Advanced Drug Delivery Reviews xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Multidrug resistance: Physiological principles and nanomedical solutions☆

Sijumon Kunjachan [a,1], Błażej Rychlik [b,1], Gert Storm [d,c], Fabian Kiessling [a], Twan Lammers [a,c,d,*]

[a] Department of Experimental Molecular Imaging, Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Pauwelsstrasse 30, 52074 Aachen, Germany
[b] Cytometry Lab, Department of Molecular Biophysics, University of Lodz, Banacha Street 12/16, 90-237 Lodz, Poland
[c] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands
[d] Department of Controlled Drug Delivery, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands

ARTICLE INFO

Article history:
Accepted 30 September 2013
Available online xxxx

Keywords:
Nanomedicine
Drug targeting
Multidrug resistance
MDR
Drug efflux pumps
ABC transporters
Pgp
MRP
Pluronics
siRNA

ABSTRACT

Multidrug (MDR) resistance is a pathophysiological phenomenon employed by cancer cells which limits the prolonged and effective use of chemotherapeutic agents. MDR is primarily based on the over-expression of drug efflux pumps in the cellular membrane. Prominent examples of such efflux pumps, which belong to the ATP-binding cassette (ABC) superfamily of proteins, are Pgp (P-glycoprotein) and MRP (multidrug resistance-associated protein), nowadays officially known as ABCB1 and ABCC1. Over the years, several strategies have been evaluated to overcome MDR, based not only on the use of low-molecular-weight MDR modulators, but also on the implementation of 1–100(0) nm-sized drug delivery systems. In the present manuscript, after introducing the most important physiological principles of MDR, we summarize prototypic nanomedical strategies to overcome multidrug resistance, including the use of carrier materials with intrinsic anti-MDR properties, the use of nanomedicines to modify the mode of cellular uptake, and the co-formulation of chemotherapeutic drugs together with low- and high-molecular-weight MDR inhibitors within a single drug delivery system. While certain challenges still need to be overcome before such constructs and concepts can be widely applied in the clinic, the insights obtained and the progress made strongly suggest that nanomedicine formulations hold significant potential for improving the treatment of multidrug-resistant malignancies.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Nanotechnology and drug resistance".

* Corresponding author at: Department of Experimental Molecular Imaging, Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Pauwelsstrasse 30, 52074 Aachen, Germany. Tel.: +49 241 8036681.
E-mail address: tlammers@ukaachen.de (T. Lammers).

[1] Equal contribution.

0169-409X/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.addr.2013.09.018

1. Introduction

In spite of significant advances in understanding the etiology and progression of cancer, and in developing novel diagnostics and therapeutics, both the incidence and the mortality rates of malignancy remain to be extremely high. One of the main reasons for this is chemoresistant cancer recurrence. Chemoresistance may either be innate, i.e. existing since the beginning of therapy, or acquired, i.e. developed during the course of treatment. Its significance can be illustrated by the fact that almost all non-small cell lung cancer patients treated with chemotherapy eventually develop resistance against the anticancer agents used [1]. The biological background of chemoresistance is complex and generally includes one or more of the following mechanisms: inhibition of apoptosis, induction of DNA repair mechanisms, alterations of drug target structure, modifications in cell membrane composition (leading to reduced drug uptake), and last but not least, elevated expression levels of drug efflux pumps. Regarding the latter, a major problem is cross-resistance, which relates to an increased expression of broad-spectrum drug transporters present within the cancer cell membrane, which are not only active against a single drug or chemically-related drugs, but against a whole range of chemotherapeutic agents, even to agents which have not yet been administered to the patient. This phenomenon is referred to as multidrug resistance (MDR), and the proteins involved in this process are called MDR proteins.

2. Physiological principles of MDR

The history of MDR proteins started in 1974, when Victor Ling and Larry Thompson described a stable colchicine-resistant cell clone derived from a CHO cell line by a single-step selection, and discovered that the resistant cells did not allow colchicine to enter the cytoplasm [2]. The selected cells were also found to be resistant to demecolcine, actinomycin D and vinblastine. It was furthermore observed that although colchicine uptake by sensitive cells was passive, resistance was an active process, as it could be inhibited by cyanides, azides and dinitrophenol [3]. It was further proven that the main difference between naive and resistant cells was the expression of a 170 kDa plasma membrane glycoprotein called P-glycoprotein (Pgp; with the first P referring to permeability) [4]. It rapidly became apparent that there are other active membrane transporters, distinct from Pgp, which are involved in multidrug resistance. In 1990, for instance, a 95 kDa membrane protein responsible for anthracycline resistance in MCF-7/AdrVp(100) cells was described [5], which later became known as BCRP (Breast Cancer Resistance Protein), and in 1992, Cole and coworkers identified and cloned another phosphoglycoprotein which was highly overexpressed in doxorubicin-resistant H69AR cells and named it MRP (Multidrug Resistance-associated Protein) [6]. It was soon clear that all of these proteins share some sequence- and functional homology, and belong to ATP-binding cassette (ABC) superfamily of proteins.

2.1. ABC of MDR transporters

ABC proteins are P-type membrane ATPases, distinguished by highly conserved amino acid sequences located in their nucleotide-binding domain (so called Walker A and Walker B motifs), separated by the 'ABC signature' motif LSGGQQ/R/KQR [7]. They constitute one of the largest protein families identified to date, are present in almost all cells of all taxonomic groups of organisms, and are engaged in various membrane transport processes, such as substrate uptake, product excretion and osmoregulation (including transmembrane ion movement). In prokaryotes, ABC proteins form oligomeric complexes, while eukaryotic ABC proteins are usually composed of a single polypeptide [8]. The inventory of human ABC genes contains 48 elements, and to fulfill standards of human genetic nomenclature, they were subdivided into seven families, A to G, each labeled as ABC followed by a family letter and a number [8]. Using this system, Pgp is now generally referred to as ABCB1, while BCRP and MRP are known as ABCG2 and ABCC1, respectively. It should be mentioned in this regard that there are several more ABC proteins, especially from the ABCC subfamily, which are involved in multidrug resistance, but we here primarily focus only ABCB1, ABCC1 and ABCG2, as their clinical significance is broadly accepted and extensively documented.

One of the key characteristic features of ABC transporters is their molecular architecture. The basic unit of the protein is a set of 6 hydrophobic membrane-spanning helical fragments forming a so called transmembrane domain (TMD), followed by a hydrophilic cytoplasmic nucleotide-binding domain (NBD) harboring amino acid sequences distinctive for ABC proteins. Such a structure is doubled in most eukaryotic transporters, forming a TMD1–NBD1–TMD2–NBD2 single polypeptide assembly. ABCB1 is a good example of a canonical eukaryotic transporter [9] (Fig. 1B), but the molecular structure of other MDR proteins can vary quite a bit. ABCC1 protein contains an additional N-terminal transmembrane domain (TMD_0), consisting of five helical fragments linked to the core of the molecule by a L_0 loop (Fig. 1A) [10]. This fragment of the protein is important for its stable expression and function [11], as well as for proper membrane trafficking [12]. ABCG2 is a representative example of the so-called 'half-transporters', consisting of a single TMD and a single NBD domain, but in reverse order (i.e. NBD is the N-terminal domain; see Fig. 1C) [13]. Unlike ABCB1 or ABCC1, which function as monomers, ABCG2 requires homo-oligomerization, most likely octamerization, to form an active transport unit [14].

MDR transporters, as all ABC proteins, are vanadate-sensitive ATPases [8]. Both NBDs are involved in ATP-binding and hydrolysis, which is coupled to a conformational change in the protein (with hydrolysis being the rate-limiting step of the catalytic cycle [15]). The ATPase activity of MDR proteins is azide- and ouabain-insensitive, and can be stimulated by drugs to which a given protein confers resistance, as was clearly shown for ABCB1 [16] and later on also for other family members. MDR transporters are located in apical (ABCG2 [17] and ABCB1 [18]) or basolateral (ABCC1 [19]) domains of the plasma membrane of polarized cells. The lipid milieu is an important factor influencing protein activity. It was clearly shown that ABCG2 is located in lipid rafts, as its activity significantly decreases in cholesterol-depleted cells [20]. Furthermore,

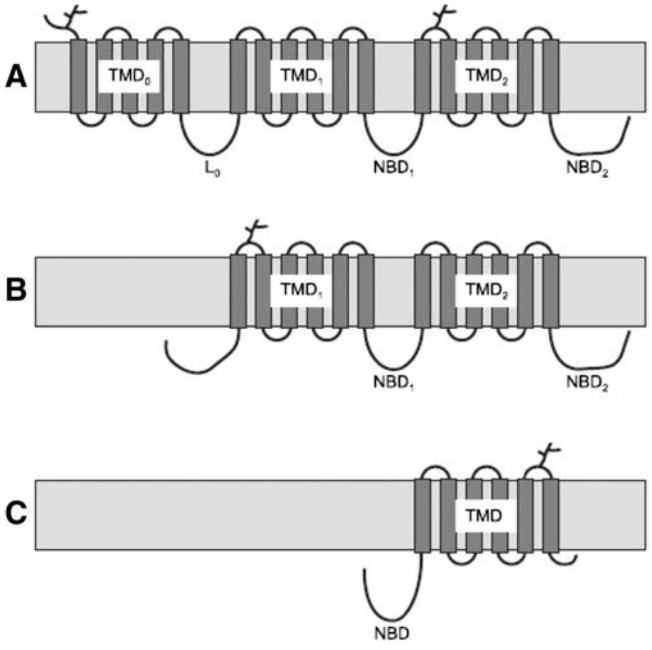

Fig. 1. Schematic molecular architecture of prototypic human ABC transporters. A. ABCC1, B. ABCB1, C. ABCG2. TMD — transmembrane domain, NBD — nucleotide-binding domain, L_0 — loop 0.

Please cite this article as: S. Kunjachan, et al., Multidrug resistance: Physiological principles and nanomedical solutions, Adv. Drug Deliv. Rev. (2013), http://dx.doi.org/10.1016/j.addr.2013.09.018