We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

10

Signalling Pathways Leading to TRAIL Resistance

Roberta Di Pietro
G. d'Annunzio University
Department of Medicine and Aging Sciences, Chieti
Italy

1. Introduction

One fundamental problem of most malignancies, including those of haematological origin, is the development of multiple mechanisms of resistance, which progressively reduce or suppress the therapeutic efficacy of conventional radio-chemotherapy. In recent years novel compounds have been identified to overcome this major hurdle. Among these, TNF-related apoptosis-inducing ligand (TRAIL) generated considerable enthusiasm for its anticancer therapeutic effectiveness, selectively targeted to a wide range of cancer cells without affecting cells derived from normal tissues and organs. A number of preliminary studies sustain the use of TRAIL-Receptors agonistic antibodies (TRAs) instead of rTRAIL (recombinant TRAIL) in the treatment of tumour cells protected from rTRAIL-induced apoptosis by the expression of cell surface decoy receptors. Although the early clinical trials are promising and well tolerated, the efficacy of these novel approaches is restricted to patients with TRAIL-sensitive tumours. In addition, TRAIL-Rs loss or mutations that can often occur in neoplastic diseases can compromise expected therapeutic results. To overcome TRAIL resistance, novel strategies based on the combination of TRAIL with radio-chemotherapy, or with proteasome or histone deacetylase or NF-κB inhibitors have been developed. In light of this complex background, this chapter will discuss the current knowledge of the signalling pathways leading to TRAIL resistance and promising targeted therapies in the treatment of haematological malignancies.

2. The TRAIL/TRAIL-Rs system as a novel avenue in anticancer treatment

In recent years novel compounds have been identified to overcome emergence of cancer cells resistance to conventional radio-chemotherapy. Among these, TRAIL generated considerable enthusiasm for its anticancer therapeutic effectiveness, selectively targeted to a wide range of cancer cells without affecting cells derived from normal tissues and organs. TRAIL, also known as Apo-2 Ligand (Apo-2L) (Pitti et al., 1996), is a member of the TNF super-family of cytokines including structurally related proteins that play important roles in regulating cell death, immune response and inflammation. The story of TRAIL begins when a new member of the TNF super-family capable of inducing apoptosis was identified and characterized by virtue of its sequence homology to CD95/Fas/Apo1L (FasL) and TNF (Wiley et al., 1995).

TRAIL/Apo-2L consists of 281-291 amino acids in the human and murine forms, respectively, which share 65% amino acid identity. Like other members of the TNF family, TRAIL is a type II membrane protein having an intracellular amino-terminal portion, an internal trans-membrane domain, and an external carboxyl-terminus that forms a soluble molecule upon proteolytic cleavage (Mariani & Krammer, 1998). To facilitate biological studies, an epitope-tagged soluble form of TRAIL was constructed and identified with SDS-PAGE with an apparent molecular weight of 28 kDa (Wiley et al., 1995). Gel filtration analysis of the purified soluble TRAIL disclosed that the native molecule was multimeric in solution with a size of ~80 kDa. Since then, a lot of rTRAIL preparations have been obtained and commercialized so that the variety of techniques of construction/purification employed may justify some data inconsistencies (LeBlanc & Ashkenazi, 2003). Both TRAIL and FasL exist as full-length membrane-bound molecules and as shorter soluble forms (Almasan & Ashkenazi, 2003) and induce apoptosis in a wide variety of transformed cell lines of diverse origin (Walczak & Krammer, 2000), although the membrane-bound form with a greater efficiency (Schneider et al., 1997). What immediately distinguished TRAIL both from FasL and TNFα was its ability to induce apoptosis in various continuous cell lines and primary tumour cells, displaying minimal or no toxicity on most normal cells and tissues (Ashkenazi & Dixit, 1999). Significant levels of TRAIL transcripts have been detected in many human tissues and expressed constitutively in some cell lines (Wiley et al., 1995; Pitti et al., 1996), suggesting that TRAIL, unlike FasL, must not be cytotoxic to most tissues *in vivo*. Actually, accumulating evidences indicate that TRAIL plays important roles in the immune response to viruses, self-antigens and allergens and in the immune surveillance of tumours and metastases (Falschlehner et al., 2009).

2.1 The TRAIL receptor family and regulation

The biological effects induced by TRAIL are mediated by interactions with cell surface TRAIL-Receptors (TRAIL-Rs). Several studies have demonstrated an extreme complexity of TRAIL-Rs expression and function (Mellier et al., 2010; Mahmood & Shukla, 2010)). In fact, at least five TRAIL receptors belonging to the apoptosis-inducing TNF-receptor family have been described so far. TRAIL-R1 (death receptor DR4; TNFRSF10a) (Pan et al., 1997a) and TRAIL-R2 (DR5; TNFRSF10b) (Pan et al. 1997b) transduce apoptotic (Ozören & El-Deiry, 2003; Huang & Sheikh, 2007) as well as non-apoptotic signals (Di Pietro & Zauli, 2004; Park et al., 2005) upon TRAIL binding, while TRAIL-R3 (DcR1; TNFRSF10c) (Pan et al., 1997b) and TRAIL-R4 (DcR2; TNFRSF10d) (Marsters et al., 1997) as well as osteoprotegerin (OPG; TRAIL-R5; TNFRSF11b) (Emery et al., 1998) are homologous to TRAIL-R1 and TRAIL-R2 in their cysteine-rich extracellular domain but they lack the intracellular death domain (DD) and apoptosis inducing capability (Almasan & Ashkenazi, 2003).

2.1.1 TRAIL death receptors

TRAIL-R1 (Pan et al., 1997a) and TRAIL-R2 (Pan et al. 1997b) are type I trans-membrane proteins exposing the N-terminal TRAIL-binding domain and exerting pro-apoptotic signals through the cytoplasmic death domain. The cytoplasmic domain of TRAIL-R1/R2 shares a significant homology to the DD of different death receptors (DRs), such as CD95 and TNF-R1. Upon TRAIL binding to appropriate cognate receptors (TRAIL-R1 and TRAIL-R2), there is aggregation of TRAIL-Rs on the cell surface followed by the activation of both extrinsic and intrinsic intracellular death signalling pathways (Cretney et al., 2007). Shortly after addition of the ligand, the death-inducing signalling complex (DISC) is assembled (Kischkel et al., 1995). TRAIL DISC resembles that of Fas since the adaptor protein Fas associated

death domain (FADD) and the apoptosis initiator caspase-8 are recruited to TRAIL-R1 and/or TRAIL-R2 (Sprick et al., 2000). Although initial studies have attributed a central role to caspase-8 in mediating the apoptotic signal of TRAIL, subsequent studies have demonstrated that apoptosis can be triggered independently through TRAIL-R1 or TRAIL-R2 and proteolytic activation of effector caspases either directly by apical caspase-8 or -10 (Kischkel et al., 2001) and/or indirectly through Apaf-1-mediated activation of caspase-9 (Green, 2000). Similarly to CD95L, the response to TRAIL is cell-type specific and might be characterized by two distinct cell death pathways (LeBlanc & Ashkenazi, 2003). In the type I extrinsic pathway, extrinsic signals lead to the activation of large amounts of caspase-8 and to the rapid cleavage of executioner caspase-3 prior to loss of mitochondria trans-membrane potential. As a consequence, in type I cells (including leukaemia cells) Bcl-2 over-expression blocks the mitochondrial changes associated with cell death but does not prevent apoptosis that occurs upon death receptors activation. Recent studies have suggested that death receptor induction of apoptosis may depend on the degree of receptor aggregation/multimerization, which may, in turn, depend on the concentration of the death ligand, its form (i.e. soluble versus membrane-bound), the relative DR expression on the cell surface and the array of growth factors and cytokines to which the cells are exposed (Abdulghani & El-Deiry, 2010; Mellier et al., 2010). In the type II intrinsic pathway of apoptosis, intrinsic signals, like DNA damage, growth factor withdrawal or cytokine deprivation, affect the function of Bcl-2 family members (Roos & Kaina, 2006). In fact, in type II cells the extrinsic pathway activated by death receptors is ineffective to recruit, at the DISC level, enough caspase-8 to activate effector caspases. However, through homotypic aggregation at the DISC, caspase-8 is stabilized in an active form and released into the cytosol, where it cleaves its target proteins, most notably the pro-apoptotic Bcl-2 homology domain (BH3)-only protein Bid (BH3-interacting-domain death agonist) (Kelley & Ashkenazi, 2004), thus connecting the "intrinsic" mitochondrial pathway to the "extrinsic" DR pathway (Sprick & Walczak, 2004). In turn, truncated Bid (tBid) is able to bind anti-apoptotic Bcl-2 family members like Bcl-2, Bcl-XL, Bcl-W and A1 allowing the pro-apoptotic Bcl-2 family members Bax and Bak to engage the mitochondria and induce the release of mitochondrial cytochrome c and Smac (second mitochondria-derived activator of caspases)/DIABLO into the cytosol, where these latter factors promote caspase activation. Actually, cytochrome c forms the "apoptosome" complex with the adaptor protein Apaf-1 resulting in the activation of the apoptosis-initiating protease caspase-9, which then stimulates effector caspases (Green, 2000). Instead, Smac/DIABLO binds to inhibitors of apoptosis proteins (IAPs), preventing their negative-regulatory binding to caspase-9 and -3 and then augmenting apoptosis induction (Salvesen & Abrams, 2004). In this scenario, oncogenic mutations affecting molecules involved in the intrinsic mitochondrial pathway might cause resistance emergence in type II cells, while mutations in the DR pathway could confer resistance to DR-dependent apoptosis especially in type I cells (Fig. 1).

It has been demonstrated that the expression pattern of the two killer receptors is broad and partly overlapping, suggesting that they may serve as an alternate or "backup" system, allowing the immune system to control aberrant cells even if one of the two receptors had failed (Greil et al., 2003). Although further investigations are needed to assess differences between DR signalling and regulation, some interesting observations have been reported so far. For example, it has been shown that TRAIL-R1 is activated both by the soluble and the membrane-bound form of the ligand (MacFarlane et al., 2005) whereas TRAIL-R2 is activated by cross-linked soluble and membrane-bound TRAIL ligand but not by the soluble non-cross-

linked ligand (Wajant et al., 2001). We previously found a selective radiation-induced up-regulation of TRAIL-R1 in different cell lines of haematological origin that sensitized cells to TRAIL cytotoxic activity (Di Pietro et al., 2001), whereas other investigators have suggested a key role for TRAIL-R2 in p53-dependent apoptosis in response to DNA damage both *in vitro* and *in vivo* (Burns et al., 2001). Similarly, TRAIL-R2 was up-regulated in B-CLL (chronic lymphocytic leukaemia) cells in response to the small molecule Nutlin-3 in a p53-dependent manner (Coll-Mulet et al., 2006). By means of receptor-selective TRAIL mutant ligands, MacFarlane et al. (2005) demonstrated that CLL cells signal to apoptosis primarily through TRAIL-R1, whereas cross-linked agonistic TRAIL-R2 antibodies facilitate signalling via TRAIL-R2 (Natoni et al., 2007). Other authors have shown different responses of DRs in their cytoplasmic domains that may account for the differences in the activation of these receptors (Thomas et al., 2004). These authors postulated that the binding of ligand and agonistic antibody to the extracellular domain exposes the FADD-binding region differently in the cytoplasmic domain of TRAIL-R1 and TRAIL–R2 to enhance caspase-8 binding and cleavage while promoting recruitment of ancillary proteins. In addition, one more recent paper indicates TRAIL-R2 as a mediator of anoikis of colorectal carcinoma cell lines through the preferential recruitment of the "extrinsic" pathway of apoptosis (Laguinge et al., 2008).

2.1.2 TRAIL decoy receptors

Unlike TRAIL death receptors, TRAIL-R3 and TRAIL-R4 have been originally proposed as "decoy" receptors able to inhibit apoptosis by sequestering TRAIL from the death-inducing TRAIL-Rs or by aggregating with TRAIL death receptors upon binding to the trimeric ligand (Ashkenazi & Dixit, 1999). Nevertheless, it has been demonstrated in primary human CD8+ T cells that the inhibition of TRAIL-induced apoptosis by TRAIL-R4 critically depends on its ligand-independent association with TRAIL-R2 via the NH2-terminal preligand assembly domain overlapping the first partial cysteine-rich domain of both receptors (Clancy et al., 2005). In addition, it has been shown that expression of TRAIL-R1 and/or TRAIL-R2 was necessary but not always sufficient to mediate apoptosis, while expression of TRAIL-R3 and/or TRAIL-R4 often did not correlate with normal and tumour cells resistance to TRAIL effects (Di Pietro & Zauli, 2004). In fact, a number of evidences, based on the use of TRAIL-R agonists rather than over-expression models, have pointed at the primary role of intracellular mechanisms in controlling TRAIL resistance in a number of cell types (Griffith & Linch, 1998; Leverkus et al., 2000), thus cutting down the importance of control at decoy receptors level, whose ability to modulate TRAIL-mediated apoptosis is still controversial. In this regard, particularly intriguing is the role of soluble OPG (Corallini et al., 2008; Secchiero & Zauli, 2008). OPG was initially characterized for its ability to inhibit RANKL-stimulated osteoclastogenesis (Emery et al., 1998), but more recent studies highlighted the capability of OPG to counteract the pro-apoptotic activity of TRAIL in a variety of neoplastic cell types, at least *in vitro* (Schaefer et al., 2007). Of note, the interplay between OPG, RANKL and TRAIL is an important issue in bone and bone marrow biology as well as in the physiopathology of haematological malignancies and in particular of multiple myeloma (MM) (Secchiero & Zauli, 2008).

3. Molecular mechanisms of TRAIL resistance

The response of individual leukaemia cell lines to TRAIL may depend on which of pro-apoptotic or pro-survival pathways is dominant. In fact, the sensitivity of leukaemia cell

lines to TRAIL is highly variable, with some cell lines demonstrating a marked resistance. In this respect, the large majority of primary haematological tumours are TRAIL-resistant, basically due to the activation of anti-apoptotic signalling pathways (such as Akt and NF-κB), over-expression of anti-apoptotic proteins (such as FLIP, Bcl-2, X-IAP), reduced expression of TRAIL death receptors or increased expression of decoy receptors (Testa, 2010; Mellier et al., 2010) (Fig. 1).

3.1 TRAIL-receptors

Defects in either of the different molecules involved in TRAIL signalling can lead to TRAIL resistance. With regard to the relationship between TRAIL-receptors expression and TRAIL-resistance, the data reported in the literature are often contradictory (Russo et al., 2010). An interesting characteristic in the family of DRs is that normal cells are TRAIL resistant, but the molecular basis for TRAIL tumour selectivity is still unclear. In fact, as many chemotherapeutic drugs, TRAIL is not universally active against tumour cell lines, especially primary tumour cells, even expressing DRs on their surface. Dysfunctions of TRAIL-R1/R2 due to oncogenic mutations have been found in different tumours and in different cancer patients (breast, lung, head and neck cancer and non-Hodgkin lymphoma) (Lee et al., 1999; 2001). In particular, most tumours mutations map the intracellular domain of TRAIL-R2 that binds the adaptor protein FADD (Shin et al., 2001), known for being essential together with caspase-8 for the DISC assembly. It has been reported that post-translational modifications, such as O-glycosylation, at the receptor level are essential for TRAIL-R1/R2 full functionality (Wagner et al., 2007), since protein glycosylation could enhance ligand-mediated receptor clustering. Thus, the glycosylation status of TRAIL-R1/R2 has been proposed as a marker of TRAIL sensitivity (Russo et al., 2010).

TRAIL resistance has also been linked to the presence of decoy receptors, although their physiological role as well as their impact on normal and cancer cells signalling is still poorly understood. It has been demonstrated that under experimental conditions decoy receptors over-expression could sequester TRAIL, decreasing the functional binding to TRAIL-R1/R2 and attenuating the apoptotic signalling, but experiments under physiological conditions are still missing (Russo et al., 2010). Initially, the preferential expression of TRAIL-R3/-R4 mRNA in normal cells, including peripheral blood lymphocytes, spleen and thymus, was related to the absence of TRAIL cytotoxicity in normal cells, but subsequent studies, using specific monoclonal antibodies (MoAbs), demonstrated that TRAIL sensitivity was not correlated with the relative expression of TRAIL death or decoy receptors (Griffith et al., 1999). It is now accepted that the simple expression of a death or decoy receptor is not an essential feature for apoptosis sensitivity.

3.2 PI3K/Akt, MAPK, c-FLIP

Another molecular mechanism responsible for TRAIL resistance emergence is considered the constitutive activation of pro-survival pathways, including Akt pathway. Akt, also known as protein kinase B (PKB), is a serine/threonine kinase that acts as a transducer of many functions initiated by the growth factor receptors that activate phosphatidylinositol 3-kinase (PI3K). In this respect, Akt and PTEN (phosphatase and tensin homologue deleted on chromosome 10) constitutive phosphorylation has been linked to TRAIL resistance of acute lymphoblastic leukaemia (ALL) cell lines (Didaa et al., 2008) and acute myeloid leukaemia (AML) patient blasts (Martelli et al., 2006). Interestingly, TRAIL itself can paradoxically

activate Akt and downstream targets, like CREB/ATF transcription factors, in leukaemia cells sensitive to its cytotoxic action, loosing to some extent its pro-apoptotic effect (Zauli et al., 2005; Caravatta et al., 2008). Constitutively active Akt is an important regulator of TRAIL sensitivity in prostate cancer (Chen et al., 2001) and protects HL60 leukaemia cells from TRAIL-induced apoptosis by activating the transcriptional factor NF-κB and up-regulating c-FLIP (cellular FADD-like interleukin-1beta-converting enzyme-inhibitory protein) (Bortul et al., 2003). c-FLIP acts as an important intracellular inhibitor of TRAIL sensitivity and delivers growth signals by activating NF-κB and ERK signalling pathways (Almasan & Ashkenazi, 2003). It is structurally related to caspase-8 (but is devoid of enzymatic activity) since it possesses two death domains that facilitate the binding to the death domain of FADD, thereby preventing association of caspase-8 with the DISC. Over-expression of c-FLIP correlates with TRAIL resistance in several types of cancer, especially in type I cells (MacFarlane et al., 2002). Although it is considered as one of the non-apoptotic NF-κB target genes, downwards the anti-apoptotic PKB/Akt and MAPK pathways, it has not been confirmed as a molecular switch between life and death in the same cell (Park et al., 2005).
More controversial is the TRAIL-R1/R2-induced activation of PKB/Akt and MAP kinases (Falschlehner et al., 2007). The three key enzymes of MAPK pathway, i.e. ERK, p38-MAPK and JNK, have been often associated with the anti-apoptotic function of TRAIL receptors. Paradoxically, pro-apoptotic effects connect TRAIL and MAPK pathways in different cell models (Frese et al., 2003; Jurewicz et al., 2006). The existence of a death domain alternative DISC complex has also been suggested to explain the TRAIL-dependent activation of MAPK pathways. FADD, caspase-8, RIP (receptor-interacting protein) and TRAF2 (TNF receptor-associated factor 2) might form a cytoplasmic complex upon TRAIL stimulation leading to p38 and JNK activation (Lin et al. 2000). In HUVECs (human umbilical vein endothelial cells) (Zauli et al., 2003) and synovial fibroblasts (Morel et al., 2005), TRAIL has a direct effect on cell survival and proliferation stimulating the PI3K-dependent phosphorylation and activation of PKB/Akt kinase without activation of NF-κB. Of note, the activation of an ERK-dependent pathway has been linked to TRAIL-induced maturation of erythroid cells (Secchiero et al., 2004a).

3.3 NF-κB/ IκB

A number of studies of different groups of investigators, including ourselves, have outlined the importance of NF-κB activation in determining the resistance/susceptibility of target cells to TRAIL cytotoxicity (Ehrhardt et al., 2003; Zauli et al., 2005), possibly by modulating c-FLIP levels (Bortul et al., 2003). Mounting experimental evidences highlight in TRAIL-resistant cells the activation of NF-κB following engagement of TRAIL-R1, -R2, or -R4 (Zauli et al. 2005; Henson et al., 2008). TRAIL activation of NF-κB is mediated via TRADD (TNF-R1-associated death domain protein), TRAF2 and RIP and occurs independently of caspase-8/-10 activation (Mühlenbeck et al., 1998; MacFarlane, 2003) (Fig. 1). Importantly, the level of NF-κB activation has been related to resistance of leukaemia (Ehrhardt et al., 2003) and neuroblastoma cell lines (Yang & Thiele, 2003) to TRAIL cytotoxicity and its aberrant activation has been involved in promoting tumour migration and dissemination. These findings are consistent with the pleiotropic activity of NF-κB transcription factors, which are implicated in the control of cell survival and tumorigenesis (Rayet & Gelinas, 1999). Activation and regulation of Rel/NF-κB proteins are tightly controlled by IκB proteins, which mask the nuclear localization signal (NLS) of NF-κB family members, thereby preventing their nuclear translocation (Baeuerle &

Baltimore, 1996). In response to many stimuli, such as TNFα, lipopolysaccharide (LPS) or interleukin-1 (IL-1), IκB kinase (IKK) is activated and can phosphorylate IκBs, which, in turn, can be poly-ubiquitinated and rapidly degraded by the proteasome, allowing the release of sequestered NF-κB. After its translocation into the nucleus, NF-κB is able to activate its target genes, which, depending on the physiological circumstances (Barkett & Gilmore, 1999), can mediate cell survival or apoptosis. It has been reported, for example, that the TRAIL-mediated recruitment of apical caspase-8/-10 was able to induce the simultaneous activation of both effector caspases (-3, -6, -7) and of NF-κB pathway in TRAIL-sensitive myeloid leukaemia cells (Secchiero et al., 2002). As a consequence, the TRAIL cytotoxic/cytostatic activity mediated by effector caspases was reduced by the concomitant pro-survival effect exerted by NF-κB. Interestingly, NF-κB activation was causally linked to the induction of maturation of the surviving leukaemia cells along the monocytic pathway (Secchiero et al., 2002). Moreover, NF-κB activation was paralleled by the absence of degradation and by the nuclear translocation of IκBα in Jurkat T leukaemia cell lines sensitive to the cytotoxic action of TRAIL (Zauli et al., 2005), whereas in TRAIL-resistant primary human erythroblasts NF-κB activation was concomitant with IκBα cytoplasmic localization (unpublished observations). The dual function of NF-κB, as an inhibitor or activator of apoptosis, would depend on the relative levels of RelA and c-Rel subunits (Chen et al., 2003). In fact, over-expression of RelA or a transcriptional-deficient mutant of c-Rel inhibits TRAIL-induced apoptosis in mouse embryonic fibroblasts, whereas depletion of RelA increases cytokine-induced apoptosis (Chen et al., 2003).

3.4 IAP, Bcl-2, caspases

Among the anti-apoptotic genes up-regulated by NF-κB are included cellular inhibitors of apoptosis proteins 1 and 2 (c-IAP1 and c-IAP2), TRAF1 and TRAF2, c-FLIP and Bcl-XL (Wang et al., 1998). Some of these (e.g. survivin, X-IAP, Bcl-2, Bcl-XL) have been shown to be associated with poor prognosis in AML (Tamm et al., 2000; Paydas et al. 2003). Over-expression of Bcl-2, Bcl-XL, or Mcl-1, loss of Bax or Bak function, increased expression of IAPs and reduced release of Smac/DIABLO from the mitochondria to the cytosol are all events resulting in TRAIL resistance in type II cancer cells (Vogler et al., 2008)(Fig. 1) . Another important mechanism through which haematological malignancies can escape TRAIL cytotoxicity is inactivation of the intracellular pro-apoptotic pathways (Ashkenazi et al., 2008). This allows malignant cells not only to escape from TRAIL-induced apoptosis, but also to take advantage of the pro-survival signals induced by TRAIL, which paradoxically may act as a survival cytokine (Fig. 1). In this respect, a possible interplay between the Akt and caspase pathways has been already described in several cell systems (Cardone et al., 1998; Jones et al., 2002; Milani et al., 2003). The picture emerging from these studies is that, when survival signals dominate, Akt impairs the activation of the apical caspases, by directly phosphorylating caspase-9 (Cardone et al., 1998) or by inhibiting the recruitment of procaspase-8/-10 to the DISC (Jones et al., 2002). On the other hand, when pro-apoptotic signals prevail, apical caspase-8/-10 activates downstream caspases, which cleave and inactivate Akt as well as other anti-apoptotic molecules (Milani et al., 2003).

All these findings underline the complexity of the TRAIL-mediated intracellular signals, which simultaneously activate pro-apoptotic and anti-apoptotic pathways. The fate of individual malignant cells would depend on which of these pathways prevail within the cell. These effects should be carefully evaluated in the individual assessment of eligibility of cancer patients to TRAIL-based therapy.

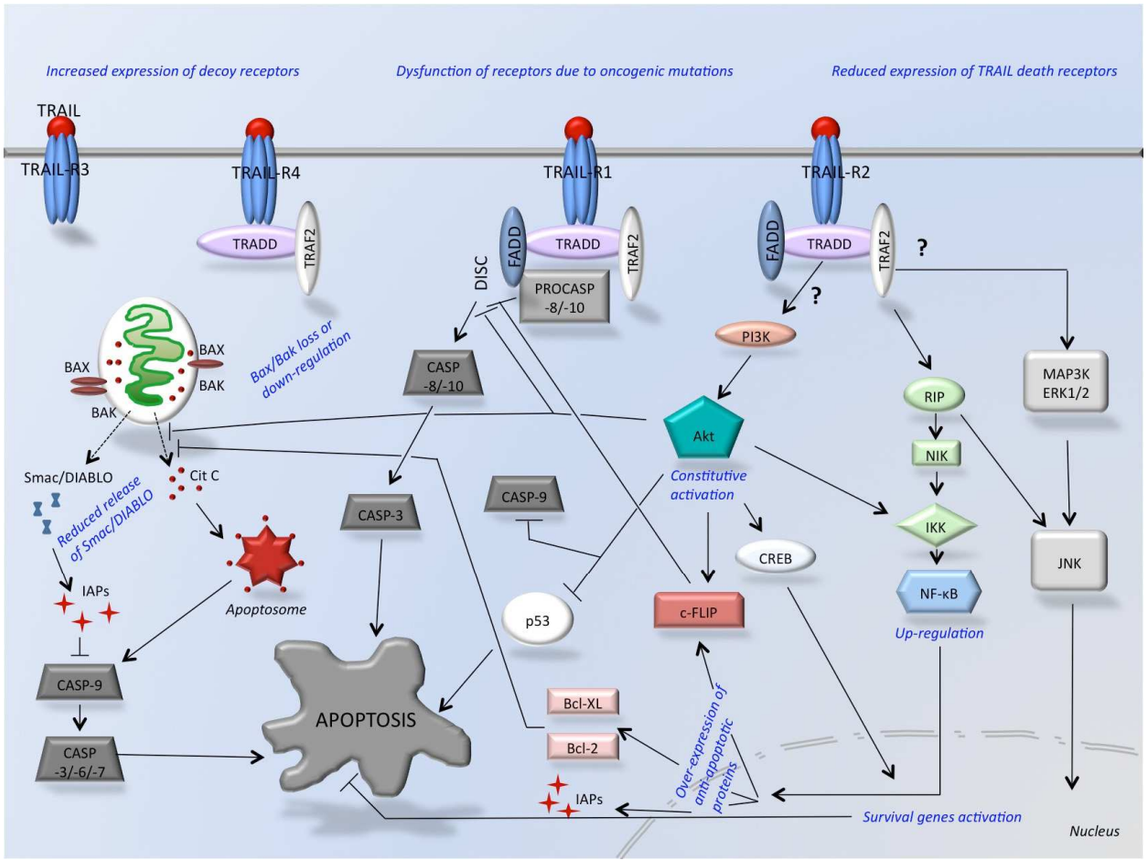


Fig. 1. Schematic representation of key mechanisms involved in TRAIL resistance of haematological malignancies. Activation of TRAIL-Rs can trigger both death and survival pathways, depending on the cell system and environmental conditions. TRAIL-R1 and TRAIL-R2 can lead to apoptotic cell death through the recruitment of FADD and the following cleavage of caspase-8 and –10. Both DRs together with the "decoy" TRAIL-R4 are also involved in the priming of survival genes through the activation of a) NF-κB and JNK pathways triggered by the engagement of TRAF2 and RIP; b) PI3K/Akt and MAPK/ERK1/2 pathways, by means of still unclear mechanisms (highlighted with a question mark). Other mechanisms leading to TRAIL resistance include different caspase or PI3K physiological inhibitors.

4. TRAIL treatment of haematological malignancies

It has been shown that TRAIL induces growth arrest and apoptosis in cancer cells independently of ^{wt}p53 function, Bcl-2 and Bcl-XL (Walczak & Krammer, 2000) and MDR gene expression (Snell et al., 1997). Thus, TRAIL may offer an alternative or complementary approach to conventional anticancer therapy. Unlike other members of the TNF super-family, such as CD95L and TNFα, that are precluded from use in systemic anticancer therapy due to their severe toxic side effects (Tartaglia and Goeddel, 1992), TRAIL is effective in selectively killing both *in vitro* and *in vivo* a vast array of tumour cells from lung, breast, kidney, colon, prostate, thyroid and skin cancers (Walczak & Krammer, 2000; Papenfuss et al., 2008), without causing significant organ toxicity and inflammation *in vivo*.

Moreover, TRAIL exerts a variable cytotoxic activity on haematological malignancies (Snell et al., 1997) and synergistically cooperates with: i) chemotherapeutic drugs, such as etoposide, campthotecin-11, doxorubicin, 5-fluorouracil, taxol (Sabatini et al., 2004; Henson et al., 2008); and ii) ionizing radiation (Chinnaiyan et al., 2000; Di Pietro et al., 2001), causing substantial regression or complete ablation of solid (colon and mammary) cancers in animal models. Besides acting as a tumour suppressor *in vivo* in primary tumours, TRAIL could play a substantial role in suppressing tumour metastasis. In fact, it has been observed that this cytokine may partially limit the formation of hepatic metastases of a variety of mouse tumours (Seki et al., 2003). A study performed in TRAIL -/- null mice demonstrated that the incidence of spontaneous lymphoid malignancies was increased by 25% in comparison with control animals (Zerafa et al., 2005), suggesting a crucial role of TRAIL in the immune-surveillance against lymphoid malignancies. Although it is not established whether TRAIL causes liver toxicity in humans (Jo et al., 2000; Lawrence et al., 2001), pre-clinical studies performed in mice and non-human primates indicated that rTRAIL protein promotes potent apoptosis-inducing activity against tumour cells without a relevant systemic toxicity (Walczak et al., 1999). Phase I and phase II clinical trials in patients with advanced solid tumours or non-Hodgkin lymphoma (NHL) appeared to go in the same direction, indicating that both rTRAIL and TRAs are safe and well tolerated (Koschny et al., 2007; Tolcher et al., 2007). Therefore, TRAIL ligand and TRAs are strong candidates for an effective but tolerable treatment of solid cancers, either used alone or in combination with radio-chemotherapy.

4.1 Acute myeloid leukaemia (AML)

The cytotoxic activity of TRAIL has been evaluated in haematological diseases by different groups of investigators, including our research group (Secchiero & Zauli, 2008; Sancilio et al., 2008; Impicciatore et al., 2010). Overall the activity of TRAIL as a single treatment in acute and chronic leukaemia is poor. Unlike the poor outcome of TRAIL treatment in primary AML blasts, continuous cell lines derived from AML display a pronounced sensitivity to the apoptotic action of TRAIL (Snell et al., 1997; Secchiero et al., 2004b). Moreover, when TRAIL is used in combination with chemotherapeutic agents (fludarabine, cytosine arabinoside or daunorubicin) additive or super-additive apoptotic effects are obtained, due to the ability of these agents to activate apical caspase-8/-10 (Jones et al., 2003). In line with these findings, other authors demonstrated that triterpenoids, natural and synthetic compounds with demonstrated anti-tumour activity, induced a substantial increase in cell death in both B-CLL and AML blasts, by inducing a concentration-dependent decrease in the levels of FLIP protein (Suh et al., 2003; Pedersen et al., 2002). A recent report has related the poor response of AML to the simultaneous expression of death and decoy receptors (Inukai et al., 2006), whereas co-expression of death receptors with the decoy receptor TRAIL-R3 resulted in significant shortened overall survival of AML patients (Chamuleau et al., 2011). Another weak point in leukaemia treatment is represented by p53 gene deletions or mutations, that usually occur in less than 15% of AML cases. To augment the poor response of AML to TRAIL cytotoxicity, Secchiero et al. (2007) have recently adopted the strategy to combine rTRAIL with Nutlin-3, a potent non-genotoxic activator of the p53 pathway (Impicciatore et al., 2010). In this investigation Nutlin-3 synergized with TRAIL in inducing apoptosis both in AML cell lines and primary M4-type and M5-type AML blasts, but not in ^{mut}p53 AML cells, suggesting that the combined treatment of Nutlin-3 plus TRAIL might offer a novel therapeutic strategy for ^{wt}p53 AML cells.

4.2 Acute lymphoblastic leukaemia (ALL)

Clodi et al. (2000) demonstrated that TRAIL has a modest activity in primary ALL since it killed a maximum of 29% of precursor-B-cell blasts within 18 hours treatment against the 75% of the sensitive Jurkat cell line. Childhood T-ALL is frequently accompanied by hyperleukocytosis at disease presentation, suggesting that T-ALL tends to acquire mechanisms for escaping immune surveillance of the hosts that promote its rapid clonal expansion. Clinically, dramatic advances have been made in the treatment of childhood T-ALL. However, despite the use of intensive risk-adapted chemotherapy, treatment failure occurs in approximately 25% of patients (Goldberg et al., 2003). Since the prognosis of relapsed T-ALL remains dismal, the development of a new therapeutic modality is urgently required. In a recent report on T-ALL cell lines and primary samples of childhood T-ALL the failure of anti-leukaemic activity of soluble rTRAIL was linked to the low cell surface expression levels of TRAIL-R1 and TRAIL-R2, which could not be modified by the demethylating agent 5-aza2'deoxycytidine (Akahanea et al., 2010).

4.3 Chronic myeloid leukaemia (CML)

Only few studies have investigated the effects of rTRAIL on CML blasts. An interesting study of Tanaka et al. (2007) demonstrated increased levels of serum TRAIL and TRAIL mRNA in neutrophils of CML patients during IFNα therapy, suggesting a novel antineoplastic role of neutrophils mediated by the expression/release of TRAIL. Since neutrophils, unlike activated lymphocytes, display a low susceptibility to TRAIL cytotoxicity (Meurette et al., 2006), these findings are of particular value. Other studies have shown that TRAIL, used as a single agent, significantly reduces the number of myeloid colonies and clusters from patients affected with CML and myelodysplastic syndromes (MDS) (Zang et al., 2001; Uno et al., 2003), while normal human stem cells treated with high doses of TRAIL maintain a repopulating potential when transplanted into NOD/SCID mice (Zang et al., 2001). Moreover, it was recently demonstrated that the loss of Bcr-Abl in imatinib-resistant CML cells leads to the down-regulation of c-FLIP and the subsequent increase in TRAIL sensitivity, suggesting that TRAIL could be an effective strategy for the treatment of imatinib-resistant CML with loss of Bcr-Abl (Park et al., 2009).

4.4 B-type chronic lymphocytic leukaemia (B-CLL)

A pressing need for the identification of novel therapeutic approaches regards B-CLL disease. It is known in fact that B-CLL patients may have initial clinical responses to alkylating agents, such as chlorambucil, or adenosine analogs, such as fludarabine, but they ultimately become refractory to therapy. Preliminary studies, carried out on cell lines and a modest number of primary samples, have shown a low cytotoxic activity of TRAIL on low-grade B-CLL (MacFarlane et al., 2002). Collectively, low-grade B-cell malignancies constitute one of the most common form of potentially lethal cancer in Europe and North America, with B-CLL representing the most prevalent of these disorders (Reed et al., 2002). B-CLL is characterized by the accumulation of mature non-proliferating B cells defective in apoptotic mechanisms and resistant to anticancer therapy. A number of molecular defects and biologic features have been identified in this pathology. Olsson et al. (2001) revealed a higher constitutive expression of the long form of FLIP (FLIP-L) in B-CLL as compared to normal tonsillar B cells. MacFarlane et al. (2002) demonstrated that resistance to TRAIL was upstream of caspase-8 activation, since little or no caspase-8 was processed in TRAIL-treated

B-CLL cells. As a consequence, the possibility of sensitizing B-CLL cells to TRAIL-mediated cytotoxicity could reside in the modulation of c-FLIP levels or in the up-regulation of DR surface expression. In consistence with this hypothesis, the combination of TRAIL with anti-CD95 ligand has proved effective in inducing apoptosis of CD40-activated B-CLL cells (Dicker et al., 2005). A more recent study, aimed at evaluating molecular mechanisms of TRAIL resistance of B-CLL, identified a different TRAIL sensitivity of Zap-70low and Zap-70high B-CLL subsets, proposing this negative prognostic marker as responsible to redirect TRAIL signallling from pro-apoptotic to pro-inflammatory pathway (Richardson et al., 2006).

4.5 Lymphoma

The potential therapeutic use of TRAIL has been also explored in the therapy of refractory diffuse large B-cell lymphoma (DLBCL) (Cillessen et al., 2006), cutaneous T-cell lymphoma (CTCL) (Braun et al., 2007), mantle B cell lymphoma (MCL) (Roue et al., 2007) and plasmacytoid dendritic cell (PDC) leukaemia (Blum et al., 2006), which shows a particularly aggressive clinical course. In particular, Cillessen et al. (2006) have shown that 12 out of a total of 22 DLBCL samples, including 7 clinically chemotherapy-refractory lymphomas, were sensitive to TRAIL-mediated apoptosis. TRAIL cytotoxic effects were also detected in CD4+CD56+ PDC leukaemia (Blum et al., 2006), as well as in the majority of MCL cell lines and primary cultures investigated by Roue et al. (2007), whose research group used TRAIL in combination with the IκB kinase inhibitor BMS-345541 to overcome resistance of MCL samples. In a recent review (Sancilio et al., 2008), we suggested the possibility to combine two biologically active and well-tolerated agents with different mechanisms of action, such as rituximab and agonist MoAbs against DRs, as an attractive treatment strategy for patients affected with B-cell lymphoma. The *in vivo* mechanisms through which rituximab mediates its effects have not been fully elucidated, though ADCC (antibody-dependent cellular cytotocxicity), CMC (complement-mediated cytotoxicity) and apoptosis have been suggested and supported by several studies (Bonavida, 2007). By contrast, a number of *in vitro* experimental evidences have been obtained in B-NHL cell lines as a model system (Bonavida, 2007). The findings here described demonstrate that rituximab treatment is able to modulate different signalling pathways, like p38-MAPK, Raf-1/MEK/ERK1/2 and NF-κB, leading to the down-regulation of Bcl-2/Bcl-XL gene products, known players of the intrinsic apoptotic pathway. Through this mechanism, chemo-sensitization of drug-resistant B-NHL cell lines to various drug-induced apoptosis could be achieved.

4.6 Multiple myeloma (MM)

A number of studies from several groups of investigators have allowed clearly establishing that myeloma is the most susceptible haematological malignancy to rTRAIL used as a single agent (Secchiero et al., 2004b). In particular, Gazitt (1999) demonstrated for the first time that TRAIL induces substantial apoptosis in freshly isolated, flow-sorted myeloma cells obtained from different MM patients. Subsequently, the same group of investigators (Liu et al., 2003) demonstrated that TRAIL is a potent inducer of apoptosis, independent of Bcl-2. Moreover, consistently with the potential role of NF-κB and Akt pathways in counteracting apoptosis induction by either chemotherapy or TRAIL, the cell permeable nuclear factor NF-κB inhibitor SN50 sensitized TRAIL-resistant MM cells to TRAIL cytotoxicity (Mitsiades et al., 2002) and the Akt inhibitor IL-6-Hydroxymethyl-chiro-inositol 2-(R)-2-O-methyl-3-O-octadecylcarbonate-induced cell death of both Dex- and Doxo-sensitive and -resistant cell

clones. Interestingly, also thalidomide, which holds great promise as a new anti-neoplastic agent for the treatment of refractory MM, triggers activation of caspase-8 and down-regulates NF-κB activity and c-FLIP (Mitsiades et al, 2002). These studies form the basis for clinical trials of these agents, alone and coupled with conventional and novel therapies, to improve outcome in MM. It is worth underlining that while the potential therapeutic use of rTRAIL or TRAs in most myeloid and lymphoid malignancies is still to be evaluated, rTRAIL appears to be a very promising candidate for the therapy of MM, either alone or in combination with valproic acid, a histone deacetylase inhibitor, arsenic trioxide, IFNγ, or with the low-molecular-weight Smac mimetic LBW242 (Secchiero & Zauli, 2008). Moreover, the use of specific anti-TRAIL-R1 or anti-TRAIL-R2 agonistic antibodies, more than the treatment with TRAIL itself (Locklin et al., 2007), has proved an effective strategy to counteract OPG-mediated effects and increase TRAIL-induced apoptosis of MM cells (Secchiero & Zauli, 2008). Of note, other preclinical studies aimed at targeting the RANK/RANKL/OPG pathway have paved the way to clinical experimentation likely to lead to new therapeutic approaches (Buckle et al., 2010).

5. Novel strategies to overcome TRAIL resistance

During the last decades, a better understanding of cancer biology has led to the development of new promising therapeutic approaches, based on "molecular targeted" drugs, directed against specific "target" molecules playing a key role in tumour maintenance (Urruticoechea et al., 2010). Based on the principle that inhibiting as many targets as possible reduces the emergence of drug resistance, the use of combined therapies or multi-target inhibitors is gaining field in the design of new treatments. A number of chemical and physical anticancer strategies have been developed to bypass TRAIL resistance, based on the combination of rTRAIL or agonistic antibodies with chemotherapeutic agents, irradiation, or targeted small molecules, like proteasome, histone deacetylase or NF-κB inhibitors (Testa, 2010; Russo et al., 2010). The agents used in combination with TRAIL either enhance TRAIL-R1/-R2 expression or decrease expression of anti-apoptotic proteins (c-FLIP, X-IAP, Bcl-2) (Mellier et al., 2010) (Fig. 2). Many of these combinatorial therapies hold promise for future developments in the treatment of haematological malignancies since they may reduce excessive systemic toxicity toward normal cells and resistance of tumour cells after recurrent treatments. We and other authors have demonstrated that TRAIL-mediated cytotoxicity is increased by ionizing radiation and chemotherapy in both myeloid and erythroid leukaemia cell lines as well as in T lymphoma cell lines (Gong & Almasan, 2000; Di Pietro et al., 2001; Sabatini et al., 2004; Zauli et al., 2005; Caravatta et al., 2008; Impicciatore et al., 2010; Signore et al., 2011). Although an increasing number of drugs warrant further investigation as potential new strategies for the treatment of solid tumours or AML in combination with soluble rTRAIL (Suh et al., 2003), only in few cases the efficacy of the combined treatments has been proved *in vivo* and a general consensus on how chemotherapy and radiotherapy may synergize with TRAIL therapy is far to be reached (Russo et al., 2010).

5.1 TRAIL-death receptor-targeted treatment

A number of receptor-specific TRAIL-variants and agonistic antibodies have been recently developed. Some of these soluble rTRAIL and MoAbs targeting TRAIL-R1 and/or TRAIL-R2 (TRAIL receptor agonists, TRAs) are progressing to phase I/II clinical trials (Mahmood &

Shukla, 2010). The clinical use of TRAs is a very promising and innovative approach to increase selectivity and reduce undesired toxicity of cancer treatments in comparison with modern anticancer drugs (protein kinase inhibitors or MoAb agonists for growth receptors) (Russo et al., 2010). These compounds were generated to selectively bind and activate their respective DRs without affecting decoy receptors or OPG. DRs engagement, using recombinant death ligands or agonistic antibodies, leads to the activation of both extrinsic and intrinsic apoptosis pathways, while, generally, chemotherapy or radiotherapy triggers the mitochondrial/intrinsic pathway (Fig. 2). Therefore, the conventional therapeutic approach could be implemented by DR-induced apoptosis when DRs are expressed and functional on tumour cells. As already mentioned, although soluble rTRAIL as well as TRAs are not completely free from toxicity, both reagents elicit a significant lower hepatotoxicity when administered systemically compared to CD95 receptor agonists (Lawrence et al., 2001). Besides the advantage of an improved specificity and a lower toxicity of TRAs over TRAIL ligand, pharmacokinetic studies performed in primates and humans have shown that these agents have a longer half-life (around 15 days) than soluble TRAIL (30 min) that makes them easier to dose and administer (Duiker et al., 2006). Preclinical studies performed *in vitro* in cultured human cell lines and *in vivo* in murine xenograft cancer models (Cretney et al., 2007) showed favourable results when TRAs were used as single agents and enhanced cytotoxicity when they were combined with chemotherapy or radiotherapy (Marini et al., 2006). In particular, HGS-ETR1 (anti-TRAIL-R1, mapatumumab) as well as HGS-ETR2 (anti-TRAIL-R2, lexatumumab) was able to induce apoptosis in primary and cultured lymphoma cells increasing cell death when associated either with conventional chemotherapy (doxorubicin) or novel drugs like proteasome inhibitors (bortezomib) (Georgakis et al., 2005). As well, multiple solid tumours including lung, colon and renal carcinoma were found responsive to TRAs treatment used alone or in combination with chemotherapy (Pukac et al., 2005). To date, the fully humanized MoAbs HGS-ETR1, HGS-ETR2 and HGS-TR2J (anti-TRAIL-R2) (all three from Human Genome Sciences, Rockville, MD) are used in ongoing trials for the treatment of advanced solid tumours, lymphoma or MM (Mahmood & Shukla, 2010). A number of excellent reviews on different therapeutic approaches to specifically target TRAIL and DR pathways have been recently published (Ashkenazi et al., 2008; Papenfuss et al., 2008; Mahmood & Shukla, 2010; Russo et al., 2010).

Of particular interest is the current use of rTRAIL and TRAs for the treatment of B cell malignancies (Mahmood & Shuka, 2010). As shown by other authors, the DR pathway is intact and functional in various types of cancers, including B-cell lymphomas (Snell et al., 1997; Georgakis et al., 2005). B-cell sensitivity to TRAs is a fundamental requirement for therapeutic efficacy, since TRAIL-R1 and TRAIL–R2 mutations, observed in NHL as well as in other human tumours (Lee et al., 2001), make neoplastic B cells insensitive to TRAIL and, presumably, to agonistic antibodies mimicking its action. TRAIL-R1 and TRAIL-R2 map to human chromosome 8p21-22, a site of frequent allelic loss in tumours. This led to the hypothesis that, as potential tumour suppressors, TRAIL-Rs may also harbour somatic mutations in human tumours. The most frequent mutations identified so far concern TRAIL-R2 and affect the intracellular domain of the receptor, i.e. the FADD-binding domain, and, as a consequence, its capability of inducing apoptosis (Bin et al., 2007). Although still poor is the knowledge of how TRAIL-Rs mutations affect signalling events, it is predictable that a patient displaying a TRAIL-R2 mutation would not benefit from treatment with either rTRAIL or an anti-TRAIL-R2 antibody but from treatment with mapatumumab or a

modified version of rTRAIL able to target only one death receptor (MacFarlane et al., 2005). Similarly to TRAIL, TRAs (mapatumumab and lexatumumab) are capable of inducing anti-lymphoma effects both *in vitro* and *in vivo* (Motoki et al., 2005). In particular, it has been recently demonstrated that mapatumumab can trigger apoptosis through caspase-8 activation via the extrinsic apoptotic pathway (Maddiplata et al., 2007). Various groups of investigators have shown that the activation of the TRAIL-Rs by either ligands or MoAbs sensitizes cancer cells to the effects of various chemotherapeutic and/or biological agents (Secchiero et al., 2007), although in a recent report no correlation between the degree of anti-tumour *in vitro* activity of mapatumumab and TRAIL-R1 antigen density has been shown (Maddiplata et al., 2007). To explain their results these investigators hypothesized differences in the receptor hetero-dimerization between various B-cell lymphoma cells upon TRAIL-R1 binding to mapatumumab. In fact, it has been published that following *in vitro* exposure to TRAIL ligand or TRAIL-R1 agonists, other DRs are recruited via trimerization, leading to signal transduction and apoptosis (Cretney et al., 2007). Depending on the type of DR undergoing trimerization upon TRAIL-R1 binding the intensity and type of response could change. In theory, the same principle could justify the absence of anti-tumour activity of lexatumumab despite ample surface expression of TRAIL-R2 in the cell lines tested *in vitro* (Maddiplata et al., 2007). Further studies are needed to address this issue.

5.2 Proteasome and histone deacetylase inhibitors

Inhibition of NF-κB (e.g. with mutant forms of IκBα or proteasome inhibitors) has also been shown to increase TRAIL responsiveness (Sayers & Murphy, 2006). In this respect, besides the clinical use of bortezomib (Velcade, PS-341) for the treatment of multiple myeloma (Sayers & Murphy, 2006), it has been recently reported that treatment with the proteasome inhibitors MG-132 and PS-341 is associated with the up-regulation of TRAIL and its death receptors, TRAIL-R1/TRAIL-R2, in primary B-CLL cells and in the Burkitt lymphoma cell line, BJAB (Kabore et al., 2006). Interestingly, the combined treatment with TRAIL or TRAs and proteasome inhibitors leads to a significant apoptosis induction in B-CLL but not in normal B cells (Kabore et al., 2006). DRs up-regulation by PS-341 was attributed to TRAIL-R2 mRNA stabilization and the consequent increased receptor half-life (Kamdasamy & Kraft, 2008). In addition to the proteasome inhibitors, inhibition of histone deacetylase (HDAC) class I sensitizes B-CLL to TRAIL-induced apoptosis (Hamilton et al., 2010). An aberrant regulation of gene expression due to alterations in histone acetyltransferase (HAT) or HDAC recruitment and activity has been constantly found in both solid and haematological tumours (Mai & Altucci, 2009). Therefore HDAC can be considered as potential therapeutic targets of human malignancies. Interestingly, the reduction of TRAIL protein degradation has been recently observed in thyroid cancer cells and proposed as a novel action of HDAC inhibitors (Borbone et al., 2010). It is worth outlining that HDAC inhibitors exert anti-tumour effects at doses that are well tolerated by the patients. Hydroxamic acids, such as SAHA (Vorinostat, Zolinza), were recently approved by the US FDA for the treatment of cutaneous manifestations in patients affected with advanced refractory CTCL. In fact, similarly to what has been seen in B-CLL, CTCL cell lines show pronounced resistance to TRAIL cytotoxicity (Braun et al., 2007). Lastly, a phase I study, currently recruiting participants, will use vorinostat in combination with cytarabine and etoposide for the treatment of patients with relapsed and/or refractory acute leukaemia, MDS or myeloproliferative disorders (see for details http://clinicaltrials.gov).

5.3 Small molecules and natural compounds

Besides oncogenes over-expression and cell cycle control mechanisms disruption, mutations in apoptotic regulators (namely p53) are very frequent in cancer cells and represent for them a way to escape toxic effects inducible with radio-chemotherapy. As an alternative strategy to restoring transcriptional activation to mutant p53 proteins in solid tumours, small molecule selective inhibitors of p53/MDM2 interaction (Nutlins) are emerging as an innovative tool in the treatment of malignancies expressing ^{wt}p53 including haematological disorders (Secchiero et al., 2008; Impicciatore et al., 2010). Nutlins were the first potent and selective small molecules, antagonists of the p53/MDM2 interaction, to be identified 7 years ago (Vassilev et al., 2004). Since then several classes of small-molecule inhibitors with distinct chemical structure have been reported (Shangary & Wang, 2009), although only Nutlin-3 has been extensively evaluated for its therapeutic potential and mechanism of action in human cancer and represents a promising therapeutic candidate for drug development (Shangary & Wang, 2009). Several authors have investigated the effects of Nutlins, used alone or in combination with other therapeutic agents, on primary cells, different cell lines and tumour xenografts (Kojima et al., 2005; Lehmann et al., 2007). In particular, it has been reported that the active enantiomer Nutlin-3a induces i) increased levels of p53, ii) p53- and p21-dependent cell cycle arrest and iii) p53-dependent apoptosis in a number of solid tumours and haematological malignancies including primary AML (Kojima et al., 2005), MM (Stuhmer et al., 2005), B-CLL (Coll-Mulet et al., 2006) and Hodgkin lymphomas (HL) (Drakos et al., 2007). Unlike radiation and conventional chemotherapy, MDM2 inhibitors induce accumulation and activation of p53 in cancer and normal cells without inducing DNA damage or post-translational modifications of p53. Nutlins in fact restore p53 function in ^{wt}p53 tumour cells without inducing p53 phosphorylation and with limited effects on primary cells (Vassilev et al., 2004). Interestingly, when used at concentrations higher than 10 mM, Nutlin-3, MI-63 and MI-219 are able to inhibit cell proliferation even in cancer cells lacking ^{wt}p53 (Shangary & Wang, 2009). In response to Nutlin-3 treatment TRAIL-R2 is up-regulated in B-CLL cells in a p53-dependent manner (Coll-Mulet et al., 2006). Moreover, Nutlins reduce the MDM2 ability to stimulate p53 degradation and represent a promising approach for improving radiotherapy effects especially for tumours over-expressing MDM2 such as sarcomas, solid tumours (Momand et al., 1998) and NHL (Finnegan et al., 1994).

Besides the use of a broad range of protein inhibitors, chemotherapeutic agents or irradiation to exert synergistic effects with TRAIL action (Mahalingam et al., 2009), more recently the use of natural compounds, including polyphenols, has gained increasing interest due to their relative safety and anti-tumour efficacy in preclinical models (Jacquemin et al., 2010). Actually, it has been demonstrated that a number of natural compounds are able to enhance TRAIL-induced apoptosis in leukaemia cells (Fas et al., 2006; Russo et al., 2007; Hussain et al., 2008; Sung et al. 2010). In particular, it has been shown that wogonin, derived from a popular Chinese herb, attenuates NF-κB activity (Fas et al., 2006), whereas curcumin, responsible for the yellow colour of the spice turmeric, up-regulates TRAIL-R2 expression and inactivates NF-κB in a ROS-dependent manner in a number of solid tumours including Burkitt's lymphoma (Hussain et al., 2008). Moreover, Russo et al. (2007) reported that leukaemia cell lines were efficiently sensitized by quercetin and TRAIL co-treatment through the inhibition of the Akt pathway, while Sung et al., 2010 observed other survival proteins down-regulation after TRAIL and triterpenoids co-

treatment. Taken together, these findings demonstrate that nongenotoxic natural molecules or small compounds enhance TRAIL-mediated killing of tumour cells with reduced side effects compared to conventional radio-chemotherapy.

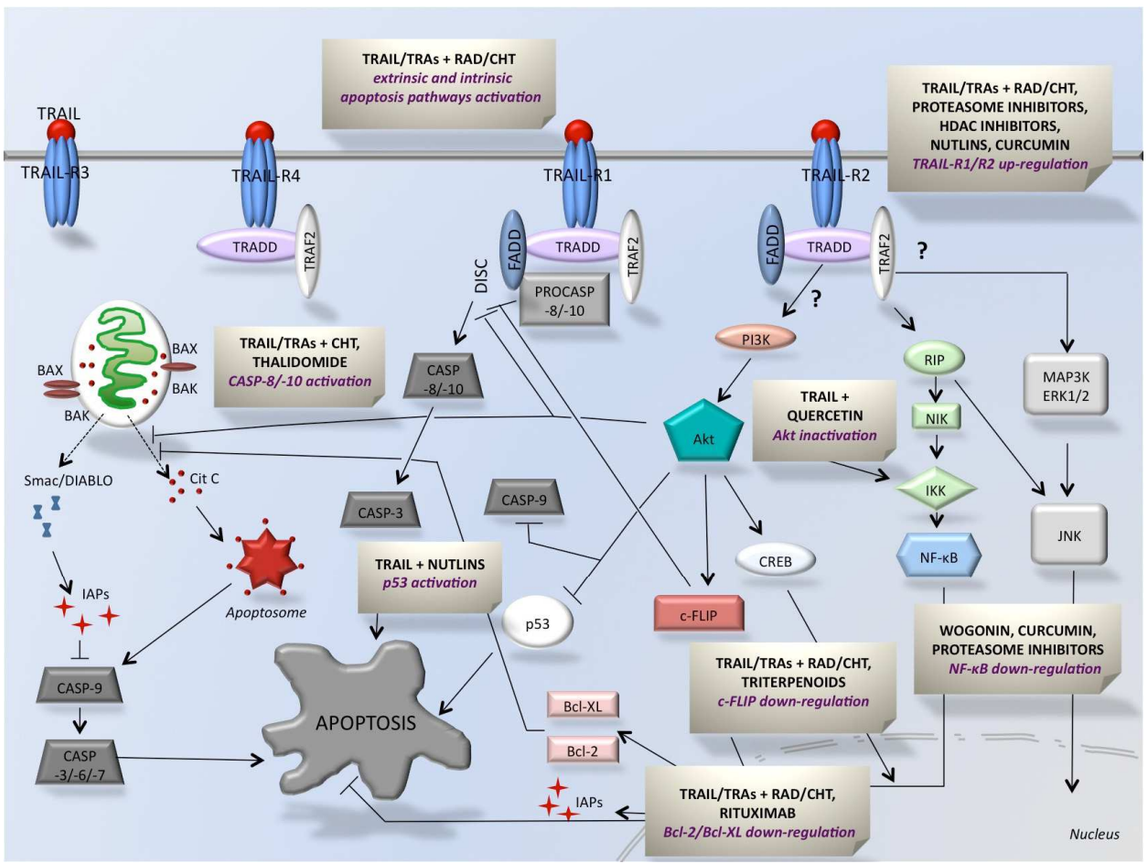


Fig. 2. Schematic representation of molecular mechanisms of novel strategies aimed at restoring TRAIL sensitivity of haematological malignancies. RAD: radiotherapy; CHT: chemotherapy. See text for other abbreviations.

6. Conclusion

A number of preliminary studies sustain the use of TRAs instead of rTRAIL in the treatment of tumour cells protected from TRAIL-induced apoptosis by the expression of cell surface decoy receptors. Although the early clinical trials are promising and well tolerated, it is worth outlining that the utility of both rTRAIL and agonistic anti-TRAIL-Rs antibodies therapies is restricted to patients with TRAIL-sensitive tumours. To restore TRAIL sensitivity in cancer cells novel compounds have been identified and are currently used in combined protocols with TRAIL ligand/TRAs or conventional radio-chemotherapy. Once mechanisms of action/resistance to TRAIL signalling are better understood, approaches to predict patient response and optimize combination regimens may be developed to overcome primary and acquired resistance on the trail to a personalized treatment of cancer.

7. Acknowledgment

The author wishes to thank Dr. Francesca Gambacorta for helpful assistance in the artwork.

8. References

Abdulghani, J. & El-Deiry, W.S. (2010). TRAIL receptor signaling and therapeutics, *Expert Opin Ther Targets* 14(10):1091-1108.

Akahanea, K., Inukaia, T., Zhanga, X., Hirosea, K., Kurodaa, I., et al. (2010). Resistance of T-cell acute lymphoblastic leukemia to tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis, *Exp Hematol* 38:885–895.

Almasan, A. & Ashkenazi, A. (2003). Apo2L/TRAIL: apoptosis signaling, biology, and potential for cancer therapy, *Cytokine Growth Factor Rev* 14(3-4):337-348.

Ashkenazi, A. & Dixit, V.M. (1999). Apoptosis control by death and decoy receptors, *Curr Opin Cell Biol* 11:255-260.

Ashkenazi, A, Holland, P. & Eckhardt, S.G. (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* 26:3621–3630.

Baeuerle, P.A. & Baltimore, D. (1996). NF-kappa B: ten years after, *Cell* 87(1):13-20.

Barkett, M. & Gilmore, T.D. (1999). Control of apoptosis by Rel/NF-kappaB transcription factors, *Oncogene* 18:6910-6924.

Bin, L., Thorburn, J., Thomas, L.R., Clark, P.E., Humphreysm R. & Thorburn, A. (2007). Tumor-derived mutations in the TRAIL receptor DR5 inhibit TRAIL signalling through the DR4 receptor by competing for ligand binding, *J Biol Chem*, 38: 28189-28194.

Blum, A., Chaperot, L., Molens, J.P., Foissaud, V., Plantaz, D. & Plumas, J. (2006). Mechanisms of TRAIL-induced apoptosis in leukemic plasmacytoid dendritic cells, *Exp Hematol* 34:1655–1662.

Bonavida, B. (2007). Rituximab-induced inhibition of antiapoptotic cell survival pathways: implications in chemo/immunoresistance, rituximab unresponsiveness, prognostic and novel therapeutic interventions, *Oncogene* 26(25): 3629-3636.

Borbone, E., Berlingieri, M.T., De Bellis, F., Nebbioso, A., Chiappetta, G. et al., (2010). Histone deacetylase inhibitors induce thyroid cancer-specific apoptosis through proteasome-dependent inhibition of TRAIL degradation, *Oncogene* 29:105-116.

Bortul, R., Tazzari, P.L., Cappellini, A., Tabellini, G., Billi, A.M., et al. (2003). Constitutively active Akt1 protects HL60 leukemia cells from TRAIL-induced apoptosis through a mechanism involving NF-kappaB activation and cFLIP(L) up-regulation, *Leukemia* 17:379–389.

Braun, F.K., Fecker, L.F., Schwarz, C., Walden, P., Assaf, C., et al. (2007). Blockade of death receptor-mediated pathways early in the signaling cascade coincides with distinct apoptosis resistance in cutaneous T-cell lymphoma cells, *J Invest Dermatol* 127(10):2425-2437.

Buckle, C.H., Neville-Webbe, H.L., Croucher, P.I. & Lawson, M.A. (2010). Targeting RANK/RANKL in the treatment of solid tumours and myeloma, *Curr Pharm Des* 16(11):1272-1283.

Burns, T.F., Bernhard, E.J. & El-Deiry, W.S. (2001). Tissue specific expression of p53 target genes suggests a key role for KILLER/DR5 in p53-dependent apoptosis in vivo, *Oncogene* 20(34):4601-4612.

Caravatta, L., Sancilio, S., di Giacomo, V., Rana, R., Cataldi, A. & Di Pietro, R. (2008). PI3-K/Akt-dependent activation of cAMP-response element-binding (CREB) protein in Jurkat T leukemia cells treated with TRAIL, *J Cell Physiol* 214(1):192-200.

Cardone, M.H., Roy, N., Stennicke, H.R., Salvesen, G.S., Franke, T.F., et al. (1998). Regulation of cell death protease caspase-9 by phosphorylation, *Science* 282(5392):1318-1321.

Chamuleau, M.E., Ossenkoppele, G.J., van Rhenen, A., van Dreunen, L., Jirka, S.M., et al. (2011). High TRAIL-R3 expression on leukemic blasts is associated with poor outcome and induces apoptosis-resistance which can be overcome by targeting TRAIL-R2, *Leuk Res* 35(6):741-749.

Chen, X., Thakkar, H., Tyan, F., Gim, S., Robinson, H., et al. (2001). Constitutively active Akt is an important regulator of TRAIL sensitivity in prostate cancer, *Oncogene* 20:6073–6083.

Chen, X., Kandasamy, K. & Srivastava, R.K. (2003). Differential roles of RelA (p65) and c-Rel subunits of nuclear factor kappa B in tumor necrosis factor-related apoptosis-inducing ligand signalling, *Cancer Res* 63:1059-1066.

Chinnaiyan, A.M., Prasad, U., Shankar, S., Hamstra, D.A., Shanaiah, M., et al. (2000). Combined effect of tumor necrosis factor-related apoptosis-inducing ligand and ionizing radiation in breast cancer therapy, *Proc Natl Acad Sci U S A* 97:1754–1759.

Cillessen, S.A., Meijer, C.J., Ossenkoppele, G.J., Castricum, K.C., Westra, A.H., et al. (2006). Human soluble TRAIL/Apo2L induces apoptosis in a subpopulation of chemotherapy refractory nodal diffuse large B-cell lymphomas, determined by a highly sensitive in vitro apoptosis assay, *Br J Haematol* 134:283–293.

Clancy, L., Mruk, K., Archer, K., Woelfel, M., Mongkolsapaya, J., et al. (2005). Preligand assembly domain-mediated ligand independent association between TRAIL receptor 4 TR4 and TR2 regulates TRAIL-induced apoptosis, *PNAS* 102(50):18099–18104.

Clodi, K., Wimmer, D., Li, Y., Goodwin, R., Jaeger, U., et al. (2000). Expression of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptors and sensitivity to TRAIL-induced apoptosis in primary B-cell acute lymphoblastic leukaemia cells, *Br J Haematol* 111:580-586.

Coll-Mulet, L., Iglesias-Serret, D., Santidrián, A.F., Cosialls, A.M., de Frias, M., et al. (2006). MDM2 antagonists activate p53 and synergize with genotoxic drugs in B-cell chronic lymphocytic leukemia cells, *Blood* 107(10):4109-4114.

Corallini, F., Rimondi, E. & Secchiero, P. (2008). TRAIL and osteoprotegerin: a role in endothelial physiopathology?, *Front Biosci* 1;13:135-147.

Cretney, E., Takeda, K. & Smyth, M.J. (2007). Cancer: novel therapeutic strategies that exploit the TNF-related apoptosis-inducing ligand (TRAIL)/TRAIL receptor pathway, *Int J Biochem Cell Biol* 39(2):280-286.

Di Pietro, R., Secchiero, P., Rana, R., Gibellini, D., Visani, G., et al. (2001). Ionizing radiation sensitizes erythroleukemic cells but not normal erythroblasts to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated cytotoxicity by selective up-regulation of TRAIL-R1, *Blood* 97(9): 2596-2603.

Di Pietro, R. & Zauli, G. (2004). Emerging non-apoptotic functions of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)/Apo2L, *J Cell Physiol* 201: 331-340.

Dicker, F., Kater, A.P., Fukuda, T. & Kipps, T.J. (2005). Fas-ligand (CD178) and TRAIL synergistically induce apoptosis of CD40-activated chronic lymphocytic leukemia B cells, *Blood* 105(8):3193-3198.

Didaa, F., Lia, Y., Iwaoa, A., Deguchia, T., Azumaa, E. & Komada, Y. (2008). Resistance to TRAIL-induced apoptosis caused by constitutional phosphorylation of Akt and PTEN in acute lymphoblastic leukemia cells, *Exp Hematol* 36:1343–1353.

Drakos, E., Thomaides, A., Medeiros, L.J., Li, J., Leventaki, V., et al. (2007). Inhibition of p53-murine double minute 2 interactions by nutlin 3-A stabilizes p53 and induces cell cycle arrest and apoptosis in Hodgkin Lymphoma, *Clin Cancer Res* 13: 3380-3387.

Duiker, E.W., Mom, C.H., de Jong, S., Willemse, P.H., Gietema, J.A., et al. (2006). The clinical trail of TRAIL, *Eur J Cancer* 42(14):2233-2240.

Ehrhardt, H., Fulda, S., Schmid, I., Hiscott, J., Debatin, K-M. & Jeremias, I. (2003). TRAIL induced survival and proliferation in cancer cells resistant towards TRAIL-induced apoptosis mediated by NF-kappaB, *Oncogene* 22:3842-3852.

Emery, J.G., McDonnell, P., Burke, M.B., Deen, K.C., Lyn, S., et al. (1998). Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL, *J Biol Chem* 273:14363-14367.

Falschlehner, C., Emmerich, C.H., Gerlach, B. & Walczak, H. (2007). TRAIL signalling: decisions between life and death, *Int J Biochem Cell Biol* 39:1462–1475.

Falschlehner, C., Schaefer, U. & Walczak, H. (2009). Following TRAIL's path in the immune system, *Immunology* 127(2):145-154.

Fas, S.C., Baumann, S., Zhu, J.Y., Giaisi, M., Treiber, M.K. et al. (2006). Wogonin sensitizes resistant malignant cells to TNFalpha- and TRAIL-induced apoptosis, *Blood* 108:3700-3706.

Finnegan, M.C., Goepel, J.R., Royds, J., Hancock, B.W. & Goyns, M.H. (1994). Elevated levels of MDM-2 and p53 expression are associated with high grade non-Hodgkin's lymphomas, *Cancer Lett* 11: 215-221.

Frese, S., Pirnia, F., Miescher, D., Krajewski, S., Borner, M.M., et al. (2003). PG490-mediated sensitization of lung cancer cells to Apo2L/TRAIL-induced apoptosis requires activation of ERK2, *Oncogene* 22:5427–5435.

Gazitt, Y. (1999). TRAIL is a potent inducer of apoptosis in myeloma cells derived from multiple myeloma patients and is not cytotoxic to hematopoietic stem cells, *Leukemia* 13(11):1817-1824.

Georgakis, G.V., Li, Y., Humphreys, R., Andreeff, M., O'Brien, S., et al. (2005). Activity of selective fully human agonistic antibodies to the TRAIL death receptors TRAIL-R1 and TRAIL-R2 in primary and cultured lymphoma cells: induction of apoptosis and enhancement of doxorubicin- and bortezomib-induced cell death, *Br J Haematol* 130: 501–510.

Goldberg, J.M., Silverman, L.B., Levy, D.E., Dalton VK, Gelber RD, et al. (2003). Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber cancer institute acute lymphoblastic leukemia consortium experience, *J Clin Oncol* 21:3616–3622.

Gong, B. & Almasan, A. (2000). Genomic organization and transcriptional regulation of the human Apo2L/TRAIL gene, *Biochem Biophys Res Commun* 278:747-752.

Green, D. (2000). Apoptotic pathways: paper wraps stone blunts scissors. *Cell* 102:1-4.

Greil, R., Anether, G., Johrer, K. & Tinhofer, I. (2003). Tracking death dealing by Fas and TRAIL in lymphatic neoplastic disorders: pathways, targets, and therapeutic tools, *J Leukoc Biol* 74(3):311-330.

Griffith, T.S. & Lynch, D.H. (1998). TRAIL: a molecule with multiple receptors and control mechanisms, *Curr Opin Immunol* 10(5):559-563.

Griffith, T.S., Rauch, C.T., Smolak, P.J., Waugh, J.Y., Boiani, N., et al. (1999). Functional analysis of TRAIL receptors using monoclonal antibodies, *J Immunol* 162:2605.

Hamilton, A., Gallipoli, P., Nicholson, E. & Holyoake, T.L. (2010). Targeted therapy in haematological malignancies, *J Pathol* 220:404-418.

Henson, E.S., Johnston, J.B. & Gibson, S.B. (2008). The role of TRAIL death receptors in the treatment of hematological malignancies, *Leukemia & Lymphoma* 49(1): 27–35.

Huang, Y. & Sheikh, M.S. (2007). TRAIL death receptors and cancer therapeutics, *Toxicol Appl Pharmacol*; 224(3):284-289.

Hussain, A.R., Ahmed, M., Al-Jomah, N.A., Khan, A.S., Manogaran, P., et al. (2008). Curcumin suppresses constitutive activation of nuclear factor-kappa B and requires functional Bax to induce apoptosis in Burkitt's lymphoma cell lines, *Mol Cancer Ther* 7:3318–3329.

Impicciatore, G., Sancilio, S., Miscia S. & Di Pietro, R. (2010). Nutlins and ionizing radiation in cancer therapy, *Curr Pharm Des* 16(12):1427-1442.

Inukai, T., Zhang, X., Goto, M., Hirose, K., Uno, K., et al.(2006). Resistance of infant leukemia with MLL rearrangement to tumor necrosis factor-related apoptosis-inducing ligand: a possible mechanism for poor sensitivity to antitumor immunity, *Leukemia* 20(12):2119-2129.

Jacquemin, G., Shirley, S. & Micheau, O. (2010). Combining naturally occurring polyphenols with TNF-related apoptosis-inducing ligand: a promising approach to kill resistant cancer cells? *Cell Mol Life Sci* 67:3115–3130.

Jo, M., Kim, T.H., Seol, D.W., Esplen, J.E., Dorko, K., et al. (2000). Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand, *Nat Med* 6:567.

Jones, R.G., Elford, A.R., Parsons, M.J., Wu, L., Krawczyk, C.M., et al. (2002). CD28-dependent activation of protein kinase B/Akt blocks Fas-mediated apoptosis by preventing death-inducing signaling complex assembly, *J Exp Med* 196(3):335-348.

Jones, D.T., Ganeshaguru, K., Mitchell, W.A., Foroni, L., Baker, R.J., et al. (2003). Cytotoxic drugs enhance the ex vivo sensitivity of malignant cells from a subset of acute myeloid leukaemia patients to apoptosis induction by tumour necrosis factor receptor-related apoptosis-inducing ligand, *Br J Haematol* 121(5):713-720.

Jurewicz, A., Matysiak, M., Andrzejak, S. & Selmaj, K. (2006). TRAIL-induced death of human adult oligodendrocytes is mediated by JNK pathway, *Glia* 53:158–166.

Kabore, A.F., Sun, J., Hu, X., McCrea, K., Johnston, J.B. & Gibson, S.B. (2006). The TRAIL apoptotic pathway mediates proteasome inhibitor induced apoptosis in primary chronic lymphocytic leukemia cells, *Apoptosis* 11:1175–1193.

Kamdasamy, K. & Kraft, A.S. (2008). Proteasome inhibitor PS-341 (VELCADE) induces stabilization of the TRAIL receptor DR5 mRNA through the 3′-untranslated region, *Mol Cancer Ther* 7(5):1091-1100.

Kelley, S.K. & Ashkenazi, A. (2004). Targeting death receptors in cancer with Apo2L/TRAIL, *Curr Opin Pharmacol* 4(4):333-339.

Kischkel, F.C., Hellbardt, S., Behrmann, I., Germer, M., Pawlita, M., et al. (1995). Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor, *EMBO J* 14:5579-5588.

Kischkel, F.C., Lawrence, D.A., Tinel, A., Virmani, A., Schow, P., et al. (2001). Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8, *J Biol Chem* 276:46639-46646.

Kojima, K., Konopleva, M., Samudio, I.J., Shikami, M., Cabreira-Hansen, M., et al. (2005). MDM2 antagonists induce p53-dependent apoptosis in AML: implication for leukemia therapy, *Blood* 106: 3150-3159.

Koschny, R., Walczak, H. & Ganten, T.M. (2007). The promise of TRAIL-potential and risks of a novel anticancer therapy, *J Mol Med* 85(9):923-935.

Laguinge, L.M., Samara, R.N., Wang, W., El-Deiry, W.S., Corner, G., et al. (2008). DR5 receptor mediates anoikis in human colorectal carcinoma cell lines, *Cancer Res* 68(3):909-917.

Lawrence, D., Sdhahroth, Z., Marsters, S., Achilles, K., Shih, D., et al. (2001). Differential hepatocyte toxicity of recombinant Apo2L/TRAIL versions, *Nat Med* 7:383-385.

LeBlanc, H.N. & Ashkenazi, A. (2003). Apo2L/TRAIL and its death and decoy receptors, *Cell Death Differ* 10:66-75.

Lee, S.H., Shin, M.S., Kim, H.S., Lee, H.K., Park, W.S., et al. (1999). Alterations of the DR5/TRAIL receptor 2 gene in non-small cell lung cancers, *Cancer Res* 59:5686.

Lee, S.H., Shin, M.S., Kim, H.S., Lee, H.K., Park, W.S., et al. (2001). Somatic mutations of TRAIL-receptor 1 and TRAIL-receptor 2 genes in non-Hodgkin's lymphoma, *Oncogene* 20:399–403.

Lehmann, B.D., McCubrey, J.A., Jefferson, H.S., Paine, M.S., Chappel, W.H. & Terrian, D.M. (2007). A dominant role for p53 dependent cellular senescence in radiosensitization of human prostate cancer cells, *Cell Cycle* 6: 595-596.

Leverkus, M., Neumann, M., Mengling, T., Rauch, C.T., Bröcker, E.B., et al. (2000). Regulation of tumor necrosis factor-related apoptosis-inducing ligand sensitivity in primary and transformed human keratinocytes, *Cancer Res* 60(3):553-559.

Lin, Y., Devin, A., Cook, A., Keane, M.M., Kelliher, M., et al. (2000). The death domain kinase RIP is essential for TRAIL (Apo2L)-induced activation of IkappaB kinase and c-Jun N-terminal kinase, *Mol Cell Biol* 20:6638–6645.

Liu, Q., Hilsenbeck, S. & Gazitt, Y. (2003). Arsenic trioxide-induced apoptosis in myeloma cells: p53-dependent G1 or G2/M cell cycle arrest, activation of caspase-8 or caspase-9, and synergy with APO2/TRAIL, *Blood* 101(10):4078-4087.

Locklin, R.M., Croucher, P.I., Russell, R.G. & Edwards, C.M. (2007). Agonists of TRAIL death receptors induce myeloma cell apoptosis that is not prevented by cells of the bone marrow microenvironment, *Leukemia* 21(4):805-812.

MacFarlane, M., Harper, N., Snowden, R.T., Dyer, M.J., Barnett, G.A., et al. (2002). Mechanisms of resistance to TRAIL-induced apoptosis in primary B cell chronic lymphocytic leukaemia, *Oncogene* 21(44):6809-6818.

MacFarlane, M. (2003). TRAIL-induced signaling and apoptosis, *Toxicol Lett* 139:89-97.

MacFarlane, M., Kohlhaas, S.L., Sutcliffe, M.J., Dyer, M.J. & Cohen, G.M. (2005). TRAIL receptor-selective mutants signal to apoptosis via TRAIL-R1 in primary lymphoid malignancies, *Cancer Res* 65(24):11265-11270.

Maddipatla, S., Hernandez-Ilizaliturri, F.J., Knight, J. & Czuczman MS. (2007). Antibodies Targeting TRAIL-R1and CD20, *Clin Cancer Res* 13(15): 4556-4564.

Mahalingam, D., Szegezdi, E., Keane, M., Jong, S. & Samali, A. (2009). TRAIL receptor signalling and modulation: are we on the right TRAIL? *Cancer Treat Rev* 35:280–288.

Mahmood, Z. & Shukla, Y. (2010). Death receptors: targets for cancer therapy, *Exp Cell Res* 316:887-899.

Mai, A. & Altucci, L. (2009). Epi-drugs to fight cancer: from chemistry to cancer treatment, the road ahead, *Int J Biochem Cell Biol* 41: 199-213.

Mariani, S.M. & Krammer, P.H. (1998). Differential regulation of TRAIL and CD95 ligand in transformed cells of the T and B lymphocyte lineage, *Eur J Immunol* 28(3):973-982.

Marini, P., Denzinger, D., Schiller, S., Kauder, S., Welz, R., et al. (2006). Combined treatment of colorectal tumours with agonistic TRAIL receptor antibodies HGS-ETR1 and HGS-ETR2 and radiotherapy: enhanced effects in vitro and dose-dependent growth delay in vivo, *Oncogene* 25: 5145-5154.

Marsters, S.A., Sheridan, J.P., Pitti, R.M., Huang, A., Skubatch, M., et al. (1997). A novel receptor for Apo2L/TRAIL contains a truncated death domain, *Curr Biol* 7:1003-1006.

Martelli, A.M., Nyakern, M., Tabellini, G., Bortul, R., Tazzari P.L. et al. (2006). Phosphoinositide 3-kinase/Akt signaling pathway and its therapeutical implications for human acute myeloid leukemia, *Leukemia* 20, 911–928.

Mellier, G., Huang, S., Shenoy, K. & Pervaiz, S. (2010). TRAILing death in cancer, *Mol Aspects Med* 31(1):93-112.

Meurette, O., Fontaine, A., Rebillard, A., Le Moigne, G., Lamy, T., et al. (2006). Cytotoxicity of TRAIL/anticancer drug combinations in human normal cells, *Ann N Y Acad Sci* 1090:209-216.

Milani, D., Zauli, G., Rimondi, E., Celeghini, C., Marmiroli, S., et al. (2003). Tumour necrosis factor-related apoptosis-inducing ligand sequentially activates pro-survival and pro-apoptotic pathways in SK-N-MC neuronal cells, *J Neurochem* 86(1):126-135.

Mitsiades, N., Mitsiades, C.S., Poulaki, V., Chauhan, D., Richardson, P.G., et al. (2002). Biologic sequelae of nuclear factor-kappaB blockade in multiple myeloma: therapeutic applications, *Blood* 99(11):4086.

Momand, J., Jung, D., Wilczynski, S. & Niland, J. (1998). The MDM2 gene amplification database, *Nucleic Acid Res* 26: 3453-3459.

Morel, J., Audo, R., Hahne, M. & Combe, B. (2005). Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) induces rheumatoid arthritis synovial fibroblast proliferation through mitogen-activated protein kinases and phosphatidylinositol 3-kinase/Akt, *J Biol Chem* 280:15709–15718.

Motoki, K., Mori, E., Matsumoto, A., Thomas, M., Tomura, T., et al. (2005). Enhanced apoptosis and tumor regression induced by a direct agonist antibody to tumor necrosis factor-related apoptosis-inducing ligand receptor 2, *Clin Cancer Res* 11:3126-3135.

Mühlenbeck, F., Haas, E., Schwenzer, R., Schubert, G., Grell, M., Smith, C., et al. (1998). TRAIL/Apo2L activates c-Jun NH2-terminal kinase (JNK) via caspase-dependent and caspase-independent pathways, *J Biol Chem* 273:33091-33098.

Natoni, A., MacFarlane, M., Inoue, S., Walewska, R., Majid, A., et al. (2007). TRAIL signals to apoptosis in chronic lymphocytic leukaemia cells primarily through TRAIL-R1

whereas cross-linked agonistic TRAIL-R2 antibodies facilitate signalling via TRAIL-R2, *Br J Haematol* 39(4):568-577.

Olsson, A., Diaz, T., Aguilar-Santelises, M., Osterborg, A., Celsing, F., et al. (2001). Sensitization to TRAIL-induced apoptosis and modulation of FLICE-inhibitory protein in B chronic lymphocytic leukemia by actinomycin D, *Leukemia* 15(12):1868-1877.

Ozören, N. & El-Deiry, W.S. (2003). Cell surface death receptor signaling in normal and cancer cells, *Semin Cancer Biol* 13(2):135-147.

Pan, G., O'Rourke, K., Chinnaiyan, A.M., Gentz, R., Ebner, R., et al. (1997a). The receptor for the cytotoxic ligand TRAIL, *Science* 276:111-113.

Pan, G., Ni, J., Wei, Y.F., Yu, G., Gentz, R. & Dixit, V.M. (1997b). An antagonist decoy receptor and a new death domain-containing receptor for TRAIL, *Science* 277:815-818.

Papenfuss, K., Cordier, S.M. & Walczak, H. (2008). Death receptors as targets for anti-cancer therapy, *J Cell Mol Med* 12:2585.

Park, S.M., Schickel, R. & Peter, M.E.(2005). Nonapoptotic functions of FADD-binding death receptors and their signaling molecules, *Curr Opin Cell Biol* 17(6):610-616.

Park, S.J., Kim, M.J., Kim, H.B., Kang, C.D. & Kim, S.H. (2009). Sensitization of imatinib-resistant CML cells to TRAIL-induced apoptosis is mediated through down-regulation of Bcr-Abl as well as c-FLIP, *Biochem J* 28;420(1):73-81.

Paydas, S., Tanriverdi, K., Yavuz, S., Disel, U., Sahin, B. & Burgut, R. (2003). Survivin and aven: two distinct antiapoptotic signals in acute leukemias. *Ann Oncol* 14(7):1045.

Pedersen, I.M., Kitada, S., Schimmer, A., Kim, Y., Zapata, J.M., et al. (2002). The triterpenoid CDDO induces apoptosis in refractory CLL B cells, *Blood* 100(8):2965-2972.

Pitti, R.M., Marsters, S.A., Ruppert, S., Donahue, C.J., Moore, A. & Ashkenazi, A. (1996). Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family, *J Biol Chem* 271(22):12687-12690.

Pukac, L., Kanakaraj, P., Humphreys, R., Alderson, R., Bloom, M., et al. (2005). HGS-ETR1, a fully human TRAIL receptor 1 monoclonal antibody, induces cell death in multiple tumour types in vitro and in vivo, *Br J Cancer* 92: 1430–1441.

Rayet, B. & Gelinas, C. (1999). Aberrant *rel/nfkb* genes and activity in human cancer, *Oncogene* 18:6938-6947.

Reed, J.C., Kitada, S., Kim, Y. & Byrd, J. (2002). Modulating apoptosis pathways in low-grade B-cell malignancies using biological response modifiers, *Semin Oncol* 29(1 Suppl 2):10-24.

Richardson, S.J., Matthews, C., Catherwood, M.A., Alexander, H.D., Carey, B.S., et al. (2006). ZAP-70 expression is associated with enhanced ability to respond to migratory and survival signals in B-cell chronic lymphocytic leukemia (B-CLL), *Blood* 107:3584–3592.

Roos, W.P. & Kaina, B. (2006). DNA damage-induced cell death by apoptosis, *Trends Mol Med* 12:440–450.

Roue, G., Perez-Galan, P., Lopez-Guerra, M., Villamor, N., Campo, E. & Colomer, D. (2007). Selective inhibition of IkappaB kinase sensitizes mantle cell lymphoma B cells to TRAIL by decreasing cellular FLIP level, *J Immunol* 178:1923–1930.

Russo, M., Mupo, A., Spagnuolo, C. & Russo G.L. (2010). Exploring death receptor pathways as selective targets in cancer therapy, *Biochem Pharmacol* 80:674–682.

Russo, M., Nigro, P., Rosiello, R., D'Arienzo, R. & Russo, G.L. (2007). Quercetin enhances CD95- and TRAIL-induced apoptosis in leukemia cell lines, *Leukemia* 21:1130–1133.

Sabatini, N., Di Pietro, R., Rapino, M., Sancilio, S., Comani, S. & Cataldi, A. (2004). PI-3-kinase/NF-κB mediated response of Jurkat T leukemic cells to two different chemotherapeutic drugs, Etoposide and TRAIL, *J Cell Biochem* 93(2):301-311.

Salvesen, G.S. & Abrams, J.M. (2004). Caspase activation - stepping on the gas or releasing the brakes? Lessons from humans and flies, *Oncogene* 23(16):2774-2784.

Sancilio, S., Grill, V. & Di Pietro, R. (2008). A combined approach with rituximab plus anti-TRAIL-R agonistic antibodies for the treatment of haematological malignancies, *Curr Pharm Des* 14(21):2085-2099.

Sayers, T. J. & Murphy, W. J. (2006). Combining proteasome inhibition with TNF-related apoptosis-inducing ligand (Apo2L/TRAIL) for cancer therapy. *Immunol Immunother* 55:76–84.

Schaefer, U., Voloshanenko, O., Willen, D. & Walczak, H. (2007). TRAIL: a multifunctional cytokine, *Front Biosci* 12:3813-3824.

Schneider, P., Bodmer, J.L., Thome, M., Hofmann, K., Holler, N. & Tschopp, J. (1997). Characterization of two receptors for TRAIL, *FEBS Lett* 416:329-334.

Secchiero, P., Gonelli, A., Mirandola, P., Melloni, E., Zamai, L., et al. (2002). Tumor necrosis factor-related apoptosis-inducing ligand induces monocytic maturation of leukemic and normal myeloid precursors through a caspase-dependent pathway, *Blood* 100:2421-2429.

Secchiero, P., Melloni, E., Heikinheimo, M., Mannisto, S., Di Pietro, R., et al. (2004a). TRAIL regulates normal erythroid maturation through an ERK-dependent pathway, *Blood* 103(2):517-522.

Secchiero, P., Vaccarezza, M., Gonelli, A. & Zauli, G. (2004b). TNF-related apoptosis-inducing ligand (TRAIL): a potential candidate for combined treatment of hematological malignancies, *Curr Pharm Des* 10(29):3673-3681.

Secchiero, P., Zerbinati, C., Melloni, E., Milani, D., Campioni, D., et al. (2007). The MDM-2 antagonist nutlin-3 promotes the maturation of acute myeloid leukemic blasts, *Neoplasia* 9(10):853-861.

Secchiero, P. & Zauli, G. (2008). Tumor-necrosis-factor-related apoptosis-inducing ligand and the regulation of hematopoiesis, *Curr Opin Hematol* 15(1):42-48.

Seki, N., Hayakawa, Y., Brooks, A.D., Wine, J., Wiltrout, R.H., et al. (2003). Tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis is an important endogenous mechanism for resistance to liver metastases in murine renal cancer, *Cancer Res* 63(1):207-213.

Shangary, S. & Wang, S. (2009). Small-molecule inhibitors of the MDM2-p53 protein-protein interaction to reactive p53 function: a novel approach for cancer therapy, *Annu Rev Pharmacol Toxicol* 49: 223-241.

Shin, M.S., Kim, H.S., Lee, S.H., Park, W.S., Kim, S.Y., et al. (2001). 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* 61:4942–4946.

Signore, M., Ricci-Vitiani, L & De Maria, R. (2011). Targeting apoptosis pathways in cancer stem cells, *Cancer Lett* [Epub ahead of print], doi:10.1016/j.canlet.2011.01.013

Snell, V., Clodi, K., Zhao, S., Goodwin, R., Thomas, E.K., et al. (1997). Activity of TNF-related apoptosis-inducing ligand (TRAIL) in haematological malignancies, *Br J Haematol* 99:618-624.

Sprick, M.R., Weigand, M.A., Rieser, E., Rausch, C.T., Juo, P., et al. (2000). FADD/MORT1 and caspase-8 are recruited to TRAIL receptors 1 and 2 and are essential for apoptosis mediated by TRAIL receptor 2, *Immunity* 12:599-609.

Sprick, M.R. & Walczak, H. (2004). The interplay between the Bcl-2 family and death receptor-mediated apoptosis, *Biochim Biophys Acta* 1644:125–132.

Stuhmer, T., Chatterjee, M., Hildebrandt, M., Herrmann, P., Gollasch, H., et al. (2005). Nongenotoxic activation of the p53 pathway as a therapeutic strategy for multiple myeloma, *Blood* 106: 3609-3617.

Suh, W.S., Kim, Y.S., Schimmer, A.D., Kitada, S., Minden, M., et al. (2003). Synthetic triterpenoids activate a pathway for apoptosis in AML cells involving downregulation of FLIP and sensitization to TRAIL, *Leukemia* 17(11):2122-2129.

Sung, B., Park, B., Yadav, V.R. & Aggarwal, B.B. (2010). Celastrol, a triterpene, enhances TRAIL-induced apoptosis through the down-regulation of cell survival proteins and up-regulation of death receptors, *J Biol Chem* 285:11498–11507.

Tamm, I., Kornblau, S.M., Segall, H., Krajewski, S., Welsh, K., et al. (2000). Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias, *Clin Cancer Res* 6(5):1796-1803.

Tanaka, H., Ito, T., Kyo, T. & Kimura, A. (2007). Treatment with IFNalpha in vivo up-regulates serum-soluble TNF-related apoptosis inducing ligand (sTRAIL) levels and TRAIL mRNA expressions in neutrophils in chronic myelogenous leukemia patients, *Eur J Haematol* 78(5):389-398.

Tartaglia, L.A. & Goeddel, D.V. (1992). Two TNF receptors. *Immunol Today* 13:151-153.

Testa, U. (2010). TRAIL/TRAIL-R in hematologic malignancies, *J Cell Biochem* 110(1):21-34.

Thomas, L.R., Henson, A., Reed, J.C., Salsbury, F.R. & Thorburn, A. (2004). Direct binding of Fas-associated death domain (FADD) to the tumor necrosis factor-related apoptosis-inducing ligand receptor DR5 is regulated by the death effector domain of FADD, *J Biol Chem* 279(31):32780-32785.

Tolcher, A.W., Mita, M., Meropol, N.J., von Mehren, M. & Patnaik, A. et al. (2007). Phase I pharmacokinetic and biologic correlative study of mapatumumab, a fully human monoclonal antibody with agonist activity to tumor necrosis factor-related apoptosis inducing ligand receptor-1, *J Clin Oncol* 25:1390–1395.

Uno, K., Inukai, T., Kayagaki, N., Goi, K., Sato, H., et al. (2003). TNF-related apoptosis-inducing ligand (TRAIL) frequently induces apoptosis in Philadelphia chromosome-positive leukemia cells, *Blood* 101(9):3658-3667.

Urruticoechea, A., Alemany, R., Balart, J., Villanueva, A., Viñals, F. & Capellá G. (2010). Recent advances in cancer therapy: an overview, *Curr Pharm Des* 16(1):3-10.

Vassilev, L.T., Vu, B.T., Graves, B., Carvajal, D., Podlaski, F., et al. (2004). In vivo activation of the p53 pathway by small-molecule antagonists of MDM2, *Science* 303: 844-848.

Vogler, M., Walczak, H., Stadel, D., Haas, T.L., Genze, F., et al. (2008). Targeting XIAP bypasses Bcl-2-mediated resistance to TRAIL and cooperates with TRAIL to suppress pancreatic cancer growth in vitro and in vivo, *Cancer Res* 68:7956–7965.

Wagner, K.W., Punnoose, E.A., Januario, T., Lawrence, D.A., Pitti, R.M., et al. (2007). Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL, *Nat Med* 13:1070–1077.

Wajant, H., Moosmayer, D., Wüest, T., Bartke, T., Gerlach, E., et al. (2001). Differential activation of TRAIL-R1 and -2 by soluble and membrane TRAIL allows selective surface antigen-directed activation of TRAIL-R2 by a soluble TRAIL derivative, *Oncogene* 20(30):4101-4106.

Walczak, H., Miller, R.E., Ariail, K., Gliniak, B., Griffith, T.S., et al. (1999). Tumoricidal activity of tumor-necrosis factor-related apoptosis-inducing ligand in vivo, *Nat Med* 5:157-163.

Walczak, H. & Krammer, P.H. (2000). The CD95 (APO-1/Fas) and the TRAIL (APO-2L) Apoptosis Systems, *Exp Cell Res* 256:58-66.

Wang, C.Y., Mayo, M.W., Korneluk, R.G., Goeddel, D.V. & Baldwin Jr, A.S. (1998). NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation, *Science* 274:782-784.

Wiley, S.R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C.P., et al. (1995). Identification and characterization of a new member of the TNF family that induces apoptosis, *Immunity* 3(6):673-82.

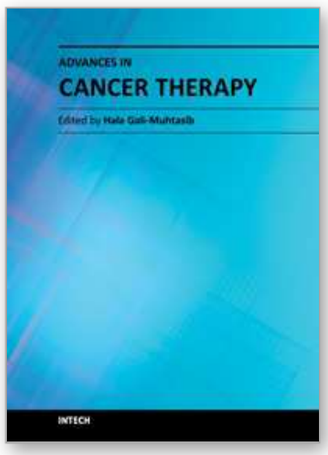
Yang, X. & Thiele, C.J. (2003). Targeting the tumor necrosis factor-related apoptosis-inducing ligand path in neuroblastoma, *Cancer Lett* 197(1-2):137-143.

Zang, D.Y., Goodwin, R.G., Loken, M.R., Bryant, E. & Deeg, H.J. (2001). Expression of tumor necrosis factor-related apoptosis-inducing ligand, Apo2L, and its receptors in myelodysplastic syndrome: effects on in vitro hemopoiesis, *Blood* 98(10):3058-3065.

Zauli, G., Pandolfi, A., Gonelli, A., Di Pietro, R., Guarnieri, S., et al. (2003). Tumor necrosis factor-Related Apoptosis-Inducing Ligand (TRAIL) sequentially upregulates nitric oxide and prostanoid production in primary human endothelial cells, *Circ Res* 92(7):732-740.

Zauli, G., Sancilio, S., Cataldi, A., Sabatini, N., Bosco, D. & Di Pietro, R. (2005). PI-3K/Akt and NF-kappaB/IkappaBalpha pathways are activated in Jurkat T cells in response to TRAIL treatment, *J Cell Physiol* 202: 900-911.

Zerafa, N., Westwood, J.A., Cretney, E., Mitchell, S., Waring, P., et al. (2005). TRAIL deficiency accelerates hematological malignancies, *J Immunol* 175: 5586–5590.



Advances in Cancer Therapy

Edited by Prof. Hala Gali-Muhtasib

ISBN 978-953-307-703-1
Hard cover, 568 pages
Publisher InTech
Published online 21, November, 2011
Published in print edition November, 2011

The book "Advances in Cancer Therapy" is a new addition to the Intech collection of books and aims at providing scientists and clinicians with a comprehensive overview of the state of current knowledge and latest research findings in the area of cancer therapy. For this purpose research articles, clinical investigations and review papers that are thought to improve the readers' understanding of cancer therapy developments and/or to keep them up to date with the most recent advances in this field have been included in this book. With cancer being one of the most serious diseases of our times, I am confident that this book will meet the patients', physicians' and researchers' needs.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Roberta Di Pietro (2011). Signalling Pathways Leading to TRAIL Resistance, Advances in Cancer Therapy, Prof. Hala Gali-Muhtasib (Ed.), ISBN: 978-953-307-703-1, InTech, Available from: http://www.intechopen.com/books/advances-in-cancer-therapy/signalling-pathways-leading-to-trail-resistance

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen