Aberrant DNA hypermethylation and apoptotic defects in pediatric neuroblastomas
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Abstract
TRAIL (TNF-Related Apoptosis Inducing Ligand) induces apoptosis in a large variety of cancer cells, but not in most normal human cells. This feature makes TRAIL a potential anti-tumor agent. TRAIL can bind to four different receptors, two pro-apoptotic death receptors DR4 and DR5 and two anti-apoptotic decoy receptors DcR1 and DcR2. Normal cells express all four receptors. The increased TRAIL sensitivity of tumor cells has been postulated to result from the lack of decoy receptor expression. We studied the tumor specific down-regulation of the TRAIL receptors DcR1 and DcR2, as well as DR4 and DR5 in a group of pediatric tumor cell lines (9 neuroblastoma and 3 peripheral PNET’s) and 3 cell lines from adult tumors. Lack of expression of DcR1 and DcR2 was widespread (13/15 and 10/15 respectively), both in the adult tumor cell lines as well as in the pediatric tumor lines. DR4 and DR5 were expressed in 8/15 and 12/15 cell lines respectively. To understand the tumor specific down-regulation of the TRAIL receptors, the promoter regions were studied for possible methylation changes of their CpG islands. All normal tissues were completely unmethylated, whereas in the tumor cell lines we found frequent hypermethylation of the promoter. For DcR1 and DcR2 we found dense hypermethylation in 9/13 (69 %) and 9/10 (90 %) of non-expressing cell lines respectively. DR4 and DR5 were methylated in 5/7 (71%) and 2/3 (67%) non-expressing cell lines respectively. Treatment with the demethylating agent 5-aza-2’ deoxycytidine resulted in partial demethylation and restored mRNA expression. In addition, we performed mutation analysis of the death domains of DR4 and DR5 by sequencing exon 9. Mutations were not present in any of the neuroblastoma or PNET cell lines.

A panel of 28 fresh neuroblastoma tumor samples also lacked expression of DcR1 and DcR2 in 85 % and 74 % of cases respectively. Hypermethylation was observed in 6/28 (21 %) for DcR1 and 7/28 (25 %) for DcR2. DR4 and DR5 were both expressed in 22/28 tumors, and no promoter methylation was observed.

These data suggest that hypermethylation of the promoters of DcR1 and DcR2 is important in the down-regulation of expression in neuroblastoma and other tumor types.
Hypermethylation of DcR1 and DcR2 in tumors

Introduction

TRAIL is a TNF related ligand of receptors which modulate programmed cell death\(^1\)\(^2\). Pro-apoptotic TRAIL signaling is mediated through death receptor 4 (DR4, also TNFRSF10A, Apo-2, TRAIL-R1) and death receptor 5 (DR5, also TNFRSF10A, KILLER/DR5, TRICK2, TRAIL-R2). These receptors signal apoptosis by association of their intracellular death domain with similar domains in intracellular adapter proteins like FADD and pro-caspase \(^3\)\(^4\). Subsequently, caspase 3 and the common route to programmed cell death are activated. This pro-apoptotic effect of TRAIL is counteracted by the decoy receptors 1 (DcR1, also TNFRSF10C, TRID, TRAIL-R3) and 2 (DcR2, also TNFRSF10D, TRUNDD, TRAIL-R4), which are structural homologues of the death receptors but defective in their death domains. DcR1 completely lacks the intracellular death domain and DcR2 contains a truncated, non-functional death domain\(^5\)\(^9\). Both receptors bind TRAIL, but are unable to associate with the intracellular signaling molecules of apoptosis. They thus act as dominant negative receptors for TRAIL. All TRAIL receptors have been mapped to the same chromosomal locus 8p21-22, suggesting that they have evolved as a result of gene duplication\(^9\).

Normal tissues usually express all four TRAIL receptors and this balance prevents TRAIL induced apoptosis. Cancer cells on the other hand, often lack expression of the decoy receptors \(^5\)\(^7\)\(^10\). The unbalance in favor of pro-apoptotic receptors was postulated to determine their increased sensitivity to TRAIL induced apoptosis. Support for this hypothesis was found in transfection assays in which re-expression of DcR1 in melanoma cells lacking endogenous DcR1 altered their TRAIL sensitive phenotype into a TRAIL resistant one\(^11\). However, later studies involving multiple cancer cell lines of varying origin, and leukemia samples from patients, could not establish a correlation between down-regulation of the decoy receptors and TRAIL sensitivity, or even a reverse correlation \(^12\)\(^14\). These conflicting data concerning the role of the decoy receptors in apoptosis have not been clarified yet.

Apoptotic defects are believed to play a major role in pediatric neuroblastomas. Neuroblastomas are exceptional tumors because they are resistant to TRAIL induction of apoptosis. It has been shown that down-regulation of caspase 8 is important in this TRAIL resistant phenotype\(^15\)\(^18\). However, not all neuroblastomas have down-regulated caspase 8, and little is known about the correlation between expression of the TRAIL receptors and TRAIL sensitivity in neuroblastomas. Here, we studied the expression and methylation status of all four TRAIL receptors in a group of pediatric tumor cell lines (9 neuroblastomas and 3 peripheral PNET’s; primitive neuro-ectodermal tumors) and cell lines from adult brain, colon and skin tumors. Our data suggest that hypermethylation of DcR1 and DcR2 is involved in down-regulation of gene expression in tumor cell lines and fresh neuroblastoma tumors.
**Materials and methods**

**Cell Culture**
Cell lines were cultured at 37 °C, 5% CO₂, using Dulbecco’s Modified Essential Medium (DMEM; Gibco) containing 10% Fetal Calf Serum, 292 μg/ml L-Glutamine, 1% 100x MEM (Non-essential amino acids medium, Gibco) and 0.5% Penicillin/Streptomycin solution. Freshly prepared 5-aza-2’deoxycytidine (5-AZA; Sigma) was added three times (2 μM) in the last week before harvest, simultaneously with fresh medium. Cells were harvested 24 hours after last addition of 5-AZA, and used for DNA and RNA isolation.

**TRAIL treatment, propidium iodide staining and FACS analysis**
TRAIL (R&D systems Europ Ltd., UK; final concentration 20 ng/ml) treatment was performed in 24-well plates (1-2 x 10^6 cells / ml medium). After 24 hours, cells were harvested, the medium removed, and washed once with PBS and centrifuged at 200 x g. Apoptotic cells were determined by the propidium iodide method 19. Briefly, 500 μl of a hypotonic buffer (50 μg/ml propidium iodide in 0.1 % sodium citrate plus Triton X-100; Sigma) was added directly to the cell pellet. The tubes were placed at 4 °C in the dark overnight before flow cytometry analyzes. The propidium iodide fluorescence of individual nuclei was measured using a FACSscan flow cytometer (Beckman). At least 1x10^4 cells of each sample were analyzed in triplicates for each sample. Apoptotic nuclei appeared as a broad hypodiploid DNA peak, as compared to the diploid DNA peak (G0 or resting cells) or hyperdiploid DNA peak (G2 or dividing cells). Induction of apoptosis after stimulation with TRAIL was defined as a two-fold induction of base line apoptosis or more.

**RT-PCR detection of mRNA**
Total RNA was isolated from cell lines using RNAzol™ B (Cinna, Biotechx Laboratories Inc) according to manufacturers protocol. First strand cDNA synthesis was performed on 2 μg of total RNA in a volume of 20 μl using Superscript™ II (GibcoBRL) and oligo (dT). The specific primers used for mRNA amplification were as follows:

**DR4** (accession nr GI:2460427) F(315): CCAACAAGACCTAGCTCCCCAGC; R(793): AAGA CTACGGGCTGCAACTGACTCC; **DR5** (accession nr GI:1945071) F(295): GTCTTGCTGCAAG TCGTACC; R(681): GATGTCACCCAGGGCGTAC

**DcR1** (accession nr GI2338421) F(205)20: CCCAAAGACCTAAAGGTTGC; R(447) GCAAGAAGGT TCATGGTGGA. **DcR2** (accession nr GI4106963) F(183): ACCCCAGATCTTAAGTTGC; R(426) CAAGAGGCAATGTTTGAGAA **Caspase 8** (accession nr GI4502582) F(516): GGAAGGGGAACT TCAGCAC; R(850) TCAGCAGGCTCTTGTGATTT.

Analysis of expression was performed in a 25 μl PCR reaction containing 1 μl of cDNA, 1 μl dNTP’s (2.5 mmol/l each), 0.5 μl each of the specific primers (150 ng/μl), and 0.25 μl Taq DNA polymerase (5 U/μl; Boehringer). PCR conditions were as follows: 1 cycle, 5 min/95 °C; 35 cycles, 1 min/95 °C,
1 min/60 0C, 1 min/72 0C, and one cycle 5 min/72 0C. PCR products were loaded on a 4% agarose gel (Metaphor; BioWhittaker Molecular Applications, USA), stained with Gelstar nucleic acid gel stain (BioWhittaker Molecular Applications, Rockland, ME, USA) and directly visualized under UV illumination.

Methylation specific PCR (MSP)
Genomic DNA was isolated from cell lines and primary tissues, using standard procedures. Approximately 1 µg of DNA was bisulfite-modified, as described 21. This treatment converts all unmethylated cytosines into uracil. In the subsequent Methylation Specific PCR reaction all uracils become thymidines. The PCR requires primer pairs that specifically recognize methylated or unmethylated sequences. These primers were designed in the 5'-UTR CpG island of the published sequences. The primer sequences are (5' to 3'):

**DcR1**: GAATTTTTTATGTATGAATTTAGTTAAT (unmethylated sense); TTACGCGTA CGAATTAGTTAAC (methylated sense); CCATCAAACCAACAAAACA (unmethylated antisense); ATCAACGACCGACCAGAAA (methylated antisense).

**DcR2**: TTGGGGATAAAAGTGTTTTGATT (unmethylated sense); GGGATAAAGCGTTCGATC (methylated sense); AAACCAACACAAAACCACA (unmethylated antisense); CGACCAACA AAACCGCG (methylated antisense).

**DR4**: GTAGTGATTTTGCAATTGGGAGTGATGTT (unmethylated sense); TTCGAATTTCGG GAGCGTAGC (methylated sense); CTCATACATCTATCCCAACAA (unmethylated antisense); GTAACCAAATCTCCCGCGA (methylated antisense).

**DR5**: TGTTTGAGTAGTGAAGGGTAGATTGTGTT (unmethylated sense); GAGTAGTGAAAGA TTAGTTCGGGTCA (methylated sense); ACAACCAACACATTCTATCCCA (unmethylated antisense); CCGAACGTCTATCCCGCG (methylated antisense).

**Caspase 8**: TAGGGGATTTGGAGATGTGA (unmethylated sense); TAGGGGATTCCGAGATTGC (methylated sense); CCATATATCATTCTAATCCAAACA (unmethylated antisense); CGTATATCT ACTTCGAAACAC (methylated antisense).

PCR reactions are hot started at 95 0C for 15 min, by using 0.25 µl (5 U/µl) of HotStarTaq DNA polymerase (Qiagen). Reactions were performed at 60 0C annealing temperature. Each PCR reaction was loaded on a 6% non-denaturing polyacrylamide gel, stained with ethidium bromide and directly visualized under UV illumination. Genomic DNA treated with Sss1 methylase (New England Biolabs; as instructed by manufacturers protocol) and after bisulfite modification was used as positive control for methylated DNA.

**Results**

Expression of the decoy receptors DcR1 and DcR2
We analyzed the mRNA expression of the decoy receptors DcR1 and DcR2 and death receptors
Chapter 2

Table 1. TRAIL sensitivity of tumor cell lines, in relation to expression and methylation profiles of DcR1, DcR2 and caspase 8

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<th>Cell Lines</th>
<th>Tumor Type</th>
<th>TRAIL Response</th>
<th>DcR1 Methyl</th>
<th>DcR1 mRNA</th>
<th>DcR2 Methyl</th>
<th>DcR2 mRNA</th>
<th>DR4 Methyl</th>
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NBL: Neuroblastoma; PNET: Primitive Neuro-Ectodermal Tumor; Methyl.: Hypermethylation; mRNA: mRNA expression; ND: Not done; No*: Besides the unmethylated product, a methylated product was detected.

DR4 and DR5 in a panel of pediatric neuroblastoma cell lines, peripheral PNET’s and adult tumor cell lines (see table 1). Non-transformed, cultured fibroblasts were used as controls. Expression of the receptors was measured by RT-PCR. In the fibroblasts, we found expression of both DcR1 and DcR2. In the tumor cell line panel, DcR1 is down regulated in 13/15 (87%) and DcR2 in 10/15 (66%) cell lines (fig. 1). This frequent down-regulation of expression of DcR1 and DcR2 in pediatric tumor cell lines was previously reported for cell lines of adult type of cancers. DR4 and DR5 expression was variable. DR4 was expressed in 8/15 (53%) cell lines and DR5 was expressed in 12/15 (80%) cell lines. Almost all cell lines (13/15) therefore express at least one of the death receptors DR4 or DR5.

TRAIL sensitivity was measured by the ability of the tumor cells to undergo apoptosis after co-culturing for 24 hours with TRAIL. Apoptosis was measured by FACS analysis for apoptotic bodies after nuclear staining with propidium iodide. Eight of 15 cell lines are sensitive to TRAIL, ranging from 2 fold (S18A, SK-N-AS) to 10 fold (CHP100) increase in apoptotic bodies compared to the controls (data summarized in table 1). However, in 6 neuroblastoma cell lines, we did not observe any induction of apoptosis after stimulation with TRAIL. Recently, it was shown that many neuroblastomas lack expression of caspase 8 and therefore are unable to respond to TRAIL. This caspase 8 down-regulation was found to be associated with promoter hypermethylation. We therefore checked the caspase 8 expression and promoter methylation status for the complete panel. Indeed, caspase 8 was not expressed in the TRAIL resistant neuroblastoma cell lines, while robust expression was observed in all TRAIL responsive cell lines (SK-N-AS, GI-ME-N, SJNB-8, and all
Figure 1. RT-PCR expression analysis of DcR1, DcR2, DR4, DR5 and caspase 8 in human cancer cell lines. Analysis of GAPDH mRNA expression serves as a control.

Figure 2. Methylation analysis of the DcR1, DcR2, DR4, DR5 and caspase 8 gene promoter. Top panels (U): Amplified products with primers recognizing unmethylated sequences for each gene. Bottom panels (M): Amplified products with primers recognizing methylated sequences for each gene. IVD, In vitro methylated DNA serves as a positive control for methylated sequence. Completely methylated cell lines correspond with down-regulation of the mRNA expression (see fig. 1).

non-neuroblastoma cell lines) (fig. 1 and table 1). We used methylation specific PCR (MSP) to analyze promoter hypermethylation. Cell lines IMR32, LA-N-1, NMB, KCNR, and LA-N-5 were found to be completely methylated and lacked expression of caspase 8. All other cell lines with partially, or completely unmethylated promoters expressed caspase 8, and responded to TRAIL (fig. 2 and table 1). LA-N-6 is an exception since it is not completely methylated and yet it also does not express caspase 8.
This analysis shows that TRAIL resistance correlates very well with down-regulation of caspase 8. To analyze a possible correlation between TRAIL sensitivity and TRAIL receptor expression, we further analyzed the group of caspase 8 positive cell lines (SK-N-AS, GI-MEN, SJNB-8, CHP100, NN-1, TC32, S18A, and SW837; see table 1). All cell lines in this sub-group are TRAIL sensitive. They all lack DcR1 expression and 4/8 lack DcR2 expression. This means that in this subgroup, down-regulation of DcR1 correlates with TRAIL sensitivity, and DcR2 does not.

Methylation of the TRAIL receptors
The almost complete absence of either of the two decoy receptors DcR1 and DcR2 in many different tumor types and the variable expression of the death receptors urged us to analyze the mechanisms involved in their down-regulation. We looked for promoter hypermethylation as a mechanism, which can selectively down-regulate gene expression. DcR1 and DcR2 both contain CpG rich areas, near the translation start site. We first analyzed the methylation status of the promoter regions in normal human tissues (heart, liver, lung, muscle, ovary, spleen, kidney) and untransformed fibroblasts (fig. 3). All normal tissues and fibroblasts were completely unmethylated for all four TRAIL receptors, except for a faint methylated DcR2 product in liver tissue, which represented less that 5% of the total DNA. In the tumor cell lines we found dense promoter methylation (>95%) for DcR1 in 9/13 (69%) of non-expressing cell lines (6/7 neuroblastoma cell lines and 3/6 non- neuroblastoma cell lines) (fig. 2 and table 1). DcR2 was densely methylated in 9/10 (90%) of non-expressing cell lines (6/7 neuroblastoma cell lines and 3/3 non- neuroblastoma cell lines). Also, methylation of DR4 and DR5 was frequent. DR4 was methylated in 5/7 (71%) non-expressing cell lines, and DR5 in 2/3 (66%). Partially methylated gene promoters did not correlate well with down-regulation of expression, as was also true for caspase 8. However, complete promoter methylation correlated in all cases with lack of expression.

Demethylation of the TRAIL receptors
To further establish the role of methylation in the down-regulation of DcR1 and DcR2, we treated
Figure 4. Demethylation and re-expression of DcR1 and DcR2 after treatment of cell lines with 5-AZA. (A) DcR-1-u and DcR2-u: Methylation analysis of DcR1 and DcR2 in tumor cell lines with primers recognizing unmethylated gene sequences. Cell lines have been pretreated with the demethylating agent 5-AZA. All cell lines contain unmethylated sequences (compare also fig. 2). (B) RT-DcR1-AZA and RT-DcR2-AZA: Corresponding RT-PCR expression analysis of DcR1 and DcR2 after 5-AZA pre-treatment. Partial demethylation restores mRNA expression of DcR1 or 2 in cell lines which did not express DcR1 or 2 prior to 5-AZA treatment (see also fig. 1). RT-GAPDH-AZA serves as a control.

the nine non-expressing, hypermethylated cell lines with the demethylating agent 5-AZA. Addition of 5-AZA to the cell culture induced partial demethylation of the decoy receptors in all cell lines tested. In addition, all demethylated cell lines restored mRNA expression of these genes to varying degrees (fig 4). The same 5-AZA-treated cell lines also showed demethylation and re-expression of the TRAIL receptor promoters plays a causative role in down-regulation of expression. Thus, in neuroblastoma cell lines, 5 genes within the TRAIL pathway (DcR1, DcR2, DR4, DR5, and caspase 8) are subject to epigenetic down-regulation of expression.

Sequence analysis of the intracellular death domains of DR4 and DR5
Mutations in the DR5 gene have been described for head and neck cancer and lung cancer\cite{22,24}, and they were exclusively found in the intracellular death domain. Since not all cell lines with down regulated DR4 and DR5 are hypermethylated, we performed a sequence analysis of the death domain spanning exon 9 for both genes. The analysis was performed on all neuroblastoma and PNET cell lines. Our analysis did not reveal any mutation (data not shown).

Methylation of Dcr1, Dcr2, DR4 and DR5 in fresh neuroblastoma tumors
To establish the role of promoter methylation and expression of DcR1 and DcR2 in fresh tumors, we analyzed a panel of 28 neuroblastoma tumors. The neuroblastoma tumor panel contains a variety of all INSS (International Neuroblastoma Staging System) stages 1-4 and 4S, and was randomly chosen from our neuroblastoma tumor bank. Areas of dense tumor tissue (>90%) were
selected. To this purpose, we made serial sections of tumor samples and did a microscopic analysis of each 5th section. Sections without detectable normal infiltrating tissue were marked and DNA and RNA were isolated from the sections in between them. Expression of the studied genes in the tumors was comparable to the cell lines. DcR1 was weakly expressed in 5/28 (18%) and DcR2 in 8/28 (29%) tumors. DcR1 was methylated in 6/28 tumors (21%) and DcR2 was methylated in 7/28 (25%) tumors (figure 5). Five of six tumors with methylated DcR1 did not express this gene, as assessed by rt-PCR. The sixth sample showed weak expression only. For DcR2, three of the four methylated samples did not express the gene. These data show that methylated tumor samples have an absent or very weak DcR1 or DcR2 expression. However, also many tumors without methylation of the promoter of DcR1 or DcR2 lack expression of these genes. This suggests that other mechanisms beside methylation operate in tumors to mediate DcR1 and 2 down-regulation. In addition, we analyzed DR4 and DR5 methylation and expression in the tumor series. DR4 and DR5 were both expressed in 22/28 (78%) of the tumors. Methylation of DR4 and DR5 was not detected in any of the tumor samples (data not shown).

**Discussion**

Carcinomas have been reported to lack expression of the decoy receptors, which may render them more susceptible to TRAIL induced apoptosis. Here, we show that a series of pediatric tumor cell lines also shows a frequent abrogation of decoy receptor expression. Considering the mechanisms responsible for this tumor specific down-regulation of the decoy receptors, we found complete DcR1 and DcR2 promoter hypermethylation in 69% and 90% of non-expressing cell lines respectively. DR4 and DR5 were also frequently down-regulated and methylated in the cell lines. After treatment of the cell lines with the demethylating agent 5-AZA, we observed partial demethylation and restoration of mRNA expression. These experiments strongly suggest that promoter methylation is responsible for the down-regulation of the TRAIL receptors DcR1 and DcR2 in the tumor cell lines tested. Promoter hypermethylation of DcR1 and DcR2 was also found
in fresh neuroblastoma tumors, although in a smaller percentage (21-25%) of samples. DR4 and DR5 were also frequently down-regulated in the cell lines, which is in agreement with earlier observations\textsuperscript{18}. Here, we report the association between down-regulation of DR4 and DR5 and promoter hypermethylation in the cell lines. However, in most of the fresh tumors, DR4 and DR5 were expressed, and we did not observe promoter hypermethylation in the non-expressing tumors. The observed differences between the fresh tumors versus the cell lines may in part be explained by the fact that neuroblastoma cell lines are raised from aggressive neuroblastomas, invariably stage 3 or 4, often associated with amplification of MYCN and/or loss of heterozygosity for chromosome 1p36. The freshly obtained neuroblastoma tumor samples used here also contained specimens from the less aggressive stages 1, 2, and 4S. However, the limited number of cases for each different stage did not permit a conclusive analysis of a possible relation between tumor stage and DcR1 or DcR2 methylation.

Methylation of promoters was assessed by MSP (Methylation Specific PCR). MSP has established itself as a robust and highly reproducible technique, which allows the screening of large tumor panels. However, only a limited number of CpG-dinucleotides within the PCR primers can be investigated. This limitation can be overcome by using multiple primer pairs within the same CpG-island, as we did for DR4 and DR5. The results were identical (data not shown). Alternatively, sequencing of areas of the CpG-island after bisulfite treatment of the DNA will give a broader insight in the methylation pattern of the island of interest. However, this technique is not suitable for screening of a large tumor panel, as described in this study.

The frequent down regulation and key position of caspase 8 in the apoptosis pathway complicates the analysis of the functional importance of TRAIL receptor expression in apoptosis. Considering only caspase 8 expressing cell lines, we could establish a correlation between DcR1 down regulation and TRAIL sensitivity. The next step would be to functionally test the effect of regained DcR1 and/or DcR2 expression on apoptosis after demethylation in a TRAIL induction assay. Unfortunately, when we demethylated the cell lines by adding 5-AZA to the cell cultures, the background apoptosis level increased from 2-8% to more than 50%. This obviously precluded a reliable comparison of the TRAIL sensitivity between 5-AZA treated and non-treated cell lines. Even a 3-fold reduction of the 5-AZA concentration could not bring the background apoptosis back to normal levels (data not shown).

The down-regulation of the decoy receptors in cancer is a puzzling feature, since it renders cancer cells more susceptible to TRAIL induced apoptosis, and thus would counteract tumorigenesis. This could be seen as a protective response against tumor formation or progression. In this view, DcR1 and DcR2 down regulation represents a ‘physiological’ response of the (pre-) cancerous cell to a cellular state in which a higher level of apoptotic sensitivity is warranted. Considering the many cancer types with downregulated decoy receptors, it may be an important threshold against cancer formation. It will be interesting to test whether DcR1 and DcR2 down-regulation is inducible
in vitro by cellular transformation with exogenous oncogenes. A precedent to such a regulatory principle is provided by MYC oncogenes, which are known to render cells prone to apoptosis\textsuperscript{25-27}. Currently, we have no clue as to the identity of the genes responsible for down regulation of DcR1 and 2. However, our results suggest that promoter methylation plays an important role in the mechanism of down regulation. The mechanistic involvement of the methylation machinery in a ‘physiological’ cellular response that counteracts carcinogenesis has not been observed previously. Aberrant methylation and subsequent down-regulation of potential tumor suppressor genes (TSGs) is found in many different cancer types (reviewed in\textsuperscript{28, 29}) and is comparable to genetic mutations or deletions of TSGs. In contrast to the down-regulation of the decoy receptors, these changes contribute to the malignant tumor phenotype. In neuroblastomas, caspase 8 hypermethylation and down-regulation has also been postulated to be such an oncogenic event\textsuperscript{15}. Caspase 8 is a downstream target of the TRAIL route to apoptosis. Absence of caspase 8 prevents cleavage and activation of pro-caspase 3 and decreases the apoptotic potential of the neuroblast. It therefore appears that promoter hypermethylation in cancer has two faces. The data presented in this paper suggest a regulatory role for decoy receptor methylation in the activation of important steps of the apoptosis pathway. This may render potential tumor cells prone to apoptosis and thus protect the organism against cancer. In neuroblastomas, it appears that cancer cells have escaped from this fate by methylation and down-regulation caspase 8, which blocks the apoptotic pathway downstream of the TRAIL receptors.
References


