ACS NANO

www.acsnano.org

LETTER TO THE EDITOR

Comment on "Self-Illuminating Nanoagonist Simultaneously Induces Dual Cell Death Pathways via Death Receptor Clustering for Cancer Therapy"

Olivier Micheau* and Sylvie Fournel

ACS Nano **2024**, *18* (26), 17119−17134. DOI: 10.1021/acsnano.4c03767
ACS Nano **2025**, *19*. DOI: 10.1021/acsnano.4c17726

Cite This: *ACS Nano* 2025, 19, 1−3

Read Online

ACCESS | Metrics & More | Article Recommendations

The study describes a self-illuminating nanoparticle that displays selective antitumoral activity.[1] The nanovector's antitumoral properties are attributed to the TRAIL receptor 2 (DR5) peptidomimetic, whose cross-linking, induced by a smart H_2O_2-responsive self-illuminating nanoagonist, strongly activates the extrinsic pro-apoptotic signaling pathway. This activation was evidenced *in vitro* by flow cytometry. The antitumoral efficacy of this smart system was also assessed *in vivo* using the syngeneic 4T1 tumor breast cancer model. The study shows that the smart nanovector not only prevents tumor growth, achieving more than 82% tumor suppression compared to controls, but also displayed anti-metastatic properties.

The manuscript is based on the assumption that the DR5-specific peptide used (WDCLDNRIGRRQCVL), which is known to target human DR5, cross-reacts with the unique mouse TRAIL receptor agonist.[2] While it is true that most ligands of the tumor necrosis factor superfamily (TNFSF), including TRAIL,[3] cross-react between human and mouse orthologues,[4] it should be kept in mind that some members such as GITR/GITRL or APRIL/BAFF-R are strictly species-specific.[4]

The first peptide described to display TRAIL-like pro-apoptotic activity was obtained from a peptide scan library composed of 8 amino acids derived from TRAIL itself (see Table 1). Out of the 6 peptides found to trigger apoptosis the most efficient candidate, RNSCWSKD, corresponded to TRAIL aa227-234.[5] Subsequent single amino acid substitution of this sequence revealed a potent peptide CNSCWSKD whose pro-apoptotic activity was shown to engage both DR4 and DR5 (ref 6; see also Table 1), which is consistent with the fact that this peptide derives from TRAIL.

However, the DR5-specific peptide used in You et al.'s study is unrelated to TRAIL,[7] and as shown in Table 1, all studies reporting its use,[7−20] so far, only described the use of human cells to assess the biological activity of their formulation (Table 1). In addition, this peptide has, early on, been described to be highly specific for human DR5 and not able to bind to mouse TRAIL receptor.[11,16] Likewise, surface plasmon resonance (SPR) assessments found this peptide unable to bind to human DR4 and the mouse TRAIL receptor.[11] In You's manuscript, the only evidence of a potential interaction between their smart nanoparticle and the mouse TRAIL receptor is provided by confocal immunofluorescence staining and a FRET assay that show the *vicinity* of the formulation with the murine TRAIL receptors. While these experiments are interesting, and despite the fact their nanovector displays antitumoral activity, the mere coincident proximity of the receptor and the nanovector is not a strong argument for demonstrating the interaction. The antitumoral efficacy of the formulation may not even rely on the ability of the peptide to engage mouse TRAIL receptor aggregation. Addressing the selective binding of this peptide to the mouse TRAIL receptor would strengthen the author's conclusions and broaden the potential use of this DR5 peptidomimetic for experimental design requiring the use of mouse cell lines or models.

AUTHOR INFORMATION

Corresponding Author

Olivier Micheau − *Université de Bourgogne, U1231 INSERM, CTM, Equipe DesCarTes, UFR Sciences de Santé, 21078 Dijon Cedex, France*; orcid.org/0000-0001-8499-7984; Email: omicheau@u-bourgogne.fr

Author

Sylvie Fournel − *Université de Strasbourg UMR _S 1121, EMR CNRS 7003, Biomatériaux & Bioingénierie, CRBS, Centre de Recherche en Biomédecine de Strasbourg, 67084 Strasbourg Cedex, France*

Received: September 18, 2024
Revised: October 12, 2024
Accepted: December 12, 2024
Published: January 14, 2025

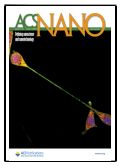

Table 1

Study	peptide	cell death assay	Human cells tested	*in vivo*	Murin cell tested	Specificity (affinity) Assay	Affinity for hDR5 (nM)	Comment
Okochi et al. 2006	RNSCWSKD	calcein AM/ Hoechst / Annexin V	Jurkat	NO	NO	NR	NR	
Kaga et al. 2007	CNSCWSKD	Hoechst / Annexin V / Caspase activation	Jurkat; HeLa; HepG2; HT-1080; DU-145	NO	NO	binds both hDR4 and hDR5	NR	Pro-apoptotic activity reduced in Jurkat deficient for Caspase-8. Binding-affinity not tested forthe other TRAIL receptors (human or mouse)
Angell et al. 2009	WDCLDNRIGxRQCVxL	MTT	HCT116	NO	NO	NR	50	
	WDCLDNRIGRRQCVkL (dimer)						6	
	WDCLDNRIGkRQCVRL (dimer)						0.6	
	WDCLDkRIGRRQCVRL (dimer)						NR	
Pavet et al. 2010	WDCLDNRIGRRQCVkL (monomer; dimer or trimer)	APO 2.7 / Annexin V / Caspase activation / DISC analysis	BJAB; BJAB-DR5-/-; HCT116; HEK; BJ; HA1ER and BJELR	YES	Yes (Affinity)	Selective to hDR5	0.88 / 1.24 / 129	Do not bind other TNF receptors, including hDR4 (shown) nor mouse TRAIL receptor (not shown)
	WDCLDNRIGkRQCVRL (monomer; dimer or trimer)			YES			0.5 / 0.114 / 664	
	WDCLDkRIGRRQCVRL (monomer; dimer or trimer)			No			0.05 / 9.74 / 226	
Lamanna et al. 2013	WDCLDNRIGRRQCVkL (monomer; trimer or hexamer)	MTS	BJAB	NO	Yes (Affinity)	Selective to hDR5	129 / 0.09 / 0.007	Do not bind mouse TRAIL receptor (Supplementary information)
Pulka-Ziach et al. 2015	WDCLDNRIGRRQCVkL (cyclic peptides -dimers)	APO 2.7 / Annexin V / Caspase activation	BJAB; BJAB-DR5-/-	NO	NO	NR	NR	
Beyrath et al. 2016	WDCLDNRIGkRQCVRL (dimer)	APO 2.7 / Annexin V / Caspase activation	BJAB; HCT116; Jurkat	NO	NO	NR	NR	
Valldorf et al. 2016	WDCLDNRIGRRQCVkL (monomer)	Annexin V / Caspase activation	Colo205; HEK293	NO	NO	NR	240	
	WDCLDNRIGRRQCVkL (heptamer)						25	
Madhumathi et al. 2017	WDCLDNRIGRRQCVkL linked to IL2	MTT / Annexin V / JC1 / Caspase activation	HL60; MOLT4; primary AML, CML, ALL and CLL	NO	NO	NR	NR	Validated in humans. Efficacy of 81.25% in AML and 100% in CML, ALL and CLL
Masum et al. 2018	WDCLDNRIGRRQCVkL (trimer)	MTT / Annexin V	Jurkat; Molt-4; K562	NO	NO	NR	NR	
Moyer et al. 2019	WDCLDNRIGRRQCVEL (dimer, polymers)	Annexin V	MDA-MB-231; T47D	YES	NO	NR	NR	
Schneider et al. 2019	WDCLDNRIGRRQCVkL (monomer, tetramer, mumltivalent)	CellTiter96® AQueous One Solution Assay	Colo205; Jurkat	NO	NO	NR	NR	
Wang et al. 2021	WDCLDNRIGRRQCVkL (origami, DNA-peptide conjugate)	CellTiter96® Glo-Luminescent Assay / Annexin V / Caspase activation	MDA-MB-231; SK-BR3; MCF-7	NO	NO	NR	NR	
Li et al. 2022	WDCLDNRIGRRQCVkL (dimer, trimer, tetramer)	Cell Counting Kit-8 / Caspase activation	Colo205; HCT116; Jurkat	NO	NO	NR	NR	
Han et al. 2023	WDCLDNRIGRRQCVkL (trimer)	Cell Counting Kit-8 / Caspase activation	Colo205	NO	NO	NR	NR	
Han et al. 2024	WDCLDNRIGRRQCVkL (trimer)	Cell Counting Kit-8 / CaspGLOW	Colo205	NO	NO	NR	NR	
You et al. 2024	WDCLDNRIGRRQCVkL (monomer conjugated to NP PEG-PLGA)	Cell Counting Kit-8 / Annexin V / Caspase activation	HUVEC	YES (4T1)	4T1	NR	NR	The binding to mouse cells 4T1 is only supported by immunofluorescence. Absence of proper binding control

Not reported (NR)

Complete contact information is available at: https://pubs.acs.org/10.1021/acsnano.4c13100

ACKNOWLEDGMENTS

O.M. is supported by grants from the ANR (Agence Nationale de la Recherche) program “Investissements d’Avenir” Labex LipSTIC (ANR-11-LABX-0021-01), ANR ISITE-BFC (ANR-15-IDEX-0003), and ANR LabCom IAM-IT (ANR-22-LCV1-0005-01), the Conseil Regional de Bourgogne, the European commission’s Horizon 2020 Research and Innovation Program DISCOVER (777995), and CHIRON (101130240). The DesCarTes team is supported by the INSERM and the Université de Bourgogne.

REFERENCES

(1) You, Y.; Zhu, L.; Song, Y.; Hu, J.; Chen, M.; Zhang, J.; Xu, X.; Huang, X.; Wu, X.; Lu, J.; et al. Self-Illuminating Nanoagonist Simultaneously Induces Dual Cell Death Pathways via Death Receptor Clustering for Cancer Therapy. *ACS Nano* **2024**, *18* (26), 17119−17134.

(2) Wu, G. S.; Burns, T. F.; Zhan, Y.; Alnemri, E. S.; El-Deiry, W. S. Molecular cloning and functional analysis of the mouse homologue of the KILLER/DR5 tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptor. *Cancer Research* **1999**, *59* (12), 2770−2775.

(3) Dufour, F.; Rattier, T.; Shirley, S.; Picarda, G.; Constantinescu, A. A.; Morle, A.; Zakaria, A. B.; Marcion, G.; Causse, S.; Szegezdi, E.; et al. N-glycosylation of mouse TRAIL-R and human TRAIL-R1 enhances TRAIL-induced death. *Cell Death Differ.* **2017**, *24* (3), 500−510.

(4) Bossen, C.; Ingold, K.; Tardivel, A.; Bodmer, J. L.; Gaide, O.; Hertig, S.; Ambrose, C.; Tschopp, J.; Schneider, P. Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. *J. Biol. Chem.* **2006**, *281* (20), 13964−13971.

(5) Okochi, M.; Nakanishi, M.; Kato, R.; Kobayashi, T.; Honda, H. High-throughput screening of cell death inducible short peptides from TNF-related apoptosis-inducing ligand sequence. *FEBS Lett.* **2006**, *580* (3), 885−889.

(6) Kaga, C.; Okochi, M.; Nakanishi, M.; Hayashi, H.; Kato, R.; Honda, H. Screening of a novel octamer peptide, CNSCWSKD, that induces caspase-dependent cell death. *Biochem. Biophys. Res. Commun.* **2007**, *362* (4), 1063−1068.

(7) Angell, Y. M.; Bhandari, A.; De Francisco, M. N.; Frederick, B. T.; Green, J. M.; Leu, K.; Leuther, K.; Sana, R.; Schatz, P. J.; Whitehorn, E. A.; et al. Discovery and optimization of a TRAIL R2 agonist for cancer therapy. *Advances in experimental medicine and biology* **2009**, *611*, 101−103.

(8) Beyrath, J.; Chekkat, N.; Smulski, C. R.; Lombardo, C. M.; Lechner, M. C.; Seguin, C.; Decossas, M.; Spanedda, M. V.; Frisch, B.; Guichard, G.; et al. Synthetic ligands of death receptor 5 display a cell-selective agonistic effect at different oligomerization levels. *Oncotarget* **2016**, *7* (40), 64942−64956.

(9) Han, Z.; Li, Z.; Raveendran, R.; Farazi, S.; Cao, C.; Chapman, R.; Stenzel, M. H. Peptide-Conjugated Micelles Make Effective Mimics of the TRAIL Protein for Driving Apoptosis in Colon Cancer. *Biomacromolecules* **2023**, *24* (11), 5046−5057.

(10) Han, Z.; Li, Z.; Stenzel, M. H.; Chapman, R. Collapsed Star Copolymers Exhibiting Near Perfect Mimicry of the Therapeutic Protein ″TRAIL. *J. Am. Chem. Soc.* **2024**, *146* (31), 22093−22102.
(11) Lamanna, G.; Smulski, C. R.; Chekkat, N.; Estieu-Gionnet, K.; Guichard, G.; Fournel, S.; Bianco, A. Multimerization of an apoptogenic TRAIL-mimicking peptide by using adamantane-based dendrons. *Chemistry* **2013**, *19* (5), 1762−1768.
(12) Li, Z.; Han, Z.; Stenzel, M. H.; Chapman, R. A High Throughput Approach for Designing Polymers That Mimic the TRAIL Protein. *Nano Lett.* **2022**, *22* (7), 2660−2666.
(13) Madhumathi, J.; Sridevi, S.; Verma, R. S. CD25 targeted therapy of chemotherapy resistant leukemic stem cells using DR5 specific TRAIL peptide. *Stem Cell Res.* **2017**, *19*, 65−75.
(14) Masum, A. A.; Yokoi, K.; Hisamatsu, Y.; Naito, K.; Shashni, B.; Aoki, S. Design and synthesis of a luminescent iridium complex-peptide hybrid (IPH) that detects cancer cells and induces their apoptosis. *Bioorg. Med. Chem.* **2018**, *26* (17), 4804−4816.
(15) Moyer, T. J.; Chen, F.; Toft, D. J.; Ruff, Y.; Cryns, V. L.; Stupp, S. I. Self-assembled peptide nanostructures targeting death receptor 5 and encapsulating paclitaxel as a multifunctional cancer therapy. *ACS Biomater Sci. Eng.* **2019**, *5* (11), 6046−6053.
(16) Pavet, V.; Beyrath, J.; Pardin, C.; Morizot, A.; Lechner, M. C.; Briand, J. P.; Wendland, M.; Maison, W.; Fournel, S.; Micheau, O.; et al. Multivalent DR5 peptides activate the TRAIL death pathway and exert tumoricidal activity. *Cancer Res.* **2010**, *70* (3), 1101−1110.
(17) Pulka-Ziach, K.; Pavet, V.; Chekkat, N.; Estieu-Gionnet, K.; Rohac, R.; Lechner, M. C.; Smulski, C. R.; Zeder-Lutz, G.; Altschuh, D.; Gronemeyer, H.; et al. Thioether analogues of disulfide-bridged cyclic peptides targeting death receptor 5: conformational analysis, dimerisation and consequences for receptor activation. *Chembiochem* **2015**, *16* (2), 293−301.
(18) Schneider, H.; Yanakieva, D.; Macarron, A.; Deweid, L.; Becker, B.; Englert, S.; Avrutina, O.; Kolmar, H. TRAIL-Inspired Multivalent Dextran Conjugates Efficiently Induce Apoptosis upon DR5 Receptor Clustering. *Chembiochem: a European journal of chemical biology* **2019**, *20* (24), 3006−3012.
(19) Valldorf, B.; Fittler, H.; Deweid, L.; Ebenig, A.; Dickgiesser, S.; Sellmann, C.; Becker, J.; Zielonka, S.; Empting, M.; Avrutina, O.; et al. An Apoptosis-Inducing Peptidic Heptad That Efficiently Clusters Death Receptor 5. *Angew. Chem., Int. Ed. Engl.* **2016**, *55* (16), 5085−5089.
(20) Wang, Y.; Baars, I.; Fordos, F.; Hogberg, B. Clustering of Death Receptor for Apoptosis Using Nanoscale Patterns of Peptides. *ACS Nano* **2021**, *15* (6), 9614−9626.

https://doi.org/10.1021/acsnano.4c13100
ACS Nano 2025, 19, 1−3