T.R.N.N.

NEAR EAST UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

MULTIDRUG RESISTANCE IN CANCER – THE ROLE OF ABC TRANSPORTERS

Narin KANBER

BIOCHEMISTRY PROGRAM

MASTER of SCIENCE (without Thesis)

LEFKOŞA

2020

T.R.N.C.

NEAR EAST UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

MULTIDRUG RESISTANCE IN CANCER – THE ROLE OF ABC TRANSPORTERS

Narin KANBER

Advisor

Prof. Dr. İzzet Hamdi Öğüş

BIOCHEMISTRY PROGRAM

MASTER of SCIENCE (without Thesis)

LEFKOŞA

2020

The Directorate of Graduate School of Health Sciences,

This study has been accepted by the project committee in Medical Biochemistry Program as a Master Project.

Project committee:

Chair:	Professor Nevbahar Turgan Near East University
Supervisor:	Professor İzzet Hamdi Öğüş Near East University
Member:	Professor Levent Kayrın University of Kyrenia

Approval:

According to the relevant articles of the Near East University Postgraduate Study-Education and Examination Regulations, this project has been approved by the above-mentioned members of the project committee and the decision of the Board of Graduate School of Health Sciences.

Professor Hüsnü Can Başer
Director of the Graduate School of Health Sciences

SUMMARY

Kanber, N. Multidrug Resistance in Cancer: The Role of ABC Transporters. Near East University, Institute of Health Sciences. Biochemistry Program, MsC Graduation Project, Lefkoşa, 2020.

The ABC transporters is an important membrane protein family which either import or export of substrates across the membranes. They are found in all living organisms. ABC transporters hydrolyze ATP to power a vast array of allocrites across cell membranes. ABC exporters present in all organisms, but ABC importers found exclusively in bacteria .

Some ABC transporters are responsible for multidrug resistance and a number of inherited or acquired disorders in humans. The acquired diseases are the result of either dysfunctional proteins or overexpression under certain stimuli.

Increased efflux of chemotherapeutic agents may cause failure of cancer chemotherapy, leading to the reduction of intracellular drug levels and consequent drug insensitivity, often to multiple agents- multidrug resistance. Well-established cause of multidrug resistance (MDR) involves the increased expression of members of the ABC transporter superfamily. The most extensively characterized MDR transporters include ABCB1, ABCC1, and ABCG2.

MDR inhibition studies include some methods including the use of drug derivatives. fluorescent drug (which become fluorescent after esteratic cleavage following the entry to cell) accumulation in cells, southern blotting of amplified MDR genes, northern blotting to detect mRNA overexpression of MDR protein genes, western blotting of overexpressed MDR proteins, and use of radioactive 99mTc-labeled MDR inhibitor for the imaging of patient body to evaluate the distribution and inhibition of transporter .

Many MDR inhibitors have been detected and developed, but few MDR inhibitors were tested in clinical investigation. Characterization of the MDR expression status of each cancer patient may give a chance of application of a specific treatment way with more satisfactory results.

Expression levels of MDR transporter genes of cancer patients show a great variability. Because of the presence of very high level of expression of MDR transporters (up to 50-100x fold of normal levels), these studies are important for the planning of a proper strategy for the therapy and diminish the drug resistance if it is necessary and available. Thus, there is no ideal chemotherapy schedule for all cancer patients.

Although a personalized pharmacology concept has been extensively discussed as an ideal approach to cancer therapy, it has some handicaps. It requires a well-equipped laboratory and well-trained personal, expensive instruments, and laborious tests. Although the presence of many promising inhibitors for MDR, only a few molecules have been approved by FDA as cancer drug. Nevertheless, resistance determination and measurement experiments should not be neglected in the management of cancer patients.

ÖZET

Kanber, N. Kanserde Çoklu İlaç Direnci: ABC Taşıyıcılarının Rolü. Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü. Biyokimya Programı, Tezsiz Yüksek Lisans Mezuniyet Projesi, Lefkoşa, 2020.

ABC taşıyıcıları, biyolojik zarlarda substratların içealım ve dışaatımından sorumlu önemli bir zar proteini ailesidir. Tüm canlı organizmalarda bulunurlar. ABC taşıyıcıları, işlevlerini ATP enerjisini kullanarak başarırlar. ABC taşıyıcı proteinler tüm organizmalarda bulunur, ancak substratları hücre içine alan taşıyıcılar bakterilerle sınırlıdır. Bazı ABC taşıyıcıları, çoklu ilaç direncinden ve insanlarda kalıtsal veya edinsel bozukluklardan sorumludur. Edinilen hastalıklar, işlevsiz proteinlerin veya belirli uyaranlar altında aşırı ekspresyonun sonucudur.

Kemoterapötik ilaçların dışaatımında artma, kanser tedavivinde başarısızlığa neden olarak, hücre içi ilaç seviyelerinin azalmasına yol açar. Bu, genellikle birden fazla ilaca dirençli bir ilaç duyarsızlığı biçiminde görülür. Çoklu ilaç direncinin (MDR) iyi bilinen nedeni, ABC taşıyıcı süper ailesi üyelerinin artan ekspresyonudur. En kapsamlı karakterize edilmiş MDR taşıyıcıları ABCB1, ABCC1 ve ABCG2'dir.

MDR inhibisyon çalışmaları, ilaç türevlerinin kullanımını içeren bazı yöntemleri içerir. Fluoresan ilacın (hücreye giren ilaç ester bağının enzimatik kırılmasından sonra fluoresan hale gelir) hücrelerde birikmesi, çoğaltılmış MDR genlerinin Southern blottingle ve MDR protein genlerinin aşırı ekspresyonunu tespit etmek için mRNA'nın Northern blottingle analizi, radyoaktif 99mTc- ile işaretli taşıyıcı inhibitörünün hasta vücudunda dağılımını ve inhibisyonunu değerlendirmek gibi çeşitli ileri yöntemler bulunmaktadır.

Birçok MDR inhibitörü tespit edilmiş ve geliştirilmiştir, ancak klinik araştırmalarda çok az MDR inhibitörü test edilmiştir. Her kanser hastasının MDR ekspresyon durumunun karakterizasyonu, daha tatmin edici sonuçlarla spesifik tedavi yolunun uygulanması şansını verebilir.

Kanser hastalarının MDR taşıyıcı genlerinin ekspresyon seviyeleri büyük bir değişkenlik gösterir. MDR taşıyıcılarının ekspresyon düzeylerinin çok yüksek olabilir (normal seviyelerin 50-100 katına kadar). Bu nedenle, tüm kanser hastaları için ideal bir kemoterapi programı yoktur. Bu nedenle, MDR'den sorumlu ABC taşıyıcılarla yapılacak çalışmalar, tedavi için uygun bir stratejinin planlanması ve gerekli ve mevcutsa ilaç direncinin azaltılması için önemlidir.

Kişiselleştirilmiş bir farmakoloji kavramı, kanser tedavisine ideal bir yaklaşım olarak kapsamlı bir şekilde tartışılsa da bazı engelleri vardır. İyi donanımlı bir laboratuvar ve iyi eğitilmiş personel , pahalı aletler ve zahmetli testler gerektirir. Ayrıca MDR için umut verici birçok inhibitör olmasına rağmen, sadece birkaç molekül FDA tarafından kanser ilacı olarak onaylanmıştır. Tüm bunlara rağmen, direnç belirleme ve ölçüm deneyleri ihmal edilmemelidir.

CONTENTS

ABREVIATIONS

ABC ATP-binding cassette

ALD Adrenoleukodystrophy

AUC Area under the curve

BCRP Breast cancer resistance protein

BSEP Bile salt export pump

CFTR Cystic fibrosis transmembrane conductance regulator

CLL Chronic lymphoblastic leukemia

CNS Central nervous system

CSC Cancer stem-like cell

EFS Event-free survival

EGFR Epidermal growth factor receptor

ESCC Esophageal squamous cell carcinoma

ESFT Ewing's sarcoma family of tumors

FGFR Fibroblast growth factor receptor

GSH Glutathione

HER2 Human epidermal growth factor receptor 2

JAK Janus kinase

LBAT Liver bile acid transporter

MATE Multidrug and toxic compound extrusion

MCT Monocarboxylate transporter 1

MD Multidrug resistance

MDR Multi-drug resistance

miRNA MicroRNA

MRP	Multi-drug resistance related protein
mTOR	Mammalian target of rapamycin
MXR	Multi-xenobiotic resistance
MXR	Multi-xenobiotic resistance
NSCLC	Non-small cell lung cancer
NTCP	Na+-taurocholate co-transporting polypeptide
OAT	Organic anion transporter
OATP	Organic anion transporter protein
OCT	Organic cation transporter
OCTN	Organic zwitterions/cation transporter
OST	Organic substrate transporter
P-gp	P-glycoprotein
PDGFR	Platelet-derived growth factor receptor
PEPT	Human peptide transporter
PXMP	Peroxysomal membrane protein
RET	Rearranged during transfection
SUR	Sulfonylurea receptor
TAP	Transporter associated with antigen processing
TKI	Tyrosine kinase inhibitor
VEGFR	Vascular endothelial growth factor receptor
WT	Wild type

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

The ABC transporter family is a group of important membrane protein family which either import or export of substrates across the membranes. All the living organisms have these membrane proteins. ABC transporters actively transport many substrates across the cell membranes and hydrolyse ATP to power the transport function.

ABC transporters are divided into ABC importers and exporters. The ABC importers are limited to bacteria while the ABC exporters are present in all organisms. Importers are mainly responsible for nutrient uptake into bacterial cells. ABC exporters are commonly involved in the extrusion of substances from the cis- to the trans-side of the lipid bilayer. Some ABC transporters are responsible for multidrug resistance and some inherited or acquired disorders in humans. These disorders are the result of either dysfunctional proteins or overexpression under certain stimuli.

Increase of ABC transporter activity cause the reduction of intracellular drug levels. This situation may lead to drug insensitivity, often to multiple agents (multidrug resistance), even to a failure of cancer chemotherapy. The increase of expression of the ABC superfamily members is a well-established cause of multidrug resistance (MDR).

ABCB1, ABCC1 and ABCG2 are the most extensively characterized MDR transporters. Actually, there are nearly a dozen transporters are connected to MDR.

Treatment of cancer cells with a mixture of anticancer drugs and inhibitors of ABC transporters is a well-known strategy. Anti-cancer drugs are exported with different ABC transporters or groups according to their chemical characteristics. So, it is important to determine the expression levels of these MDR transporters and their kinetic properties such as efflux capacity and inhibition characteristics, because of the presence of very high level of expression of MDR transporters. These studies are important for the planning of a proper strategy for therapy and diminish drug resistance.

2. GENERAL INFORMATION

2.1. Overview of the ABC proteins

The ATP-binding cassette (ABC) superfamily involves many functionally diverse transmembrane proteins. The name ABC transporters was introduced in 1992 by Chris Higgins. The designation ABC is based on the highly conserved ATP-binding cassette, the most characteristic feature of the superfamily. All living organisms have ABC exporters while ABC importers are limited with prokaryotes (Higgins 1992).

These ATP-driven active transporters have various substrates (inorganic ions, saccharides, metal ions, drugs, amino acids, lipids, and proteins. They share a high degree of sequence and structural homology, although differences are observed in molecular mechanisms, in vivo localizations, substrate specificities, and their functions. Transport functions of these proteins are not limited with export out of the cell but are also involved in intracellular compartmental transport in eukaryotic cells. (Higgins 1992).

2.2. Classification of ABC transporters

In the human genome, 48 ABC genes are present, and they are arranged in seven subfamilies and are designated A to G. The genes that encode ABC proteins with a high degree of homology among eukaryotes and they are widely dispersed in the genome. There are 22 ABC pseudogenes which have been identified and localised to chromosomal regions (Piehler et al. 2008). Each subfamily has one or more nicknames: A; ABC1, B; MDR/TAP, C; MRP/CFTR, D; ALD, E; OABP, F; GCN20, and G; White .(Neumann et al. 2016).

Human ABC gene subfamilies with genes and pseudogenes are shown in Table 2.1. Members of each subfamily of human ABC transporters are listed in Table 2.2. (Neumann et al. 2017).

Subfamily name	Alias	Number of genes	Number of pseudogenes
ABCA	ABC1	12	5
ABCB	MDR	11	4
ABCC	MRP	12	3
ABCD	ALD	4	4
ABCE	OABP	1	2
ABCF	GCN20	3	2
ABCG	White	5	2
Total		48	22

Table 2.1. Human ABC gene and pseudogene subfamilies (Vasiliou et al. 2009)

ABCA (ABC1)	ABCB (MDR/TAP)	ABCC (MRP/CTFR)	ABCD (ALD)	ABCE (OABP)	ABCF (GCNN20)	ABCG (White)
ABCA1	**ABCB1 (MDR1/P-GP)**	**ABCC1 (MRP1)**	ABCD1 (ALDP)	ABCE1 (OABP)	ABCF1	ABCG1
ABCA2	ABCB2	**ABCC2 (MRP2)**	ABCD2 (ALDR)		ABCF2	**ABCG2 (BCRP)**
ABCA3	ABCB3	**ABCC3 (MRP3)**	ABCD3		ABCF3	ABCG4
ABCA4	**ABCB4 (MDR3)**	**ABCC4 (MRP4)**	ABCD4			ABCG5
ABCA5	ABCB5	**ABCC5 (MRP5)**				ABCG8
ABCA6	ABCB6	**ABCC6 (MRP6)**				
ABCA7	ABCB7	ABCC7 (CFTR)				
ABCA8	ABCB8	ABCC8 (SUR1)				
ABCA9	ABCB9	ABCC9 (SUR2)				
ABCA10	ABCB10	**ABCC10 (MRP7)**				
ABCA12	**ABCB11 (BSEP/SGPG)**	**ABCC11 (MRP8)**				
ABCA13		**ABCC12 (MRP9)**				

cMOAT, canalicular multispecific organic anion transporter, **ALD**, adreno-leukodystrophy; **ALDR**, adrenoleukodystrophy protein; **OABP**, organic anion-binding transporter; **SPGP**, sister of P-glycoprotein.

Table 2.2. Members of human ABC transporter superfamily. Cell in bold type represent ABC transporter having recognized activity in the transport of drugs (Neumann et al. 2016).

2.3. Structure of ABC transporters

ABC genes are generally organized as either full or half transporters.The full transporters contain two trans-membrane domains (TMD) and two nucleotide binding domains (NBD). The half transporters have one TMD and one NBD domain. The half transporter proteins have to dimerized to be a functional transporter. These dimers may be either homodimer or heterodimer (Figure 2.1.).

The transmembrane domains (TMD) contain six hydrophobic alpha-helices (transmembrane domain). These domains take role in substrate recognition and its translocation across the lipid membrane.The molecules pump substrates out of the cytoplasm. Hydrophobic compounds, often move this from the inner leaf of the bilayer to the outer layer (Hyde et al. (1990).

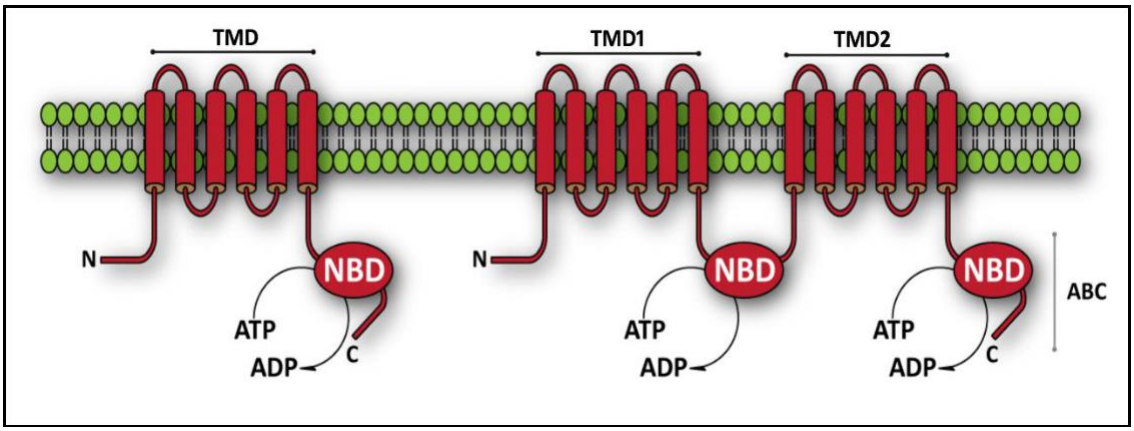


Figure 2.1. Typical structures of half- and full ABC transporters.

The NBD domains located in cytoplasm and two NBD together function. They bind and hydrolyze ATP to provide the driving energy for transport.

The NBDs contain several highly conserved A and B motifs, the turn and loop regions, and an ABC signature motif (C). This ABC motif is specific to ABC transporters and other ATP-binding proteins have no similar motif (Kerr 2002).

Nevertheless, ABC proteins generally do not obey these simplified TMD-NBD or TMD-NBD-TMD-NDB models. Although the TMD domains of a typical full ABC

transporter have a total of 12 transmembrane hydrophobic helices, some of the full type ABC transporters have an additional TMD, with up to five hydrophobic transmembrane helices (TMD0). The variation of the transporter topology of ABC superfamily are summarized in the Figure 2.2. Subfamily A and some members of subfamily B and C are the examples for the typical full transporter definition with TM domains of a total 12 helices. Members of subfamily D and G, and some ABCB transporters are typical half transporters. Some ABCB and ABCC transporters have additional variable lengthtransmembrane domain (TMD0) with up to 5 hydrophobic helices in addition to TMD1.

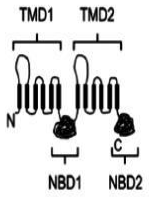
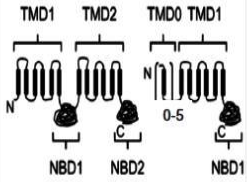
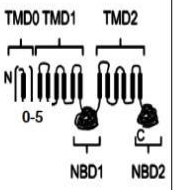
Human ABC Family	A	B	C	D	G
Transporter Topology					
Subcellular Localization	Plasma mebrane (A1, A4,A7) Lysosome (A2, A5) Lameller bodies (A3, A12)	Plasma membrane (B1, B4, B11) (G2,G5,G8)	Plasma membrane (C1, C2, C3, C10)	Peroxsome (D1-D4)	Plasma membrane (G2,G5,G8) Endosome (G1, G4)

Figure 2.2. Structures and subcellular localizations of the ABC half- and fulltransporters in ABC subfamilies (Neuman et al. 1990).

Two subfamilies of ABC superfamily (ABCE and ABCF) are quite different in structure from the others. These members which have no TMD are in soluble form in cytoplasm and can not function as a transporter. (Domenichini et al. 2018)

2.3.1. Subcellular localization of ABC transporters

Many tissues have a characteristic intracellular ABC transporter location pattern which involves some organelles. Intracellular localizations are defined by functions and requirements of cells such as synthesis, secretion, intracellular transport, and defense

against xenobiotics. A brief distribution pattern is given in Figure 2.2. (Neuman et al. 2016).

2.4. The Mechanism of action of ABC transporters

ABC transporters can any one of nucleotides or transport substrate firstly to one face open-inward conformation (Figure 2.3) and then the TMDs or NBDs could cometogether in a orientation. Both NBD and TMD halves form complete binding sites for ATPs and substrate molecules, respectively (Higgins and Linton 2004).

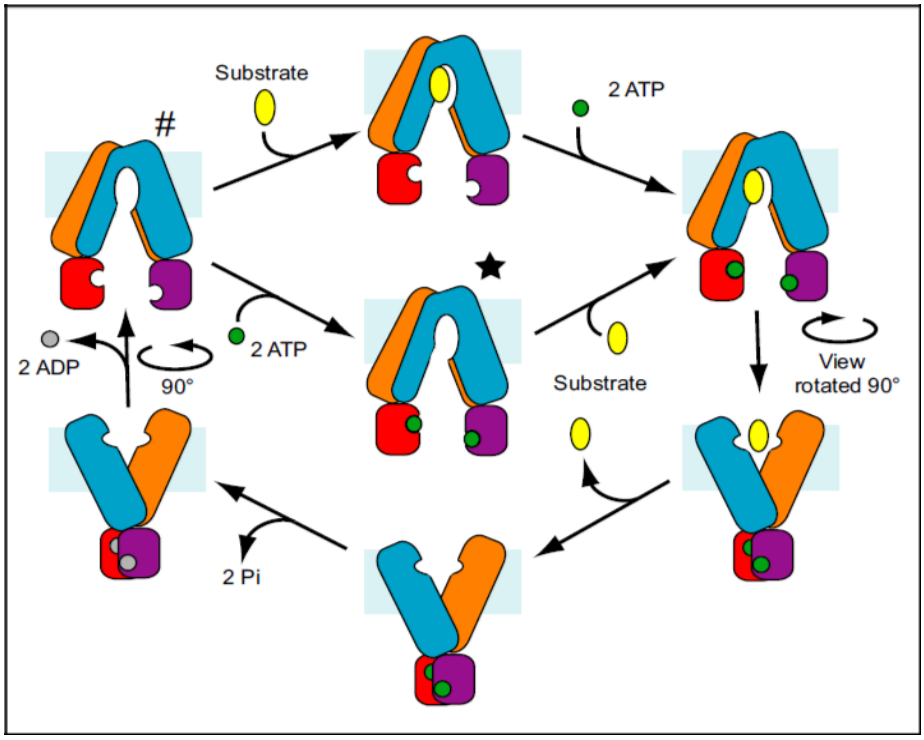


Figure2.3. Mechanism of action of ABC transporters. The blue colored TMDs are associated with purple and red NBDs, ADP and transport substrate are shown in green, gray, and yellow, respectively.The symbol of number (#) and asterisk (*)indicate nucleotide-free and nucleotide bound conformations ABCB10 (Shintre et al. 2013).

2.5. Some characteristics and functions of ABC transporters

ABC transporters show a widespread tissue distribution in humans. Some characteristics of ABC transporters including their genes, chromosome locations, the number of amino acids, and tissue distributions are given in Table 2.3. Mutations in ABC genes cause or accompany many diseases. These ABC transporters which are involved in diseases are listed in Table 2.4.

2.5.1. ABCA Subfamily

In sub-family A, there are 12 full transporters which play a key role in lipid trafficking in many diverse organs (Vasiliou 2009).

The ABCA1 (ABC1) protein is to exports the cholesterol and phospholipids across the cell membrane to the outside of the cell (Adamska, 2018). There is a pure confirmation that ABCA1 always flips phospholipids and cholesterol from the inside leaflet to the outside leaflet of the plasma membrane. The mutations and deficiencies of ABCA1 cause a deficiency of cholesterol efflux onto HDL (Tangier disease) (Oram, 2000).

ABCA2 is extensively located in the brain and also exists in the kidney, the heart andthe lung with lower levels. It is observed that the level of ABCA2 mRNA increases in cholesterol-rich macrophages. Early onset forms of Alzheimer disease in humans was reported to be linked to ABCA2 gene (a single nucleotide polymorphism in exon 14) (Mack et al. 2007).

ABCA3 (ABC3) is connected to alveolar type II pneumocytes in the lung. It isresponsible for the continuous secretion of pulmonary surfactant (a complex combination of proteins, phospholipids, and cholesterol) and concentrated storage (Tarling, 2013).

ABCA4 (ABCR) and is responsible for the transport of N-retinylidene-phosphatidylethanolamine (NRPE), an intermediate product of vision cycle. It is dedicated to retinal cell photoreceptors and ABCA4 is also named ABCR. ABCA4 mutations are responsible for Stargardt's macular degeneration (cone and rod cell dystrophy), and recessive retinitis pigmentosa (Tsybovsky et al. 2010)).

Some characteristics, tissue distributions and functions of ABC transporters

Gene	Alias	Gene location	AA	Tissue distribution	Function
ABCA1	ABC1	9q31.1	2261	Most tissues	Cholesterol efflux onto HDL
ABCA2	ABC2	9q34	2436	Brain, monocytes	Drug resistance
ABCA3	ABC3, ABCC	16p13.3	1704	Lung	Multidrug resistance
ABCA4	ABCR	1p22.1-p21	2273	Rod photoreceptors	N-retinylidene-phospha-tidylethanolamine (PE) efflux
ABCA5		17q24	1642	Muscle, heart, testes	Urinary diagnostic marker for prostatic intraepithelial neoplasia
ABCA6		17q24	1617	Liver	Multidrug resistance
ABCA7		19p13.3	2146	Spleen, thymus	Cholesterol efflux
ABCA8		17q24	1581	Ovary	Transports certain lipophilic drugs
ABCA9		17q24	1624	Heart	Monocyte differentiation and macrophage lipid homeostasis?
ABCA10		17q24	1543	Muscle, heart	Cholesterol-responsive gene
ABCA12		2q34	2595	Stomach	Has implications for prenatal diagnosis
ABCA13		7p11-q11	5058	Low in all tissues	Inherited disorder affecting the pancreas
ABCB1	P-PG, MDR1	7p21	1280	Kidney, brain, liver, intestine, placenta, adrenal	Multidrug resistance
ABCB2	TAP1	6p21	808	All cells	Peptide transport
ABCB3	TAP2	6p21	703	All cells	Peptide transport
ABCB4	MDR3	7q21.1	1279	Liver	Phosphatidylcholine transport
ABCB5		7p14	812	Most tissues	Melanogenesis
ABCB6	MTABC3	2q36	842	Most tissues	Iron transport
ABCB7	ABC7	Xq12-q13	753	Most tissues	Fe/S cluster transport
ABCB8	MABC1	7q36	718	Most tissues	Intracellular trafficking of peptides across membranes
ABCB9		12q24	766	Heart, brain	Located in lysosomes
ABCB10	MTABC2	1q42	738	Most tissues	Export of peptides derived from proteolysis of inner-membrane proteins

Some characteristics and tissue distributions of ABC transporters (continued)

ABCB11	BSEP, SPGP	2q24	1321	Liver	Bile salt transport
ABCC1	MRP1	16p13.1	1531	All tissues	Drug resistance
ABCC2	MRP2, cMOAT	10q24	1545	Liver, kidney, intestine	Organic anion efflux
ABCC3	MRP3	17q21.3	1527	Lung, intestine, liver, pancreas, kidney, adrenal glands	Drug resistance
ABCC4	MRP4	13q32	1325	Prostate, testis, ovary, intestines, pancreas, lung	Nucleoside transport
ABCC5	MRP5	3q27	1437	Most tissues	Nucleoside transport
ABCC6	MRP6	16p13.1	1503	Kidney, liver	Expressed primarily in liver and kidney
ABCC7	CFTR	7q31.2	1480	Exocrine tissues	Chloride ion channel (same as CFTR gene in cystic fibrosis)
ABCC8	SUR1	11p15.1	1581	Pancreas	Sulfonylurea receptor
ABCC9	SUR2	12p12.1	1549	Heart, muscle	Encodes the regulatory SUR2A subunit of the cardiac K? (ATP) channel
ABCC10	MRP7	6p21	1464	Low in all tissues	Multidrug resistance
ABCC11	MRP8	16q11-q12	1382	Low in all tissues	Drug resistance in breast cancer
ABCC12	MRP9	16q11-q12	1359	Low in all tissues	Multidrug resistance
ABCD1	ALDP	Xq28	745	Most tissues	Very-long-chain fatty acid (VLCFA) transport
ABCD2	ALDR	12q11-q12	740	Most tissues	Major modifier locus for clinical diversity in X-linked ALD (X-ALD)
ABCD3	PXMP1, PMP70	1p22-p21	659	Most tissues	Import of fatty acids and/or fatty acyl-coenzyme A into the peroxisome
ABCD4	PMP69, P70R	14q24.3	606	Most tissues	May modify the ALD phenotype
ABCE1	OABP, RNS4I	4q31	599	Ovary, testes, spleen	Oligoadenylate-binding protein

Some characteristics and tissue distributions of ABC transporters (continued)

ABCF1	ABC50	6p21.33	845	Most tissues	Susceptibility to auto-immune pancreatitis
ABCF2		7q36	634	Most tissues	Tumor suppression at metastatic sites and in endocrine pathway for breast cancer/drug resistance
ABCF3		3q25	709	Most tissues	
ABCG1	ABC8, White1	21q22.3	678	Most tissues	Cholesterol transport
ABCG2	ABCP, MXR, BCRP	4q22	655	Placenta, liver, intestine, breast	Toxicant efflux, drug resistance
ABCG4	White2	11q23	646	Liver	Found in macrophage, eye, brain and spleen
ABCG5	White3	2p21	651	Liver, intestine	Sterol transport
ABCG8		2p21	673	Liver, intestine	Sterol transport

Table 2.3. Some characteristics, tissue distributions and functions of ABC transporters. (Dean et al. 2001, Vasiliou et al. 2009).

ABCA5 (ABC13) mutations may cause excessive hair overgrowth (DeStefano etal. 2014). **ABCA10** is a mitochondria located half transporter and its function is unknown.

ABCA12 proteins are mostly found in the stomach and on the skin. The*ABCA12*gene share same chromosomal region which is previously linked to congenital cataracts, diabetes mellitus and skin diseases. ABCA12 functions as an important skin homeostasis factor. Patients with *ABCA12* gene mutations suffer from lamellar ichthyosis type 2 (Albrecht, 2007).

2.5.2. ABCB (MDR, TAP) subfamily

A few of the B family members present multidrug resistance in cancer and have been named the “MDR family of ABC transporters” (Štefková, 2004). Modifications in ABCB genes are linked to some diseases; ankylosing spondylitis, Coeliac disease, X-linked sideroblastic anemia with ataxia, lethal neonatal syndrome and cholestatic liver disease of infancy (Vasiliou 2009).

ABC transporter	Disease associated	Implication
ABCA1	Tangier disease	Inability to transport the cholesterol to HDL
	Familial HDL deficiency	
ABCA4	Stargardt's disease Age-related macular degeneration Retinitis pigmentosa	Degeneration of photoreceptors due to defective transport of N-retinylidene-phosphatidylethanolamine
ABCB1	Cancer	Overexpression of ABCB1 causing MDR
ABCB4	Progressive familial intrahepatic cholestasis	Inability to transport phospholipids
ABCB7	Sideroblastic anemia and cerebellar ataxia	Inability to produce heme
ABCB11	Progressive familial intrahepatic cholestasis	Reduction of bile salt transport
ABCC1	Cancer	Overexpression of MRP1
	Chronic obstructive pulmonary disease	Reduced MRP1 expression levels in lung
	Cystic fibrosis	
ABCC2	Dubin–Johnson syndrome	Reduced expression of ABCC2 levels in liver
ABCC6	Pseudoxanthoma elasticum	Reduced expression of ABCC6
ABCC7	Cystic fibrosis	Absence / reduced expression of ABCC7 in liver, respiratory and intestinal tract
ABCC8	Persistent hypoglycemia of infancy	Increased and continuous insulin secretion
ABCC9	Persistent hypoglycemia of infancy	Increased and continuous insulin secretion
ABCD1	Adrenoleukodystrophy	Inability to transport very long chain fatty acids into peroxisomes
ABCG2	Cancer	Overexpression of ABCG2 causing MDR
	Gout	Reduced expression of *ABCG2* in renal tubules

Table 2.4. ABC transporters involved in diseases (Patel et al. 2015).

ABCB1 is mainly expressed in blood-brain barrier and in the liver and functions for the protection of the cells from toxic agents (Dean, 2001). ABCB4 is the key source of progressive familiar intrahepatic cholestasis type 3 (Annilo, 2006).

Mutations in **ABCB4** and **ABCB11** genes cause various progressive clinical forms of familial intrahepatic cholestasis. In these autosomal recessive liver disorders cholestasis starts early and puts the organ into failure and is the main reason of liver transplantations in children (Zarenezhad et al. 2017).

ABCB6, **ABCB7**, **ABCB8**, and **ABCB10** are located on the mitochondrial membrane and they have roles such as carrying iron/sulfur protein precursors and iron import to mitochondria (Liesa et al. 2012). Four mutations in the **ABCB7** gene have been recognized in X-linked sideroblastic anemia with cerebellar ataxia (XLSA/A) (D'Hooghe et al. 2014). The **ABCB9** half transporter has been detected on lysosomes (Zhang et al. 2000)

Two half transporters ABCB8 and ABCB9 are located in mitochondria and lysosomes respectively and their functions are unknown.

2.5.3. ABCC (MRP, CFTR) Subfamily

MRP-like proteins are organic anion (glucuronate glutathione or sulfate) transporters and have a major function in resistance to nucleoside analogs (Štefková, 2004).

ABCC2 mutations cause Dubin-Johnson disease, an autosomal recessive syndrome. Carrying bilirubin from the liver into the biliary system is defective and there is bilirubin storage in liver (Okada et al. 2014).

ABCC6 mutations result in pseudoxanthoma elasticum (PXE). In this progressive disorder the deposits of calcium and other minerals are accumulated (mineralization) in elastic fibers (Le Saux et al. 2001).

Mutations in ***ABCC7*** (also named CFTR, cyctic fibrosis transmembrane regulator) cause cystic fibrosis, which is a genetic autosomal recessive disorder that

influences the pancreas mainly, and leading to severe digestive complications (Vasilliou, 2009).

2.5.4. ABCD (ALD) Subfamily

The ABCD transporters are peroxisomally located half transporters. Mutations of ***ABCD1*** gene is the cause of a peroxisomal disorder, drenoleukodystrophy (ALD). van Roermund et al. showed that ALDP (human peroxisomal ABC half transporter) is functional in its dimer form and accepts acyl-CoA esters (van Roermund et al. 2008). In ALD, there is an accumulation of unbranched and saturated very long chain fatty acids (in form of cholesterol esters).

ABCD1, ABCD2, ABCD3, and ABCD4 are the genes of peroxysomal related transporters. These mammalian genes are highly conserved in evolution. In mouse, the expression of ALD gene, but it predominates in brain and adrenals (Lombard-Plat et al., 1996).

The ABCD2 gene is induced by cholesterol-lowering drugs (fibrates) and maintains a contingent therapeutic strategy to treat X-ALD (Netik et al.,1999)

2.5.5. ABCE (OABP) Subfamily

The ABCE1 protein is a negative regulator of the 2-5A/RNase L system and it may be involved in some biological functions such as tumor cell proliferation and antiapoptosis (Tian Y. et al. 2012). *ABCE1* has only two nucleotide binding domain (NBD) and the transmembrane domains (TMD) is missing. This member is in soluble form in cytoplasm and does not function as a transporter. ABCE members are in soluble form and have no transporter function.

2.5.6. ABCF (GCN20) Subfamily

ABCF members are similar to ABCE in structure (no TMD) and do not functionas transporter. They seem to be involved in translational regulation (Xiong et al. 2015). ABCF genes are thought to be upregulated by tumor necrosis factor. The members of this ABCF subfamily have an important function in inflammatory processes. (Vasilliou, 2009).

2.5.7. ABCG (White) Subfamily

ACBG transporters take role in the efflux of lipids such as cholesterol and derivatives, hydrophobic xenobiotics and drugs. **ABCG1** and **ABCG4** together have functions in macrophage lipid metabolism(Woodward et al., 2011).

ABCG2 has an important role in tissueprotection against several xenobiotics and itis located on the apical membranes (Mansoori etal. 2017).

ABCG2 is identified to promote multidrug resistance (MDR) in chemotherapy of cancer (Allen et al. 2002). Expression of ABCG2 in tumor cell is related through resistance to multiple chemotherapeutic agents (Ishikawa, 2005).

Cancer cells which have ABCG2 can carry out some photosensitizers such as porphyrins including benzoporphyrin and verteporfin (Ishikawa, 2005). This ability protects the cancer cells against phototoxicity of porphyrin based drugs in photodynamic therapy

Also **ABCG2** is a physiologically important urate transporter. A positive connection between ABCG2 dysfunction and hyperuricemia and gout is shown (Toyoda, 2019). Gout attacks and high serum urate concentrations may arise through the possible side effects of ABCG2 inhibitors (Woodward et al., 2011).

The ***ABCG5***and ***ABCG8*** genes are adjacent and in anti-parallel arrangement on the chromosome 2p21. Both ABCG5 and ABCG8 genes are mutated in phytosterolemia. An **ABCG5/ABCG8** heterodimer seems to be a principal transporter for sitosterol. They limit intestinal absorption and remove sterols. (Vasilliou, 2009).

3. CANCER AND MULTIDRUG RESISTANCE

3.1. General characteristics of cancer cells

According to WHO reports, cancer caused 9.6 million deaths and 18.1 million new cases in 2018, and it is the second leading cause of death globally (WHO, 2020). Typical properties of cancer cells are evading apoptosis, immortality, loss of cell cycle control, and to be independent of external growth signals (Hanahan and Weinberg, 2000). Uncontrollable division and aggressive invasion of cancer cells is probably the most important problems in medicine.

Cancer differs from the other troublesome diseases with its variable and autonomous character and behavior. There is no standard long-life therapy strategy or prescription for the patients with any type of cancer, even for the same patient during the therapy. Cancer cells can acquire some capabilities such as more resistant to drugs and more insensitive to tumor suppressing factors as a result of continuous mutations (Table 3.1.) (Hanahan and Weingberg, 2000).

Acquired Capabilities of Cancer	
Acquired Capability	***Example of Mechanism***
Self-sufficiency in growth signals	*Activate H-Ras oncogene*
Insensitivity to anti-growth signals	*Lose retinoblastoma suppressor*
Evading apoptosis	*Produce IGF survival factors*
Limitless replicative potential	*Turn on telomerase*
Sustained angiogenesis	*Produce VEGF inducer*
Tissue invasion & metastasis	*Inactivate E-cadherin*

Table 3.1. Acquired capabilities of cancer cells (Hanahan and Weinberg, 2000).

3.2. Multidrug Resistance

Multi-drug resistance (MDR) the cancer chemotherapy can been defined as the capability of cancer cells to survive against manyanti-cancer drugs.

Biedler and Riehm defined the "cross-resistance" term in the investigation of cellular resistance to actinomycin D in Chinese hamster cells in vitro. (Biedler and Riehm 1970). In 1976, Juliano and Ling used the term "pleiotropic drug resistance" in the study of drug permeability of a surface glycoprotein in chinese hamster ovary cell mutants (Juliano and Ling 1976).Beginning in the 1980s, the term "multidrug resistance" has been widely used. The main cause of MDR seems to be the release of the cancer drug outside the cells is (Zahreddine & Borden, 2013).

Cancer cells can develop some resistance mechanisms against cancer drugs to survive (Figure 3.1.).

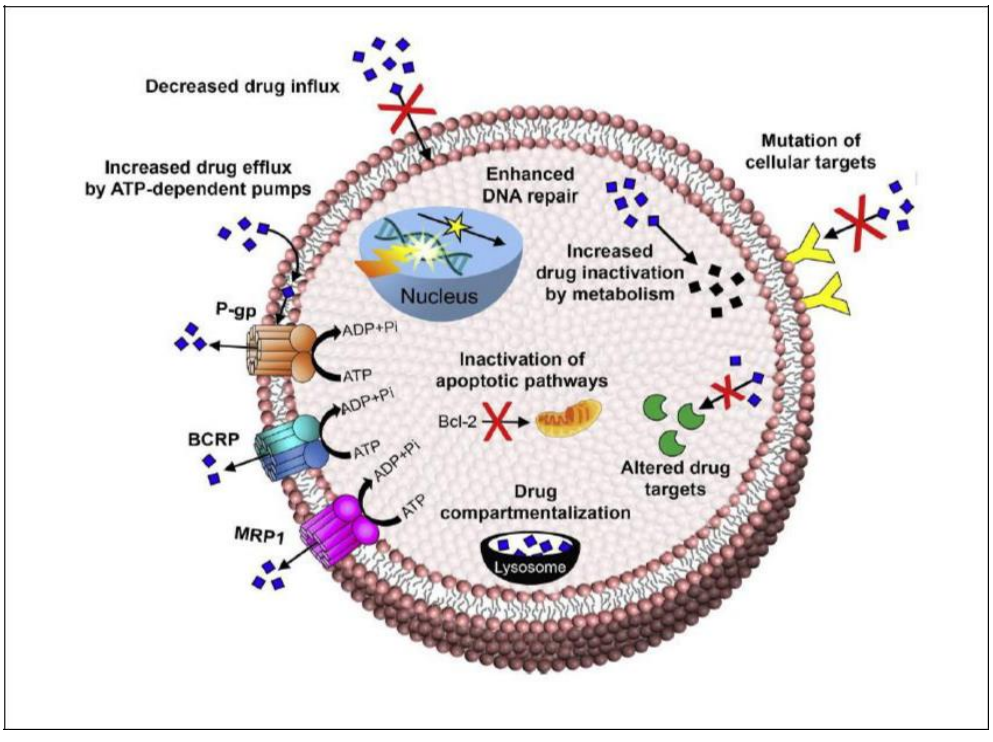


Figure 3.1. Resistance tools and mechanisms of cancer cells. (modified from Liu et al. 2018).

.They can be classified into eight major categories (Liu et al. 2018).

a) increased drug efflux by ATP-dependent pumps,

b) decreased drug influx,

c) increased drug inactivation by metabolism,

d) drug compartmentalization,

e) altered drug targets,

f) enhanced DNA repair,

g) mutation of cellular targets, and

h) inactivation of apoptotic pathways.

The first four mechanisms maintain the cytoplasmic drug concentrations at low levels in cancer cells. The other mechanisms will be effective on the cytotoxicity of chemotherapeutics on the cancer cells.

The accumulating evidence about multidrug resistance has shown that the most important mechanism in multidrug resistance is the increased excretion of cancer drugs into the cell by active transport. Increased expression of drug transport proteins is a common condition. These drug transporters belong to the ATP-binding cassette (ABC) transporter gene family (Ishikawa, 2005)

Although 48 transporters of ABC superfamily were identified in seven subfamilies, only 15 transporters were found to export chemotherapeutic agents (Huang and Sadée, 2006; Wu et al., 2011). These are given in Table 3.2.

Nevertheless, the number of major transporters for MDR is limited. Actually, **ABCB1**, **ABCC1, ABCC2,** and **ABCG2** are the major responsible transporters for MDR.. The other members of these three subfamilies may contribute to multidrug resistance in some cases.

The first drug export protein identified as responsible for multiple drug resistance is P-glycoprotein (ABCB1). The second multi-drug resistance protein identified later is M RP1 (ABCC1). However, the drug exclusion activity of these two proteins is far from fully explaining the resistance to cancer drugs in some canser cases. This suggests the presence of other carriers responsible for multiple drug resistance (Natarajan et al. 2012).

As a result of studies, a drug efflux protein was discovered by using a cell line devoid of Pgp or MRP1 expression [Chen et al., 1990). This protein (ABCG2) was named as breast cancer resistance protein (BCRP) (Natarajan et al. 2012).

Nowadays only ABCB1 (P-gp), ABCC1 (MRP1), and ABCG2 (BCRP) are used for routine measurements and monitoring of MDR in clinics and laboratories.

Selected ABC transporters, cancer-related substrates and their expression in tumoursç

ABC family	Chemotherapy substrates	Expression in cancer cell populations
ABCA		
ABCA2	Estramustine and mitoxantrone	Lung cancer cell lines and AML
ABCA3	Anthracyclines	Neuroblastoma
ABCB		
ABCB1	Colchicine, epipodophyllotoxins, taxanes, anthracyclines, vinca alkaloids, camptothecins, imatinib, mitoxantrone, saquinivir, methotrexate and actinomycin D	AML and lung cancer cell lines
ABCB5	Anthracyclines, camptothecins and thiopurines	Melanoma
ABCC		
ABCC1	Anthracyclines, mitoxantrone, vinca alkaloids, epipodophyllotoxins, camptothecins, imatinib, colchicine, saquinivir and methotrexate	Squamous cell carcinoma lines, lung cancer cell lines, glioma and AML
ABCG		
ABCG2	Mitoxantrone, camptothecins, anthracyclins, bisantrene, imatinib, methotrexate, flavopiridol and epipodophyllotoxins	Lung cancer, AML, oesophageal carcinoma, glioma, melanoma, neuroblastoma, squamous cell carcinoma cell lines, ovarian cancer and nasopharyngeal carcinoma cell lines

Table 3.2. Characteristics of some cancer related ABC transporters (Fletcher et al., 2010).

3.3. ATP transporter regulators and inhibitors in cancer

ATP transporters responsible for MDR have been investigated. Overexpression of MDR transporters are induced by cancer drugs. 100- to 200-fold expression of ABCC1 in doxorubicin-selected lung cancer cells(line H69AR) has been reported (Cole et al., 1992).

ABC transporters require phosphorylation by some protein kinases. Is was shown that the phosphorylation of ABCG2 is necessary for its proper function (Xie et al.,2008). They propose that ABCG2 is phosphorylated at Thr362 by a protein kinase (Pim-1) and this phosphorylation modulates ABCG2 molecule to form a dimer with proper function.

ATP transporters which are synthetized in endoplasmic reticulum are reconstructed and transported to cytoplasmic membranes or organelles. These processes are under the control of protein kinase systems (Crawford et al., 2018). ABC transporters are degraded by proteosomal system.

4. DISCUSSION

In chemotherapy, most tumor cells of patients become resistant to the drug by following the administration of a certain drug. Drug resistance is responsible of 90% of failures during invasion and metastasis of cancer. This resistance is not only to a certain drug, but also to a group of drugs. So, the drug resistance seems to be the most serious problem in cancer therapy.

The causes of multiple drug resistance have been extensively studied. The best-known cause is increased expression of members of the ABC transporter family. This causes a decrease in intracellular cancer drug level and insensitivity to these drugs, and consequently failure in cancer treatment (Fletcher et al., 2010).

Using anticancer drugs and inhibitors of ABC transporters as a mixture is one of the cancer therapy strategies. Anti-cancer drugs are exported with different ABC transporters or groups according to their chemical characteristics. Consequently, the determination of expression levels of these ABC transporters including efflux capacity and inhibition characteristics is very important. Because of a very high level of expression of MDR transporters (up to 50-100x fold of normal levels), these studies are important for the planning of a proper strategy for the therapy and to diminish the drug resistance if it is necessary and available (Roundhill at al. 2015).

Nooter et al. showed the multidrug resistance-associated protein (MRP) was frequently overexpressed in primary breast cancer. They suggest that MRP expression might be of prognostic significance in breast cancer and more important in altered cell biological behaviour with more aggressive phenotype (Nooter et al. 1997).

Many MDR inhibitors have been detected and developed, but few MDR inhibitors were tested in clinical investigation. Characterization of the MDR expression status ofeach cancer patient may give a chance of application of specific treatment way with more satisfactory results.

Expression levels of MDR transporter genes of cancer patients show a great variability. Thus, there is no ideal chemotherapy schedule for all cancer patients.

Although a personalized pharmacotherapy concept has been extensively discussed as an ideal approach to cancer therapy, it has some handicaps. It requires a well-equipped laboratory and personal, expensive instruments, and laborious tests. Although there is the presence of many promising inhibitors for MDR, only a few molecules have been approved by FDA as cancer drug.Nevertheless, resistance determination and measurement experiments should not be neglected in the management of cancer patients.

5. REFERENCES

Allen JD, Jackson SC, Schinkel AH (2002) A mutation hot spot in the Bcrp1 (Abcg2) multidrug transporter in mouse cell lines selected for Doxorubicin resistance. Cancer Res 62(8):2294–2299.

Biedler JL and Riehm H (1970) Cellular resistance to actinomycin D in Chinese hamster cells in vitro : Cross-resistance, radioaytographic, and cytogenetic studies. *Cancer Res*. 30:1174-1184.

Bisbal C, Martinand C, Silhol M, Lebleu B, Salehzada T.(1995) Cloning and characterization of a RNase L inhibitor. A newcomponent of the interferon-regulated 2-5 A pathway. *J. Biol. Chem.*270, 13308–13317.

Broccardo C, Troffer-Charlier N, Savary S, Mandel JL, Chimini G. (1998) Exon organisation of the mouse gene encoding the adrenoleukodystrophy related protein (ALDRP). *Eur J Hum Genet* 6:638–641.

Chen YN, Mickley LA, Schwartz AM, Acton EM, Hwang JL, Fojo AT (1990) Characterization of adriamycin-resistant human breast cancer cells which display overexpression of a novel resistance-related membrane protein. *J Biol Chem*, 265:10073–80.

Cole SPC, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV, Deeley RG (1992) Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science*, 258:1650-1654.

Croop JM, Raymond M, Haber D, Devault A, Arceci RJ, Gros P, et al. (1989) The three mouse multidrug genes of resistance (mdr) are expressed in a tissue-specific manner in normal mouse tissues. *Mol Cell Biol*, 9(3):1346-50. [doi: 10.1128/MCB.9.3.1346].

Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, et al.(1998)A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA*, 95:15665–70.

Fletcher JI, Haber M, Henderson MJ, Norris MD (2010) ABC transporters in cancer: More than just drug efflux pumps. *Nature Reviews Cancer*, 10: 147-156. [doi 10.1038/nrc2789]

Hanahan and Weinberg, (2000) The Hallmarks of Cancer Review. *Cell*, 100;57–70. [doi: 10.1016/s0092-8674(00)81683-9].

Higgins CF (1992) ABC transporters: from microorganisms to man. *Ann. Rev. Cell Biol*.,8: 67-113.

Higgins CF, Linton KJ (2004) The ATP switch model for ABC transporters. *Nat StructMol Biol* 11(10): 918–926.

Huang Y and Sadée W (2006) Membrane transporters and channels in chemoresistance and sensitivity of tumor cells, *Cancer Letters* 239:168-182.

Hyde, S et al. (1990) Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. *Nature*. 346: 362–365.

Ishikawa T (2005) Multidrug Resistance in Cancer: Genetics of ABC Transporters. *Encyclopedia of Life Sciences.* [doi: 10.1038/npg.els.0006083].

Juliano RL, Ling V. (1976) A surface glycoprotein modulating drug permeability in chinese hamster ovary cell mutants. *Biochim Biophys Acta,* 455(1):152-62. [doi: 10.1016/0005-2736(76)90160-7].

Kerr ID (2002) Structure and association of ATP-binding cassette transporter nucleotide-binding domains. *Biochim Biophys Acta* 1561(1):47–64

Koshiyama M, Yoshida M, Fujii H, Konishi M, Nanno H, Hayashi M, et al. (1999) Expression of multidrug resistance-associated protein in endometrial carcinomas: correlation with clinicopathology and prognosis. *Ann Diagn Pathol.* 3:81–7.

Lage, H (2008) An overview of cancer multidrug resistance: a still unsolved problem. *Cellular and Molecular Life Sciences*, 65(20), 3145.

Le Saux O, Beck K, Sachsinger C, Silvestri C, Treiber C, Goring HH, Johnson EW, De Paepe A, Pope FM, Pasquali-Ronchetti I, Bercovitch L, Marais AS, Viljoen DL, Terry SF, Boyd CD (2001) A spectrum of ABCC6 mutations is responsible for pseudoxanthoma elasticum. Am J Hum Genet. 69(4):749-64.

Li J, Liang H, Liu J, Wang Z (2018) Poly (amidoamine) (PAMAM) dendrimer mediated delivery of drug and pDNA/siRNA for cancer therapy. Int. J. Pharmaceutics. [DOI: 10.1016/j.ijpharm.2018.05.045].

Lombard-Platet G, Savary S, Sarde CO, Mandel JL, Chimini G. (1996) A close relative of the adrenoleukodystrophy (ALD) gene codes for a peroxisomal protein with a specific expression pattern. *Proc Natl Acad Sci USA*, 93:1265–1269.

Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B (2017) The Different Mechanisms of Cancer Drug Resistance: A Brief Review. *Advances PharmaceuticalBulletin,* 7(3): 339-348. [doi: 10.15171/apb.2017.041].

Mosser J, Douar AM, Sarde CO, Kioschis P, Feil R, Moser H, Poustka AM, Mandel JL, Aubourg P. (1993) Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature*, 361:726–30.

Natarajan K, Xie Y, Baer MR, Ross DD (2012) Role of breast cancer resistance protein (BCRP/ABCG2) in cancer drug resistance. *Biochemical Pharmacology* 83: 1084–1103. [doi:10.1016/j.bcp.2012.01.002].

Netik A, Forss-Petter S, Holzinger A, Molzer B, Unterrainer G, Berger J. (1999) Adrenoleukodystrophy-related protein can compensate functionally for adrenoleukodystrophy protein deficiency (X-ALD): implications for therapy. *Hum Mol Genet*, 8:907–913.

Neumann J, Rose-Sperling D, Hellmich UA (2016) Diverse relations between ABC transporters and lipids: An overview. *Biochimica et Biophysica Acta*. [doi.org/10.1016/j.bbamem.2016.09.023].

Nooter K, de la Riviere GB, Look MP, van Wingerden KE, Henzen-Logmans SC,Scheper RJ, Flens MJ, Klijn JG, Stoter G, Foekens JA (1997) The prognostic significance of expression of the multidrug resistance-associated protein (MRP) in primary breast cancer. *Br J Cancer.* 76(4): 486–493.

Okada H, Kusaka T, Fuke N, Kunikata J, Kondo S, Iwase T, Nan T, Hirota T, Ieiri I, Itoh S (2014) Neonatal Dubin-Johnson Syndrome: Novel Compound Heterozygous Mutation in the ABCC2 Gene. *Pediatr Int* 56(5): e62-4. [DOI: 10.1111/ped.12404]

Oram JF (2000) Tangier Disease and ABCA1. *Biochim Biophys Acta,*1529 (1-3):321-30 [DOI: 10.1016/s1388-1981(00)00157-8].

Piehler A, Kaminski WE, Wenzel JJ, Langmann T, Schmitz G (2002) Molecular structure of a novel cholesterol-responsive A subclass ABC transporter, ABCA9. *Biochem. Biophys. Res. Commun*. 295: 408-416.

Piehler AP, Hellum M, Wenzel JJ, Kaminski E, KBF Haug, Kierulf P, Kaminski WE (2008) The human ABC transporter pseudogene family: Evidence for transcription and gene-pseudogene interference. *BMC Genomics* 9: 165 [doi: 10.1186/1471-2164-9-165].

Ross DD, Doyle LA, Schiffer CA, Lee EJ, Grant CE, Cole SP, et al.(1996) Expression of multidrug resistance-associated protein (MRP) mRNA in blast cells from acute myeloid leukemia (AML) patients. *Leukemia* 1996;10:48–55.

Sharom FJ (2008) ABC multidrug transporters: Structure, function and rolein chemoresistance. *Pharmacogenomics*, 9: 105–127[doi: 10.2217/14622416.9.1.105].

Tian Y, Han X, Tian D. (2012) The Biological Regulation of ABCE1. *IUBMB Life*, 64(10): 795-800.

Tsybovsky Y, Molday RS, Palczewski K. (2010) The ATP-binding cassette transporter ABCA4: structuraland functional properties and role in retinal disease. *Adv Exp Med Biol.* 2010;703:105-25. [doi:10.1007/978-1-4419-5635-4_8.].

Vlaming ML, Pala Z, van Esch A, Wagenaar E, de Waart DR, van de Wetering K, et al. (2009a) Functionally overlapping roles of Abcg2 (Bcrp1) and Abcc2 (Mrp2) in the elimination of methotrexate and its main toxic metabolite 7-hydroxymethotrexate in vivo. *Clin Cancer Res*, 15:3084–93.

Vlaming ML, van Esch A, Pala Z, Wagenaar E, van de Wetering K, van Tellingen O, et al. (2009b) Abcc2 (Mrp2), Abcc3 (Mrp3), and Abcg2 (Bcrp1) are the main determinants for rapid elimination of methotrexate and its toxic metabolite 7-hydroxymethotrexate in vivo. *Mol Cancer Ther* , 8:3350–9.

WHO Report on Cancer (2020) Setting Ppiorities, Inversting Wisely And Providing Care For All. World Health Organisation, *ISBN 978-92-4-000129-9* .

Woodward OM, Köttgen A, Köttgen M. (2011) ABC transporters and disease. *FEBS Journal* 278: 3215-3225.

Wu CP, Hsieh CH, Wu YS (2011) The Emergence of Drug Transporter-Mediated Multidrug Resistance to Cancer Chemotherapy, *Mol Pharmaceut* 8:1996- 2011.

Xie Y, Lin DE, Yang X, Guo Z, Shimelis H, Nakanishi T et al. (2008) Pim-1 kinase phosphorylates BCRP/ABCG2 and thereby promotes its multimerization and drug-resistant activity in human prostatic cancer cells. *J. Biol. Chem*. 283:3349-3356.

Xiong J, Feng J, Yuan D, Zhou J, Miao W (2015) Tracing the structural evolution of eukaryotic ATP binding cassette transporter superfamily, *Scientific Reports*, 5:16724. [doi: 10.1038/srep16724.].

Yamada A, Ishikawa T, Ota I, Kimura M, Shimizu D, Tanabe M, et al. (2013) High expression of ATP-binding cassette transporter ABCC11 in breast tumors is associated with aggressive subtypes and low disease-free survival. *Breast Cancer Res Treat.* 137:773–82.

Zahreddine H, Borden KL (2013) Mechanisms and insights into drug resistance in cancer. *Front Pharmacol*, 4:28. [doi: 10.3389/fphar.2013.00028].