Reviewer's report

Title: Modulation of TRAIL resistance in colon carcinoma cells: Different contribution of DR4 and DR5

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The manuscript by van Geelen et al. entitled “Modulation of TRAIL resistance in colon carcinoma cells: Different contribution of DR4 and DR5” points at different pro-apoptotic activities of two apoptotic signaling-inducing TRAIL receptors - TRAIL-R1/DR4 and TRAIL-R2/DR5 in the colorectal carcinoma-derived cell line SW948 and its selected TRAIL-resistant variant – SW948-TR. Two major findings in the manuscript in principle are only minor contribution of TRAIL-R2/DR5 to TRAIL-induced apoptosis in SW948 cells (despite apparently effective early DISC formation and in-DISC activation of caspase-8) and MG132- or interferon gamma-assisted re-sensitization of SW948-TR cells to TRAIL- or TRAIL receptors agonistic antibodies-mediated apoptosis. Variations in the efficacy of either of the pro-apoptotic TRAIL receptors has been observed in a number of previous publications but the molecular mechanism(-s) of these cells- or conditions-specific variations remains unexplained. Selective requirements of TRAIL-R1/DR4-mediated apoptotic signaling in this colorectal cancer cell line is even more interesting with the regard to apparently preferential use of TRAIL-R2/DR5 in colon cancer-derived cell lines (see e.g. Nawrocki ST et al., 2007 or Marini P et al., 2006). The authors also showed that the TRAIL-sensitizing effects of IFN-g on SW948-TR are being restricted to TRAIL-R1/DR4-mediated pro-apoptotic signaling, pointing to either late DISC or post-DISC events that might discriminate between effective use of TRAIL-R1/DR4 and TRAIL-R2/DR5. The data in the manuscript and the author’s conclusions are relatively straightforward and self-defending (though they do not offer any explanation for this different use of TRAIL receptors) but there are still some points that would require some attention (see below).

My comments and suggestions:

a/ On pg. 10 the authors claim that possible mutation in DR5 extracellular domain could affect TRAIL-DR5 interaction and thus they should address this possibility by sequencing of DR5 in SW984 cells.

b/ The authors claim that at the DISC level DR4 and DR5 signaling in ETR1- vs ETR2-treated cells is comparable (Fig. 4A), i.e. DISC assembly and processing of caspase-8 proceeds with similar efficacy. However, they just analyzed an early time point – 15 min, and it is rather possible that the differences in ETR1 vs. ETR2 signaling could unveil at later time point. Thus, they should examine DISC composition e.g. 45 min after adding the agonistic antibodies. This might also explain significant differences in caspase-8 processing observed in the total...
lysate (Fig. 4B)

c/ According the authors the acquired TRAIL resistance of SW948-TR cells is caused by downregulation of caspase-8 expression in these cells (Fig. 1C), which is being reversed by IFNg treatment. But IFNg could have additional effects on these cells (e.g. by downregulating FLIP expression etc.). Thus it would be instrumental to reconstitute (or better to say increase to the level in SW948 cells) pro-caspase-8 levels in SW948-TR and analyze an effect on TRAIL-induced apoptosis.

d/ The authors mention that DEAD-box helicase DDX3 could negatively influence DR5-triggered apoptotic signaling via its interaction with the intracellular part of DR5. Thus it might be worthy to examine DDX3 association with ETR2-DR5 DISC.

In general, the manuscript adds to a quite interesting area of the research on specificity and regulation of signaling from individual TRAIL receptors and should be considered for further processing.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.