
Bacterial Toxins for Oncoleaking Suicidal Cancer Gene Therapy

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Abstract

For suicide gene therapy, initially prodrug-converting enzymes (gene-directed enzyme-producing therapy, GDEPT) were employed to intracellularly metabolize non-toxic prodrugs into toxic compounds, leading to the effective suicidal killing of the transfected tumor cells. In this regard, the suicide gene therapy has demonstrated its potential for efficient tumor eradication. Numerous suicide genes of viral or bacterial origin were isolated, characterized, and extensively tested in vitro and in vivo, demonstrating their therapeutic potential even in clinical trials to treat cancers of different entities. Apart from this, growing efforts are made to generate more targeted and more effective suicide gene systems for cancer gene therapy. In this regard, bacterial toxins are an alternative to the classical GDEPT strategy, which add to the broad spectrum of different suicide approaches. In this context, lytic bacterial toxins, such as streptolysin O (SLO) or the claudin-targeted *Clostridium perfringens* enterotoxin (CPE) represent attractive new types of suicide oncoleaking genes. They permit as pore-forming proteins rapid and also selective toxicity toward a broad range of cancers. In this chapter, we describe the generation and use of SLO as well as of CPE-based gene therapies for the effective tumor cell eradication as promising, novel suicide gene approach particularly for treatment of therapy refractory tumors.

Keywords

Cancer gene therapy · Suicide gene therapy · Bacterial toxin · Solid tumors

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1 Introduction

Finding novel and efficient therapies that target malignancies is still important as the incidence of cancer diseases is constantly increasing. Conventional treatment modalities for cancer such as surgery, radiation therapy, and chemotherapy, which are usually combined for a better treatment effect, remain the therapeutic backbone of cancer therapy. However, these therapies do have their limitations, mainly caused by tumor heterogeneity and development of therapy refractory tumor cell populations. During the last decades, anticancer therapy has been continuously improved to overcome these drawbacks, but problems with adverse effects and drug resistance still constitute a main obstacle for successful cancer treatment. Therefore, alternative treatment options are urgently required to efficiently target and eradicate tumors.

Cancer gene therapy represents one such promising strategy, an approach where selective tumor cell killing and tumor growth inhibition can be achieved by introducing foreign nucleic acid (DNA or RNA) as therapeutic agent to tumor cells (Walther and Stein 1999). Genetic therapy can be approached from different directions, such as insertion of a normal gene into cancer cells to replace a mutated or altered gene (Lu et al. 2012; Senzer et al. 2013), selective eradication of tumor cells by suicide mechanisms, induced apoptosis using additive gene insertion (Di Stasi et al. 2011; Zarogoulidis et al. 2013), gene suppression by intervention of gene transcription and translation using, e.g., antisense-oligonucleotides (ASO) (Moulder et al. 2008; Fidiias et al. 2009), micro-RNA (miRNA) (Croce 2009) or small interfering double-stranded RNA (siRNA) (Santel et al. 2010; Strumberg et al. 2012). Furthermore, many approaches involve inoculation of immune cells (namely engineered T cells) for immunotherapy. These cells are specifically modified to either replace the immune system to enhance the anti-tumoral response or to boost the patient's own immune system to efficiently kill cancer cells (Kantoff et al. 2010; Sharpe and Mount 2015).

Gene transfer technology comprises a diverse set of therapeutic options and provides promising frontiers for treatment. During the last decades, a broad variety of viral and non-viral vectors have been developed (Gillet et al. 2009). In this regard, replicating and non-replicating viral vectors were improved using retroviral or DNA-virus technology platforms (e.g., lentivirus, adenovirus, AAV, Herpes simplex virus) to increase transfer efficiencies and to improve vector targeting and transgene expression complemented with transcriptional targeting or conditional gene (Walther and Stein 2000). Non-viral systems have entered a new level of quality represented by novel vector types (e.g., minicircle, miniplasmid, dumbbell-shaped minimalistic vectors, sleeping-beauty), transfer technologies including nanoparticles/lipofection and physical technologies (e.g., sonoporation, electroporation, particle bombardment/gene-gun, jet injection). One basic obstacle in cancer gene therapy is the specific targeting directly to a solid tumor. Since particularly in its advanced stages cancer is a metastasizing disease, systemic gene delivery is still one major challenge in cancer gene therapy. Insufficient selectivity and transfer

efficiency especially for clinical applications are limiting factors for successful gene therapy and demand improvements in targeting of vector delivery, transgene transcription, and/or translation. In this context, local gene therapy of cancer for local control of the disease is still of some attractiveness and about 20 % of all clinical cancer gene therapy trials are performed as local viral or non-viral gene transfer (Walther et al. 2011b).

Apart from the development of improved transfer technologies, an appropriate therapeutic gene is decisive for a successful cancer treatment. The choice of the respective and most suited gene is often determined by the specific gene therapeutic strategy used for cancer treatment, such as immunogene therapy, suicide gene therapy, gene correction therapy, or gene suppression therapy.

Since long time of the evolution of cancer gene therapy bacterial toxins have complemented the list of therapeutic genes and are attractive candidates as they have demonstrated efficient cell killing capacity in several *in vitro* and *in vivo* studies and have shown their potential for effective cancer treatment (Richardson et al. 1999). In this chapter, we will focus on the bacterial toxin-based suicide gene therapy, which is currently gaining increasing attention as treatment option.

2 Suicide Gene Therapy for Cancer Treatment

The major application of suicide gene therapy is focused on treatment of cancer. For this, initially different so-called prodrug-converting enzymes (gene-directed enzyme-producing therapy, GDEPT) of bacterial or viral origin were used for expression in tumor cells, which convert non-toxic prodrugs into toxic metabolites to kill tumor cells and neighboring cells (bystander effect). Most prominent members of these suicide genes are still the cytosine deaminase (CD), Herpes simplex virus thymidine kinase (HSV-tk), cytochrome P450-2B1, and nitroreductase and variants thereof. The CD- and HSV-tk-expressing vectors have long entered clinical phases I and II (Zarogoulidis et al. 2013). Recent developments for these classical suicide genes are aiming at their optimization via mutated variants or fusion proteins for more efficient generation of the toxic metabolites. The term suicide gene therapy has meanwhile broadened toward the delivery of genes that are either directly toxic or pro-apoptotic (Fig. 1).

Even though the ability to kill cancer cells is powerful, there are two major drawbacks of this enzyme-prodrug system: the mentioned bystander effect, which can cause unwanted side effects and a reduced effectiveness in slow-dividing cancer cells. As alternative, suicide gene therapy based on apoptotic genes, such as p53, Bax, or FasL, has been extensively studied but also revealed limitations as cancer cells develop resistance to apoptosis induction (Reed 2002; Igney and Krammer 2002).

Therefore, novel suicide gene therapeutics such as bacterial toxins came into focus, which can overcome the obstacles of resistance and proliferation dependence of the classical suicidal systems.

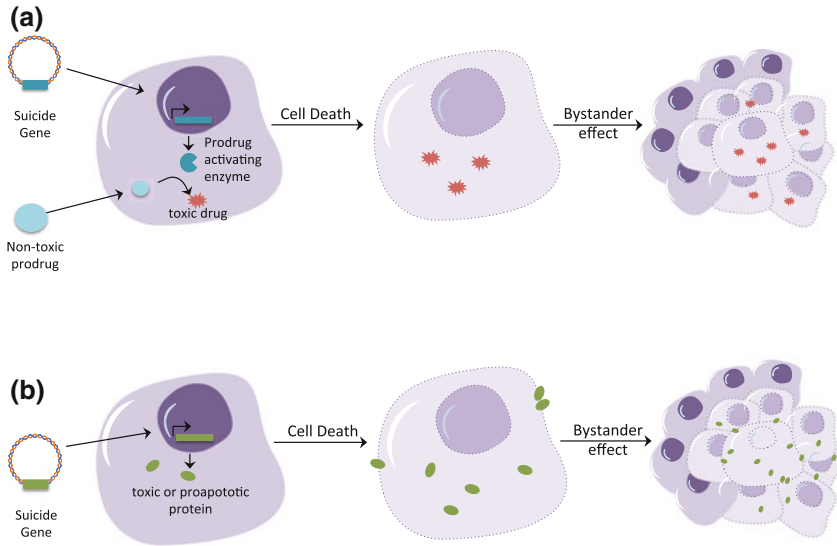


Fig. 1 Targeted killing of cancer cells by using suicide gene therapy. **a** This approach involves the transfer of a therapeutic gene encoding a prodrug-activating enzyme into tumor cells followed by treatment with a specific prodrug. The expression of the therapeutic gene (prodrug-activating enzyme) enables the conversion of the inactive non-toxic prodrug into an active cytotoxic drug. Toxic metabolites can then pass to neighboring cancer cells causing cell killing via the bystander effect. **b** Direct cell killing is also possible if the inserted gene is expressed to produce a toxin-inducing cytotoxicity. As the transfected cells undergo cell death, the expressed toxin can affect neighboring non-transfected cells

3 Bacterial Toxins in Cancer Therapy

The concept of using bacterial toxins as anticancer agents is actually not new as their therapeutic potential was recognized and explored almost 100 years ago (Richardson et al. 1999; Strebhardt and Ullrich 2008). Meanwhile, a continuously growing number of promising experimental *in vitro* and *in vivo* studies, using bacterial toxins for cancer treatment, has been published, which reveal their capability of effective cell killing (McCarthy 2006; Patyar et al. 2010; Felgner et al. 2016). In the last decades, the processing and manipulation of toxic bacterial proteins, such as diphtheria toxin, streptolysin O, or clostridium perfringens enterotoxin, and their encoding genes were facilitated, leading to the establishment of “toxin-based therapy” for cancer treatment introducing novel features to suicide gene therapy such as rapid and quite effective pore-forming cell lysis as novel oncoleaking strategy.

3.1 Diphtheria Toxin

One prominent bacterial toxin, which has been extensively used for therapeutic approaches including gene therapies, is the diphtheria toxin (DT). The DT, a 62-kDa exotoxin, secreted by pathogenic strains of *Corynebacterium diphtheria*, binds to the heparin-binding epidermal growth factor precursor (HB-EGF) on the cell surface (Louie et al. 1997). DT consists of 535 amino acids and belongs to the group of AB toxins as it can be cleaved into 2 major fragments (DTA and DTB). The fragment DTB mediates cell entry by binding to a surface receptors and subsequent translocation into cytoplasm by undergoing endocytosis. By contrast, DTA is responsible for the cytotoxic enzymatic activity and inactivates the ADP-ribosylation of elongation factor 2 (EF2), causing inhibition of protein syntheses and cell death (Thorburn et al. 2004; Deng and Barbieri 2008). It is known that the delivery of a single molecule of the catalytic DTA is sufficient to kill a cell, but it is not able to enter a neighboring cell in the absence of DTB (Yamaizumi et al. 1978) (Fig. 2).

3.1.1 DT-Based Suicide Gene Therapy

As mentioned above, DTA is not able to enter a neighboring cell in the absence of DTB. Therefore, it only specifically kills the actual targeted cell, restricting its toxicity. These features of high therapeutic potency, the locally restricted toxic effect, the additional advantages of the evasion of anti-DT immunity, as it is endogenously expressed within the tumor, and the absence of cellular resistance to the toxin supports the great potential of DT-A as gene therapeutic agent.

Nevertheless, this potent bacterial toxin requires efficient and reliable selective targeting, mainly to avoid any unintended side effects on normal cells, which is an essential requirement for the use of the toxin in cancer gene therapy. Several attempts to limit the toxicity of DTA by using modified metallothionein promoter (Maxwell et al. 1986) or by replacement of the wild type DTA sequence with attenuated mutant variants of DTA (Maxwell et al. 1987) were still not able to generate targeted tumor cell killing. To minimize damage to healthy tissue, a specific targeting mechanism was an essential requirement to ensure further use of the toxin.

In the last decades, tissue- and tumor-specific promoter elements were identified, which are critically important for more effective and transcriptionally targeted application of gene therapy (Haviv and Blackwell 2001; Dorer and Nettelbeck 2009). With this knowledge, transcriptional targeting, a method based on positioning the therapeutic gene (e.g., suicide gene) under the transcriptional regulation of a promoter which is specifically or preferentially activated in targeted tumor tissue, was developed (Fukazawa et al. 2004; Saukkonen and Hemminki 2004; Danda et al. 2013). Until today, numerous tissue-specific promoters have been cloned, molecularly characterized, and applied for the controlled DTA expression in different cancer entities.

One example of such promoter is originated from the human H19 RNA gene that is highly expressed in a wide range of cancers and is important for cell proliferation,

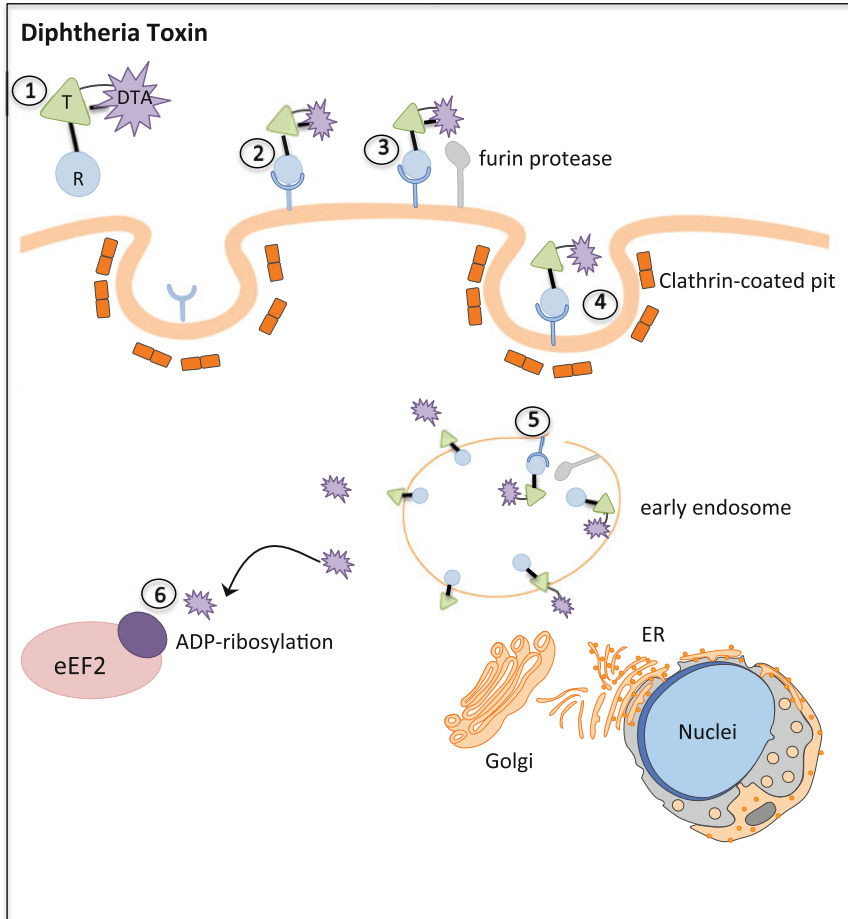


Fig. 2 Mechanism of action of diphtheria toxin. 1 The secreted toxin consists of three functional domains: the N-terminal catalytic domain (DTA), the translocation domain (T), which is bridged by a disulfide bond to the receptor-binding domain (R). 2 DT binds its receptor (heparin-binding epidermal growth factor precursor). 3 The cell surface furin protease cleaves the polypeptide chain between the C and T domains. 4 The toxin-receptor complex is internalized into clathrin-coated pit. 5 Inside the early endosome, furin protease cleaves toxin molecules and T domain undergoes conformational change, inserts into endosome membrane and forms a channel, which leads to translocation of catalytic domain into the cytoplasm, followed by reduction of the disulfide bond. 6 DTA inactivates eukaryotic translation elongation factor 2 (eEF2) by ADP-ribosylation, causing inhibition of translation and consequently cell death

genetic instability, vascular angiogenesis, multiple drug resistance, metastasis as well as secondary tumor progression and dissemination (Matouk et al. 2013). Mizrahi et al. reported the use of then H19 gene promoter to drive the targeted expression of DTA in ovarian cancer and demonstrated significant tumor growth

inhibition of ovarian cancer xenograft-bearing mice after intratumoral injection of DTA-H19 (Mizrahi et al. 2009). This great potential has been further confirmed in a variety of tumor entities, such as pancreatic cancer (Scaiewicz et al. 2010; Sorin et al. 2012), colon adenocarcinoma (Sorin et al. 2011), or primary lung cancer (Hasenpusch et al. 2011). Thus, DTA-H19 became a “multi-potent vector” (Smaldone and Davies 2010; Amit and Hochberg 2012; Amit et al. 2013) and has entered multiple clinical studies. A phase I and II clinical trial in patients with invasive bladder cancers, receiving intravesical DTA-H19 (namely BC-819) revealed partial and complete response rates as well as prevention of tumor recurrence in two-thirds of treated patients (Gofrit et al. 2014).

Another very recent example for transcriptionally targeted therapy of DT was shown by Tholey et al. They generated DNA-vector constructs with either the pancreatic cancer-specific mesothelin (MSLN) or Mucin 1 (MUC1) promoter linked to DTA coding sequence and combined it with a highly efficient and biodegradable polymer to deliver the vector DNA to pancreatic cancer cells (Tholey et al. 2015). MSLN and MUC1 gene promoters represent promising transcriptional control elements, as they are active at low level in normal cells and highly active a diversity of tumor types, particularly in pancreatic cancer cells, mainly in the most aggressive form that are typically resistant to conventional therapy (Singh and Bandyopadhyay 2007; Showalter et al. 2008; Winter et al. 2012). With this knowledge on promoter activities, MSLN and MUC1 promoter-driven DTA constructs were generated, demonstrating its specific and selective activity as it preferentially kills MSLN/MUC1-expressing pancreatic cancer cells *in vitro*. A further analysis of matched primary and metastatic tumors in patients showed the great potential of MUC1-targeted therapy as targeting strategy, since this expression is observed consistently in primary tumors and metastasis.

3.2 Streptolysin O

Apart from the strategy of, e.g., of intervention in protein translation by bacterial toxin like DTA, the approach of cell lysis by pore-forming toxins is of attractiveness to eradicate tumor cells. Particularly in light of additional immunostimulation, tumor cell lysis might add to tumor therapy as it deliberates tumor antigens, which could in turn contribute to the activation of the patient’s immune response against the tumor. One such pore-forming toxin is streptolysin O (SLO). SLO is a 62-kDa toxin secreted by many strains of *Streptococcus* bacteria and belongs to the family of pore-forming toxins called cholesterol-dependent cytolysins (CDCs) (Bhakdi et al. 1996). SLO consists of four domains D1–D4, which are rich in β -sheet proportions. The most important protein domains are D3 and D4, as domain D3 provides the transmembrane spanning regions for the toxin and domain D4 directly interacts with cholesterol of the cell membranes. After specifically binding to membrane cholesterol, SLO monomers oligomerize to form homotypic aggregates, which insert into membrane to form a large pore whose diameters can reach up to 35 nm (Shatursky et al. 1999; Sierig et al. 2003) (Fig. 3a).

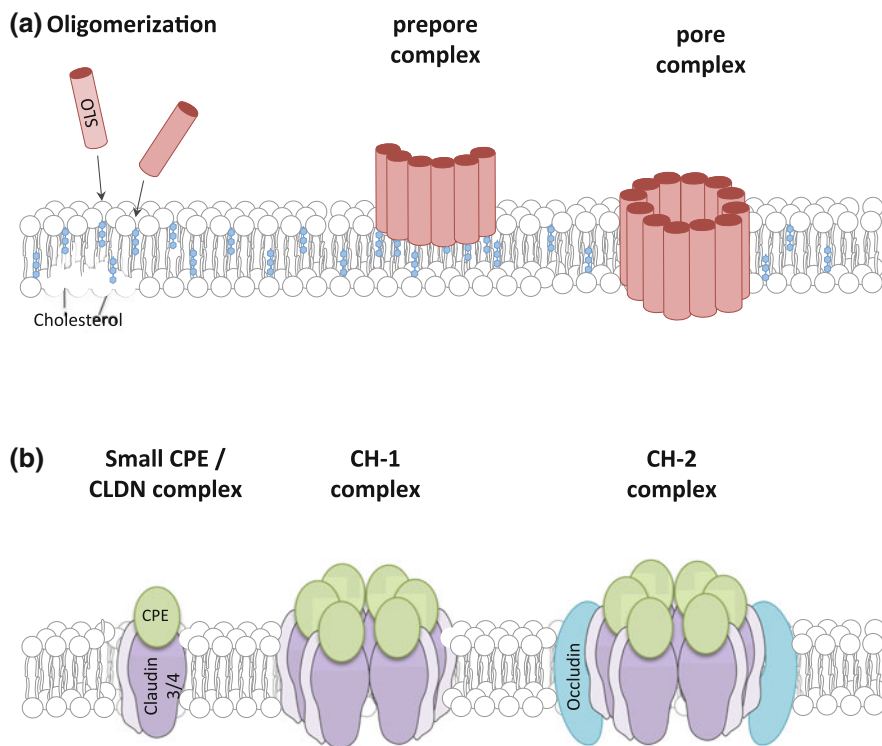


Fig. 3 **a** The mechanism of SLO action. SLO binds specifically to membrane cholesterol and oligomerizes to create a ring structure, which contains 45–50 units and inserts into the membrane to create a large pore, leading to loss of balance between in- and effluxes across the cell membrane. This pore formation further induces cytolysis. **b** The mechanism of CPE binding and mediated cytotoxicity. CPE binds directly to its receptor, preferably claudin-3 or claudin-4, at the plasma membrane of intestinal epithelium. The small CPE/claudin complexes may also include other claudins, e.g., claudin-1, via an indirect interaction. Six small complexes oligomerize to form a large hexameric complex (CH-1), which increases permeability of the cell membrane. CH-1 complexes eventually incorporate occluding, resulting in an even larger complex, namely CH-2, which disrupts epithelial tight junctions resulting in a breakdown of colloid-osmotic equilibrium of affected cells. In consequence, cells undergo cell death by lysis

3.2.1 SLO-Mediated Cytolytic Suicide Gene Therapy

While recombinant SLO protein has been used in several studies for its pore-forming properties, Yang et al. thought of exploiting this particular property of SLO to kill malignant tumor cells and to take the advantage to overcome the anti-apoptotic resistance of cancer cells as well proliferation rate dependence (Yang et al. 2006). Most bacterial toxins, such as diphtheria toxin or pseudomonas exotoxin, tested so far in suicide gene therapy acted “inside” the targeted tumor cell (Martín et al. 2000; Kawakami et al. 2001). Conversely, pore-forming toxins are known to act at the cell membrane and have formerly been used as immunotoxins or recombinant proteins for anticancer treatment. In the study of Yang et al., a

conventional plasmid expression vector carrying the SLO gene was used in a liposome-mediated transfection system. Initially, HEK293T cells (human embryonic kidney fibroblasts) were transiently transfected with the SLO vector, leading to cell death caused by cell membrane permeabilization and disintegration. SLO secreted by bacteria usually creates large pores in target cell membrane, allowing large molecules to pass through. To determine whether the observed cytotoxicity in SLO transfected cells was caused by pore formation of expressed SLO lactate dehydrogenase (LDH) release, caspase activity was measured and cells were monitored under electron microscopy. High level of LDH but no caspase activation was observed, indicating that SLO protein expressed within HEK cells induces necrosis. In further studies, they extended their approach by developing an adenoviral expression vector as high-efficiency gene transfer system, which significantly reduced cell viability in several human cancer cell lines (cervical carcinoma cells, C33; lung carcinoma cells, A549; breast cancer cells, MCF-7; and prostate cancer cells, Hep3B). After SLO gene transfer, significant anti-tumoral activity by SLO-mediated cytotoxicity was observed in treated CA33 xenograft-bearing mice.

This study demonstrated the successful use of the SLO gene as anticancer agent *in vitro* and more importantly *in vivo*. However, these studies reveal limitations, as the use is limited to local treatment otherwise massive unwanted side effects could occur, since cholesterol is certainly also present in cell membranes of healthy normal cells. Therefore, further modifications like attachment of rather tumor-specific promoters upstream the SLO gene or changing adenoviral fiber proteins, which bind to specific tumor cell surface proteins, are required.

3.3 Clostridium Perfringens Enterotoxin

Another promising pore-forming bacterial toxin for the suicide gene therapy is the clostridium perfringens enterotoxin, which is produced by the anaerobic gram-positive bacterium *Clostridium perfringens* and mainly associated with food poisoning (Minton 2003; Johnson 1999). This 35-kDA single protein contains 319 amino acids with a unique primary sequence. CPE is a two-domain protein that consists of (1) C-terminal receptor-binding domain (amino acid residues 184-319), which recognizes and binds to certain members of the claudin family and an N-terminal domain that is involved in oligomerization and pore formation (Kitadokoro et al. 2011; Briggs et al. 2011).

The C-terminal fragment of CPE (c-CPE) reveals a high-affinity binding to its receptors, for example, claudin-3 or claudin-4; however, it is not able to initiate or form pores (Hanna et al. 1991). The N-terminal residues 80–160 also referred to as TM1 region consist of hydrophilic and hydrophobic amino acids, which resemble the β -loops, which then mediate membrane insertion and pore formation. The mechanism of action of CPE is initiated by the binding to its natural receptors, the transmembrane proteins claudin. In particular, claudin-3, claudin-4, claudin-6, claudin-6, and claudin-14 are proven CPE receptors (Katahira et al. 1997; Fujita et al. 2000; Lal-Nag et al. 2012; Shrestha and McClane 2013; Shrestha et al. 2016).

The claudin family consists of at least 27 proteins that are essential for tight junction formation in epithelial and endothelial cells. They also play an important role in controlling paracellular transport and maintenance of cell polarity (Gumbiner 1987). Claudins are comprised of four transmembrane domains; a C-terminal cytoplasmic tail; and two extracellular loops, ECL1 and ECL2 (Günzel and Fromm 2012). The binding of CPE to its claudin receptor triggers the formation of a “small complex” (90 kDa), containing CPE and the receptor (Tsukita and Furuse 2000; Smedley et al. 2007). This small complex by itself is not able to mediate cytotoxicity, but several small complexes interact and oligomerize to a prepore on the membrane surface, which results in a 450-kDa “large complex”—named CH-1 complex (Robertson et al. 2007). The CH-1 complex that comprises a CPE hexamer and claudins subsequently forms a pore into the membrane, causing membrane permeability alterations, and permits a calcium influx, inducing cell death (Matsuda and Sugimoto 1979; Freedman et al. 2016). The morphological damage leads to exposure of the basolateral cell surface, allowing additional binding of the toxin to form an even larger ~600-kDa complex, known as CH-2, which consists of claudins as well as occludin (Singh et al. 2000) (Fig. 3b).

So far it is known that high CPE concentration causes formation of many pores, leading to a massive calcium influx and consequently to necrotic cell death, whereas low CPE concentration results in formation of low number of pores, rather causing apoptosis (Chakrabarti et al. 2003).

3.3.1 CPE-Based Oncoleaking Suicide Gene Therapy

Numerous studies have shown that certain cancer entities, particularly epithelial cancers, such as colon, breast, prostate, ovarian, and pancreatic cancer, possess a high expression of claudin-3 and/or claudin-4 (Rangel et al. 2003; Hewitt et al. 2006; Santin et al. 2007; Takala et al. 2007; Kominsky et al. 2007; Saeki et al. 2009; Neesse et al. 2012; Lu et al. 2013). Due to this fact, considerable effort has been made to develop a CPE-based approach for cancer therapy and its potential clinical benefit in targeting claudin-3- and claudin-4-expressing tumors has been evaluated. The application of recombinant CPE protein demonstrated a dose-dependent cell killing of claudin-3 and claudin-4-overexpressing pancreatic, breast or colon cancer cells *in vitro* and *in vivo* (Litkouhi et al. 2007; Saeki et al. 2009; Gao and McClane 2012; Kojima et al. 2012). In addition to that, the intratumoral application in tumor bearing mice did not induce unwanted toxin-associated side effects. However, the use of recombinant CPE protein requires repeated application of the toxin to achieve significant therapeutic effect (Michl et al. 2001; Kominsky et al. 2004). Alternatively, the gene transfer of a CPE-expressing vector could be sufficient to significantly prolong toxin availability and improve intratumoral dispersion and subsequently amplify the cytotoxic effect.

Based on this idea, we established an eukaryotic translation optimized CPE vector (optCPE), which combines both target specificity and efficient cytotoxicity (Walther et al. 2011a). The intracellular CPE expression and accumulation after gene transfer led to effective eradication of claudin-3 and claudin-4 high-expressing cells, such as the mammary carcinoma cell line MCF-7 or the human pancreatic

cancer cells Panc1, whereas claudin-negative cells like the melanoma cell line Sk-Mel5 remained unaffected. This study further demonstrated that even though CPE is produced inside the transfected cell, its outside action of binding to the claudins and mediating pore formation and cell lysis is not hampered.

More importantly, it was shown that non-viral intratumoral gene transfer of CPE-expressing plasmid-vector does induce extensive tumor necrosis in HCT116 human colon carcinoma and in MCF-7 human mammary carcinoma xenotransplanted mice. This was associated with significant reduction in tumor growth and showed improved efficacy over treatments with the recombinant CPE, which was well tolerated by the animals.

In our very recent study, we employed the optCPE gene therapy to selectively eradicate claudin-3 and claudin-4-expressing pancreatic carcinomas and demonstrated again the successful use of this suicide gene therapy approach as CPE expression permitted rapid tumor destruction *in vitro* (Pahle et al. 2015).

In both studies, we observed the presence of released biological active CPE in the media of all transfected cells (claudin-positive and claudin-negative cells), suggesting a cytotoxicity-independent deliberation of CPE, which further supports the concept of bystander effect that strongly contributes to the efficiency of this gene therapy approach (Fig. 1b).

For the improved and more effective use of the toxin, the mode of cell death, induced by transfected CPE is of interest. The analysis on cell death mechanism revealed that delayed activation of the caspases 3/7 was induced, indicating rather CPE-mediated necrosis than apoptosis. This was further supported by the dramatic increase of LDH release after CPE transfection and appearance of necrotic cell morphology, such as cell membrane and nuclear rupture. As mentioned above and reported by others, cell death mechanism is dependent on CPE concentration, number of pores generated by CPE and claudin localization (tight junctions, cell membrane, cytoplasm) of targeted cell, which determines accessibility for CPE binding.

Taken together, CPE oncoleaking gene therapy is of great value for the targeted eradication of therapy refractory tumors, which is further improved by the bystander effect of this particular suicide approach.

4 Conclusions

As gene therapy comes of age, it has shown its efficacy for the treatment of cancer diseases reflected by the application of this strategy in clinical trials. In fact, more than 7 % of all gene therapy clinical trials worldwide are employing suicide approaches either as monotherapy or in combination with other, conventional therapies such as chemo- and radiotherapy. Numerous suicidal systems have been established and successfully employed and among them bacterial toxins might experience some thorough re-evaluation as potential tools for more effective and to some extent more targeted gene therapies. In this regard, pore-forming, oncoleaking

bacterial toxins such as SLO or CPE hold promise for the efficient and rapid tumor cell killing. In particular CPE action is associated with selective tumor cell killing properties targeting the claudin-3 and claudin-4 tight junction proteins, which are often deregulated in epithelial cancers. As shown for meanwhile classical suicidal systems (e.g., CD, HSV-tk), bacterial toxins like CPE do also possess the feature of bystander effect, which is important if not essential for effective in vivo use of this suicide gene therapeutic. These initial studies for the use of bacterial toxins for oncoleaking suicide therapies might further promote the directed search for novel, similarly or even more effective and tumor-targeted toxins that can potentially be used for cancer gene therapy.

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