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Expression Profiles of TRAIL and Its Receptors in Normal, Hyperplastic, and Malignant Endometrial Tissues: Hints on Endometrial Cancer Biology

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ABSTRACT

Endometrial cancer is the sixth most common neoplasm in women worldwide, with a rising incidence largely attributed to the ongoing obesity epidemic. TNF-Related Apoptosis-Inducing Ligand (TRAIL) and TRAIL receptors have been tested for their predictive, diagnostic, and prognostic values in various cancers, as well as for possible use in combination therapies. The roles of TRAIL and its receptors in endometrial tissue biology has not yet been cleared, and the potential of these molecules as biomarkers in endometrial cancer is yet to be defined. We investigated the expression profiles of TRAIL and its transmembrane receptors during endometrial carcinogenesis to evaluate their potential as prognostic markers. Paraffin-embedded normal endometrium (n=18), endometrial hyperplasia (n=27), and endometrioid endometrial adenocarcinoma tissues (n=100) were analysed for TRAIL and receptor expression profiles via immunohistochemical staining. Apoptotic indexes in the corresponding tissues were defined by TUNEL assay.

Endometrial carcinoma displayed decreased TRAIL and DR4 expressions compared to the normal endometrium, while increased DR5 and decoy receptor (DcR1 and DcR2) expressions were evident. The complex atypical hyperplasia displayed the most similar expression profiles to the endometrial carcinoma, in accordance with the greatest risk of progression to endometrial carcinoma attributed to this tissue type. TRAIL/TRAIL receptor expression levels did not correlate with the prognostic factors of tumor stage or grade, or depth of myometrial invasion.

Overall, distinct profiles of TRAIL and its receptor expressions were evident in progression from normal endometrium to hyperplasia and cancer, which may indicate significance of TRAIL signaling in the course of endometrial carcinoma development.

Key Words: Normal endometrium, Endometrial Hyperplasia, Endometrial Carcinoma, TRAIL, DR4, DR5, DcR1, DcR2.

Introduction

Endometrial cancer (EC) is the most common gynaecological cancer in North America and Europe (1). Together with cancers of the breast, cervix, ovary, lung, liver, and colorectum, it accounts for 60% of the cancer burden among women throughout the world (2). Morever, as a cancer type highly associated with obesity, EC has been related to rapidly increasing death rates in a recent statistical report, also in relation with the ageing population (3).

EC is traditionally classified into two major groups based on clinical, epidemiological, and endocrine criteria. The estrogen-dependent Type 1 (EEC) is associated with endometrial hyperplasia, and the estrogen-independent Type 2 (NEEC) is associated with endometrial atrophy (4). The common subtypes in the histopathological classification include endometrioid carcinoma, serous carcinoma, carcinosarcoma, and clear-cell carcinoma. EECs mostly display endometrioid histology, whereas the NEECs are correlated with serous carcinomas (1). Of all the endometrial carcinomas, 75-80% display the endometrioid histological subtype (5,6). The high level of biological, pathological, and molecular heterogeneity in these tumors is now opening the way for an improved classification scheme with the genomic features also incorporated (1). Endometrial hyperplasia, which is frequently associated with EEC, is commonly classified into

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Table 1. Demographic and clinical features of patients included in the study

Group/Classification	Endometrial carcinoma	Endometrial hyperplasia
Age	36-74 (mean 57)	33-70 (mean 48)
Tumor grade (FIGO)		
Grade 1	63(63%)	-
Grade 2	26(26%)	-
Grade 3	11(11%)	-
Depth of myometrial invasion		
No invasion	9(9%)	-
50% or less	45(45%)	-
50% or more	46(46%)	-
Stage		
IA	40(40%)	-
IB	28(28%)	-
IIB	6(6%)	-
IIIA	8(8%)	-
IIIB	2(2%)	-
IIIC	14(14%)	-
IV	1(1%)	-
Unclassified	1(1%)	-
Histological type		
Simple	-	1(3.7%)
Complex	-	7(25.93%)
Complex atypical	-	19(70.37%)

3 main types as simple, complex, and atypical hyperplasia, with low, intermediate, and greatest risk of progression to endometrial carcinoma, respectively (7).

Among the prognostic parameters of EC are uterine factors including but not limited to the histological type and grade and depth of myometrial invasion, besides extrauterine factors such as positive peritoneal cytology, adnexal involvement, and pelvic and para-aortic lymph node metastasis (8). Identification of new molecular markers may contribute to improved prognosis and management of the disease, as well as improved schemes of classification.

TNF-Related Apoptosis-Inducing Ligand (TRAIL) has attracted great attention in cancer research as a selective apoptotic agent inducing apoptotic cell death in various cancers but not in non-transformed cells (9). Further investigations revealed many different roles for TRAIL besides antitumor cytotoxicity, including proliferative effects on vascular endothelial and smooth muscle cells, and a putative protective role in diabetes [10, 11]. Although discovered through its homology to FasL and TNF-alpha, TRAIL differs from the other TNF superfamily members with its wide expression pattern in human tissues, and the 5 different receptors it can bind to (12-14). Of these, four are transmembrane receptors (TR-1/DR4, TR-2/DR5, TR-3/DcR1, and TR-4/DcR2), and one is a soluble receptor (OPG; osteoprotogerin). DR4 and DR5 are death receptors with death domains that can initiate apoptosis, while DcR1 and DcR2 are defined as decoy receptors with no death domains, and are often related to anti-apoptotic pathways. All four transmembrane receptors in fact have the ability to initiate intracellular survival and proliferative pathways.

Expression levels of both the ligand itself and its receptors are known to influence the actions of TRAIL in a particular tissue. We have previously found correlations of high TRAIL expression levels with increased cell death in human pancreas, and high DR4 and DcR2 expressions correlating with significant cell death in pancreatic ductal adenocarcinoma (15,16). DcR2 expression also correlated with high Gleason scores, prostate specific antigen recurrence, and decreased survival in prostate carcinoma (17). Furthermore, a

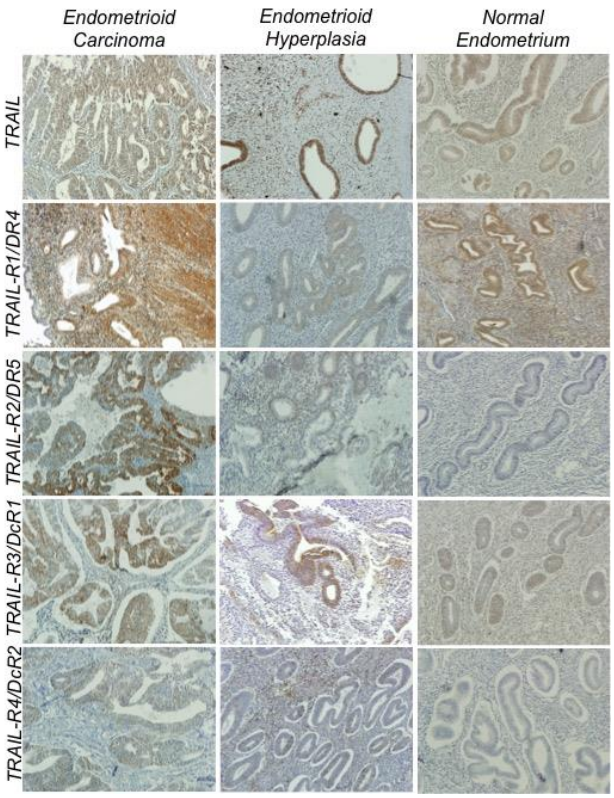

Fig. 1. Immunohistochemical analyses of normal, hyperplastic, and malignant endometrioid tissues. Representative examples of stainings for TRAIL and receptors are shown, provided from different patients. Antibodies used for the stainings are specific for the proteins listed to the left of each panel (Magnification =x200)

positive correlation between DR4 expression and the tumor grade was also evident in invasive ductal breast carcinoma (18). Expression patterns of TRAIL and its receptors may provide significant clues on the ligand's possible role in malignant or hyperplastic development, as well as its potential as a prognostic biomarker. Here we studied the expression patterns of TRAIL and its transmembrane receptors in endometrioid carcinoma, and in hyperplastic and normal endometrium, in relation with the apoptotic indexes and histopathological characteristics defined in the corresponding tissues.

Materials and Methods

Patients and Tissue Samples: Formalin-fixed, paraffin-embedded normal, hyperplastic, or malignant endometrial tissues that had been archived in Akdeniz University Faculty of Medicine, Department of Pathology between the years of 2000 and 2009 were analysed by immunohistochemistry. Of the studied samples, 100 belonged to cases with endometrioid type endometrial adenocarcinoma, while 27 samples were acquired from endometrial hyperplastic tissue. Control samples consisted of 18 normal endometrial samples. Sections of 5 μm thickness were studied.

All patients had been treated surgically with total abdominal hysterectomy, bilateral salpingo-oophorectomy, pelvic lymph node dissection and omentectomy. Follow-ups were performed clinically via abdominopelvic ultrasonography, as well as gynecological and physical examinations. Data on tumor stage, tumor grade and depth of myometrial invasion were collected retrospectively. AJCC/FIGO criteria had been used in determination of the tumor stages and grades in the archived material. Most cases with endometrioid endometrial adenocarcinoma had early stage carcinoma: 40 cases were Stage IA (40%), 28 cases were Stage IB (28%), 6 cases were Stage IIB (6%), 8 cases were Stage IIIA (8%), 2 cases were Stage IIIB (2%), 14 cases were Stage IIIC (14%), 1 case was Stage IV (1%), while 1 case was unclassified (Table 1). Using the FIGO grading system, 63 cases out of 100 were classified as Grade 1 (63%), 26 cases as Grade 2 (26%), and 11 cases as Grade 3 (11%) by two independent pathologists. Myometrial invasion rate was ≤50% in 45 patients, and >50% in 46 patients. Tumors of 9 patients were noninvasive.

Demographic and clinical features of the patients included in the study are listed in Table 1.

Immunohistochemistry: Sections for immunohistochemistry (5 μm) were first deparaffinized in xylene followed by rehydration in decreasing concentrations of ethanol. Antigen retrieval was performed via microwave treatment for 15 min at 700W in 0.01 M citrate buffer (pH 6.0). Endogenous peroxidase was blocked with 3% H_2O_2 for 20 min. Nonspecific antibody binding was blocked with Ultra V Block solution (Lab-Vision, UK) for 5 min. Next, slides were incubated overnight at +4°C with primary antibodies against TRAIL (ALX-804-326), TRAIL-R1 (ALX-804-297), TRAIL-R2 (ALX-210-743), TRAIL-R3 (ALX-210-744), and TRAIL-R4 (ALX-804-299), with working dilutions of 1:50, 1:50, 1:200, 1:300 and 1:100, respectively. Immune reactions were detected with a labeled streptavidin-biotin-peroxidase method (LSAB+ System-HRP, Dako-K0690). Diaminobenzidine (DAB) chromogen staining was then performed in hematoxylin-counterstained tissues. Lymph node sections were used as positive controls. Tissue samples stained with the secondary antibody alone constituted the negative controls.

Immunohistochemical analyses were performed in a double-blinded manner by 2 independent

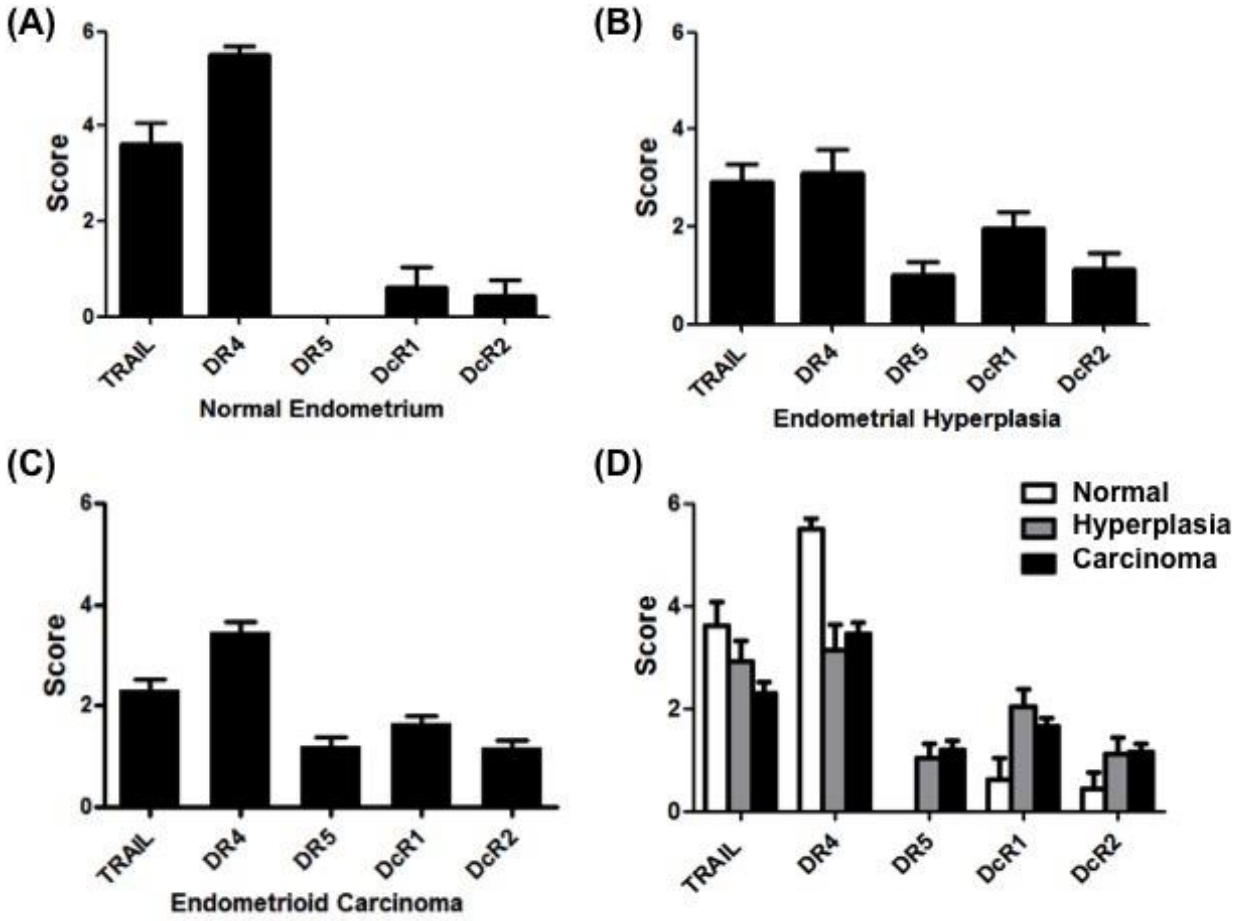

Fig. 2. Comparative analysis of TRAIL and TRAIL receptor expressions in the normal endometrium (A), and in patients with endometrial hyperplasia (B), and endometrioid carcinoma (C). Each bar represents the mean (± SEM) of total corresponding numbers of tissues analysed (18 normal endometrial, 27 endometrial hyperplastic, and 100 endometrial carcinoma samples). Section (D) displays quantitative analysis of the immunohistochemical stainings, with open bars representing normal endometrial tissue samples, solid gray bars representing hyperplastic tissue samples, and solid black bars representing endometrioid carcinoma tissue samples (mean ±SEM)

pathologists (EP and GE). Staining intensities and marker distribution patterns were both taken into consideration in calculation of the immunostaining scores. Intensity of staining was classified as 0 = negative, 1 = weak, 2 = moderate, and 3 = strong; for positivity in nuclei and/or cytoplasm. Marker distribution was scored as 0 = less than 10%, 1 = between 10-40%, 2 = between 40-70%, and 3 = for more than 70% of the cells stained positive. Sum of the total intensity and marker distribution scores constituted the final staining score.

TUNEL Assay: Apoptotic cell counts in tissues were detected via Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End-Labeling (TUNEL) assay, according to the manufacturer's instructions (In Situ Cell Death Detection Kit-AP, Roche Applied Science). Negative controls consisted of sections where no terminal deoxynucleotidyl transferase enzyme was used. For each sample, 10 randomly selected fields were evaluated for positive staining under light microscope, and mean values were obtained. Proportion of the positively stained cells to the total number of cells in the area of analysis constituted the apoptotic index.

Statistical Analysis: Statistical Software Package for the Social Sciences (SPSS), Version 13.0 for Windows was used for statistical analysis. The Kolmogorov-Smirnov test was utilized to test for normal distribution of the data; Friedman test was used to document the statistical significance among the markers; and groups were compared in pairs by the Wilcoxon signed rank test. Statistical significance was considered at the $p<0.05$ level. GraphPad Software (Prism 5.0, San Diego, CA) was used to plot data.

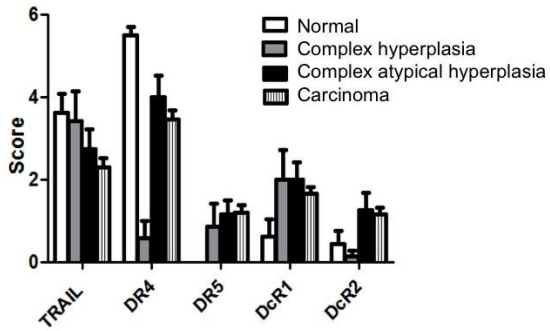

Fig. 3. Differential levels of TRAIL ligand and receptors in normal, complex hyperplasia, complex atypical hyperplasia, and carcinoma tissues. Open bars represents normal endometrial tissue samples, solid gray bars represent complex hyperplastic tissue samples, solid black bars represent complex atypical hyperplasia samples, and striped bars represent endometrioid carcinoma tissue samples (mean ±SEM)

Results

Comparative expression patterns of TRAIL ligand and its receptors in normal, hyperplastic, and malignant endometrium: Immunohistochemical analysis for TRAIL ligand and its transmembrane receptor expressions was performed in 18 normal endometrial tissue, 27 endometrial hyperplasia, and 100 endometrial carcinoma samples, as defined in the Materials and Methods section. Overall, expression of all markers were evident in all three types of tissues, except for the lack of DR5 in the normal endometrium (Figure 1, 2). Among all the markers, DR4 is generally the highest expressed receptor in the tissues studied, although its expression appears quite low in the complex hyperplasia, when the complex and complex atypical subtypes are evaluated separately (Figure 3). Normal tissue displays the highest DR4 expression. DcR1 is increased in the hyperlastic and malignant tissues compared to the normal endometrium (Figure 2). DcR2 reaches its lowest level of expression in the complex subtype of hyperplasia (Figure 3). On the other hand, a gradual decrease in the TRAIL ligand expression is evident as the tissue progresses from normal to cancerous.

All the marker expressions analyzed in the endometrial carcinoma samples were statistically significantly different from the marker levels expressed in the normal endometrium. Compared to the normal tissue, TRAIL and DR4 expressions were significantly lower in the endometrial carcinoma (p=0,019 and p=0,000, respectively); while DR5, DcR1, and DcR2 expressions were significantly higher (p=0,003, p=0,000, p=0,021, respectively). On the other hand, the complex atypical hyperplastic tissue displayed the most similar marker expression profile to the endometrioid carcinoma. Spearman-Rho correlation test revealed positive correlation between DcR1 and DR5, and between DcR1 and DcR2; while a negative correlation was evident between the two death receptors DR4 and DR5 in the carcinoma tissue (Data not shown).

Differential apoptotic indexes between normal, hyperplastic, and cancer tissues: Normal endometrium, endometrial hyperplasia and endometrial carcinoma samples from a total of 145 subjects were analysed for apoptotic indexes by TUNEL method, as described briefly in Materials and Methods. The apoptotic index was the lowest in the normal endometrium, and highest in the cancer tissue (Figure 4). No correlations were evident between the apoptotic indexes and the TRAIL ligand and receptor expression profiles, as defined by the Spearman Rho correlation test (Data not shown).

Correlation analysis of TRAIL/TRAIL receptor expressions with histopathological characteristics: Possible correlation between TRAIL ligand and receptors and histopathological characteristics such as the tumor stage or grade, or myometrial invasion was analysed by Spearman Rho correlation test. No statistically significant correlation was found (Data not shown).

Discussion

As a major actor in cancer immunosurveillance, TNF-related apoptosis-inducing ligand (TRAIL) is well known for its selective apoptotic effect on many transformed cells but not on most non-transformed cells (19,20). It attracted attention as a promising potential therapeutic agent against cancer, and various TRAIL-mediated therapeutic approaches have been proposed (21-25). Among many other functions attributed to the TRAIL/TRAIL receptor pathway to date are regulation of T cell survival and activation, and anti-inflammatory and protective effects on various inflammatory disease settings such as obesity, diabetes, and cancer (10,26-28). In fact, TRAIL is pronounced in relation to a possible link between obesity, diabetes, cancer, and inflammation (10). While there is a proposed association between TRAIL's anti-inflammatory effect and suppression of tumor development, accumulated evidence suggest a direct role for

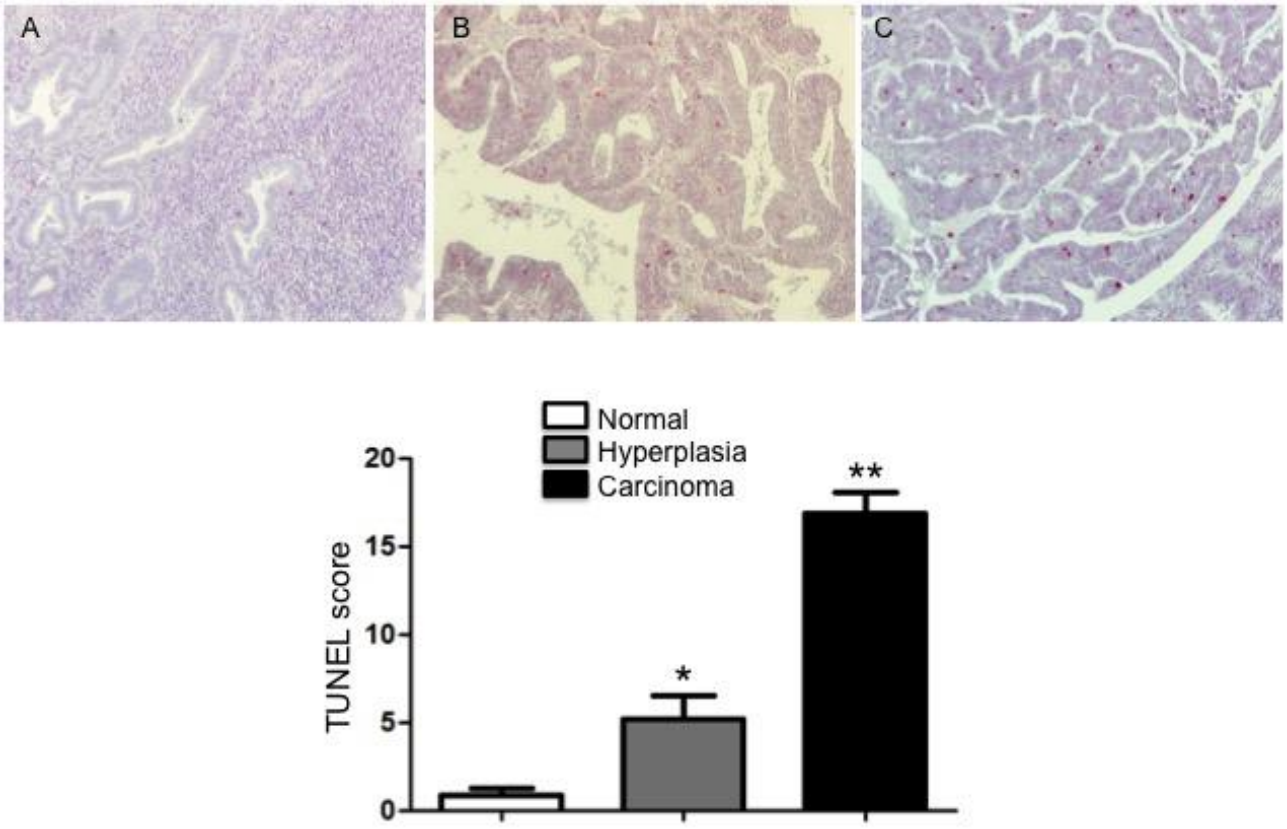

Fig. 4. Representative images of normal tissue (A), hyperplastic tissue (B), and carcinoma (C) stained for TUNEL. Below is the quantitative analysis of TUNEL assay results, with open bar representing normal endometrium, solid gray bar representing endometrial hyperplasia, and solid black bar representing endometrioid carcinoma tissues

TRAIL in regulation of tumor initiation and development (22). The significance of the possible effect of TRAIL signaling on shaping the immune microenvironment is yet to be defined. Overall, altered expression profiles of the ligand itself and its receptors usually correlate with their particular roles in tissues; thus the expression profiles of TRAIL/TRAIL receptors are thoroughly studied in different disease settings in order to test their predictive, diagnostic, prognostic, and therapeutic potential, such as colon, cervical, ovarian, pancreatic, mammary, and non-small cell lung carcinoma (15,29-33). The fact that TRAIL ligand and receptor expression profiles vary in different cancer types suggests that these molecules may have potential as biomarkers in defining the prognosis of disease, while providing hints on cancer biology.

Endometrial cancer is the sixth most common malignancy in women worldwide, the rising incidence of which is attributed greatly to the recent elevation in obesity rates. The biology of endometrial cancer has not been clearly elucidated yet, and the need for novel biomarkers is pointed out, in particular to help in personalized identification of disease and adjuvant treatment options (34). Although 75-88% of cases can be diagnosed at an early stage, disease may recur in about 15-20% of patients, leading to death, as is the situation in advanced disease (35). Thus, it is emphasized that any additional factors that may be present, related to the risk of tumor progression and dissemination should be defined.

The role of TRAIL ligand and receptors in endometrial carcinogenesis has not yet been fully understood, which obviates the need for further studies. Deregulation of apoptosis-regulatory molecules and resistance to TNF family members such as Fas ligand and TRAIL have been reported in endometrial carcinomas (36-41). We investigated differential expression profiles of TRAIL ligand and its transmembrane receptors in normal (n=18), hyperplastic (n=27), and malignant (n=100) endometrial tissues via immunohistochemical analysis, and determined the apoptotic indexes of each tissue using TUNEL assay. Expression of TRAIL and all of its receptors was evident although in varying degrees in all types of tissues, except for the absence of DR5 expression in the normal epithelium (Figures 1,2).

While DR4 death receptor expression was generally the highest in all tissues compared to the other markers, complex hyperplasia displayed

much lower levels, when analysed seperately from the complex atypical subtype (Figure 3). Similar to our previous findings in breast cancer patients, DR4 expression was the prominent TRAIL receptor expressed in endometrial carcinoma patients (18). In fact, our overall analysis indicated that TRAIL ligand and DR4 death receptor was down-regulated, while other markers were up-regulated in endometrial carinoma compared to the normal endometrium tissue. Elevated TRAIL death receptor expressions are reported in many other cancer types as well, including hepatocellular, renal, and ovarian cancer; which may suggest a possible benefit provided to tumor cells by expression of death receptors (42).

The expression and role of decoy receptors in malignant tumors are much less well defined. There are various hypotheses regarding their action. One is the classical hypothesis, whereby decoy receptors are concomitantly expressed with the death receptors and compete for binding to TRAIL, which results in inhibition of the apoptotic signal. Alternatively, they may activate NFkB within the cell to drive antiapoptotic signals, or they can form mixed receptor complexes with the two death receptors, leading to an ineffective death inducing signalling complex (DISC), resulting in blockage of subsequent TRAIL signaling. Our results revealed upregulated expression of both decoy receptors DcR1 and DcR2 in endometrial carcinoma. This may refer to a correlation between decoy receptor expressions and resistance to TRAIL-mediated apoptosis as suggested in many previous reports, which should be functionally tested (43-46)(47-50).

Tarragona et al. examined DcR1 expression levels in 80 normal endometrial tissue samples (NE) and 62 endometrial carcinoma tissues (EC) by IHC. This study reported frequent cytoplasmic DcR1 expression in NE (79.6%), varying according to the menstrual cycle. EC also displayed positive DcR1 immunostaining (98.1% of the cases), but no statistical association could be found with the histological type, grade, and stage of the carcinoma (51).

In a recent series of studies by Gottwald et al., membrane expressions of DR4, D5, DcR1, and DcR2 were studied in NE, atypical endometrial hyperplasia (AEH), and endometrioid adenocarcinoma (EAC). One study included 20 NE, 18 AEH, and 159 EAC samples in a tissue microarray study (53). The expression levels of all markers were found to be higher in NE than EAC, with receptor expression levels not correlating with grading or staging. Also, receptor expression levels could not be defined as independent predictors of survival. Same group reported in another study a strong correlation between type of endometrial tissue and total scores of DR4 and DR5 receptor expressions (54). They have reported reduction of membrane DR4 and DR5 expressions along with malignant transformation. This result is in consistence with ours in terms of DR4 expression only, as we have not detected DR5 expression in normal epithelium. Yet another study by the same group examined DcR1 and DcR2 membrane expressions in 20 NE, 14 AEH, and 67 endometrioid endometrial cancer (EEC) (55). Similar to our results, membrane expressions of DcR1 and DcR2 were both higher in EEC compared to NE. In addition, this group defined a strong correlation between endometrial tissue type and total scores of DcR1 and DcR2, with negative correlation of DcR1 but not DcR2 with staging. None of the markers were related to grading and survival.

Thus our results, similar, but also different in some aspects to other reports on the topic, display reduced levels of TRAIL ligand and DR4 death receptor expressions and increased decoy receptor expression levels in hyperplastic and malignant tissue compared to the normal endometrial tissue. This may suggest a possible role for TRAIL ligand and its receptors in hyperplastic and malignant progression, the nature of which should be defined via further investigations. The TRAIL/TRAIL receptor system has been suggested in many reports as a disease activation marker in both cancer and autoimmune diseases (16-18, 52). Yet on the other hand, we could not find any correlation between TRAIL ligand and receptor expressions and histopathologic characteristics such as tumor stage, tumor grade and depth of myometrial invasion. TRAIL and its receptors' widespread expression patterns in humans, unlike other TNF superfamily members such as FasL and TNF-alpha, and the fact that it has 5 receptors it can bind to already adds up to the complex nature of TRAIL molecule and its signaling mechanism, as a significant member of the immune system. More evidence is required to solve the path behind what appears as a possible role for TRAIL and its receptors, either protective or destructive, in hyperplastic and malignant development in endometrial tissue. Elucidating the molecular biology of endometrial cancer is significant for possible detection of novel therapeutic directions.

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References

1. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. Lancet Oncol 2014; 15: e268-278.
2. Torre LA, Islami F, Siegel RL, Ward EM, Jemal A. Global Cancer in Women: Burden and Trends. Cancer Epidemiol Biomarkers Prev 2017; 26: 444-457.
3. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
4. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983; 15: 10-17.
5. Ryan AJ, Susil B, Jobling TW, Oehler MK. Endometrial cancer. Cell Tissue Res 2005; 322: 53-61.
6. Bansal N, Yendluri V, Wenham RM. The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. Cancer Control 2009; 16: 8-13.
7. Reed SD, Newton KM, Clinton WL et al. Incidence of endometrial hyperplasia. Am J Obstet Gynecol 2009; 200: 678.e1-6.
8. Prat J. Prognostic parameters of endometrial carcinoma. Hum Pathol 2004; 35: 649-662.
9. Nagane M, Huang HJ, Cavenee WK. The potential of TRAIL for cancer chemotherapy. Apoptosis 2001; 6: 191-197.
10. Harith HH, Morris MJ, Kavurma MM. On the TRAIL of obesity and diabetes. Trends Endocrinol Metab 2013; 24: 578-587.
11. Secchiero P, Gonelli A, Carnevale E et al. Evidence for a proangiogenic activity of TNF-related apoptosis-inducing ligand. Neoplasia 2004; 6: 364-373.
12. Griffith TS, Lynch DH. TRAIL: a molecule with multiple receptors and control mechanisms. Curr Opin Immunol 1998; 10: 559-563.
13. Wiley SR, Schooley K, Smolak PJ et al., Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995; 3: 673-682.
14. Pitti RM, Marsters SA, Ruppert S et al., Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J Biol Chem 1996; 271: 12687-12690.
15. Sanlioglu AD, Dirice E, Elpek O et al. High TRAIL death receptor 4 and decoy receptor 2 expression correlates with significant cell death in pancreatic ductal adenocarcinoma patients. Pancreas 2009; 38: 154-160.
16. Sanlioglu AD, Dirice E, Elpek O et al. High levels of endogenous tumor necrosis factor-related apoptosis-inducing ligand expression correlate with increased cell death in human pancreas. Pancreas 2008; 36: 385-393.
17. Koksal IT, Sanlioglu AD, Karacay B, Griffith TS, Sanlioglu S. Tumor necrosis factor-related apoptosis inducing ligand-R4 decoy receptor expression is correlated with high Gleason scores, prostate-specific antigen recurrence, and decreased survival in patients with prostate carcinoma. Urol Oncol 2008; 26: 158-165.
18. Sanlioglu AD, Korcum AF, Pestereli E et al. TRAIL death receptor-4 expression positively correlates with the tumor grade in breast cancer patients with invasive ductal carcinoma. Int J Radiat Oncol Biol Phys 2007; 69: 716-723.
19. Ashkenazi A, Pai RC, Fong S et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 1999; 104: 155-162.
20. Walczak H, Miller RE, Ariail K et al. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nat Med 1999; 5: 157-163.
21. Koschny R, Walczak H, Ganten TM. The promise of TRAIL--potential and risks of a novel anticancer therapy. J Mol Med (Berl) 2007; 85: 923-935.
22. Johnstone RW, Frew AJ, Smyth MJ. The TRAIL apoptotic pathway in cancer onset, progression and therapy. Nat Rev Cancer 2008; 8: 782-798.
23. Aydin C, Sanlioglu AD, Bisgin A et al. NF-kappaB targeting by way of IKK inhibition sensitizes lung cancer cells to adenovirus delivery of TRAIL. BMC Cancer 2010 27; 10: 584.
24. Terzioglu E, Bisgin A, Sanlioglu AD et al. Concurrent gene therapy strategies effectively destroy synoviocytes of patients with rheumatoid arthritis. Rheumatology (Oxford) 2007; 46: 783-789.
25. Dirice E, Sanlioglu AD, Kahraman S et al. Adenovirus-mediated TRAIL gene (Ad5hTRAIL) delivery into pancreatic islets prolongs normoglycemia in streptozotocin-induced diabetic rats. Hum Gene Ther 2009; 20: 1177-1189.
26. Di Pietro R, Zauli G. Emerging non-apoptotic functions of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/Apo2L. J Cell Physiol 2004; 201: 331-340.
27. Secchiero P, Zerbinati C, Rimondi E et al. TRAIL promotes the survival, migration and proliferation of vascular smooth muscle cells. Cell Mol Life Sci 2004; 61: 1965-1974.
28. Janssen EM, Droin NM, Lemmens EE et al. CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. Nature 2005; 434: 88-93.

29. Ganten TM, Sykora J, Koschny R et al. Prognostic significance of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor expression in patients with breast cancer. J Mol Med (Berl) 2009; 87: 995-1007.
30. Sträter J, Hinz U, Walczak H et al. Expression of TRAIL and TRAIL receptors in colon carcinoma: TRAIL-R1 is an independent prognostic parameter. Clin Cancer Res 2002; 8: 3734-3740.
31. Maduro JH, Noordhuis MG, ten Hoor KA et al. The prognostic value of TRAIL and its death receptors in cervical cancer. Int J Radiat Oncol Biol Phys 2009; 75: 203-211.
32. Lancaster JM, Sayer R, Blanchette C et al. High expression of tumor necrosis factor-related apoptosis-inducing ligand is associated with favorable ovarian cancer survival. Clin Cancer Res 2003; 9: 762-766.
33. Spierings DC, de Vries EG, Timens W et al. Expression of TRAIL and TRAIL death receptors in stage III non-small cell lung cancer tumors. Clin Cancer Res 2003; 9: 3397-3405.
34. Binder PS, Mutch DG. Update on prognostic markers for endometrial cancer. Womens Health (Lond) 2014; 10: 277-288.
35. Appel ML, Edelweiss MI, Fleck J et al. P53 and BCL-2 as prognostic markers in endometrial carcinoma. Pathol Oncol Res 2008; 14: 23-30.
36. Dolcet X, Llobet D, Pallares J et al. FLIP is frequently expressed in endometrial carcinoma and has a role in resistance to TRAIL-induced apoptosis. Lab Invest 2005; 85: 885-894.
37. Llobet D, Eritja N, Encinas M et al. CK2 controls TRAIL and Fas sensitivity by regulating FLIP levels in endometrial carcinoma cells. Oncogene 2008; 27: 2513-2524.
38. Llobet D, Pallares J, Yeramian A et al. Molecular pathology of endometrial carcinoma: practical aspects from the diagnostic and therapeutic viewpoints. J Clin Pathol 2009; 62: 777-785.
39. Pallares J, Martínez-Guitarte JL, Dolcet X et al. Survivin expression in endometrial carcinoma: a tissue microarray study with correlation with PTEN and STAT-3. Int J Gynecol Pathol 2005; 24: 247-253.
40. Dolcet X, Llobet D, Pallares J et al., NF-kB in development and progression of human cancer. Virchows Arch 2005; 446: 475-482.
41. Pallares J, Llobet D, Santacana M et al. CK2beta is expressed in endometrial carcinoma and has a role in apoptosis resistance and cell proliferation. Am J Pathol 2009; 174: 287-296.
42. Cullen SP, Martin SJ. Fas and TRAIL 'death receptors' as initiators of inflammation: Implications for cancer. Semin Cell Dev Biol 2015; 39: 26-34.
43. Sanlioglu AD, Koksal IT, Karacay B et al. Adenovirus-mediated IKKbetaKA expression sensitizes prostate carcinoma cells to TRAIL-induced apoptosis. Cancer Gene Ther 2006; 13: 21-31.
44. Sanlioglu AD, Dirice E, Aydin C et al. Surface TRAIL decoy receptor-4 expression is correlated with TRAIL resistance in MCF7 breast cancer cells. BMC Cancer 2005; 5: 54.
45. Zhang XD, Franco A, Myers K et al. Relation of TNF-related apoptosis-inducing ligand (TRAIL) receptor and FLICE-inhibitory protein expression to TRAIL-induced apoptosis of melanoma. Cancer Res 1999; 59: 2747-2753.
46. Nimmanapalli R, Perkins CL, Orlando M et al. Pretreatment with paclitaxel enhances apo-2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of prostate cancer cells by inducing death receptors 4 and 5 protein levels. Cancer Res 2001; 61: 759-763.
47. Sanlioglu AD, Karacay B, Koksal IT, Griffith TS, Sanlioglu S. DcR2 (TRAIL-R4) siRNA and adenovirus delivery of TRAIL (Ad5hTRAIL) break down in vitro tumorigenic potential of prostate carcinoma cells. Cancer Gene Ther 2007; 14: 976-984.
48. Aydin C, Sanlioglu AD, Karacay B et al. Decoy receptor-2 small interfering RNA (siRNA) strategy employing three different siRNA constructs in combination defeats adenovirus-transferred tumor necrosis factor-related apoptosis-inducing ligand resistance in lung cancer cells. Hum Gene Ther 2007; 18: 39-50.
49. Pan G, Ni J, Wei YF et al. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. Science 1997; 277: 815-818.
50. Zhang XD, Franco AV, Nguyen T et al. Differential localization and regulation of death and decoy receptors for TNF-related apoptosis-inducing ligand (TRAIL) in human melanoma cells. J Immunol 2000; 164: 3961-3970.
51. Tarragona J, Llecha N, Santacana M et al. DcR1 expression in endometrial carcinomas. Virchows Arch 2010; 456: 39-44.
52. Bisgin A, Terzioglu E, Aydin C et al. TRAIL death receptor-4, decoy receptor-1 and decoy receptor-2 expression on CD8+ T cells correlate with the disease severity in patients with rheumatoid arthritis. BMC Musculoskelet Disord 2010; 11: 192.

53. Gottwald L, Piekarski J, Kubiak R et al. Membrane expression of TRAIL receptors DR4, DR5, DcR1 and DcR2 in the normal endometrium, atypical endometrial hyperplasia and endometrioid adenocarcinoma: a tissue microarray study. Arch Gynecol Obstet 2013; 288: 889-899.
54. Gottwald L, Szwalski J, Piekarski J et al. Membrane expression of the death ligand trail receptors DR4 and DR5 in the normal endometrium, endometrial atypical hyperplasia and endometrioid endometrial cancer. J Obstet Gynaecol 2013; 33: 512-518.
55. Gottwald L, Pasz-Walczak G, Piekarski J et al. Membrane expression of trail receptors DcR1 and DcR2 in the normal endometrium, endometrial atypical hyperplasia and endometrioid endometrial cancer. J Obstet Gynaecol 2014; 34: 346-349.