Molecular crosstalk between TRAIL and natural antioxidants in the treatment of cancer.

Stuart A. Rushworth, Olivier Micheau

COMMENTARY

Molecular crosstalk between TRAIL and natural antioxidants in the treatment of cancer

[1]Stuart A Rushworth and [2]Olivier Micheau

[1]School of Chemical Sciences and Pharmacy

University of East Anglia

Norwich

NR4 7TJ, UK

[2] INSERM, U866, Dijon, F21079, France; Univ. Bourgogne, Dijon, F-21079, France

Correspondence to:

Email: S.Rushworth@uea.ac.uk or omicheau@u-bourgogne.fr

Keywords: TRAIL, antioxidants, apoptosis, wogonin, ROS, cell death, tumour, flavonoid.

Abbreviations: TRAIL, TNF receptor apoptosis inducing ligand; ROS, reactive oxygen species; cFLIP, cellular FLICE-like inhibitory protein.

Summary

TRAIL induces apoptosis in a wide variety of transformed and cancer cells but has little or no effect on normal cells. Therefore, TRAIL is considered to be a tumour-selective, apoptosis-inducing cytokine and a promising new candidate for cancer prevention and treatment. Some cancer cells are however resistant to TRAIL induced apoptosis, but treatment in combination with conventional chemotherapeutic drugs or irradiation generally restores TRAIL sensitivity in those cells. A novel class of molecules exhibiting synergy with TRAIL but devoid of major side effects are emerging as alternative approaches to cure resistant cancer cells, including natural antioxidants such as sulforaphane or the flavonoids curcumin, quercetin, resveratrol, baicalein and wogonin. In this issue, Lee and co-workers demonstrate that treatment of TRAIL resistant cancer cells with wogonin restores TRAIL-induced cell death in a ROS-dependent manner through p53- and puma-upregulation.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily and has been shown to induce apoptosis in cancer cells but not normal cells, for this reason TRAIL has become a recognised target for cancer therapy (Ashkenazi *et al.*, 2008). TRAIL binds to TRAIL-R1 or TRAIL-R2, two death domain-containing receptors also coined DR4 and DR5 to trigger apoptosis. Unfortunately considerable numbers of cancer cells, especially some highly malignant tumours, are resistant to apoptosis induction by TRAIL. Resistance to TRAIL can occur at different points in the signalling pathways of TRAIL-induced apoptosis, including mutations in the death receptors, defects in the molecules involved in DISC formation, dysregulation of DISC activation by TRAIL Receptor antagonists or by overexpression of cFLIP (Merino *et al.*, 2007). Therefore, developing strategies to overcome such resistance are important for the successful use of TRAIL for cancer therapy.

A large amount of studies have revealed that combination of TRAIL with chemo or radiotherapy significantly enhances cytotoxicity to tumours or reverses the resistance to monotherapy (Merino *et al.*, 2007). However, the use of conventional chemotharapeutic drugs or radiotherapy in association with TRAIL could be limited due their severe toxic side effects and their potential to induce cell death processes in both malignant and non-malignant cells (Meurette *et al.*, 2006). In recent years, efforts have been focused on the development of biological based strategies to enhance the anti-tumour activity of TRAIL without the toxic side effects of chemo or radiotherapy (Ashkenazi *et al.*, 2008). Amongst these, flavonoids and glucosinolates, which are "natural antioxidants" known to scavenge free radicals, have emerged as promising

compounds to overcome resistance of cancer cells to TRAIL, while having little or no effect on normal cells (Ishibashi *et al.*, 2008). For example, curcumin which is the active component of tumeric, has been shown to enhance TRAIL induced apoptosis in a variety of in vitro cancer models including breast, ovarian, prostate and pancreatic (Reuter et al., 2008). Resveratrol which is found in numerous plant species, including mulberries, peanuts and grapes can also enhance TRAIL induced apoptosis in prostate, colon and melanoma cancer cells (Ishibashi *et al.*, 2008). Resveratrol-induced sensitization to TRAIL was shown to occur through multiple mechanisms including cFLIP downregulation in melanoma cells (Ivanov *et al.*, 2008) or redistribution of agonistic TRAIL receptors within lipid rafts in colon carcinoma cells (Delmas *et al.*, 2004). Other antioxidants found to enhance the apoptotic properties of TRAIL include sulforaphane, indole-3-carbinol, apigenin and quercetin (Ishibashi *et al.*, 2008).

Induction of procaspase-3, procaspase-8, and procaspase-9 cleavage followed by Bid truncation and cytochrome c release from the mitochondria is probably the most frequent mechanism by which these antioxidants are found to enhance TRAIL induced apoptosis. Strikingly, in a large number of reports, TRAIL-induced sensitization by flavonoids or sulforaphane was also associated with ROS production. Likewise, sulforaphane which is found in broccoli was found to enhance TRAIL-induced apoptosis in a prostate cancer cell line (PC-3) through the generation of intracellular ROS, leading to collapse of mitochondrial membrane potential, activation of caspase-3 and caspase-9, and up-regulation of DR4 and DR5 (Ishibashi *et al.*, 2008). Another study showed that resveratrol in combination with TRAIL resulted in generation of ROS, translocation of

Bax to mitochondria and subsequent drop in mitochondrial membrane potential, release of mitochondrial proteins to cytosol, activation of effector caspase-3 and caspase-9, and induction of apoptosis. More recently, ROS-induced upregulation of DR5 was also reported to account for TRAIL-induced sensitization by baicalein (Taniguchi *et al.*, 2008). The common theme from these studies is that the range of antioxidants, in combination with TRAIL, show that generation of reactive oxygen species (ROS) seems to trigger signal transduction culminating in cell cycle arrest and/or apoptosis.

In this issue, Lee and co-workers provide strong experimental evidences that wogonin, which is one of the main active compounds of Scutellaria baicalensis, enhances TRAIL-induced cytotoxicity through ROS-mediated DNA damage-induced p53 and puma up-regulation, leading to enhanced Bax activation and cell death. Blocking ROS generation, like deficiency in p53, puma or Bax failed to restore TRAIL-sensitivity in cells stimulated with wogonin. ROS generation was however not inhibited in cells lacking p53, Bax or puma nor in cells in which p53 was mutated indicating that ROS production drives p53 regulation. Furthermore, authors show in addition that phosphorylation of H2Ax at serine 139 occurs before p53 upregulation. This demonstration is in line with a previous report showing that several flavonoids such a quercetin, genistein, but not biochaninA nor daidzein activate the DNA-Damage Response pathway (Ye *et al.*, 2004). This finding is particularly interesting as TRAIL itself was recently shown to activate the Chk2 DNA-Damage Response pathway leading to bax activation in a caspase-dependent feed-back loop- but p53-independent manner (Solier *et al.*, 2009). Altogether, the findings reported by Lee et al. highlight a novel

molecular cross-talk between TRAIL and compounds exhibiting sensitizing activities like natural antioxidants, irradiation or conventional chemotherapeutic drugs.

Work is however clearly needed to assess the reality of using these natural antioxidants in combination therapy with TRAIL to treat patients suffering from cancer. TRAIL-based agonists are being assessed in clinical trials (Ashkenazi *et al.*, 2008). Most recombinant humain TRAIL (rhTRAIL) or agonistic antibodies targeting DR4 (mapatumumab) and DR5 (lexatumumab, mapatumumab, AMG-655, LBY135, CS-1008) have now been assessed in phase I and shown to be generally well tolerated with minimum toxicity. Phase II are now in progress in association with conventional chemotherapeutic compounds and results are being eagerly awaited.

Importantly, antioxidants such as the one reported in this review may be an alternative to chemo or radiotherapy for the use of TRAIL agonists. However, it remains to be determined whether these findings will be applicable since a large number of tumor cells, corresponding to approximately 50 % of the tumors found in patients, are p53 mutated. Besides, pharmocokenitic studies suggest that oral administration of many of these natural antioxidants results in low bioavailability. However, pharmacologically active concentrations maybe achievable in tissues that are directly exposed to such compounds including the colon and skin. With this in mind, additional efforts appear to be required to address the therapeutic potential of combination therapy using TRAIL and natural antioxidants in cancer animal models. The findings reported here may

nevertheless pave the way to novel alternative therapeutic approaches using TRAIL agonists.

Ashkenazi, A, Holland, P, Eckhardt, SG (2008) Ligand-based targeting of apoptosis in cancer: the potential of recombinant human apoptosis ligand 2/Tumor necrosis factor-related apoptosis-inducing ligand (rhApo2L/TRAIL). *J Clin Oncol* **26**(21)**:** 3621-3630.

Delmas, D, Rébé, C, Micheau, O, Athias, A, Gambert, P, Grazide, S, laurent, G, Latruffe, N, Solary, E (2004) Redistribution of CD95, DR4 and DR5 in rafts accounts for the synergistic toxicity of resveratrol and death receptor ligands in colon carcinoma cells. *Oncogene* **23**(55)**:** 8979-8986.

Ishibashi, M, Ohtsuki, T (2008) Studies on search for bioactive natural products targeting TRAIL signaling leading to tumor cell apoptosis. *Med Res Rev* **28**(5)**:** 688-714.

Ivanov, VN, Partridge, MA, Johnson, GE, Huang, SX, Zhou, H, Hei, TK (2008) Resveratrol sensitizes melanomas to TRAIL through modulation of antiapoptotic gene expression. *Exp Cell Res* **314**(5)**:** 1163-1176.

Merino, D, Lalaoui, N, Morizot, A, Solary, E, Micheau, O (2007) TRAIL in cancer therapy: present and future challenges. *Expert Opin Ther Targets* **11**(10)**:** 1299-1314.

Meurette, O, Fontaine, A, Rebillard, A, Le Moigne, G, Lamy, T, Lagadic-Gossmann, D, Dimanche-Boitrel, MT (2006) Cytotoxicity of TRAIL/anticancer drug combinations in human normal cells. *Annals of the New York Academy of Sciences* **1090:** 209-216.

Reuter, S, Eifes, S, Dicato, M, Aggarwal, BB, Diederich, M (2008) Modulation of anti-apoptotic and survival pathways by curcumin as a strategy to induce apoptosis in cancer cells. *Biochem Pharmacol* **76**(11)**:** 1340-1351.

Solier, S, Sordet, O, Kohn, KW, Pommier, Y (2009) Death receptor-induced activation of the Chk2- and histone H2AX-associated DNA damage response pathways. *Molecular and cellular biology* **29**(1)**:** 68-82.

Taniguchi, H, Yoshida, T, Horinaka, M, Yasuda, T, Goda, AE, Konishi, M, Wakada, M, Kataoka, K, Yoshikawa, T, Sakai, T (2008) Baicalein overcomes tumor necrosis factor-related apoptosis-inducing ligand resistance via two different cell-specific pathways in cancer cells but not in normal cells. *Cancer Res* **68**(21)**:** 8918-8927.

Ye, R, Goodarzi, AA, Kurz, EU, Saito, S, Higashimoto, Y, Lavin, MF, Appella, E, Anderson, CW, Lees-Miller, SP (2004) The isoflavonoids genistein and quercetin

activate different stress signaling pathways as shown by analysis of site-specific phosphorylation of ATM, p53 and histone H2AX. *DNA Repair (Amst)* **3**(3)**:** 235-244.