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The TRAIL-receptors 1 (DR4) and 2 (DR5) contribute differently to TRAIL-mediated apoptosis

Abstract:
Cancer is a major disease which had 12.7 million cases worldwide in 2008 of which 82.000 were in The Netherlands. Because of this high incidence it is important to develop new therapies. The TNF-related apoptosis inducing ligand (TRAIL) is a promising new drug that induces cell death or apoptosis in tumor cells. Important advantages of TRAIL are that is does not affect healthy cells and that it can be used in many different cell types making it a wide-range drug. TRAIL can induce apoptosis through two receptors: TRAIL-R1 (DR4) and TRAIL-R2 (DR5). In this review we analysed whether TRAIL-R1 and TRAIL-R2 contribute differently to TRAIL-mediated apoptosis and what the underlying mechanisms are. This review showed that there are different contributions of TRAIL-R1 and TRAIL-R2 to TRAIL-mediated apoptosis which might be cell type specific.TRAIL-R1 induced apoptosis predominantly in leukemic cancer cells, melanoma cancer cells and pancreatic cancer cells, whereas TRAIL-R2 induced apoptosis predominantly in colon cancer cells, breast cancer cells and glioblastoma cancer cells. The underlying mechanisms are not well understood and it is important that they are identified. That way it might be possible to determine whether a tumor is a TRAIL-R1 type or a TRAIL-R2 type. Treatment with specific TRAIL receptor variants will be useful, because it maximizes the success of the therapy due to the higher affinity.

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1. Introduction

1.1 Cancer

In 2008 there were 12.7 million cases of cancer worldwide. It is expected that in 2030 the number of cases will reach up to 21 million worldwide (1). For men the most common cancers are lung cancer, prostate cancer and colorectal cancer, whereas women suffer the most of breast cancer, colorectal cancer and cervical cancer. In The Netherlands, 82.000 cases of cancer were observed in 2008, which covers over 25 types of cancer (1). Because of the high incidence of cancer, worldwide as well as in The Netherlands, it is important to develop new drugs to treat this disease.

Because there are many different types of cancer, this often leads to the development of cancer-specific drugs. These drugs focus on characteristics which are specific for a certain type of cancer, therefore it can only be used in that type of cancer. Although these specific drugs are often successful, it is disadvantageous that it can only be used in a small percentage of all cancers. Another problem with treatments like chemotherapy and radiation is that they often have many side effects. Not only cancer cells are affected by these treatments, but also cells of healthy tissue are affected. Therefore it should be useful to develop drugs that only affect cancer cells and can be applied in multiple forms of cancer.

To develop drugs that only affect cancer cells, the drugs should be based on the properties that cancer cells have and by which they are distinguished of normal cells. Characteristic for cancer cells are the high proliferation, production of angiogenic factors, invasiveness and apoptosis inhibition, which all contribute to the carcinogenesis (2).

1.2 TRAIL

A promising new therapeutic drug is the homotrimeric protein TRAIL (TNF-related apoptosis inducing ligand), also known as apoptosis-inducing ligand2, which is able to induce apoptosis in cancer cells. TRAIL is a promising drug because it does not induce apoptosis in healthy cells. Another advantage of TRAIL is that it can be used in many types of cancer, because most cancers express the receptors to which TRAIL can bind. TRAIL is a member of the TNF family which are cytokines. It is able to bind to five different receptors (3). TRAIL can induce apoptosis by binding to TRAIL receptor 1 (Death Receptor 4) (35) and TRAIL receptor 2 (Death Receptor 5) (29) which are membrane bound receptors. TRAIL can also bind to Decoy Receptor 1 (DcR1 or TRAIL-R3) and Decoy Receptor 2 (DcR2 or TRAIL-R4) which are also expressed on the cell surface. Because these two receptors do not have a functional intracellular death domain, they do not induce apoptosis. The last receptor to which TRAIL can bind is osteoprotegerin (OPG) which is a soluble receptor (3).

Although TRAIL seems to be a promising drug, it also faces some problems. First of all there are cancer cells which showed to be resistant towards TRAIL treatment, whereby apoptosis is not induced (8, 9, 10, 11). This resistance can be developed in multiple ways. Another problem is the observed dual activity of TRAIL; many experiments showed the apoptosis-inducing properties of TRAIL, but there are also experiments that showed TRAIL to contribute to cell survival and cell proliferation (5, 16). The resistance and dual activity of TRAIL will be discussed further below.

The presence of two TRAIL receptors that can mediate signalling following ligand

binding have raised questions whether they elicit similar or perhaps different responses and with possible different functional outcomes. New observations showed that TRAIL might induce apoptosis predominantly by only one of the two death receptors. Even though there is not very much known about this mechanism, there have been different studies showing TRAIL-induced apoptosis by TRAIL-R1 activation (17, 18, 19, 20). However, there have also been studies that showed TRAIL-induced apoptosis by TRAIL-R2 activation (21, 22, 23). It is important to know which receptor contributes mostly to TRAIL-mediated apoptosis to develop receptor-specific therapies. Which receptor induces apoptosis could differ between cancer cells types.

In this review, a short overview will be given about mechanisms of TRAIL signalling and functional consequences in tumor cells. However, the main question addressed in this review will be whether TRAIL-R1 and TRAIL-R2 contribute differently to the induction of apoptosis and what the possible underlying mechanisms are.

2. TRAIL

2.1 Apoptotic pathways

As mentioned before, TRAIL is able to induce apoptosis by binding to TRAIL-R1 or TRAIL-R2 (3). This activates the extrinsic pathway of apoptosis (4). Binding of TRAIL to TRAIL-R1 or TRAIL-R2 leads to receptor trimerization. The receptor is activated by a conformational change in the death domain of the receptor which is located intracellular. Next is the binding of an adapter molecule: Fas-associated protein with death domain (FADD) to the receptor. The death effector domain (DED) of FADD binds to the DED of pro-caspase-8, which leads to the auto-activation of this caspase. The complex of the death receptor, FADD and caspase-8 is named DISC; death inducing signalling complex. Caspase-8 activates other caspases which eventually leads to apoptosis (3, 4, 5).

Besides the activation of the extrinsic apoptotic pathway via caspase-8, caspase-8 can also activate the intrinsic apoptotic pathway. Therefore caspase-8 cleaves the pro-apoptotic protein Bid. Bid activates two proteins; Bax and Bak. These proteins lead to the formation of pores in the mitochondrial membrane. By these pores, cytochrome c is released into the cytosol which activates caspase-9 and eventually leads to apoptosis (3, 4). In Figure 1, the intrinsic and extrinsic pathways are described.

Tumor cells can be distinguished by the apoptotic pathway they use. Cancer cells that only need the extrinsic pathway to induce apoptosis are Type-I cells. Cancer cells that need the extrinsic and intrinsic pathway to induce apoptosis are Type-II cells (5, 6)

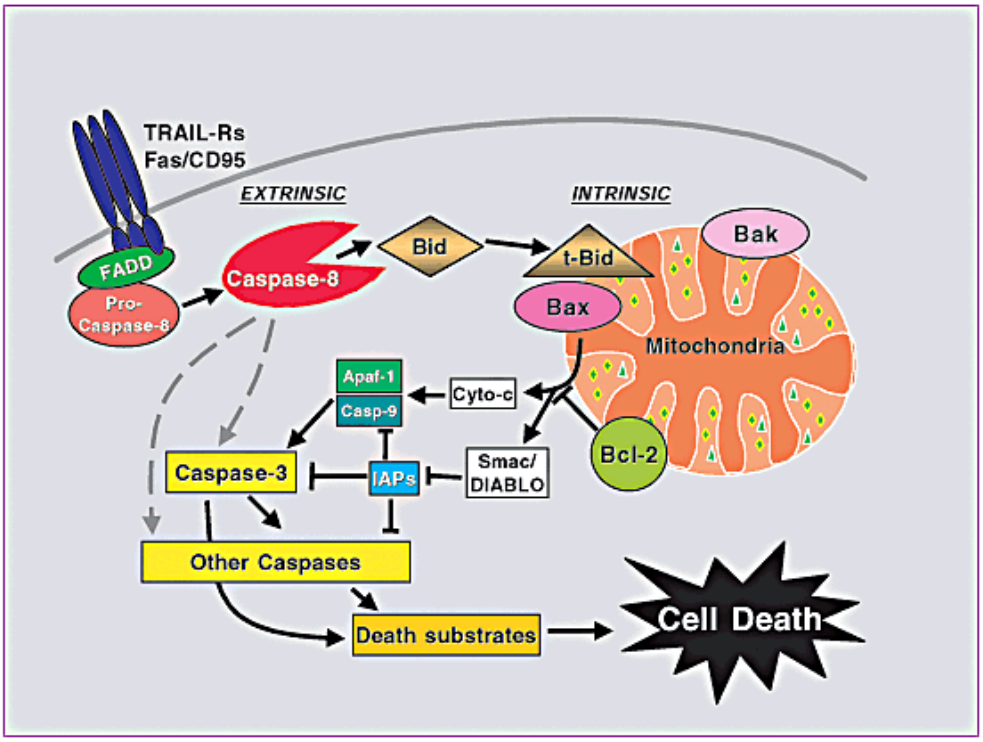

Figure 1: The TRAIL-induced apoptosis extrinsic and intrinsic pathway. Caspase-8 plays a role in both pathways. It either activates other caspases which eventually leads to the induction of apoptosis (extrinsic) or it activates Bid leading to cytochrome-c release which eventually leads to apoptosis (intrinsic). (Roth W, Reed JC. 2002. *Apoptosis and Cancer: When BAX is TRAILing away.* Nature Medicine. 8: 216-218)

2.2 TRAIL resistance

Although TRAIL-treatment seems to be a promising therapy, one of the problems that TRAIL therapy faces is the development of resistance by tumor cells. Many studies showed different tumor cell types to be TRAIL-resistant. This led to the identification of apoptosis inhibiting pathways or proteins.

Resistance can arise at different sites in the apoptotic pathway. High expression of the decoy receptors DcR1 and DcR2 leads to competition for TRAIL-binding by TRAIL-R1, TRAIL-R2, DcR1 and DcR2 (3, 7). Although DcR1 and DcR2 have a lower affinity for TRAIL than TRAIL-R1 and TRAIL-R2 (34), they are able to bind TRAIL. Thereby, TRAIL binds less TRAIL-R1 and TRAIL-R2, which decreases the TRAIL-induced apoptosis. Resistance could also be induced by high expression of cFLIP (cellular FLICE inhibitory protein) which regulates apoptosis. A study by Geserick et al (8) showed an increase in TRAIL-sensitivity when cFLIP is downregulated. cFLIP competes with caspase-8, whereby less caspase-8 is activated and less apoptosis is induced (8). Another way of TRAIL resistance is the high expression of IAP (inhibitor of apoptosis) which is often activated in cancers and inhibits apoptosis (9) Another experiment showed that cells were resistant for TRAIL-treatment when NFκB or PKC was upregulated (10).

It is also possible that due to mutations, deletions or epigenetic silencing the expression of certain proteins, which are involved in apoptosis, is changed. This was shown for caspase-8 which was not or low expressed in some TRAIL-resistant cells (11).

It is clear that this TRAIL resistance can be caused by many different proteins, which makes TRAIL-treatment less favourable. Fortunately, by combining TRAIL-treatment with sensitizing agents, we can often overcome this resistance. These agents are able to inhibit the anti-apoptotic proteins, like cFLIP and IAP, or increase the expression of TRAIL-R1 and TRAIL-R2, making cells more sensitive for TRAIL-treatment (8, 9, 10, 11).

2.3 Dual activity

As mentioned in section 2.1, TRAIL can activate TRAIL-R1 and TRAIL-R2 which eventually leads to apoptosis by an extrinsic (and intrinsic) pathway. But research showed that activation of the death receptors could also lead to non-apoptotic signals, known as the non-canonical pathway. Different studies showed that TRAIL promoted migration (12, 13), invasiveness (12), proliferation (13, 14, 15) and survival (14, 15) in different TRAIL-resistant cancer cells. These anti-apoptotic effects indicate that TRAIL might have dual activity, which could explain the observed TRAIL-resistance.

This dual activity is mostly induced by the formation of a secondary complex, next to the formation of the DISC complex. This secondary complex exists of RIP1, TRAF2, NEMO, caspase-8 and FADD which leads to the activation of specific pathways (16), see Figure 2. It was shown that RIP1 could induce the activation of p38 and NFκB and that inhibition of RIP1 increased TRAIL-mediated apoptosis (16). RIP1 is able to phosphorylate IκB leading to the activation of NFκB. NFκB can travel to the nucleus where it functions as a transcription factor for non-apoptotic genes (5). RIP1 also phosphorylates p38 which eventually leads to an increase in Mcl-1, an oncogene which also promotes the carcinogenesis (5). TRAF2 was able to activate JNK which also showed to have non-apoptotic effects (5, 16). Besides non-apoptotic effects caused by the formation of this secondary complex, other pathways, like ERK can also be involved in causing these effects (5, 13, 15)

It was shown that NFκB, p38 and JNK can also have apoptotic effects, suggesting that the observed dual effect of TRAIL might be caused by the dual effect of these proteins (5, 16). The observed apoptotic effects in TRAIL-resistant cancer cells induced by TRAIL can be due to a shift in balance between apoptotic and non-apoptotic signals.

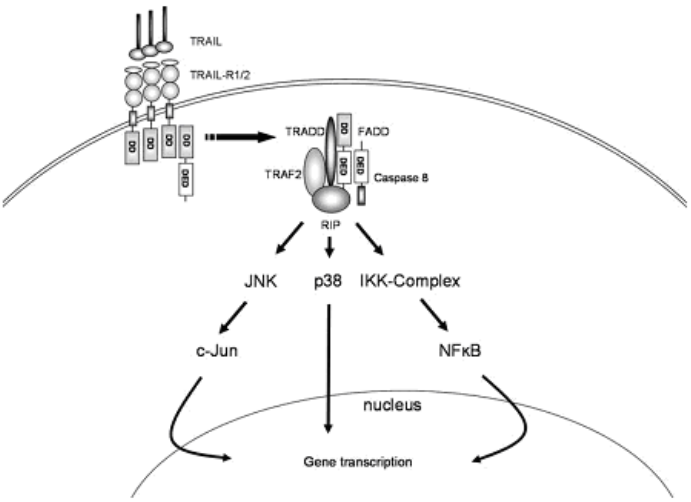

Figure 2: TRAIL leads to the activation of non-apoptotic pathways like JNK, p38 and NFκB. The gene transcription can either support or inhibit apoptosis. (Newsom-Davis T, Prieske S, Walczak H. 2009. *Is TRAIL the holy grail of cancer therapy?* Apoptosis. 14: 607-623)

2.4 TRAIL receptors 1 and 2

As mentioned before, TRAIL is able to bind five different receptors of which two are able to induce apoptosis. Although these apoptosis-inducing receptors, TRAIL-R1 and TRAIL-R2, are homologous, they are only identical for 58% (29). The differences between TRAIL-R1 and TRAIL-R2 are due to differences in their amino acid sequence, which are shown in Figure 3. Figure 3 also shows the amino acid sequence of TRAIL-R3. It is clear that TRAIL-R1 and TRAIL-R2 show more similarity compared to TRAIL-R3. The transmembrane segment (TM) is indicated within the figure. The death domain (DD) which is in the intracellular part, is also indicated in the figure. It is shown that there is not much difference between TRAIL-R1 and TRAIL-R2 in the transmembrane region, but that there are many differences in amino acid sequence in the death domain region. This might influence the function of both receptors causing differences in function between them. The two cysteine domains are also indicated, these are important for ligand binding.

Research showed that only TRAIL-R2 contained sequences adjacent to the transmembrane region. Another difference was the presence of N-linked glycosylation sites in TRAIL-R1 which TRAIL-R2 did not have (29). This glycosylation is a posttranslational process which occurs in the endoplasmic reticulum. It was shown that TRAIL-R1 can also function without the glycosylation (32).

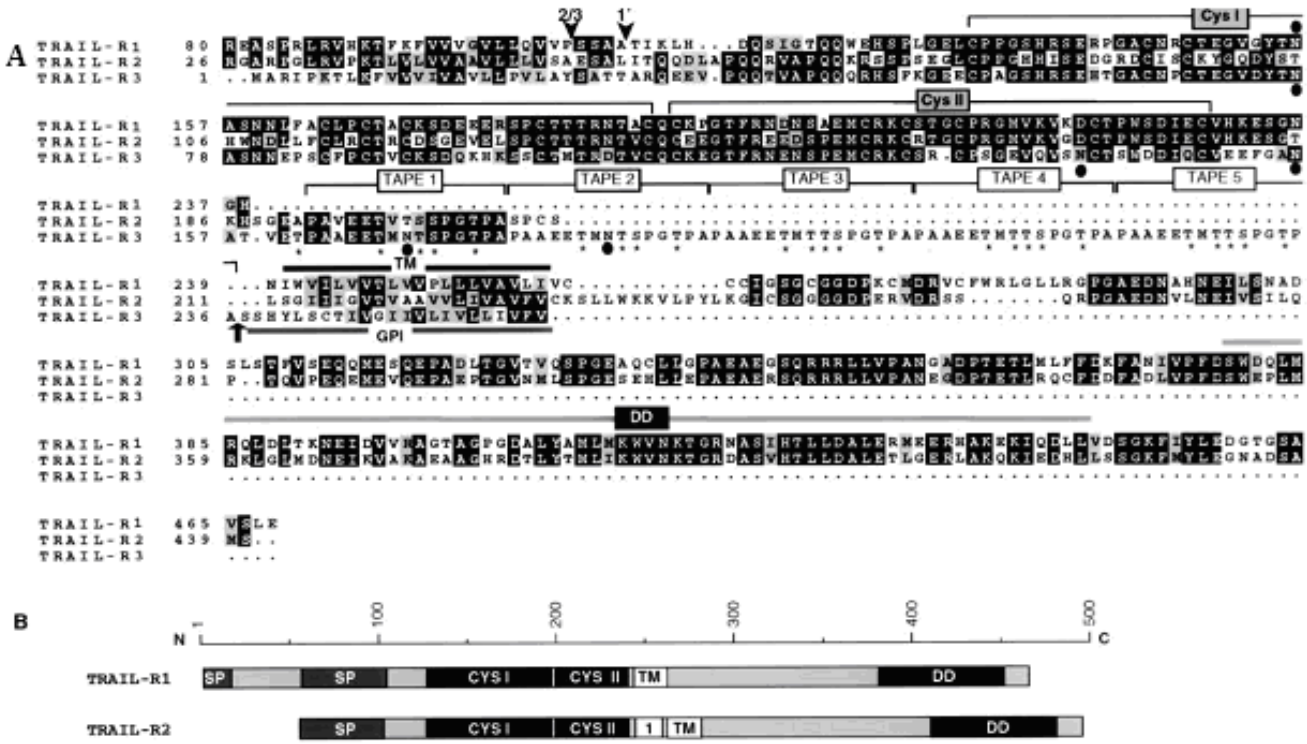

Figure 3: Amino acid sequence of TRAIL-R1 and TRAIL-R2. TRAIL-3 is also shown. A: Amino acid sequence of TRAIL-R1, TRAIL-R2 and TRAIL-R3. Cys I and Cys II are cysteine-rich domains. TM is transmembrane segment and DD is death domain. B: Schematic representation of the TRAIL receptors. (Schneider P, Bodmer J-L, Thome M, Hofmann K, Holler N, Tschopp J. 1997. *Characterization of two receptors for TRAIL*. FEBS Letters. 416: 329-334.)

3. TRAIL receptor specific apoptosis activation

Although many studies showed TRAIL-induced apoptosis by activation of TRAIL-R1 and TRAIL-R2, it was not clear whether these receptors had a different contribution to this apoptosis induction (8, 9, 10, 11). It was shown that in cells that express both receptors, TRAIL-R2 has a higher affinity for TRAIL than the other receptors at 37 °C which resulted in more TRAIL-TRAIL-R2 interactions (27). Therefore, it was thought that TRAIL-R2 contributes more to apoptosis when both receptors are expressed. This was confirmed by multiple studies that showed TRAIL-mediated apoptosis was mainly induced by TRAIL-R2 activation in different cell types. But lately there have also been studies that showed that TRAIL-mediated apoptosis was mainly induced by TRAIL-R1 activation which is contradictory with the suggestion that TRAIL-R2 predominantly induces apoptosis when both receptors are expressed. Table 1 shows the receptor-dependent apoptosis in different cell types. Next, an overview will be given about the different contributions of TRAIL-R1 and TRAIL-R2 to TRAIL-mediated apoptosis in multiple cancer cell types and the possible underlying mechanisms will be discussed.

TRAIL-mediated apoptosis by:	**Tumor cell type**	**References**
DR4 (TRAIL-R1)	AML	28
DR5 (TRAIL-R2)	Breast	21
DR4 (TRAIL-R1)	CLL	17, 18
DR5 (TRAIL-R2)	Colon	21
DR5 (TRAIL-R2)	Glioblastoma	22
DR5 (TRAIL-R2)	Hepatocarcinoma	23
DR4 (TRAIL-R1)	MCL	17, 18
DR4 (TRAIL-R1)	Melanoma	19
DR4 (TRAIL-R1)	Pancreas	20

Table 1: Overview of TRAIL-mediated apoptosis in different cell types by either TRAIL-R1 or TRAIL-R2.

3.1 Apoptosis by TRAIL-R1

There are studies that showed that TRAIL-mediated apoptosis predominantly is induced by TRAIL-R1 activation. This was shown in leukemic cells (17, 18, 28), melanoma cells (19) and pancreatic adenocarcinoma cells (20).

The studies by MacFarlane et al (17, 18) were performed with chronic lymphocytic leukemia (CLL) cells and with mantle cell lymphoma (MCL) cells. They used TRAIL mutants that were developed to specifically bind to either TRAIL-R1 or TRAIL-R2. For TRAIL-R1, HGS-ETR1 was used, also known as mapatumumab. For TRAIL-R2, HGS-ETR2 was used, also known as lexatumumab. These mutants are currently in Phase I and II studies. In the study by MacFarlane et al, DISC formation was analysed as measurement for apoptosis. For both CLL and MCL cells it was shown that, after sensitizing the cells for TRAIL-treatment, the TRAIL-R1 specific TRAIL mutant induced more apoptosis compared to the TRAIL-R2 specific TRAIL mutant. This confirmed their hypothesis that apoptosis in CLL cells was mainly induced by activation of the TRAIL-R1 receptor.

Another study performed by Kurbanov et al (19) tested TRAIL-R1 and TRAIL-R2 for inducing apoptosis in seven melanoma cell lines. All cell lines showed TRAIL-R2

expression, only two (A-375 and SK-Mel-13) showed TRAIL-R1 expression. When cell lines were treated with TRAIL, TRAIL-R1-positive cells were more sensitive for TRAIL-mediated apoptosis. TRAIL-R1-negative (but TRAIL-R2-positive) cells showed a lower response to apoptosis induced by TRAIL. Whereas MacFarlane et al studied the effects of agonistic TRAIL-receptor specific antibodies, this study examined the effects of TRAIL-R1- and TRAIL-R2-specific antagonistic antibodies on the TRAIL-R1-positive cell lines. It was shown that TRAIL-R1-specific antibodies blocked apoptosis for 70% (A-375) and 83% (SK-Mel-13), while TRAIL-R2-specific antibodies could only block apoptosis for 23% (A-375) and 44% (SK-Mel-13). Although not all melanomas show TRAIL-R1 expression, the melanomas that do express TRAIL-R1 show a greater contribution of TRAIL-R1 to TRAIL-mediated apoptosis than TRAIL-R2 (19).

The effects of TRAIL-R1 and TRAIL-R2 on pancreatic ductal adenocarcinoma (PDAC) was examined by Lemke et al (20). For their experiments they used receptor specific agonistic antibodies; mapatumumab which is TRAIL-R1-specific and lexatumumab which is TRAIL-R2-specific. These are the same antibodies there were used in the study by MacFarlane et al (17, 18). The used cell lines, Colo357, Panc89 an PT45, all expressed both TRAIL-R1 and TRAIL-R2. Although it was shown that both receptors were able of inducing TRAIL-mediated apoptosis, Lemke et al showed that apoptosis in PDAC cells was mostly induced by TRAIL-R1. An interesting observation they made was the 'reactivation' of apoptosis induced by TRAIL-R2 by treating cells with Goe6983, a PKC inhibitor.

The study by Szegezdi et al (28) analysed TRAIL-mediated apoptosis in acute myelogenous leukaemia (AML) by testing four AML cell lines (HL-60, ML-1, MOLM-13, OCI-AML3) which all expressed both TRAIL-R1 and TRAIL-R2. This study showed that apoptosis is predominantly induced by TRAIL-R1. For the development of a specific TRAIL-R1 mutant, three substitutions were made; glycine to arginine at position 131, asparagine to arginine at position 199 and lysine to histidine at position 201. This mutant, rhTRAIL-C3, showed to have a threefold increase in affinity for TRAIL-R1. Testing rhTRAIL-C3 in the cell lines showed an increase in apoptosis compared to normal TRAIL in three cell lines (OCI-AML3 showed to be completely resistant for TRAIL-treatment). And TRAIL-R1 activation and apoptosis were faster induced compared to normal TRAIL. Treating the cell lines with a TRAIL-R2 selective TRAIL led to a weak apoptotic response. Therefore, this research showed apoptosis is predominantly induced by TRAIL-R1 in AML (28). Together with the results of MacFarlane et al (17, 18) it might be that TRAIL-R1 mediated apoptosis is specific for leukemic cell lines.

Altogether, these data suggest that TRAIL receptor 1 predominantly induces apoptosis when cells are treated with TRAIL. This was shown for leukemic cells, melanoma cell lines and pancreatic adenocarcinomas.

3.2 Apoptosis by TRAIL-R2

Besides apoptosis that is predominantly induced by TRAIL-R1, there is also evidence that TRAIL-R2 induces apoptosis predominantly. This was shown in colon carcinomas and breast carcinomas (21), glioblastomas (22) and hepatocarcinomas (23).

Kelley et al (21) showed that TRAIL-R1 and TRAIL-R2 induce apoptosis independently of each other. They selected ligand variants that selectively bound to either TRAIL-R1 or TRAIL-R2. For TRAIL-R1 FLAG-Apo2L.DR4-8 was used and for TRAIL-R2 they used FLAG-Apo2L.DR5-8. The response of the cells towards these

variants was analysed in two colon carcinoma cell lines, Colo205 and Colo320, and in a breast cancer cell line, MDA-MB-231. The TRAIL-R2-specific variant showed higher levels of apoptosis compared to the TRAIL-R1-specific variant in all cell lines. Some cells even showed reduced apoptosis by the TRAIL-R1-specific variant. Therefore they suggested that TRAIL-R2 contributes more to apoptosis in breast and colon cancer cells than TRAIL-R1.

The study by Bellail et al (22) analysed the apoptotic pathway in glioblastoma cells. They showed that most of these cell lines did not or very low expressed TRAIL-R1 and that TRAIL-R1 could not interact with TRAIL. Knock-down of TRAIL-R2 inhibited TRAIL-mediated apoptosis, whereas TRAIL-R1 knock-down had no effect. This study showed that TRAIL-mediated apoptosis is induced by TRAIL-R2 in glioblastoma cells.

Charette et al (23) performed an experiment with hepatocarcinoma cell lines which are TRAIL resistant. Therefore, cells were treated with salirasib or YM155 which sensitized cells for TRAIL treatment. TRAIL-R1 was expressed in two cell lines (HepG2 and Hep3B) and TRAIL-R2 expression was observed in three cell lines (HepG2, Hep3B and Huh7). They showed that TRAIL-R1 inhibition partially inhibited the apoptotic effects of TRAIL, whereas TRAIL-R2 inhibition almost completely inhibited the effects of TRAIL. This was confirmed by treating these cells with a TRAIL-R2 agonistic antibody (and salirasib) which induced apoptosis. Thus, apoptosis is predominantly induced by TRAIL-R2 in hepatocarcinomas (23).

Besides the differences in apoptosis induction by TRAIL-R1 and TRAIL-R2, the earlier described dual activity (section 2.3) might also be influenced by the apoptosis inducing TRAIL receptors in different ways, meaning that non-apoptotic signals can also be predominantly induced by either TRAIL-R1 or TRAIL-R2. Although there is not many evidence for this hypothesis, a study by Azijli et al (24) showed the induction of non-apoptotic signals by TRAIL-R2. They used selective TRAIL-R1 and TRAIL-R2 TRAIL-variants, respectively 4CT and DHER, which they tested in three cell lines of non-small cell lung cancer (NSCLC). Their results showed that DHER led to an increase in migration and invasiveness in one of the cell lines (A549). Because TRAIL-R1 activation by 4CT did not show these effects it is suggested that this is mediated by TRAIL-R2 activation. Another study by Belyanskaya et al (15) showed TRAIL-R2-mediated proliferation in small cell lung cancer cell lines. Four of six tested cell lines showed increased proliferation (up to 40%) after TRAIL-treatment. This was mediated by TRAIL-R2 which led to activation of the ERK-pathway. Their results showed almost no expression of TRAIL-R1 on all tested cell lines, therefore it might be that TRAIL-R1 has the same effects when it is expressed.

It is possible that these non-apoptotic effects are specific for lung cancer cells.

4. Underlying mechanisms

In the previous part different studies showed TRAIL-mediated apoptosis being induced by either TRAIL-R1 or TRAIL-R2. These studies confirmed the hypothesis that TRAIL-R1 and TRAIL-R2 differ in their contribution to TRAIL-mediated apoptosis. But it is unclear how these differences are regulated. The TRAIL-mediated apoptotic pathway can be affected at multiple levels leading to these differences.

First of all, the TRAIL receptors, decoy receptors as well as apoptosis-inducing receptors, could be involved in the differences of apoptosis induction. Because TRAIL-R1 and TRAIL-R2 are only identical for 58%, they could differ in function. As mentioned before, only TRAIL-R2 contains a sequence adjacent to the transmembrane region. It was suggested that this region possibly had a regulatory function (29), which could be an explanation for the differences between TRAIL-R1 and TRAIL-R2. Also decoy receptor 2 (TRAIL-R4) could influence the TRAIL-mediated apoptosis. Different studies (33, 34) showed that high expression of the decoy receptors led to the inhibition of apoptosis and low expression of the decoy receptors sensitized cells for TRAIL-mediated apoptosis. The apoptosis inhibition by decoy receptor 1 was mostly induced by the earlier mentioned competition. The apoptosis inhibition by decoy receptor 2 is obtained by the formation of heterotrimeric complexes which exist of TRAIL-R2 and TRAIL-R4. Because of the presence of TRAIL-R4, the formation of the DISC complex is disrupted and there is no apoptosis induction. It was also shown that TRAIL-R4 was able to interact with TRAIL-R1, but this interaction was much weaker (33). Therefore, it might be possible that cells that highly express TRAIL-R4, are not able to induce apoptosis by TRAIL-R2 and thereby TRAIL-mediated apoptosis is predominantly induced by TRAIL-R1 in these cells.

A second explanation for the differences in TRAIL-mediated apoptosis could be the gene expression of TRAIL-R1 and TRAIL-R2. It is possible that due to mutations or epigenetic silencing the expression of one of the receptors is lost. Although loss of gene expression of one of the TRAIL receptors leads to apoptosis induction by the other receptor, most studies showed that both receptors are expressed but one predominantly induces apoptosis. Therefore the differences in apoptosis could not be a result of gene silencing or mutations in either one of the receptors, because in that case there would be no expression of that gene.

Third, it might be that there are a few mutations in the TRAIL-R1 or TRAIL-R2 encoding gene, whereby there is still a TRAIL-receptor protein, but with a slightly different conformation. This might influence TRAIL binding, receptor trimerization or DISC formation which might lead to a less active receptor.

Another possibility which might cause differences between the receptors are posttranslational modifications. These modifications include O-linked glycosylations and the earlier mentioned N-linked glycosylations. O-linked glycosylation is important for protein function and this is often deregulated in cancer. Wagner et al (39) studied the effects of GALNT14, a O-glycosylation initiating enzyme, in different cancer cell lines. It was shown that GALNT14 mRNA expression was significantly higher in TRAIL-sensitive cells compared to TRAIL-resistant cells. Conversely, when GALNT14 was knocked down with the use of siRNA, TRAIL-sensitivity decreased. It was also shown that both TRAIL-R1 and TRAIL-R2 contain O-glycosylation sites. Altogether, it was shown that O-glycosylation of TRAIL-R1 and TRAIL-R2 led to an increased sensitivity for TRAIL which was promoted by receptor clustering and the activation of caspase-8 (39). It might be

that there are differences in glycosylation between TRAIL-R1 and TRAIL-R2, thereby altering their function.

The most important explanation for the differences in apoptosis by TRAIL-R1 and TRAIL-R2 is that they activate different apoptotic pathways. When one of the pathways or receptors is affected, TRAIL can still induce apoptosis via the other receptor and pathway. This leads to TRAIL-mediated apoptosis predominantly via one receptor although both receptors are expressed. The explanation that TRAIL-R1 and TRAIL-R2 are able to activate different pathways is supported by a study that analysed these differences in receptors towards TRAIL-mediated apoptosis (25).

In this study by Ren et al (25) specific TRAIL-R1 and TRAIL-R2 antibodies were used and the effect of many siRNAs (small interfering RNA) on TRAIL-R1- and TRAIL-R2-induced apoptosis was analysed. They found that treatment with certain siRNAs led to an increase or decrease in apoptosis. They focussed on the siRNA that silenced SRP72, a component of the SRP (signal recognition particle) complex. The SRP complex is a ribonucleoprotein that transports specific proteins from the ribosome towards the endoplasmic reticulum (26). Silencing SRP72 with siRNA led to the inhibition of apoptosis by TRAIL-R1. It did not affect TRAIL-R2-mediated apoptosis. The next step was to examine whether this effect was caused by silencing SRP72 or the whole SRP complex. They showed that silencing the SRP complex led to a decrease in TRAIL-R1-mediated apoptosis, suggesting this effect is due to silencing the SRP complex and not SRP72. It was shown that the SRP complex is necessary for TRAIL-R1 cell surface localization but not for TRAIL-R2. Silencing SRP decreased the TRAIL-R1 cell surface localization, but TRAIL-R1 expression was not affected (25). The results of this study contribute to the hypothesis that underlying pathways differ between TRAIL-R1 and TRAIL-R2.

There are also other studies that provided evidence that the underlying pathways differ between TRAIL-R1 and TRAIL-R2. It was shown that both TRAIL-R1 and TRAIL-R2 used caspases to activate their death pathway and that TRAIL-R2 used FADD in this pathway (29). Pan et al (35) and Yeh et al (36) showed that TRAIL-R1 was able to induce apoptosis, but that TRAIL-R1 did not use FADD suggesting that TRAIL-R1 used other mechanisms to induce apoptosis. This might be necroptosis, whereby apoptosis is induced via receptor –interacting protein RIP1 or RIP3 (5). Although these studies showed that FADD is involved in TRAIL-R2 mediated apoptosis and not in TRAIL-R1 mediated apoptosis, there also have been studies that showed that both TRAIL-R1 and TRAIL-R2 use FADD in their apoptotic signalling pathway (37, 38).

5. Discussion:

This review showed that TRAIL is a promising drug but that the use of it is not as easy as it seems. It was shown that tumor cells show resistance towards TRAIL and that TRAIL has pro-survival effects. With the use of combination therapy, whereby a sensitizing agents together with TRAIL is used, we can overcome this resistance and dual activity. In this review we focussed on the question whether TRAIL-R1 and TRAIL-R2 contribute differently to the induction of apoptosis and what the possible underlying mechanisms are. It was shown that the TRAIL-receptors indeed differ in their contribution to apoptosis induction, which might be cell type specific. TRAIL-R1 predominantly induces apoptosis in leukemic cancer cells, pancreatic cancer cells and melanoma cancer cells, whereas TRAIL-R2 induces apoptosis predominantly in colon cancer cells, breast cancer cells, glioblastoma cancer cells and hepatocarcinoma cells.

Besides the differences in apoptosis induction between the receptors that we have showed, we have tried to identify underlying mechanisms. It was suggested that the apoptotic pathway can be affected at multiple levels and that a promising method to identify the different apoptotic pathways is with the use of siRNAs. As the study by Ren et al (25) already showed, treatment with siRNAs can show which proteins are important for both TRAIL receptors. Therefore it is important that the effects of other siRNAs on TRAIL-R1 and TRAIL-R2 are analysed. This might results in the identification of other proteins that are involved in the TRAIL-R1- or TRAIL-R2-mediated apoptosis.

The study by Ren et al also showed the importance of the SRP complex for TRAIL-R1-mediated apoptosis. Therefore it might be useful to analyse cells that induce apoptosis predominantly by TRAIL-R2 for mutations in the SRP complex or pathway.

It was also shown that decoy receptor 2 might play a role in the different contributions of TRAIL-R1 and TRAIL-R2 to apoptosis. To confirm or reject the hypothesis that high decoy receptor 2 expression inhibits TRAIL-R2 mediated apoptosis whereby apoptosis is mostly induced by TRAIL-R1, it is important that the effects of decoy receptor 2 will be studied in detail.

It was also discussed whether TRAIL-R1 uses FADD to induce apoptosis or that it induces apoptosis by a necroptotic pathway. Because studies are contrary about this, it is important that this will be studied.

Because there is not much evidence about the way TRAIL-R1 and TRAIL-R2 contribute differently to TRAIL-mediated apoptosis, it will be necessary to identify the TRAIL-R1 and TRAIL-R2 pathways to see where they exactly differ. That way it might be possible to predict which TRAIL receptor will induce apoptosis by analysing which pathway is affected. When we can identify proteins that are involved in TRAIL-receptor mediated-apoptosis, these proteins could possibly work as biomarkers to predict which receptor induces apoptosis.

Besides studying the different pathways of TRAIL-R1 and TRAIL-R2 it is also important to determine whether this receptor-dependent apoptosis is cell type specific. As the results of TRAIL-R1 dependent apoptosis showed (section 3.1), it is possible that leukemic cells predominantly induce apoptosis by activating TRAIL-R1. Whether this is the same in all leukemic cell types should be analysed. Also of other cell types it should be analysed whether apoptosis is being induced by one TRAIL receptor and whether this is consistent in cells. Besides determining the active TRAIL receptor in cell types it is important to understand why TRAIL-R1 is most active in one cell type whether TRAIL-R2 is most active in the other cell type. This might be due to mutations, which inhibit one of the underlying pathways, but no evidence is found yet to support this hypothesis.

To determine which receptor induces apoptosis predominantly, new techniques should be developed. They should not measure expression, but functionality of both receptors. The technique should be able to quantify how much each receptor contributes to the TRAIL-mediated apoptosis. A possible way is with an assay in which certain tumor cells are treated with either specific TRAIL-R1 or TRAIL-R2 variants. That way, it can be determined which patient must be treated with which specific TRAIL-receptor variant.

We have seen in the discussed studies that many TRAIL-receptor specific variants were used. To develop these variants, small changes in the amino acid sequence are being made. Therefore it is important that the new variant is still functional and that it has an increased affinity for one of the TRAIL receptors. These specific TRAIL-R1 and TRAIL-R2 TRAIL mutants are developed by changing a few amino acids. By changing the protein it can have a slightly different conformation, making it more specific for one of both receptors. In a study by van der Sloot et al (30), a TRAIL-R2 specific variant was developed. They showed that the amino acid at position 269 is very important for TRAIL-R2 selective binding. In normal TRAIL, there is a aspartic acid at this position. But substituting this amino acid for a histidine and substituting threonine for a arginine at position 214 results in a TRAIL-R2 specific variant. The aspartic acid of normal TRAIL interacts with a lysine at position 120 of the receptor. But TRAIL-R2 does not have a lysine at that position, but an aspartic acid. By using the TRAIL-R2 variant, the histidine does not interact with the lysine making the affinity for the other receptors lower, but the histidine does interact with the aspartic acid leading to a higher affinity for TRAIL-R2 (30). They showed that only two mutations are already enough to develop a specific TRAIL-R2 variant.

Because there are differences in the apoptosis-inducing capacity of the receptors, it is clear that it is necessary to develop TRAIL-R1 and TRAIL-R2 specific TRAIL mutants, which only activate one of both receptors. These TRAIL-R1 and TRAIL-R2 specific variants are important for research were they are used to determine which receptor is most active. Furthermore, they are important as therapy which will probably optimize TRAIL-treatment and will replace the normal TRAIL protein. Currently, there are many TRAIL-receptor variants being developed (3).

TRAIL-receptor specific variants will be very useful as therapy when it is possible to determine which receptor contributes most to TRAIL-mediated apoptosis. Patients will then only be treated with the specific TRAIL variant instead of the normal TRAIL. Due to the high affinity of the specific TRAIL variant, success of a treatment can be maximized. This will lead to person-specific treatments.

It will still be necessary to use a combination therapy and treat patients also with a sensitizing agent to decrease TRAIL resistance and the dual activity.

Most of the discussed studies examined the apoptotic effect of the death receptors in cell lines (19, 20, 23). It might be that tumor cells from patients show other results. Therefore it is important that these experiment are repeated with humane tumor cells. This might also provide more information about whether the differences of TRAIL-R1 and TRAIL-R2 are cell type specific.

Besides the advantages that TRAIL does not affect healthy cells and that it is a wide-range drug, another advantage of TRAIL therapy is the possibility to induce apoptosis in a different way than the often used p53 pathway. P53 is a tumor suppressor gene which is often mutated in cancer cells. Therefore, apoptosis cannot be induced by p53. By treating these p53-deficient cells with TRAIL, apoptosis might be induced again.

Altogether, this review showed that there are different contributions of TRAIL-R1 and TRAIL-R2 to TRAIL-mediated apoptosis which might be cell type specific. Although the underlying mechanisms are not well understood, the discussed studies show an important role for TRAIL-treatment in cancer cells , although TRAIL will probably be replaced for receptor-specific TRAIL mutants whereby only the active receptor will be affected. It was shown that receptor-specific TRAIL mutants can be used in many different tumor cell types, which makes TRAIL a wide-range drug.

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