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Implications of a Newly Discovered DR5 Specific Antagonistic Peptide for Neurodegenerative Disorders

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PharmSight on Vrielink JV et al., Synthetic constrained peptide selectively binds and antagonizes death receptor 5. FEBS J 2010;277:1653-65.

Abstract

Most neurodegenerative disorders are the result of inflammation and neuronal cell death. Although many cytokines have been implied to be involved in the pathogenesis, recent studies have shown TRAIL to be responsible for neuronal apoptosis. TRAIL is best known for its ability to induce apoptosis in many cancer cells. Normally TRAIL is not present in the CNS. However, it is induced by β-amyloid protein and upregulated on infected macrophages which can infiltrate the CNS. TRAIL is able to induce apoptosis via death receptors DR4 and DR5. DR5 is shown to be expressed on neuronal cells. The identification of an antagonistic peptide that specifically binds DR5 provides us with a useful investigative tool. Small peptides can bind their targets with high affinity and specificity. In addition, they are easily modified and further developed for clinical application. So the peptide R2C16 might even be used as a lead peptide for the development of therapeutic agents in neurodegenerative disorders.

Keywords: Neurodegenerative disorders; TRAIL; DR5; Apoptosis; R2C16

The tumor necrosis factor super family (TNFSF) consists of a large group of cytokines that regulate cell survival, proliferation, differentiation and apoptosis. In addition, the members of the TNFSF share a typical jelly roll topology and they interact with their receptors as trimers. One of its members is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). TRAIL induces apoptosis via its death receptors, DR4 and DR5. Upon binding the extracellular part of these receptors the cytoplasmic death domains cluster and thus the death inducing signaling complex (DISC) is formed. The DISC activates the caspase cascade which eventually results in apoptosis (1). TRAIL is considered to be an interesting therapeutic molecule, because it can specifically induce apoptosis in many malignant cells while it leaves most untransformed cells unharmed (2).

Recently it has been shown that TRAIL is also involved in several neurodegenerative disorders as studies have demonstrated that neuronal loss in multiple sclerosis (MS), Alzheimer's disease (AD) and HIV-1 associated dementia (HAD) is related to TRAIL induced apoptosis. In normal, healthy CNS. TRAIL is not expressed, but its receptors are. However, under pathological conditions macrophages with upregulated expression of TRAIL have been found to infiltrate the brain (3). Another scenario is that TRAIL production is induced in cells as neurons, microglia and astrocytes by pathogens or immune activation (4-6).

MS and its animal model, experimental autoimmune encephalitis (EAE), are characterized by demyelination, loss of oligodendrocytes and chronic inflammation of the CNS. Due to the inflammation macrophages expressing TRAIL might infiltrate the brain, leading to neuronal loss. Indeed, TRAIL induces apoptosis in neurons and additionally promotes demyelination in murine EAE (7). Contrarily, earlier it was demonstrated that inhibition of TRAIL outside the CNS worsened EAE in mice (8). Most recently however, TRAIL expressed on invaded T cells was found in close vicinity of dying neurons in MS patients. In EAE, this TRAIL was identified as the mediator of the immune attack against spinal cord neurons (9).

Deposits of ß-amyloid protein in the brain are characteristic for AD onset (10). It was demonstrated that expression of both TRAIL and

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one of its death receptors, DR5, were induced in neuroblastoma cells by ß-amyloid protein. These SH-SY5Y cells are sensitive to TRAIL induced apoptosis, while treatment of the cells with TRAIL-neutralizing or DR5 blocking antibody protected them from ß-amyloid protein neurotoxicity (6, 11). In addition, it was shown by immunostaining of cerebral cortex sections that TRAIL is expressed in patients with AD while it is absent in the brain of non-demented patients (12).

HAD typically occurs during the later stages of viral disease. Monocytes and macrophages infiltrate the CNS and cause neuronal damage which leads to disabling cognitive impairment and motor dysfunction (13). HIV upregulates TRAIL expression in macrophages of infected patients (14). Both DR4 and DR5 are expressed on HIV-1 encephalitic brain tissue. This indicates a role for the expressed TRAIL in HAD (3).

Depending on the cell type TRAIL initiates its apoptotic signal either via DR4, via DR5 or via both (15, 16). For DR5 it is also known that depending on the reaction with different receptor agonists it can mediate various cell signals (17, 18). To elucidate these differences our laboratory has already designed DR4 and DR5 selective agonistic variants of TRAIL (19, 20, 21). For the identification of a selective antagonist of DR5 we decided to use a phage library displaying a cystein constrained heptamer. Earlier attempts to identify a selective binder to DR5 by phage display have been made by others (22). Here, the researchers used phage displayed random libraries of peptides, single chain variable fragments (scFv's) and antigen binding fragments (Fab's). However, their objective was to identify agonists of DR5 induced apoptosis and not antagonists of DR5. Also, there have been antibodies developed that bind DR5 specifically (23). Again, they were looking for agonists, which induce apoptosis and can be used in cancer treatment.

Selection of an antagonist starts with identifying a binder to the elected target. A frequently used method is to test a library consisting of continuous parts of the natural ligand. Any part of the ligand that binds the death receptor, but does not trimerize nor activate the DISC would be a suitable candidate. However, by using a randomized library instead of continuous parts of the natural ligand our chances of success increase. In this way we can also identify the discontinuous amino acids that would be in a naturally occurring binding patch of folded protein. For expression and selection of our random peptide library we chose to use phage display, an elegant

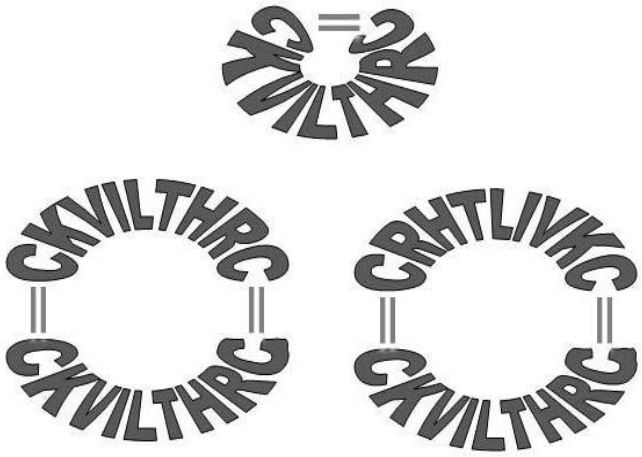

Figure 1. Schematic representation of monomeric peptide R2C16 (top) and dimeric peptide R2C16 in both the parallel (bottom left) and anti-parallel (bottom right) orientation of the two monomers.

and well-established technique linking genotype and phenotype. It has been demonstrated that peptides bind their targets with high affinity and specificity (24). An additional advantage of using peptides is that they are easily modified and further developed for clinical application. By simply adding a scaffold, such as a leucine zipper, they can be multimerized. Another, frequently used modification is linking of an antibody or specific protein to the peptides so they can be targeted, for instance to a specific organ (25).

We were able to identify a cystein constrained heptameric peptide that is able to bind specifically to DR5 with high affinity: CKVILTHRC. Synthesis of this peptide yielded both monomeric and dimeric forms of the peptide. Measurements by MALDI-TOF indicated the dimeric peptides consist of two monomers linked via disulfide bonds. These monomers were shown to be oriented both parallel and anti-parallel (Figure 1) (26). It can be seen that the dimeric peptides are much longer compared to the monomeric peptide which might increase flexibility, influencing the binding of the peptide to the receptor. Therefore we decided to investigate the effects of both the monomeric and the dimeric peptides. Both forms of the peptide were able to bind to DR5 in a dose-dependent manner. This was confirmed in ELISA and SPR studies where the binding towards Fc-DR5 was investigated, and also for FACS studies, where the binding towards Jurkat cells was evaluated. The apparent Kd value of the dimers is more than twice as good as that of the monomers, 40nM compared to 272nM. In addition, the dimers bind the Jurkat cells much better than

the monomers do, as is reflected by the higher fluorescent PE signal at equimolar concentrations (26). The observed higher avidity for the dimeric peptide might well be a result of the increased flexibility compared to the monomeric peptide, which would allow the dimers to adapt a more favourable confirmation for binding. The antagonizing ability of the peptides was shown in the colon carcinoma cell line Colo205. These cells are sensitive to TRAIL-induced apoptosis and its death signal is transmitted via DR5 (19). Treatment with the peptides caused hardly any cell death, indicating the peptides are not agonistic. Preincubation with the peptides before TRAIL treatment showed a reduction in apoptosis as compared to treatment with only TRAIL. Pre-incubation with the dimeric peptides was more efficient than with the monomers in rescuing the cells. These combined data demonstrate the peptides are able to act as antagonists and can inhibit TRAIL induced apoptosis, which is mediated via DR5 (26).

Recognizing the importance of a better understanding of neurodegenerative diseases we think we have identified a useful research tool and perhaps even a candidate for drug development. The first step towards this would be demonstrating the affinity of the peptides towards brain cells expressing DR5. We chose the neuroblastoma cell line SH-SY5Y to perform flow cytometry binding analyses with biotinylated monomeric and dimeric peptide (Figure 2). Similarly to the binding studies on the Jurkat cells, we found that also for the SH-SY5Y cells the peptides bound in a dose-dependent manner to DR5. Compared to control the increasing amounts of biotinylated monomeric and dimeric peptide showed an increase in fluorescence signal. The fluorescence obtained with the dimeric peptides is much higher than that with an equal molarity of monomeric peptide. Again, with higher concentrations of peptide the fluorescence signal drops back to control levels. This might be a result of the hydrophobic nature of the peptides, as we hypothesized before. While this hydrophobic nature complicated our studies towards the characteristic of the peptides, it might prove to be beneficial when used in the CNS, which is known to be lipophilic.

Now that we know the peptides are able to bind DR5 on SH-SY5Y neuroblastoma cells, we should investigate whether the peptides can block TRAIL induced apoptosis. In addition, since we have established the dimeric peptides to have a higher

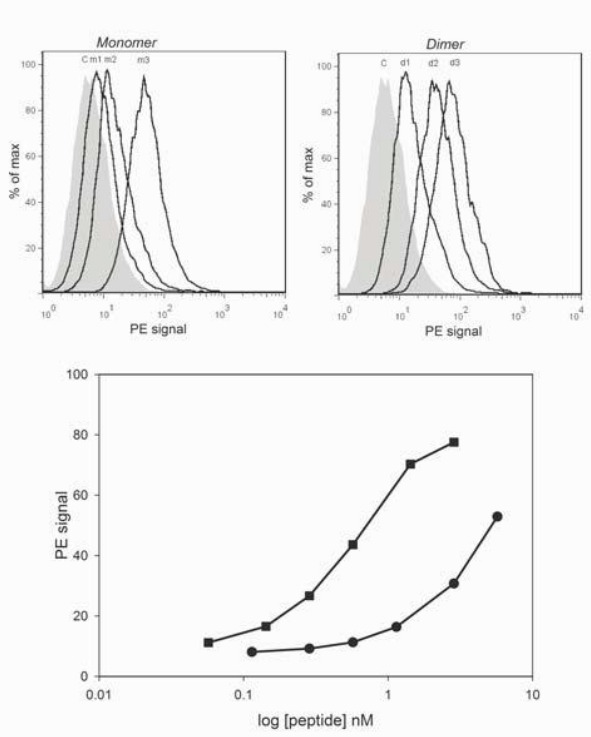

Figure 2. Dose-response histograms of biotinylated peptide R2C16, monomeric (left panel) or dimeric (right panel) to SH-SY5Y neuroblastoma cells. Increasing amounts of both monomeric (m1 = 0.29 nM, m2 = 1.14 nM, m3 = 5.71 nM) and dimeric (d1 = 0.14 nM, d2 = 0.57 nM, d3 = 2.86 nM) biotinylated peptide cause a right shift in the fluorescent PE signal compared to the control (C, filled grey).The curves represent the relative fluorescent PE signal compared to control for both monomeric (●) and dimeric (■) biotinylated peptide R2C16.

avidity than the monomeric peptides, we could imagine trimers or even higher multimers to be even more effective. And finally, it would be interesting to see whether we can improve the efficiency of the DR5 specific peptides by linking them to a scaffold that can target them towards the CNS.

Acknowledgements

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Conflicts of Interest

No potential conflicts of interest to disclose.

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