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Common death receptor 4 (DR4) polymorphisms do not predispose to ovarian cancer

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Abstract

Objective. Polymorphisms of death receptor 4 (DR4) might impair the apoptotic signal transduction and lead to dysregulation of the homeostasis between cell survival and cell death, promoting tumor development and progression.

Methods. We performed an analysis of known DR4 polymorphisms, namely G442A, C626G, and A1322G, in germ line DNA of 97 ovarian cancer patients and controls as well as in established ovarian cancer cell lines.

Results. Patient and matched control populations were not differing significantly in case of G442A ($P = 0.736$) and C626G alterations ($P = 0.699$). For the A1322G transversion, we generated population data for the first time and could find a rate of 19% heterozygotes and 3% homozygotes. Again, we could not detect any significant difference between patients and controls ($P = 0.326$).

Conclusion. To summarize, alterations of the DR4 gene do not lead to clinically relevant ovarian cancer predisposition and are therefore most unlikely to contribute to familial ovarian cancer.

Keywords: Ovarian cancer; DR4 protein; Polymorphism; RFLP

Introduction

Ovarian cancer is the most lethal gynecologic malignancy and the fourth most frequent cause of cancer-related death in women. Mostly to late diagnosis, most patients have a poor prognosis despite frequent initial response to chemotherapeutic regimens. Ovarian cancer cells were shown to be sensitive to TRAIL-induced apoptosis and combination with chemotherapeutic drugs enhances the apoptotic effect of TRAIL [1–3]. TRAIL induces apoptosis by ligation to death receptor DR4 and death receptor 5 (DR5, TRAIL-R2, KILLER) [4–6] and subsequent activation of the apoptotic cascade through caspase 8 and FADD [7], forming the death inducing signaling complex (DISC). Two other, truncated, TRAIL receptors, decoy receptor 1 (DcR1, TRAIL-R3, TRID) and decoy receptor 2 (DcR2, TRAIL-R4, TRUNDD), can interfere with the programmed cell death induction by TRAIL [4,6,8,9].

Latest evidence for the involvement of the TRAIL system in ovarian cancer is the fact that TRAIL expression was shown to correlate closely with the prognosis of ovarian cancer patients [10]. DR4 may play an important role during tumor development in ovarian cancer either as an endogenous factor in the regulation of apoptosis of tumor cells or as an exogenous factor in the immune escape of tumor cells [11]. Moreover, all four receptors for TRAIL were mapped to chromosome 8p22–p21 [8,9], a region we and other research groups found frequently deleted in ovarian cancer [12,13], suggesting that genes located in this area may be crucially involved in the pathogenesis of this disease.

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Missense mutations at the 5′ end of the DR4 gene may interfere with the ligand–receptor interaction of TRAIL, whereas 3′ terminal mutations may influence DISC formation. Identification of these mutations in an ovarian cancer population and definition of their impact on TRAIL sensitivity in this population would be needed in terms of future TRAIL-based therapy. Mutations in the ectodomain of DR4, namely G422A (R141H) and C626G (T209R), were found in a higher frequency in primary tumors of different origin (SCLC, NSCLC, HNSCC, gastric adenocarcinoma) as compared to matched controls [14]. Additionally, these alterations were suggested to be mostly germline in origin and having influence on tumorigenesis of some cancer entities, particularly in association with additional environmental influences, like smoking [15]. In addition, another DR4 polymorphism (A1322G, K441R) in the death domain of DR4 was reported, and this time the genotype was functionally linked to resistance against TRAIL-induced apoptosis in several cancer cells [16]. This prompted us to investigate the possible influence of DR4 polymorphisms on genetic predisposition to ovarian cancer in a population-based study comparing the frequency of genotypes in ovarian cancer patients and matched controls as well as in ovarian cancer cell lines.

Materials and methods

Study population and statistical analysis

Ninety-seven patients (mean age at diagnosis 55.8 (SD ± 12.0) years) with pathologically confirmed epithelial ovarian cancer diagnosed between 1981 and 2000 were included in our observation (Table 1). Written informed consent was obtained from all patients before drawing blood samples.

Table 1
Patients' characteristics ($N = 92$)[a,b]

	n	%
Histology		
Serous	51	55.4
Mucinous	9	9.8
Endometrioid	15	16.3
Clear cell	3	3.3
Undifferentiated	11	12.0
Mixed	3	3.3
Grading		
1	26	28.3
2	28	30.4
3	39	41.3
FIGO		
I	33	35.9
II	5	5.4
III	45	48.9
IV	9	9.8

[a] All patients were of Caucasian ethnicity.
[b] For 5 patients, information regarding the above criteria was not available (except for epithelial ovarian cancer).

One hundred subjects without a history of malignant disease, but corresponding by age, sex, and race, were chosen as controls. Deviations from Hardy Weinberg equilibrium were calculated for all groups and the goodness of fit χ^2 test was used to compare the observed allele frequencies between patient and control populations. Risk of ovarian cancer associated with DR4 polymorphisms was assessed using odds ratio estimate (OR) and 95% confidence interval (CI). Our study had >80% power to detect the protective effect (OR = 0.45) of the homozygous C626G alteration as described by Hazra et al. [15]. The G422A allele cosegregates with C626G in about 96%, so similar odds ratios were expected. For the A1322G polymorphism, we had a more than 80% power to detect an OR = 2 for the heterozygous and an OR = 6 for the homozygous population as based on our results.

Cell culture and genotype analysis

Epithelial ovarian cancer cell lines ES-2 and Caov-3 were obtained from ATCC (Rockville, MD, USA), cell lines A2780 and A2780ADR from ECACC (Salisbury, Wiltshire, UK), and cell lines OV-MZ-15 and OV-MZ-26 were established from ascitic fluid of ovarian cancer patients. All cell lines were cultured with appropriate media and kept at standard conditions. Genomic DNA was extracted from peripheral blood lymphocytes of ovarian cancer patients and controls. For the A1322G polymorphism, we sequenced the death domain of DR4 using an automated fluorescence-based cycle sequencer (ABI PRISM® 310 Genetic Analyzer, Applied Biosystems). PCR products generated with primers DR4-11 and DR4-12 [16] were subjected to sequencing. To detect the G422A and the C626G polymorphism, a PCR with subsequent RFLP analysis was performed according to Fisher et al. [14]. The A1322G polymorphism was sequenced from both sides in all patients and the RFLP results were also validated by sequencing in ten cases.

Results

G422A polymorphism

The G to A transversion results in an amino acid change of a histidine for an arginine (R141H) and creates a new and unique *Fok*I restriction site and therefore yields a 160-bp and a 70-bp fragment upon incubation with *Fok*I. From the six cell lines studied, two had the homozygous GG variant (OV-MZ-26 and ES-2), one was heterozygous (OV-MZ-15) and three had the homozygous CC variant (A2780, A2780ADR and Caov-3) (Fig. 1). In the study population, both cohorts fulfilled the HW equilibrium criteria. In direct comparison, the GG, GA, and AA alleles were almost equally distributed in both, patients and controls ($P = 0.736$; Table 2).