DR4 and DR5

Target: TRAILR1 and TRAILR2 Death Receptors

TRAIL has two intact functional receptors, termed as TRAIL receptor 1 and 2. TRAIL receptor 1 (TRAILR1) isalso known as DR4, TNFRSF10A,APO2, TR10A_HUMAN, was identified in 1997 (1), and possesses 468 amino acids(accession number U90875). TRAIL receptor 2 (TRAILR2) is also known as TNFRSF10B, DR5,KILLER, TRICK2, was also identified in 1997 and has two isoforms resulting from alternative splicing, TRAILR2a and TRAILR2b are encoded by412 and 441 amino acids respectively(accession numbers AF018657 and AF018658)(2, 3). The extracellular and intracellular domains for TRAILR2 have 58% and 65% similarity to TRAILR1 (2).TRAILR1and TRAILR2 mRNAs were found to be distributed in almost all tissues including spleen, thymus, prostate, testis, ovary, small intestine, heart, lung, liver and peripheral blood leukocytes. TRAILR2 expression was particularly high in peripheral blood lymphocytes, pancreas and heart (2). Interestingly, the genes encoding TRAILR1 and TRAILR2 are tightly clustered on human chromosome 8p21-22, a region frequently deleted in cancer.Notably, in mice it has been shown that there is only one functional death-inducing receptor homologous to human TRAILR2 (mTRAIL-R2/mDR5).

Biology of the Target

Apoptotic signaling through death receptors is regulated by the recruitment of receptors into lipid rafts, where receptors, signaling enzymes, and adaptor proteins assemble into a complex. Ligation of TRAILR1/2 leads to their localization in lipid rafts, resulting in assembly of the DISC and activation of the intracellular apoptotic machinery. Induction of TRAILR1/2 redistribution and clustering into lipid rafts is essential for mediating apoptosis signals. In TRAIL-resistant cells, TRAILR1/2 remain localized in non-lipid rafts, and are associated with the inhibitor protein cellular (FLICE)-like inhibitory protein(c-FLIP) after stimulation with TRAIL. On the other hand, the histone deacetylase inhibitor (HDACi) depsipeptide, and polyphenol resveratrol induces the distribution of TRAILR1/2 in the lipid raft leading to an increase of apoptosis and inhibition of tumor development.

At the transcriptional level, there are various transcriptional factors that tightly regulate expression of TRAILR1 and TRAILR2. The TRAILR1 promoter encodes an activator protein 1 (AP-1) binding site, which was shown to be important for promoter activation upon AP-1 activator phorbol 12-myristate 13-acetate (TPA) treatment (4). Moreover, TRAILR1 promoter encodes a p53 responsive element, and functional p53 was shown to be important for expression of TRAILR1 (5). TRAILR2 is known to be a transcriptional target for p53, since, similar to TRAILR1, the TRAILR2 gene encodes a p53-responsive element in the first intronic region in which p53 binds and enhances expression of TRAIL2 (6). Nevertheless, TRAILR2 expression may also be regulated in a p53-independent manner; it has been shown that the treatment of various cancer cells, harboring mutated p53, with carboplatin or interferon-gamma and glucocorticoids increases TRAILR2 expression independent from the p53 status of the treated cells (7, 8). It was suggested that this upregulation may be mediated by STAT1. Moreover, there are several transcriptional factors found to regulate TRAILR2 expression including NFkB (9), Myc (10), CCAAT/enhancer binding protein homologous protein(CHOP) (11), and SP1 (12).

Post-translational modification of TRAILR1 and TRAILR2 has been shown to be important in inducing the intracellular apoptotic machinery. O-glycosylation of TRAILR1 and TRAILR2 is essential for inducing ligand-mediated receptor clustering and subsequent DISC formation and caspase-8 activation. Small interfering RNA (siRNA)-mediated downregulation of genes encodes enzymes carryout O-glycosylation (GALTNT14 or FUT16) suppressed TRAIL-mediated apoptosis (13).

Target Assessment

TRAILR1/2are characterized by an extracellular cystein-rich domain and an intracellular death domain giving them the ability to trigger the assembly of the death-inducing signaling complex (DISC) upon ligand stimulation which initiates the apoptotic machinery. Trimerization of TRAILR1 and TRAILR2 by TRAIL on the surface of target cells leads torecruitment of adaptor molecule Fas-associated death domain protein(FADD), which in turn leads to recruitment and activation of caspase-8. In certain cell types, type I activation of caspase-8 is sufficient for subsequent activation of the effector caspase-3 to execute cellular apoptosis (extrinsic pathway). In other cell types, type II, amplification occurs through the mitochondrial pathway (intrinsic pathway), which is initiated by cleavage of Bid by caspase-8. The truncated Bid (tBid) translocates to the mitochondria and leads to Bax and Bak-mediated release of cytochrome-c (cyt-c) and Smac/DIABLO from mitochondria. The released cyt-c binds to Apaf-1 to activate caspase-9, which in turn activates caspase-3. The Smac/DIABLO promotes caspase-3 activation by preventing IAPs from attenuating caspases.

Role of the Target in Cancer

The TRAIL-TRAILRs system has been proposed to regulate tumor onset and development. Genetic and epigenetic mutations in functional TRAIL receptors have been observed in several cancers. Mutation in TRAILR1 was detected in lung cancer and head and neck cancer (14). Hypermethylation of the TRAILR1 promoter could be found in 27.7% of ovarian cancer patients (15). Mutations in the intracellular domain, a region that mediate the intracellular signaling, of TRAILR2 were found in 10.6% of non-small cell lung cancer (NSCLC) (16). Somatic mutations in TRAILR1 and 2 were found in 6.8% of non-Hodgkin's lymphoma (NHL) cases (17). Moreover, mutations in the intracellular domain of TRAILR1 and 2 were identified in patients with metastatic breast cancer (18).In the light of recent data from in vitro and in vivo studies, upregulation of TRAILR1 or TRAILR2 expression has a clear effect on enhancing the sensitivity of cancer cells to apoptosis (7, 19). Interestingly, it has been suggested that apoptotic signalling through TRAILR2 may be more potent than through TRAILR1 in cancer cells that express both receptors (20). Different studies have shown that TRAILR2 is more efficiently activated by secondary cross-linked trimers of soluble TRAIL than by non-cross-linked molecules, whereas TRAILR1 is stimulated with the same efficiency by cross-linked and non-cross-linked TRAIL (20)
TRAIL and its functional receptors have been shown to be key effectors in mediating host immune surveillance against cancer progression, and loss of function of TRAILR1 and TRAILR2 may confer resistance to TRAIL-induced apoptosis
Overall, at this stage, human TRAILR1 and TRAILR2 play a fundamental role in the development of various cancers and therefore are promising targets for cancer therapy. The ability of the agonistic molecules targeting these death receptors to induce apoptosis in cancer cells has become attractive candidates for anticancer treatment, and are currently being tested in clinical trails. Accordingly, we rank TRAILR1 and TRAILR2 eight on a scale one "unknown" to ten "known".

High Level Overview

A. Diagnostic, Prognostic, Predictive

Several recent studies have demonstrated the correlation between TRAILR1 and TRAILR2 expression and development of different cancers. In a clinical study of 376 stage III colon cancer patients who treated with adjuvant chemotherapy, TRAILR1 expression was found to be associated with worse disease-free and overall survival (21). In Other study, 90 breast cancer patients with invasive ductal carcinoma showed that TRAILR1 expression positively correlates with the tumor grade (22). In colorectal cancer study of 82 patients, TRAILR2 expression was found to be decreased with increased colorectal cancer stage (23).

B. Therapeutics

So far, a number of therapeutic strategies involving agonistic antibodies to the TRAILR1 and TRAILR2 have been developed. It has been reported that agonistic anti-human TRAILR1 or TRAILR2 monoclonal antibodies (mABS) exhibited potent tumoricidal activities against human tumor xenografts in nude or SCID mice without apparent toxicity. These agonistic antibodies may be more effective than the ligand at eradicating tumors for several reasons. First the fact, that there is a prolonged half-life time in vivo when compared to the recombinant proteins. In human, the half time of agonistic antibody in serum is about 15-20 days, whereas the recombinant soluble TRAIL in serum is only 20-30 minutes. Second, agonistic antibodies possess a Fc domain, which can recruit and activate FcR-expressing immune cells like NK cells, macrophages, and dendritic cells. Accordingly, administration of either TRAILR1 or 2 agonistic antibody kills TRAIL-sensitive tumor cells as well as induce specific T cells that eliminate the TRAIL-resistant cells. Induction of T cells produce also memory T cells, providing an ideal environment for a longterm protection from tumor recurrence. Third, the decoy receptors, which have been implicated in modulating response to TRAIL, are not targeted by these agonistic antibodies. On the other hand agonistic antibodies may for the same reason be more toxic to normal tissue because decoy receptors have also been proposed to protect normal tissue cells from apoptosis mediated by TRAIL.

Pre-clinical Summary

To date, several agonistic antibodies have been reported to exhibit a notable degree of apoptosis, growth inhibition, and cytotoxicity in a broad range of human cancer cell lines and tumor xenografts. Of these approaches, HGS-ETR1 (mapatumumab; developed by Human Genome Sciences), a fully human agonistic mAB targeting TRAILR1; HGS-ETR2 (lexatumumab) and HGS-TR2J (developed by Human Genome Sciences) a fully human agonistic mABs targeting TRAILR2; AMG 655 (developed by Amgen) a fully human agonistic mAB targeting TRAILR2; Apomab (developed by Gentech) a fully human mAB targeting TRAILR2;LBY135 (developed by Novartis)a chimeric agonistic mAB targeting TRAILR2; TRA-8 (24)mouse mAB targeting TRAILR2.Further encouraging results were obtained in recent years by developing AD5-10, anti-human agonistic mAB against TRAILR2 that has exhibited a clear tumorcidial activity in various cancer mouse models (7, 25). In contrast to Apomab, AD5-10 characterized by its unique binding site that does not compete with TRAIL for binding to TRAILR2, and has no toxic effect on human normal hepatocytes (25).
Interestingly, numerous reports have noted more favourable interactions following treatment of tumors with a combination of TRAILR1/2 agonistic antibodies and distinct classes of pharmaceutical and cellular anti-cancer agents. For example, combination of AD5-10 with carboplatin eradicates ovarian tumors in xenograft mouse model (7). HGS-ETR1 has been demonstrated to augment apoptosis in vitro in combination with cisplatin, camptothecin, topotecan and doxorubicin.

Clinical Summary

Clinical Trials have been started in patients using agonistic antibodies targeting TRAILR1 and TRAILR2. In this approach, TRAILR1 human agonistic antibody (HGS-ETR1), and TRAILR2 human agonistic antibodies (HGS-ERT2, HGS-TR2J,CS-1008, Apomab, and AMG 6555) in addition to chimeric (LBY135) antibody against TRAILR2 are in phase I/II clinical trials (Table 1). Overall, data from these clinical trials indicate anti-tumor activity against a range of different tumors as both monotherapy and in combination with different anticancer agents. These early clinical findings support the safety of these agonistic antibodies and further studies are required alone and in combination with pharmaceutical and cellular anti-cancer agents. All in all, agonistic antibodies targeting TRAILR1 and TRAILR2 seem to be a promising regimen for future cancer therapy.

Anticipated High-Impact Results

Table 1. Summary of clinical trials of existing agonistic TRAILR1 and TRAILR2 specific antibodies. This table is adapted and updated from several tables contained in refs (26-30).

Molecule tested	Targeted receptor	Clinical trials	N	Tumor type	Status	Comment

		Phase I	49	Solid tumors	Completed	39% of patients showed stable disease
		Phase I	24	Solid tumors	Completed	33% of patients showed stable disease
HGS-ETR1 (Mapatumumb)	TRAILR1	Phase I	15	Solid and NHL	Ongoing	
		Phase II	40	NHL	Completed	7.5% of patients showed response, and 27 % stable disease
		Phase II	38	Relapsed or refractory colorectal cancer	Completed	32% of patients showed stable disease
		Phase II	32	NSCLC	Completed	29% of patients showed stable disease
HGS-ETR1 in combination with paclitaxel and carboplatin	TRAILR1	Phase I	28	Solid tumors	Completed	14% of patients showed partial response
HGS-ETR2 (Lexatumumab)	TRAILR2	Phase I	31	Solid tumors	Completed	32.3% of patients showed stable disease
		Phase I	37	Solid tumors	Completed	29.7% of patients showed stable disease
HGS-ETR2 in combination with gemcitabine, pemetrexed, doxorubicin, or FOLFIRI.	TRAILR2	Phase Ib	41	Solid tumor and hematological malignancies	Completed	Patients showed partial response
HGS-TR2J	TRAILR2	Phase I		Non reported	Ongoing	
CS-1008 (TRA-8)	TRAILR2	Phase I	17	Solid tumors or lymphomas	Completed	41.1% of patients showed stable disease
CS-1008 in combination with chemotherapy	TRAILR2			TRAILR2 positive epithelial tumors	Ongoing	
Apomab	TRAILR2	Phase I	26	Solid tumors	Completed	3.8% of patients showed stable disease
		Phase II		Soft tissue sarcomas	Ongoing	
Apomab in combination with different anticancer drugs	TRAILR2	Phase I/II		NHL, NSCLC, colorectal cancer	Ongoing	
AMG 655	TRAILR2	Phase I	16	Solid tumors	Completed	6% of patients showed partial response, and 25% of patients showed stable disease
AMG 655 in combination with panitumumab	TRAILR2 and EGFR	Phase Ib/II	15	Solid tumors	Ongoing	
AMG 655 in combaintion with gemcitabine	TRAILR2	Phase II	13	Metastatic pancreatic cancer.	Ongoing	Preliminary data indicated that 23% of patients showed partial response, and 46% stable disease.
LBY135	TRAILR2	Phase I	32	Solid tumors	Completed	43.7% of patients showed a minor response
LBY135 in combination with capecitabine	TRAILR2	Phase I	24	Solid tumors	Completed	8.3% of patients showed partial response

N, number of patients; NSCLC, non-small cell lung cancer; NHL, non-Hodgkin lymphoma

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DR4 and DR5

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