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RESEARCH ARTICLE

STRUCTURE AND FUNCTION OF MULTIDRUG TRANSPORTER PROTEINS AND MECHANISM OF RESISTANCE TO AVAILABLE CHEMOTHERAPY.

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Abstract

Simple prokaryotic cells and complex eukaryotic cells constantly monitor their environment and try to maintain the chemical homeostasis and composition inside their cells' liquid broth (cytoplasm). Cells express, on or across their lipid-bilayer membrane, various proteinaceous receptors, in order to communicate with extra-cellular matrix system by exchange of nutrients, ions, solutes and other molecules of diverse nature and polarity. Majority of compounds, including natural toxins or drugs, can move in or out of cell via passive transport depending on the permeability of membrane by either simple diffusion (through protein-lined channel), facilitated diffusion (using carrier proteins) or osmosis (through membrane) based on their small size, polarity and concentration gradient across the membrane. Whereas, the remaining category of compounds - mainly toxins, metabolites and xenobiotic - which cells sense as poisonous, harmful and unwanted is transported out of the cell through specialized membrane transporters at the expense of energy molecules, called Adenosine Tri-Phosphate (ATP), as they do not follow the normal mechanism of transport. These membrane proteins have evolved as a multidrug transporter, which belongs to the superfamily of ATP-binding cassette (ABC) transporters, are able to expel a wide range of substrates from cells assisted by ATP hydrolysis. These proteins are responsible for efflux of antimicrobials and anticancer drugs out of their target cells' membrane rendering therapy ineffective and development of resistance to available drugs. Understanding the structure, function and mechanism of these proteins holds the key to designing and develop right drug and therapy to combat the problem of drug resistance.

Introduction:-

The perpetuation of life on our planet lies in the complex, yet simple and stable, combination of four nitrogenous bases, one deoxygenated sugar (five carbon) group, and one to three phosphate groups to form a phenomenal molecule called DNA (Deoxyribonucleic acid) which carries the genetic instructions for the development and functioning of all forms of life on earth. Life evolved on earth 3.5 billion years back in the form of the single-celled organism, with a possible simple structure and function. Since then the physical and chemical properties of surrounding environment has been the most influential factor in constant changes in DNA (called mutation), and in accordance with Charles Darwin's theory of adaptation and natural selection, the highest competent form of life survives and evolve into more advanced and complex living system, different than its ancestors yet leaving a trail behind to track the genetic similarities (homology or conserved domains) and disparities to assign an evolutionary relationship on tree of life. All forms of life have always strived to resist change and develop some genetically driven bio-molecular modifications by expressing functional macromolecules like proteins to adapt to the situation and find a way to flourish.

A very common example is the evolution of pathogenic bacteria and cancer cells, resistant to current antimicrobial and chemotherapy respectively, which were sensitive to these therapies before. These cells have developed a basic

mechanism, well conserved from bacteria to man, to bypass or counteract the poisonous effects of cytotoxic chemicals. Out of many know mechanisms one mechanism has been well studied and characterized that involves the jettison of cytotoxic substances outside of their cell system through different members of a transmembrane protein that mainly fall into the category of ATP-binding cassette (ABC) transporters superfamily (Doshi and van Veen, 2013). ABC transporter proteins are among the top largest families of structurally related and functionally similar membrane receptor proteins harnessing energy, for active transport of substrates across a biological membrane, from adenosine triphosphate (ATP)-binding and hydrolysis (Kos and Ford, 2009). Some well-studied protein like multidrug resistance (MDR) permeability-glycoprotein (P-gp), multidrug resistance protein (MRP), and breast cancer resistance protein (BRCP or ABCG2) belongs to the ABC transporter family and their overexpression has found to be associated with many diseases and disorders including cancer, and are important to the development of resistance against plethora of drugs and chemotherapy (Kuo, 2009).

Structure of multidrug transport proteins:-

Exploring the structure of proteins is the first step to understanding and comprehend the mechanism of their functions. X-ray crystallographic studies on many membrane transporters have helped in obtaining high-resolution three-dimensional structures which are now being utilized to understand the functional mechanism of transport process (Dahl *et al*., 2004).

Histidine permease, involved in the transport of histidine amino acids into the cell, was the first ATP-binding cassette transporter sequenced and cloned in 1982 in the laboratory of Giovanna Ames (Higgins *et* al., 1982). The first high-resolution structure of an ABC domain, HisP (the nucleotide-binding domain [NBD] of histidine permease), was explained in the year 1998 (Hung *et al*., 1998). Common to all ATP-binding cassette (ABC) transporters are two easily distinguishable domains: membrane-spanning domain (MSD) and nucleotide-utilization domain (NUD) (Figure 1). The MSD is also known as transmembrane domain (TMD) or integral membrane (IM) domain, consists of at least six transmembrane α-helices rooted in the bi-lipid membrane (van Veen and Konings, 1998). Whereas NBD is located in cytoplasm attached with TMD by either covalent or non-covalent single polypeptide chain (Saier *et* al., 1998).

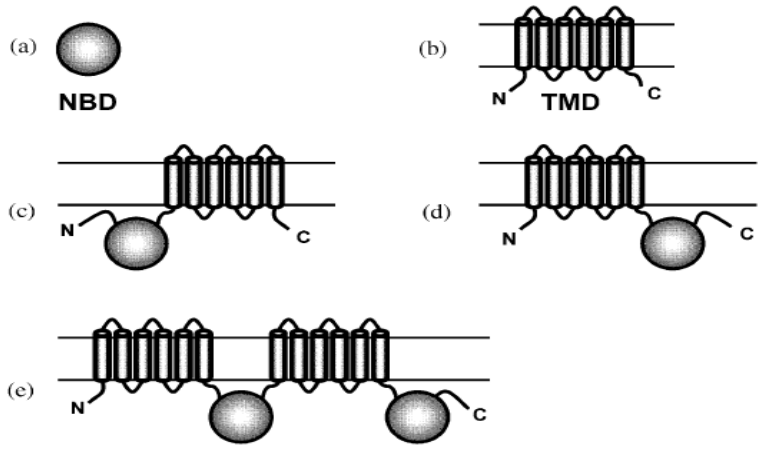

Figure 1: Model representation of basic components of membrane transporter proteins. (a) Nucleotide binding domain [NBD] containing a Walker A and a Walker B motif, and the ABC signature C motif. (b) Transmembrane domain [TMD] consisting of six transmembranes (TM) α-helices with N-terminal and C-terminal. (c) and (d) non-functional unit structure of ABC transporters with NBT-TMD and TMD-NBT configurations, respectively. (e) Dimer configuration of a fully-functional ABC transporter (Lage, 2003).

The NBDs of all ABC transporters, from prokaryotes or eukaryotes, and irrespective of the transport substrate, share extensive amino acid sequence identity and several characteristic motifs. Sharam (2008) and Lage (2003) mentioned in their review the presence of three highly conserved sequence motifs, in all ABC transporters, crucial for the role in ATP binding and hydrolysis which are two short peptide motifs, a glycine-rich Walker A and a hydrophobic Walker B, found in number of proteins which bind to ATP or GTP, and a signature C motif which is also known as

the LSGGQ motif or the linker peptide unique to the ABC superfamily (Davidson *et al*., 2008). Figure 2 shows conserved sequence motifs: Walker A, Walker and Signature C for ABC transporter HlyB, a haemolysin B exporter from Escherichia coli: H662A mutant (Hanekop et al., 2006).

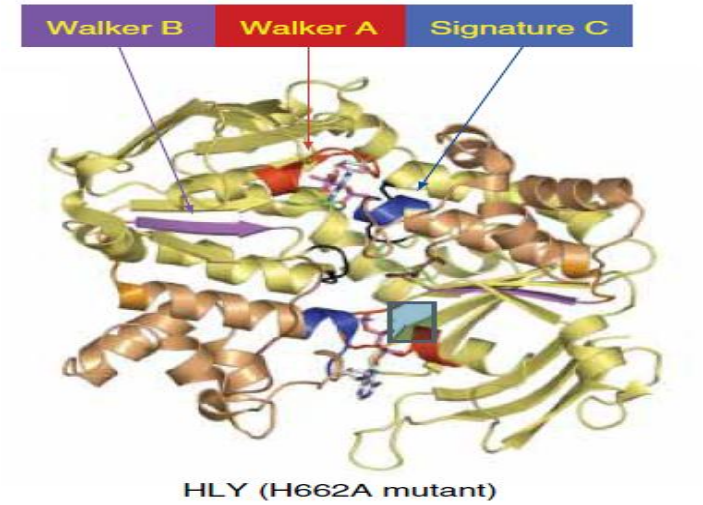

Figure 2: ABC transporter HlyB (haemolysin B exporter from Escherichia coli: H662A mutant) with conserved sequence motifs: Walker A, Walker and Signature C (Hanekop et al., 2006).

Bacterial multidrug transporter protein:-

A Large number of prokaryotes, including Lactococcus lactis, Bacillus subtilis, and Escherichia coli, express ABC exporter proteins – like LmrA, BmrA, and MsbA, respectively - on their cell membrane. These functional proteins exist as a dimer; where each individual subunit contains one nucleotide-binding domain attached to the transmembrane domain (6 α-helices) (Eckford and Sharom, 2008). van Veen et al. (2000) postulated LmrA protein to be half-molecule (half-transporter) consisting of an N-terminal TMD with six membrane-spanning segments fused to one ATP-binding domain and predicted that LmrA functions as a homodimer to form a full transporter (Figure 3) with four core domains. Likewise, MsbA and BmrA have been reported in many articles to function as a homodimer to transport a vast variety of substrate across the membrane (Linton and Higgins, 1998; Dalmas et al., 2005). Therefore, for a fully functional transporter protein, the half-transporter must form a dimer: homo or heterodimer (Dean, 2002).

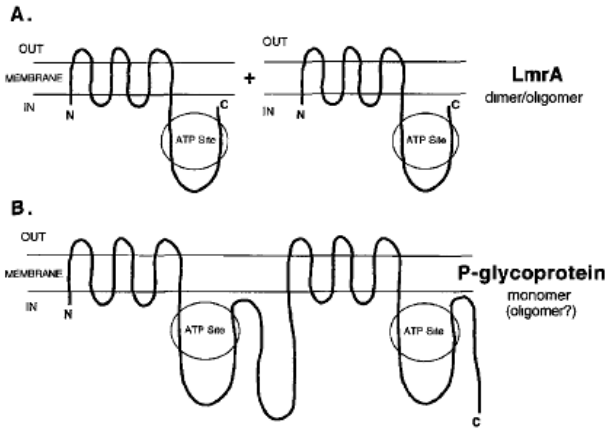

Figure 3: Schematic diagram of the hypothetical models of bacterial LmrA (A) and human P-glycoprotein (B).

(A) A functional unit of LmrA transporter consists of a dimer or higher order oligomer in which per subunit contains six TMs and one ATP site. (B) P-glycoprotein has 12 TMs and 2 ATP sites and can function as a monomer (Hrycyna and Gottesman, 1998).

Human multidrug transporter protein:-

Unlike prokaryotes, eukaryotes do not have ABC importers in their cell membrane (Davidson *et al*., 2008). There are 49 (48 + 1?) human ATP-binding cassette transporter (Table 1) genes known so far which are divided into seven distinct subfamilies of proteins based on phylogenetic analysis (Dean *et al.,* 2001). Table 2 (after reference list) gives the classification of 49 human ABC transporters, their phenotype, tissue regulation and function or substrate (Human ABC, 2006).

Table 1: Broad classification of ABC transporters (Human ABC, 2006).

49 Human ATP-Binding Cassette Transporters							
Name	ABC1	MDR	MRP	ALD	OABP	GCN20	White
Subfamily	ABCA	ABCB	ABCC	ABCD	ABCE	ABCF	ABCG
Members	12	11	13	4	1	3	5 (+ 1?)

One of the well-studied ABC-type drug transporters in human is multidrug resistance P-glycoprotein (van Veen and Konings, 1997) which function as a monomer, and has 12 TMs and 2 NBDs (Figure 3). Central to all ABC transporters functional structure are four core domains: two hydrophobic transmembrane domains (TMDs), which form the drug translocation pathways across the phospholipid bilayer, and two hydrophilic nucleotide binding domains (NBDs), which bind and hydrolyze ATP to derive energy for transport reaction (Hardwick *et al*., 2007). These proteins can be categorized into three groups, based on the structure and arrangement of NBD and MSD, 'full-transporters', 'half-transporters' and non-transporters (Mo *et al*., 2012). A typical 'full-transporter' such as ABCB1 (belongs to MDR/TAP (subfamily B) (Kolwankar *et al*., 2005)) is comprised of two homologous halves and characterized by two MSDs and two NBDs with an arrangement of MSD_1-NBD_1-MSD_2-NBD_2. Other types of full-transporters, for example, ABCC1 (belongs to CFTR/MRP (subfamily C) (Pan *et al*., 2013)), have an extra MSD (MSD_0) at the amino terminus with a domain structure of MSD_0- MSD_1-NBD_1-MSD_2-NBD_2. Half-transporters are about half the size of a full transporter and contain only one MSD and one NBD. Members of ABCD subfamily (belongs to ALD subfamily D (Hillebrand *et al*., 2007)) and few of the ABCB subfamily are half-transporters with a domain structure of MSD-NBD, and members of the ABCG subfamily (belongs to White subfamily G (Lorkowski and Cullen, 2002)) with a reversed NBD-MSD topology. Members of the ABCE (belongs to OABP subfamily E (Human ABC, 2006)) and ABCF subfamilies (belongs to GCN20 subfamily F (Human ABC, 2006)) are non-transporters that do not have MSDs (Mo and Zhang, 2012).

Function of multidrug transporter protein:-

As the name suggests these proteins' primary function is to translocate huge list of different substrates of diverse chemical nature across the membrane. In prokaryotes, they are primarily engaged in the uptake of essential compounds – like sugars, vitamins, metal ions, and other nutrients – that cannot be obtained by diffusion into the cell. In eukaryotes, ABC pumps are mainly unidirectional, the majority of ABC genes move compounds from the cytoplasm to the outside of the cell or into an intracellular compartment viz, peroxisome, mitochondria, endoplasmic reticulum (ER) and few other cells (Dean, 2002). Commonly known roles of eukaryotic ABC transporters include the movement of hydrophobic compounds either within the cell as part of a metabolic process or outside the cell for transport to other organs, or for secretion from the body (Dean *et al*., 2001). However, some other ABC transporters are capable of translocating highly hydrophilic substances including sugars and inorganic ions (Higgins and Linton, 2001). Therefore, in general ABC transporters can translocate a wide range of substrates across cellular membranes, e.g. lipids, sugars, peptides, ions and xenobiotics such as anticancer drugs (Piehler *et al*., 2008). Several studies suggest that the directionality of transport (import or export) purely depends on the presence or absence of an identifiable PBP (periplasmic-binding protein) associated with the coding sequences for the ABC and TMD domains (Linton and Higgins, 1998).

Mechanism of transport in ABC transporter proteins:-

Many theories and models (Figure 4) have been proposed to explain the function of multidrug transport pump for the translocation of a broad spectrum of chemically unrelated compounds (van Veen and Konning, 1998). '**Conventional transport hypothesis**' that gives a simple explanation of substrate transport from cytoplasm to the extracellular matrix via aqueous pore with a flexible 'enzyme-like' substrate recognition site (Altenberg *et al.,* 1994). '**Hydrophobic vacuum cleaner hypothesis**' explains multidrug transporter recognize the lipophilic drugs by their physical property to intercalate into the lipid bilayer, and transport drugs from the lipid bilayer to the exterior (Raviv *et al.,* 1990). '**Lipid flippase hypothesis**' supports the transport of less stable lipid substrate, within inner leaflet, to the outer lipid bilayer leaflet, into an energetically more stable state (Higgins and Gottesman, 1992).

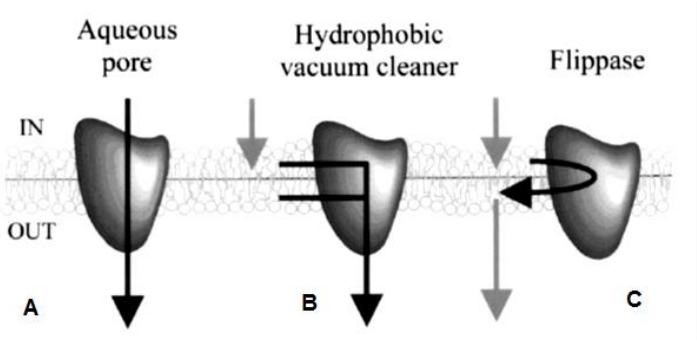

Figure 4: Molecular Model for drug efflux by multidrug transporter by three different mechanisms based on substrate recognition and hydrophobic or -philic nature. Partitioning of drugs between the water phase and phospholipid bilayer is indicated by a grey arrow. Protein-mediated drug transport is indicated by a black arrow. A drug transport protein may function as: (A) an aqueous pore which transports drugs from the intracellular to the extracellular water phase. (B) A hydrophobic vacuum cleaner which transports drugs from the membrane to the extracellular environment. (C) A flippase which transports drugs from the inner to the outer leaflet of the phospholipid bilayer (van Veen and Konings, 1997).

Inward- and outward-facing conformation model:-

The successful crystallization of various ABC multidrug transporters, from prokaryotes and eukaryotes, and X-ray crystal structure study in recent past has helped in better understanding of the mechanism of transport in ATP-binding cassettes based on structural conservation and functional similarities including overlapping substrate specificities (Doshi and van Veen, 2013).

Many studies have come up with a model based on the dynamic movement of ABC exporters flipping between an inward-facing and outward-facing conformation to expose the substrate-binding site in the membrane domains (MDs) to the inside and outside of the cell, respectively. The inward and outward conformation represents the cytoplasmic and periplasmic side of the plasma membrane, respectively (Ward *et al.*, 2007).

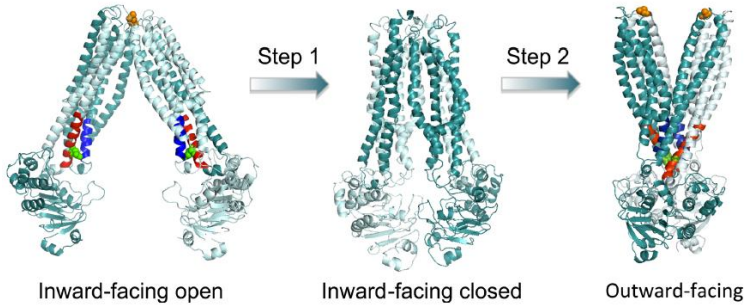

Figure 5: Proposed conformational changes of the MsbA dimer in the transition from inward facing to outward-facing (Doshi and van Veen, 2013).

Doshi and van Veen (2013) explained in their mechanistic model for substrate transport by the MsbA dimer that substrate binding to MsbA in Step 1 stabilizes an intermediate state that precedes the outward-facing conformation. In this intermediate state both pairs of A281C/A281C' and E208C/E208C' residues are in close proximity. ATP binding to this intermediate state in Step 2 switches MsbA into the outward-facing conformation by allowing the formation of stabilizing tetra helix bundle interactions (helices in blue and red). ATP hydrolysis is then required to resolve the outward-facing conformation back to an inward- facing conformation. The observed (Figure 5), outward-facing conformation reflects the ATP-bound state, with the two nucleotide-binding domains in close contact and the two transmembrane domains forming a central cavity—presumably the drug translocation pathway—that is shielded from the inner leaflet of the lipid bilayer and from the cytoplasm, but exposed to the outer leaflet and the extracellular space (Dawson and Locher, 2006).

Ni *et al.,* (2010) in their study on the structure and function of the Human Breast Cancer Resistance Protein (BCRP/ABCG2) presented three homology models (Figure 6), similar to that presented by Doshi and van Veen (2013) (Figure 5), BCRP representing different conformational states: the first model using the MsbA structure as template (PDB code 3B5W) (Ward *et al.,* 2007) represents the substrate-unbound nucleotide-free inward facing open apo conformation (Figure 6A). The second model using the mouse P-gp structure as a template (PDB code 3G60) (Aller *et al.,* 2009) represents the substrate-bound nucleotide-free inward-facing closed apo conformation (Figure 6B). The third model using the Sav1866 structure as a template (PDB code 2HYD) (Dawson and Locher, 2006) represents the nucleotide-bound outward-facing conformation (Figure 6C).

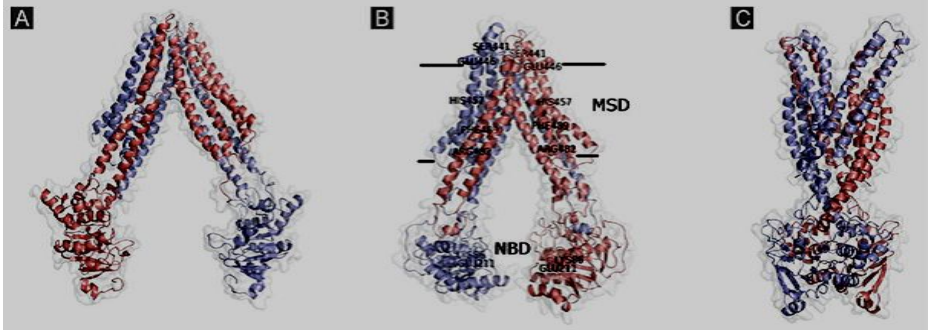

Figure 6: Schematic representation of the homology models of BCRP. A, the substrate-unbound nucleotide-free inward-facing open apo conformation based on the MsbA structure (PDB code 3B5W); B, the substrate-bound nucleotide-free inward-facing closed apo conformation based on the mouse P-gp structure (PDB code 3G60). The approximate locations of several amino acid residues in the MSD (Ser441, Glu446, His457, Phe489, and Arg482) or the NBD (Lys86 and Glu211) that could be important for substrate specificity and/or overall transport activity are indicated; C, the nucleotide-bound outward-facing conformation based on the Sav1866 structure (PDB code 2HYD). Two monomers in the BCRP dimer are shown in different colors (Ni *et al.,* 2012).

ABC transporter proteins being active transporters pump their substrates up a concentration gradient using the energy of ATP hydrolysis (Sharam, 2008). Regardless of the nature of the substrate, the transport process is fuelled by ATP hydrolysis in all these systems. Stoichiometric analysis of ATP hydrolysis per molecule of substrate indicated that roughly one molecule of ATP is consumed in case of MDR1 (Shapiro and Ling, 1998). But it is still unclear that from where, namely the substrate-dependent stimulation, the export mechanism start or at which stage of the transport cycle ATP is hydrolysed or how the chemical energy is converted into the 'power stroke', which finally shuttles the substrate across the membrane; in other words, is the binding of ATP, its hydrolysis or the dissociation of inorganic phosphate the triggering step? But various studies suggest that the binding and hydrolysis of ATP (ATPase cycle) cause alternating dimerization and dissociation, respectively, of the two NBDs (Gutmann *et al.,* 2010).

Phenomena of resistance against chemotherapy:-

There are two possible general causes for the failure of a patient's response to a specific chemotherapy: host factors and specific genetic or epigenetic alterations in the cancer cells (Gottesman, 2002). Leaving the host factor (age, sex, drug tolerance, ADME, and individual's genetic factors) at the moment, cancer cells, unlike normal cells, in general, responds to chemotherapy in their own way. For a given patient each cancer cell has a different genetic make-up based on a different tissue of origin and the pattern of activation of oncogenes and /or deactivation of tumor suppressor genes. As a result, every cancer expresses a different array of drug-resistance genes, and cells within cancer, even though clonally derived, exhibit an enormous amount of heterogeneity with respect to drug resistance. The genetic and epigenetic heterogeneity, under the powerful dictatorship of evolution, selection and survival of the fittest, leaves resistant cancer cells exposed to chemotherapy agents in the body. From the past several decades researchers have come up with several mechanisms by which cancer cells develop resistance to anticancer drugs, as depicted in Figure 7.

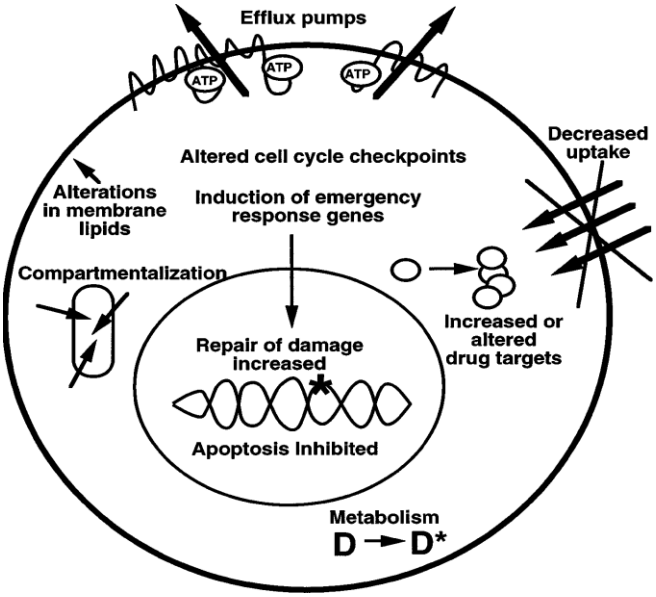

Figure 7: This figure presents several ways in which cultured cancer cells have been shown to become resistant to cytotoxic anticancer drugs. The efflux pumps shown schematically at the plasma membrane include MDR1, MRP family members, and MXR (ABC G2), which is presumed to function as a dimer (Gottesman, 2002).

Among various mechanisms, one mechanism of prime importance here is increased drug efflux from cancer cells, alternatively know as P-glycoprotein (P-gp) or the multidrug transporter, the product of *MDR1* gene in the humans (Chen *et al.*, 1986). This efflux pump was one of the first members described of a large family of ATP-dependent transporters known as the ATP-binding cassette (ABC) family (Figure 8). Members of this family can efflux wide variety of antimicrobial and anti-cancerous drugs from the cell membrane of bacteria and cancer cells which grant resistant phenotype to these cells (Higgins, 1992).

Common Names	Official Name	Structure	Substrates	Normal location
P-gp, MDR1	ABC B1		Neutral and cationic organic compounds	intestine, liver, kidney, blood-brain barrier
MRP1	ABC C1		GS-X and other conjugates, organic anions	widespread
MRP2, cMOAT	ABC C2		GS-X and other conjugates, organic anions	liver, kidney, intestine
MRP3, MOAT-D	ABC C3		GS-X conjugates, anti-folates, bile acids, etoposide,	pancreas, kidney, intestine, liver, adrenal
MRP4, MOAT-B	ABC C4		nucleoside analogs, methotrexate	prostate, testis, ovary, intestine, pancreas, lung
MRP5, MOAT-C	ABC C5		nucleoside analogs, cyclic nucleotides, organic anions	widespread
MRP6, MOAT-E	ABC C6		anionic cyclic pentapeptide	liver, kidney
MXR, BCRP, ABC-P	ABC G2		anthracyclines, mitoxantrone	placenta, intestine, breast, liver

Figure 8: ABC transporters with known drug substrates. Curved lines represent transmembrane domains, and the ATP in the ovals represents the ATP-binding cassettes in these ABC transporters. GS-X represents glutathione conjugates of drugs (Gottesman, 2002).

Table 2: Classification of 49 human ABC transporters, their phenotype, tissue regulation and function or substrate (Human ABC, 2006).

Name/Symbol	Phenotype	Tissue Regulation	Function/ Substrate
ABC1 (Subfamily A)			
ABCA1, TGD, HDLDT1, CERP	Mutations in this gene have been associated with Tangier Disease T1 and familial high-density lipoprotein deficiency.	Many tissues	ABCA1 is a major regulator of cellular cholesterol and phospholipid homeostasis. It mediates e.g. the efflux of phospholipids (PS) and cholesterol from macrophages to apoA-I, reversing foam cell formation. Likely not involved in hepatic cholesterol secretion and intestinal apical cholesterol transport (→ ABCG5/G8).
ABCA2		Brain, Kidney, Lung Heart	
ABCA3, ABC-C		Lung, and other tissues	
ABCA4, ABCR, RP19, ABC10, FFM, STGD1, STGD, RIM, RMP	Mutations in this gene are found in patients diagnosed with Stargardt disease-1 and are associated with Age-related Mac.dyst.2 Ret. Pigmentosa, Retina Mut. Db	Retina, photoreceptor cells	This protein is a retina-specific ABC transporter with N-retinylidene-PE as a substrate. It is expressed exclusively in retina photoreceptor cells, indicating the gene product mediates transport of an essential molecule across the photoreceptor cell membrane.
ABCA5		Muscle, Heart, Testes upregulated in cultured hepatocytes	
ABCA6		Liver	
ABCA7, ABCX		Peripheral leukocytes, Thymus, Spleen, Bone marrow	This full transporter has been detected predominantly in myelo-lymphatic tissues with the highest expression in peripheral leukocytes, thymus, spleen, and bone marrow. The function of this protein is not yet known; however, the expression pattern suggests a role in lipid homeostasis in cells of the immune system. Alternative splicing of this gene results in two

			transcript variants.
ABCA8		Ovary	This gene is clustered among 4 other ABC1 family members on 17q24, but neither the substrate nor the function of this gene is known.
ABCA9		Heart	This gene is clustered among 4 other ABC1 family members on 17q24 and may play a role in monocyte differentiation and macrophage lipid homeostasis.
ABCA10		Muscle, Heart also in monocytes and M-CSF differentiated macrophages	This gene is clustered among 4 other ABC1 family members on 17q24, but neither the substrate nor the function of this gene is known. ABCA10 expression is suppressed by cholesterol import into macrophages, indicating that it is a cholesterol-responsive gene.
ABCA12		Stomach	
ABCA13		Major transcript shows highest expression in human trachea, testis, and bone marrow.	The predicted ABCA13 protein consists of 5,058 amino acid residues making it the largest ABC protein described to date. ABCA13 contains a hydrophobic, predicted transmembrane segment at the N-terminus, followed by a large hydrophilic region.
MDR/TAP (subfamily B)			
ABCB1, PGY1, MDR1, P-GP, GP170		Many tissues (especially those with barrier functions such as liver, BBB, kidney, intestine, placenta) apical membranes	The protein (also called P-glycoprotein) is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. It is responsible for decreased drug accumulation in multidrug-resistant cells and often mediates the development of resistance to anticancer drugs.
ABCB2, TAP1, PSF1, RING4, ABC17, APT1, D6S114E	Mutations in this gene may be associated with ankylosing spondylitis, insulin-dependent diabetes mellitus, and	Most cells ER	The protein is a half-ABC transporter functioning as peptide transporter involved in the pumping of degraded cytosolic peptides

	celiac disease.		across the endoplasmic reticulum into the membrane-bound compartment where class I molecules assemble.
ABCB3, TAP2, PSF2, RING11, D6S217E, ABC18	Mutations in this gene may be associated with ankylosing spondylitis, insulin-dependent diabetes mellitus, and celiac disease.	Most cells ER	The protein is a half-ABC transporter functioning as peptide transporter involved in the pumping of degraded cytosolic peptides across the endoplasmic reticulum into the membrane-bound compartment where class I molecules assemble.
ABCB4, PGY3, NDR2/3, MDR3, PFIC-3, ABC21		Hepatocyte apical membranes	
ABCB5		Ubiquitous	
ABCB6, ABC14, UMAT, MTABC3	This gene is considered a candidate gene for lethal neonatal metabolic syndrome, a disorder of mitochondrial function.	Mitochondria	This half-transporter likely plays a role in mitochondrial function and possibly transports iron.
ABCB7, ATM1P, ASAT	Mutations in this gene have been implicated in X-linked sideroblastic anemia with ataxia.	Mitochondria	This gene encodes a half-transporter involved in the transport of heme from the mitochondria to the cytosol. With iron/sulfur cluster precursors as its substrates, this protein may play a role in metal homeostasis.
ABCB8, M-ABC1		Mitochondria	The function of this half-transporter has not yet been determined; however, it may involve the compartmentalization and transport of heme, as well as peptides, from the mitochondria to the nucleus and cytosol. This protein may also play a role in the transport of phospholipids into mitochondrial membranes.
ABCB9, TAPL		Heart, Brain Lysosomes	The function of this half-transporter has not yet been determined; however, this protein may play a role in lysosomes. Alternative splicing of this gene results in two known products which are likely to have different substrate

			specifications.
ABCB10, M-ABC2 MTABC2		Mitochondria	Peptides?
ABCB11, BSEP, SPGP, PFIC2, PGY4, ABC16	Mutations in this gene cause a form of progressive familial intrahepatic cholestases (PFIC-2) which are a group of inherited disorders with severe cholestatic liver disease from early infancy.	Hepatocytes apical membranes	BSEP is the major canalicular bile salt export pump in man responsible for active transport of bile salts across the hepatocyte canalicular membrane into bile. It represents the molecular basis of the bile-salt-dependent bile flow. BSEP activity is necessary for PC secretion via PGY3/ABCB4.
CFTR/MRP (subfamily C)			
MRP1**,** MRP, ABCC, GS-X, ABC29	This transporter is involved in multi-drug resistance.	Many tissues, Lung Testes, PBMC lateral membranes	MRP1 functions as a multispecific organic anion transporter, with (oxidized) glutathione, cysteinyl leukotrienes, and activated aflatoxin B1 as substrates. This protein also transports glucuronides and sulfate conjugates of steroid hormones and bile salts. It also transports drugs and other hydrophobic compounds in presence of glutathione.
MRP2, CMOAT	Several different mutations in this gene have been observed in patients with Dubin-Johnson syndrome (DJS), an autosomal recessive disorder characterized by conjugated hyperbilirubinemia.	Liver, Intestine Kidney apical membranes	MRP2 is expressed in the canalicular (apical) part of the hepatocyte and functions in biliary transport of mainly anionic conjugates with glutathione, with sulfate or with glucuronosyl e.g. glucuronosyl bilirubin. Other substrates include anticancer drugs such as vinblastine (similar specificity as MRP1/ABCC1); appears to contribute to drug resistance.
MRP3, ABCC3		Intestine Kidney up-regulated in cholestatic livers lateral membranes	The specific function of this protein has not yet been determined; however, this protein may play a role in the transport of biliary and intestinal excretion of organic anions including bile salts.
MRP4, ABCC4		Many tissues	The human multidrug

			resistance protein MRP4 is an organic anion transporter that transports cyclic nucleotides and some nucleoside monophosphate analogs including nucleoside-based antiviral drugs (specificity similar to MRP5) MRP4 also transports prostaglandins.
MRP5, ABCC5		Many tissues, Liver	The human multidrug resistance protein MRP5 is an organic anion transporter that transports cyclic nucleotides and some nucleoside monophosphate analogs including nucleoside-based antiviral drugs (specificity similar to MRP4)
MRP6, ABCC6	Pseudoxanthoma elasticum	Kidney, Hepatocyte lateral membranes	In humans, MRP6 is highly expressed in the liver and kidney. Lower expression was found in tissues affected by pseudoxanthoma elasticum, including skin, retina, and vessel walls. Functional studies suggests that small peptides (BQ123) are transported by rat Mrp6. Recent studies show also transport of glutathione conjugates.
CFTR,ABCC7	Mutations in this gene have been observed in patients with the autosomal recessive disorders Cystic Fibrosis (CF) and congenital bilateral aplasia of the vas deferens (CBAVD).	Lung Intestine (crypt) Cholangiocytes apical membranes	This protein functions as a chloride channel and controls the regulation of other transport pathways.
SUR1, ABCC8	Mutations and deficiencies in this protein have been observed in patients with hyperinsulinemic hypoglycemia of infancy, an autosomal recessive disorder of unregulated and high insulin secretion. Mutations have also been associated with	Pancreas	This protein functions as a modulator of ATP-sensitive potassium channels and insulin release.

	non-insulin-dependent diabetes mellitus type II, an autosomal dominant disease of defective insulin secretion.		
SUR2, ABCC9	No disease has been associated with this gene thus far.	Heart and skeletal muscle, with lower levels in all other tissues	This protein is thought to form ATP-sensitive potassium channels in cardiac, skeletal, and vascular and non-vascular smooth muscle. Protein structure suggests a role as the drug-binding channel-modulating subunit of the extrapancreatic ATP-sensitive potassium channels.
MRP7, ABCC10		Low in all tissues	MRP7/ABCC7 has shown to transport estradiol(2)17beta glucuronide and less well also LTC4. E(2)17betaG transport was saturable, with Km and Vmax values of 57.8 microM and 53.1 pmol/mg/min.
MRP8, ABCC11		Low in all tissues Liver Breast	MRP8/ABCC11 is expressed at low levels in all tissues, except kidney, spleen, and colon. This gene and family member ABCC12 are determined to be derived by duplication and are both localized to chromosome 16q12.1. Their chromosomal localization, potential function, and expression patterns identify them as candidates for paroxysmal kinesigenic choreoathetosis, a disorder characterized by attacks of involuntary movements and postures, chorea, and dystonia. Multiple alternatively spliced transcript variants have been described for this gene.
ABCC12	candidate for paroxysmal kinesigenic choreoathetosis, a disorder characterized by attacks of	Low in all tissues	ABCC12 is expressed at low levels in testes, ovary + prostate tissues. This gene and family member ABCC11 are determined to

	involuntary movements and postures, chorea, and dystonia.		be derived by duplication and are both localized to chromosome 16q12.1. Multiple alternatively spliced transcript variants encoding different isoforms have been described for this gene but some of their full-length sequences are not available.
ABCC13		High expression in the fetal liver also bone marrow in peripheral blood leukocytes of adult humans much lower and no detectable levels in differentiated hematopoietic cells	
ALD (subfamily D)			
ALD**,** ALDP, ABCD1	Defects in this gene have been identified as the underlying cause of Adrenoleuko-dystrophy, an X-chromosome recessively inherited demyelinating disorder of the nervous system. Mutation DB GeneClinics	Peroxisomes	This protein is a member of the ALD subfamily, which is involved in peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle. All known peroxisomal ABC transporters are half transporters which require a partner half transporter molecule to form a functional homodimeric or heterodimeric transporter. This peroxisomal membrane protein is likely involved in the peroxisomal transport or catabolism of very long chain fatty acids.
ALD1, ALDR, ASCD2	Mutations in this gene have been observed in patients with adrenoleukodystrophy, a severe demyelinating disease. This gene has been identified as a candidate for a modifier gene, accounting for the extreme variation among adrenoleukodystrophy phenotypes. This gene is also a candidate for a complement group of Zellweger syndrome, a genetically heterogeneous disorder	Peroxisomes	This protein is a member of the ALD subfamily, which is involved in peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle. All known peroxisomal ABC transporters are half transporters which require a partner half transporter molecule to form a functional homodimeric or heterodimeric transporter. The function of this peroxisomal membrane protein is unknown; however this protein is speculated to function as a

	of peroxisomal biogenesis.		dimerization partner of ABCD1 and/or other peroxisomal ABC transporters.
PXMP1, PMP70, ABCD3	This peroxisomal membrane protein likely plays an important role in peroxisome biogenesis. Mutations have been associated with some forms of Zellweger syndrome, a heterogeneous group of peroxisome assembly disorders	Peroxisomes	This protein is a member of the ALD subfamily, which is involved in peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle. All known peroxisomal ABC transporters are half transporters which require a partner half transporter molecule to form a functional homodimeric or heterodimeric transporter.
PXMP1L, P70R, ABCD4		Peroxisomes	This protein is a member of the ALD subfamily, which is involved in peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle. The function of this peroxisomal membrane protein is unknown. However, it is speculated that it may function as a heterodimer for another peroxisomal ABC transporter and, therefore, may modify the adrenoleukodystrophy phenotype. It may also play a role in the process of peroxisome biogenesis.
OABP (subfamily E)			
RNASELI, OABP, ABCE1		Ovary Testes Spleen	This protein is a member of the OABP subfamily. Alternatively referred to as the RNase L inhibitor, this protein functions to block the activity of ribonuclease L. Activation of ribonuclease L leads to inhibition of protein synthesis in the 2-5A/RNase L system, the central pathway for viral interferon action.
GCN20 (subfamily F)			
ABC50, ABCF1		All	Unlike other members of the superfamily, this protein lacks the transmembrane domains which are characteristic of most ABC transporters.

			This protein may be regulated by tumor necrosis factor-alpha and play a role in the enhancement of protein synthesis and the inflammation process.
ABCF2		All	Iron-inhibited ABC-transporter?
ABCF3		All	
White (subfamily G)			
ABCG1, ABC8, White		Brain, Spleen, Lung	ABCG1 is involved in macrophage cholesterol efflux and may regulate cellular lipid homeostasis in other cell types.
ABCG2, BCRP1, MXR1, ABCP		Placenta, Breast Liver, Intestine apical membranes	This protein functions as a xenobiotic transporter which may play a major role in multi-drug resistance. It likely serves as a cellular defense mechanism in response to mitoxantrone and anthracycline exposure. Recently it has been shown to transport organic anions but also steroids (cholesterol, estradiol, progesterone, testosterone) and certain chlorophyll metabolites.
ABCG3? No human gene?		In mouse high in spleen and thymus	No human gene was found until now. High levels of expression in the thymus and spleen in mice suggest a potential role in the transport of specific peptides or hydrophobic compounds from lymphocytes.
ABCG4, White 2		Macrophage, Brain Eye, Spleen	
ABCG5, White 2, Sterolin1	Mutations in this gene may contribute to sterol accumulation and atheroschlerosis, and have been observed in patients with Sitosterolemia.	Liver, Small Intestine Apical	ABCG5 functions as a half-transporter to limit intestinal absorption and promote biliary excretion of sterols. It is expressed in a tissue-specific manner in the liver, colon, and intestine. This gene is tandemly arrayed on chromosome 2, in a head-to-head orientation with family member ABCG8.

ABCG8, White 4, Sterolin2	Mutations in this gene may contribute to sterol accumulation and atheroschlerosis, and have been observed in patients with Sitosterolemia.	Liver, Small Intestine Apical	ABCG8 functions as a half-transporter to limit intestinal absorption and promote biliary excretion of sterols. It is expressed in a tissue-specific manner in the liver, colon, and intestine. This gene is tandemly arrayed on chromosome 2, in a head-to-head orientation with family member ABCG5.

Conclusion and future prospects:-

ABC transporters belong to one of the biggest family of multidrug transport proteins, conserved from prokaryotic cell system to eukaryotes, that confers resistance to the vast majority of antimicrobials and anticancer drugs, leaving one of the most challenging problems to current scientists and researchers. Several years of intensive research work, through the use of technologies like advanced molecular cloning, gene sequencing, robust spectroscopic analysis, x-ray crystallography, generation of high-resolution three-dimensional structures and many more, in understanding the structure and function of these ATP-driven transporter proteins has undoubtedly given some shocking and interesting results and further hope to develop sensitivity in cancer cells to available drugs. But stills there are several gaps and question marks in current research which need to be filled and answered. One of the biggest questions which need to be answered is that what kind of stimulation or chemical effect drifts ATP molecules towards NBDs, how even a normal healthy cells can sense at first instance the presence of xenobiotic in their cytoplasm - is it genetic or molecular response?, or it is completely associated with physical or chemical properties of substrates which make these proteins so non-selective and overly expressed on cancerous cells membrane? Is it possible to block the formation of functional dimer? In order to answer these questions, deep study of mammalian proteins (in particular), their structure, biochemical information, and very close homology to prokaryotic proteins will be necessary. Understanding complete mechanism and pathology of these transporters are the keys to the development of right drug and therapy that can suppress the multidrug resistance at the clinical stage on a huge population of genetically diverse phenotypes. Complete understanding of the dynamics of the conformational changes in ABC exporters is crucial in designing a molecule that can halt the protein in one conformational state or block the protein in inward facing at high-affinity ATP-binding site. An understanding of the exporter/importer, structure/function relationship will have implications for the mechanisms of transport, specifically whether a common structure can function in both directions depending on whether or not a PBP interacts with it, or whether specialized TMDs are a prerequisite for uptake or export.

Efforts are required not only in designing the efficient pump blocker but also in understanding the reason behind the generation of fast-dividing cancerous cells in response to genetic damage to cells caused by a plethora of environmental, genetic or behavioral factors. The generation of tumor cells inside the body could be a defensive or curative response, which can be linked to the presence of cancer stem cells, which tries to replace damaged cells or tissue mass by enormously increasing the speed of cell proliferation and growth. Why these transporters are present in normal cells and why they are overly expressed by cancer cells?

Pharmacogenomics personalized or gene therapy could be another area which surely has the answer to all the diseases and disorder in the medical history. Cellular genetics targeting at the differences, cellular and/or molecular, between normal cells and cancer cells can provide targeted therapy employing which cancer cells transport system can selectively be targeted without even affecting any single normal and healthy cell in the body. Answering questions like these hold the future of chemotherapy without resistance.

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