The death receptors: signaling and modulation
The extrinsic cell death pathway

Death receptors

Belong to the tumor necrosis factor (TNF) receptor gene superfamily

All family members of TNFR superfamily contain a similar, cysteine-rich extracellular domains

In addition to the similar extracellular domain, death receptors (DRs) contain an cytoplasmic death domain

Death domains typically enable death receptors to engage the core cell death machinery, but in some instances they mediate functions other than cell death

Nature reviews cancer 2002, 2:277
Members of death receptors

CD95/Fas/Apo1
TNFR1/p55/CD120a
CAR1, avain
Death receptor 3/DR3/Apo3/Wsl-1/TRAMP/LARD
DR4
DR5/Apo2/TRAIL-R2/TRICK 2/KILLER
The ligands of death receptors

CD95----CD95 ligand/CD95L/FasL

TNFR1----TNF and lymphotoxin α

DR3----Apo3L

DR4 and DR5----Apo2L/TRAIL
Expression of CD95 and CD95L

CD95

A 48 KDa transmembrane receptor

Expressed in activated lymphocytes, tissues of lymphoid origin or nonlymphoid origin and some tumor cells

CD95L

A 40 KDa transmembrane molecule, occurs in a membrane-bound and in a soluble form, generated through cleavage by metalloproteases

CD95L is produced by activated T cells and plays a crucial role in triggering autocrine suicide or paracrine death in neighboring lymphocytes or other target cells
CD95 and CD95L

CD95/Fas and CD95L/FasL

Involved in:

- Peripheral deletion of activated mature T cells
- Killing of virus-infected cells and cancer cells
- Killing of inflammatory cells at immune-privileged sites such as the eye

Mutations of CD95 or CD95L in patients or mouse model result in accumulation of peripheral lymphoid cells and a fetal autoimmune syndrome.
CD95 signaling

A homotrimeric CD95L complex binds to three CD95

Binding of CD95L leads to clustering of the receptors’ death domains

FADD (Fas-associated death domain), an adaptor protein, binds through its own death domain to the clustered receptor death domains

The death effector domain (DED) of the FADD then binds to the DED of procaspase 8 (or named FLICE/MACH)

Clustering of procaspase 8 promoted by associations of DED domains leads to self-activation of caspase 8

Science 281:1305-1308, 1998
Tumor necrosis factor (TNF)

Mainly produced by activated macrophages and T cells in response to infection

Usually activates NF-kB and AP-1, and leads to induction of proinflammatory and immunomodulatory genes

In some circumstances, e.g., inhibition of protein synthesis (treated with cycloheximide), TNF also induces apoptosis
The TNF-TNFR signaling

TNFR is a transmembrane protein as well as a soluble protein cleaved by proteolytic cleavage.

The function of the soluble form of TNFR is unknown.

Trimeric TNF binding to TNFR induces trimerization of TNFR, promoting association of the receptors’ death domains.

Subsequently, an adaptor protein TRADD (TNFR-associated death domain) binds through its own death domain to the clustered receptor death domains.

TRADD function as a platform adaptor that recruits several signaling molecules to the activated receptor.

Science 281:1305-1308, 1998
The TNF-TNFR signaling, cont.

Signaling molecules downstream of the TRADD include TNFR-associated factor-2 (TRAF2), receptor interacting protein (RIP), and FADD

Association of RIP and TRAF2 leads to activation of NF-kB and of JNK/AP-1, respectively

Association of FADD mediates activation of caspase 8 and apoptosis
DR3-Apo3L signaling pathway

DR3 shares the most homology with TNF-R1

DR3 binds to Apo3L, which is related most closely to TNF

Apo3L activates NF-κB through TRADD, TRAF2, RIP and NIK, just like TNF

Apo3L induces apoptosis through TRADD and FADD

Science 281:1305-1308, 1998
DR4, DR5,-Apo2L/TRAIL

The crystal structure of TRAIL and three extracellular domains of DR5

DR4, DR5,-Apo2L/TRAIL signaling pathway

Apo2L/TRAIL was found to be most similar to CD95L

Apo2L mRNA expression is constitutive in many tissues

Apo2L binds to DR4 and DR5 and induces apoptosis

Expression of DR4 and DR5 was found in many tissues

Decoy receptors, such as DcR1 and DcR2, also bind to Apo2L, but the binding leads to no apoptosis

Therefore, decoy receptors appear to have a protective effect against DR4 and DR5-induced apoptosis

Science 281:1305-1308, 1998
DcR-Apo2L/TRAIl signaling pathway

DcR1/TRID/TRAIl-R3/LIT

A glycosyl phosphatidylinositol (GPI)-anchored cell surface protein that resembles DR4 and DR5, but lacks a cytoplasmic tail

Treatment of DcR1-bearing cells with a phospholipase that cleaves the GPI anchor results in marked sensitization to Apo2L-induced apoptosis

DcR2/TRAIl-R4/TRUNDD

Another receptor resembles DR4 and DR5, and has a substantially truncated cytoplasmic death domain

Overexpression of DcR2 inhibits apoptosis induction by Apo2L
Regulation of the death receptor signaling pathway
The extrinsic cell death pathway

C-FLIP (cellular FLICE inhibitor protein)

c-FLIP, as a cellular inhibitor of caspase 8

Caspase 8 also named FLICE, FADD-like interleukin-1-β-converting enzyme)

Two variants: c-FLIPs and c-FLIP_L

- c-FLIPs only contains two N-terminal DEDs very similar to the prodomains of caspase-8/-10
- c-FLIP_L is identical in length with caspase 8, but its caspase domain is altered, rendering it enzymically inactive
- c-FLIP_L is similar to a dominant negative inhibitor
- c-FLIPL⁻⁻ MEFs are more sensitive to death-receptor induced cell death

However, recent reports showed that c-FLIP_L is not only an inhibitor of apoptosis, but also an activator of pro-caspase-8
C-FLIP (cellular FLICE inhibitor protein), cont.

c-FLIP$_L$ forms heterodimers with caspase8/10 more efficiently than caspase8/10 to form homodimers

In heterodimerization with c-FLIP$_L$, procaspase 8 can be activated without processing

Recently, Caspase 8 was shown to be required in cell cycle progression and proliferation. This function of caspase 8 may be regulated by c-FLIP$_L$

When FLIP is high, it will efficiently activate procaspase 8, but will not allow full processing to the mature enzyme

Active Caspase 8 or first cleavage intermediates may stay tethered to the Disc

Biochem. J. 382, e1-e3, 2004
TNFR1-induced pathways modulating apoptosis

J. Cell Sci. 117, 5197-5208, 2004
NF-κB induces multiple downstream targets to inhibit apoptosis
Factors affect direction of TNF signaling pathway going life or death decisions

Cell types

Microenvironment of the TNFR

Timing of recruitment of signaling complex

Deficiency in NF-κB signaling pathway or apoptosis machinery
Lipid rafts modify early events in TNFR family signaling

Immunity, 21:461-465, 2004
TRAIL as a cancer therapy target

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clinical development stages</th>
<th>Company</th>
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<td>HGS-ETK1 (anti-TRAIL-R1 mAb)</td>
<td>Phase II completed; NHL, colorectal cancer, NSCLC</td>
<td>Human Genome Science</td>
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<tr>
<td>HGS-ETK1 _ paclitaxel _ carboplatin</td>
<td>Phase I: advanced solid tumors</td>
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<tr>
<td>HGS-ETK1 _ gemcitabine _ cisplatin</td>
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<td>HGS-ETK1 _ bortezomib</td>
<td>Phase I: advanced multiple myeloma</td>
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<td>HGS-ETK2 (anti-TRAIL-R2 mAb)</td>
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<td>HGS-ETK2 _ chemotherapy</td>
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<td>LBV135 _ capecitabine</td>
<td>Phase I/II: advanced solid tumors (recruiting since 2006)</td>
<td>Genentech</td>
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<td>Apomat (anti-TRAIL-R2)</td>
<td>Phase I: advanced solid tumors</td>
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<td>Apomat _ avastin</td>
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<td>TRA-8 (anti-TRAIL-R2)</td>
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<td>AMG555 (anti-TRAIL-R2)</td>
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<td>Genentech/Amgen</td>
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<td>Apo2L/TRAIL _ rituximab</td>
<td>Phase Ib/II: NHL (recruiting since 2006)</td>
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<tr>
<td>ADS-TRAIL</td>
<td>Phase Ia: organ-confined prostate cancer</td>
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NHL indicates non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer.
Pathways mediating sensitization to TRAIL by down-regulation of Mcl-1

References


