain [44, 183].

STIM resting state is characterized by the EF-Sam close conformation due to the Ca^{2+} ions bound to the EF domain (Fig. 12). The decrease of Ca^{2+} levels into the ER lumen induces structural changes which move the EF away from the Sam domain and thus exposes hydrophobic surfaces on both EF and Sam domains giving rise to a rather unstable unfolded conformation [44, 183]. Next, to gain stability, STIM1 forms oligomers which translocate to the ER-PM (Plasma Membrane) junction where they associate into larger aggregates originating the so called STIM1 punctae [44]. As a consequence of store depletion and Ca^{2+} unbinding from EF, other conformational changes occur [183]. In this way STIM1 can be trapped at the ER-PM punctae where it ends up close to ORAI1 [183-184]. Thus, multimerization of STIM1 appears crucial for CRAC channels activation [183-184].

It has been reported that function-disrupting mutations of ORAI1 (p.R91W and p.A88SfsX25) and STIM1 (p.R429C, p.P165Q and p.R426C), found in human patients, impair CRAC channel and SOCE activities bringing to SCID-like diseases (characterized by increased susceptibility for the infections in a way similar to canonical SCID, although with a normal number of T cells and other lymphocytes) [184]. In this context, it is interesting to note that one out of three STIM1 loss-of-function mutations: p.P165Q (Fig. 12), is located in the Sam domain and close to residues L167 and T172 belonging to the hydrophobic core (Fig. 12) [184]. Mutagenesis analyses revealed the importance of L167 and T172 (Fig. 12) in the aggregation process of EF-Sam domains and consequently in STIM1 activation [44].

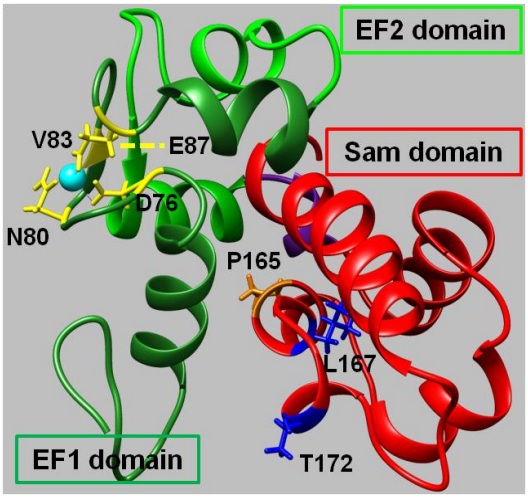

Fig. (**12**). NMR structure of calcium-loaded STIM1 EF-SAM (PDB entry: 2K60 [44]). The Sam domain is colored in red. The EF1 and EF2 domains are colored in dark green and light green, respectively. The short linker helix between Sam EFs domains is colored violet. Residues in the Sam domain, which are aggregation-stimulating, if substituted by arginine, are highlighted in blue (i.e., T172 and L167). Residue P165 whose mutation is linked to

SCID-like diseases, is shown in orange. The Ca^{2+} ion is represented by the cyan sphere and its coordinating residues are colored yellow.

On the opposite hand, in the STIM1 structure (PDB entry: 2K60 [44]), the P165 residue extends its side chain towards the EF-hand domain (Fig. 12) [184]. For this reason, the substitution of this residue by a glutamine is potentially able to interfere with the dimerization capacity of the EF-Sam domains and therefore hamper STIM1 activation and SOCE [184].

In the next few years the knowledge of additional structural insights will be essential to more precisely determine the mechanism by which the P165Q mutation abolishes STIM1 function and determines SCID-like disease. In this context it may be interesting to investigate in cell if compounds able to favor EF-Sam oligomerization may be implemented for the development of potential specific therapeutic routes against SCID diseases.

11. USHER SYNDROME I

Usher syndrome (USH) is a hereditary disease which is associated with a double sensory deprivation (auditory and visual) defined as deafblindness [185]. From clinical and genetical points of views, USH is described as a heterogeneous disease [186]. Indeed, this pathology can be characterized by three forms: USH1, with six associated genes (MYO7A, USH1C, CDH23, PCDH15, USH1G and CIB2); USH2, related to three identified genes (USH2A, USH2C, and USH2D); USH3 with only one associated gene (USH3A) [186-187].

Patients affected by the most severe form of USH (i.e., USH1), usually manifest complete deafness since birth or an almost complete hearing loss during the first few years of their life, or sensorineural hearing impairment [187]. These pathological conditions are later accompanied by the retinitis pigmentosa (RP) which is responsible for the gradual degeneration of retina photoreceptor [187].

MYO7A is the gene of USH1 encoding for the actin-based molecular motor described as carrier and positioner of the sensory hair cell stereocilia Upper Tip-Link Insertion (UTLI) complex and thus responsible for the tensing of the tip-link [187]. The central organizer of this complex is the PDZ domain-containing protein Harmonin, which is encoded by the USH1C gene [187-188]. The USH1G gene encodes for another UTLI scaffolding protein named "Scaffold protein containing ankyrin repeats and SAM domain" (or Sans) [189]. These along with other cadherin-related proteins [190-191], form an “interactome” in the inner ear and the retina, which play a fundamental role in the development, maintenance, and correct function of the sensorineural cells [187].

A considerable attention has been given to the Harmonin/Sans interaction [187]. Syndromic mutations found in human patients affected by USH1 syndrome are associated with a reduced stability of this complex [186]. Harmonin interacts with Sans through the first out of two PDZ domains which characterize all Harmonin isoforms [186].

Sans presents four ankyrin repeats at the N-terminus, a central region and a Sam domain along with a PDZ binding motif (PBM) at the C-terminus [186].

Concerning the Sans and Harmonin complex, it has been reported that the N-terminal (red in Fig. 13A) and PDZ1 (NPDZ1) (dark green in Fig. 13A) domains of Harmonin constitute, together with a C-terminal mini-domain (magenta in Fig. 13A), the structural and functional supramodule by which Harmonin can bind Sans with high affinity [186].

Sans participates to the complex with Harmonin by the synergic contribution of its Sam and PBM domains (= Sam-PBM) [186]. Hydrophobic and electrostatic interactions (Fig. 13B) are critical for the binding of Sans Sam-PBM to the NPDZ1 domain of Harmonin [186].

Interesting, the D458V (in the Sans Sam-PBM domain, Fig. 13B) and R103H (in the Harmonin PDZ1 domain, Fig. 13B) mutations have a disrupting effect on Sam-PBM/Harmonin interaction and are correlated with the deafblindness of patients affected by USH1 [186, 192].

In this context, it may be interesting to target the Sam-PBM/Harmonin interaction surface with small molecules and identify compounds able to stabilize this complex. Such compounds may represent potential therapeutic tools to hinder the negative effects of mutations, such as D458V and R103H found in USH1.

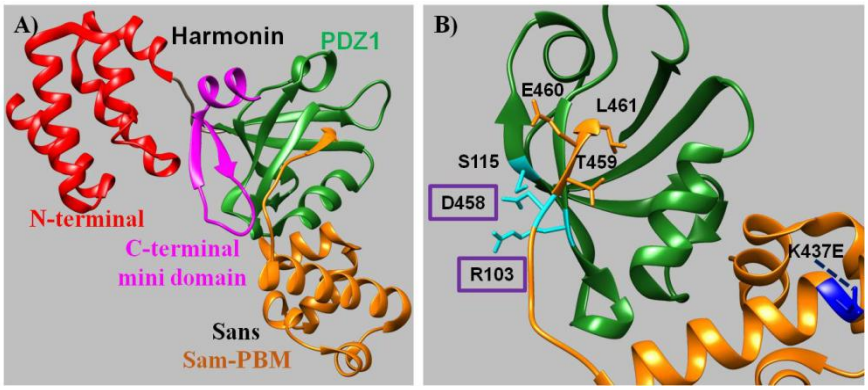

Fig. (**13**). X-ray structure of NPDZ1 domain of Harmonin in complex with the Sam-PBM domain of Sans (PDB entry: 3K1R [186]). (**A**) The N-terminal domain of Harmonin is shown in red, the PDZ1 domain in dark green and the C-terminal mini-domain in magenta. The Sam-PBM domain of Sans is reported in orange. (**B**) Structural details of the interaction surface between NPDZ1 and Sam-PBM. The side chains of residues of Sam-PBM (= T459, E460 and L461) mostly involved in binding to the PDZ1 domain of Harmonin are shown in orange as well. In addition, the residue D458 (colored in cyan) of Sam-PBM domain is involved into crucial electrostatic and H-bond interactions respectively with R103 and S115 (colored in cyan as well) located in the PDZ1 domain of Harmonin (dark green). The violet rectangle highlights residues mutated in patients with USH1 syndrome (D458 in Sam-PBM domain and R103 in PDZ1). In the Sam-PBM domain of Sans the K437E mutation (blue) was introduced to reduce aggregation effects.

CONCLUSION

This review focuses the attention on the pathologies that can be linked directly or indirectly to Sam domains.

The possible connection between cancer and the Sam-Sam interaction between the EphA2 receptor and the lipid phosphatase Ship2 has been well described [7, 19]. In addition, the presence of mutations in the Sam domain of EphA2 have been largely correlated to the formation of cataracts [9-10]. Sam domains of a few proteins, such as Anks6, Anks3, Bicc1 have been associated to renal pathologies [48]. The Sam module in the protein TEL is linked to hematological malignancies [45]. Moreover, most of the mutations in p63-Sam have a destabilizing effect on the 3D fold of this Sam domain and are related to the pathogenesis of AEC syndrome [144]. Another valuable example is given by the protein STIM1. Indeed, a deregulation of STIM1-Sam activity is correlated with SCID-like disease [44]. Nevertheless, Sam domains may be associated to neurological disorders as well [11].

It's worth noting that most Sam domains have shallow global surfaces along with large-dynamic interaction areas thus lacking pockets where to accommodate small molecules/peptides. These features make the search for selective and potent inhibitors of Sam-domain mediated interactions very challenging. However, due the relevance in so many diverse diseases, it is surely meaningful to put efforts in original drug discovery campaigns aiming at discovering Sam-domain ligands. Compounds able to interfere with homo- and hetero-typical interactions, in which Sam domains are engaged, may be implemented to design novel therapeutic strategies or be tested in cell as innovative molecular tools to better comprehend the role of Sam domains in the onset and progression of the above mentioned pathologies. Indeed, Sam-domain targeting molecules may have a tremendous positive impact on human health.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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