

University of Groningen

Trail receptor-targeted therapy : strategies to enhance DR4- and DR5-induced apoptosis
van Roosmalen, Ingrid

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:
2014

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

van Roosmalen, I. (2014). *Trail receptor-targeted therapy : strategies to enhance DR4- and DR5-induced apoptosis*. [Thesis fully internal (DIV), University of Groningen]. [S.n.].

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

SUMMARIZING DISCUSSION AND
FUTURE PERSPECTIVES

7

SUMMARIZING DISCUSSION

Cancer is one of the leading causes of death in the world and the number of new cases and deaths continues to increase [1]. Presently, standard treatment of cancer consists of surgical resection in combination with radiotherapy and chemotherapeutic agents. Unfortunately, radiation and chemotherapy result in unwanted side effects to healthy tissues and, moreover, these therapies depend for a major part on activity of the tumour suppressor protein p53 that is often mutated or inactivated in cancer. Therefore, these conventional therapies are often not curative and lead eventually to tumour resistance and relapse.

TRAIL is a promising biological anti-tumour therapeutic as it was shown that rhTRAIL selectively induced apoptosis in a variety of tumour cells *in vitro* and *in vivo* regardless of p53 status [2, 3], and proved to be well tolerated in patients [4-6]. TRAIL can bind to five different receptors, of which DR4 (TRAIL-R1) and DR5 (TRAIL-R2) activate the extrinsic, or death receptor-mediated, apoptotic pathway. In contrast, the other three receptors, DcR1, DcR2 and OPG can diminish apoptosis induction by sequestering TRAIL [7, 8] or by forming non-signalling heterotrimeric complexes [9]. Agonistic DR-selective rhTRAIL variants and mAbs have been generated to specifically target one DR and reduce DcR-binding and, thereby, enhance anti-tumour efficacy [10, 11].

Although pre-clinical and clinical studies have proven that TRAIL is a very interesting anti-cancer therapeutic [12, 13], apoptosis induction is hampered in approximately 50% of tumour cells [14-16] and this obstruction can occur at different levels in the apoptotic pathway [17]. Many studies have focused on designing strategies that combine rhTRAIL or mAbs with other agents in order to enhance TRAIL-induced apoptosis in a wide range of different tumour types, such as GBM [18], colorectal cancer [19] and NSCLC [16].

In this thesis, we focused on investigating the molecular mechanisms controlling TRAIL resistance in several cancer types in order to establish rational combination strategies of rhTRAIL with other agents to provide an effective strategy to enhance TRAIL-induced apoptosis. Moreover, we were interested in unravelling the contribution of either DR4 or DR5 in apoptosis activation in order to assess the prospective of using receptor-targeted agents in clinical studies. **Chapter 2** provides an overview of the differences and similarities in function and regulation of DR4 and DR5. In this review we compared the structure and amino acid sequences of both receptors, provided an overview of sensitivity of different cancer types to TRAIL receptor-selective agonists and discussed the mechanisms that could account for DR preference in these different tumour types. Studies that examined the effect of DR4 and DR5-selective agents *in vitro* showed that leukemic cells are mainly sensitive via activation of DR4, whereas most other tumours exhibit heterogeneity in receptor preference. Moreover, we concluded that mainly DR4 cell surface expression is highly regulated at the level of promoter methylation, post-translational modification

and cellular trafficking processes, while DR5 expression is predominantly regulated at the transcriptional level, which may explain why mainly DR5 mediates intracellular stress-induced apoptosis. Interestingly, DR5 was also found to mediate non-apoptotic signalling, such as proliferation, migration and invasion. Although it is clear that there is much diversity between, and even within, tumour types, the findings mentioned in this review can help with designing rational combination strategies to enhance DR-mediated apoptosis.

In **Chapter 3**, combination treatment with 2,5-dimethyl-celecoxib (DMC) was used to enhance TRAIL sensitivity in TRAIL-sensitive A172 and -resistant U87 cells. GBM cells have been found to be highly resistant to TRAIL-induced apoptosis because of different factors, including moderate to low expression of DR4 and DR5 [20, 21], up-regulation of c-Flip, Bcl-2 and survivin [20, 22-25], or down-regulation of caspase-8 and Bak [20, 23, 26, 27]. ER stress induction has been reported to counteract these modulations. DMC is a specific ER stress inducer that was demonstrated to block cell proliferation in several tumour cell culture models [28, 29] and demonstrated anti-tumorigenic activity *in vivo* [29]. Using the DR4-specific rhTRAIL variant 4C7 and the DR5-specific rhTRAIL variant D269H/E195R, we found that A172 cells were sensitive via activation of DR5 and resistant to TRAIL-induced apoptosis via DR4, while U87 cells were resistant via both DRs. Ectopic overexpression of DR5 did not increase TRAIL sensitivity of U87 cells. We demonstrated that DMC reduced proliferation and cell viability in a panel of GBM cell lines. ER stress induction upon DMC treatment, and subsequent survivin down-regulation, was confirmed in A172 and U87 cells, but enhanced TRAIL sensitivity was only seen in the TRAIL sensitive cell line. Depletion of survivin using a siRNA approach induced apoptosis in A172 cells and partially affected apoptosis-induction by rhTRAIL WT and rhTRAIL variant D269H/E195R. These findings indicate that the synergistic effects observed for TRAIL in combination with DMC may be due to other, perhaps ER-stress independent, mechanisms, such as cell cycle arrest [30, 31]. Our data indicates that DMC might be a potential anti-cancer therapeutic for GBM treatment, and combined treatment with rhTRAIL can further potentiate apoptosis induction in a subset of GBM.

DMC is a close structural analogue of the cyclooxygenase-2 (COX-2)-selective non-steroidal anti-inflammatory drug (NSAID) celecoxib, a FDA-approved drug that is used for treating various forms of arthritis and acute or chronic pain. Both celecoxib and DMC demonstrate anti-tumour properties [29] and inhibited angiogenesis in a COX-2-independent manner *in vitro* and *in vivo* [32], suggesting that ER stress might represent an interesting strategy to target both the tumour and its vasculature. Interestingly, DMC lacks the COX-2 inhibitory function present in celecoxib, while demonstrating enhanced ER stress-activating potential [28, 33]. Since long-term use of selective COX-2 inhibitors might lead to life-threatening risks such as cardiovascular events and gastrointestinal

risks [34], DMC appears to be the more preferred drug. However, to date, the effects of DMC have not yet been evaluated in clinical studies.

In **Chapter 4** we evaluated the effect of fucosylation on DR4- and DR5-mediated apoptosis in colon adenocarcinomas by overexpression of fucosyltransferases (FUT) 3 and 6 or L-fucose treatment using the DR-specific rhTRAIL variants 4C7 (DR4) and D269H/E195R (DR5). Fucosylation was reported to enhance TRAIL sensitivity in colon cancer [14, 35-37] and it was demonstrated that FUT3 and FUT6 expression correlated with TRAIL sensitivity in a large panel of colorectal adenocarcinoma cell lines [14]. We show that low FUT3 and FUT6 expression levels render colon adenocarcinoma cells completely resistant to DR5- but not DR4-mediated apoptosis, and that overexpression of either fucosyltransferases showed a synergistic effect on TRAIL-induced apoptosis mainly via DR5, without altering DR surface expression levels. Moreover, L-fucose treatment reduced proliferation of cells and augmented TRAIL sensitivity also primarily via DR5 without affecting DR surface expression levels. TRAIL-induced caspase-8 and PARP activation was more rapid in DLD-1 cells overexpressing FUT3 and FUT6 and correlated with DR4 and DR5 membrane-clustering. As lipid rafts are known to enhance DISC formation and TRAIL-induced apoptosis by localization of TRAIL receptors [38], it is likely that fucosylation-induced clustering of DRs can enhance the efficacy of TRAIL-induced apoptosis in the same manner. Thus, fucosylation by FUT3 and FUT6 overexpression, or by L-fucose treatment, restored TRAIL-DR5-mediated apoptosis in colon adenocarcinoma cells, which is, at least in part, due to pre-clustering of DR4 and DR5 at the cell membrane. However, since overexpression of FUT3 and FUT6 can induce pre-clustering of both DRs, further experiments are needed to investigate the specific molecular mechanisms that underlie the observed DR-selective sensitization upon enhanced fucosylation. In conclusion, our data clearly show that modulation of fucosylation represents a promising novel approach for restoring TRAIL sensitivity in DR5-resistant colon adenocarcinoma cells.

Since fucosylation is often upregulated in specific cancers [39], these tumours might be especially interesting for TRAIL-induced apoptosis. Currently, immunohistochemistry assays are being developed to assess FUT3 and FUT6 expression levels in human tumour material [40, 41], which might lead to the pre-selection of patients that could benefit of DR5-targeted therapy. In case of mutation of the GDP-mannose-4,6-dehydratase (GMDS) gene, leading to the inactivation of the *de novo* GDP-fucose pathway [35], dietary fucose therapy could increase fucosylation and thereby enhance TRAIL-mediated apoptosis. However, although overexpression of FUT3 and FUT6 enhances TRAIL-mediated apoptosis, these fucosyltransferase enzymes can also have a negative effect in cancers. FUT3 and FUT6, among other fucosyltransferases, are involved in the synthesis of the Lewis antigen. In colon cancer, elevated levels of Lewis antigens are correlated with a

poor prognosis [42]. Therefore, care should be taken using L-fucose treatment in colon cancer patients.

In **Chapter 5**, we tested the novel thymidylate synthase inhibitor trifluorothymidine (TFT) in combination with rhTRAIL in a panel of NSCLC cell lines, i.e. A549, H292, H322 and H460. TFT is a thymidine analogue that can be phosphorylated by thymidine kinase (TK) leading to thymidylate synthase (TS) inhibition in its active monophosphate form, and DNA damage and eventually cell death by incorporation of its triphosphate form TF-TTP into the DNA [43]. TFT was found to enhance TRAIL sensitivity in all cell lines, including resistant A549 cells, when following the combination schedule of 24h TFT and TRAIL followed by 48h of TFT alone. TFT inhibited cell cycle progression at the G2/M phase, which was confirmed by activated Chk2 and reduced Cdc25c levels, while rhTRAIL treatment halted cell cycle progression at the G1 phase, as was shown by a slight increase in phosphorylated Chk1, whereas Chk2 and Cdc25c phosphorylation levels remained similar. TFT enhanced TRAIL-induced apoptosis, whereas TFT as a single agent mainly induced caspase-independent cell death. Furthermore, it was demonstrated that TFT increased p53, p21, Bax and p53-mediated DR5 surface expression, while down-regulating c-Flip and XIAP. Thus, the efficacy of TRAIL-induced apoptosis can be increased by TFT due to the regulation of multiple pro- and anti-apoptotic proteins of the extrinsic apoptotic pathway. Based on our findings, combined use of TFT and TRAIL is highly interesting and indicates a possible therapeutic strategy for treating NSCLC.

TAS-102, a novel oral formulation which comprises TFT and the potent thymidine phosphorylase inhibitor (TPI), which increases the bioavailability of TFT, is currently tested in phase II and phase III clinical trials in patients with metastatic colorectal cancer refractory or intolerable to standard chemotherapies. TFT as single treatment is currently tested in several phase I clinical trials, including studies in which the pharmacokinetics and the mass balance of orally administered TFT as a component of TAS-102 is evaluated in patients with advanced solid tumours (www.clinicaltrials.gov). This suggests that combination treatment of rhTRAIL with TFT might be feasible in the future.

Apart from activating the canonical apoptotic pathway, DR activation can also lead to non-canonical kinase cascades that can be involved in cell proliferation, survival, migration/invasion and angiogenic properties in TRAIL-resistant tumour and normal non-transformed cells [44, 45]. In **Chapter 6**, we investigated the activation of p38 and JNK upon TRAIL treatment and evaluated the role of these kinases in modulating TRAIL-induced apoptosis in TRAIL-sensitive and -resistant NSCLC cells. Previously, it was determined that TRAIL stimulation activated JNK and p38 through the formation of the secondary complex, which consists of FADD, caspase-8, RIP1 and TRAF2 [46]. TRAIL treatment led to p38 and JNK1/2/3 phosphorylation in TRAIL-sensitive H460, but not

in TRAIL-resistant A549 cells. Chemical inhibition and siRNA-dependent knockdown experiments demonstrated the involvement of JNK and p38 in anti- and pro-apoptotic TRAIL signalling, respectively. Although RIP1 kinase, a kinase that has been reported to mediate MAPK activation [46], was expressed in both cell lines, only H460 cells demonstrated clear TRAIL-induced RIP1 cleavage, which correlated with detectable JNK phosphorylation. Down-regulation of RIP1 by shRNA or inhibition by necrostatin-1 demonstrated RIP1-dependent and -independent phosphorylation of p38, while phosphorylation of JNK only occurred independently of RIP1. Interestingly, RIP1 cleavage is suppressed by JNK. What molecular mechanism is causing RIP1-independent p38 and JNK activation by TRAIL and suppression of RIP1 cleavage by JNK is currently unknown. Inhibition of caspase-8 activation by ectopic overexpression of the caspase-8 inhibitor CrmA demonstrated that TRAIL-induced caspase-8 activity only affected JNK activation in H460 cells. The anti-apoptotic protein Mcl-1 was identified as a downstream target of p38 and JNK, as siRNA-mediated depletion of Mcl-1 strongly enhanced TRAIL-induced apoptosis in H460 cells. In conclusion, using NSCLC cells, we demonstrated opposing activities of TRAIL-induced activation of p38 and JNK on Mcl-1 expression. Therefore, combination therapy of TRAIL with p38-stimulating agents, or inhibitors of JNK or Mcl-1 may enhance the efficacy of TRAIL-induced apoptosis in NSCLC.

Intriguingly, TRAIL-induced activation of p38 and JNK can have dual effects. While we have shown that activation of p38 can have pro-apoptotic effects by down-regulation of Mcl-1, others demonstrated that activation of p38 up-regulates the expression of Mcl-1 [47], or can lead to increased catalytic and invasive activities of Akt [48]. In addition, TRAIL-induced activation of JNK can not only lead to cell survival by up-regulation of Mcl-1 or TRAIL-induced cytoprotective autophagy [49], but can also contribute to apoptosis [50].

Presently, the pro- and anti-apoptotic effects of TRAIL-induced kinase activation have not yet been thoroughly studied. However, combining these results with data of other treatments [51-54], it is clear that p38 and JNK, like other kinases [45], can have dual effects on TRAIL-induced apoptosis. In conclusion, TRAIL-based combination strategies with p38-stimulating agents, or inhibitors of JNK or Mcl-1 could be promising in specific situations but caution is required due to the dual nature of these MAPK kinases.

The studies described in **Chapter 3, 4 and 5** were all focused on enhancing TRAIL-mediated apoptosis by using combination strategies that work at different levels of the TRAIL signalling pathway. While fucosylation was found to enhance apoptosis induction mainly via DR5, at least in some extent, due to pre-clustering of the receptors on the membrane, DMC and TFT were found to alter the ratio of pro- and anti-apoptotic proteins by down-regulation of survivin, c-Flip and XIAP and up-regulation of p53, p21, Bax and DR5 cell surface levels. In addition, in **Chapter 6** we found that stimulation of p38, or depletion of JNK or Mcl-1 may enhance TRAIL-induced apoptosis in NSCLC cells.

The up-regulation of p53, DR5 and other pro-apoptotic proteins that lead to sensitization for TRAIL-induced apoptosis can be accomplished by treatment with chemotherapeutics and radiation. However, these treatments affect not only tumour cells but also healthy cells, leading to unwanted side effects. Furthermore, the efficacy of standard treatments is often hampered by blockades in the intrinsic pathway, most frequently by inactivation of the p53 pathway. Therefore, the use of targeted agents that more specifically enhance TRAIL-mediated apoptosis in tumour cells and induce less toxic side effects would be preferable. Currently available epigenetic drugs, such as histone deacetylase (HDAC) inhibitors, although known to induce expression of pro-apoptotic proteins are unfortunately also relative non-specific in their mode of action. Therefore, drugs that specifically target the anti-apoptotic machinery, such as YM155 (survivin), CMH (c-Flip), AEG35156 (XIAP) and GX15-070 (Mcl-1), might have a higher therapeutic value. However, it has to be kept in mind that also these small molecule inhibitors are able to modulate signalling in both healthy and cancer cells and, thereby, might lead to toxic side effects.

Overall, we demonstrated that several combination therapies can enhance TRAIL-induced apoptosis in different cancer types. It remains to be investigated if these combination therapies are of interest for treatment of other tumour types. Furthermore, the anti-tumorigenic properties of these combination strategies have to be verified *in vivo*.

FUTURE PERSPECTIVES

Cancer is one of the leading causes of death in the world and cancer mortality continues to rise. Biological therapeutics have been developed as alternative strategies for radiation and chemotherapeutic agents since these conventional therapies cause unwanted side effects and, moreover, depend for a major part on activity of p53, which is often mutated or inactivated in cancer. RhTRAIL (dulanermin) and DR-specific agonists are biological therapeutics that have gathered considerable interest since they selectively target apoptosis activation in tumour cells. However, as TRAIL resistance occurs in approximately half of the tumour cells, and TRAIL can even induce non-canonical signalling such as migration and invasion in TRAIL-resistant cancer cells, it is vital to increase our understanding of the molecular machineries that control TRAIL activity. Studies aiming to elucidate the mechanisms behind DR preference and functioning of the TRAIL pro- and anti-apoptotic pathways, such as the studies presented in this thesis, are necessary to further design rational combination strategies to enhance TRAIL-mediated apoptosis in cancer cells. Given that DR preference and regulation of pro- and anti-apoptotic proteins differ between, and even within, tumour types it will be essential to identify biomarkers that allow for customized TRAIL-based combination strategies that will enhance the efficacy of TRAIL-induced apoptosis.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. *CA Cancer J Clin* 61:69-90
2. Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, Blackie C, Chang L, McMurtrey AE, Hebert A, DeForge L, Koumenis IL, Lewis D, Harris L, Bussiere J, Koeppen H, Shahrokh Z, Schwall RH (1999) Safety and antitumor activity of recombinant soluble Apo2 ligand. *J Clin Invest* 104:155-162
3. Lawrence D, Shahrokh Z, Marsters S, Achilles K, Shih D, Mounho B, Hillan K, Totpal K, DeForge L, Schow P, Hooley J, Sherwood S, Pai R, Leung S, Khan L, Gliniak B, Bussiere J, Smith CA, Strom SS, Kelley S, Fox JA, Thomas D, Ashkenazi A (2001) Differential hepatocyte toxicity of recombinant Apo2L/TRAIL versions. *Nat Med* 7:383-385
4. Herbst RS, Eckhardt SG, Kurzrock R, Ebbinghaus S, O'Dwyer PJ, Gordon MS, Novotny W, Goldwasser MA, Tohnya TM, Lum BL, Ashkenazi A, Jubb AM, Mendelson DS (2010) Phase I dose-escalation study of recombinant human Apo2L/TRAIL, a dual proapoptotic receptor agonist, in patients with advanced cancer. *J Clin Oncol* 28:2839-2846
5. Soria JC, Smit E, Khayat D, Besse B, Yang X, Hsu CP, Reese D, Wiezorek J, Blackhall F (2010) Phase 1b study of dulanermin (recombinant human Apo2L/TRAIL) in combination with paclitaxel, carboplatin, and bevacizumab in patients with advanced non-squamous non-small-cell lung cancer. *J Clin Oncol* 28:1527-1533
6. Soria JC, Mark Z, Zatloukal P, Szima B, Albert I, Juhasz E, Pujol JL, Kozielski J, Baker N, Smethurst D, Hei YJ, Ashkenazi A, Stern H, Amler L, Pan Y, Blackhall F (2011) Randomized phase II study of dulanermin in combination with paclitaxel, carboplatin, and bevacizumab in advanced non-small-cell lung cancer. *J Clin Oncol* 29:4442-4451
7. Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, Ramakrishnan L, Gray CL, Baker K, Wood WI, Goddard AD, Godowski P, Ashkenazi A (1997) Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science* 277:818-821
8. Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, Yuan J, Gurney A, Goddard AD, Godowski P, Ashkenazi A (1997) A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr Biol* 7:1003-1006
9. Clancy L, Mruk K, Archer K, Woelfel M, Mongkolsapaya J, Screaton G, Lenardo MJ, Chan FK (2005) Preligand assembly domain-mediated ligand-independent association between TRAIL receptor 4 (TR4) and TR2 regulates TRAIL-induced apoptosis. *Proc Natl Acad Sci U S A* 102:18099-18104
10. Reis CR, van Assen AH, Quax WJ, Cool RH (2011) Unraveling the binding mechanism of trivalent tumor necrosis factor ligands and their receptors. *Mol Cell Proteomics* 10:M110.002808
11. Szegezdi E, van der Sloot AM, Mahalingam D, O'Leary L, Cool RH, Munoz IG, Montoya G, Quax WJ, de Jong S, Samali A, Serrano L (2012) Kinetics in signal transduction pathways involving promiscuous oligomerizing receptors can be determined by receptor specificity: apoptosis induction by TRAIL. *Mol Cell Proteomics* 11:M111.013730
12. Mahalingam D, Szegezdi E, Keane M, de Jong S, Samali A (2009) TRAIL receptor signalling and modulation: Are we on the right TRAIL?. *Cancer Treat Rev* 35:280-288
13. Wiezorek J, Holland P, Graves J (2010) Death receptor agonists as a targeted therapy for cancer. *Clin Cancer Res* 16:1701-1708
14. Wagner KW, Punnoose EA, Januario T, Lawrence DA, Pitti RM, Lancaster K, Lee D, von Goetz M, Yee SF, Totpal K, Huw L, Katta V, Cavet G, Hymowitz SG, Amler L, Ashkenazi A (2007) Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. *Nat Med* 13:1070-1077
15. Saturno G, Valenti M, De Haven Brandon A, Thomas GV, Eccles S, Clarke PA, Workman

- P (2013) Combining trail with PI3 kinase or HSP90 inhibitors enhances apoptosis in colorectal cancer cells via suppression of survival signaling. *Oncotarget* 4:1185-1198
16. Stegehuis JH, de Wilt LH, de Vries EG, Groen HJ, de Jong S, Kruyt FA (2010) TRAIL receptor targeting therapies for non-small cell lung cancer: current status and perspectives. *Drug Resist Updat* 13:2-15
 17. Zhang L, Fang B (2005) Mechanisms of resistance to TRAIL-induced apoptosis in cancer. *Cancer Gene Ther* 12:228-237
 18. Kuijlen JM, Bremer E, Mooij JJ, den Dunnen WF, Helfrich W (2010) Review: on TRAIL for malignant glioma therapy?. *Neuropathol Appl Neurobiol* 36:168-182
 19. Van Geelen CM, de Vries EG, de Jong S (2004) Lessons from TRAIL-resistance mechanisms in colorectal cancer cells: paving the road to patient-tailored therapy. *Drug Resist Updat* 7:345-358
 20. Knight MJ, Riffkin CD, Muscat AM, Ashley DM, Hawkins CJ (2001) Analysis of FasL and TRAIL induced apoptosis pathways in glioma cells. *Oncogene* 20:5789-5798
 21. Kuijlen JM, Mooij JJ, Platteel I, Hoving EW, van der Graaf WT, Span MM, Hollema H, den Dunnen WF (2006) TRAIL-receptor expression is an independent prognostic factor for survival in patients with a primary glioblastoma multiforme. *J Neurooncol* 78:161-171
 22. Kouri FM, Jensen SA, Stegh AH (2012) The role of Bcl-2 family proteins in therapy responses of malignant astrocytic gliomas: Bcl2L12 and beyond. *ScientificWorldJournal* 2012:838916
 23. Song JH, Song DK, Pyrzynska B, Petruk KC, Van Meir EG, Hao C (2003) TRAIL triggers apoptosis in human malignant glioma cells through extrinsic and intrinsic pathways. *Brain Pathol* 13:539-553
 24. Xie D, Zeng YX, Wang HJ, Wen JM, Tao Y, Sham JS, Guan XY (2006) Expression of cytoplasmic and nuclear Survivin in primary and secondary human glioblastoma. *Br J Cancer* 94:108-114
 25. Fulda S, Meyer E, Debatin KM (2002) Inhibition of TRAIL-induced apoptosis by Bcl-2 overexpression. *Oncogene* 21:2283-2294
 26. Capper D, Gaiser T, Hartmann C, Habel A, Mueller W, Herold-Mende C, von Deimling A, Siegelin MD (2009) Stem-cell-like glioma cells are resistant to TRAIL/Apo2L and exhibit down-regulation of caspase-8 by promoter methylation. *Acta Neuropathol* 117:445-456
 27. Qi L, Bellail AC, Rossi MR, Zhang Z, Pang H, Hunter S, Cohen C, Moreno CS, Olson JJ, Li S, Hao C (2011) Heterogeneity of primary glioblastoma cells in the expression of caspase-8 and the response to TRAIL-induced apoptosis. *Apoptosis* 16:1150-1164
 28. Chuang HC, Kardosh A, Gaffney KJ, Petasis NA, Schonthal AH (2008) COX-2 inhibition is neither necessary nor sufficient for celecoxib to suppress tumor cell proliferation and focus formation in vitro. *Mol Cancer* 7:38
 29. Pyrko P, Soriano N, Kardosh A, Liu YT, Uddin J, Petasis NA, Hofman FM, Chen CS, Chen TC, Schonthal AH (2006) Downregulation of survivin expression and concomitant induction of apoptosis by celecoxib and its non-cyclooxygenase-2-inhibitory analog, dimethyl-celecoxib (DMC), in tumor cells in vitro and in vivo. *Mol Cancer* 5:19
 30. Kardosh A, Wang W, Uddin J, Petasis NA, Hofman FM, Chen TC, Schonthal AH (2005) Dimethyl-celecoxib (DMC), a derivative of celecoxib that lacks cyclooxygenase-2-inhibitory function, potently mimics the anti-tumor effects of celecoxib on Burkitt's lymphoma in vitro and in vivo. *Cancer Biol Ther* 4:571-582
 31. Ehrhardt H, Wachter F, Grunert M, Jeremias I (2013) Cell cycle-arrested tumor cells exhibit increased sensitivity towards TRAIL-induced apoptosis. *Cell Death Dis* 4:e661
 32. Lin HP, Kulp SK, Tseng PH, Yang YT, Yang CC, Chen CS, Chen CS (2004) Growth inhibitory effects of celecoxib in human umbilical vein endothelial cells are mediated through G1 arrest via multiple signaling mechanisms. *Mol Cancer Ther* 3:1671-1680

33. Pyrko P, Kardosh A, Liu YT, Soriano N, Xiong W, Chow RH, Uddin J, Petasis NA, Mircheff AK, Farley RA, Louie SG, Chen TC, Schonthal AH (2007) Calcium-activated endoplasmic reticulum stress as a major component of tumor cell death induced by 2,5-dimethyl-celecoxib, a non-coxib analogue of celecoxib. *Mol Cancer Ther* 6:1262-1275
34. Schonthal AH, Chen TC, Hofman FM, Louie SG, Petasis NA (2008) Celecoxib analogs that lack COX-2 inhibitory function: preclinical development of novel anticancer drugs. *Expert Opin Investig Drugs* 17:197-208
35. Moriwaki K, Noda K, Furukawa Y, Ohshima K, Uchiyama A, Nakagawa T, Taniguchi N, Daigo Y, Nakamura Y, Hayashi N, Miyoshi E (2009) Deficiency of GMDS leads to escape from NK cell-mediated tumor surveillance through modulation of TRAIL signaling. *Gastroenterology* 137:188-98, 198.e1-2
36. Moriwaki K, Narisada M, Imai T, Shinzaki S, Miyoshi E (2010) The effect of epigenetic regulation of fucosylation on TRAIL-induced apoptosis. *Glycoconj J* 27:649-659
37. Moriwaki K, Shinzaki S, Miyoshi E (2011) GDP-mannose-4,6-dehydratase (GMDS) deficiency renders colon cancer cells resistant to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor- and CD95-mediated apoptosis by inhibiting complex II formation. *J Biol Chem* 286:43123-43133
38. Rossin A, Derouet M, Abdel-Sater F, Hueber AO (2009) Palmitoylation of the TRAIL receptor DR4 confers an efficient TRAIL-induced cell death signalling. *Biochem J* 419:185-92, 2 p following 192
39. Christiansen MN, Chik J, Lee L, Anugraham M, Abrahams JL, Packer NH (2013) Cell surface protein glycosylation in cancer. *Proteomics*
40. Stern HM, Padilla M, Wagner K, Amler L, Ashkenazi A (2010) Development of immunohistochemistry assays to assess GALNT14 and FUT3/6 in clinical trials of dulanermin and drozitumab. *Clin Cancer Res* 16:1587-1596
41. de Albuquerque Vasconcelos JL, de Almeida Ferreira S, de Lima AL, de Melo Rego MJ, Bandeira AR, de Lima Bezerra Cavalcanti C, de Melo Lira MM, Beltrao EI (2013) Comparing the Immunoeexpression of FUT3 and FUT6 between Prostatic Adenocarcinoma and Benign Prostatic Hyperplasia. *Acta Histochem Cytochem* 46:105-109
42. Miyoshi E, Moriwaki K, Nakagawa T (2008) Biological function of fucosylation in cancer biology. *J Biochem* 143:725-729
43. Temmink OH, Emura T, de Bruin M, Fukushima M, Peters GJ (2007) Therapeutic potential of the dual-targeted TAS-102 formulation in the treatment of gastrointestinal malignancies. *Cancer Sci* 98:779-789
44. Gonzalez F, Ashkenazi A (2010) New insights into apoptosis signaling by Apo2L/TRAIL. *Oncogene* 29:4752-4765
45. Azijli K, Weyhenmeyer B, Peters GJ, de Jong S, Kruyt FA (2013) Non-canonical kinase signaling by the death ligand TRAIL in cancer cells: discord in the death receptor family. *Cell Death Differ* 20:858-868
46. Varfolomeev E, Maecker H, Sharp D, Lawrence D, Renz M, Vucic D, Ashkenazi A (2005) Molecular determinants of kinase pathway activation by Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand. *J Biol Chem* 280:40599-40608
47. Son JK, Varadarajan S, Bratton SB (2010) TRAIL-activated stress kinases suppress apoptosis through transcriptional upregulation of MCL-1. *Cell Death Differ* 17:1288-1301
48. Sun BK, Kim JH, Nguyen HN, Kim SY, Oh S, Lee YJ, Song JJ (2011) TRAIL-induced caspase/p38 activation is responsible for the increased catalytic and invasive activities of Akt. *Int J Oncol* 38:249-256
49. He W, Wang Q, Xu J, Xu X, Padilla MT, Ren G, Gou X, Lin Y (2012) Attenuation of TNFSF10/TRAIL-induced apoptosis by an autophagic survival pathway involving TRAF2- and RIPK1/RIP1-mediated MAPK8/JNK activation. *Autophagy* 8:1811-1821

50. Herr I, Wilhelm D, Meyer E, Jeremias I, Angel P, Debatin KM (1999) JNK/SAPK activity contributes to TRAIL-induced apoptosis. *Cell Death Differ* 6:130-135
51. Liu X, Qiu F, Liu Z, Lan Y, Wang K, Zhou PK, Wang Y, Hua ZC (2014) Urokinase-type plasminogen activator receptor regulates apoptotic sensitivity of colon cancer HCT116 cell line to TRAIL via JNK-p53 pathway. *Apoptosis*
52. Song IS, Jun SY, Na HJ, Kim HT, Jung SY, Ha GH, Park YH, Long LZ, Yu DY, Kim JM, Kim JH, Ko JH, Kim CH, Kim NS (2012) Inhibition of MKK7-JNK by the TOR signaling pathway regulator-like protein contributes to resistance of HCC cells to TRAIL-induced apoptosis. *Gastroenterology* 143:1341-1351
53. Lamy V, Bousserouel S, Gosse F, Minker C, Lobstein A, Raul F (2011) Lupulone triggers p38 MAPK-controlled activation of p53 and of the TRAIL receptor apoptotic pathway in human colon cancer-derived metastatic cells. *Oncol Rep* 26:109-114
54. Lepage C, Leger DY, Bertrand J, Martin F, Beneytout JL, Liagre B (2011) Diosgenin induces death receptor-5 through activation of p38 pathway and promotes TRAIL-induced apoptosis in colon cancer cells. *Cancer Lett* 301:193-202

