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Review

Glioblastoma multiforme targeted therapy: The Chlorotoxin story

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ABSTRACT

Glioblastoma multiforme (GBM) is the most common malignant primary brain neoplasm having a mean survival of <24 months. Scorpion toxins are considered promising cancer drug candidates, primarily due to the discovery of hlorotoxin, derived from the venom of the Israeli yellow scorpion. This intriguing short peptide of only 36 amino-acids length and tight configuration, possess the ability to bind to GBM cells in a grade-related manner with ~100% of GBM cells staining positive and no cross reactivity to normal brain. Chlorotoxin has an anti-angiogenic effect as well. Molecular targets for Chlorotoxin include voltage gated chloride channels (GCC), calcium-dependent phospholipid-binding protein Annexin-2, and the inducible extracellular enzyme Matrix Metalloproteinase-2 (MMP-2). Of all its targets, MMP-2 seems to bear the most anti-neoplastic potential. Chlorotoxin is a promising tumortargeting peptide. Its small size and compact shape are convenient for intracranial delivery. We present a short discussion on Chlorotoxin. The structure, biological activity, molecular targets and possible clinical role of Chlorotoxin are discussed. Chlorotoxin can be utilized as a targeting domain as well, attaching different effector functions to it. Clinical applications in GBM therapy, intraoperative imaging, nano-probes and nano-vectors based technology; targeted chemotherapy and immunotherapy are discussed as well. Chlorotoxin is likely to play a significant role in effective GBM immunotherapy in the future.

1. Introduction

Grade-IV astrocytoma, better known as glioblastoma multiforme (GBM) is unfortunately ranked as both the most common and the most malignant glioma in adults. GBM is associated with a median overall survival of 1–2 years and a 5-year survival rate of less than 10% [1–3]. The unique nature of Glioblastoma multiforme (GBM), featuring both local and distant aggressive recurrence, and its inherent challenging features was evident as early as 80 years ago. Patients with GBM exhibit altered and suppressed function spanning different elements of the immune system [4–21]. These reports set forth the basic premise that GBM cells have cloaking abilities in addition to suppressive mechanism which allow them to persevere. Another premise laid by these reports, is that homing of immune elements to the tumor site may prove sufficient for an effective anti-tumor response.

Targeted immunotherapy is rapidly becoming one of the pillars of anti-cancer therapy. Its targeted nature and reduced treatment related toxicity, stemming from recruiting and activating own cells, selective cytotoxic mechanisms, and effector responses, makes it intuitively an attractive option [22,23]. Metastatic melanoma treatment is one such example. FDA approval of cytokine based therapy with interleukin-2 in 1998 and checkpoint blockade with Ipilimumab in 2011 were major developments in the treatment of melanoma [24,25]. Ipilimumab was shown to increase survival in patients with unresectable stage III/IV melanoma [25–27]. GBM has not received similar clinical successes as of yet [4–8]. This may be attributed to its relative inaccessibility (its location beyond the confines of the blood–brain barrier), any of the multiple mechanisms of active or passive immunosuppression reported [4,20,21] which result in overall poor immunogenicity, or the lack of characterized cancer antigens. One should note that the immune response against tumors is limited by the fact that these arise from the organism's own tissue, and, therefore, mainly express self-antigens. The patient's T cells are rendered tolerant to these self-antigens via either central or peripheral tolerance [4–8,28,29], and thus do not act to eradicate these cells.

Preclinical studies suggest that targeted therapies can elicit significant antitumor responses in GBM, overcoming some of the barriers and tumor-related escape mechanisms, while others fail at specific points [4]. We will next briefly describe the structure,

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biological activity. Molecular target and possible clinical role of Chlorotoxin (ClTx), an intriguing and short peptide derived from the venom of the Israeli Yellow Scorpion. We will present both pre-clinical laboratory data as well as clinical data from our own laboratory as well as from other colleagues, supporting the premise that ClTx will play a significant role in effective GBM targeted therapy in the future. ClTx is a promising tumortargeting peptide. Its small size and compact shape are convenient for intracranial delivery.

2. Classification, structure and synthesis of Chlorotoxin

Scorpion toxins are considered promising cancer drug candidates [30,31], primarily due to the discovery of ClTx, a peptide from the venom of the giant Israeli yellow scorpion Leiurus quinquestriatus [32], which can preferentially bind to cancer cells [30]. Its venom is known to inhibit reconstituted small-conductance chloride channels, when applied to the cytoplasmic surface [33] (discussed later). Of note, this scorpion's venom is rich in many potent physiologically active substances in addition to ClTx. An array of bio-polysaccharides, hyaluronic acid derivate, serotonin, histamine, histamine-releasing factors and protease inhibitors are known to comprise the crude venom [34,35]. These bioactive polypeptides selectively bind to and modulate specific ion channels of excitable cell membranes. Scorpion-derived peptide toxins can be classified according to their specificity in inhibiting various ion channel receptors. So far scorpion-derived toxins identified specifically target either Na^+ [36,37], K^+ [38], Cl^- [39] and Ca^{2+} channels [40]. Since its original description in 1992, a number of Chlorotoxin related peptides have been isolated and identified [32].

ClTx is 4070 Da and 36 amino acids basic peptide with considerable sequence homology to the class of small insectotoxins [30,32,41,42]. Its amino acids sequence includes eight cysteines and four disulfide bonds, and it is therefore classified as a short-chain, disulfide-containing peptide. Other scorpion peptides with a similar primary structure to Chlorotoxin have been collectively referred to as Chlorotoxin-like peptides (i.e. ClTx-a, -b, -c, -d, BmKCL1, Lqh-8:6, Be I5A, BeI1, AmmP2 and GaTx1) [43–45]. The secondary structure of ClTx was first determined using nuclear magnetic resonance (NMR) spectroscopy in 1995 [46]. The NMR data showed that ClTx comprises an α-helix, formed by amino acids 11–21, and three β-sheets, formed by amino acids 1–4, 26–29, and 32 –36 (Fig. 1) [46,47].

The amount of ClTx present in venom is limited. Thus, an alternative or synthetic source of the peptide is necessary for biochemical and biophysical studies, as well as for potential therapeutic applications. Chlorotoxin has been successfully synthesized and folded in vitro, using classical molecular biology laboratory techniques. Ojeda et al. [30] described synthesis using a solid-phase peptide synthesis (SPPS). An alternative and simple method utilized in our laboratory entailed cloning the ClTx gene [7]. The widely published and available amino-acid sequence of the 36-amino-acids ClTx, was converted into a nucleic acids sequence empirically. Next, this sequence was commercially optimized for expression in *Escherichia coli* (GENEART Inc.). In the optimization process, the codon usage is adapted to the codon bias of *E. coli* genes. In addition, regions of very high (>80%) or very low (<30%) GC (Guanine-thymine) has been avoided when possible. The expressed protein can then be purified and refolded in vitro. This allows for a cheap, easy and highly reliable method of ClTx production. It also allows for unique chimeric molecular designs to be planned and produced from the DNA level and then expressed in *E. coli* (discussed later) [7].

3. Chlorotoxin activity

ClTx is assigned two clinical effects; a tumor binding activity and an antiangiogenic activity. Soroceanu et al. [48] first described in 1998 the tumor binding activity of ClTx using a ^{125}I-labeled peptide (^{125}I-ClTx) [48]. The authors showed that upon ^{125}I-Cltx administration to GBM tumor-bearing mice, the peptides accumulated within the tumor. Soroceanu et al. [48] also showed the specific binding of ^{125}I-Cltx to glioma cells, sparing normal rat astrocytes or neurons [48]. ClTx was initially thought to bind only to the so-called glioma-specific chloride ion channel (GCC) [49–53] which was found to be abundantly expressed in glioma cells but absent in normal brain tissue. Ullrich et al. [49] reported a ClTx-sensitive Cl−current, present in human astrocytoma (glioma) cell lines [49]. From then, ClTx has been widely used as a Cl−-channel blocker to analyze Cl−channels [50–52] and has been proposed as a glioma-specific marker with diagnostic and therapeutic potential [53,54]. GCC expression in situ using labeled ClTx was found to correlate with the tumor's grade, with only 40–45% of low-grade astrocytoma's (World Health Organization [WHO] grade I–II) binding it, as opposed to over 90% of high-grade tumors (WHO grade III) and essentially all of the GBM samples, binding labeled ClTx. Comparison tissues including normal human brain, kidney, and colon were consistently negative for ClTx immunostaining [51,53,54].

Sontheimer et al. [55] also showed ClTx binding to tumors of neuro-ectodermal descent (sharing a common embryonic origin

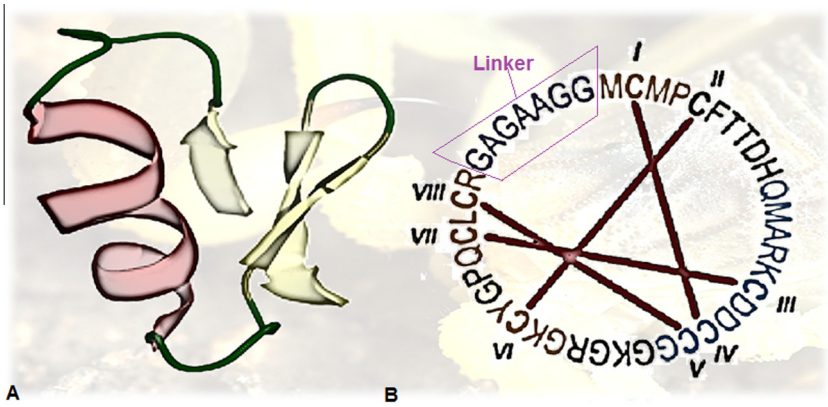

Fig. 1. Chlorotoxin (Cltx). (A) The three-dimensional structure of Cltx. α-Helix is in red, β-sheets are in yellow arrows. The disulfide bonds are not shown. Toxicon 38 (2000). (B) Amino-acids sequence of Cltx. The Disulfide bones are shown as black lines, the cysteines are labeled with roman letters. The cyclic configuration is allowed using an extra amino-acids linker sequence, marked in purple. Adapted from Ojeda et al. [14].