Chapter 7
Trail Receptors: Targets for Cancer Therapy

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Abstract A human tumor cell's ability to avoid the normal regulatory mechanisms of cell growth, division, and death are the hallmarks of transformation and cancer. Numerous novel therapeutic agents currently in preclinical or clinical evaluation aim to revive the normal regulation or evade these regulatory defects and induce growth arrest and cell death. One of the cell death pathways that has garnered significant interest, as a potential target for therapeutic intervention, is the programmed cell death pathway regulated by the tumor necrosis factor-related apoptosis-inducing ligand receptors (TRAIL-RS). Receptor agonist molecules including forms of the native ligand and monoclonal antibodies are being developed and tested as therapeutics in the treatment of human cancer.

Keywords apoptosis, monoclonal antibody, agonist, TRAIL, TRAIL receptor

1 Introduction

This review will focus on the tumor necrosis factor-related apoptosis-inducing ligand receptor (TRAIL-R) signaling pathway and the therapeutic agents currently in development that activate this cell death pathway as a treatment for cancer. The TRAIL receptors are an attractive therapeutic target because of their relatively restricted expression on tumor cells, their capacity, when activated, to induce cell death in a spectrum of human tumor cells and their ability to act in concert with various chemotherapeutic agents to promote tumor cell death. TRAIL agonists, including various forms of the ligand and agonist antibodies, have demonstrated significant antitumor activity in preclinical studies across a spectrum of different

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human tumors. Recently, some of these agents have begun evaluation in the clinical setting. In addition, emerging molecular therapies are being developed to act on specific key regulatory molecules in the TRAIL-R apoptosis pathway to complement the action of TRAIL-R agonists. The combination of receptor agonist activation and attenuation of the anti-apoptotic threshold with targeted molecular therapy holds promise as a rational approach to cancer treatment.

2 Trail Receptor Signaling

The ability to induce programmed cell death is mediated in all eukaryotic cells through distinct signaling pathways that are responsive to both external and internal inputs. A spectrum of sources can induce programmed cell death, including secreted and membrane-bound proteins, DNA damage, radiation, Ca^{+2} stress, viral and oncogenic transformation, and serum and growth factor starvation.

The active induction of cell death is effected through a family of related cell surface proteins and their cognate ligands. One of these death-inducing ligands, TRAIL, can instigate cell death through a cell surface receptor-mediated catalytic activation of a series of cysteine proteases, leading to cleavage of key cellular structural and signaling components. Binding to either of the competent TRAIL-R's by ligand or antibody agonists can activate this protease cascade through two distinct but intersecting pathways; an extrinsic receptor-mediated pathway and an intrinsic pathway associated with the mitochondria. TRAIL binds to five cognate cell surface receptors, but only two of these receptors TRAIL-R1 and TRAIL-R2, are death receptors that have the ability to transmit a complete death signal. TRAIL-R1 (DR4, TNFSFR10a) and TRAIL-R2 (DR5, TNFSF10b) are members of the TNF receptor superfamily (TNFRSF). Only these two receptors possess the capability to competently transmit a TRAIL death signal. The other members of this family capable of binding to the ligand, TRAIL; DcR1, DcR2, and osteoprotegerin, lack a required cytoplasmic signaling domain, known as a death domain (DD) (Table 7.1).

TRAIL-Rs exist as a functional homotrimeric subunit. Members of the TNFSFR can form and function as heterotrimers. The TRAIL-Rs have been identified in a heteromeric structure in cells transfected with TRAIL-R1 and TRAIL-R2 expression constructs. It is unclear whether this is a physiologically relevant formation as this heterotrimer has not been isolated in immunoprecipitation experiments from nontransfected cells. (Kischkel et al., 2000; Schneider et al., 1997). Although initial reports suggested that ligand is required for receptor trimerization, studies of

Table 7.1 TRAIL receptors

Receptor	TNFSF	Other names	Death domain
TRAIL-R1	10A	DR4, Apo2	Complete
TRAIL-R2	10B	DR5, TRICK, KILLER	Complete
TRAIL-R3	10C	Decoy receptor 1	None
TRAIL-R4	10D	Decoy receptor 1	Truncated
Osteoprotegerin	11B	OPG, OCIF, TR1	None

TNFR1 and Fas have demonstrated the presence of a preligand association domain (PLAD) that is required for ligand-independent trimerization. Interestingly, the PLAD domains interactions are very specific and only permit homotrimeric formations (Chan et al., 2000). This data suggests that TRAIL-R1 and TRAIL-R2 only form homotrimers. However, a recent report suggested that the TRAIL-binding decoy receptor, DcR1, may regulate TRAIL-R2 activity by forming a heterocomplex through the PLAD (Clancy et al., 2005). A common structural feature present in all TNFSF receptors is a series of extracellular cysteine-rich domains (CRD). The number of these domains can vary between different TNFSFRs from 1 to 6. Each CRD domain is defined by six highly conserved cysteines that form three intrachain disulfide bridges. TRAIL-R1 and TRAIL-R2 possess three such CRD repeats that contain seven intrachain disulfide bridges (Hymowitz et al., 1999; Locksley et al., 2001; Marsters et al., 1992; Mongkolsapaya et al., 1999). The TRAIL- Rs also possess a structural feature that is unique to death-inducing receptors in the TNFSFR. Each of the receptors in this class possesses a short (65–80 aa) cytoplasmic protein–protein domain that is required for interaction with a key adaptor protein that is required for transmission of the death signal. Consequently, this structure is known as the DD. Seven members of the TNFSFR, including TRAIL-R1 and TRAIL-R2, possess DD. (Igney and Krammer, 2002)

3 Trail Receptor Expression

Two of the most intriguing and attractive features of the TRAIL-Rs are that TRAIL-R1 and TRAIL-R2 are proapoptotic and that these two receptors are expressed on many types of tumor cells. These features make the proapoptotic TRAIL-Rs an extremely appealing target for the generation of therapeutic agents.

Surface expression of TRAIL receptors has been reported for both normal (Atkins et al., 2002; Dorr et al., 2002; Jo et al., 2000; Leverkus et al., 2000b; Mundt et al., 2003) and tumor cells (Arts et al., 2004; Ashkenazi et al., 1999; Bouralexis et al., 2004; Clodi et al., 2000; Cuello et al., 2001; Frank et al., 1999; Frese et al., 2002; Ibrahim et al., 2001; Mitsiades et al., 2000; Odoux et al., 2002; Shin et al., 2001; Song et al., 2003a; van Geelen et al., 2003; Vignati et al., 2002). Weak but detectable TRAIL-R1 and TRAIL-R2 expression has been identified by flow cytometry on the surface of a limited number of normal (diploid) cell types, including hepatocytes, keratinocytes, astrocytes, and osteoblasts (Atkins et al., 2002; Dorr et al., 2002; Jo et al., 2000; Leverkus et al., 2000a; Mundt et al., 2003). However, a broad spectrum of tumor cell types has been identified with variable levels of TRAIL-R1 and /or TRAIL-R2 including some examples of relatively high expression. Cells isolated from primary tumors of the lung (Odoux et al., 2002), blood (Cappellini et al., 2005; Clodi et al., 2000), skin (Song et al., 2003a), bone (Bouralexis et al., 2004), and the brain (Ciusani et al., 2005) have detectable cell surface expression of TRAIL-R1 and TRAIL-R2 by flow cytometry. Likewise, human tumor cell lines derived from carcinomas of the colon (van Geelen et al.,

2003), breast (Ashkenazi et al., 1999), ovary (Cuello et al., 2001; Vignati et al., 2002), thyroid (Mitsiades et al., 2000), lung (Frese et al., 2002), pancreas (Ibrahim et al., 2001), and liver (Griffith et al., 1998), as well as from melanomas (Song et al., 2003a), sarcomas (Bouralexis et al., 2003), and tumors of the brain (Song et al., 2003b), have variable and high-level FACS-detectable TRAIL-R1 and TRAIL-R2. In many tumor cell lines where resistance to TRAIL-R agonism was observed, the relevance of cell surface expression of the TRAIL-Rs was complicated by the fact that receptor levels did not have a role in regulating response. A clear relationship between receptor expression level and potential for activation of apoptosis through proapoptotic TRAIL-Rs has not been established. However, evaluation of receptor expression in a tissue context is desirable in understanding more about the TRAIL-Rs as targets of systemic therapies.

Antibody reagents specific for linear peptides of the C-terminal, intracellular portion of TRAIL-R1 and TRAIL-R2 have been utilized in studies of TRAIL-R distribution in tissues (Arts et al., 2004; Koornstra et al., 2003; Reesink-Peters et al., 2005; Spierings et al., 2003; Spierings et al., 2004). Arts et al. demonstrated that most ovarian tumors expressed one or both proapoptotic TRAIL receptors, and that TRAIL-R2 expression was increased after chemotherapy in paired samples collected pre-therapy and post-therapy. Likewise, Koornstra et al. highlighted that expression of these death receptors was increased in colon tumors vs normal colon, and that both TRAIL-R1 and TRAIL-R2 were detected on all adenomas and carcinomas evaluated. In parallel, Spierings et al. (2003) evaluated a large panel of stage III non-small-cell lung (NSCL) tumors ($n = 87$) and related the staining to available clinical outcome data. In this study, TRAIL-R1 was identified on essentially all specimens (99%), with staining often strongest at the basal cell layers in tumors with squamous differentiation. TRAIL-R2 was also identified on the majority of the specimens (82%); interestingly, TRAIL-R2 expression was correlated with increased risk of death (odds ratio 5.76). A second study by Spierings et al. (2004) evaluated distribution of TRAIL and TRAIL-RS on normal tissues from humans and chimpanzees. In this study, as with the tumor panels, there was fairly widespread labeling of tissues evaluated for both TRAIL-R1 and TRAIL-R2, but staining patterns were similar across the two species. Finally, a recent study by Reesink-Peters et al. evaluated the distribution of TRAIL-Rs and markers of proliferation and apoptosis in cervical neoplasia. TRAIL-R1 and TRAIL-R2 were each identified in >80% of the specimens evaluated, with slightly more staining for TRAIL-R2; however, there was no correlation of TRAIL-Rs to either proliferation or ongoing apoptosis in these specimens.

Interestingly, in several of the studies listed above, staining was often restricted to the cytoplasmic compartment; therefore, it is unclear whether this distribution is relevant to the potential activity of therapeutics that target the extracellular portion of the receptor. It should also be noted that the peptides used for immunization to produce these polyclonal antibodies include considerable homology between the published TRAIL-R1 and TRAIL-R2 sequences. Although these antibody reagents perform well for specific recognition of the linear peptide in a western blot, it may be difficult to demonstrate highly specific staining in an immunohistochemical

assay format where the receptor protein has not been denatured and stabilized as a linear peptide target.

Others have reported tissue distribution of the proapoptotic TRAIL-Rs using monoclonal antibody reagents raised against the extracellular domains of these TRAIL-Rs, including use on formalin-fixed tissues (Daniels et al., 2005), frozen sections (Strater et al., 2002a), or a fluorescence-based method of quantitative tissue staining (McCarthy et al., 2005). Daniels et al. reported widespread staining of TRAIL-R1 and TRAIL-R2 in both tumor and normal tissues, with tumors staining more intensely than the adjacent normal tissue, but noted that the staining was often patchy in breast carcinomas, and that there was much less staining than expected on lymphoid tumors. Strater reported widespread TRAIL-R1 and TRAIL-R2 staining in tumors of the colon, but reported also that there was a positive correlation between TRAIL-R1 expression and survival. In contrast, McCarthy et al. identified a strong negative correlation between TRAIL-R2 expression and survival in breast cancer, with TRAIL-R2 expression associated with increased node-positive tumors. The TRAIL-R2 specific monoclonal antibody described in these studies can also be used for flow cytometry applications for determination of surface receptor levels, but is not currently recommended by the manufacturer for immunohistochemical studies in tissue specimens.

In evaluating the distribution of potential targets for agonist TRAIL receptor antibodies, it was considered critical to focus efforts specifically on detection of the extracellular portion of these receptors in order to understand the distribution of the part of the receptor recognized by TRAIL-R agonists. Antibodies have been developed to TRAIL-R1 and TRAIL-R2, respectively and the specificity of these antibodies has been tested by western blotting, flow cytometry, and immunohistochemistry utilizing fixed and embedded cell pellets and xenografts. These antibodies have been utilized for development of sensitive and specific immunohistochemical tests for TRAIL-R1 and TRAIL-R2 in formalin-fixed tissue specimens as described (Roach et al., 2004). To evaluate TRAIL-R distribution using these tests, approximately 270 tumor and normal tissue specimens have been evaluated. A summary of the expanded results is presented in Figs. 7.1–7.3.

After screening several proprietary and commercially available antibodies selected for specificity to TRAIL-R1 or TRAIL-R2, we concluded that, for both TRAIL-R1 and TRAIL-R2, rabbit polyclonal antibodies represented the best option for developing these tests (Roach et al., 2004). These antibodies performed well on formalin-fixed paraffin-embedded tissues and had minimal background staining. Importantly, staining of sectioned cell pellets or xenografts of cell lines was consistent with receptor expression levels identified by other methods such as flow cytometry, using different TRAIL-R antibodies, and TaqMan to quantitate RNA levels ((Roach et al., 2004) and hierarchical genetic search [HGS] data not shown).

The mean staining scores were determined for the 10 tumor types for which there were at least 10 evaluable specimens, and are presented in Figs. 7.1 and 7.2. Like the other studies reported, we identified stronger staining for both TRAIL-R1 and TRAIL-R2 in tumor specimens overall than in normal tissues. In addition, for most tumor types, although granular to diffuse cytoplasmic staining was noted,

Fig. 7.1 General tumor survey for TRAIL-R1: Tumor types with at least 10 samples. Immunohistochemical score for TRAIL-R1 staining, using a subjective scale of 0–4+, is indicated for 10 general tumor types for which at least 10 specimens were evaluated. Circles indicate individual specimen scores, and the mean score is indicated by a bar for each tumor type. Abbreviations in tumor type: carcinoma (Ca), non-small-cell (NSC)

distinct membrane staining was also observed and was often the stronger pattern. We identified general patterns of relatively high staining in colorectal carcinomas and relatively little staining in most lymphomas, as has been reported by others (Daniels et al., 2005; Koornstra et al., 2003). However, we identified less staining overall, in both tumor and normal tissues, than in most other reports in the literature. Instead of >80% staining for each receptor in tumor tissues, an average of 43% of tumors scored at least a 2+ for TRAIL-R1 ($N = 235$), and an average of 64% of tumors scored at least a 2+ for TRAIL-R2 ($N = 227$). These scores were based upon both distribution and intensity of receptor staining (Fig. 7.3). It was noted that there was often heterogeneous staining of tumor cells within individual specimens, as well as occasionally between specimens from a single individual. There was more staining identified for TRAIL-R2 than for TRAIL-R1 in both normal and tumor tissues using this method. This was in agreement with other nonclinical data suggesting more widespread distribution of TRAIL-R2 than TRAIL-R1 on several human tumor cell lines.

Importantly, the potential clinical relevance of TRAIL receptor expression has yet to be determined. Agonist monoclonal antibodies specific for TRAIL receptors can cause decreases in cell line viability even in cell lines with very low surface TRAIL receptor as determined by flow cytometry (HGS data not shown). It is possible

Fig. 7.2 General tumor survey for TRAIL-R2: Tumor types with at least 10 samples. Immunohistochemical score for TRAIL-R2 staining, using a subjective scale of 0–4+, is indicated for 10 general tumor types for which at least 10 specimens were evaluated. Triangles indicate individual specimen scores, and the mean score is indicated by a bar for each tumor type. Abbreviations in tumor type: carcinoma (Ca), non-small-cell (NSC)

that immunostaining methodology on formalin-fixed tissues will not be sensitive enough to detect functionally meaningful levels of the extracellular portion of the receptor, which would be targeted by TRAIL-R agonists. However, this possibility cannot be fully evaluated in the absence of clinical outcome data. Therefore, the immunohistochemical staining of tumor tissue specimens from subjects enrolled in the ongoing clinical trial programs represents a critical next step in the development of these tests. In addition, further studies of the downstream signaling pathways will likely contribute to our understanding of the appropriate application of TRAIL-R agonist therapies.

4 The Extrinsic Pathway and the Death-Inducing Signaling Complex

There are two essential pathways for TRAIL-mediated cell death (see also Chapter 3): one “extrinsic,” which is dependent on the signal from the ligand and activation of a TRAIL-R protein complex, which leads directly to cell death; the other,

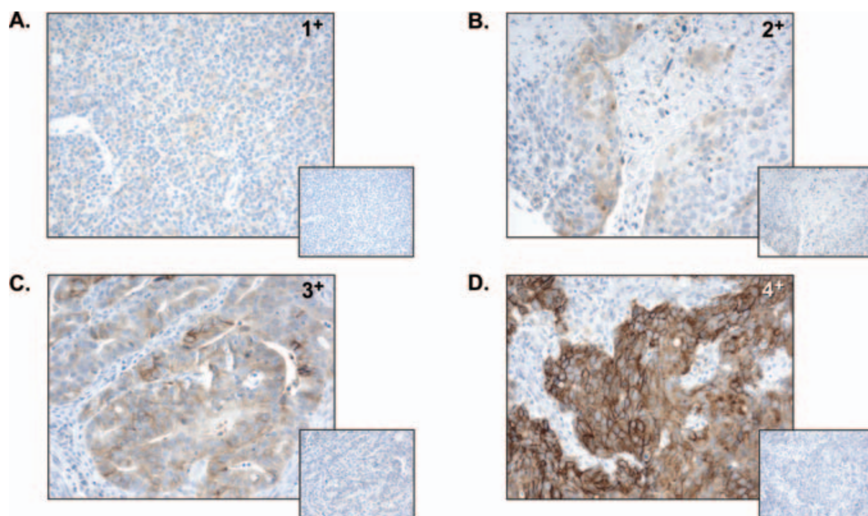

Fig. 7.3 Examples of IHC scoring scale for TRAIL-receptors. Panels A–D indicate examples of the 0–4+ scale used to evaluate TRAIL-R1 and TRAIL-R2 staining, and illustrate some of the typical patterns observed. Panel A, illustrating 1+ staining, has weak, but widespread, staining (non-Hodgkin's lymphoma); panel B, illustrating 2+ staining, has focally stronger staining of the tumor population (cervical carcinoma); panel C, illustrating 3+ staining, has widespread staining with variability in intensity (gastric carcinoma); and panel D, illustrating 4+ staining, considered exceptional, has uniformly strong staining of the tumor cell population, highlighting membrane areas and excluding nuclei (colon carcinoma). The smaller image includes the same field stained with a non-specific IgG as a control (scored 0). All photomicrographs were taken using a 20× objective

"intrinsic," which is activated by TRAIL binding to the TRAIL-Rs, but initiation of cell death is mediated through the mitochondria.

The extrinsic pathway of TRAIL-R cell death mediated through the formation of a ligand–receptor complex. Each DD on a TRAIL-R molecule interacts with a similar DD on a cytoplasmic adaptor protein, Fas-associating protein with a death domain (FADD). FADD acts as a bridge between the ligand–receptor complex and the receptor proximal caspase-8, through the death effector domain (DED). Transfection of dominant negative forms of FADD, or wild-type TRAIL-R1 or TRAIL-R2 into cells lacking FADD, blocks apoptosis demonstrating that FADD is a critical component of TRAIL-R signaling. Recently, it has been shown that the C-terminal tails of TRAIL-R1 and TRAIL-R2 are required for efficient FADD binding, caspase cleavage, and TRAIL-dependent apoptosis. (Ashkenazi, 2002; Bodmer et al., 2000a; Kuang et al., 2000; Luschen et al., 2000; Muhlenbeck et al., 1998; Thomas et al., 2004a; Yeh et al., 1998). The multiprotein complex of ligand, death receptor, adaptor, and protease is known as the death-inducing signaling complex (DISC). This signaling structure is unique amongst cell surface receptor signaling pathways for its threefold symmetry. The formation of this complex is a critical regulatory event in the process of apoptosis. Inactive caspase-8 molecules

are recruited into the DISC by FADD and are cleaved into active proteases through an unknown mechanism. It has been suggested in the “induced proximity” model that inactive initiator caspases brought into close proximity during DISC formation, promotes mutual cleavage and activation. (Boatright et al., 2003; Boatright and Salvesen, 2003; Muzio et al., 1998; Salvesen and Dixit, 1999). Autocleavage and activation of the receptor-associated caspase-8 leads to its release from the DISC and formation of heterodimeric active subunits. The initiator caspase is now able to target the “effector caspases” 3, 6, and 7. These terminal caspases, once activated, cleave key structural and signaling components of the cell and begin the physical destruction of apoptosis. This relatively short signaling cascade emphasizes the potential for rapid induction of cell death. Various apoptosis assays have demonstrated cellular and molecular changes associated with apoptosis appearing within 30 min after TRAIL-R engagement (Houghton, 1999; Walczak and Sprick, 2001). Importantly, the TRAIL-R pathway can activate cell death independently of p53, a primary target for apoptosis regulation by tumor cells (Galligan et al., 2005; Igney and Krammer, 2002; Wang and El-Deiry, 2003). Interestingly, p53 regulates expression of TRAIL-R2, suggesting p53 can increase sensitivity to TRAIL-R agonists in response to other apoptotic stimuli (Sheikh and Fornace, 2000).

5 The Intrinsic Pathway

The bridge from the DISC to the intrinsic pathway is formed through an intervening catalytic event. One of the cytoplasmic targets for the TRAIL-R-activated initiator caspases is the cytoplasmic protein Bid. Bid is a member of the Bcl-2 family of proteins responsible for regulating the mitochondrial pathway of apoptosis. In addition, the intrinsic pathway is also activated through several molecular monitors of cellular health such as p53 and AKT. In response to apoptotic stimuli from various metabolic and structural insults, including DNA damage, serum starvation and radiation, there is a loss of mitochondrial membrane integrity that precipitates the activation of another initiator caspase, caspase-9, and subsequently the effector caspases. Here, at the mitochondria the two pathways of apoptosis intersect emphasizing the importance of the regulation of this intersection.

The cleavage of Bid by caspase-8 creates a truncated form of Bid (tBid) that can translocate to the mitochondrial membrane (Srivastava, 2001). Bid is thought to form a heteromeric complex with other apoptosis-promoting molecules, Bax and Bak (Luo et al., 1998; Wei et al., 2000). Bax is liberated from its complex with the antiapoptotic protein Bcl-2 in response to apoptotic stimuli. This translocation of Bax or Bid to the mitochondrial membrane disrupts membrane integrity and induces release of cytochrome *c* and the formation of a protein complex known as the apoptosome (Adams and Cory, 2002). The apoptosome is comprised of cytochrome c, pro-caspase-9 and a scaffolding protein, apoptotic protease activating factor (APAF1). APAF1 forms a heptamer after binding cytochrome *c* and recruits several molecules of pro-caspase-9 through reciprocal caspase recruitment domains

(CARD) present in APAF1 and caspase-9 (Pan et al., 1998). This recruitment and oligomerization leads to caspase-9 activation and suggests again a role for the induced proximity model of caspase activation. Deletion of APAF1 demonstrates its necessary role in caspase-9 activation (Yoshida et al., 1998). Active caspase-9 can now cleave and activate the executioner caspase-3, caspase-6, and caspase-7.

The intrinsic pathway can be activated independent of the TRAIL-R pathway through other signals such as those transmitted by p53. One of the dominant mechanisms of chemotherapeutic resistance in cancer cells is the gene deletion or acquisition of inactivating mutations in TP53. Conversely, the ability of the TRAIL-R pathway to bypass the loss or inactivation of p53, via Bid cleavage, and still induce apoptosis through the mitochondria is one of the distinct advantages of targeting the TRAIL-Rs. Therefore, there are two pathways, extrinsic and intrinsic, for activation and execution of the TRAIL-R-mediated signals that lead to cell death.

6 Regulation of Death Signaling

Not surprisingly, given the activation of the death signal and its resulting dire consequences for the cell, the apoptotic pathway is highly regulated at several key points. Importantly, tumor cells have exploited these normal regulatory check points through acquired or induced modifications to attenuate the activity of caspases, alter the formation or composition of the DISC, or alter the interaction of intrinsic apoptosis regulatory proteins Bcl-2 and Bax, or their family members (Igney and Krammer, 2002).

FLICE-like inhibitory protein (FLIP) is a dominant negative form of caspase-8 that competes with caspase-8 for binding in the DISC. FLIP plays an important role in regulating sensitivity to TRAIL signaling (Griffith et al., 1998). Chemotherapy or FLIP siRNA can modify FLIP levels in tumors and promote TRAIL-induced apoptosis (Chawla-Sarkar et al., 2004; Galligan et al., 2005; Kang et al., 2005; Song et al., 2003a; Xiao et al., 2005). Interestingly, in support of the data that FLIP receptor complexes exist prior to ligand binding, a peptide sequence at the COOH terminus of FLIP (L) and TRAIL-R2 interact preventing FADD binding to TRAIL-R2. Upon ligand binding, FLIP is dislodged and a competent DISC is formed (Jin et al., 2004). The intimate interaction of FLIP with the receptor makes it an attractive target for pharmacologic intervention (Roth and Reed, 2004).

All of the apoptotic caspases described are regulated not only by a requirement for death receptor- or mitochondrial-mediated cleavage, but also by endogenous inhibitory proteins as well. These caspase-inhibitory proteins contain a protein interaction domain that classifies them as inhibitor of apoptosis proteins (IAPs). Their baculovirus IAP repeat (BIR) domains are zinc-binding folds that play a role in forming binding grooves for the active caspase. Once bound within the groove, caspase-9 cannot self-activate. Several members of this family are overexpressed in tumors (Igney and Krammer, 2002). Interestingly, the protein SMAC/DIABLO

Table 7.2 Tumor modifications of the extrinsic and intrinsic pathways

Location	Target	Modification and consequence	References
Upstream of mitochondria	AKT	AKT constitutive activity promotes Bad phosphorylation	Bortul et al. (2003); Cenni et al. (2004); Chen et al. (2001); Whang et al. (2004)
Upstream of mitochondria	PTEN	Loss of PTEN yields an inability to dephosphorylate AKT	Deocampo et al. (2003); Nesterov et al. (2001)
Upstream of mitochondria	Bcl-2	Overexpression, blocks apoptosis	Nencioni et al. (2005)
Upstream of mitochondria	Bcl-XL	Overexpression, blocks apoptosis	Dole et al. (1995); Foreman et al. (1996); Nagane et al. (1998)
Upstream of mitochondria	Mcl-1	Overexpressed in AML, blocks apoptosis	Kaufmann et al. (1998); Taniai et al. (2004); Yu et al. (2005)
Downstream of mitochondria	Survivin	Overexpressed in neuroblastoma blocks apoptosis	Adida et al. (2000); Kim et al. (2005); Wang et al. (2005); Yamaguchi et al. (2005b)
Downstream of mitochondria	cIAP2	Gene rearranged in MALT	Dierlamm et al. (1999)
Downstream of mitochondria	ML-IAP	Overexpressed in melanoma	Vucic et al. (2000)
Downstream of mitochondria	APAF1	Loss of APAF1 Blocks Caspase 9	Soengas et al. (2001); Soengas et al. (2006)
Downstream of mitochondria	XAF	Binds to XIAP	Leaman et al. (2002)
Receptor complex	Caspase-8	Methylation of gene represses expression, blocks cytoplasmic apoptosis signal	Ashley et al. (2005); Poulaki et al. (2005); van Noesel et al. (2003); Zuzak et al. (2002)
Receptor complex	TRAIL-R1, TRAIL-R2	Point mutations and genetic deletion	Fisher et al. (2001); Kuraoka et al. (2005); McDonald et al. (2001); Ozoren et al. (2000); Pai et al. (1998); Wolf et al. (2006)
Receptor complex	Decoy receptors, DcR1, DcR2	Occasional elevated expression	Meng et al. (2000)
Upstream of mitochondria	Bax	Inactivating mutation prevents apoptosis	Rampino et al. (1997); Zhang et al. (2000); Ionov et al. (2000)
Upstream of mitochondria	c-Myc	Represses FLIP expression	Ricci et al. (2004)
Downstream of mitochondria	SMAC/ Diablo	Reduced release of SMAC/Diablo	Zhang and Fang (2005)

is released from the mitochondria and antagonizes the binding of caspase-9 to the IAP family member, X-linked IAP (XIAP), thereby promoting apoptosis (Ng and Bonavida, 2002). A summary of the known modifications of proteins involved in regulating apoptosis found in tumor cells is described in Table 7.2.

7 Agonists of the Trail-R Apoptotic Pathway

7.1 Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand

Members of the tumor necrosis factor (TNF) superfamily have demonstrated the ability to induce apoptosis in virally and oncogenically transformed cells, human tumor cell lines and activated lymphocytes, NK, and monocytes (TRAIL, TNFα, TNFβ, and FASL). The ability to induce cell death, 14 other TNF ligands possess a diverse array of immunomodulatory and growth-stimulatory capabilities, including stimulation and proliferation of B-cells (BLyS) and T-cells (CD40L, LIGHT, OX40L, and 4–1-BBL) and regulation of bone metabolism (RANKL) (reviewed in Locksley et al. (2001) and Ashkenazi (2002)). TRAIL is a type II membrane-bound protein which exists as a self-assembling homotrimeric molecule that possesses apoptotic activity in a membrane bound or soluble form. The membrane form can be cleaved from the cell surface by an extracellular cysteine protease (Lawrence et al., 2001; Mariani and Krammer, 1998). TRAIL exists as a trimer in solution and requires elemental Zn+2 and a cysteine residue to coordinate and properly organize the trimeric structure. (Hymowitz et al., 2000) TRAIL that is generated in the absence of zinc permits the formation of cysteine disulfide bonds that result in an asymmetric molecule, which is less stable and insoluble in solution (Ashkenazi, 2002; Lawrence et al., 2001). Crystal studies of the ligand bound to TRAIL-R2 have revealed that the inverted pyramid-shaped trimeric ligand binds in the pocket between three receptor molecules (Hymowitz et al., 2000; Mongkolsapaya et al., 1999).

Cell surface expression of the ligand TRAIL has been observed on a variety of immune cells including IL-15- or IL-2-activated NK cells, virally infected T-cells, interferon gamma-activated monocytes, and dendritic cells, as well as CD4+ and CD3+ T-cells. TRAIL can confer tumoricidal activity to monocytes and NK cells and plays a role in immune surveillance against tumor development (Kayagaki et al., 1999a, b; Mariani and Krammer, 1998; Nieda et al., 2001; Takeda et al., 2002). Recently, a "window of TRAIL sensitivity" was observed in CD34 erythroid progenitor cells that is promoted initially by the expression of TRAIL-Rs and then inhibited by intercellular expression of Bcl-2 (Mirandola et al., 2006a). TRAIL has also been detected on the surface of colonic epithelium (Strater et al., 2002b). The soluble and membrane-bound form of TRAIL-induced apoptosis in a wide variety of human tumor cells both in vitro and in vivo without affecting the viability of normal cells.

Several forms of recombinant TRAIL have been generated to evaluate the ligand in preclinical studies. Histidine-tagged (Pitti et al., 1996), leucine zipper (Walczak et al., 1999), Flag-tagged (Bodmer et al., 2000b; Schneider and Tschopp, 2000), and Zn+2-stabilized versions (Ashkenazi and Dixit, 1999; Kelley et al., 2001) have all been generated and tested for activity against tumor and normal cells in preclinical studies. These different forms of the ligand have

displayed a spectrum of antitumor activity in human cell lines in vitro, in xenograft models and primary tissues transplanted into nude mice. TRAIL, either alone or in combination with chemotherapeutic agents, has demonstrated apoptosis activity in tumor cell lines derived from a broad array of human tumors including colon, brain, uterus, ovary, liver, breast, prostate, kidney, liver, lung, thyroid, and blood (Asakuma et al., 2003; Ashkenazi et al., 1999; Bouralexis et al., 2003, 2004; Chen et al., 2003; El-Zawahry et al., 2005; Jazirehi et al., 2001; Jeon et al., 2003; Keane et al., 1999; Kelly et al., 2002; LeBlanc and Ashkenazi, 2003; Miao et al., 2003; Mitsiades et al., 2001a; Muhlethaler-Mottet et al., 2004; Nagane et al., 2001; Naka et al., 2002; Ohtsuka et al., 2003; Pitti et al., 1996; Secchiero et al., 2002; Singh et al., 2003; Srivastava, 2001). TRAIL can overcome chemoresistance or radioresistance when administered in combination with chemotherapy in adriamycin-resistant myeloma, radio-resistant lymphoma, and taxane- and platinum-insensitive breast and osteosarcoma cell lines (Belka et al., 2001; Clayer et al., 2001; Cuello et al., 2001; Evdokiou et al., 2002; Frese et al., 2002; Jazirehi et al., 2001; Johnston et al., 2003; Keane et al., 1999; Liu et al., 2001; Mitsiades et al., 2001b; Nagane et al., 2000, 2001; Voelkel-Johnson, 2003).

While the epitope-tagged forms of the ligand assisted the isolation and purification of the recombinant protein, and in many instances enhanced the activity of TRAIL, they also enhanced the toxicity on normal cells. HIS-tagged, leucine-zipper or Flag-tagged antibody cross-linked forms of TRAIL-induced apoptosis in normal hepatocytes in vitro(Jo et al., 2000; Lawrence et al., 2001). Conflicting results were obtained when no apoptosis was observed with soluble TRAIL administered to normal primary cells from the lung, bone, liver, endothelium, breast, brain, and kidney (Ashkenazi et al., 1999). Safety studies of Zn2+-stabilized TRAIL administered in short-term treatment of mouse, monkey, and chimpanzees showed no detectable toxicities (Lawrence et al., 2001). Additional studies with soluble TRAIL were performed in chimeric mice whose livers were reconstituted with human hepatocytes. Repeated injection of soluble nontagged form of TRAIL did not generate any hepatotoxicity (Hao et al., 2004). These conflicting results suggested that nonphysiologically or inappropriately aggregated forms of TRAIL can be toxic. Whereas a soluble, correctly organized Zn2+-stabilized TRAIL was not toxic. It is important to note that there is a role for native TRAIL in response to inflammation or infection. Acute bacterial or viral infection of the liver or pancreas or in mouse models of hepatitis or pancreatitis TRAIL can induce apoptosis (Mundt et al., 2003; Hasel et al., 2003). Membrane-bound TRAIL has been shown to induce liver damage in adenoviral-transfected hepatocytes in vivo (Ichikawa et al., 2001). These types of responses coincide with the predicted role for TRAIL in mediating an immune surveillance response to acute bacterial- or viral-induced infection or inflammation.

The substantial preclinical antitumor data observed with the ligand implied that TRAIL-R agonism could potentially yield significant clinical antitumor activity. In fact, a recombinant form of the TRAIL ligand is currently is phase 1 clinical development. Nonetheless, this optimism should be tempered with the knowledge that certain versions of the TRAIL ligand, albeit in nonphysiological forms, did induce

severe cytotoxicity of normal cells. Therefore, clinical development should be prudently conducted with awareness toward potential indicators of toxicity.

7.2 Antibodies

A spectrum of mouse and human monoclonal and polyclonal antibodies has demonstrated the ability to agonize the TRAIL-Rs and induce death in tumor cells. They have proven to be valuable tools to explore mechanism of action, define chemotherapeutic combinations agents that enhance apoptosis, and describe functional differences between the TRAIL-R1 and TRAIL-R2 pathways. Importantly, human monoclonal antibodies selected for high-affinity binding and maximal agonism have been advanced into clinical development as therapeutic cancer agents.

Experiments using antibodies, which target the TRAIL-Rs, revealed that only TRAIL-R1 and TRAIL-R2 were capable of inducing apoptosis and not the decoy receptors TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2) (Griffith et al., 1999). TRAIL-R2 specific antibodies when cross-linked, generated a distinct activation of NF-κB, apoptosis, and Jun NH2 kinase (JNK) activation compared to the NF-κB activation and apoptosis induced by cross-linking of TRAIL-R1 antibodies (Muhlenbeck et al., 2000). Mouse monoclonal antibodies against TRAIL-R1 were potent agonists in vivo but minimally active in vitro. In vitro activity was enhanced by secondary cross-linking antibodies, presumably through multimerization of receptor complexes. These antibodies, however, were very active against human xenografts when administered in vivo (Chuntharapai et al., 2001; Griffith et al., 1998). This result suggested that the mouse contributed a cross-linking function possibly through Fc receptors on immune cells. However, the use of agonist antibodies of immunoglobin isotypes that preferentially bind to Fc receptors or have the ability to fix complement were not significantly more active in vivo suggesting that this is not the mechanism that enhances these antibodies in vivo.

Receptor-specific antibodies selected for high-affinity binding and their TRAIL-R agonism have been identified and generated from phage display libraries, hybridomas, and transgenic mice containing human immunoglobin genes (Dobson et al., 2002; Ichikawa et al., 2001; Motoki et al., 2005; Pukac et al., 2005).

TRA-8, an agonist mouse monoclonal antibody to the human TRAIL-R2, was generated by immunization of mice with the extracellular domain of human TRAIL-R2 fused to the Fc portion of human IgG_1. TRA-8 bound to TRAIL-R2 specifically induced apoptosis in human T-cell leukemia, B-cell lymphoma, and glioma lines, and enhanced antitumor activity in combination with chemotherapeutic agents including Adriamycin (doxorubicin hydrochloride) and cisplatin. In TRA-8-resistant glioma lines sensitivity was restored after overexpression of Bax mediated by adenoviral transfer. Importantly, TRA-8 was also tested against hepatocytes in vitro and did not display any evidence of apoptosis (Choi et al., 2002; Ichikawa et al., 2001; Kaliberov et al., 2004; Ohtsuka et al., 2003).

Human Genome Sciences in collaboration with Cambridge Antibody Technology generated a series of fully human monoclonal antibodies, which target TRAIL-R1 or TRAIL-R2. The most active of these candidates were selected for evaluation in preclinical studies and are now advancing through clinical development. HGS-ETR1 (mapatumumab) an antibody specifically targeting TRAIL-R1, demonstrated potent in vitro apoptotic activity against human tumor cell lines derived from colon, lung, pancreas, ovary, uterus, renal, and hematologic malignancies. This in vitro activity was achieved in the absence of cross-linking agents. HGS-ETR1 enhanced the cytotoxicity of chemotherapeutic agents (camptothecin, cisplatin, carboplatin, or 5-fluorouracil) even in tumor cell lines that were not sensitive to HGS-ETR1 alone. In preestablished colon, NSCL, and renal xenografts, HGS-ETR1 treatment resulted in rapid tumor regression or repression of tumor growth. Addition of chemotherapeutic agents like topotecan, 5-fluorouracil, and irinotecan in colon xenograft models enhanced antitumor efficacy and in some models a synergistic antitumor activity was observed (Pukac et al., 2005).

Phase 1 trials of HGS-ETR1 have been conducted in advanced solid tumor patients and have demonstrated the safety and tolerability of single agent HGS-ETR1 up to 20 mg/kg. Single agent phase 2 studies were conducted in colorectal cancer, non-small-cell lung carcinoma (NSCLC), and non-Hodgkin's lymphoma (NHL). While stable disease was the best response observed in the two solid tumor studies, objective responses, including one complete response, were observed in the NHL study. Further, phase 1b studies have demonstrated that HGS-ETR1 can be safely administered in combination with standard doses of chemotherapy agents, such as carboplatin and paclitaxel. Additional phase 2 studies are planned to assess the activity of HGS-ETR1 in combination with chemotherapy.

HGS-ETR2 (lexatumumab), a fully human antibody identified via screening of phage display libraries for high-affinity, single-chain antibodies to TRAIL-R2, has been evaluated in similar human tumor cell lines for apoptotic activity. HGS-ETR2 produced potent apoptotic activity in a spectrum of human tumor cell lines including NSCL, colon, renal, and ovarian in the absence of cross-linking agents. (Alderson et al., 2003; Humphreys et al., 2003; Johnson et al., 2003, 2004). HGS-ETR2 has demonstrated the ability to enhance the activity of chemotherapeutic agents from various classes including taxanes and platinums (Georgakis et al., 2003; Humphreys et al., 2003; Johnson et al., 2004; Zeng et al., 2006). HGS-ETR2 induced cell death in two human RCC cell lines and nine human primary RCC cell cultures. This in vitro effect was enhanced with addition of a cross-linking antibody. In a renal xenograft model using primary renal carcinoma tumor cells HGS-ETR2 was able to induce tumor regression (Zeng et al., 2006). HGS-ETR1 and HGS-ETR2 were effective in cell lines from multiple myeloma, acute lymphoblastic leukemia (ALL), NHL, and chronic myelogenous leukemia and in primary hematological tumor cells from NHL, chronic lymphocytic leukemia, and multiple myeloma patients (Georgakis et al., 2003; Johnson et al., 2003). Phase 1 trials of HGS-ETR2 have been conducted in advanced solid tumor patients. This agent has demonstrated that it can be safely and repetitively administered up to 10 mg/kg. The results of the phase 1 studies support

the additional study of HGS-ETR2 in phase 2 trials to evaluate its potential for use in the treatment of cancer.

Another TRAIL-R2 mAb (HGS-TR2J, KMTR2) was identified in collaboration between Human Genome Sciences and Kirin Brewery, Inc. This agonist antibody, derived from transchromosomal mice expressing human Ig locus, showed in vitro and in vivo activity against human tumor cell lines. Importantly, HGS-TR2J generated significant apoptotic activity without cross-linking and was active in many human tumor cell lines. It was also shown that ligation of HGS-TR2J to cell surface receptors induced clustering of TRAIL-R2 (Motoki et al., 2005). HGS-TR2J is currently in phase 1 clinical development.

7.3 Agonist Signaling

Receptor oligomerization is potentially a key event in TRAIL-R signaling. In vitro and in vivo experiments have shown that cross-linking TRAIL-R agonists, including various forms of the recombinant ligand and antibodies, altered antitumor activity. Antibodies, because of their bivalent binding, have the potential to oligomerize receptor molecules, which could lead to activation of TRAIL-R signaling, DISC formation and cell death. The recombinant ligand, generated in several forms that permitted cross-linking or aggregation, demonstrated potent antiapoptotic activity. Additionally, chemotherapeutic treatment has been able to induce TRAIL-R1 and TRAIL-R2 receptor aggregation and enhance apoptosis (Bergeron et al., 2004; Delmas et al., 2004). Experiments have shown that cross-linking an agonist, including the ligand and antibodies, can improve apoptosis in vitro. Even in those experiments where cross-linking was required for activity in vitro, agonists were readily effective in vivo without cross-linking. In addition, some agonists can achieve maximal apoptosis activity without any enhancement from in vitro cross-linking. This conflicting data suggests several possible mechanisms of killing by TRAIL-R agonists.

Conceivably, both cross-linking-dependent and cross-linking-independent mechanisms may exist for TRAIL-R agonists. Where cross-linking is involved in vivo this function may be provided by the host through immune cells that can cross-link IgG molecules, i.e., Fc receptors. Alternatively, in the absence of cross-linking a TRAIL-R agonist could bind to the trimerized receptor and induce a conformational change similar to the alteration that is theorized to occur with the native ligand. Conformational change in the receptor could expose relevant binding domains on FADD and induce the apoptotic cascade. In fact, the ability to expose different protein-binding domains of FADD has been observed with TRAIL-R agonist antibodies (Thomas et al., 2004b). The ability to cross-link cell surface receptors with antibodies induces capping and increases agonistic activity that has been shown in other signaling systems including those within the TNFSFR. (Cremesti et al., 2001; Liu et al., 2003; Ludwig et al., 2003; Miller et al., 2003). While the precise nature of the interaction between TRAIL-R agonists and the formation of the DISC

remains to be determined, their ability to activate this pathway and induce tumor cell death has been proven in preclinical studies and is being validated in the clinical setting.

8 Agents Targeting the Apoptosis Pathway

The availability of human tumor cell lines that are refractory to TRAIL-R agonism has allowed exploration of potential mechanisms of resistance (Igney and Krammer, 2002; Wang and El-Deiry, 2003). Both extrinsic and intrinsic regulatory proteins have been blamed for this resistance, including FLIP (Griffith et al., 1998; Kim et al., 2000; Leverkus et al., 2000a), XIAP, survivin (Kim et al., 2004) Bcl-2 (Fulda et al., 2002a), and Bax (Deng et al., 2002; He et al., 2003; Kandasamy et al., 2003; LeBlanc et al., 2002). Genetic alterations have been identified in TRAIL-R1 and TRAIL-R2 in NSCLC, colon cancer, head and neck cancer, and lymphoma. Some of these modifications induced a loss of apoptotic signaling. Unfortunately, their role in TRAIL resistance in the clinic has not been validated. (Arai et al., 1998; Fisher et al., 2001; Jeng and Hsu, 2002; Lee et al., 1999; Ozoren et al., 2000; Pai et al., 1998; Wolf et al., 2006; Wu et al., 2000). Changes in the level of cell surface receptor expression, caspase-8/FLIP ratio and loss of caspase-8 have all been discovered as mechanisms of resistance to TRAIL-R agonism (Poulaki et al., 2005; Van Geelen et al., 2004; Wachter et al., 2004).

Consequently, many strategies have been evaluated for their ability to enhance sensitivity or maximize responsiveness to TRAIL-R agonism. Early obvious strategies involved combining standard, approved chemotherapeutic agents with TRAIL-R agonists. Chemotherapy agents or radiation improved response in breast, colorectal, and NSCLC cell lines that displayed resistance to TRAIL-R agonism (Adams and Cory, 2002; Ganten et al., 2005; Kondo et al., 2006; Wendt et al., 2005; Zhang et al., 2005). The use of chemotherapy agents modified levels of specific molecules including TRAIL-R1, TRAIL-R2, FLIP, XIAP, or the proapoptotic protein Bad and restored TRAIL-R responsiveness (Fesik, 2005; Galligan et al., 2005; Mirandola et al., 2006b; Xiao et al., 2005; Yamaguchi et al., 2005a). Other strategies have targeted specific molecules known to regulate the pathway at important catalytic or survival signaling steps. For example, many new compounds have targeted the ubiquitous, antiapoptotic protein Bcl-2, or related family members, through antisense or small molecules (Chawla-Sarkar et al., 2004; Sinicrope et al., 2004; Zhu et al., 2005a) (Table 7.3) (also see Chapter 8). Oblimersen sodium (Bcl-2 antisense) as a single agent or in combination with chemotherapy has shown some clinical activity. (Marcucci et al., 2005; O'Brien et al., 2005; Tolcher et al., 2005). Many strategies are focused on the elimination or reduction of inhibitors that block activation of the initiator caspase-8 and caspase-9, namely XIAP, survivin, and FLIP. Small-molecule and antisense techniques have yielded promising results in preclinical models. FLIP, survivin, and XIAP inhibitors in combination with TRAIL-R agonists have significantly enhanced apoptosis across

Table 7.3 Apoptosis therapeutics in development

Compound	Type	Target	Institute/company	Status
HGS-ETR1	Human agonist mAb	TRAIL-R1	Human Genome Sciences	Ph2
HGS-ETR2	Human agonist mAb	TRAIL-R2	Human Genome Sciences	Ph1
HGS-TR2J	Human agonist mAb	TRAIL-R2	Human Genome Sciences	Ph1
TRA-8	Agonist mAb TRA-8	TRAIL-R2	Sankyo	Preclinical
APO2L/TRAIL-PRO1762	Recombinant TRAIL ligand	TRAIL-R1 TRAIL-R2	Amgen/Genentech	Ph1
Genasense (oblimersen sodium)	Antisense	Bcl-2	Genta	Ph2/3
GX15–070	Small molecule	Bcl-2	GeminX	Ph1
AT101	Small molecule	Bcl-2	Ascenta	Ph1/2
ApoGossypol	Small molecule	Bcl-2	Burnham Institute/NCI	Preclinical
EGCG	Small molecule	Bcl-2	Mayo Clinic	Preclinical
ABT-737	Small molecule	Bcl-2	Abbott/Idun	Preclinical
HA14–1	Small molecule	Bcl-2	Raylight	Preclinical
CDDO	Triterpenoid	FLIP	Reata Discovery/ Dartmouth	Preclinical
ISIS 2181308	Antisense	Survivin	Isis/Lilly	Ph1
AG35156	Antisense	XIAP	Aegera	Ph1
Not defined	SMAC mimetic peptide	XIAP	Joyant Pharmaceuticals	Preclinical
Not defined	SMAC mimetic peptide	XIAP	Tetralogics	Preclinical

various cancer cell lines. (Amantana et al., 2004; Chawla-Sarkar et al., 2004; McManus et al., 2004; Ou et al., 2005; Wang et al., 2005; Yamaguchi et al., 2005a, b). There are other compounds that mimic the action of the mitochondrially released XIAP inhibitor, SMAC/DIABLO (Bockbrader et al., 2005; Fulda et al., 2002b; Li et al., 2004; Pei et al., 2004; Roa et al., 2003). There are examples of single-agent activity in tumor cell lines and xenografts for many of these targeted therapies. More importantly, where they have been evaluated, the apoptosis activity of these agents shows a dramatic enhancement in combination with TRAIL-R agonists. These data demonstrate that the use of TRAIL-R agonists and compounds that lower hurdles for active apoptosis signaling may be potent therapeutic agents and importantly active in TRAIL insensitive cells.

Another avenue that has generated encouraging results has come from the use of agents with less direct action on TRAIL-R signaling. The proteosome inhibitor, bortezomib, has broad-ranging effects on receptor expression, upregulation of proapoptotic proteins such as Bik and Bim, and TRAIL production (also see Chapter 12). Bortezomib has also shown activity in combination with the agonist antibodies HGS-ETR1 and HGS-ETR2 in hematological cell lines and primary cells from NHL and CLL patients. (Georgakis et al., 2005; Lashinger et al., 2005; Matta and Chaudhary, 2005; Nencioni et al., 2005; Nikrad et al., 2005; Papageorgiou et al., 2004; Sayers and Murphy, 2006; Zhang et al., 2004; Zhu et al., 2005b). Histone

deacetylase (HDAC) inhibitors have demonstrated significant antitumor activity in combination with TRAIL-R agonists. Effects with HDAC inhibitors include changes in TRAIL-R2 expression, decreasing levels of Bcl-2 and FLIP, and increasing the proapoptotic protein Bik. Some early HDAC inhibitors are now progressing through clinical trial development and show early signs of activity (Ganten et al., 2005; Guo et al., 2004; Kelly and Marks, 2005; Kelly et al., 2005; Marks et al., 2004; Yoshida et al., 2005; Zhu et al., 2005b) (also see Chapter 13).

The use of these apoptosis-promoting compounds as single agents or in combination with standard chemotherapy has, in those agents being advanced into clinical development, shown signs of biological activity. These strategies directly targeting the apoptosis pathway are exploiting the potential that they will confer greater effectiveness to chemotherapy. Alternatively, the elimination or obstruction of antiapoptotic molecules may lower the threshold for induction of apoptosis when used in combination with a TRAIL-R agonist. This combination strategy of TRAIL-R agonists and proapoptotic-targeted therapy has the potential to significantly enhance antitumor activity and eliminate the need for nonspecific chemotherapeutic agents that elicit toxic side effects. While a broad range of exciting preclinical data has verified the activity of this amalgamation, a combinatorial apoptotic strategy needs to be validated in a clinical setting.

9 Conclusion

Targeting the TRAIL-R pathway with therapeutic agents provides an opportunity to induce apoptosis selectively in tumor cells. In preclinical studies the use of TRAIL-R agonists like recombinant TRAIL ligand or monoclonal antibodies have demonstrated significant, potent antitumor activity and have enhanced chemotherapeutic agent activity in a spectrum of human tumor cell lines and xenografts. Several human monoclonal antibodies and a recombinant TRAIL ligand have advanced through preclinical evaluation and are now in clinical development. Hopefully, other novel agents that target the apoptotic pathway will enter and advance successfully through the clinical arena, strengthening, and diversifying the armamentarium against the tumor cell.

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