THR alpha and its subtypes alpha1 and alpha2 are prognostic markers for survival of ovarian cancer patients

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Nina Ditsch
Ludwig-Maximilians-Universitat Munchen Medizinische Fakultat

Sabine Heublein
Ruprecht Karls Universitat Heidelberg Medizinische Fakultat Heidelberg

Udo Jeschke
LMU Munich

udo.jeschke@med.uni-muenchen.de*Corresponding Author*
ORCiD: https://orcid.org/0000-0003-2623-3235

Cornelia Sattler
Ludwig-Maximilians-Universitat Munchen Medizinische Fakultat

Christina Kuhn
Ludwig-Maximilians-Universitat Munchen

Anna Hester
Ludwig-Maximilians-Universitat Munchen Medizinische Fakultat

Bastian Czogalla
Ludwig-Maximilians-Universitat Munchen Medizinische Fakultat

Fabian Trillsch
Ludwig-Maximilians-Universitat Munchen Medizinische Fakultat

Sven Mahner
Ludwig-Maximilians-Universitat Munchen Medizinische Fakultat

Jutta Engel
Ludwig-Maximilians-Universitat Munchen

Doris Mayr
Ludwig-Maximilians-Universitat Munchen

Elisa Schmoeckel
Ludwig-Maximilians-Universitat Munchen

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Abstract

Background

Ovarian cancer is most lethal in comparison to all other gynecological cancer cases. Within this study, we aimed to investigate the expression of the thyroid hormone receptor alpha and its influence on patient survival in ovarian cancer (OvCa).

Methods

The presence of the thyroid hormone receptors THRα, THRα1 and -2 were investigated in 156 ovarian cancer samples using immunohistochemistry (IHC) using semi-quantitative immunoreactivity (IR) scores and correlated to clinical, pathological data, subtype of ovarian cancer, clinical data, staining of 20 already described OvCa marker proteins and overall survival (OS).

Results

Patients with clear cell OvCa showed the highest THRα expression and nuclear THRα expression is associated with reduced survival in this group. In contrast, nuclear expressed THRα1 is a positive prognosticator for all OvCa patients. Nuclear THRα2 is a positive prognosticator for OvCa patients of the serous subtype. Cytoplasmic expression of THRα2 accompanies with reduced OS in all OvCa patients, whereas cytoplasmic expression of THRα1 is associated with reduced OS in mucinous OvCa patients only. In addition, THRα expression correlates to gonadotropin receptors, steroid hormone receptors, TA-MUC1 and glycodelin.

Conclusion

Thyroid hormones promote ovarian cancer cell proliferation. The further investigation of thyroid hormones and its specific receptors offer the possibility to investigate new endocrine targets against ovarian cancer.

Background

Thyroid-stimulating hormone (TSH) regulates thyroid function by binding to its receptor (thyroid-hormone receptor-THR) expressed at the surface of thyroid cells. Recently, it has been demonstrated that THR is abundantly expressed in several tissues apart from the thyroid, among them the normal ovarian surface epithelium. The hormone dependency of the ovaries and the functional similarity of

THRs and estrogen- (ER) and progesterone receptors (PR; both act in the nucleus as transcription factors) lead to the hypothesis that THRs may be a prognostic marker in ovarian cancer patients as demonstrated recently for breast cancer patients [1-4].

The nuclear receptors of thyroid hormones regulate the expression of specific cellular genes by interacting with distinct DNA sequences. They are ligand-activated transcription factors, which regulate the transcription of target genes. THRs are encoded by two genes – THR alpha and beta – located on human chromosomes 17 and 3 [5]. They have three major isoforms: THRa1, THRa2 and THRb1 [6] with high homology in amino acid composition. The most diversified region between THRa and THRb is located in the N-terminal area, related to their trans-activation activity [7]. Recent studies previously discovered by oligonucleotide microarray transcriptional profiling that THRα and THRβ mRNAs are among the most strongly expressed nuclear hormone receptor genes in cultured human ovarian surface epithelial (OSE) cells [8]. The presence of THRα1, THRα2, and THRβ1 transcripts in cultured OSE cells are confirmed and the presence of THRα and THRβ proteins in the OSE cell layer has been demonstrated. Although, THRα and β isoforms are encoded by separate genes, differential promoter usage gives rise to two different THRα receptors, THRα1 and THRα2 [9]. Unlike THRα1 and THRβ1, which are conventional ligand-activated receptors, THRα2 is a ligand-independent negative regulator of active THRs. Thus, the presence of different THR isoforms, in conjunction with the potential for pre-receptor metabolism of thyroid hormones through expression of activating and inactivating deiodinase enzymes, strengthens the likelihood that the OSE is a physiologically important thyroid hormone target tissue [8].

Ovulation is a recurrent inflammatory reaction causing regular and frequent local injury to the ovarian surface during follicular rupture [10, 11]. Ovarian cancer develops when a mutation or genetic change - possibly caused by repeat episodes of inflammation-associated DNA damage [12-14] - occurs in the cells on the surface of the ovaries or in the fallopian tubes and leads to uncontrolled cell growth that may often metastasize [15]. Suppression of ovulation by e.g. pregnancy, breast feeding, or oral contraception reduces the risk of ovarian cancer, whereas diseases such as endometriosis, ovarian cysts, and hyperthyroidism are associated with increased risk [16, 17].

Ovarian cancer consists of four histopathological subtypes, represents the fourth most frequent type of cancer among females, and is the leading cause of death from gynecological cancer in the western world. Besides the histopathological subtype, grading, clinical staging and the amount of residual tumor, a number of several putative prognostic markers had been suggested for monitoring this disease [4]. As ovarian cancer is also a thyroid hormone-dependent neoplasm [18], T3 has been shown to directly exert inflammatory effects on ovarian surface epithelial cell function *in vitro* and activate expression of genes associated with inflammation [19, 20]. Studies also indicate that T3 increases the expression of ERα, which strongly associates with the development of epithelial ovarian cancer, which may explain the epidemiological linkage between hyperthyroidism and ovarian cancer [20].

The current study examines possible alterations of THR expression in ovarian carcinomas and its implication in ovarian cancer survival. Little is known about the context of thyroid function in ovarian carcinogenesis and the role of THR expression outside the thyroid is not completely understood. From our knowledge of therapy modalities, anti-hormonal therapy like tamoxifen, which unfold its effect via steroid hormone receptors, can be affective in ER-positive ovarian cancers. First in this field, our examinations focuses on the prognostic impact of thyroid hormone receptors of the alpha subtype (general alpha, alpha-1 and alpha-2; respectively) on pathological different ovarian cancer tissues.

Methods

Tissue Samples

Tissue samples were obtained from 156 patients undergoing gynaecological surgery for epithelial ovarian cancer (EOC) at the Department of Obstetrics and Gynaecology of the Ludwig-Maximilians-University Munich. Experienced gynaecologic pathologists performed histopathological staining and evaluation according to the criteria of the International Federation of Gynaecologists and Obstetricians (FIGO) and the World Health Organization (WHO). EOC specimens were available in different histological subtypes: serous (n=110) thereof 84 high-grade and 26 low-grade cases, clear cell (n=12), endometrioid (n=21), mucinous (n=13). Patients with ovarian low malignant potential tumours (e.g. Borderline tumors) were excluded from the study and no patients had adjuvant

chemotherapy. Patient's clinical data were available from patient charts, aftercare files and tumour registry database information. The main outcomes assessed were disease recurrence and patient survival. For survival analysis, survival time was defined as the time between the date of primary ovarian cancer diagnosis and the date of death.

Ethical Approval

The Ethics Committee of the Ludwig-Maximilians-University, Munich, Germany (approval number 227-09) on 30 September 2009, approved the study. All tissue samples used for this study were obtained from leftover material from the archives of LMU Munich, Department Gynaecology and Obstetrics, Ludwig-Maximilians-University, Munich, Germany, initially used for pathological diagnostics. When this retrospective study was initiated all diagnostic procedures had already been fully completed, the tissue samples were classified as left-over material and underwent irreversible anonymization. Under these circumstances, no individual written informed consent was needed as per declaration of the Ethics Committee of the Ludwig-Maximilians-University. All experiments were performed according to the standards of the Declaration of Helsinki (1975).

Immunohistochemistry

Our group has extensively described immunohistochemistry of THRa, THRα1 and THRa2 on FFPE sections [4, 21]. In brief, rabbit polyclonal antibodies detecting THRα (Abcam, Cambridge, UK); Zytomed, Berlin, Germany), THRa1 (Zytomed) or THRa2 (Zytomed)) were stained by employing commercially available kits (Vectastain Elite rabbit-IgG-Kit (VectorLabs, Burlingame, CA); ZytoChem Plus HRP Polymer System (Zytomed). Appropriate positive (struma, colon and placental tissue) and negative controls were included in each experiment (**Figure 1**). Tissue sections treated with pre-immune IgGs (supersensitive rabbit negative control, BioGenex, Fremont, CA) instead of the primary antibody served as negative controls. Immunoreactivity was quantified by applying a well-established semi-quantitative scoring system (IR-score; also known as Remmele's score) by two independent observers (gynecologic pathologists (D.M. and E.S.)) by consensus. This scoring method has already been used in numerous studies [22-25] of our group. The IRS quantifies immunoreactivity by multiplication of optical staining intensity (graded as 0: no, 1: weak, 2: moderate and 3: strong

staining) and the percentage of positive stained cells (0: no staining, 1: ≤ 10% of the cells, 2: 11–50% of the cells, 3: 51–80% of the cells and 4: ≥ 81% of the cells). According to previously published data tissue samples that had been assigned an IRS higher than 1 were scored as positive [26].

Statistical Analysis Methods

The IBM statistic package SPSS (version 25) was used to test data for statistical significance. Differences in THR expression among three or more groups were tested using the non-parametric Kruskal-Wallis rank-sum test and for pairwise comparisons using the nonparametric Mann–Whitney-U rank-sum test. Correlation analysis was performed using the Spearman correlation coefficient. Survival times were compared by Kaplan-Meier graphics and differences in patient overall survival times were tested for significance by using the chi-square statistics of the log rank test. Data were assumed to be statistically different in case of $p < 0.05$.

Results

THRalpha expression according to EOC subtypes

THRα expression showed significant differences within the histological subtype, accounting nuclear as well as cytoplasmic staining. Serous carcinomas showed only faint expression of THRa in the nucleus (median IRS = 2) as well as in the cytoplasm (median IRS = 0; **Figure 2a** = 10 x lens, **Figure 2f** = 25 x lens). A more intense staining was observed in the clear cell cases in the nucleus (median IRS = 2) as well as in the cytoplasm (median IRS = 2; **Figure 2b** = 10 x lens, **Figure 2g** = 25 x lens). The endometrioid subtype showed similar expression schemas as the serous subtype in the nucleus (median IRS = 2) as well as in the cytoplasm (median IRS = 0; **Figure 2c** = 10 x lens, **Figure 2h** = 25 x lens). The lowest expression of THRa was found in the mucinous subtype in the nucleus (median IRS = 1) as well as in the cytoplasm (median IRS = 0; **Figure 2d** = 10 x lens, **Figure 2i** = 25 x lens). A summary of the staining results is shown in **Figure 2e** for the nuclear staining ($p = 0.005$) and **Figure 2j** for the cytoplasmic staining ($p = 0.037$).

THRa1 as well as THRa2 showed no significant different expression according to the histological subtype. The median expression of THRalpha1 in the nucleus was 2 and the median expression in the cytoplasm was 0. The median expression of THRa2 in the nucleus was 6 and therefore much more

intense compared to THRa and -a1, respectively. The median expression of THRa2 in the cytoplasm was 0. There was no significant different expression of the three THRa subtypes according to grading, FIGO staging or age at surgery.

Correlation analyses

By using recently published data by our institute, we were able to correlate the expression of all THRa subtypes stained with former investigation results. There are significant correlations with the gonadotropin receptors [24] and the luteinizing hormone (LH)-receptor ligand hCG [25]; specifically THRa staining in the nucleus showed a positive correlation to the follicle stimulating hormone receptor (FSHR) (correlation coefficient (cc) = 0.181; p = 0.027) and a negative correlation to hCG (cc = -0.247, p = 0.003). In opposite, THRa in the cytoplasm showed a positive correlation to the luteinizing hormone/choriogonadotropin receptor (LH/hCGR) (cc = 0.199, p = 0.014) and a positive correlation to hCG (cc = 0.187, p = 0.027). The THRa1 expression in the cytoplasm is positively correlated to hCG (cc = 0.278, p = 0.001). THRa2 in the nucleus showed a positive correlation to FSHR (cc = 0.185, p = 0.024). In addition, there are also positive correlations to the classical steroid hormone receptors, which were analysed by our research group too [26]. THRa staining in the nucleus showed a positive correlation to the ERb (cc = 0.213, p = 0.009) and to the PRA (cc = 0.172, p = 0.035). The THRa1 expression in the cytoplasm is positively correlated to ERb (cc = 0.219, p = 0.006). THRa2 in the nucleus showed positive correlation to ERa (cc = 0.247, p = 0.002) and to PRA (cc = 0.219, p = 0.007). In addition to the classical estrogen receptors, also the GPER [27-29] showed positive correlation to THRa staining in the nucleus (cc = 0.219, p = 0.007) and to THRa2 in the nucleus (cc = 0.252, p = 0.002).

Another positive correlation was found within the tumour associated mucin 1 epitop (TA-MUC1) detected with the Gatipotuzumab antibody formerly known as PankoMab [30, 31] and THRa staining in the nucleus (cc = 0.279, p = 0.001). In contrast, THRa1 expression in the cytoplasm is negatively correlated to TA-MUC1 (cc = -0.195, p = 0.019). TA-MUC1 as membrane bound protein can also be translocated to the cytoplasm of tumour cells [32]. In that case it is negatively correlated to the expression of THRa1 in the nucleus (cc = -0.166, p = 0.048) and THRa2 in the nucleus (cc = -0.268, p

= 0.001). An immunosuppressive glycoprotein that is connected to TA-MUC1 is glycodelin and its specific glycoform glycodelin A [33, 34]. Glycodelin A showed a positive correlation to THRa2 in the cytoplasm (cc = 0.170, p = 0.037). Glycodelin showed positive correlation with THRa in the nucleus (cc = 0.241, p = 0.003) as well as in the cytoplasm (cc = 0.231, p = 0.004). THRa2 expression in the nucleus is positively correlated with glycodelin (cc = 0.265, p = 0.001).

Survival analyses

The expression of the general THRa is connected to significantly reduced overall survival in the subgroup of clear cell carcinomas. The median survival for THRa-negative patients is 5.24 years in contrast to only 0.29 years for patients showing THRa expression in the nucleus (**Figure 3a**, p = 0.006).

The THRa isoforms -a1 and –a2 are in general positive prognosticators if expressed in the nucleus and negative prognosticator if expressed in the cytoplasm, respectively. In detail, THRa1 is a general positive prognosticator if expressed in the nucleus with a median survival of 4.22 years for patients positive for THRa1 and 2.08 years for patients that do not express THRa1 in the nucleus (**Figure 3b**, p = 0.024). Subgroup analyses of mucinous carcinomas showed that THRa1 is a negative prognosticator if expressed in the cytoplasm. The median survival time is 16.59 years for mucinous carcinoma patients that do not express THRa1 in the cytoplasm and 2.87 years for mucinous carcinoma patients with cytoplasmic THRa1 expression (**Figure 3c**, p = 0.037).

The THRa2 receptor in general is a negative prognosticator if expressed in the cytoplasm. The median survival time is 3.75 years for patients and 1.37 years for patients with THRa2 in the cytoplasm (**Figure 3d**, p = 0.001). Nuclear expression of THRa2 is not a general positive prognosticator. This can be found in the subgroup of serous carcinomas. The mean survival time for serous carcinoma patients with nuclear THRa2 expression is 6.21 years in contrast to 2.32 years for patients with no nuclear THRa2 expression (**Figure 3e**, p = 0.002). It is remarkable that patients with clear cell carcinomas show opposite results. The median survival time for clear cell carcinoma patients with nuclear THRa2 expression is only 1.65 years in contrast to 5.24 years for patients with no nuclear THRa2 expression (**Figure 3f**, p = 0.034).

Comparison of THRa, -a1 and –a2 expression in low-grade and high-grade serous ovarian cancer

As shown in **Figure 4**, the expression of all three a-subunits is higher in the nucleus of low grade serous ovarian cancer cases with a trend to significance in the general THRa (p=0.078), no significance for THRa1 and a significantly higher THRa2 expression in low-grade serous cancer cases compared to high-grade subtype.

Cox Regression analyses of survival

Cox regression (**Table 1**) was performed to identify independent predictors for OS. Pattern of age at surgery failed to remain significant within multivariate testing, while grading, FIGO staging, THRa1 in the nucleus (**1A**, p = 0.043) and THRa2 in the cytoplasm (**1B**, p = 0.002) was still predictive in multivariate testing sets regarding all subtypes of the study group. Due to missing clinical data in single cases cox regression analyses was available in 146 out of 156 cases.

Discussion

Within this study we analyses the prognostic value of the thyroid hormone receptor alpha forms 1 and 2. The general THRa has prognostic value only in clear cell carcinomas, where it is expressed at the highest immune scores. The differential analyses of nuclear versus cytoplasmic expression of THRa1 and THRa2 revealed striking differences concerning the overall survival of ovarian cancer patients.

The thyroid hormone receptor alpha (THRa) exhibits a dual role as an activator or repressor of gene transcription. Former studies showed that THRa, formerly thought to reside solely in the nucleus and tightly bound to the DNA, shuttles rapidly between the nucleus and the cytoplasm [35, 36].

The role of thyroid hormones and its receptors was not very well understood in ovarian cancer biology for a longer time, only very recent publication showed their tremendous roles for this deadly disease. Early investigations with ovarian cancer cell lines and T3, T4 and reversed T3 stimulation did not result in sufficient stimulation or inhibition outcomes [37]. Later it was found that messenger RNA transcripts for THRa1, THRa2, T3 activating deiodinase 2 and inactivating deiodinase 3 are present in

primary ovarian surface epithelial cell cultures [20]. A more recent study described that for ovarian cancer patients conflicting results were observed for T3 and T4 levels in the serum. Insignificant differences were found for T3 ($p = 0.209$) and T4 ($p = 0.050$) as compared to controls [15].

An actual study described that αvβ3 integrin, a plasma membrane receptor that bind the thyroid hormones T3 and T4, is overexpressed in ovarian cancer [18]. Both hormones induced cell proliferation and significantly reduced the expression of genes that inhibit cell cycle particularly in ovarian cancer cells (OVCAR-3) with high integrin expression [18]. The same group studied the expression of fifteen genes involved in DNA repair, cell cycle, apoptosis, and tumor suppression in OVCAR-3 and A2780 cell lines, using real-time PCR following short incubation with T3 or T4 [38]. The thyroid hormones downregulated the expression of the majority of genes examined, showing that these hormones influence the expression of cancer-relevant-genes in ovarian cancer [38]. The same group hypothesized that natural thyroid hormone derivatives may antagonize these actions. The three antagonists, tetraiodoacetic acid (tetrac), triiodothyroacetic acid (triac) and 3-iodothyronamine (T1AM) inhibited cell proliferation and induced cell death and DNA damage in the two ovarian cancer cell lines (OVCAR3 and A2780). Therefore, they concluded that the cytotoxic potential of thyroid hormone derivatives, tetrac, triac and T1AM, in ovarian cancer might provide a much-needed novel therapeutic approach [39].

Based on the results of the former study, another group described that thyroid hormone causes elevated phosphorylation and nuclear enrichment of ERa [40]. In addition, confocal microscopy indicated that both T4 and estradiol caused nuclear translocation of integrin αv and phosphorylation of ERa [40]. Within our study, we found a positive correlation between the THRa2 in the nucleus and ERa. We also found positive correlation of THRa in the nucleus and ERb, assuming that thyroid hormones not only elevate the nuclear enrichment of ERa but also might influence ERb. Another study showed that THRa1 inhibits the ERa transactivation from the consensus estrogen response element (ERE). In contrast, the ligand bound THRa1 facilitates ERb-mediated transactivation [41]. We also found a positive correlation between the GPER and THRa. Sheng et al. showed that the GPER together with integrin αvβ3 participate in the induction of male germ cell proliferation and thyroid transcription

disruption after low-dose Bisphenol A treatment [42]. Another correlation of our study was found between THRa in the nucleus and the FSH receptor, whereas the THRa expression in the cytoplasm showed a positive correlation to the LH/hCG receptor. It has been known for a longer time that LH, FSH, and TSH show low-level cross-reactivity between their respective receptors [43]. Vissenberg et al. explained that T3 in combination with FSH enhances granulosa cell proliferation and inhibits granulosa cell apoptosis by the PI3K/Akt pathway [44]. They also described that T3 is considered a biological amplifier of the stimulatory action of gonadotrophins on granulosa cell function [44]. Because the exclusive expression of the FSHR has already been described by our group as a negative prognosticator in ovarian cancer cases, our finding about enhanced expression of both FSHR and THRa in the nucleus might lead to new treatment strategies for this type of cancer [24]. This assumption might also apply for the antibody Gatipotuzumab and its TA-MUC1 epitope [45], which showed an inverse correlation to THRa1 and -2 expression either in the nucleus or in the cytoplasm, respectively.

In addition, T4 has been shown to promote ovarian cancer cell proliferation via integrin αvβ3. T4 also induced the activation of ERK1/2 and expression of programmed death-ligand 1 (PD-L1) in ovarian cancer cells [46]. In contrast, resveratrol binds to integrin αvβ3 at a discrete site and induces p53-dependent anti-proliferation in malignant neoplastic cells. T4 impairs resveratrol-induced anti-proliferation in human ovarian cancer cells and T4 inhibited resveratrol-induced nuclear accumulation of COX-2 [46]. Furthermore, T4 increased expression and cytoplasmic accumulation of PD-L1, which in turn acted to retain inducible COX-2 in the cytoplasm [46]. Thus, T4 inhibits COX-2-dependent apoptosis in ovarian cancer cells by retaining inducible COX-2 with PD-L1 in the cytoplasm [46]. Recently, the interplay between epithelial-mesenchymal transition (EMT) and the thyroid hormones-αvβ3 axis in ovarian cancer was investigated [47]. It was found that the transcription of mesenchymal markers, β-catenin, zeb-1, slug/snail, vimentin, and n-cadherin was hardly affected by T3 and T4, while that of the epithelial markers, e-cadherin and zo-1, and was inhibited after treatment with thyroid hormones. These results suggest a novel role for the thyroid hormone-αvβ3 axis in EMT, with possible implications for ovarian cancer metastasis [47].

Finally, a group investigated the role of the thyroid hormone receptor Interactor 13 (TRIP13) in epithelial ovarian cancer (EOC) [48]. Bioinformatics analysis showed that TRIP13 was one of the most significantly upregulated proteins in EOC. Results of the described study showed that TRIP13 acted as an onco-promotive regulator in EOC development by modulating the Notch signaling pathway [48].

A large demographic study – the “Ovarian Cancer Association Consortium” showed that hyperthyroidism within the 5 years before ovarian cancer diagnosis was associated with an increased risk of death [49]. These very recent results were accompanied by the fact that a more modest association was observed with the history of hypothyroidism (n = 624 cases) and mortality [49].

Taken together the results of the experimental and demographic studies about the roles of thyroid hormones, its receptors and interacting proteins. There is growing body of evidence that they play a major role in ovarian cancer biology and survival of ovarian cancer patients. Only recent studies were able to bring new light into this area of research.

Conclusions

With our study, we could show that there is a direct link between nuclear expression of THRa1 or -2 and better survival in EOC, except for the subgroup of clear cell carcinomas. The latter group seems to have different properties concerning THRa expression. Shifting the expression of THRa1 or -2 to the cytoplasm seems to be connected with reduced overall survival in EOC cases. Therefore, the search for THRa interacting factors that prevent this shift to the cytoplasm seems to be a useful new approach for the search of future treatment strategies against the threatening disease of Epithelial Ovarian Cancer.

Abbreviations

COX	cyclooxygenase
EOC	epithelial ovarian cancer
ER	estrogen receptor
FöFoLe	Förderung für Forschung und Lehre
LMU	Ludwig-Maximilians Universität München
THR	thyroid hormone receptor

T3	triiodothyronine
T4	thyroxine

Declarations

Ethics approval and consent to participate: The Ethics Committee of the Ludwig-Maximilians-University, Munich, Germany (approval number 227-09) on 30 September 2009, approved the study. All tissue samples used for this study were obtained from leftover material from the archives of LMU Munich, Department Gynaecology and Obstetrics, Ludwig-Maximilians-University, Munich, Germany, initially used for pathological diagnostics. When this retrospective study was initiated, diagnostic procedures had already been fully completed; the tissue samples were classified as leftover material and underwent irreversible anonymization. Under these circumstances, no individual written informed consent was needed as per declaration of the Ethics Committee of the Ludwig-Maximilians-University. All experiments were performed according to the standards of the Declaration of Helsinki (1975).

Consent to Publish: Not Applicable.

Author Contributions: N.D. S.H. U.J. D.M. conceived and designed the experiments. C.S. C.K. A.H. E.S. performed the experiments. B.S. F.T. S.M. J.E. analyzed the data. N.D. U.J. wrote the paper. All authors have read and approved the manuscript.

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Availability of data and materials: Raw data are provided as Suppl. File 1.

Tables

Table 1A: Multivariate survival analyses including age, grading, staging and THRa1 expression in the nucleus (n = 146).

Variables	P-value	Hazard ratio	95.0% Confidence Interval	
			lower	upper
THRα1 (nucleus) IRS = 0 versus IRS ≥1	0.043	0.617	0.386	0.986
FIGO I/II versus III/IV	0.000	0.360	0.207	0.626
Grading G1 versus G2/3	0.001	0.365	0.202	0.660
Age < 60 versus ≥ 60 years	0.116	0.717	0.473	1.085

Table 1B: Multivariate survival analyses including age, grading, staging and THRa2 expression in the cytoplasm (n = 146).

Variables	P-value	Hazard ratio	95.0% Confidence Interval	
			lower	upper
THRα2 (cytoplasm) IRS 0 versus IRS >0	0.002	2.783	1.463	5.294
FIGO I/II versus III/IV	0.000	0.347	0.200	0.601
Grading G1 versus G2/3	0.000	0.343	0.191	0.618
Age < 60 versus ≥ 60 years	0.210	0.769	0.509	1.160

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Figures

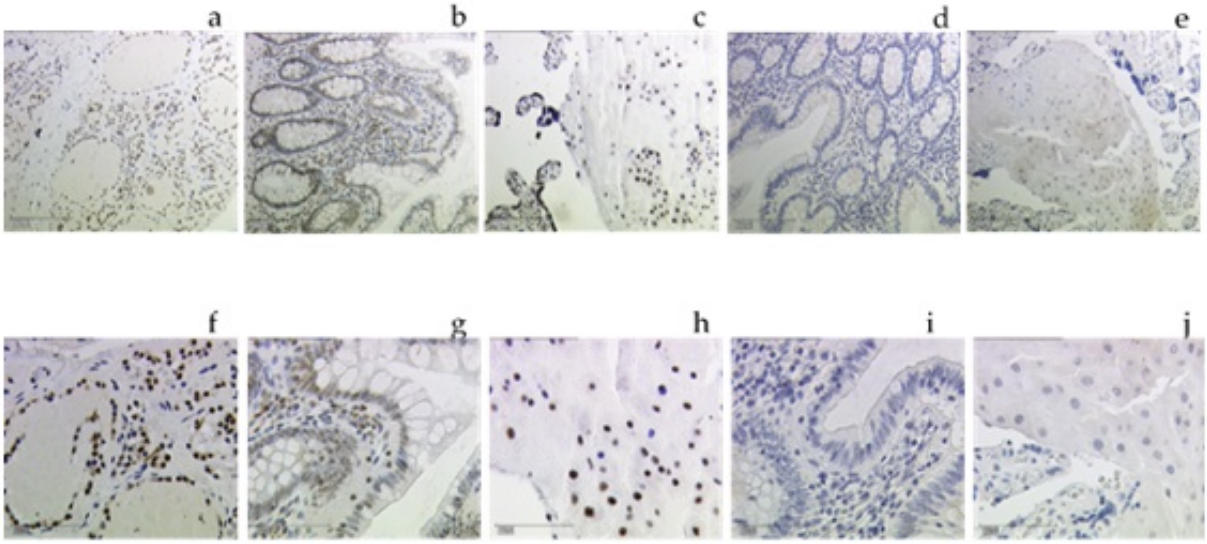

Figure 1

Positive and negative control staining for THRα antibodies used: (a) THRα staining in Struma tissue (10x lens). (b) THRα1 staining in colon tissue (10x lens). (c) THRα2 staining in placental tissue (10x lens). (d) THRα1 negative control in colon tissue (10x lens). (e) THRα2 negative control in placental tissue (10x lens). (f) THRα staining in Struma tissue (25x lens). (g) THRα1 staining in colon tissue (25x lens). (h) THRα2 staining in placental tissue (25x lens). (i) THRα1 negative control in colon tissue (25x lens). (j) THRα2 negative control in placental tissue (25x lens).

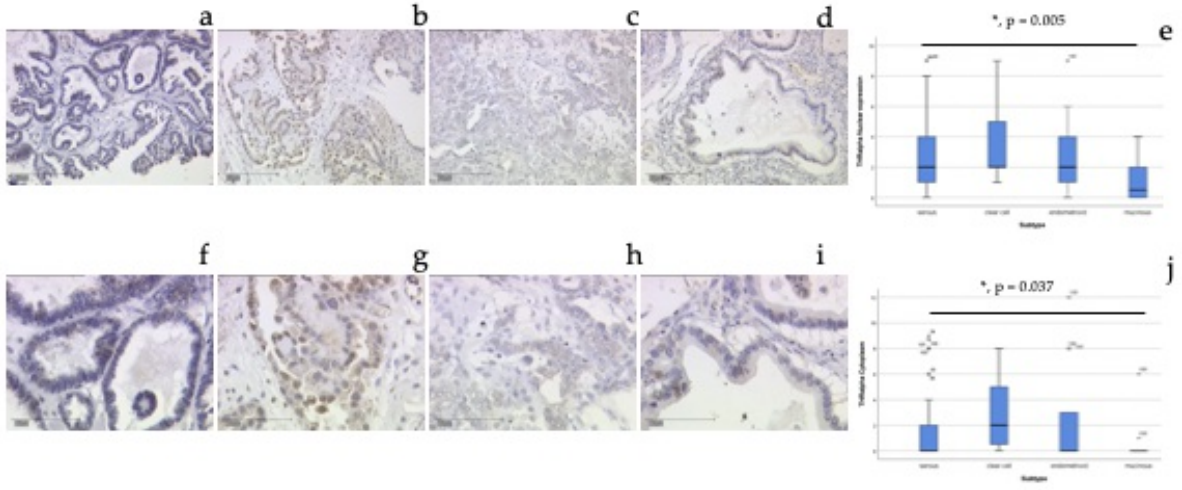

Figure 2

(a) THRα expression in serous carcinoma (10x lens). (b) THRα expression in clear cell carcinoma (10x lens). (c) THRα expression in endometrioid carcinoma (10x lens). (d) THRα expression in mucinous carcinoma (10x lens). (e) summary of THRα expression in different carcinoma subtypes (nuclear expression). (f) THRα expression in serous carcinoma (25x lens). (g) THRα expression in clear cell carcinoma (25x lens). (h) THRα expression in endometrioid carcinoma (25x lens). (i) THRα expression in mucinous carcinoma (25x lens). (j) summary of THRα expression in different carcinoma subtypes (cytoplasmic expression).

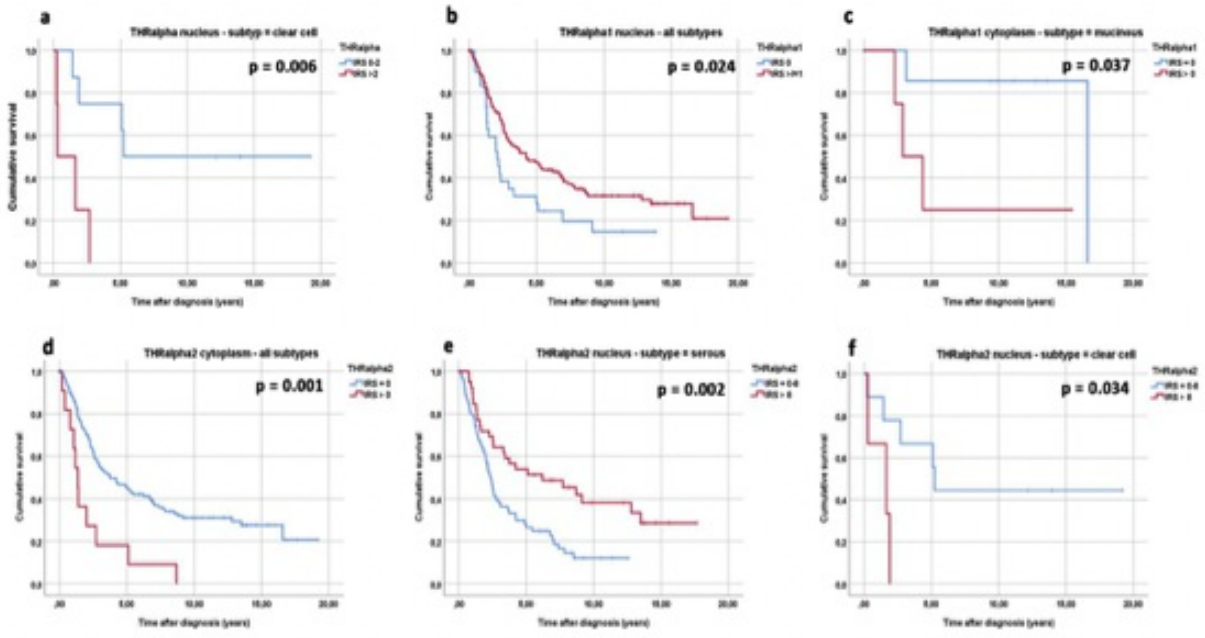

Figure 3

Kaplan–Meier estimates of THRα expression, THRα1 expression and THRα2 expression were analyzed. In the clear cell subtype, patients with a high nuclear expression of THRα showed a significantly reduced overall survival compared with patients with a low nuclear expression (a). In addition, high nuclear THRα1 expression was associated with significantly better overall survival in all ovarian cancer subtypes compared to patients with a low nuclear THRα1 expression (b). Patients with high THRα1 expression in the cytoplasm and mucinous subtype had a significantly decreased overall survival compared with those mucinous carcinoma patients with low cytoplasmic expression (c). High cytoplasmic THRα2 expression was associated with a significantly reduced overall survival in all ovarian cancer subtypes compared to patients with a low cytoplasmic THRα2 expression (d). In the serous subtype, patients with a high nuclear expression of THRα2 showed a significantly better overall survival compared with patients with a low nuclear expression (e). Finally, in the clear cell subtype, patients with a high nuclear expression of THRa2 showed a significantly reduced very low overall survival (all patients deceased within two years) compared to patients with a low nuclear expression (f).

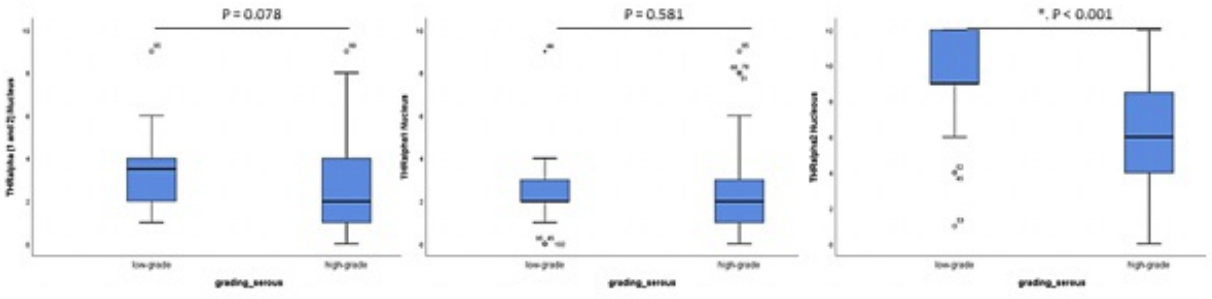

Figure 4

Comparison of Immunohistochemical staining results of the different THR (median values) in the nucleus of the high- and low-grade serous ovarian cancer subtypes. (IRS: Immunoreactive Score, THR: Thyroid Receptor). The expression of THRα2 in the nucleus is significantly different in low-grade compared to high-grade serous carcinomas (marked by an asterisk).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

Data set.xlsx