Gene Therapeutic Approaches to Overcome ABCB1-Mediated Drug Resistance

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Abstract

Multidrug resistance (MDR) to pharmaceutical active agents is a common clinical problem in patients suffering from cancer. MDR is often mediated by over expression of trans-membrane xenobiotic transport molecules belonging to the superfamily of ATP-binding cassette (ABC)-transporters. This protein family includes the classical MDR-associated transporter ABCB1 (MDR1/P-gp). Inhibition of ABC-transporters by low molecular weight compounds in cancer patients has been extensively investigated in clinical trials, but the results have been disappointing. Thus, in the last decades alternative experimental therapeutic strategies for overcoming MDR were under extensive investigation. These include gene therapeutic approaches applying antisense-, ribozyme-, RNA interference-, and CRISPR/Cas9-based techniques. Various delivery strategies were used to reverse MDR in different tumor models in vitro and in vivo. Results and conclusions of these gene therapeutic studies will be discussed.

Keywords

Multidrug resistance · Cancer · Gene therapy · RNA interference · CRISPR/Cas9

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1 Introduction

Resistance including multidrug resistance (MDR) to chemotherapy is a common clinical problem in patients suffering from cancer. Besides alterations in various cellular pathways involved in regulation of cell cycle, apoptosis, and repair, MDR is often mediated by over expression of trans-membrane xenobiotic transport molecules belonging to the superfamily of ATP-binding cassette (ABC)-transporters (Lage 2008). The first identified member of this family involved in MDR was ABCB1, a 170 kDa cytoplasm membrane-embedded glycoprotein also known as P-glycoprotein, P170, or MDR1/P-gp (Juliano and Ling 1976). Following the identification of ABCB1, a large number of drug-extruding ABC-transporters have been identified in cancer cells. Inhibition of ABC-transporters by low molecular weight compounds in cancer patients has been extensively investigated in clinical trials, but the results have been disappointing. In particular, different experimental therapeutic strategies were developed which are able to selectively switch off specific ABC-transporter encoding genes in drug-resistant cancer cells. The most promising of these efforts focussed on the design of RNA-based therapeutics.

Already in the 1980s, DNA- and RNA-based antisense oligonucleotides with different chemical modifications were tested with different model systems both in vitro and in vivo with varying degrees of success. Accordingly, at that time the synthesis of ABCB1 could be inhibited by nonionic oligonucleoside methylphosphonates in human multidrug-resistant K562 erythroleukemia cells (Vasanthakumar and Ahmed 1989).

During the 1990s, ribozymes with both antisense and catalytic properties were successfully applied for specific knock down of gene expression. Ribozymes were demonstrated to selectively inhibit the expression of various genes including the ABCB1 encoding gene in human cancer cell lines (Kobayashi et al. 1994; Holm et al. 1994) as well as in tumors grown in mice (Gao et al. 1999). However, unsolved problems with delivery issues for these therapeutic RNA molecules limited their clinical exploitation.

In the 2000s, advances have revealed new opportunities for the development of RNA therapeutics in particular those based on the utilization of the RNA interference (RNAi) pathway. The observation that synthetic RNA molecules, i.e., double-stranded small interfering RNA (siRNA) molecules, could be used to specifically silence genes in mammalian cells (Elbashir et al. 2001) initiated an explosion of research on the mechanisms and application of the RNAi phenomenon. Following the first evidence of the in vivo efficacy of the RNAi technology in an animal model (Soutschek et al. 2004), RNA-based drugs were developed efforts and several clinical trials, including phase III trials, started with RNAi-based therapeutics (Whitehead et al. 2009). Following the first proofs that this technology is useful to circumvent MDR by inhibition of ABCB1 synthesis (Nieth et al. 2003; Wu et al. 2003), a huge number of studies were published applying this technique in different cancer cell models in vitro and in vivo.

At the beginning of the 2010s, the development of the CRISPR/Cas9-gene editing technology offered new possibilities as a general tool for the precise regulation of gene expression in eukaryotic cells (Gilbert et al. 2013). In cancer research, firstly this technique was applied in vitro to target and destroy the HPV16-encoded E6 or E7 genes in human cervical carcinoma cells resulting in cell cycle arrest leading to cancer cell death (Kennedy et al. 2014). So far, only a single study was published in which this new technology was applied to silence ABCB1 expression in a canine in vitro model (Simoff et al. 2016). In this study, Madin–Darby canine kidney cells (MDCK II wt) showing high expression of ABCB1 were transfected with CRISPR/Cas9 plasmid vectors, targeting three specific regions of the ABCB1 encoding gene. The treated cells completely lacked detectable levels of ABCB1 expression and no ABCB1-specific transport activity could be observed.

Just like with antisense oligonucleotides and ribozymes, the key challenge with RNAi-based or CRISPR/Cas9-based therapeutics is achieving the effective delivery. Naked RNA molecules are rapidly degraded in physiological milieu and, therefore, have very short half-life times. Former chemical modifications which were developed to prolong the half-life of antisense molecules and ribozymes have proved to be valuable in developing siRNA therapeutics. Most of these modifications concerned to the oligonucleotide backbone linkages to protect against degradation by nucleases. RNAi or CRISPR/Cas9 strategies may offer greater opportunities than those resulting from earlier approaches, potentially as a consequence of the possibility by which the hurdle of delivery will be solved. However, new approaches for delivery of therapeutic RNA molecules are continuously in development and were applied for targeting MDR-associated ABC-transporters.

2 Resistance Overcoming Gene Therapy Approaches

2.1 Delivery by Transfection

The easiest way for the delivery of therapeutic agents consisting of nucleic acids is transfection using different types of transfection reagents. These reagents consist of cationic lipids or other cationic polymers which can form complexes with negatively charged nucleic acids. These complexes are admitted by the target cells via endocytosis. Respectively to the complex-forming reagent this process is designated as lipofection or polyfection. Accordingly, the first studies using antisense oligonucleotides, ribozymes, or RNAi-mediating agents applied transfection procedures for knock down of the ABCB1-encoding mRNA. Meanwhile, a huge number of studies using different cell models were published (overview in Lage 2009). Depending on the cell models, the gene silencing agents, and the target sequences, a wide range of efficacies in gene silencing could be observed. As an example, treatment of the multidrug-resistant human gastric carcinoma cell line EPG85-257RDB and the human pancreatic carcinoma cell line EPP85-181RDB with complexes consisting of chemically synthesized siRNAs and cationic

polymers resulted in up to 90 % decrease in the ABCB1 mRNA expression level (Nieth et al. 2003). In this transient experiment, resistance against the ABCB1 substrate daunorubicin was decreased to 89 % (EPP85-181RDB) or 58 % (EPG85-257RDB). However, experiments with these cell models applying stable transfection procedures showed more efficacies in reversal of MDR. ABCB1 downregulation by transfection with anti-ABCB1 ribozyme or anti-ABCB1 short hairpin RNA (shRNA)-plasmid-based expression vectors resulted in each case in a comprehensive knock down of the ABCB1 mRNA expression and a complete reversal of the multidrug-resistant phenotype in EPP85-181RDB and EPG85-257RDB cells (Stege et al. 2004).

Different improvements of these strategies were developed. For example a "multitarget multiribozyme" (MTMR) was constructed (Kowalski et al. 2005). This MTMR was simultaneous directed against the mRNAs encoding three different ABC-transporters, i.e., against ABCB1, ABCC2, and ABCG2. In this MTMR, the three trans-acting hammerhead ribozymes directed against ABCB1, ABCC2, and ABCG2 were linked with ABCB1-homologous spacer sequences and three cis-acting ABCB1-specific ribozymes. The trans-acting hammerhead ribozymes were liberated from the MTMR through autocatalytic self-cleavage by the cis-acting ribozymes. In different cell models, the MTMR could cleave their specific substrates without loss of efficiency when compared with the corresponding monoribozymes.

Although these studies demonstrated the high potential of gene therapeutic approaches for reversal of MDR by targeting ABCB1, these experimental strategies are not suitable for therapeutic application. Thus, improved delivery protocols were developed.

2.2 Nanoparticle-Based Delivery

Different types of nanocarriers designed for gene therapy including RNA-based drugs therapy have been developed (Xu et al. 2014). These carriers include liposomes, metallic and polymeric nanoparticles, dendrimers, gelatins, and quantum dots/rods each showing distinct characteristics. Modulation of classical ABCB1-mediated MDR by nanoparticle-based siRNA delivery started in 2005 with construction of a delivery vehicle consisting of a dendrimer conjugated to Tat peptide, a cell penetrating peptide (Kang et al. 2005). However, the dendrimer–oligonucleotide complexes were poorly effective for delivery of siRNAs and expression of ABCB1 was only inhibited weakly. In the next nanoparticle-based RNAi approaches, an improvement of the efficacy of ABCB1 inhibition could be observed (Patil et al. 2010; Susa et al. 2010). Several studies followed using different multidrug-resistant cancer cell models for in vitro and in vivo studies (Yin et al. 2012; Zhao et al. 2013; Nourbakhsh et al. 2015; Yang et al. 2015). All these studies provide a proof of concept that this technique may be applicable for circumvention of ABCB1-associated MDR.

2.3 Viral Delivery

In various human trials, viral vectors have emerged as safe and effective delivery vehicles for clinical gene therapy (Kotterman et al. 2015). Accordingly, viruses were also used to design vectors encoding shRNAs directed against a disease-associated target mRNA, including the ABC-transporter-specific transcripts. For example, adenoviruses encoding anti-ABCB1 shRNAs demonstrated the high potential of this strategy in complete reversal of the multidrug-resistant phenotype in different cancer models in vitro and in vivo (Kaszubiak et al. 2007; Ahn et al. 2010). Likewise, lentiviral vectors derived from HIV-1 demonstrated an efficient downregulation of ABCB1 expression and a successful reversal of the multidrug-resistant phenotype (Ye et al. 2009).

2.4 Bacterial Delivery

In particular for gene therapeutic treatment of bowel-associated diseases, non-pathogenic invasive Escherichia coli strains have been developed (Lage and Fruehauf 2011; Ahmed et al. 2015). In this concept, DNA sequences encoding a therapeutic nucleic acid such as a shRNA directed against a specific molecule are transferred to the target cell by bacteria. Two different steps are required for bacteria to act as delivery systems for therapeutic nucleic acids into mammalian cells: (i) internalization of the microorganisms into the host cell by endocytosis, followed by (ii) escape of the therapeutic bacteria or their therapeutic nucleic acid molecules from the endocytosis vesicle to the cytosol of the target cell. For this approach, Escherichia coli were equipped with a plasmid containing sequences that encode two different proteins that can mediate these two steps. The first protein is invasin of *Yersinia pseudotuberculosis*. Invasin is localized on the bacterial surface and is able to bind to a subset of beta-1-integrins embedded in the cell membranes of mammalian cells including cancer cells. By this binding, the selective uptake by endocytosis of the bacteria by the mammalian host cell is enabled. Subsequent to internalization, *Escherichia coli* are located in a lysosomal endocytosis vesicle where lysis of the microorganisms occurs. Among the various bacterial proteins released into the phagosomal vesicle, lumen is the second protein necessary for therapeutic bacterial delivery, listeriolysin O (LLO) from *Listeria monocytogenes*. This protein is a pore-forming toxin. The cytoplasmic contents of invasive *Escherichia coli*, including therapeutic nucleic acids or proteins, can then escape into the cytosol of the mammalian host cell through the LLO-generated pores. Consequently, this concept was successfully applied for bacteria-mediated delivery of therapeutic molecules in vitro as well as in vivo (Critchley et al. 2004).

By the application of this technology to multidrug-resistant cancer cells, the expression of ABCB1 and the corresponding drug resistance level could be decreased to approximately 50 % of the initial value (Krühn et al. 2009). The technique was not

as effective as alternative strategies such as adenoviruses but showed a gentle direct delivery strategy for treatment of multidrug-resistant carcinoma cells derived from gastrointestinal tissues.

2.5 Delivery by Jet-Injection

As discussed, the efficient delivery of RNAi effectors represents the major problem for successful clinical application. In this context, the transfer of naked RNAi-triggering molecules represents an additional alternative to viral, bacterial, and liposomal gene transfer technologies which were described. Various in vitro and in vivo procedures, such as simple needle injection, particle bombardment, in vivo electroporation, or jet-injection, are employed to deliver naked RNAi-mediating nucleic acids into the desired cells or tissues (Walther et al. 2010). These techniques have numerous advantages, such as avoidance of utilization of recombinant viral particles, modified bacterial organisms, reduced or no immunostimulatory potential, and no toxicity.

Among different technologies, jet-injection has gained increasing acceptance, since this technique allows transfer into different tissues with deeper penetration of naked nucleic acids. The jet-injection technology is based on jets of small volumes, which are ejected with high velocity and generate the force to deeply penetrate the targeted tissues and to transfect the affected area. Jet-injection generates broad areas of RNAi effector encoding transgene expression within the jet-injected tissue. The in vivo application of this technology does not induce tissue damage or significant inflammatory reactions at jet-injection sites (Walther et al. 2001).

The technology was successfully applied for reversal of ABCB1-mediated multidrug resistance in cancer cell lines as well as in human cancer xenograft-bearing mice (Stein et al. 2008). Anti-ABCB1 shRNA encoding plasmid vectors decreased the ABCB1 mRNA expression level by more than 90 %. The corresponding transporter protein was no longer detectable in the tumors. By two jet injections of anti-ABCB1 shRNA vectors into the tumors, combined with two intravenous administrations of the cytotoxic drug doxorubicin, were sufficient to achieve complete reversal of the multidrug-resistant phenotype in the tumor xenografts.

3 Conclusion

Although in recent years progress has been made in the development of new gene therapeutic strategies, delivery is still the main hurdle for successful design of gene therapeutic protocols including targeting ABC-transporters for overcoming multidrug resistance. New or improved technologies including RNAi- and CRISPR/Cas9-based approaches may use the long experience obtained with different delivery

strategies in the past. Also in future, scientists and clinicians will make assiduous efforts in the development of improved delivery strategies for gene therapeutic agents to target drug-resistant cancer cells.

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