Molecular Cancer Therapeutics

A Better TRAIL Variant for Tumor Cell–Specific Targeting? – Response

Dirk Spitzer, Peter Goedegebuure, and William G. Hawkins

With great interest, we have read the letter by Bremer and Helfrich about our recent report describing the generation and initial characterization of a genetically encoded TRAIL fusion protein (TR3) as a novel platform for cancer therapy (1). We very much appreciate the feedback and acknowledge the expertise of this group in advancing the field of targeted TRAIL therapy. Here, we address the complex nature of TRAIL biosynthesis and the problems that had to be resolved in order to produce bioactive, recombinant TRAIL forms for basic research and biomedical applications.

In 2000, Bodmer and colleagues systematically investigated the critical nature of TRAIL's unique cysteine at position 230 (2). It turned out that bioactive material (sTRAIL, amino acids 95-281) could not be produced from mammalian cells because of the formation of intermolecular disulfide bridges identified by Western blotting as covalently linked dimers and trimers. This limitation is the reason we have not been able to do comparative functional studies between mammalian-produced sTRAIL and TR3. It is, however, possible to generate bioactive TRAIL by *N*-terminal addition of a trimerization domain such as an isoleucine zipper (ILZ; ref. 3). We have produced both TRAIL variants in HEK293T cells and found that sTRAIL was, indeed, nearly completely inactive, whereas ILZ-TRAIL (and TR3) was a potent inducer of cell death (1).

With respect to a comparison of scFvC54:sTRAIL (4) and scFv-TR3 (1), we would like to point out that fundamental differences exist between the two concepts. This can be readily deduced from the stoichiometry of the targeting (scFv) and effector domains (TRAIL) employed [1:1 in the former (polyvalent target antigen binding via scFv) and 1:3 in the latter (monovalent)]. Together, the data presented by the authors [Western blotting (predominantly monomers and dimers and some trimers) and size exclusion chromatography (exclusively containing trimers without providing immunostaining data)], Bodmer's studies on Cys^{230}, and our own experimental results suggest that scFvC54:sTRAIL more closely resembles disulfide-linked multimers that are completely inactive in the absence of ectopically expressed EpCAM on Jurkat targets (4), whereas monomeric scFv-TR3 (as well as all the derivatives described in our study such as TR3 and the spacer-containing scFv-*S*-TR3) is more closely related to a noncovalently associated ILZ-TRAIL trimer, because these reagents were all potent inducers of apoptosis.

It is worth mentioning that studies on the differential activation of death receptors 4 and 5 (DR4/5) suggest that on Jurkat cells (exclusively expressing DR5), only aggregated soluble TRAIL trimers seem to be capable of inducing apoptosis (5). However, this would imply that commercially available, prokaryotically produced TRAIL used in our study [amino acids 114-281, (1)], LZ-TRAIL, ILZ-TRAIL, and all of our TR3 preparations would exist as aggregates, which we think is unlikely, because, for example, TR3 did not differ in its electrophoretic mobility on Western blotting under reducing and nonreducing conditions and did not reveal evidence of higher mole cular weight aggregates on the same blots.[1] Because of the importance of these issues to the entire TRAIL field, we are currently investigating the exact nature of our TR3 variants and look forward to making new and exciting discoveries that will help better understand the complex biology of human TRAIL.

With respect to the binding affinities of single-chain antibody fragments, we and many others have shown that monovalent scFvs can successfully deliver a variety of effector proteins to an increasing number of target antigens and exert their functions *in vitro* and *in vivo*, without the requirement for higher valencies (1, 6).

Thus, future studies that assess the efficacy and mechanistic aspects of the TRAIL–death receptor pathways of both targeting concepts are eagerly awaited. We are optimistic that these results will help us to optimize either targeting strategy and move forward the best concept for the sake of the patients who suffer from cancer.

[1] D. Spitzer, unpublished observations.

Authors' Affiliation: Washington University School of Medicine, Department of Surgery, St. Louis, Missouri

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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