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ATP-ase as a Potential Drug Target for Cancer, Tumor Growth and Cellular Functions

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ABSTRACT ATP-ases are a group of enzymes that utilizes ATP hydrolysis, and the subsequent release of energy, to achieve a cellular function. The cellular functions involving ATP-ases are plentiful and diverse including initiation of DNA replication, DNA repair and remodeling, protein folding and chaperoning, protein degradation, intracellular transport, and ion transport. A large number of these enzymes represent attractive drug targets, and drugs targeting ATP-ases, such as proton pump inhibitors. Two families of molecular chaperones, heat shock protein 90 and heat shock protein 70, possess N-terminal nucleotide binding domains (NBD) and require ATP-ase activity for their functions. NBD is charged and highly polar in nature and there is no crystal structure yet published. These two families of ATP-ases represent significant therapeutic targets for the treatment of cancer. The ATP-ase activity of Hsp90, in addition to its various co-chaperones, is essential for maintaining the conformational maturation and stability of key signaling molecules involved in cell proliferation, survival, and transformation. The mechanism by which Hsp90 functions is complex, requiring the sequential binding and dissociation of various co-chaperones as well as the hydrolysis of ATP to drive the chaperone cycle. Inhibition of ATP-ase activity at nucleotide binding site of the Hsp90 leads to prevent tumor growth. Till now, only two antibiotics (ansamycin and geldanamycin) were discovered for inhibiting ATP-ase enzymes. This article discuss about types of ATP-ases, interaction with chaperons and various ATP-ase inhibitors at NBD, chaperones inhibitors and also the possibilities for discovering more antibiotics to inhibit cell proliferations and cellular functions.

INTRODUCTION

ATP-ase is a class of enzymes that hydrolyzes ATP (Adeonsine Tri Phosphate) and decomposes them into ADP (Adenosine Di Phosphate). In other words, ATP-ases are a class of enzymes that catalyze the decomposition of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and a free phosphate ion[1]. The energy released during dephosphorylation, enzyme harnesses to drive other chemical reactions. The most important fact is that this process is widely used in all known forms of life.[2]

ATP-ases are membrane-bound transporters that couples ion movement through a membrane with the synthesis or hydrolysis of a nucleotides, usually ATPs. Different forms of membrane-associated ATP-ases have evolved over time to meet specific demands of cells[3]. These ATP-ases have been classified as F-, V-, A-, P- and E-ATPases based on their functional differences. F-ATPases (F1FO-ATPases) in mitochondria, chloroplasts and bacterial plasma membranes are the prime producers of ATP, using the proton gradient generated by oxidative phosphorylation (mitochondria) or photosynthesis (chloroplasts). V-ATPases (V1VO-ATPases) are primarily found in eukaryotic vacuoles, catalysing ATP hydrolysis to transport solutes and lower pH in organelles like proton pump of lysosome. A-ATPases (A1AO-ATPases) are found in Archaea and function like F-ATPases. P-ATPases (E1E2-ATPases) are found in bacteria, fungi and in eukaryotic plasma membranes and organelles, and function to transport a variety of different ions across membranes. E-ATPases are cell-surface enzymes that hydrolyse a range of NTPs, including extracellular ATP. Some such enzymes are integral membrane proteins (anchored within biological membranes) and move solutes across the membrane, typically against their concentration gradient and are called trans-membrane[4].

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Hydrogen potassium ATP-ase

H+/K+ ATP-ase

The gastric Hydrogen Potassium ATP-ase is found in stomach. It acts as a proton pump of the

stomach and, as such, is the enzyme primarily responsible for the acidification of the stomach contents. They are found in parietal cells[5]. Parietal cells are highly specialized epithelial cells located in the inner cell lining of the stomach called the gastric mucosa[6]. The beta subunit of hydrogen-potassium ATP-ase is a major antigen recognized by sera from pernicious anemia and atrophic gastritis patients. Hydrogen-potassium ATP-ase secrets acid into the stomach and catalyzes electro-neutral exchange of cytoplasmic hydrogen ions and external potassium ions coupled with ATP hydrolysis[7]. Hydrogen-potassium ATP-ase inhibitors induce relaxation on rabbit prostatic strips in vitro. The hydrogen potassium ATP-ase, or gastric proton pump, belongs to a family of P-type cation-transporting ATP-ase. MA3-923 detects the beta-subunit of hydrogen/potassium ATP-ase from bovine, canine, porcine, rabbit, mouse, ferret, and rat tissues[8].*Structural Details*:The H+/K+ ATP-ase are a heterodimer protein. The gene *ATP4A* encodes the H+/K+ ATP-ase á subunit and is a ~1000-amino acid protein that contains the catalytic sites of the enzyme and forms the pore through the cell membrane that allows the transport of ions[9]. The gene *ATP4B* encodes the â subunit of the H+/K+ ATP-ase, which is an ~300-amino acid protein with a 36-amino acid N-terminal cytoplasmic domain, a single transmembrane domain, and a highly glycosylated extracellular domain. The H+/K+ ATP-ase â subunit stabilizes the H+/K+ ATP-ase á subunit and is required for function of the enzyme[10]. It also appears to contain signals that direct the heterodimer to membrane destinations within the cell, although some of these signals are subordinate to signals found in H+/K+ ATP-ase á subunit.*Hydrogen Potassium ATP-ase working*:Being a member of P Type ATP-ase, its basic function is to transport ions, mostly cations, across biological membranes in almost all available species in this world. It transports one H+ Hydrogen Ion from the cytoplasm of parietal cell in exchange of one K+ Potassium ion retrieved from gastric lumen[11]. Being an ion pump it can transport ions against concentration gradient using energy that is derived from hydrolysis of Adenosine Tri Phosphate and gets converted to Adenosine Di Phosphate. During this process, one phosphate group from ATP is transferred to Hydrogen Potassium ATP-ase[12]. The phosphate transfer provides power to drive the ion transport[13].

Sodium Potassium ATP-ase

Na+/K+-ATP-ase

Active transport is responsible for the well-established observation that cells contain relatively high concentrations of potassium ions but low concentrations of sodium ions. The mechanism responsible for this is the sodium-potassium pump which moves these two ions in opposite directions across the plasma membrane[14]. This was investigated by following the passage of radioactively labeled ions across the plasma membrane of certain cells. It was found that the concentrations of sodium and potassium ions on the two other sides of the membrane are interdependent, suggesting that the same carrier transports both ions[15]. This shows that the carrier is an ATP-ase and that it pumps three sodium ions out of the cell for every two potassium ions pumped in.The sodium-potassium pump was discovered in the 1950's by a Danish scientist, Jens Christian Skou, who was awarded a Nobel Prize in 1997. It marked an important step forward in our understanding of how ions get into and out of cells, and it has a particular significance for excitable cells such as nervous cells, which depend on it for responding to stimuli and transmitting impulses[16]. The Na+/K+-ATP-ase helps maintain resting potential, avail transport and regulate cellular volume. It also functions as signal transducer/integrator to regulate MAPK pathway, ROS as well as intracellular calcium[17].

Proton ATP-ase

ATP phosphohydrolase / H+ Exporting ATP-ase

Proton ATP-ase or H+ ATP-ase is found in plants and fungi. The proton ATP-ase is responsible for catalyzing the following reaction.
ATP + H2O + H+in $\rightleftharpoons$ ADP + phosphate + H+out

The 3 substrates of this enzyme are ATP, H2O and H+ where as its three products are ADP, phosphate and H+. Proton ATP-asebelong to the family of hydrolases[18], specifically those acting on acid anhydrides to catalyse transmembrane movement of substances. To be specific, the protein is a part of the P-Type ATP-ase family.

A proton-pumping ATP-ase is present in the plasma membrane of plant cells where it sustains transport-related functions. This enzyme is encoded by a family of genes that shows signs of both transcriptional and post-transcriptional regulation[19]. The regulation of *pma1*, one of the Nicotiana H+-ATP-ase genes, was characterized with the help of the [beta]-glucuronidase (gusA) reporter gene in transgenic plants. *pma1* is active in the root epidermis, the stem cortex, and guard cells. This activity depends on developmental and growth conditions[20]. For instance, *pma1* activity in guard cells was strongly enhanced when the plant material (young seedlings or mature leaves) was incubated in liquid growth medium[21]. *pma1* is also expressed in several tissues of the reproductive organs where active transport is thought to occur but where scarcely any ATP-ase activity has been identified, namely in the tapetum, the pollen, the transmitting tissue, and the ovules[22]. Several pma genes have a long 5[prime] untranslated region (leader sequence) containing an upstream open reading frame (ORF). Analysis of translational and transcriptional fusions with gusA in transgenic plants suggests that the *pma1* leader sequence might activate translation of the main open reading frame, even though the URF is translated by a large majority of the scanning ribosomes[23]. As confirmation, transient expression experiments showed that the *pma1* leader causes a fourfold post-transcriptional increase of main open reading frame expression[24]. Deletion of the URF by site-directed mutagenesis stimulated the main open reading frame translation 2.7-fold in an in vitro translational assay. These results are consistent with a regulatory mechanism involving translation re-initiation. Altogether, they suggest a fine, multilevel regulation of H+-ATP-ase activity in the plant[25]. H+-exporting ATP-ase is also known as proton ATP-ase or more simply proton pump. Other names in common use include proton translocating ATP-ase, yeast plasma membrane H+-ATP-ase, yeast plasma membrane ATP-ase, and ATP phosphohydrolase[26].

Three major structural families of ATP-ases have been identified and characterized[27]. First, Most of the ATP-ases for which structures have been described contain the classical mononucleotide binding motif known as the Walker motif[28]. In comparison, Hsp90 belongs to a smaller subset of GHKL ATP-ases whose binding site is characterized by a left handed β-R-β (Bergerat) fold. The GHKL ATP-ases are named after key family members: gyrase B, Hsp, histidine kinase, and MutL[29]. Hsp70 belongs to a third subset of ATP-ases that contain actin fold[30]. In this group of ATP-ases, the nucleotide binds in a cleft formed at the interface of two domains with a loop containing conserved residues and two β-hairpins forming interactions with adenine and the phosphate groups, respectively[31]. The very nature of the ATP binding pocket is a clear reason why targeting the NBD of Hsp70 has proved particularly challenging so far[32]. Hsp70 and Hsc70 exhibit a high degree of structural identity (>99%) in the NBD, and therefore, small molecule inhibitors targeted against the NBD of Hsp70 are likely to inhibit Hsc70 with equi-potency. The following discussion will focus on the binding site of Hsp70, but the points and ideas raised here are equally applicable and relevant to Hsc70[33].

CHAPERONS

Chaperones are proteins that assist the noncovalent folding or unfolding and the assembly or disassembly of other macromolecular structures, but do not occur in these structures when the structures are performing their normal biological functions having completed the processes of folding and/or assembly[34]. The common perception that chaperones are primarily concerned with protein folding is incorrect. The first protein to be called a chaperone assists the assembly of nucleosomes from folded histones and such assembly chaperones, especially in the nucleus, are concerned with the assembly of folded subunits into oligomeric structures[35].

Chaperones do not necessarily convey steric information required for proteins to fold: thus statements of the form ‘chaperones fold proteins’ can be misleading. One major function of chaperones is to prevent both newly synthesized polypeptide chains and assembled subunits from aggregating into nonfunctional structures[36]. It is for this reason that many chaperones, but by no means all, are also heat shock proteins because the tendency to aggregate increases as proteins are denatured by stress[37]. However, ‘steric chaperones’ directly assist in the folding of specific proteins by providing essential steric information, e.g. pro-domains of bacterial proteases, lipase-specific folds, or chaperones in fimbria adhesion systems[38].

Hsp60

Hsp60 (GroEL/GroES complex in *E. coli*) is the best characterized large (~ 1 MDa) chaperone complex. GroEL is a double-ring 14mer with a greasy hydrophobic patch at its opening; it is so large it can accommodate native folding of 54-kDa GFP in its lumen. GroES is a single-ring heptamer that binds to GroEL in the presence of ATP or ADP. GroEL/GroES may not be able to undo previous aggregation, but it does compete in the pathway of mis-folding and aggregation. Also acts in mitochondrial matrix as molecular chaperone[39].

Hsp70

Hsp70 (DnaK in *E. coli*) is perhaps the best characterized small (~ 70 kDa) chaperone. The Hsp70 proteins are aided by Hsp40 proteins (DnaJ in *E. coli*), which increase the ATP consumption rate and activity of the Hsp70s.It has been noted that increased expression of Hsp70 proteins in the cell results in a decreased tendency towards apoptosis[40]. Although a precise mechanistic understanding has yet to be determined, it is known that Hsp70's have a high-affinity bound state to unfolded proteins when bound to ADP, and a low-affinity state when bound to ATP.Hsp70 also acts as a mitochondrial and chloroplastic molecular chaperone in eukaryotes[41].

Hsp90

Hsp90 (Htp G in *E. coli*) may be the least understood chaperone. Its molecular weight is about 90 kDa, and it is necessary for viability in eukaryotes (possibly for prokaryotes as well)[42]. Heat shock protein 90 (Hsp90) isa molecular chaperone essential for activating many signaling proteins in the eukaryotic cell. Each Hsp90 has an ATP-binding domain, a middle domain, and a dimerization domain. Originally thought to clamp onto their substrate protein (also known as a client protein) upon binding ATP[43], indicate that client proteins may bind externally to both the N-terminal and middle domains of Hsp90. Hsp90 may also require co-chaperones like immunophilins, Sti1, p50 (Cdc37) and Aha1 and also cooperates with the Hsp70 chaperone system[44].

Hsp100

Hsp100 (Clp family in *E. coli*) proteins have been studied *in vivo* and *in vitro* for their ability to target and unfold tagged and misfolded proteins[45]. Proteins in the Hsp100/Clp family form large hexameric structures with unfolded activity in the presence of ATP. These proteins are thought to function as chaperones by processed threading client proteins through a small 20 Å (2 nm) pore, thereby giving each client protein a second chance to fold[46]. Some of these Hsp100 chaperones, like ClpA and ClpX, associate with the double-ringed tetra-decamericserine protease ClpP [47]; instead of catalyzing the refolding of client proteins, these complexes are responsible for the targeted destruction of tagged and unfolded proteins[48]. Hsp104, the Hsp100 of *Saccharomyces cerevisiae*, is essential for the propagation of many yeast prions. Deletion of the HSP104 gene results in cells that are unable to propagate certain prions.Hsp70 and Hsp90 have very different Nucleotide Binding Domains[49].

Na (+)/K (+)-ATPase, a plasma membrane protein abundantly expressed in epithelial tissues, linked to numerous biological events, including ion transport and reabsorption. In Na (+)/K (+)-ATPase, the â-subunit plays a fundamental role in the structural integrity and functional maturation of holo-enzyme. Estrogens are important circulating hormones that can regulate Na (+)/K (+)-ATPase abundance and activity; however, the specific molecules participating in this process are largely unknown. The characterization of N-myc downstream-regulated gene 2 (NDRG2) is an estrogen up-regulated gene. 17β-Estradiol binds with estrogen receptor â but not estrogen receptor á to up-regulate NDRG2 expression via transcriptional activation. NDRG2 interacts with the β1-subunit of Na (+)/K (+)-ATPase and stabilizes the β1-subunit by inhibiting its ubiquitination and degradation. NDRG2-induced prolongation of the β1-subunit protein half-life is accompanied by a similar increase in Na (+)/K (+)-ATPase-mediated Na (+) transport and Na (+) current in epithelial cells. In addition, NDRG2 silencing largely attenuates the accumulation of β1-subunit regulated by 17β-estradiol. Thus, estrogen/NDRG2/ Na (+)/K (+)-ATPase β1 pathway is important in promoting Na (+)/K (+)-ATPase activity and suggest this novel pathway might have

substantial roles in ion transport, fluid balance, and homeostasis[50].

CONCLUSION

Drugs targeting the Hsp70 family of chaperones have the possibility to be decisive therapeutic targets for a wide range of diseases, not only cancer. An apparent approach is targeting the substrate binding domain and here the small molecule PES is suggested to bind. However, like the NBD, this site is charged and highly polar in nature with no crystal structure yet published, and it may therefore prove as challenging to drug as the NBD. Disrupting the protein-protein interactions of Hsp70 with specific co-chaperones, such as Hop or BAG-1, is another potential area that may be worth exploring. Supporting this approach, short peptides derived from the C-terminus of BAG-1 can disrupt the interaction between Hsp70 and BAG-1 and decrease the growth of breast cancer cells. Disrupting protein-protein interactions with small molecules is notoriously challenging and an area of drug discovery that is still in its infancy, and thus, this approach will not be without its own challenges. An alternative may be to target the co-chaperones directly, as disrupting one part of the chaperone cycle may be sufficient to inhibit Hsp70 function and elicit an antitumor response. No independent enzymatic activity for any of Hsp70 co-chaperones has, however, so far been detected. A final approachcould be to target the transcription factor HSF-1 responsible for Hsp70 up-regulation in response to stress, oncogenesis, and Hsp90 inhibition. Several tool compounds that inhibit HSF-1 have been identified. These have been shown to inhibit HSF-1 activation and tri-merization, the binding of activated.HSF-1 to the DNA heat shock element, or to inhibit HSF-1 mediated transcription or translation. Genetic and biochemical data support Hsp70 as an exciting therapeutic target for a wide range of therapeutic indications and not just cancer. Similar arguments were presented in the early days for Hsp90 inhibitors, but tumor cells have been shown to have a higher dependence on the Hsp90 chaperoning pathway for survival, and these fears have so far proved and founded. Potent small molecule inhibitors with appropriate pharmaceutical and pharmacokinetic properties targeting Hsp70 and Hsc70 as well as distinctive processes such as the anti-apoptotic function of Hsp70 are needed to address these questions. In summary, discovering small molecule, ATP competitive inhibitors of the nucleotide binding domain of Hsp70 has proved extremely challenging. However, the lessons learned from attempting to drug this class of ATP-ases leave us better equipped to evaluate the drug ability of novel ATP-ases and ATP binding proteins.

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