Discovery of Peptide-based Inhibitors against Dendrotoxin B from Black Mamba through Phage Display Screening

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Discovery of Peptide-based Inhibitors against Dendrotoxin B from Black Mamba through Phage Display Screening

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Outcompeting the mammalian immune system

The black mamba (Dendroaspis polylepis) is Africa’s most feared snake due to its potent, rapid-acting venom and its speed of attack [1]. The most abundant toxins in D. polylepis venom are the Kunitz-type protease inhibitors, dendrotoxins, which are unique for mamba [1-3]. Dendrotoxins are poorly neutralized by current antivenoms, and they are almost impossible to raise an immune response against due to their similarity to homologous mammalian, non-toxic proteins [4]. Here, we report the discovery of peptide-based antitoxins against dendrotoxin B from D. polylepis through phage display screening [5].

Results – Identification of binders with phage display

Via phage display screening of phage libraries containing 7-mer, cyclic 7-mer, 12-mer, 16-mer, and 20-mer peptides displayed on coat proteins of the M13 phage, we were able to isolate 25 monoclonal phages that bound strongly to dendrotoxin B (Dtx B). Sequencing of the monoclonal phage DNA is pending and will reveal the identity of the peptides.

Figure 2: Phage display is a screening technique whereby peptides are displayed on the surface of bacteriophages, some of which bind with high affinity to snake toxins that are attached to plate wells.

Figure 3: ELISA results. Selection of peptide displaying phages against Dtx B yielded an amplification of toxin-binding phages.

Outlook – Synthetic peptides targeting dendrotoxins

If the peptides displayed on the isolated, strongly binding phages are not only able to bind, but also inhibit the toxic effects of dendrotoxin B, these could be used to reinforce existing antivenoms that do not neutralize dendrotoxin B well [1]. After the sequences of the displayed peptides has been obtained, the next steps include synthesis and precise determination of binding constants for the peptides. If good binding is observed, the peptides will be tested for inhibitory effect in vitro and in vivo.

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References

Figure 1: Dendroaspis polylepis (black mamba). Larsa D. 2011

Figure 2: Phage display is a screening technique whereby peptides are displayed on the surface of bacteriophages, some of which bind with high affinity to snake toxins that are attached to plate wells.

Figure 3: ELISA results. Selection of peptide displaying phages against Dtx B yielded an amplification of toxin-binding phages.

Figure 4: ELISA results. 25 selective dendrotoxin B binding phages were isolated.

Figure 5: Cartoon model of Dtx B compared with an overlaid cartoon models of Dtx I and α-Dtx, drawn in PyMOL. Sequence alignment between Dtx B, Dtx I, α-Dtx, and δ-Dtx shows high sequence similarity. Dtx B and Dtx I are dendrotoxins found in D. polylepis, while α-Dtx and δ-Dtx are dendrotoxins from D. angusticeps.