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Chapter 11

Multidrug Resistance Proteins: A Family of ATP Dependent Transporters and their Role in Cancer

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1. Introduction

Multidrug resistance (MDR) or chemo-resistance is a serious phenomenon utilized by cancer cells that hinder the success of cancer chemotherapy. Owing to the chemo-resistance to antineoplastic drugs, either by acquired or intrinsic mechanisms, the 5-year survival rates remain dismal despite the significant advances in the field of chemotherapy [1]. This was first demonstrated in 1973, where it was found that Ehrlich ascites cells lowered the intracellular daunorubicin concentration by active outward transport [2]. Later it was discovered that the large glycoprotein, now known as multidrug resistance proteins (MRP), in the plasma membrane of MDR cells is responsible for the active outward transport of antineoplastic drugs [3, 4]. The identification of drugs and conjugates efflux pumps of MRP family was started with the discovery of MRP1 in 1992 [5].

Multidrug resistance proteins are the subfamily of the transmembrane transporters superfamily ATP- binding cassette (ABC) [6,7]. It is the largest family of transmembrane proteins which use the energy of ATP hydrolysis to drive a wide range of organic and anionic conjugates such as sulfate, glutathione, glucuronide conjugates and leukotriene C_4 across the

cell membranes [7]. Based on the alignment and phylogenetic analysis with a number of methods, the ABC superfamily can be categorized into seven major subfamilies [6]. The multidrug resistance proteins or ATP binding cassette subfamily C (ABCC) is one of the seven major subfamilies.

MDR uses various mechanisms for the transport of drugs which can be classified as target dependent and drug dependent [8]. Target dependent multidrug resistance mechanism mainly uses factors which cause deletion, mutation and translocation to the target of drugs [9]. Drug dependent MDR is caused by the overexpression of detoxifying enzymes and efflux drug transporters which results into increased efflux of drugs from cell [10]. The aim of this chapter is to discuss the general properties such as structural and functional and to highlight the role of MRPs in cancers cells.

2. General characters of MRPs

The MRP subfamily contains nine members of drug transporters. All the members of the subfamily may have multiple names as several laboratories characterized the MRP family as displayed in **Table 1**. Based on the presence or absence of extra N-terminal membrane spanning domain (MSD), the MRPs are of two types. MRP1, MRP2, MRP3, MRP6 and MRP7 falls into one category which contains an extra N-terminal MSD as presented in **Figure 1** whereas rest of the MRPs contains only two MSDs i.e. MSD1 and MSD2 (**Figure 2**).

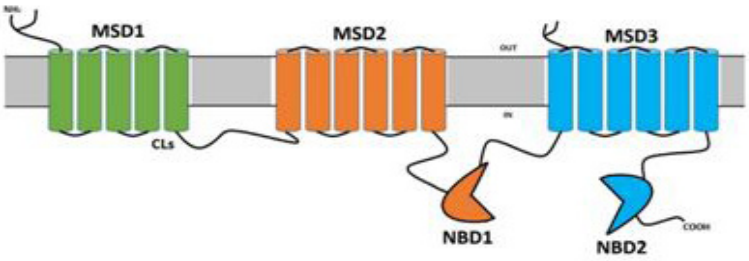

Figure 1: Domain organization of MRP1, MRP2, MRP3, MRP6 and MRP7 with extra N-terminal membrane spanning domain and 17 transmembrane α-helices (MSD–Membrane Spanning Domain; NBD–Nucleotide Binding Domain; CLs – Cytoplasmic Loops)

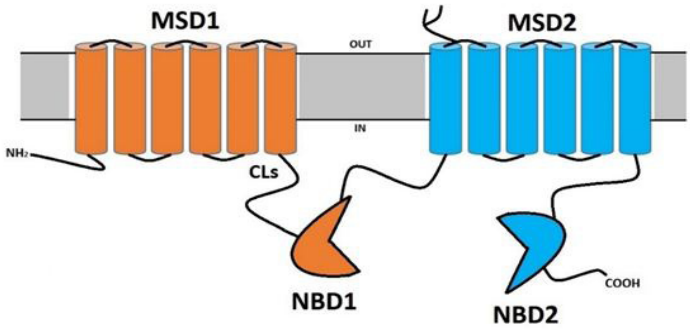

Figure 2: Domain organization of MRP4, MRP5, MRP8 and MRP9 without extra N-terminal membrane spanning domain and 12 transmembrane α-helices (MSD–Membrane Spanning Domain; NBD–Nucleotide Binding Domain; CLs – Cytoplasmic Loops)

Along with all MSDs, the MRPs also have two cytoplasmic nucleotide binding domains (NBDs) and the 17 transmembrane α-helices in case of three MSDs whereas 12 transmembrane α-helices in case of two MSDs [7,11,12]. The binding and the hydrolysis of ATP at NBDs is required for the passage of substances across membrane.

The amino acid sequence lengths of MRP subfamily range between 1325 amino acids for MRP4 to 1545 amino acids for MRP2 (**Table 1**). As compared to MRP1, the amino acid percent identity of MRP3 shares 58 % which is closest member to MRP1 along with MRP2. While the MRP4 and MRP5 shares below 40 % identity which appear to lack the extra N-terminal MSD [13,14]. Furthermore, several studies have revealed that the extra N-terminal MSD is not essential for the transport of drugs across the membrane [14].

Table 1: The human multidrug resistance protein family and some general characters

S. No.	Name	Synonyms/ Symbols	Chromosomal Localization	Amino acids	Amino acid identity	Protein Accession number	References
1.	MRP1	ABCC1, GS-X	16p13.11	1531	100	NP_004987	[5, 15]
2.	MRP2	ABCC2, cMRP, DJS	10q24.2	1545	50	NP_000383	[16, 17]
3.	MRP3	ABCC3, cMOAT2, EST90757, MLP2, MOAT-D	17q21.33	1527	58	NP_003777	[18, 19]
4.	MRP4	ABCC4, EST170205, MOAT-B, MOATB	13q32.1	1325	41	NP_005836	[19, 20]
5.	MRP5	ABCC5, EST277145, MOAT-C, SMRP	3q27.1	1437	38	NP_005679	[18, 19]
6.	MRP6	ABCC6, EST349056, MLP1, URG7	16p13.11	1503	46	NP_001162	[21, 22]
7.	MRP7	ABCC10, EST182763, SIMRP7	6p21.1	1492	35	NP_258261	[19]
8.	MRP8	ABCC11	16q12.1	1382	33	NP_149163	[7, 23]
9.	MRP9	ABCC12	16q12.1	1356	36	NP_150229	[7, 23]

3. Overview of the MRP Family

The localization and distribution of multidrug resistance proteins vary in different human tissues as their expression pattern is cell and tissue type specific such as kidney, lung, skeletal and cardiac muscles specific. To understand the function of the MRPs efflux pump, it

is required to see the domain-specific localization of MRPs in various cell types. Along with the localization and distribution of MRPs, it is also needed to know the substrates of the members of MRP family. The amphiphilic organic anions of molecular mass between 0.3 to 1.0 kDa are the substrates of the MRP subfamily members [11,12]. **Table 2** summarizes the location of members of MRPs and their substrates.

3.1. MRP1

The MRP1 or ABCC1 is localized mainly in the cells of blood-tissue barriers which is shown by the immunofluorescence and immunohistochemical analysis [24]. It is highly detectable in several human cells and tissues such as macrophages, kidney, lung, placenta, testis, umbical cord, skeletal muscles, cardiac muscles and gestational tissue [12,25]. During pregnancy, MRP1 expression level changes have been associated with pre-term birth, growth restriction, and pre-eclampsia [26]. There is lack of detectable amount of MRP1 in normal hepatocytes but in proliferating hepatocyte-derived cells MRP1 appears to be upregulated [12, 27]. The cells that do not express MRP2, MRP1 plays an important function in detoxification from those cells [12].

The first physiological substrate of MRP1 to be identified was the cysteinyl leukotriene LTC4. This finding was discovered during the search for the efflux pump that cause the release of LTC4 from mastocytoma cells [28]. Later by the studies in Abcc1-/- mice it was confirmed that LTC4 is a physiologically relevant substrate [29]. MRP1 can identify a wide range of substrates by making a single bipartite substrate-binding site. The substrate binding site of MRP1 can be categorized into two parts – one with the positively charged region that directs the GSH moiety and other with the hydrophobic area that incorporates the lipid tail [30]. Glutathione containing LTC4, which is high affinity MRP1 substrate, discovery preceded the finding of several glucuronosyl and S-glutathionyl substrates for MRP1 as displayed in Table 2. Another MRP1 substrate, oxidized glutathione (GSSG) with comparatively low affinity suggests the role of MRP1 against oxidative stress [12,31]. GHS plays various role in MRP1-mediated transport such as it act as co-substrate together with the other compounds like Vinca alkaloids. Moreover, it plays a role as transport enhancer without being co-transported itself in case of glucuronidated and sulfated conjugates [32].

3.2. MRP2

The second member of MRP subfamily, MRP2, was first localized in the canalicular membrane of the human and rat hepatocytes [33] and afterward in the apical membrane of rat and human kidney proximal tubules [34,35], placenta [36], small intestine [37], colon [38], gall bladder [39] and bronchi segments [38]. In various human cells and tissues such as blood-brain barrier, pancreas and skin the expression of MRP2 protein either remain below detection limit or remain absent. The apical localization of MRP2 remain in line with its function in the

efflux of many endogenous substance and phase II conjugation product of drugs into extracellular fluids including urine, bile and intestinal fluid.

Table 2: Tissue distribution and substrates of human multidrug resistance proteins

S. No.	Members of MRP subfamily	Location of MRPs in human body	Substrates of MRP transporters
1.	MRP1	Macrophages, kidney, lung, testis, placenta, umbical cord, skeletal and cardiac muscles	Leukotriene C4, Leukotriene D4, Glutathione disulphide, GSH, S-Glutathionyl prostaglandin A2, Glutathionyl melphalan, Estrone 3-sulphate, Bisglucuronosyl bilirubin, folate, cobalamin-OH
2.	MRP2	Liver, kidney, small intestine, colon, gall bladder, placenta, segment of bronchi	Leukotriene C4, Mono- and Bisglucouronosyl bilirubin, 17β-glucuronosyl estradiol, cholecystokinin peptide, Estrone 3-sulfate
3.	MRP3	Gut, Liver, kidney, adrenals, colon, spleen and pancreas	Leukotriene C4, Mono- and Bisglucouronosyl bilirubin, 17β-glucuronosyl estradiol, Cholyglycine, Dehydroepiandrosterone 3-sulfate
4.	MRP4	Prostate, testis, ovary, lung, muscle, gall bladder and pancreas	Leukotriene C4, B4, Prostaglandin E2, F2α, Thromboxane B2, , 17β-glucuronosyl estradiol, cGMP, cAMP, Cholyltaurine (+GSH), cholate (+GSH), Folate, Urate, ADP
5.	MRP5	Urethra, heart, placenta, blood brain barrier	Methotrexate, cGMP, cAMP, Folate, 2'-Deoxyuridine 5'-monophosphate, 9-(2-Phosphonomethoxyethyl)adenine (PMEA)
6.	MRP6	Kidney and Liver	Leukotriene C4, S-Glutathionyl N-ethylmaleimide
7.	MRP7-9	Cerebral cortex and Secretory cells	17b-Glucuronosyl estradiol, Leukotriene C4, Dehydroepiandrosterone 3-sulfate, cGMP, cAMP, Folate, Cholylglycine

MRP2 and MRP1 substrates are quite similar, however the kinetic properties are different i.e. the Km values of MRP1 for 17β-glucuronosyl estradiol and LTC4 are five and tenfold lower, respectively, than those for MRP2 [40]. Similarly, MRP2 have higher affinity for the mono- and bisglucuronosyl bilirubin as compared to the MRP1 [41]. Additionally, as compared to MRP1, MRP2 have low affinity for the transport of GSH and GSSG [42]. The amino acid identity of both MRPs i.e. MRP1 and MRP2, is only 50% (**Table 1**). Thus the similar substrate specificity was initially unexpected, however advancement in the research leads to the finding of the similar structural determinants for substrate binding of both proteins which are responsible for the similar substrates [12].

3.3. MRP3

ATP-dependent efflux transporter mainly MRP3 localized to basolateral (sinusoidal) hepatocyte membrane which transport compounds from hepatocytes to sinusoidal blood [43]. It was initially demonstrated in the human and rat hepatocytes [44,45] and now localized in several human cells and tissues including cholangiocytes, pancreas, kidney, enterocytes, spleen, gall bladder and adrenal cortex [12,43]. The level of MRP3 in human liver may fluctuate upto

80 fold among people. In hereditary MRP2 deficiency and different types of cholestatic liver disease, the MRP3 level increases which leads to elevated serum concentration of bilirubin glucuronosides [46].

MRP3 transports a broad range of xenobiotics and endogenous organic anions, mostly conjugated as presented in **Table 2**. Mono- and bisglucuronosyl bilirubin efflux across the basolateral membrane of hepatocytes into sinusoidal blood is MRP3-mediated transport [47]. It also transports methotrexate in addition to LTC4 and S-(2, 4-dinitrophenyl) glutathione. Human MRP3 transports bile acids (e.g., cholylglycine, cholyltaurine, and sulfatolithocholyltaurine) with low affinity in case of bile acid cholylglycine or below detectability in case of bile acid cholyltaurine [48]. However, the MRP3 of rat transports bile acids with high affinity [49]. This indicates that the MRP3 substrates are species specific, particularly for bile acids.

3.4. MRP4

The MRP4 protein is expressed in a variety of polarized cells and localized in the apical and basolateral membrane domain [43]. Initially it was localized in the glandular epithelial cells of the prostate gland in basolateral membrane [50]. Additionally MRP4 protein express in platelets [51], erythrocytes, astrocytes, adrenal glands and in many cultured cell lines used for the transfection studies such as V79, HEK293, HL60 and HeLa [12,52]. Moreover, MRP4 localization is detected in human and rat hepatocytes, choroid plexus epithelial cells and in polarized MDCKII cells [53-56].

The substrates first identified for MRP4 protein were the nucleosides monophosphate analogs used as antiretroviral drugs, mainly the 9-(2-phosphonylmethoxyethyl)adenine (PMEA) [57]. In delta granules of human platelets, MRP4 mediates the ADP transport which results into accumulation of ADP in delta granules [58]. In addition, the cGMP, cAMP and LTC4 are the important physiological substrate for the MRP4. Other MRP4 protein substrate includes eicosanoids such as prostaglandins E1, E2 and F2α and leukotrienes C4 and B4 [52].

3.5. MRP5

Localization of MRP5 has been detected in basolateral membrane of polarized epithelial cells. However in brain capillary endothelial cells, MRP5 aka ABCC5 is detected in apical membrane [59]. Relatively higher level of MRP5 protein has been demonstrated in smooth muscle cells, astrocytes and in various tissues of human genitourinary system [59]. Furthermore MRP5 protein expresses in epithelial cells of urethra [60], endothelial cells of heart [61] and in fetal vessels of placenta [62].

MRP5 protein substrates includes anionic dye fluorescein diacetate, a number of nucleosides monophosphate analogs, the cyclic nucleotides cGMP and cAMP and some GSH

S-conjugates [63,64]. MRP5 mediated transport is inhibited by various phosphodiesterase inhibitors some of which are structurally similar to cGMAP. MRP5 along with the MRP4 may contribute to regulation of cAMP and cGMP tissue level. Moreover, the affinity of MRP5 to cAMP and cGMP seems to vary depending upon the cellular system [11].

3.6. MRP6

MRP6 protein is highly expressed in the basolateral membrane of human and rodent hepatocytes and epithelial cells of proximal tubule of kidney [65]. Recently it has been identified that MRP6 acts as basolateral efflux pump for nucleotides mainly ATP which after hydrolysis by ecto-enzymes leads to extracellular pyrophosphate [43].

MRP6 protein of inside-out vesicles transports the glutathione S-conjugates LTC4 and NEM-SG and BQ-123 with low affinities [66]. MRP6 mutation leads to a serious genetic disorder, Pseudoxanthoma elasticum (PXE), with ectopic mineralization affecting eye, skin and cardiovascular system. This is hypothesized that PXE is a consequence of hepatic accumulation of MRP6 substrate(s) as it seems that MRP6 remain absent in the affected organs whereas a high expression is seen in hepatocytes [67].

3.7. MRP7-9

On the basis of mRNA analysis it is assumed that the MRP7-9 are expressed widely in various human cell types and tissues [12]. MRP8 was detected in axonal membrane of neurons in human cerebral cortex and in the HepG2 apical membrane [68]. Additionally, MRP8 protein express in the luminal membrane and large vacuoles of secretory cells such as apocrine sweat glands [12,69,70].

A number of substrates are mediated by the MRP7 protein including 17β-glucuronosyl estradiol and LTC4 [12]. It also confers the low level of resistance to Vinca alkaloids and paclitaxel [71]. MRP8 mediates the transport of a number of physiological substrates including dehydroepiandrosterone 3-sulfate, LTC4, cholylglycine, cyclic nucleotides, 17β-glucuronosyl estradiol and folate. Recently a new substrate, Tenofovir disoproxil fumarate, of MRP8 is identified which is a nucleotide reverse transcriptase inhibitor [72]. The substrate of MRP9 has not been detected so far [12].

4. Role of MRPs in Cancer

MRPs are the proteins which are responsible for the resistance of cancer cells to a broad variety of mechanistically and structurally anticancer drugs. The chemotherapeutic failure is the result of either intrinsic resistance or acquired resistance of cancers cell which leads to the malignant tumor progression [73]. The major mechanism of multidrug resistance can be categorized into various groups such as inhibition of apoptosis pathways, metabolic modifica-

tion, activation of DNA repair, decreased drug influx, altered drug targets, and detoxification, increased drug efflux mainly via MRP subfamily transporters and via higher expression levels of these efflux transporters [8,74]. These efflux transporters or efflux pumps reduce the concentration of several intracellular exo- and endotoxins via the above-mentioned mechanisms.

Recently, an overexpression of members of MRP subfamily, particularly MRP1 and MRP8, was reported in the aggressive breast carcinoma subtypes [75]. Similarly overexpression of MRP1, MRP2 and MRP3 was observed in lung cancer patients [76]. The expression level of these pumps varies based on the lung cancer subtypes i.e. In non-small cell lung cancer (NSCLC) cell lines, higher expression of MRP1, MRP2, and MRP3 was found than small cell lung cancer (SCLC) cell lines, with the highest level of MRP3 [76]. Additionally, the decrease in drug sensitivity towards etoposide, cisplatin, vincristine and doxorubicin in lung cancer patients is owing to overexpression of MRP1 and MRP3. Likewise in colorectal carcinoma, the levels of MRP1 and MRP2 are found to be higher [77]. As compared to the patients who respond to chemotherapy, there is higher expression of MRP6 and MRP8 in non-responders. The overexpression of MRPs in drug resistance of specific types of cancer are summarized in **Table 3**.

RT-PCR and northern blotting exhibited the MRP1 overexpression in prostate cancer lines resistant to doxorubicin. Similarly, overexpression has also been detected by immunohistochemistry in pancreatic carcinoma cell lines and in renal cell carcinoma patients [78–80]. Recently, MRP7 and docetaxel-treatment failure were confirmed by *ex vivo* study where MRP7 was greatly expressed in ER and Her2 breast cancers and to reverse MDR in chemotherapy, inhibition of MRP7 was suggested [81]. Therefore, in patients with high expression level of MRPs, to inhibit drug efflux function by developing modulators is a feasible approach.

Table 3: MRPs overexpressed in drug resistance of specific types of cancer

S. No.	Specific type of Cancer	MRPs Overexpressed	Reference
1.	Breast cancer	MRP1, MRP8	[75]
2.	Non-small cell lung cancer	MRP1, MRP2, MRP3	[76]
3.	Small cell lung cancer	MRP3, MRP5	[76,82]
4.	Colorectal cancer	MRP1, MRP2	[77]
5.	Prostate cancer	MRP1, MRP4	[78,83]
6.	Pancreatic cancer	MRP1	[79]
7.	Renal cancer	MRP1	[80]

4.1. Current strategies for MRP modulators

In order to reverse the MRP-mediated MDR, several attempts have been performed. Recently a few approaches are being used to develop MRPs modulators to reverse MDR in chemotherapy such as off-target small molecular inhibitors as modulators and miRNA based

therapy [73].

MicroRNA (miRNA) is small RNA molecules of approximately 20-25 nucleotides in length which bind directly to 3'UTR of targeted mRNAs. Various miRNA based modulators are being used such as miR-326, miR-297, miR-534 and miR-134. The overexpression of MRP1 mRNA and its protein was observed in the breast cancer cell line MCF-7/VP. On MCF-7/VP and MCF-7 cells, 17 of miRNAs were distinctly expressed by utilizing a microarray consisting of human mature miRNA probes. All the expressed miRNA showed increased expression but miR-429, miR-92-2, miR-7, miR-187 and miR-326 exhibited decreased expression [84]. Quantitative RT-PCR result revealed that the decreased expression of miR-326 was 3.3 fold less as compared to MCF-7 and the expression was inversely correlated with the MRP1 mRNA. It was observed that expression of MRP1 were lowered in miR-326 miRIDIAN mimic-transfected MCF-7/ VP cells [84]. From this finding, it was suggested that by blocking the MRP1, the miR-326 could strengthen the cytotoxic effect of doxorubicin on MCF-7/VP cells.

A number of small off-target molecular inhibitors are used as modulators of MRPs i.e. Ibrutinib as modulator of MRP1, Masitinib, Lapatinib, Imatinib, Erlotinib, Nilotinib and Tandutinib as Modulators of MRP7. MRP1 modulator ibrutinib can potentially block the efflux of doxorubicin in HL60/Adr cells which leads to increased intracellular doxorubicin accumulation [85]. In recent years, it has been reported that Masitinib, Lapatinib, Imatinib, Erlotinib, Nilotinib and Tandutinib could reverse MDR in transfected HEK/MRP7 cells [73,86,87].

5. Summary

This chapter summarizes about the multidrug resistance proteins which are a subfamily of ATP dependent transporters, ABC family. The MRP family is the transmembrane proteins which use the energy of ATP hydrolysis to drive a wide range of organic and anionic conjugates such as sulfate, glutathione, glucuronide conjugates and leukotriene C4 across the cell membranes. The MRP subfamily contains nine members of drug transporters i.e. MRP1-9. All the members of the subfamily may have multiple names as several laboratories characterized the MRP family. The localization and distribution of multidrug resistance proteins vary in different human tissues as their expression pattern is cell and tissue type specific such as kidney, lung, skeletal and cardiac muscles specific. The amphiphilic organic anions of molecular mass between 0.3 to 1.0 kDa are the substrates of the MRP subfamily members. The major mechanism of multidrug resistance can be categorized into various groups such as inhibition of apoptosis pathways, metabolic modification, activation of DNA repair, decreased drug influx, altered drug targets, and detoxification, increased drug efflux mainly via MRP subfamily transporters and via higher expression levels of these efflux transporters. The chemotherapeutic failure is the result of either intrinsic resistance or acquired resistance of cancers cell

which leads to the malignant tumor progression. Recently a few approaches are being used to develop MRPs modulators to reverse MDR in chemotherapy such as off-target small molecular inhibitors as modulators and miRNA based therapy. These recent strategies to engage the MRP transporters to enhance the cancer treatment reflect the creativity of cancer researchers and hopefulness that at least this basis of MDR can be defeated.

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