Free Radical Research, August 2012; 46(8): 996–1003

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.3109/10715762.2012.690514

informa
healthcare

REVIEW ARTICLE

The three Rs along the TRAIL: Resistance, re-sensitization and reactive oxygen species (ROS)

Gregory Mellier[3] & Shazib Pervaiz[1,2,3,4]

[1]*Department of Physiology, Yong Loo Lin School of Medicine, Singapore,* [2]*NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore,* [3]*Duke-NUS Graduate Medical School, Singapore, and* [4]*Singapore-MIT Alliance, Singapore*

Abstract

Ligation of the Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) death receptors has been associated with cancer specific apoptotic execution in a number of model systems. This has generated tremendous interest in the use of TRAIL as a potential therapeutic modality. However, recent evidence indicates that resistance to TRAIL might present with a therapeutic challenge. In this short report, we review the basic biology of TRAIL signalling in cancer cells, highlight the mechanisms underlying resistance to TRAIL and the ability of small molecule compounds to re-sensitize cells to TRAIL-mediated apoptosis. In particular, we provide evidence that intracellular reactive oxygen species could be critical in regulating the response of cancer cells to TRAIL.

Keywords: apoptosis, death receptors, oxidative stress, redox, TNF family

TRAIL and TRAIL-mediated apoptosis

Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) belongs to a large family of proteins grouped under the TNF superfamily [1,2]. Since its discovery, TRAIL has generated a lot of interest due, notably, to its ability to specifically target tumour cells both *in vitro* and *in vivo* with minimal toxicity towards normal cells [1–4]. However, numerous studies have since then showed that many human tumours are resistant or could easily acquire resistance to TRAIL-induced apoptosis [5–7]. Hence, the focus was switched towards the identification and development of novel small molecule compounds that could restore cancer cells sensitivity to TRAIL.

TRAIL is expressed as a type II transmembrane protein or as a cleaved soluble form (extracellular domain). TRAIL interacts with the death receptors (DRs) DR4/TRAIL-R1/TNFRSF10A [8] and DR5/TRAIL-R2/KILLER/TNFRSF10B [9,10], the decoy receptors DcR1/TRAIL-R3/TNFRSF10C [9,10] and DcR2/TRAIL-R4/TNFRSF10D [11] and with a soluble receptor called osteoprotegerin although with low affinity [12]. The first step of the death receptor apoptotic pathway, also known as the extrinsic pathway, is the binding of a trimerized form of TRAIL to the DRs, which induces a conformational change in the death domain of the receptors. In association with the DRs' clustering and oligomerization, the TRAIL/DR ligation constitutes the functional activation of the receptors. Once activated, the receptors, via their death domains, recruit the adaptor protein Fas-associated protein with death domain (FADD). In turn, FADD recruits caspases-8/10 through interaction of their death effector domains [13]. This complex is termed as the death-inducing signalling complex (DISC) [14] and promotes the activation of initiator caspases [15]. Caspases-8/10 then triggers the activation of the downstream effector caspase-3 leading to the subsequent cleavage of caspase substrates, which are important regulatory and structural proteins [16,17], and ultimately, apoptosis as evidenced by the appearance of its associated hallmarks (e.g. nuclear shrinkage, DNA fragmentation and membrane blebbing [18]). As an alternative, the initiator caspases can engage the intrinsic or mitochondrial apoptotic pathway by cleaving the protein Bid (a pro-apoptotic member of the Bcl-2 family). Bid, in its truncated form, then binds to BAX and BAK leading to their oligomerization and translocation to the mitochondrial outer membrane as well as the subsequent decrease in the mitochondrial membrane potential and the formation of pores responsible for the permeabilization of the mitochondrial outer membrane [19]. Disruption of mitochondria structural integrity permits the release into the cytosol of cytochrome *c* and Smac/DIABLO, among other pro-apoptotic proteins. Once in the cytosolic compartment, cytochrome *c* interacts with ATP and APAF-1 to form the apoptosome, thus enabling the activation of caspase-9. Caspase-9 can in turn activate caspase-3, -6 and -7 [20] and commit the cells to apoptosis as described earlier. Interestingly, according to the two cell types defined by Ozeren and El-Deiry [21], the cleavage of Bid can either serve as an amplification loop for the apoptotic

Correspondence: Shazib Pervaiz, M.B.B.S., Ph.D., Professor, ROS, Apoptosis and Cancer Biology Laboratory, Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore. Tel: +65-96614732. Fax: +65-67788161. E-mail: phssp@nus.edu.sg

(Received date: 22 March 2012; Accepted date: 30 April 2012; Published online: 13 June 2012)

signal in cells that do not depend on the intrinsic pathway for apoptosis induction (type I cells, e.g. thymocytes, colorectal adenocarcinoma cell line SW480, B lymphoblastoid cell line SKW6.4) or as the primary mechanism of TRAIL-mediated cell death, by engaging the intrinsic pathway, in cells where the extrinsic pathway is impaired or insufficient to induce cell death alone (type II cells, e.g. hepatocytes, colorectal carcinoma cell line HCT116, T lymphocyte cell line Jurkat).

TRAIL resistance and re-sensitization

As alluded to earlier, the promising debut of TRAIL as a potential therapeutic agent was hampered by a growing number of studies showing that numerous cancer cell lines exhibit or acquire resistance to TRAIL. No consensus regarding the development of TRAIL resistance has yet been reached, however, several mechanisms by which cancer cells evade TRAIL-mediated apoptosis at several levels of the apoptotic pathway have been described. Fortunately, the growing understanding of such mechanisms has helped to put in place novel approaches permitting the identification of sensitizing drugs. Indeed, several studies have shown that in many cases, TRAIL-resistant cancer cells could reacquire sensitivity to TRAIL when treated with such compounds in combination with TRAIL, often with a synergistic drug interaction [22]. In the following section, we discuss the most common apoptotic blockades involved in TRAIL resistance as well as the mechanisms of re-sensitization offered by small compounds used in combination with TRAIL.

Defects in death receptors and DISC

As the ligation of TRAIL to DR4 and DR5 and formation of the DISC are the first steps of TRAIL-induced apoptosis, changes in the protein structure of the DRs and/or their expression at the cell surface can dramatically decrease the efficiency of the apoptotic signal conveyed by TRAIL. Interestingly, many of the studies on TRAIL re-sensitization have focused on this first critical step of TRAIL-induced apoptosis transduction.

In this regard, it was shown that the epigenetic silencing of DR4 [23,24], loss-of-function mutations in DR4 and DR5 [25,26], deficient transport of the DRs to the cell surface [27], an increased TRAIL-decoy to death receptors ratio [28,29] and overall low or no expression of the DRs were all correlated to resistance to TRAIL. Our own group has demonstrated that LY303511, an inactive analogue of the PI3K inhibitor LY294002, has the ability to amplify the sensitivity of neuroblastoma cells to TRAIL via a MAPK-dependent upregulation of both DR4 and DR5 [30]. In the similar manner, drugs such as curcumin, sulphoraphane and trichostatin A sensitize tumour cells of different origins by increasing DR5 surface expression [31–33]. Additionally, modulation of the DRs mRNA levels has been shown to contribute to DRs upregulation. The proteasomal inhibitor Bortezomid increases DR5 half-life by stabilizing its mRNA [34], while MG132 – another proteasomal inhibitor, silibinin and withaferin A enhance TRAIL sensitivity by upregulating DR5 in a C/EBP Homologous Protein (CHOP)-dependent manner [35–37]. Taken together, these findings support the critical importance of DRs upregulation in the re-sensitization process.

Similarly, deficiency of DRs clustering and oligomerization that can, for example, occur via post-translational modifications of the DRs such as O-glycosylation and S-palmitoylation, can promote resistance to TRAIL [38,39]. Indeed, aggregation of the receptors in lipid rafts – sphingolipid – and cholesterol-enriched microdomains in the plasma membrane facilitates the transduction of the apoptotic signal [40]. Martin *et al*. have presented evidence of enhanced apoptosis following redistribution of DR5, FADD and caspase-8 to lipid rafts upon co-treatment of TRAIL with cyclooxygenase-2 (Cox-2) inhibitors [41]. Likewise, compounds, such as quercetin, its derivative LY303511 or aplidin, were shown to facilitate the clustering and oligomerization of the DRs and hereby sensitize cells to TRAIL-induced apoptosis [42–44]. Interestingly, the disruption of lipid rafts using methyl-β-cyclodextrin or nystatin abrogated the DRs redistribution and TRAIL sensitization in another research study [45], hence substantiating the critical role played by raft-associated receptors in TRAIL sensitization.

Finally, slightly downstream of TRAIL-DRs interaction and activation, a change in the expression of caspase-8 protein, through silencing, point mutation or increased protein degradation [24,46], or its inhibitor, cellular FLICE Inhibitor Protein (c-FLIP) [47–49] has been shown to significantly decrease cancer cells sensitivity to TRAIL-mediated cell death. C-FLIP, and its two main isoforms c-$FLIP_S$ and c-$FLIP_L$, is very efficient at inhibiting the apoptotic signal provided by TRAIL due to its structural similarity to caspase-8 and its over-expression is commonly observed in cells resistant to TRAIL. As an example, treatment of cells with the PPARγ agonist rosiglitazone enhances TRAIL sensitivity by selectively down-regulating c-$FLIP_S$, and such re-sensitization is abrogated if c-FLIP is over-expressed [50]. C-FLIP down-regulation can either be transcriptional, as it is the case upon treatment with withaferin A, or post-translational via proteasomal degradation as shown with quercetin [36,50]. Alternatively, prevention of caspase-8 degradation by bortezomib was also shown to re-sensitize [51]. Taken together, these results indicate a strong reliance of TRAIL-resistant cancer cells on keeping caspase-8 inhibited and the viability of using small molecules targeting this dependence.

Resistance at the mitochondrial level

As stated earlier, TRAIL-induced cell death is also able to engage the intrinsic apoptotic pathway to serve either as an amplification mechanism for the extrinsic pathway or as the primary pathway leading to the demise of the targeted cells.

In regards to the involvement of mitochondria in TRAIL-mediated cell death, members of the Bcl-2 family, and more specifically the ratio of pro-apoptotic to anti-apoptotic members, have been shown to play a crucial role [52]. Indeed, on one hand, cancer cells lacking pro-apoptotic proteins BAX and/or BAK failed to undergo apoptosis following treatment with TRAIL [53–56]. On the other hand, an increased expression of anti-apoptotic proteins, such as Bcl-2, Bcl-X_L and Mcl-1 confers resistance to TRAIL [47,57,58]. To further illustrate the role Bcl-2 can play in TRAIL resistance, Sun *et al*. over-expressed Bcl-2 in TRAIL-sensitive human non-small cell lung carcinoma cells and observed that those cells had acquired a resistant phenotype [59]. In the light of those studies, the pro-apoptotic members of the Bcl-2 are, with reason, valid therapeutic targets. In this regard, the BH3-mimetic ABT-737, which specifically inhibits Bcl-2, Bcl-X_L and Bcl-w, could efficiently restore sensitivity to TRAIL, either by releasing Bim and Bak from their inhibitory interaction with anti-apoptotic members of their family, or by affecting Bax distribution [60,61]. Similar reversals of resistance were obtained with the use of daidzein or flavopiridol through down-regulation of Bcl-2 or Mcl-1, respectively [57,62].

Members of the inhibitor of apoptosis protein (IAP) family, upregulated in a variety of cancers, represent another major apoptotic block downstream of the mitochondria. IAPs are able to bind and inhibit caspases and are themselves inhibited by Smac/DIABLO when it is released from the mitochondria into the cytosol. Hence, a defective release of Smac/DIABLO would impede the transduction of the apoptotic signal through the intrinsic pathway. Indeed, over-expression of Smac/DIABLO was shown to overcome X-linked IAP (XIAP) apoptotic block [63], an effect mirrored when XIAP, c-IAP-1/2 were knocked-down [64,65]. More interestingly, while TRAIL can induce Smac/DIABLO release when used as a single agent, it was suggested that the extent of such release can determine whether cancer cells are sensitive or resistant to TRAIL [66]. Incidentally, countering over-expression of IAPs, such as XIAP, c-IAP-1/2 or survivin has been associated with encouraging results. Smac mimetics and small molecules XIAP inhibitors have been used to significantly increase cancer cells sensitivity to TRAIL [67–69]. Likewise, a number of drugs such as roscovitine and rottlerin have been shown to down-regulate both XIAP and survivin, thereby restoring caspases' activation and sensitivity to TRAIL [70,71]. To note, the flavonoids quercetin and kaempferol target survivin through upregulation of its proteasomal degradation [72,73].

Reactive oxygen species and cell fate signalling

ROS production and regulation

As the site where oxidative phosphorylation occurs, mitochondria are a major source of intracellular ROS. During this process, electrons are transferred from electron donors to electron acceptors along the electron transport chain (ETC). Due to the high flux of electrons through the ETC, electron leakage, principally from the complexes I (NADH-ubiquinone oxidoreductase) and III (ubiquinol-cytochrome c oxidoreductase) onto oxygen (O_2) is not avoidable and superoxide ($O_2^{\bullet -}$) is produced as a by-product [74]. Other significant sources of mitochondrial ROS include complexes from the Krebs cycle such as α-ketoglutarate dehydrogenase (α-KGDH) and pyruvate dehydrogenase [75] (Figure 1).

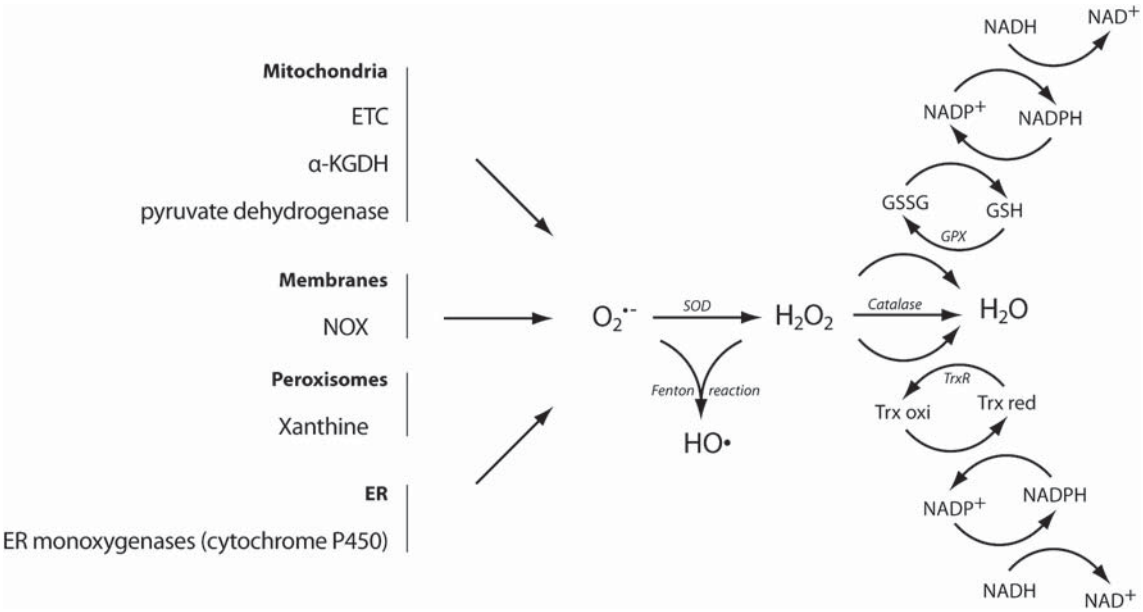

Figure 1. Redox flux. The redox flux shows the most common cellular enzymes involved in ROS production and the scavenging cellular systems in place. NAD + /NADH: nicotinamide adenine dinucleotide, NADP + /NADPH: nicotinamide adenine dinucleotide phosphate, GSH: glutathione (reduced form), GSSG: glutathione disulphide (oxidized form), GPX: glutathione peroxidase, Trx oxi/red: thioredoxin reduced/oxidized, TrxR: Trx reductase, SOD: superoxide dismutase, ETC: electron transport chain, α-KGDH: α-ketoglutarate dehydrogenase, NOX: NADPH oxidases, ER: endoplasmic reticulum.

Among the most prevalent sources of cytosolic ROS are NADPH oxidases (Nox). Members of the Nox family are transmembrane proteins localized in specific subcellular compartments, such as lipid rafts, membrane ruffles, caveolae, endosomes and the nucleus [76,77]. Nox produces O_2^- when using NADPH as the source of electron and molecular oxygen as the acceptor [78]. Xanthine oxidase, in peroxisomes [79], and monooxygenases, in the endoplasmic reticulum [80], are also sources of cytosolic ROS and contribute to increased cellular hydrogen peroxide (H_2O_2) and O_2^-.

In addition, H_2O_2 and O_2^- can lead to the generation of other reactive species. For example, both are involved in the Fenton reaction that allows for the generation of the highly reactive hydroxyl radical ($^{\bullet}OH$). Superoxide can also react with nitric oxide ($^{\bullet}NO$) to form peroxynitrite ($ONOO^-$).

If left unchecked, excessive accumulation of ROS could be responsible for cell and tissue injury and damage, such as lipid peroxidation, DNA damage and protein oxidation. However, under physiological conditions, intracellular levels of ROS are tightly regulated and maintained within tolerable limits. Cellular elimination of ROS is done mainly in two ways, by antioxidant enzymes that scavenge specific forms of ROS or by non-enzymatic molecules.

The glutathione system includes glutathione (GSH), glutathione reductase, glutathione peroxidase (GPX) and glutathione S-transferase (GST). GSH is the most abundant free thiol in the cell and, as such, is responsible in large part for the maintenance of an adequate intracellular redox milieu. During oxidative stress, cellular protection is achieved through two different mechanisms. One is the inactivation of electrophilic compounds by oxidization of glutathione to glutathione disulphide (GSSG) catalyzed by GSTs. The other is the GPX driven reduction of H_2O_2 to H_2O where glutathione also acts as an enzymatic substrate [81]. Glutathione reductase favours a high GSH:GSSG ratio by returning glutathione to its reduced form [82].

Similarly, the thioredoxin (Trx) system is the other main family of proteins involved in cellular thiol reduction and redox regulation. It is composed of three oxidoreductases (Trx-1/3) and the Trx reductase (TrxR). Trx interacts with oxidized proteins and reduces them. TrxR activity then regenerates the reduced/active form of Trx using NADPH as an electron donor [83,84].

In addition to the GSH and Trx systems, other enzymes play a role in scavenging ROS in the cell. On the one hand, dismutation of O_2^- into H_2O_2 is done by the copper/zinc superoxide dismutase (Cu/Zn SOD, SOD1) in the cytoplasm and the manganese superoxide dismutase (MnSOD/SOD2) in the mitochondrial matrix. And, on the other hand, decomposition of H_2O_2 into $H_2O + O_2$ is promoted by the activity of the enzyme, catalase [85].

Redox balance

Tissue homeostasis depends on a tight balance between the rates of cell proliferation and cell death. This balance is itself a highly regulated process, through a multitude of intracellular signalling networks. Interestingly, it has now been well established that most, if not all, of these signalling pathways involved in cell fate decision are impacted in one way or another by the cellular redox status. Hence, the existence of a tight control over ROS intracellular levels is crucial in maintaining cell homeostasis. For a long time, the conventional consensus was that abnormally elevated levels of ROS and their derivatives were linked solely to cell and tissue damage and cell death [86,87]. Nevertheless, an alternate theory has been gathering increasing support in the last decade [88]. According to that theory, the effects of a moderate increase in ROS levels are much more diverse than simply cell death induction and can in fact include cell proliferation, gene transcription or DNA damage [89]. Furthermore, the effects of an increase in intracellular ROS levels seem to be dependent on the nature of the ROS species. Indeed, a slight increase in O_2^- has been shown to activate pro-survival signalling as well as, but not necessarily concomitantly, to inhibit the induction of cell death [90–92]. Conversely, a similar mild increase in H_2O_2 promotes death execution by creating an intracellular milieu permissive for proteases activation [93–95], although it is important to note that, under certain circumstances, a low level of hydrogen peroxide can cause cell proliferation [96]. Additional works on oncogenic cell transformation have further strengthened the link between a pro-oxidant state and cell survival [97,98]. These studies substantiate a critical role of the intracellular redox status in processes involved in cell survival and cell death evasion (Figure 2).

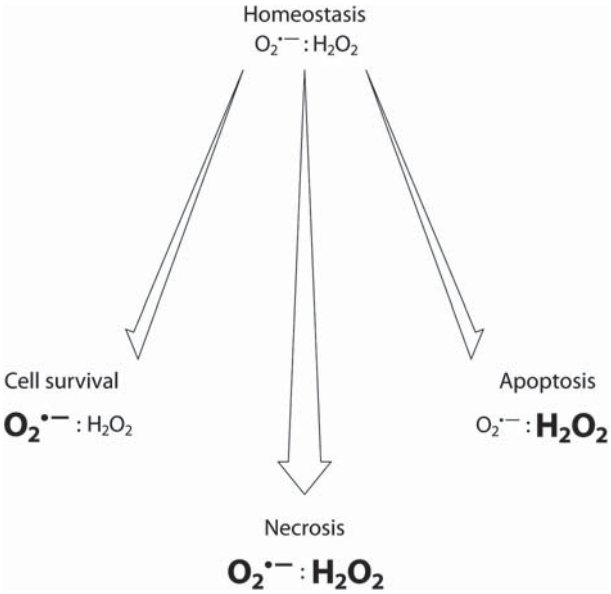

Figure 2. A ROS balance. Hypothetical schematic representation of the role on cell fate of the fine balance between two major reactive oxygen species.

TRAIL sensitization and ROS

Current studies

As detailed in the previous section, a rather large variety of compounds have shown the ability to modulate sensitivity

to TRAIL-induced apoptosis through action on different targets. Interestingly, several of those molecules are known to be efficient inducer of ROS and a number of recent studies have investigated the role of ROS in the regulation TRAIL-mediated apoptosis.

One particular area where the involvement of ROS in TRAIL-mediated apoptosis has been highlighted is the modulation of gene expression. Our group has recently demonstrated that the DRs upregulation following treatment with LY303511 was dependent on ROS as pre-incubation with catalase abrogated said upregulation as well as the subsequent sensitization [30]. Likewise, Kim *et al*. observed an inhibition of DR5 upregulation when using sulforaphane as the sensitizing agent and N-acetyl-cysteine (NAC) or catalase over-expression to scavenge ROS [32]. Additional studies have shed some light on the mechanism(s) by which ROS modulate the expression of proteins involved TRAIL-mediated apoptosis. For example, MG132-induced upregulation of DR5 was shown to involve a ROS-dependent upregulation of p53 and its binding to an intronic region of *DR5*, a phenomenon inhibited in the presence of GSH and NAC [99]. In the same way, withaferin A is reported to act in a ROS-dependent manner to increase DR5 and decrease c-FLIP levels, via CHOP and NFκB, respectively [36]. Other compounds have displayed a similar transcriptional multi-target facet such as dibenzylideneacetone (DBA), which was shown to down-regulate DcR2 dependently of ROS and upregulate DR4 and DR5 in a ROS-dependent CHOP-mediated manner [100]. Though DR5 regulation is more than likely to be scrutinized during such studies, it is interesting to note that most, if not all, of these studies have reported a ROS-mediated upregulation of DR5 expression, alone or among other genes, which hints that it might be a common feature of ROS-mediated sensitization to TRAIL.

Speculations

A very recent paper by Park *et al.* highlighted a new relation between the death receptors DR4 and DR5 and ROS [101]. In this study, they showed that activation of DR4 and/or DR5 with an agonistic protein (KD548-Fc) promotes the production of superoxide and the accumulation of intracellular ROS through direct activation of NOX1, which then leads to a ROS-mediated apoptotic cell death. In the case of the sensitizing drugs reviewed in the previous paragraph, it is then possible to hypothesize that the ROS-mediated upregulation of the death receptors, due to the sensitizing compounds, could lead to a further increase in ROS production through activation of NADPH oxidases, thereby fully committing the tumour cells to apoptosis. Depending on the sensitization mechanisms and the sensitizing drug itself, the production of ROS displays a constant increase over time or, possibly, a two-wave pattern. A slight change of focus to discover where and when ROS are produced during sensitization to TRAIL, as well as which ROS are produced, would indeed greatly improve our current understanding of the cellular mechanisms involved.

Concluding remarks

Over the last two decades, the ever-growing interest in TRAIL has allowed to characterize its signalling components as well as the resistance mechanisms developed by cancer cells. These mechanisms have been shown to spread along the entirety of TRAIL signalling pathway, from the apical signal mediated by TRAIL interaction with its cognate receptors, through the extrinsic apoptotic pathway and the mitochondrial amplification loop to the activation of executioner caspases. In parallel to the characterization of those apoptotic blockades, there has been a thorough search for small molecules able to enhance cancer cells sensitivity to TRAIL-mediated apoptosis that has led to numerous compounds. Among them, several have been shown to achieve TRAIL re-sensitization through a ROS-dependent mechanism. However, most of the current studies have only scratched at the surface and more thorough ones are needed to elucidate the intricate cellular processes leading to and originating at the ROS production during re-sensitization to TRAIL.

Acknowledgements

The authors would like to acknowledge all authors whose work could have been inadvertently omitted. S.P. is supported by grants from the National Medical Research Council, Biomedical Research Council, and Ministry of Education, Singapore.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Pitti RM, Marsters SA, Ruppert S, Donahue CJ, Moore A, Ashkenazi A. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J Biol Chem 1996;271:12687–12690.
[2] Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995;3:673–682.
[3] Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, et al. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nat Med 1999;5:157–163.
[4] Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 1999;104:155–162.
[5] Koschny R, Walczak H, Ganten TM. The promise of TRAIL—potential and risks of a novel anticancer therapy. J Mol Med 2007;85:923–935.
[6] LeBlanc HN, Ashkenazi A. Apo2L/TRAIL and its death and decoy receptors. Cell Death Differ 2003;10:66–75.

[7] Joy AM, Beaudry CE, Tran NL, Ponce FA, Holz DR, Demuth T, Berens ME. Migrating glioma cells activate the PI3-K pathway and display decreased susceptibility to apoptosis. J Cell Sci 2003;116(Pt 21):4409–4417.

[8] Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, Dixit VM. The receptor for the cytotoxic ligand TRAIL. Science 1997;276:111–113.

[9] Pan G, Ni J, Wei YF, Yu G, Gentz R, Dixit VM. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. Science 1997;277:815–818.

[10] Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. Science 1997;277: 818–821.

[11] Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, et al. A novel receptor for Apo2L/TRAIL contains a truncated death domain. Curr Biol 1997;7:1003–1006.

[12] Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, et al. Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. J Biol Chem 1998;273:14363–14367.

[13] Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH, Peter ME. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J 1997;16:2794–2804.

[14] Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH, Peter ME. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J 1995;14:5579–5588.

[15] Kischkel FC, Lawrence DA, Chuntharapai A, Schow P, Kim KJ, Ashkenazi A. Apo2L/TRAIL-dependent recruitment of endogenous FADD and caspase-8 to death receptors 4 and 5. Immunity 2000;12:611–620.

[16] Danial NN, Korsmeyer SJ. Cell death: critical control points. Cell 2004;116:205–219.

[17] Jin Z, El-Deiry WS. Overview of cell death signaling pathways. Cancer Biol Ther 2005;4:139–163.

[18] Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvogel L, Kroemer G. Cell death modalities: classification and pathophysiological implications. Cell Death Differ 2007;14:1237–1243.

[19] Waterhouse NJ, Ricci JE, Green DR. And all of a sudden it's over: mitochondrial outer-membrane permeabilization in apoptosis. Biochimie 2002;84:113–121.

[20] Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1997;90:405–413.

[21] Ozoren N, El-Deiry WS. Defining characteristics of Types I and II apoptotic cells in response to TRAIL. Neoplasia 2002;4: 551–557.

[22] Bonavida B, Ng CP, Jazirehi A, Schiller G, Mizutani Y. Selectivity of TRAIL-mediated apoptosis of cancer cells and synergy with drugs: the trail to non-toxic cancer therapeutics (review). Int J Oncol 1999;15:793–802.

[23] Horak P, Pils D, Haller G, Pribill I, Roessler M, Tomek S, et al. Contribution of epigenetic silencing of tumor necrosis factor-related apoptosis inducing ligand receptor 1 (DR4) to TRAIL resistance and ovarian cancer. Mol Cancer Res 2005;3:335–343.

[24] Hopkins-Donaldson S, Ziegler A, Kurtz S, Bigosch C, Kandioler D, Ludwig C, et al. Silencing of death receptor and caspase-8 expression in small cell lung carcinoma cell lines and tumors by DNA methylation. Cell Death Differ 2003;10:356–364.

[25] Lee SH, Shin MS, Kim HS, Lee HK, Park WS, Kim SY, et al. Somatic mutations of TRAIL-receptor 1 and TRAIL-receptor 2 genes in non-Hodgkin's lymphoma. Oncogene 2001;20: 399–403.

[26] Shin MS, Kim HS, Lee SH, Park WS, Kim SY, Park JY, et al. Mutations of tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2) genes in metastatic breast cancers. Cancer Res 2001;61: 4942–4946.

[27] Jin Z, McDonald ER, 3rd, Dicker DT, El-Deiry WS. Deficient tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptor transport to the cell surface in human colon cancer cells selected for resistance to TRAIL-induced apoptosis. J Biol Chem 2004;279:35829–35839.

[28] Merino D, Lalaoui N, Morizot A, Schneider P, Solary E, Micheau O. Differential inhibition of TRAIL-mediated DR5-DISC formation by decoy receptors 1 and 2. Mol Cell Biol 2006;26:7046–7055.

[29] Bernard D, Quatannens B, Vandenbunder B, Abbadie C. Rel/NF-kappaB transcription factors protect against tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced apoptosis by up-regulating the TRAIL decoy receptor DcR1. J Biol Chem 2001;276:27322–27328.

[30] Shenoy K, Wu Y, Pervaiz S. LY303511 enhances TRAIL sensitivity of SHEP-1 neuroblastoma cells via hydrogen peroxide-mediated mitogen-activated protein kinase activation and up-regulation of death receptors. Cancer Res 2009;69:1941–1950.

[31] Jung EM, Park JW, Choi KS, Lee HI, Lee KS, Kwon TK. Curcumin sensitizes tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis through CHOP-independent DR5 upregulation. Carcinogenesis 2006;27: 2008–2017.

[32] Kim H, Kim EH, Eom YW, Kim WH, Kwon TK, Lee SJ, Choi KS. Sulforaphane sensitizes tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistant hepatoma cells to TRAIL-induced apoptosis through reactive oxygen species-mediated up-regulation of DR5. Cancer Res 2006;66: 1740–1750.

[33] Earel JK, Jr., VanOosten RL, Griffith TS. Histone deacetylase inhibitors modulate the sensitivity of tumor necrosis factor-related apoptosis-inducing ligand-resistant bladder tumor cells. Cancer Res 2006;66:499–507.

[34] Kandasamy K, Kraft AS. Proteasome inhibitor PS-341 (VELCADE) induces stabilization of the TRAIL receptor DR5 mRNA through the 3'-untranslated region. Mol Cancer Ther 2008;7:1091–1100.

[35] Yoshida T, Shiraishi T, Nakata S, Horinaka M, Wakada M, Mizutani Y, et al. Proteasome inhibitor MG132 induces death receptor 5 through CCAAT/enhancer-binding protein homologous protein. Cancer Res 2005;65:5662–5667.

[36] Lee TJ, Um HJ, Min do S, Park JW, Choi KS, Kwon TK. Withaferin A sensitizes TRAIL-induced apoptosis through reactive oxygen species-mediated up-regulation of death receptor 5 and down-regulation of c-FLIP. Free Radic Biol Med 2009;46:1639–1649.

[37] Son YG, Kim EH, Kim JY, Kim SU, Kwon TK, Yoon AR, et al. Silibinin sensitizes human glioma cells to TRAIL-mediated apoptosis via DR5 up-regulation and down-regulation of c-FLIP and survivin. Cancer Res 2007;67:8274–8284.

[38] Wagner KW, Punnoose EA, Januario T, Lawrence DA, Pitti RM, Lancaster K, et al. Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. Nat Med 2007;13:1070–1077.

[39] Rossin A, Derouet M, Abdel-Sater F, Hueber AO. Palmitoylation of the TRAIL receptor DR4 confers an efficient TRAIL-induced cell death signalling. Biochem J 2009;419:185–192, 2 p following 192.

[40] Cremesti AE, Goni FM, Kolesnick R. Role of sphingomyelinase and ceramide in modulating rafts: do biophysical properties determine biologic outcome? FEBS Lett 2002;531:47–53.

[41] Martin S, Phillips DC, Szekely-Szucs K, Elghazi L, Desmots F, Houghton JA. Cyclooxygenase-2 inhibition sensitizes human colon carcinoma cells to TRAIL-induced apoptosis through clustering of DR5 and concentrating death-inducing signaling complex components into ceramide-enriched caveolae. Cancer Res 2005;65:11447–11458.

[42] Psahoulia FH, Drosopoulos KG, Doubravska L, Andera L, Pintzas A. Quercetin enhances TRAIL-mediated apoptosis in colon cancer cells by inducing the accumulation

of death receptors in lipid rafts. Mol Cancer Ther 2007;6:2591–2599.
[43] Poh TW, Huang S, Hirpara JL, Pervaiz S. LY303511 amplifies TRAIL-induced apoptosis in tumor cells by enhancing DR5 oligomerization, DISC assembly, and mitochondrial permeabilization. Cell Death Differ 2007;14:1813–1825.
[44] Gajate C, Mollinedo F. Cytoskeleton-mediated death receptor and ligand concentration in lipid rafts forms apoptosis-promoting clusters in cancer chemotherapy. J Biol Chem 2005;280: 11641–11647.
[45] Delmas D, Rebe C, Micheau O, Athias A, Gambert P, Grazide S, et al. Redistribution of CD95, DR4 and DR5 in rafts accounts for the synergistic toxicity of resveratrol and death receptor ligands in colon carcinoma cells. Oncogene 2004;23:8979–8986.
[46] Zhang L, Zhu H, Teraishi F, Davis JJ, Guo W, Fan Z, Fang B. Accelerated degradation of caspase-8 protein correlates with TRAIL resistance in a DLD1 human colon cancer cell line. Neoplasia 2005;7:594–602.
[47] Wang X, Chen W, Zeng W, Bai L, Tesfaigzi Y, Belinsky SA, Lin Y. Akt-mediated eminent expression of c-FLIP and Mcl-1 confers acquired resistance to TRAIL-induced cytotoxicity to lung cancer cells. Mol Cancer Ther 2008;7:1156–1163.
[48] Dolcet X, Llobet D, Pallares J, Rue M, Comella JX, Matias-Guiu X. FLIP is frequently expressed in endometrial carcinoma and has a role in resistance to TRAIL-induced apoptosis. Lab Invest 2005;85:885–894.
[49] Geserick P, Drewniok C, Hupe M, Haas TL, Diessenbacher P, Sprick MR, et al. Suppression of cFLIP is sufficient to sensitize human melanoma cells to TRAIL- and CD95L-mediated apoptosis. Oncogene 2008;27:3211–3220.
[50] Kim YH, Jung EM, Lee TJ, Kim SH, Choi YH, Park JW, et al. Rosiglitazone promotes tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by reactive oxygen species-mediated up-regulation of death receptor 5 and down-regulation of c-FLIP. Free Radic Biol Med 2008;44: 1055–1068.
[51] Thorpe JA, Christian PA, Schwarze SR. Proteasome inhibition blocks caspase-8 degradation and sensitizes prostate cancer cells to death receptor-mediated apoptosis. Prostate 2008;68:200–209.
[52] Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ. BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell 2001;8:705–711.
[53] Kandasamy K, Srinivasula SM, Alnemri ES, Thompson CB, Korsmeyer SJ, Bryant JL, Srivastava RK. Involvement of proapoptotic molecules Bax and Bak in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced mitochondrial disruption and apoptosis: differential regulation of cytochrome c and Smac/DIABLO release. Cancer Res 2003;63:1712–1721.
[54] Ravi R, Bedi A. Requirement of BAX for TRAIL/Apo2L-induced apoptosis of colorectal cancers: synergism with sulindac-mediated inhibition of Bcl-x(L). Cancer Res 2002;62: 1583–1587.
[55] Deng Y, Lin Y, Wu X. TRAIL-induced apoptosis requires Bax-dependent mitochondrial release of Smac/DIABLO. Genes Dev 2002;16:33–45.
[56] LeBlanc H, Lawrence D, Varfolomeev E, Totpal K, Morlan J, Schow P, et al. Tumor-cell resistance to death receptor—induced apoptosis through mutational inactivation of the proapoptotic Bcl-2 homolog Bax. Nat Med 2002;8:274–281.
[57] Taniai M, Grambihler A, Higuchi H, Werneburg N, Bronk SF, Farrugia DJ, et al. Mcl-1 mediates tumor necrosis factor-related apoptosis-inducing ligand resistance in human cholangiocarcinoma cells. Cancer Res 2004;64:3517–3524.
[58] Zhang L, Fang B. Mechanisms of resistance to TRAIL-induced apoptosis in cancer. Cancer Gene Ther 2005;12:228–237.
[59] Sun SY, Yue P, Zhou JY, Wang Y, Choi Kim HR, Lotan R, Wu GS. Overexpression of BCL2 blocks TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in human lung cancer cells. Biochem Biophys Res Commun 2001;280: 788–797.
[60] Huang S, Sinicrope FA. BH3 mimetic ABT-737 potentiates TRAIL-mediated apoptotic signaling by unsequestering Bim and Bak in human pancreatic cancer cells. Cancer Res 2008;68:2944–2951.
[61] Sinicrope FA, Penington RC, Tang XM. Tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis is inhibited by Bcl-2 but restored by the small molecule Bcl-2 inhibitor, HA 14 - 1, in human colon cancer cells. Clin Cancer Res 2004;10: 8284–8292.
[62] Siegelin MD, Gaiser T, Habel A, Siegelin Y. Daidzein overcomes TRAIL-resistance in malignant glioma cells by modulating the expression of the intrinsic apoptotic inhibitor, bcl-2. Neurosci Lett 2009;454:223–228.
[63] Ng CP, Bonavida B. X-linked inhibitor of apoptosis (XIAP) blocks Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of prostate cancer cells in the presence of mitochondrial activation: sensitization by overexpression of second mitochondria-derived activator of caspase/direct IAP-binding protein with low pl (Smac/DIABLO). Mol Cancer Ther 2002;1:1051–1058.
[64] Connolly K, Mitter R, Muir M, Jodrell D, Guichard S. Stable XIAP knockdown clones of HCT116 colon cancer cells are more sensitive to TRAIL, taxanes and irradiation in vitro. Cancer Chemother Pharmacol 2009;64:307–316.
[65] Gill C, Dowling C, O'Neill AJ, Watson RW. Effects of cIAP-1, cIAP-2 and XIAP triple knockdown on prostate cancer cell susceptibility to apoptosis, cell survival and proliferation. Mol Cancer 2009;8:39.
[66] Zhang XD, Zhang XY, Gray CP, Nguyen T, Hersey P. Tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of human melanoma is regulated by smac/DIABLO release from mitochondria. Cancer Res 2001;61:7339–7348.
[67] Vogler M, Walczak H, Stadel D, Haas TL, Genze F, Jovanovic M, et al. Small molecule XIAP inhibitors enhance TRAIL-induced apoptosis and antitumor activity in preclinical models of pancreatic carcinoma. Cancer Res 2009;69: 2425–2434.
[68] Fakler M, Loeder S, Vogler M, Schneider K, Jeremias I, Debatin KM, Fulda S. Small molecule XIAP inhibitors cooperate with TRAIL to induce apoptosis in childhood acute leukemia cells and overcome Bcl-2-mediated resistance. Blood 2009;113:1710–1722.
[69] Secchiero P, Zerbinati C, Rimondi E, Corallini F, Milani D, Grill V, et al. TRAIL promotes the survival, migration and proliferation of vascular smooth muscle cells. Cell Mol Life Sci 2004;61:1965–1974.
[70] Kim EH, Kim SU, Choi KS. Rottlerin sensitizes glioma cells to TRAIL-induced apoptosis by inhibition of Cdc2 and the subsequent downregulation of survivin and XIAP. Oncogene 2005;24:838–849.
[71] Kim EH, Kim SU, Shin DY, Choi KS. Roscovitine sensitizes glioma cells to TRAIL-mediated apoptosis by downregulation of survivin and XIAP. Oncogene 2004;23:446–456.
[72] Siegelin MD, Reuss DE, Habel A, Herold-Mende C, von Deimling A. The flavonoid kaempferol sensitizes human glioma cells to TRAIL-mediated apoptosis by proteasomal degradation of survivin. Mol Cancer Ther 2008;7:3566–3574.
[73] Siegelin MD, Reuss DE, Habel A, Rami A, von Deimling A. Quercetin promotes degradation of survivin and thereby enhances death-receptor-mediated apoptosis in glioma cells. Neuro Oncol 2009;11:122–131.
[74] Orrenius S, Gogvadze V, Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. Annu Rev Pharmacol Toxicol 2007;47:143–183.
[75] Starkov AA, Fiskum G, Chinopoulos C, Lorenzo BJ, Browne SE, Patel MS, Beal MF. Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. J Neurosci 2004;24:7779–7788.

[76] Ushio-Fukai M. Localizing NADPH oxidase-derived ROS. Sci STKE 2006;2006:re8.
[77] Zhang AY, Yi F, Zhang G, Gulbins E, Li PL. Lipid raft clustering and redox signaling platform formation in coronary arterial endothelial cells. Hypertension 2006;47: 74–80.
[78] Leto TL, Morand S, Hurt D, Ueyama T. Targeting and regulation of reactive oxygen species generation by Nox family NADPH oxidases. Antioxid Redox Signal 2009;11:2607–2619.
[79] Bonekamp NA, Volkl A, Fahimi HD, Schrader M. Reactive oxygen species and peroxisomes: struggling for balance. Biofactors 2009;35:346–355.
[80] Zangar RC, Davydov DR, Verma S. Mechanisms that regulate production of reactive oxygen species by cytochrome P450. Toxicol Appl Pharmacol 2004;199:316–331.
[81] Ursini F, Maiorino M, Brigelius-Flohe R, Aumann KD, Roveri A, Schomburg D, Flohe L. Diversity of glutathione peroxidases. Methods Enzymol 1995;252:38–53.
[82] Carlberg I, Mannervik B. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem 1975;250:5475–5480.
[83] Mustacich D, Powis G. Thioredoxin reductase. Biochem J 2000;346(Pt 1):1–8.
[84] Arner ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 2000;267: 6102–6109.
[85] Goyal MM, Basak A. Human catalase: looking for complete identity. Protein Cell 2010;1:888–897.
[86] Clement MV, Pervaiz S. Reactive oxygen intermediates regulate cellular response to apoptotic stimuli: an hypothesis. Free Radic Res 1999;30:247–252.
[87] Halliwell B, Gutteridge JM, Cross CE. Free radicals, antioxidants, and human disease: where are we now? J Lab Clin Med 1992;119:598–620.
[88] Clement MV, Pervaiz S. Intracellular superoxide and hydrogen peroxide concentrations: a critical balance that determines survival or death. Redox Rep 2001;6:211–224.
[89] Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47–95.
[90] Ahmad KA, Clement MV, Pervaiz S. Pro-oxidant activity of low doses of resveratrol inhibits hydrogen peroxide-induced apoptosis. Ann N Y Acad Sci 2003;1010:365–373.
[91] Clement MV, Hirpara JL, Pervaiz S. Decrease in intracellular superoxide sensitizes Bcl-2-overexpressing tumor cells to receptor and drug-induced apoptosis independent of the mitochondria. Cell Death Differ 2003;10:1273–1285.
[92] Clement MV, Stamenkovic I. Superoxide anion is a natural inhibitor of FAS-mediated cell death. EMBO J 1996;15: 216–225.
[93] Clement MV, Ponton A, Pervaiz S. Apoptosis induced by hydrogen peroxide is mediated by decreased superoxide anion concentration and reduction of intracellular milieu. FEBS Lett 1998;440:13–18.
[94] Ahmad KA, Iskandar KB, Hirpara JL, Clement MV, Pervaiz S. Hydrogen peroxide-mediated cytosolic acidification is a signal for mitochondrial translocation of Bax during drug-induced apoptosis of tumor cells. Cancer Res 2004;64:7867–7878.
[95] Hirpara JL, Clement MV, Pervaiz S. Intracellular acidification triggered by mitochondrial-derived hydrogen peroxide is an effector mechanism for drug-induced apoptosis in tumor cells. J Biol Chem 2001;276:514–521.
[96] Rao GN. Hydrogen peroxide induces complex formation of SHC-Grb2-SOS with receptor tyrosine kinase and activates Ras and extracellular signal-regulated protein kinases group of mitogen-activated protein kinases. Oncogene 1996;13: 713–719.
[97] Lim S, Clement MV. Phosphorylation of the survival kinase Akt by superoxide is dependent on an ascorbate-reversible oxidation of PTEN. Free Radic Biol Med 2007;42:1178–1192.
[98] Pervaiz S, Cao J, Chao OS, Chin YY, Clement MV. Activation of the RacGTPase inhibits apoptosis in human tumor cells. Oncogene 2001;20:6263–6268.
[99] Chen JJ, Chou CW, Chang YF, Chen CC. Proteasome inhibitors enhance TRAIL-induced apoptosis through the intronic regulation of DR5: involvement of NF-kappa B and reactive oxygen species-mediated p53 activation. J Immunol 2008;180: 8030–8039.
[100] Prasad S, Yadav VR, Ravindran J, Aggarwal BB. ROS and CHOP are critical for dibenzylideneacetone to sensitize tumor cells to TRAIL through induction of death receptors and downregulation of cell survival proteins. Cancer Res;71: 538–549.
[101] Park KJ, Lee CH, Kim A, Jeong KJ, Kim CH, Kim YS. Death receptors 4 and 5 activate Nox1 NADPH oxidase through riboflavin kinase to induce reactive oxygen species-mediated apoptotic cell death. J Biol Chem 2012;287:3313–3325.